BEHAVIORAL FUNCTIONS OF THE RAT VENTRAL AND DORSAL HIPPOCAMPUS: EFFECTS OF LOCAL PHARMACOLOGICAL MANIPULATIONS ON SENSORIMOTOR AND MEMORY PROCESSES

A dissertation submitted to the
SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH
for the degree of
Doctor of Natural Sciences

presented by

Tobias Bast
Dipl.-Biochemist, University of Bochum

born March 28th, 1974

citizen of Germany

accepted on the recommendation of

Prof. Dr. Joram Feldon, examiner
Prof. Dr. John P. Aggleton, co-examiner

2002
ACKNOWLEDGEMENTS

The work for the present thesis was conducted in the Laboratory of Behavioral Neurobiology at the Research Unit Schwerzenbach of the Swiss Federal Institute of Technology Zurich (ETHZ). It was financially supported by the Swiss National Science Foundation and the ETHZ and strongly benefited from the excellent infrastructure provided by the ETHZ as well as from the outstanding facilities available in the Laboratory of Behavioral Neurobiology.

I thank Joram Feldon, the head of the laboratory, for providing these excellent facilities and for the supervision of the present thesis, and I am grateful to John Aggleton for being willing to act as a co-referee of the thesis.

Thanks are due to several people from the support staff of the Lab and of the Research Unit Schwerzenbach for their excellent work: Peter Schmid for his outstanding help with any kind of technical equipment; Liz Weber for histological preparations; Christian Schlatter for cannulae manufacturing; Oliver Asprion, Pascal Guela, Sepp Torlucci, and Jeanne von Arx for animal care; Bonnie Strehler and Jane Fotheringham for help with manuscript preparation and administrative matters.

Most of the studies included in the present thesis were conducted in collaboration with Wei-Ning Zhang, and several with Marie Pezze. In addition, Boris Ferger, Daria Peleg, and Ilsun White were collaborators in some of the studies. This collaboration is gratefully acknowledged.

I also thank Ilsun White for strongly supporting me and teaching me a lot during the first time of the work for my thesis. Wei-Ning Zhang was also very helpful in getting started with experiments.

Particular thanks are due to Marie Pezze. The work presented in the present thesis strongly benefited from the fact that her way to deal with scientific subjects is very complementary to mine.

Andreas Leng was always very willing to help and really extremely helpful when problems occurred related to HPLC equipment and computer matters.

Finally, I want to thank all current and former colleagues in the Lab for occasional help, advice, or support, in particular Holger Russig, who started working on his Ph.D. when I started working on mine, and Ben Yee, with whom I have had interesting discussions about hippocampus, behavior, and statistics.
# TABLE OF CONTENTS

**ABSTRACT** ..................................................................................................................1

**ZUSAMMENFASSUNG** ...............................................................................................3

**INTRODUCTION:**
Objectives and outline of the thesis .............................................................................6

**PART I:**
Hippocampus and sensorimotor processes: The effects of pharmacological manipulations of the ventral and dorsal hippocampus on locomotor activity, prepulse inhibition (PPI), and startle reflex ..........................................................................................................................12

I.1. Effects of MK801 and neuroleptics on prepulse inhibition: Re-examination in two strains of rats .................................................................................................................................13

I.2. Microinfusion of the noncompetitive N-methyl-D-aspartate antagonist MK-801 (dizocilpine) into the dorsal hippocampus of Wistar rats does not affect latent inhibition and prepulse inhibition but increases startle reaction and locomotor activity ............25

I.3. Hyperactivity and disruption of prepulse inhibition induced by N-methyl-D-aspartate stimulation of the ventral hippocampus and the effects of pretreatment with haloperidol and clozapine. .........................................................................................................................36

I.4. Hyperactivity, decreased startle reactivity, and disrupted prepulse inhibition following disinhibition of the rat ventral hippocampus by the GABA<sub>A</sub> receptor antagonist picrotoxin. .........................................................................................................................47

I.5. Effects of hippocampal N-methyl-D-aspartate infusion on locomotor activity and prepulse inhibition: differences between the dorsal and ventral hippocampus ........57

I.6. Prepulse inhibition in rats with temporary inhibition/inactivation of ventral or dorsal hippocampus .................................................................................................................................70
Table of contents

I.7. Hippocampal modulation of sensorimotor processes: Effects of pharmacological manipulations of the rat ventral and dorsal hippocampus on locomotor activity, prepulse inhibition (PPI), and startle reflex ................................................ 82
[Bast, Feldon: in preparation for submission]

PART II:

Hippocampus and simple associative or relational memory: The effects of pharmacological manipulations of the ventral and dorsal hippocampus on classical fear conditioning to discrete and contextual cues ........................................ 140

II.1. The ventral hippocampus and fear conditioning in rats: different anterograde amnesias of fear after tetrodotoxin inactivation and infusion of the GABA<sub>A</sub> agonist muscimol .......................................................... 141

II.2. The ventral hippocampus and fear conditioning in rats: different anterograde amnesias of fear after infusion of N-methyl-D-aspartate or its noncompetitive antagonist MK-801 into the ventral hippocampus ........................................ 155
[Zhang, Bast, Feldon (2001) Behav Brain Res 126:159-174]

II.3. Dorsal hippocampus and classical fear conditioning to tone and context in rats: the effects of local NMDA-receptor blockade and stimulation ...................... 171
[Bast, Zhang, Feldon Hippocampus: in press]

II.4. Hippocampus and classical fear conditioning ........................................ 216

PART III:

Extrahippocampal mechanisms mediating the hippocampal control of sensorimotor processes and fear conditioning: the role of dopamine transmission in the medial prefrontal cortex ........................................ 220

III.1. Dopamine receptor blockade in the rat medial prefrontal cortex reduces spontaneous and amphetamine-induced activity and does not affect prepulse inhibition .......... 221
[Bast, Pezze, Feldon Behav Pharmacol: in press]

III.2. Significance of dopamine transmission in the rat medial prefrontal cortex for conditioned fear ........................................................... 233
[Pezze, Bast, Feldon Cereb Cortex: in press]
Table of contents

III.3. Post mortem study of changes in forebrain dopamine and serotonin transmission induced by ventral hippocampal N-methyl-D-aspartate stimulation in rats: evidence for an activation of dopamine transmission in the medial prefrontal cortex. 265
[Bast, Zhang, Ferger, Feldon: submitted]

III.4. The effects of ventral hippocampal stimulation by N-methyl-D-aspartate on dopamine transmission in the medial prefrontal cortex: preliminary results of an in vivo microdialysis study in freely moving rats. 287
[Bast, Pezze, Peleg, Ferger, Feldon: preliminary report]

MAIN CONCLUSIONS 307

CURRICULUM VITAE 310

LIST OF PUBLICATIONS 311
ABSTRACT

The mammalian hippocampus has been implicated in a wide variety of behavioral processes comprising basic sensorimotor processes, i.e. innate processes involving control of motor responses to sensory stimuli, and memory processes, i.e. processes serving the acquisition, retention, and use of information about past experiences. In humans, for example, certain aberrations of normal hippocampal function have been associated with schizophrenia and anxiety disorders, whose cardinal symptoms primarily reflect deficits in the processing of and responding to sensory information, while hippocampal mechanisms also appear to be of particular importance for episodic memory, i.e. the recollection of everyday events, which is severely disrupted by hippocampal lesions.

The research for the present thesis has, therefore, been aimed at a broad characterization of how hippocampal mechanisms contribute to behavior in rats, considering basic sensorimotor processes as well as processes related to memory. Specific pharmacological manipulations by local microinfusions of neuroactive drugs have been used to induce temporary alterations of neurotransmission in the ventral or dorsal hippocampus. The fact that drug microinfusions allowed separate manipulation of the ventral and dorsal hippocampus is important given that these two parts of the hippocampus differ in their neuroanatomical connections and, thus, may also have different behavioral functions. In behavioral pharmacological experiments, the effects of the pharmacologically induced temporary alterations in hippocampal neurotransmission on particular sensorimotor (Part I of the thesis) and memory processes (Part II of the thesis) have been characterized. The sensorimotor processes under study were locomotor activity, sensorimotor gating, as measured by prepulse inhibition (PPI) of the acoustic startle reflex, and the startle reflex itself. Alterations of these basic sensorimotor processes in rats have been related to psychosis and anxiety disorders in humans. As to the memory processes, the hippocampal contribution to both simple associative and to relational memory – the latter is linked to episodic memory – has been examined. For this purpose, the effects of hippocampal drug microinfusions on classical fear conditioning to tone and context have been investigated. Classical fear conditioning is simple associative learning when the conditioned stimulus (CS) is a discrete cue, such as a tone, but may involve relational learning when a context serves as CS. Additional experiments have been conducted in order to identify pathways and extrahippocampal mechanisms that possibly mediate the hippocampal modulation of behavior (Part III of the thesis). These experiments, using behavioral pharmacological as well as post mortem and in vivo neurochemical (microdialysis) approaches, focused on dopamine transmission in the medial prefrontal cortex (mPFC).
Abstract

The effects of hippocampal drug microinfusions on locomotor activity, PPI, and startle reflex, presented and discussed in Part I of the thesis, corroborate that hippocampal mechanisms directly regulate these three sensorimotor processes and that hippocampal dysfunction may contribute to neuropsychiatric diseases, in particular schizophrenia. The roles of the ventral and dorsal hippocampus, as well as the ways each of these hippocampal subdivisions influences different sensorimotor processes, can partly be dissociated. Thus, the hippocampal modulation of sensorimotor processes appears to involve multiple operations, rather than to represent one unitary operation. Interestingly, the results presented in Part I suggest that mechanisms in the ventral hippocampus are more closely linked to the regulation of two of the examined sensorimotor processes (locomotor activity and PPI) than dorsal hippocampal mechanisms. Thus, they complement previous data indicating that the dorsal hippocampus, as compared to the ventral hippocampus, plays a more important role for a particular form of hippocampus-dependent memory, spatial memory.

From the effects of hippocampal drug microinfusions on classical fear conditioning to tone and context, presented and discussed in Part II of the thesis, it can be concluded that some mechanisms [for example those requiring activation of N-methyl-D-aspartate (NMDA) receptors] in both ventral and dorsal hippocampus selectively contribute to relational memory. This corroborates prevalent concepts that the hippocampus is of eminent importance for this specific type of memory. However, some pharmacological manipulations of the ventral and dorsal hippocampus (pharmacological deactivation, stimulation by NMDA) also impaired fear conditioning to tone, i.e. simple associative learning, indicating that the hippocampal contribution to memory is not restricted to relational memory.

Importantly, as discussed extensively in the present thesis, the microinfusion studies presented in Part I and II suggest considerable modifications of prevalent notions that have emerged from studies examining the effects of hippocampal lesions. Thus, one methodological implication of the present thesis is that intracerebral drug microinfusions should increasingly be used in order to further the current understanding of structure-function relationships.

Finally, the studies presented in Part III of the thesis suggest that the ventral hippocampus may exert a positive control on dopamine transmission in the mPFC, and that this control may partly mediate the hippocampal modulation of locomotor activity and also be important for the expression of conditioned fear formed with participation of the hippocampus.
ZUSAMMENFASSUNG


Zusammenfassung

Furchtkonditionierung auf einen Ton oder einen Kontext, d.h. eine bestimmte Umgebung, hat. Klassische Furchtkonditionierung ist einfaches assoziatives Lernen, wenn der konditionierte Reiz (CS) ein diskreter Stimulus ist, beispielsweise ein Ton, während auch relationales Lernen an der Furchtkonditionierung beteiligt sein kann, wenn ein Kontext als CS dient. Zusätzliche Experimente wurden durchgeführt, um Verbindungen und extrahippokampale Mechanismen zu bestimmen, die möglicherweise die Verhaltensmodulation durch den Hippokampus vermitteln (Teil III der Doktorarbeit). Diese Experimente, die verhaltenspharmakologische sowie post mortem und in vivo neurochemische (Mikrodialyse) Ansätze verwendeten, konzentrierten sich auf die Dopamintransmission im medialen präfrontalen Kortex (mPFC).


Zusammenfassung

NMDA) beeinträchtigten jedoch auch Furchtkonditionierung auf einen Ton, d.h. einfaches assoziatives Lernen. Dies deutet darauf hin, daß der Beitrag des Hippokampus zu Gedächtnis nicht auf relationales Gedächtnis beschränkt ist.

Es ist von Bedeutung, und dieser Punkt wird in der vorliegenden Doktorarbeit ausgiebig diskutiert, daß die Mikroinfusionsstudien, die in Teil I und II vorgestellt sind, deutliche Änderungen gängiger Vorstellungen nahelegen, die aufgrund von Studien entstanden sind, welche die Effekte von hippokampalen Läsionen untersucht haben. Somit ist eine methodologische Schlußfolgerung der vorliegenden Arbeit, daß intracerebrale Mikroinfusionen vermehrt zur Anwendung kommen sollten, um das gegenwärtige Verständnis von Struktur-Funktionsbeziehungen zu erweitern.

Die Studien, die in Teil III der Doktorarbeit vorgestellt werden, legen schließlich nahe, daß der ventrale Hippokampus eine positive Kontrolle auf Dopamintransmission im mPFC ausübt und daß diese Kontrolle teilweise die hippokampale Modulation lokomotorischer Aktivität vermitteln könnte sowie möglicherweise für die Expression von konditionierter Furcht, die unter Beteiligung des Hippokampus gebildet wurde, von Bedeutung ist.
INTRODUCTION:
Objectives and outline of the thesis
It has been recognized that the mammalian hippocampus is involved in a wide variety of behavioral processes comprising basic sensorimotor processes, i.e. innate processes involving control of motor responses to sensory stimuli (e.g., Altman et al., 1973; Gray and McNaughton, 1983; Mogenson and Yang, 1991; Bland and Oddie, 2001; Vinogradova, 2001), and particular memory processes, i.e. processes serving the acquisition, retention, and use of information about past experiences (e.g., O'Keefe and Nadel, 1979; Olton et al., 1979; Rawlins, 1985; Morris and Frey, 1997; Eichenbaum, 2000; Fanselow, 2000; Aggleton and Pearce, 2001). Accordingly, in humans, certain aberrations of hippocampal function have been associated, for example, with schizophrenia, whose cardinal symptoms primarily reflect deficits in the processing of and responding to sensory information (e.g., Gray et al., 1991; Harrison and Eastwood, 2001; Heckers, 2001), while hippocampal mechanisms appear also to be of particular importance for episodic memory, i.e. the recollection of everyday events, which is severely impaired by hippocampal lesions (e.g., Tulving and Markowitsch, 1998; Buckner, 2000; Eichenbaum, 2000). The research for the present thesis has, therefore, been aimed at a broad characterization of how hippocampal mechanisms contribute to behavior in rats, considering basic sensorimotor processes as well as memory processes. First, we have characterized the effects of temporary alterations in ventral or dorsal hippocampal activity, induced by local microinfusions of neuroactive drugs, on particular sensorimotor (Part I of the thesis) and memory processes (Part II of the thesis). The fact that drug microinfusions allow separate manipulation of the ventral and dorsal hippocampus is of importance given that these two parts of the hippocampus differ in their neuroanatomical connections and, thus, may also have different behavioral functions (Witter, 1986; Moser and Moser, 1998). Second, we have conducted experiments in order to identify possible pathways and extrahippocampal mechanisms that might mediate the hippocampal modulation of behavior (Part III of the thesis). These experiments focused on dopamine transmission in the medial prefrontal cortex (mPFC).

The present thesis consists of research and review articles, originally written for separate peer-reviewed journal publications. All research papers constitute parts of a coherent research project and are linked by extensive cross-references. Moreover, the thesis includes two review articles (I.7., II.4.), which concisely and comprehensively describe and discuss the background, as well as the implications, of the different studies contributing to the present thesis. The research and review articles have been arranged in three parts.

Part I combines the work concerned with the hippocampal modulation of sensorimotor processes. It comprises six studies examining the effects of pharmacological manipulations of
the ventral or dorsal hippocampus on locomotor activity, sensorimotor gating, as measured by prepulse inhibition (PPI) of the startle reflex, and the startle reflex itself (I.1.-I.6.). Alterations of these sensorimotor processes in rats are related to neuropsychiatric diseases in humans, in particular to psychosis (Geyer et al., 1990; Lipska and Weinberger, 2000; for further explanation and additional references see I.1.-I.7.), and one main objective of these studies was to examine whether and what kind of hippocampal dysfunction may contribute to psychosis. Two experiments complementing the six studies of Part 1 and examining the effects of pharmacological deactivation of the ventral hippocampus, as well as of local N-methyl-D-asparte (NMDA)-receptor blockade, on locomotor activity are included in studies presented in Part 2 of the thesis (II.1., II.2.). Part 1 concludes with a review article (I.7.). This article presents the picture of the hippocampal modulation of locomotor activity, PPI, and startle reflex emerging from experiments examining the effects of pharmacological manipulations of the rat dorsal and ventral hippocampus, and identifies the implications and future research directions suggested by this work. One can also refer to this review article for a concise and comprehensive introduction to the background of the six studies presented in Part 1.

Part II presents three studies examining the effects of ventral and dorsal hippocampal pharmacological manipulations on classical fear conditioning to tone and context (II.1.-II.3.). Classical fear conditioning is simple associative learning when the conditioned stimulus (CS) is a discrete cue, such as a tone, but may involve relational learning, linked to episodic memory (Eichenbaum, 1996), when a context serves as CS. Thus, studying the effects of pharmacological manipulations of the hippocampus on classical fear conditioning to tone and context allows examining and possibly dissociating hippocampal mechanisms contributing to simple associative and to relational learning (Fanselow, 2000; for further explanation and additional references see II.1.-II.4.). This was the aim of the studies presented in Part II. Part II concludes with a commentary that concisely presents the implications that the effects of hippocampal drug microinfusions on classical fear conditioning have for views on the role of the hippocampus in fear conditioning, as well as for prevalent theories on hippocampus-dependent memory (II.4.).

Finally, Part III combines work concerned with possible pathways and extrahippocampal mechanisms mediating the behavioral effects of hippocampal drug microinfusions on sensorimotor processes and fear conditioning. This work focused mainly on the role of dopamine transmission in the mPFC. There is some evidence that dopamine in the mPFC is involved in the regulation of sensorimotor processes and conditioned fear (Morrow et al.,
1999a,b; Feenstra et al., 2001; Tzschentke, 2001; for further details and additional references see III.1., III.2.). Neuroanatomical characteristics of the hippocampo-prefrontal cortex pathway and related neuronal circuits suggest that prefrontal dopamine transmission may be intimately related to and interact with hippocampal activity (Thierry et al., 2000). The studies presented in Part III, two behavioral pharmacological (III.1., III.2.) and two neurochemical (III.3., III.4.) studies, were aimed at clarifying the relation between dopamine in the mPFC and sensorimotor processes and conditioned fear, as well as the relation between hippocampal activity and prefrontal dopamine transmission. The two behavioral pharmacological studies investigated the effects of pharmacological manipulations of prefrontal dopamine transmission on the regulation of sensorimotor processes (III.1.) and on fear conditioning (III.2.). The two neurochemical studies examined the effects of ventral hippocampal NMDA infusion on post mortem measures of dopamine and serotonin transmission in several forebrain areas, including the mPFC (III.3.), as well as on dopamine transmission in the mPFC of freely moving rats using in vivo microdialysis (III.4.). Combining the information from the behavioral pharmacological and the neurochemical experiments allows the determination of whether or not hippocampal mechanism may influence sensorimotor processes or conditioned fear via a modulation of prefrontal dopamine transmission. Respective considerations are made in the discussions of the studies presented in Part II and are also the subject of the paragraphs on possible extrahippocampal mechanisms mediating the hippocampal modulation of sensorimotor processes contained in the review article in Part I of this thesis (I.7.).
References


Introduction


Morrow BA, Elsworth JD, Rasmusson AM, Roth RH (1999a) The role of mesoprefrontal dopamine neurons in the acquisition and expression of conditioned fear in the rat. Neuroscience 92:553-564.


PART I:

Hippocampus and sensorimotor processes: The effects of pharmacological manipulations of the ventral and dorsal hippocampus on locomotor activity, prepulse inhibition (PPI), and startle reflex
Effects of MK801 and neuroleptics on prepulse inhibition: re-examination in two strains of rats

Tobias Bast, Weining Zhang, Joram Feldon, Ilsun M. White*

Behavioural Neurobiology Laboratory, The Swiss Federal Institute of Technology Zurich, Schorenstrasse 16, Postfach, CH-8093 Zurich, Switzerland

Received 20 June 2000; received in revised form 30 August 2000; accepted 31 August 2000

Abstract

Disruption of prepulse inhibition (PPI) induced by NMDA receptor antagonists, such as MK801, has been used as an animal model of positive and negative symptoms of schizophrenia. Previous studies suggested that atypical, but not typical, neuroleptics can selectively restore MK801-induced PPI disruption and that such selectivity may depend on strain differences. The present study re-examined PPI disruption by systemic MK801 in Wistar (WS) and Sprague-Dawley (SD) strains, and addressed the issue whether clozapine (atypical), compared to haloperidol (typical), effectively antagonizes MK801-induced PPI disruption. In addition, we tested the effects of bilateral microinfusion of MK801 into the ventral hippocampus in WS. Systemic MK801 disrupted PPI in both strains. Neither clozapine nor haloperidol antagonized MK801-induced PPI in either strain. Our clozapine data do not agree with previous reports of clozapine’s ability to antagonize MK801-induced PPI disruption. Similar to previous results with SD, MK801 infusion into the ventral hippocampus failed to affect PPI in WS. In our view, the selective ability of atypical neuroleptics to restore PPI disruption by NMDA antagonists, and to serve as a tool for identifying possible atypical neuroleptics, requires further examination. PPI disruption with systemic MK801 may be due to the blockade of NMDA receptors in multiple brain sites. © 2000 Elsevier Science Inc. All rights reserved.

Keywords: Prepulse inhibition; NMDA; Dizocilpine; Hippocampus; Clozapine; Haloperidol; Schizophrenia

The phenomenon of prepulse inhibition (PPI) is the reduction of an acoustic startle response to an intense acoustic stimulus (pulse) that is immediately preceded by a stimulus of lower intensity (prepulse). The prepulse itself does not elicit a startle response, but is thought to activate inhibitory neuronal processes which attenuate or gate the startle response. Thus, PPI is a frequently used measure of sensorimotor gating in both humans and rats. Schizophrenic patients exhibit disruption of PPI and related measures, suggesting disruption of sensorimotor gating [5–7,10]. In rats, disruption of PPI can be induced by administration of either NMDA antagonists or dopamine agonists [13,18,19,26,28,34]. The similarity between pharmacologically induced PPI disruption in rats and PPI disruption exhibited by schizophrenic patients suggests that PPI disruption is an animal model of sensorimotor gating deficits with construct validity for schizophrenia.

Although NMDA antagonists and dopamine agonists produce a comparable disruption of PPI, the ability of neuroleptics to antagonize PPI disruption induced by these drugs may differ. Pretreatment with either a typical or an atypical neuroleptic, haloperidol and clozapine, respectively, antagonized PPI disruption produced by dopamine agonists, such as apomorphine [19,22,28,29,30,32]. In contrast, PPI disruption induced by NMDA antagonists, such as phencyclidine (PCP), MK801, and ketamine, was unaffected by pretreatment with a typical neuroleptic, haloperidol [8,11,12,14,26,34]. Pretreatment with atypical antipsychotics, however, has yielded conflicting results. For example, clozapine, olanzapine, seroquel, and remoxipride antagonized PPI disruption by PCP or MK801 in Sprague-Dawley (SD) rats [1,4,12,25]. On the other hand, studies using SD and Wistar (WS) rats found that clozapine was as ineffective as haloperidol in antagonizing PPI disruption induced by MK801 and PCP [11,12,34,37]. It has been suggested that strain differences may contribute to such discrepancy in clozapine effects [34]. Thus, the question whether atypical neuroleptics possess the ability to antag-
onize PPI disruption by NMDA receptor antagonists in different strains of rats requires further examination. Indeed, such selective effects of atypical neuroleptics on PPI can be used as a possible measure for identifying potential atypical neuroleptics.

Assessing PPI disruption induced by noncompetitive NMDA receptor antagonists is worthwhile for another reason: it may reveal whether different neural structures are involved in mediating PPI disruption by NMDA antagonists and by dopamine agonists (for review, see Ref. [15]). One theory emphasizes that disrupted PPI can be used as a possible measure for identifying potential atypical neuroleptics. Indeed, such selective effects of atypical neuroleptics on different strains of rats requires further examination of the specific role of the ventral hippocampus in PPI disruption by NMDA receptor antagonists in the nucleus accumbens [17,28,29,39]. This notion is supported by findings that haloperidol, a potent dopamine D2 receptor antagonist, antagonizes apomorphine-induced PPI disruption. Given that haloperidol failed to antagonize PPI disruption produced by noncompetitive NMDA receptor antagonists, the reversibility by haloperidol appears to be limited to PPI changes mediated by the dopaminergic system. Thus, it is conceivable that brain areas involved in NMDA-mediated expression of PPI may differ from the areas involved in dopamine-mediated effects. Although the precise target site for systemic MK801 is not clear, the hippocampus, which has a higher NMDA receptor expression and PCP binding sites than any other brain regions [21,24], may be critically involved in modulation of PPI [17,29]. Moreover, stimulation of the NMDA receptors in the ventral hippocampus by NMDA infusion disrupts PPI [16,35,40]. Interestingly, however, bilateral infusion of NMDA receptor antagonist, MK801, into the dorsal, but not the ventral, hippocampus disrupted PPI in SD rats [2,3]. These findings suggest that within the hippocampus, there may exist a regional difference in NMDA receptor-mediated processes. Within the ventral hippocampus, overstimulation of NMDA receptors disrupts PPI, whereas a blockade of NMDA receptors in the same region may not affect PPI expression.

The present study examined MK801-induced PPI disruption. In the first part of the study, we re-examined the disruption of PPI by systemic MK801 in both WS and SD rats, addressing the issue whether clozapine, compared to haloperidol, is effective in antagonizing MK801-induced PPI disruption. Emphasis was placed on two issues: First, Bakshi et al.'s [4] findings suggested that a narrow range of effective doses of clozapine antagonized the PPI disruption by MK801 and PCP in SD rats. We approached this issue by testing various combinations of MK801 (0.05–0.1 mg/kg) and clozapine (5 and 10 mg/kg) on PPI in WS rats and by testing MK801 (0.1 mg/kg) and clozapine (5 and 10 mg/kg) in SD rats. Second, findings of Bakshi et al. [4] suggested that clozapine's effects may be limited to one half of the session and the higher prepulse intensities. We conducted additional statistical analyses to detect any subtle changes in the magnitude of MK801 effects that could be restricted to a narrow time window after drug administraton and in response to different prepulse intensities. In the second part of the study, the effects on PPI of systemic administration of the lowest effective dose of MK801 (0.05 mg/kg) were compared with the effects of bilateral microinjection of MK801 (0.65 μg/0.5 μl/site) into the ventral hippocampus in WS rats. This experiment was conducted in order to assess whether the lack of effect of ventral hippocampal infusion of MK801 (0.65 μg/0.5 μl/site) on PPI reported in SD rats [2] is strain specific, or more general. Given the evidence that NMDA transmission within the ventral hippocampus is critical for PPI [16,35,40], we tested the hypothesis that the blockade of NMDA transmission in the ventral hippocampus would affect PPI in WS rats.

1. Method

1.1. Part 1. PPI disruption by systemic MK801 and pretreatment with neuroleptics

1.1.1. Subjects

Sixty-one male adult WS rats [Zur-Wist(Haibm)] and 24 SD rats [Zur:SD(Crl:CD(SD)BR)], weighing 300–450 g, were used in this experiment. These two strains were bred in the same facility at the Research Unit Schwerzenbach, Switzerland. Animals were housed four to a cage under a reversed light–dark cycle (lights on: 19:00–07:00 hours) with free access to food and water. Before testing, rats were handled 5 min per day for at least 3 days. All experimental procedures occurred during the dark phase of the cycle. The experiments were carried out in accordance with Swiss federal regulations for animal experimentation.

1.1.2. Drug administration

MK801 (Merck, Sharp, and Dohme, UK) was dissolved in 0.9% saline and administered subcutaneously (0.05, 0.075, and 0.1 mg/kg, sc) 15 min prior to PPI testing. Haloperidol (5 mg/ml, Janssen-Cilag, Baar, Switzerland) was diluted with 0.9% saline. Clozapine (Novartis, Basel, Switzerland) was dissolved in 0.9% saline adjusted to pH 5.5 by addition of 0.1 M HCl and Na₂CO₃. Haloperidol (0.2 mg/kg, ip) and clozapine (5 and 10 mg/kg, ip) were administered intraperitoneally 45 and 30 min, respectively, prior to PPI testing. Saline (1 ml/kg) was used for control injections.

1.1.3. Experimental design

Each experiment included three groups of rats. The first group was pretreated with saline, followed by another saline injection (saline + saline). The second group received a saline pretreatment, followed by a single dose of MK801 (saline + MK801). The third group was pretreated with a single dose of either clozapine or haloperidol, followed by a single dose of MK801 (haloperidol + MK801 or clozapine + MK801). MK801 effect on PPI was measured by...
comparing the saline + saline group with the saline + MK801 group. Pretreatment effects of neuroleptics were tested by comparing the neuroleptic-pretreated group (haloperidol + MK801; clozapine + MK801), and the saline-pretreated group (saline + MK801). All animals used in Part 1 were experimentally naive subjects. None of these animals was reused in other experiments.

1.1.4. PPI testing

The animals were tested in squads of four with startle chambers counterbalanced across the different experimental groups. The testing was conducted in four ventilated startle chambers (SR-LAB, San Diego Instruments, San Diego, CA), which contained a transparent Plexiglas tube (diameter 8.2 cm, length 20 cm) mounted on a plexiglas frame. Acoustic pulses and prepulses were delivered via a speaker, which was mounted 24 cm above the tube. Movement inside the tube was detected by a piezoelectric accelerometer below the frame. The amplitude of the whole body startle to an acoustic pulse was defined as the average of 100 one-millisecond accelerometer readings collected from pulse onset. Delivery of the acoustic stimuli and recording of startle responses was controlled by a computer. Once the animals were placed inside the tube, the startle session started with a 5-min acclimatization period, with a background noise level of 68 dB(A), which was maintained throughout the session. Following the acclimatization period, four startle pulses [30 ms, 120 dB(A)] were presented. The four initial startle pulses served to achieve a relatively stable level of startle reactivity for the remainder of the test session because the most pronounced habituation of the startle response occurs during the first four pulse presentations [2, 17]. The prepulses were broad band noise bursts of either 72, 76, 80, or 84 dB(A) and were 20 ms in duration. The interval between the prepulse and pulse was 80 ms.

The animals were placed inside the tube, the startle session started with a 5-min acclimatization period, with a background noise level of 68 dB(A), which was maintained throughout the session. Following the acclimatization period, four startle pulses [30 ms, 120 dB(A)] were presented. The four initial startle pulses served to achieve a relatively stable level of startle reactivity for the remainder of the test session because the most pronounced habituation of the startle response occurs during the first four pulse presentations [2, 17]. The prepulses were broad band noise bursts of either 72, 76, 80, or 84 dB(A) and were 20 ms in duration. The interval between the prepulse and pulse was 80 ms.

Each session consisted of six blocks of 11 trials. Each block included four different trial types: two pulse alone trials, four prepulses at different intensities followed by pulse, four prepulses alone at four intensities, and one no stimulus trial. The different trial types were presented pseudorandomly with a variable intertrial interval of 10–20 s. One session lasted about 23 min.

1.1.5. Data analysis

For each of the four ‘prepulse–pulse’ trial types, the percentage PPI (%PPI) was calculated: \( \text{%PPI} = 100 \times \left[ 1 - \frac{\text{startle amplitude in prepulse trial}}{\text{startle amplitude in pulse alone trial}} \right] \). The overall mean %PPI was calculated for the four prepulse intensities. The mean startle amplitude was calculated as the average response to the 12 ‘pulse-alone’ trials across the entire six blocks. The PPI values were calculated for the total duration of the test session as well as for the first and second half of the test session. Statistical analysis was conducted by ANOVA with the different combinations of injections for pretreatment and treatment as between-subjects factors and prepulse types and session halves as repeated measures. Pair-wise comparisons were made by Fisher’s PLSD test.

1.2. Part 2. Effects of MK801 microinfusion into the ventral hippocampus on PPI: comparison with the effects of systemic MK801

1.2.1. Subjects

Twenty-six male adult WS rats (300–450 g) from the same breeding facility as in Part 1 were used in this experiment. All animals used in Part 2 were drug-naïve but had been used in previous experiments involving saline infusion and PPI or open-field assessments. Of 26 rats, 10 rats were bilaterally implanted with cannulae in the ventral hippocampus and 16 unoperated rats were used as an additional comparison to assess the effects of systemic MK801 (0.05 mg/kg, sc). The animals were kept in single cages throughout the experiment. Other animal care and housing conditions were identical to those described in the Part 1 experiment.

1.2.2. Implantation of guide cannulae and intracerebral infusion

Rats were anesthetized with Nembutal (sodium pentobarbital, 50 mg/ml/kg, ip, Abbott, Chicago, IL) and placed in a stereotaxic frame. The skull was exposed, bregma and lambda were aligned in the same horizontal plane. Two small holes (1.5 mm in diameter) were drilled onto the skull and bilateral guide cannulae were implanted. The tips of the cannulae were aimed 1.5 mm above the ventral hippocampus: AP = −5.2 mm, ML = ±5 mm, DV = −5 mm [20]. Guide cannulae were constructed from 26-gauge stainless-steel tubing (0.45 mm in outer diameter) held in a 5-mm perspex block. The guide cannulae were fixed onto the skull using dental cement. Three small stainless-steel skull screws served as anchors for the guide cannulae. Stainless-steel stylets, which extended 0.5 mm beyond the tips of the guide cannulae, were placed inside the guide cannulae to prevent occlusion. At least 7 days were allowed for a recovery period, during which the animals were handled and acclimatized to a simulation of the intracerebral infusion procedure.

For intracerebral microinfusions, the animals were gently held by the experimenter, stylets were removed, injector needles were inserted into the guide cannulae, and the tip of each injector needle was allowed to protrude 1.5 mm beyond the end of the guide cannula. The injector unit consisted of 34-gauge (0.22 mm) cannulae, held in a cuff of 26-gauge stainless-steel cannulae (0.45 mm outer diameter), which were connected via polyethylene (PEEK) tubing to 10-μl Hamilton syringes. MK801 was dissolved in 0.9% sterile saline at a concentration of 6.25 μg/0.5 μl [2]. MK801 or saline was infused using an infusion pump (WPI, Sarasota, FL; model sp200i) at a speed of 0.5 μl/min. The volume of infusion was 0.5 μl per site. A 60-s diffusion period was allowed before removal of the injector needles.
and replacement of the stylets. The animals were tested on PPI immediately after infusion.

1.2.3. Experimental design

This experiment included three groups. The first group of rats (n = 10) had bilateral cannulae in the ventral hippocampus (vHippo), and received either vehicle (saline) or MK801 infusion. The dose of MK801 for infusion was the same dose used by Bakshi and Geyer [2]. The second group (n = 8) of unoperated rats received systemic injection of MK801 (UNOP-MK801) to compare the systemic effects with intracerebral infusion effects on PPI. The dose of MK801 was the lowest effective dose used in the earlier experiment. The third group (n = 8) of unoperated rats received systemic saline injection (UNOP-Sal) as a control. All systemic injections were given 15 min prior to PPI testing. MK801 (0.05 mg/ml) solution for systemic injection was prepared by diluting the solution (6.25 mg/0.5 ml in concentration) that was used for intracerebral infusion.

1.2.4. PPI testing

PPI testing spanned 4 test days, with 3 days between test days. On day 1, all rats were tested for baseline PPI without any infusions or injections. The unoperated rats were then matched by baseline PPI and divided into the UNOP-MK801 and the UNOP-Sal groups. On day 2, the vHippo group received vehicle infusion into the ventral hippocampus less than 2 min prior to a PPI session. The two UNOP groups were subjected to PPI testing without injection. On day 3, the vHippo group received MK801 infusion. A complete counterbalanced treatment sequence (vehicle-MK801, MK801-vehicle infusion) was not used. This was to avoid possible carryover effects of MK801. The UNOP-MK801 and UNOP-Sal groups received systemic injection of MK801 (0.05 mg/kg, ip) and saline, respectively, 15 min prior to the session. On day 4, all three groups received a PPI session to assess a possible residual drug effect from any infusions or injections. The unoperated rats were then matched by baseline PPI and divided into the UNOP-MK801 groups (n = 6) of unoperated rats and the saline+saline groups (n = 15) and the saline+MK801 groups of three separate experiments, using three different MK801 doses (0.05, 0.075, and 0.1 mg/kg; n = 5–6) did not yield a significant effect on the mean startle amplitude, F(3, 27) = 1.32, P > .05.

1.2.5. Histology

Upon completion of the experiment, rats were deeply anesthetized with an overdose of pentobarbital, and they were transcardially perfused with 0.9% NaCl solution, followed by 4% formalin solution. Each brain was extracted, post-fixed in 4% formalin solution, and cut in 40-μm coronal sections on a freezing microtome. Every fifth section through the ventral hippocampus was mounted on slides and stained with Cresyl violet. The sections were examined under a light microscope and the injection sites (i.e., the placement of injector tips) were verified after Paxinos and Watson [20].

1.2.6. Data analysis

The mean startle amplitude and %PPI were calculated as in Part 1. The resulting values were subjected to ANOVA with groups as a between-subjects factor and days, prepulse intensities, and session halves as repeated measures. Fisher’s PLSD test was used for pair-wise comparisons.

2. Results

2.1. Part 1. PPI disruption by systemic MK801 and pretreatment with neuroleptics

In all experiments that involved systemic injections of MK801 and combinations of MK801 and neuroleptics, there was a significant main effect of pulse alone trials on startle amplitude, indicating habituation of the startle response over the 16 pulse presentations (P < .001). Overall %PPI increased with an increase in prepulse intensity, with the greatest %PPI at the highest prepulse intensity. This was reflected by a significant main effect of prepulse intensity on %PPI (P < .001).

2.2. Effects of systemic MK801 on PPI in WS rats

2.2.1. Startle response

ANOVA of the combined startle data of the saline + saline groups (n = 15) and the saline + MK801 groups of three separate experiments using different MK801 doses (0.05, 0.075, and 0.1 mg/kg; n = 5–6) did not yield a significant effect on the mean startle amplitude, F(3, 27) = 1.32, P > .05.

2.2.2. Prepulse inhibition

Systemic injection of MK801 (0.05, 0.075, and 0.1 mg/kg) disrupted PPI (Fig. 1A). ANOVA yielded a significant main effect of MK801 treatment on %PPI, F(3, 27) = 14.96, P < .0001. Post hoc pair-wise comparisons showed that all MK801 groups significantly differed from the saline + saline group. The data in Fig. 1 are combined data from the saline + saline groups (n = 15) and the saline + MK801 groups of three separate experiments, using three different MK801 doses (n = 5–6).

In further analysis, following the precedent in Bakshi et al. [4], we divided the PPI data into two half sessions. ANOVA revealed a significant effect of session half, F(1, 27) = 4.89, P < .05 (see Fig. 1B). There was no significant interaction of treatment group and session half, F(3, 27) = 1.48, P > .05.

2.3. Effect of clozapine and haloperidol pretreatment on MK801-induced PPI disruption in WS rats

2.3.1. Startle response

Experiments involving 0.05 or 0.075 mg/kg MK801 and pretreatment with clozapine failed to show a significant drug effect on the mean startle amplitude (P > .05). However, in one experiment involving 0.1 mg/kg MK801 and pretreatment with haloperidol (0.2
mg/kg) and clozapine (5 mg/kg), there was a significant treatment effect, $F(3, 17) = 3.25, P < .05$, which was due to a higher mean startle amplitude of the saline + saline group, compared to other treatment groups ($P < .05$). There was no significant difference among the saline + MK801, haloperidol + MK801, or clozapine + MK801 groups ($P > .05$).

2.3.2. Prepulse inhibition

Neither clozapine (5 mg/kg) nor haloperidol (0.2 mg/kg) pretreatment reversed MK801 (0.1 mg/kg)-induced PPI disruption. Both the clozapine + MK801 and the haloperidol + MK801 groups showed a similar degree of PPI disruption as the saline + MK801 group, which showed a marked PPI disruption, compared to the saline + saline group (Fig 2A). ANOVA indicated a significant main effect of group on %PPI, $F(3, 17) = 6.42, P < .05$. Pair-wise comparisons indicated no significant differences between clozapine- and haloperidol-treated groups. When the PPI data were divided into two half sessions, MK801 effects on PPI during the first and the second half were comparable (Fig 2B). There was no significant half-session effect nor an interaction between session half and prepulse intensity.

Neuroleptic-pretreatment effects were further examined with different combinations of clozapine and MK801. Combinations were used to detect any narrow dose range within which clozapine might antagonize MK801-induced PPI disruption. As shown in Fig. 3, both 0.05 mg/kg and 0.075 mg/kg of MK801 markedly disrupted PPI, whereas pretreatment with either 5 mg or 10 mg/kg clozapine failed to restore PPI disruption (Fig 3A and C). ANOVA yielded a significant main effect of group [for Fig 3A, $F(3, 16) = 8.89, P < .005$; and for Fig 3C, $F(3, 16) = 6.47, P < .005$]. This was due to a difference between the saline + saline and the saline + MK801 (0.05 mg or 0.075 mg) groups ($P < .05$, all groups $n = 5$).

To further examine the time course of clozapine effects, which may contribute a subtle change to the magnitude of PPI over time, the data were divided into two half sessions. For the experiment involving 0.05 mg/kg MK801, there was no significant effect of session half. ANOVA yielded a significant interaction of session half and group, $F(3, 16) = 3.69, P < .05$, reflecting the fact that PPI disruption within the group pretreated with 5 mg/kg clozapine was more severe during the first half of the session as compared to the second half (Fig 3B). For the experiment involving 0.075 mg/kg MK801, ANOVA yielded a significant effect of session half, $F(1, 16) = 18.51, P < .001$, and a significant interaction of session half and group, $F(3, 16) = 5.82, P < .01$, which may be due to a more severe PPI disruption during the second half in the MK801 group pretreated with 5 mg/kg clozapine (Fig 3D).

The half session data were further divided by different prepulse intensities. For the experiment involving 0.05 and
Fig. 2. Effects of pretreatment with either haloperidol (0.2 mg/kg, ip) or clozapine (5 mg/kg, ip) on PPI disruption by MK801 in WS rats. The numbers on the Y axis represent the mean %PPI. The numbers on the X axis represent different pretreatment-doses of neuroleptics and MK801 (0.1 mg/kg, sc, n=5 for each group). A dose of 0.0 mg/kg (n=6) indicates the saline injection. Sal: saline; Hal: haloperidol; Clo: clozapine. Asterisks denote significant differences compared to the saline controls (saline + saline). Bars represent standard error of the mean. (A) Data collapsed over all prepulse intensities for the entire session. (B) The session data are divided into two half sessions.

0.075 mg/kg MK801, ANOVA yielded no significant interaction of session half and prepulse intensity.

2.4. Effect of MK801 and clozapine pretreatment on PPI in SD rats

MK801 effects on PPI and clozapine-pretreatment effects on MK801-induced PPI disruption were further tested in SD rats, with 0.1 mg/kg MK801 and 5 and 10 mg/kg clozapine. These were the same doses tested in WS rats in our study, as well as in the study of Bakshi et al. [4], which demonstrated clozapine's ability to antagonize MK801 (0.1 mg/kg)-induced PPI disruption in SD rats.

2.4.1. Startle response

Consistent with our recent report [36], SD rats showed a substantially lower mean startle amplitude (± SEM) (165 ± 9, n=6), compared to WS rats (850 ± 38, n=15) across 12 pulse-alone trials, F(19)=4.43, P<.001. Consistent with a previous report of Bakshi et al. [4], we found that MK801 (0.1 mg/kg) treatment produced an increase in mean startle amplitude, which was blocked by clozapine pretreatment, F(3, 20)=9.49, P<.001. The mean startle amplitudes for different groups were: 165 ± 9 for saline + saline; 404 ± 60 for saline + MK801; 193 ± 22 for clozapine (5 mg/kg) + MK801; and 183 ± 30 for clozapine (10 mg/kg) + MK801.

2.4.2. Prepulse inhibition

Like WS rats, SD rats showed disrupted PPI after systemic MK801, compared to the saline + saline group. Pretreatment with either 5 mg or 10 mg/kg of clozapine failed to reverse MK801-induced PPI disruption (Fig. 4A). ANOVA yielded a significant main effect of group on %PPI, F(3, 20)=5.62, P<.001. Pair-wise comparisons showed no significant differences between clozapine-treated groups and the saline + MK801 group. Again, drug-treatment effects on PPI during the first and the second half of the session were comparable (Fig. 4B). There was no significant interaction of session half and group. We also analyzed the half session data for different prepulse intensities and found no significant interaction of session half and prepulse intensity.

2.5. Part 2. Effects of MK801 microinfusion into the ventral hippocampus on PPI in WS rats: comparison with the effects of systemic MK801

2.5.1. Intracerebral infusion sites

Histological analysis showed that the tips of the infusion cannulae were located within or around the
Fig. 4. Effects of pretreatment with clozapine (5 mg or 10 mg/kg, ip) on startle response and PPI disruption by MK801 (0.1 mg/kg, sc) in SD rats. The numbers on the X axis represent doses of clozapine and MK801. A dose of 0.0 mg/kg indicates the saline injection. Asterisks denote significant differences compared to the saline controls (saline + saline). Bars represent standard error of the mean. All groups: n = 6. (A) Combined PPI data from all prepulse intensities for the entire session. (B) Two half session data.

Fig. 5. Histological location of infusion sites in the ventral hippocampus of WS rats. (A) Photomicrographic image of a coronal brain section with the tracks of bilateral guide cannulae. (B) Estimated location of the injector tips. Numbers on each histological section represent the distance from bregma. CA1, CA2, and CA3, CA1, CA2, and CA3 field of the hippocampus; DG, dentate gyrus; Ent, entorhinal cortex; S, subiculum. Adapted from Paxinos and Watson [20].
significant main effect of pulse alone trials on startle amplitude, indicating habituation of the startle response over the 16 pulse presentations, \( F(15, 345) = 27.0, P < .0001 \).

2.5.3. Prepulse inhibition

ANOVA with the data of all 4 days yielded a highly significant effect of prepulse intensity on %PPI, \( F(3, 23) = 122.29, P < .0001 \), reflecting a gradual increase of PPI with higher prepulse intensities. There was no interaction between prepulse intensities and days (\( P > .05 \)) or prepulse intensities and treatment groups (\( P > .05 \)).

PPI was disrupted by systemic injection of MK801 but was not affected by bilateral MK801 infusion into the ventral hippocampus on day 3 (Fig. 6). While all three groups exhibited equal levels of PPI on the days without MK801 administration (days 1, 2, and 4), PPI at the three higher prepulse intensities was significantly disrupted on day 3 in the rats that received systemic application of MK801 (UNOP-MK801). The rats that received MK801 infusion into the ventral hippocampus (vHippo), however, exhibited the same intact PPI as the unoperated rats that received a saline injection (UNOP-Sal). The ANOVA with the data from all 4 days yielded a significant effect of day, \( F(3, 23) = 7.345, P < .001 \). Analysis of PPI at the three higher prepulse intensities on day 3 yielded a significant effect of group, \( F(2, 23) = 4.11, P < .05 \). Post hoc pair-wise comparisons revealed that PPI at the three higher prepulse intensities was significantly disrupted in the UNOP-MK801 group as compared to the UNOP-Sal and vHippo groups (\( P < .05 \) and \( P < .05 \), respectively), while there was no difference between the latter two groups (\( P > .05 \)).

3. Discussion

3.1 Part 1. PPI disruption by systemic MK801 and effects of pretreatment with clozapine and haloperidol

3.1.1. Startle response

In the present study and in our previous report [36], the mean startle amplitude of WS rats was higher than that of SD rats. Such a strain difference in startle amplitude was also reported by others [33]. In the present study, the ranges of weights of the two strains were comparable. Thus, a higher startle response in WS rats compared with SD rats may reflect strain-related difference, rather than
variables such as weight. We also found strain-dependent drug effects on startle amplitude. Our results, consistent with findings of Bakshi et al. [4], showed that in SD rats MK801 (0.1 mg/kg) increased startle amplitude, and clozapine (5 mg/kg) reversed this increase. In WS rats, however, we found that MK801 had no consistent effect on mean startle. It is noteworthy that apomorphine (a dopamine agonist) increased the startle amplitude in SD rats but not in WS rats [15,33]. Thus, strain-dependent drug effects on the startle response appear to exist between these strains.

3.1.2. Prepulse inhibition

We found a robust disruption of PPI by MK801 in both WS and SD strains. This is consistent with previous studies in SD rats [1,4,8,11,13,14,18] and in WS rats [34]. In addition, we found a marked disruption of PPI following low doses of 0.05 and 0.075 mg/kg MK801 in WS rats. Our findings are in agreement with findings of Johansson et al. [13] who showed that 0.05 mg/kg MK801 (the lowest dose we used) disrupted PPI in SD rats. Our data are not in agreement with one report, showing that only a higher dose (0.1 mg/kg), but not a lower dose (0.075 mg/kg), of MK801 produced a significant PPI disruption in WS rats [34].

Neither clozapine nor haloperidol pretreatment restored MK801-induced PPI disruption in the present study. In WS rats, two doses of clozapine (5 and 10 mg/kg) consistently failed to antagonize PPI disruption induced by different doses of MK801 (0.05, 0.075, and 0.1 mg/kg). Similarly, in SD rats, clozapine (5 and 10 mg/kg) failed to reverse MK801 (0.1 mg/kg)-induced PPI disruption. Our results are in agreement with previous reports that 5 mg/kg of clozapine showed no effects on PPI disruption following 0.1 mg/kg MK801 in both SD [11] and WS rats [34]. Our results, however, are inconsistent with the findings of Bakshi et al. [4] that 5 mg/kg clozapine antagonized PPI disruption by 0.1 mg/kg MK801 in SD rats. Inconsistent results between different laboratories may be attributed to strain differences or to methodological differences between the different studies [11,15,27,31,34]. In our experiments, different strains were tested under identical conditions.

When our PPI data from WS rats were divided into two half sessions and analyzed for different intensities, we failed to observe the intensity-dependent or time-dependent clozapine effects in our results, involving either strain. In fact, we observed an opposite tendency, that is, a deterioration of PPI during the second half session in the clozapine-pretreated WS rats that received 0.075 mg/kg MK801. Considering that there was a minimal difference in methodology between Bakshi et al. [4] and the present study, we cannot rule out the possibility that the discrepancies in results may reflect subject variation due to differences between rat breeders or suppliers [31]. Nevertheless, given that the clozapine effect of MK801-induced PPI disruption reported by Bakshi et al. [4] was relatively mild, a parsimonious interpretation of our results is that the ability of clozapine to restore MK801-induced PPI disruption may not critically depend on strain differences.

Our finding that haloperidol (0.2 mg/kg) pretreatment failed to antagonize PPI disruption produced by 0.1 mg/kg MK801 in WS rats confirms and extends previous reports that 0.3 mg/kg haloperidol did not antagonize PPI disruption by 0.1 mg/kg MK801 in WS rats [34]. Similarly, pretreatment with either 0.1 mg or 0.5 mg/kg haloperidol was ineffective in antagonizing PPI disruption produced by 0.5 mg or 0.1 mg/kg MK801 in SD rats [11,14]. Taken together, these findings indicate that, unlike dopamine agonist-induced PPI disruption, which may be due to overstimulation of D2 receptors in the nucleus accumbens [17,29,39], MK801-induced PPI disruption may be mediated via a different mechanism. Nevertheless, the selective ability of atypical neuroleptics to restore PPI disruption by NMDA receptor antagonists and to serve as a tool in screening for possible atypical neuroleptic compounds [1,4,12,25,26] needs to be further examined.

3.2. Part 2. Effects of MK801 microinfusion into the ventral hippocampus on PPI in WS rats: comparison with the effects of systemic MK801

In contrast to a marked disruption of PPI with systemic MK801, bilateral infusion of a high dose of MK801 (6.25 µg/0.5 µl/site) into the ventral hippocampus did not affect PPI. Our finding in WS rats is in agreement with previous findings of Bakshi and Geyer [2] that infusion of the same dose of MK801 into the ventral hippocampus failed to affect PPI in SD rats. Though stimulation of NMDA receptor-mediated transmission in the ventral hippocampus disrupts PPI [16,35,40], blockade of NMDA transmission within the ventral hippocampus may not affect the expression of PPI.

Our results, however, do not rule out the possibility that the ventral hippocampus is involved in PPI disruption induced by systemic MK801. MK801 blocks NMDA receptors non-competitively in different brain regions, including the medial prefrontal cortex, nucleus accumbens, amygdala, and both the ventral and dorsal hippocampus [21,24], all of which are implicated in the regulation of PPI (for review, see Refs. [17,29]). Thus, it is conceivable that NMDA receptor blockade in multiple brain regions with systemic injection could affect several brain regions, and thereby act synergistically to yield a severe PPI disruption. By the same token, MK801 infusion into a specific brain region may not yield the marked changes in PPI shown after systemic injection. In fact, a recent report showed that bilateral MK801 infusions (6.25 µg/0.5 µl/site) into the amygdala [2] (see also Ref. [9]) or dorsal hippocampus produced a relatively weak, but significant PPI disruption, whereas infusions into the nucleus accumbens, ventral hippocampus, medial prefrontal cortex, or
dorsomedial thalamus had no significant effects on PPI [2]. The authors argued that a lack of infusion effect in these brain regions may have been due to the involvement of multiple limbic regions in PPI. Interestingly, results from our laboratory [38] indicate that bilateral infusions of MK801 into the dorsal hippocampus was not sufficient to disrupt PPI in WS rats. Thus, the recent view that blockade of NMDA receptors in multiple brain regions is involved in MK801-induced PPI disruption deserves further testing.

4. Conclusions

Systemic administration of MK801 yielded reliable results, with disruption of PPI in both WS and SD strains. In WS rats, the MK801-induced PPI disruption was not antagonized by pretreatment with either haloperidol or clozapine, a typical neuroleptic and an atypical neuroleptic, respectively. Similarly, in SD rats, clozapine failed to reverse MK801-induced PPI disruption. Our data do not indicate that strain difference contributed to a selective ability of clozapine to reverse MK801 effects on PPI. Although, at the present time, our data suggest that MK801-induced PPI disruption may not be a reliable tool with which to differentiate between typical and atypical neuroleptic compounds, our findings provide additional evidence that underlying mechanisms for NMDA receptor antagonist-induced PPI disruption could be essentially dopamine independent. Thus, given the construct validity of disrupted PPI for an animal model of schizophrenia [28], understanding of such a dopamine-independent mechanism may lead to new insights into treatment for the cases of schizophrenia which do not respond to any neuroleptic treatment and which constitute up to 40% of schizophrenic illnesses [23]. MK801 infusion into the ventral hippocampus did not affect PPI in the present study. The lack of effects on PPI following MK801 infusion in the ventral hippocampus, which expresses a considerably higher NMDA receptor density than other brain areas, suggests that severe PPI disruption with systemic MK801 may be due to additive effects resulting from blockade of NMDA receptors in multiple brain sites. Our view is in line with the notion that MK801-induced PPI disruption resembles schizophrenic pathology, which reflects abnormalities in several different limbic regions.

Acknowledgments

This work was supported by a grant from the Swiss Federal Institute of Technology Zurich. We thank Peter Schmid for technical support. Discussions with Dr. Mark Geyer are gratefully acknowledged. Clozapine was a gift from Novartis.

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Annotation:
A1 Updated reference:
Latent inhibition (LI) refers to the retarded conditioning to a stimulus following its repeated non-reinforced pre-exposure and is considered to index organisms' ability to ignore irrelevant stimuli. Prepulse inhibition (PPI) is the reduction in the startle response to an intense acoustic stimulus when a prepulse immediately precedes this stimulus. It reflects a central inhibitory process by which an organism protects the ongoing processing of one stimulus from interruption by another stimulus. Though there are several differences between LI and PPI, both reflect attentional mechanisms of gating that are of fundamental importance for the processing of information by the brain. The impairment of these mechanisms is observed in schizophrenia and probably causally related to this illness. The disruptions of LI and PPI in the rat are the two most prominent animal models for attentional gating deficits related to schizophrenia, and the substrates of these disruptions are considered to provide clues to the pathogenesis of schizophrenia. A large amount of evidence has accumulated demonstrating that manipulations of the mesolimbic or dopamine pathway, the hippocampal formation and the hippocampus–accumbens connections influence the expression and modulation of LI and PPI.

Non-competitive N-methyl-D-aspartate (NMDA) receptor antagonists, such as ketamine, phencyclidine (PCP) and the more specific antagonist MK-801 (dizocilpine), block NMDA receptor-mediated transmission by binding to the PCP-binding site within the ion pore of the NMDA receptor. In humans, non-competitive NMDA receptor antagonists have strong psychotomimetic effects (i.e. they induce states similar to schizophrenia). The understanding of where and how non-competitive NMDA receptor antagonists induce their behavioral effects could provide insights into the brain abnormalities which cause schizophrenia. The state induced by the systemic administration of non-competitive NMDA receptor antagonists in rats constitutes an animal model to study the mechanisms by which non-competitive NMDA receptor antagonists exert their behavioral effects. Increased locomotor activity and disruption of PPI are well-known behavioral effects induced by systemic administration of the specific non-competitive NMDA receptor antagonist MK-801 in rats.

In the rat brain, the hippocampus, especially the dorsal hippocampus, is among the regions with the highest density of NMDA receptors and PCP-binding sites. Thus, the dorsal hippocampus is a likely target site via which non-competitive NMDA receptor antagonists like MK-801 could exert their behavioral effects. In particular, the hippocampus seems to be crucially involved in the regulation of locomotor...
activity (e.g. Ref. 82) and PPI\textsuperscript{13,14,45,73} (but see Ref. 64), both of which are affected by systemic MK-801. Indeed, in two recent studies, Bakshi and Geyer\textsuperscript{5,6} found PPI disruption after microinfusion of MK-801 into the dorsal hippocampus of Sprague–Dawley rats. Studies concerning the effects of non-competitive NMDA receptor antagonists on LI are scarce and their outcomes make clear conclusions difficult. In one study, acute systemic administration of PCP by s.c. injection before pre-exposure and conditioning was found to be without effect on LI.\textsuperscript{80} Other studies confirmed this finding, but found an impairment of LI by continuous systemic long-term administration of PCP\textsuperscript{26} or by systemic administration of a very high dose of PCP (8.6 mg/kg) 20 h prior to each pre-exposure and conditioning.\textsuperscript{75} Additionally, there are two studies\textsuperscript{22,23} that found the non-competitive NMDA receptor antagonist ketamine to impair LI in a conditioned taste aversion paradigm. However, the results of one of these studies\textsuperscript{2} were controversial, as in one experiment ketamine impaired LI, whereas in another it did not.

In the present study, we investigated the effects of MK-801 microinfusion into the dorsal hippocampus of Wistar rats on LI and PPI, as well as on locomotor activity in the open field. We used the same dose of MK-801 (6.25 μg per side) which had previously been found to disrupt PPI in Sprague–Dawley rats.\textsuperscript{55} In the first experiment, we examined whether LI would be affected by MK-801 infusion into the dorsal hippocampus. This was prompted by our recent finding that another manipulation of hippocampal NMDA receptor transmission which disrupts PPI, namely NMDA receptor stimulation of the ventral hippocampus,\textsuperscript{44,78,85} also disrupted LI (Pouzet, Zhang and Feldon, unpublished observation). The high local density of PCP-binding sites and the evidence suggesting the hippocampal formation as a critical site for attentional gating processes (see above) were further reasons for examining LI. However, we did not find a disruption of LI by MK-801 infusion into the dorsal hippocampus. Therefore, in the second experiment, we tried to replicate in our Wistar rats the PPI disruption which has been found after MK-801 infusion into the dorsal hippocampus of Sprague–Dawley rats.\textsuperscript{55} The third experiment, based on the observation of increased activity during the LI experiment, tested whether MK-801 infusion into the dorsal hippocampus increases locomotor activity in an open field.

**EXPERIMENTAL PROCEDURES**

**Animals**

Sixty naïve male Wistar rats (Zur:WIST[HanBm], Research Unit Schwerzenbach, Schwerzenbach, Switzerland), aged two to three months and weighing approximately 300 g at the beginning of the experiment, served as subjects. They were housed in groups of four per cage under a reversed light–dark cycle (lights on: 19:00–07:00) in a temperature (21 ± 1°C)- and humidity (55 ± 5%)-controlled room. All animals were allowed free access to food and water. The 60 rats were divided into 20 unoperated (UNOP) animals and 40 animals to be cannulated and later to be allocated to one of two infusion groups: MK-801 infusion (MK-801) or vehicle infusion (VEH). After surgery, all 60 rats were caged individually. Starting three days before surgery and then throughout the studies, all rats were handled daily. Behavioral testing was carried out in the dark phase of the cycle. All experiments were conducted in accordance with Swiss regulations for animal testing. All efforts were made to minimize the number of animals used and their suffering.

**Surgical procedure**

Rats were anesthetized with 1 ml of Nembutal (sodium pentobarbital, 50 mg/ml; Abbott Labs, North Chicago, IL, USA) per kg body weight and their head was placed in a Kopf stereotaxic frame. After application of a local anesthetic (lidocaine), the scalp was incised to expose the skull, and bregma and lambda were aligned in the same horizontal plane. A small hole (1.5 mm in diameter) was drilled on each side of the skull to reveal the dura covering the cortex underlying the dorsal hippocampus. Stainless steel guide cannulae (7 mm, 26-gauge) were implanted bilaterally into the brain aiming at the dorsal hippocampus.

Coordinates were as follows (in mm): AP = −3.0 from bregma, LM = 1.5 from bregma, DV = −2.5 from ventral to dura.\textsuperscript{62} The guide cannulae were fixed to the skull with three anchoring skull screws and dental cement. Stainless steel stylets, which extended 0.5 mm beyond the tips of the guide cannulae, were placed inside the guide cannulae to prevent occlusion. After surgery, rats were allowed to recover for five days, during which the experimenter gave the rats daily health checks and gentle handling, and replaced missing stylets.

**Intracerebral infusion**

The cannulated rats were manually restrained, the stylets removed carefully and infusion cannulae (8.5 mm, 34-gauge, stainless steel) were inserted into the brain through the previously implanted guide cannulae. The tips of the infusion cannulae protruded into the dorsal hippocampus 1.5 mm beyond the tip of the guide cannulae, thus aiming at a field DV below dura: $4.5$ (Figs. 1A and 2B). The guides (1.5 cm beyond the tips of the guide cannulae) were connected to 10-μl Hamilton microsyringes mounted on a Kds micropipufusion pump. MK-801 (0.625 μg in 0.5 μl saline per side; MK-801 group) or saline only (0.5 μl; VEH group) was infused into the brain bilaterally over 1 min. The dose (0.625 μg/0.5 μl per side) of MK-801 used in this study was based on the dose which was reported by Bakshi and Geyer\textsuperscript{55} to be effective in disrupting PPI when infused into the dorsal hippocampus of Sprague–Dawley rats. Following infusion, the injection cannulae were kept in place for an additional 60 s to allow the injection bolus to be absorbed by the tissue. Then, the infusion cannulae were replaced by the stylets before the rats were subjected to further behavioral testing.

**Drugs**

MK-801 (Merck, Sharp & Dohme, UK) was dissolved in warm isotonic 0.9% saline at a concentration of 12.5 mg/ml by slight sonification. This solution was freshly prepared before infusion.

**Apparatus and procedures**

In all behavioral testing procedures, rats were tested in counterbalanced squads of four.

**Latent inhibition in the conditioned emotional response procedure**

Rats were put on a 23-h water deprivation schedule for five days prior to the commencement of the LI experiment and throughout the experiment. During this period, the rats were handled for 5 min daily. Water during the experimental sessions was given in addition to the daily 1-h ration given in the home cages 1 h after the end of the session.

LI was tested in four chambers (Coulbourn Instruments, Allentown, PA, USA) enclosed in ventilated sound attenuating boxes. A drinking spout, measuring 3 mm in diameter, was situated in the middle of an opening (3 cm × 4 cm) positioned in the middle of the panel wall, 1.5 cm above the grid floor. Licks were detected by an optical lickometer (Coulbourn Instruments, model E24-01). The pre-exposed and to-be-conditioned stimulus (CS) was a 5-s, 28-V, 40-mA light generated from the houselight (mounted above the hole, 2 cm from the ceiling), which was off during the rest of the session. Footshock was supplied to the grid floor by a constant direct current source (Coulbourn Instruments, Model E13-14) and a scanner (Coulbourn Instruments, Model E13-13) set at 0.5 mA intensity and 1 s duration. Activity in the chambers was detected by an infrared activity monitor (Coulbourn Instruments, model E24-61) mounted at the ceiling. The activity monitor was operated in the "movement unit" mode, in which a 10-ma pulse is produced each time the monitor detects a change in the animal’s infrared heat pattern, and counted as one unit of activity. Equipment programming and data recording were computer controlled.

The testing for LI in the conditioned emotional response procedure included the following stages.

**Baseline.** The rats were placed individually into the experimental
chambers and allowed to lick freely for 20 min each day for six days. Based on the total number of licks, the latency for the first lick, and the total activity exhibited by each rat during the last baseline sessions, the cannulated rats were allocated to one of two matched groups: VEH and MK-801. Then, the VEH, MK-801 and UNOP groups were each divided into two matched subgroups, pre-exposed (PE) and non-pre-exposed (NPE), for the next four stages of the LI testing procedure. These four stages consisted of pre-exposure, conditioning, rebaseline and test. All stages were 24 h apart. The MK-801 and VEH rats were infused bilaterally with 6.25 μg/0.5 μl MK-801 or with 0.5 μl saline, respectively, immediately before the pre-exposure and the conditioning sessions.

Pre-exposure. The pre-exposure session was conducted on the day after the last baseline session. With the water bottle in the chamber, the PE animals received 40 light presentations with a fixed inter-stimulus interval of 35 s, while the NPE animals were confined to the boxes for an equivalent period of time without receiving the stimulus. One pre-exposure session lasted around 28 min.

Conditioning. With the water bottle in the chamber, each rat received two light-shock pairings 5 and 10 min after the start of the session. Light parameters were identical to those used in pre-exposure. The 1-s, 0.5-mA shock immediately followed light termination. After the second pairing, the animals were left in the experimental chambers for an additional 5 min. Thus, one conditioning session lasted 15 min.

Rebaseline. Each rat was given a drinking session as in the baseline sessions.

Test. Each rat was placed in the chamber with the houselight off and allowed to drink. When it had completed 200 licks, the light was presented and lasted for 15 min.

The following measures were recorded during all stages of the LI experiment: the total number of activity counts, the latency for the first lick and the total number of licks. During the test session, the number of licks was recorded in 10-s blocks. The number of licks taken by the PE and the NPE animals during the first 120 s after onset of the CS in the test session were compared to assess for LI. Intact LI (i.e., diminished behavioral suppression after presentation of the aversive CS) is revealed by a higher number of licks in the PE as compared with the NPE animals.

Prepulse inhibition. The apparatus consisted of four startle chambers (SR-LAB, San Diego Instruments, San Diego, CA, USA), containing a transparent Plexiglas tube (diameter 8.2 cm, length 20 cm) mounted on a Plexiglas frame within a ventilated enclosure. Acoustic noise bursts were presented via a speaker mounted 24 cm above the tube. A piezoelectric accelerometer mounted below the frame detected and transduced motion within the tube. Startle amplitudes were defined as the average of 100 1-ms stimulimeter readings, collected from stimulus onset.

Each rat was put into the PPI chamber for a 5-min acclimatization period with a 68-dBA(A) background noise level that continued throughout the session. After the acclimatization period, four startle pulses of 120 dB(A) broad-band burst for 30 ms were presented for the basal startle responsiveness. Then, six blocks of 11 trials were presented to measure PPI. Each block consisted of four different trial types, presented pseudo-randomly throughout the session: pulse alone (two trials), prepulse alone (one trial for each prepulse intensity), prepulse followed by pulse (one trial for each prepulse intensity) or no stimulus (one trial). The four different prepulses had an intensity of either 72, 76, 80 or 84 dB(A) and a duration of 20 ms. The time interval between the prepulse offset and the pulse onset was 80 ms. The percentage of PPI induced by each prepulse intensity was calculated as: \[100 - \left(100 \times \text{startle amplitude on prepulse trial}/\text{startle amplitude on pulse-alone trial}\right)\].

Open field locomotor activity. Locomotor activity was measured in four closed square arenas (76.5 cm x 76.5 cm x 49 cm) made of dark gray plastic. Behavior in the arenas was recorded by a video camera suspended from the ceiling and relayed to a monitor and a Video Tracking, Motion Analysis & Behavior recognition system (EthoVision®, Noldus, Wageningen, The Netherlands). The room was dimly illuminated (20 ± 0.5 Lux, provided by two halogen lights) and the experimenter was out of the room during the entire experiment. The total distance the rat traveled in the complete arena was calculated by the computer for each 5-min block of testing.

Experimental design

All experiments were run with three treatment groups of animals: UNOP, VEH and MK-801. The UNOP group served as control for effects of the surgery and infusion procedure.

Experiment 1: effects of MK-801 infusion into the dorsal hippocampus on latent inhibition in the conditioned emotional response procedure. Five days after surgery, all 60 rats were subjected to the LI experiment. After matching according to the results of the last baseline session and discarding one UNOP animal, which did not lick at all during the rebaseline session, the numbers of animals in the different conditions for the LI experiment were: 10 PE and nine NPE animals for the MK-801 group, 11 PE and 10 NPE animals for the VEH group, and 10 PE and nine NPE animals for the UNOP group. The animals of the VEH and MK-801 groups were infused bilaterally immediately before the pre-exposure and the conditioning session.

Experiment 2: effects of MK-801 infusion into the dorsal hippocampus on prepulse inhibition. Ten days after the LI experiment, 42 rats were used for the PPI experiment. The number of rats in the different groups of the PPI experiment were as follows: 13 MK-801, 15 VEH and 14 UNOP rats. The MK-801 group consisted of rats which had received vehicle infusion in the LI experiment, and thus were drug naive. Eight of the 15 VEH rats for the PPI experiment came from the VEH group of the LI experiment and the other seven had received two MK-801 infusions during the LI experiment. As potential neurotoxic effects of MK-801 have been described in rodent models, these could have affected PPI, all measurements during the PPI experiment were compared between the eight drug-naive VEH rats and the seven VEH rats which had received two prior MK-801 infusions, to ensure lack of effect of prior MK-801 administration. The PPI experiment was conducted on three successive days: day 1, baseline PPI testing without infusion and then matching; day 2, PPI testing immediately after infusion with MK-801 or saline; day 3, PPI testing without infusion. The immediate effects of MK-801 infusion into the dorsal hippocampus on PPI were examined by comparing the PPI of the different groups on day 2 (between-subjects design). The aim of the PPI sessions on day 3 was to test for long-term effects.

Experiment 3: effects of MK-801 infusion into the dorsal hippocampus on open field locomotor activity. Based on the observation that MK-801 infusion into the dorsal hippocampus increased locomotor activity in the LI experiment, a final open field experiment was conducted one week after the PPI experiment. Twenty rats were subjected to the open field experiment, 14 of which were UNOP rats. The remaining 34 rats were cannulated, had received one to three MK-801 infusions throughout the previous LI and PPI experiments, and were allocated to either the MK-801 or VEH group of the open field experiment after counterbalancing and matching for baseline activity on day 1 of the experiment. The open field experiment was run on three successive days. On day 1, each rat was placed in the center of one of the arenas and allowed to habituate to the open field for 30 min. After testing for baseline locomotor activity on day 1, 18 cannulated animals were allocated to the MK-801 and 16 to the VEH group, so that the three experimental groups were matched for baseline locomotor activity. On day 2, each rat was placed in the center of one of the arenas and left in the open field for 30 min of free exploration. All rats were then returned to their home cages. After the MK-801 and VEH rats had received their infusion, all rats were replaced into the arena for an additional 30 min of free exploration to check for the immediate effect of MK-801 infusion into the dorsal hippocampus on locomotor activity. On day 3, each rat was replaced in the center of one of the arenas for 30 min of free exploration to check for possible long-term effects of the infusion.

Histology

After completion of the behavioral experiments, rats were deeply anesthetized with an i.p. injection of 2.5 mg/kg Nembutal (sodium pentobarbital, 50 mg/ml, i.p.) and transcardially perfused with 0.9% NaCl solution, followed by 250 ml of 4% formalin (4%) to fix the brain.
concentrated within or around the border of the dorsal hippocampus (Fig. 1). In nearly all cases, damage resulting from the implantation of the guide cannulae was found in the dorsal hippocampus and the cortex overlying the hippocampus. This damage was restricted to the area immediately surrounding the guide cannulae and did not extend into the remainder of the dorsal hippocampus. Based on the histological examinations, behavioral data of all cannulated rats were included in the statistical analysis.

**Experiment 1: effects of MK-801 microinfusion into the dorsal hippocampus on latent inhibition in the conditioned emotional response procedure**

For the last baseline session, there was no difference in the total number of licks, the total activity or the latency for the first lick between the subgroups (PE, NPE), reflecting successful matching, nor between the three treatment groups (MK-801, VEH, UNOP) of the LI experiment.

During the pre-exposure session, the rats infused with MK-801 exhibited enhanced activity levels (2078 ± 145) as compared with both the UNOP group (1594 ± 84, \( P < 0.005 \)) and the VEH group (1688 ± 101, \( P < 0.02 \)), which did not differ (Fig. 2A). This was supported by the significant main effect of group \( (F_{2,53} = 4.81, P < 0.015) \) in the 3×2 (treatment×pre-exposure) ANOVA.

Analysis of the conditioning session data revealed no significant differences between the three treatment groups (MK-801, VEH, UNOP) or between the two pre-exposure subgroups (PE, NPE) in any of the measured parameters (all ANOVAs: \( P > 0.1 \)). However, as can be seen in Fig. 2B, there was a tendency for the MK-801 group (794 ± 74) to be more active than the UNOP group (624 ± 62, \( P = 0.06 \) in a planned comparison).

During the rebaseal sessions, the MK-801 group (1025 ± 51) was more active than both the UNOP group (702 ± 83, \( P < 0.003 \)) and the VEH group (839 ± 65, \( P = 0.062 \)), which did not differ from each other (\( P > 0.16 \)) (Fig. 2C). This was supported by the significant main effect of treatment \( (F_{2,53} = 5.36, P < 0.008) \) in the 3×2 (treatment×pre-exposure) ANOVA.

During the test session, activity was only monitored for the time after onset of the CS. For this period of the test session, ANOVA yielded a significant main effect of treatment on the total activity counts \( (F_{2,53} = 8.96, P < 0.001) \). Post hoc tests revealed that the total activity was significantly higher in the MK-801 group (878 ± 59, \( P < 0.001 \)) and in the VEH group (773 ± 53, \( P < 0.01 \)) than in the UNOP group (524 ± 63) (Fig. 2D). The MK-801 and VEH groups did not differ (\( P > 0.5 \)).

In the test session, all three treatment groups (UNOP, VEH and MK-801) expressed LI. This was indicated by a lower number of licks in the NPE animals of all three groups during the first 120 s after onset of the CS (steady house light; Fig. 3). A 3×2×12 (treatment×pre-exposure×10-s blocks) ANOVA of the mean number of licks yielded a highly significant main effect of pre-exposure \( (F_{1,53} = 10.88, P < 0.002) \) and no interaction of treatment×pre-exposure \( (F_{2,53} = 0.03, P > 0.97) \) or treatment×pre-exposure×10-s blocks \( (F_{2,529} = 0.78, P > 0.7) \). One-tailed post hoc comparisons revealed that, for all three groups, the total number of licks during the first 120 s (i.e., the first 12 10-s blocks after CS onset) was significantly higher in the PE animals.
NMDA receptor blockade in the dorsal hippocampus

Experiment 2: the effects of MK-801 infusion into the dorsal hippocampus on prepulse inhibition

MK-801 infusion into the dorsal hippocampus increased the mean startle response (Fig. 4A). A 3 x 3 x 16 ANOVA consisting of a between-subjects factor of group (UNOP, VEH, MK-801) and two within-subjects factors of three days and 16 pulse-alone trials carried out on the startle amplitude yielded a significant main effect of days ($F_{2,78} = 17.63$, $P < 0.001$), and a significant interaction of group x days ($F_{4,78} = 2.78$, $P < 0.04$). This was due to the fact that the mean startle amplitude over 16 pulses gradually decreased over the three days in the UNOP group (1024 ± 65, 932 ± 83 and 788 ± 107) and similarly in the VEH group (1260 ± 68, 1163 ± 99 and 982 ± 106), whereas in the MK-801 group it was higher on the infusion day (1315 ± 114) than on the previous (1092 ± 104, $P < 0.05$) and the following (848 ± 108, $P < 0.001$) days. Though the groups were not matched for baseline startle on day 1, a 3 x 16 ANOVA with a between-subjects factor of group and the 16 pulse-alone trials as repeated measures carried out on the startle data of each day separately yielded a significant effect of group only for the day of infusion ($F_{2,39} = 3.44$, $P < 0.05$), but not for the day preceding ($F_{2,39} = 2.44$, $P > 0.1$) or following ($F_{2,39} = 0.91$, $P > 0.4$) infusion. On the day of infusion, the mean startle was higher in the MK-801 group as compared to the two control groups, with this difference being statistically significant between the MK-801 and the UNOP group ($P < 0.02$). However, the MK-801 infusion on day 2 did not disrupt long-term habituation of the startle response. In all three groups, startle amplitudes were markedly decreased on day 3 as compared to day 1. This decrease was more pronounced in the MK-801 (36%) as compared to the VEH (22%) and the UNOP (23%) groups. In addition, the factor of pulse-alone trials was significant ($F_{1,39} = 17.05$, $P < 0.001$), reflecting short-term habituation of the startle response over the 16 pulse-alone presentations for all three groups on all three days of testing.

MK-801 infusion into the dorsal hippocampus failed to disrupt PPI (Fig. 4B). A 3 x 3 x 4 ANOVA with a between-subjects factor of treatment (UNOP, VEH, MK-801) and two within-subjects factors of days and prepulse intensities carried out on the mean PPI yielded only a highly significant main effect of prepulse intensity ($F_{2,117} = 217.3$, $P < 0.001$), reflecting a gradual increase in PPI with higher prepulse intensities. Though there was no significant effect of day ($F_{2,78} = 0.24$, $P > 0.1$) or group ($F_{2,39} = 2.44$, $P < 0.05$), or any significant interaction involving day or group ($F_{2,39} = 2.44$, $P < 0.05$), PPI at the three higher prepulse intensities seemed to be lower in the MK-801 group as compared to the VEH and UNOP groups on the infusion day (Fig. 4B). However, all attempts to reveal a significant effect of the MK-801 infusion into the dorsal hippocampus on PPI—by analysing the percentage PPI data separately for day 2, or by examining separately the first and last half of the testing session (following Bakshi and Geyer), or by analysing separately each prepulse intensity—failed. Finally, it can be stated that the drug-naïve VEH rats and those VEH rats which received prior MK-801 infusions during the previous experiment did not differ in the percentage PPI averaged over days 1–3 (43 ± 4 vs 40 ± 5; $P > 0.8$) and specifically on day 2 (45 ± 8 vs 41 ± 6; $P > 0.8$). Thus, we have no evidence to suggest that prior MK-801 infusions affected percentage PPI due to possible neurotoxic effects.
carried out on the total distance moved by the UNOP, VEH and MK-801 groups yielded only a significant main effect of time blocks ($F_{5,225} = 153.76, P < 0.001$). This reflected the gradual reduction in activity (i.e. habituation) over time.

On day 2, the three groups did not differ in total distance moved in the entire open field arena during the 30 min prior to MK-801 or vehicle infusion (Fig. 5, left panel). As on day 1, a $3 \times 6$ (treatment $\times$ time blocks) ANOVA yielded only a significant main effect of treatment ($F_{2,45} = 9.92, P < 0.005$) and again a highly significant main effect of time blocks ($F_{5,225} = 68.08, P < 0.001$). Post hoc comparisons revealed increased locomotor activity in the MK-801 treatment group (total distance moved in cm $5695 \pm 612$) relative to both the VEH ($4283 \pm 369, F = 0.051$) and the UNOP ($4151 \pm 480, P < 0.05$) groups, which did not differ.

During the post-infusion session on day 3, there were no significant differences in the total distance moved in the entire open field arena between the three groups (Fig. 5, right panel). A $3 \times 6$ (groups $\times$ time blocks) ANOVA yielded only a significant main effect of time blocks ($F_{5,225} = 139.01, P < 0.001$), reflecting habituation of locomotor activity over time. Planned comparisons further confirmed that there were no significant differences in the total distances moved by the three groups (in cm, all $P > 0.19$): MK-801, $6272 \pm 348$; VEH, $6903 \pm 340$; UNOP, $6479 \pm 351$.

**DISCUSSION**

The present study investigated, in Wistar rats, the effects of temporary blockade of NMDA receptors within the dorsal hippocampus on LI, PPI, startle response, and locomotor activity. Deficits in LI and PPI have been associated with schizophrenia. While microinfusion of the non-competitive NMDA receptor antagonist MK-801 (6.25 μg/0.5 μl per side) into the dorsal hippocampus left both PPI and LI unaffected, it significantly increased startle magnitude and locomotor activity.

In the present experiments, blockade of NMDA receptors in the dorsal hippocampus by MK-801 during the pre-exposure and conditioning phases of the LI experiment did not affect the expression of LI in the conditioned emotional response procedure. This indicates that NMDA receptor-mediated processes within the dorsal hippocampus are not essential for the expression of normal LI. Our findings are in line with several studies that suggested that an intact hippocampus is not needed for the acquisition and expression of LI. These studies found that lesioning the hippocampus before the LI experiment did not affect the expression of LI ($15,16,24$) (Pouzet, Zhang, and Feldon, unpublished observation) or even enhanced ($37,38,46,66$) the expression of LI. The LI deficit after hippocampal lesions observed in several earlier studies ($13,33,46,47,65$) was possibly due to the lesions affecting the entorhinal cortex ($16,63$) rather than to specific damage of the hippocampus. In contrast, stimulation of the ventral hippocampus by NMDA infusion during the pre-exposure and conditioning phases of the LI experiment disrupted the expression of LI in the same procedure used in the present study (Pouzet, Zhang, and Feldon, unpublished).

### Experiment 3: the effects of MK-801 infusion into the dorsal hippocampus on open field locomotor activity

For day 1, a $3 \times 6$ ANOVA (treatment $\times$ time blocks)
NaBD receptor blockade in the dorsal hippocampus

In the present study, blockade of NaBD receptors in the dorsal hippocampus by MK-801 did not affect PPI. This is in contrast to two previous studies, which reported that bilateral infusion of MK-801 into the dorsal hippocampus, at the same dose we used in our experiments and applying a PPI procedure very similar to ours, led to PPI disruption in Sprague-Dawley rats. The discrepant findings may be due to several reasons. First, the location of the infusion sites differed slightly between our study and the studies of Bakshi and Geyer. In our study, the infusion sites were located more posterior (AP = 2.6 to 3.6 as against 2.1 to 2.8) and they were also located rather in the center of the dorsal hippocampus, whereas in Bakshi and Geyer's studies the infusion sites lay at the ventral border of the dorsal hippocampus. Second, Bakshi and Geyer used a within-subjects design, which is more sensitive in revealing relatively weak drug effects on PPI than is our between-subjects design. Indeed, the PPI disruption observed by Bakshi and Geyer after MK-801 infusion into the dorsal hippocampus was not very marked, and it is possible that the statistical power of our experimental design was too low to reveal these effects. Third, Wistar rats may be less sensitive to MK-801-induced PPI disruption than Sprague-Dawley rats. However, based on our own observations (Bast, Zhang, Feldon and White, unpublished), we think that we can rule this out, as we found marked PPI disruption in Wistar rats after systemic administration of MK-801 doses (0.05 and 0.075 mg/kg) that were considerably lower than those usually used for PPI disruption in Sprague-Dawley rats (≥0.1 mg/kg; e.g. Ref. 7). In addition, studies comparing different rat strains concerning their sensitivity to PPI disruption by systemic injection of another non-competitive NaBD receptor antagonist, PCP, did not find a considerable difference between Sprague-Dawley and Wistar rats. Fourth, it has to be mentioned that the data were analysed differently in the two studies in which Bakshi and Geyer reported the PPI disruptive effects of MK-801 infusion into the dorsal hippocampus. In one study, they analysed only the first half of the PPI session, while in the second study they analysed the entire session. For the data of our experiments, however, the conclusion that MK-801 infusion into the dorsal hippocampus of Wistar rats did not influence PPI significantly was independent of whether we considered only the first half, only the second half or both halves of the PPI testing session. Thus, our results indicate that PPI disruption after MK-801 infusion into the dorsal hippocampus is at least not a robust effect and that effects of MK-801 at NaBD receptors within the dorsal hippocampus cannot alone account for the severe PPI disruption observed after systemic administration of MK-801 in Sprague-Dawley rats and Wistar rats (Bast, Zhang, Feldon and White, unpublished observation). However, our data do not rule out that the dorsal hippocampus is involved in PPI disruption following systemic MK-801 by acting synergistically with other brain regions. Our finding is in line with the emerging picture that just depriving the brain of the normal neuronal processing provided by the dorsal hippocampus is not sufficient to disrupt PPI. Thus, selective destruction of cell bodies within the dorsal hippocampus did not disrupt or even slightly enhanced, whereas less selective aspiration lesions of the dorsal hippocampus, which also affected transversing fibers, did significantly disrupt Carbachol infusion into (i.e. chemical stimulation of) the dorsal hippocampus, however, has been reported to disrupt PPI.

Despite the lack of effect on PPI, MK-801 infusion into the dorsal hippocampus increased the startle amplitude in our experiments. This is a partial replication of the effect observed in the two infusion studies by Bakshi and Geyer. Bakshi and Geyer found an increase in startle magnitude of about 70-90%. Because of the different designs
of the studies, it is difficult to compare the increase of startle amplitude by MK-801 in our study with that found by Bakshi and Geyer. While Bakshi and Geyer applied a within-subjects design, with all test sessions being separated by four days, our design was a between-subjects design with the three test sessions run on three successive days. For our study, a within-subjects comparison (mean startle of the MK-801 group on the day of infusion as compared to the preceding and the following day) is confounded by long-term habituation of the startle amplitude, whereas a between-subjects comparison (comparison of all groups on the day of infusion) is confounded by the fact that animals were not matched for baseline startle. However, applying the within-subjects comparison, one can state that the increase in mean startle induced by MK-801 in our study was between 20% and maximally 55%, and thus, in any case, considerably lower than that found by Bakshi and Geyer. It has to be considered that this quantitative difference in the magnitude of startle increase between our study and that of Bakshi and Geyer might also account for the fact that they found a significant disruption of PPI and we did not. As discussed and detailed by Bakshi and Geyer, markedly increased startle reactivity rather than real changes in PPI occur on the background of changes in startle magnitude by MK-801 infusion into the dorsal hippocampus (middle panel), as well as during the 30-min post-infusion session one day after infusion (right panel). Values are expressed as averaged data from consecutive 5-min blocks (main graph) and total distance ± S.E.M. over the 30-min test session after infusion (inset graph). The bar represents 1 standard error (S.E.) derived from the ANOVA. Asterisks denote significant differences revealed by post hoc comparisons (P ≤ 0.05).

As expected from the observations during the LI experiment, MK-801 infusion into the dorsal hippocampus significantly increased open field locomotor activity. Thus, the dorsal hippocampus, with its high density of NMDA receptors, seems to be one of the brain sites via which MK-801, and probably also other non-competitive NMDA receptor antagonists, exert their stimulatory effects on locomotor activity. Previously, it has been found that MK-801 infusion into the nucleus accumbens and the prefrontal cortex increases locomotor activity in rats, indicating that the increased locomotor activity observed after systemic MK-801 is due to the synergistic action of the drug in multiple (at least three) brain areas. Several studies suggested that MK-801-induced hyperactivity is mediated by a stimulation of the mesoaccumbens dopamine system. Other findings have questioned this view. The dorsal hippocampus has ways to influence the mesoaccumbens dopamine system, and thus the hyperactivity following MK-801 infusion into the dorsal hippocampus may be due to an increase of dopamine in the nucleus accumbens. However, this clearly needs further investigation. A further question regards the direct effects of MK-801 on the activity of hippocampal outputs to eventually increase locomotor activity. Electrophysiological studies demonstrated that NMDA receptor antagonists have both inhibiting and activating effects on hippocampal neurons. The activating effects may be due to the inhibition of NMDA receptor-mediated excitation of inhibitory hippocampal interneurons. Furthermore, increase of extracellular concentrations of excitatory neurotransmitters like glutamate or acetylcholine is a way by which NMDA receptor hypofunction induced by NMDA receptor antagonists can lead to neuronal hyperstimulation. Thus, in principle, it is possible that MK-801 infusion into the dorsal hippocampus increases locomotor activity by inhibition or activation of outputs of the dorsal hippocampus. However, previous studies demonstrated that specific excitotoxic lesions (i.e. a total inhibition) of the dorsal hippocampus did not increase spontaneous locomotor activity. Thus, it is very likely that MK-801 infusion into the dorsal hippocampus increases locomotor activity by activating rather than by inhibiting outputs of the dorsal hippocampus.
these conditioned fear responses. Indeed, several studies suggested that the hippocampus (e.g. Refs 4, 26, 53 and 67) and NMDA receptor-mediated processes within the hippocampus are involved in the formation of conditioned fear responses.

CONCLUSIONS

Microinjection of the non-competitive NMDA receptor antagonist MK-801 (6.25 μg per side) into the dorsal hippocampus did not affect LI and PPI, but significantly increased the startle amplitude and spontaneous locomotor activity, in Wistar rats. Given also that, when applied systemically, doses of MK-801 needed to increase locomotor activity are generally appreciably higher than, or at least equal to, those needed to disrupt PPI (27,39,42,52,77) (Bast, Zhang, Feldon and White, unpublished observation), our findings appear to imply that NMDA receptor transmission within the dorsal hippocampus plays a minor role in the maintenance of attentional gating processes reflected by PPI and LI, while it is critical for the regulation of locomotor activity. Action of the non-competitive NMDA receptor antagonist MK-801 in single brain sites like the nucleus accumbens, prefrontal cortex and dorsal hippocampus (present study) seems sufficient to markedly disturb the regulation of locomotor activity. It is obviously more difficult to identify brain sites where NMDA receptor-mediated processes are similarly critical for the maintenance of sensorimotor gating. Thus, previous studies in Sprague–Dawley rats suggested two crucial brain sites, the dorsal hippocampus and amygdala. However, one of these, the dorsal hippocampus, could not be confirmed by our study in Wistar rats. The difficulties in disrupting PPI by local intracerebral administration of even high doses of MK-801 (6.25 μg/0.5 μl, bilaterally) are in striking contrast to the marked and robust PPI disruption after systemic application of quite low doses of MK-801 (0.05 mg/kg, i.p.; Bast, Zhang, Feldon and White, unpublished observation). It appears that MK-801 and other non-competitive NMDA receptor antagonists, when administered systemically, affect multiple brain sites and the resulting disturbances accumulate in a synergistic manner to eventually cause the marked breakdown of sensorimotor gating, as reflected by PPI disruption. This resembles the pathophysiology of schizophrenia, in which severe behavioral disturbances are the result of multiple minor disturbances in several brain regions, making it very difficult to identify single critical brain sites and mechanisms.

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(Accepted 1 September 2000)

Annotation:

HYPERACTIVITY AND DISRUPTION OF PREPULSE INHIBITION INDUCED BY N-METHYL-D-ASPARTATE STIMULATION OF THE VENTRAL HIPPOCAMPUS AND THE EFFECTS OF PRETREATMENT WITH HALOPERIDOL AND CLOZAPINE

T. BAST, W.-N. ZHANG, C. HEIDBREDER† and J. FELDON*
Laboratory of Behavioural Neurobiology, The Swiss Federal Institute of Technology-Zurich, Schorenstrasse 16, CH 8603 Schwerzenbach, Switzerland

Abstract—This study re-examined the hyperactivity and disruption of prepulse inhibition induced by N-methyl-D-aspartate stimulation of the rat ventral hippocampus and compared how both effects were affected by pretreatment with either haloperidol or clozapine. While the hyperactivity is thought to depend on dopamine receptor activation in the nucleus accumbens, the dopamine D2-class receptor blocker haloperidol failed to antagonize the disruption of prepulse inhibition in previous studies. However, an ameliorative effect of the atypical neuroleptic clozapine on disruption of prepulse inhibition was suggested by our previous experiments [Zhang et al (1999) NeuroReport 10, 1-6]. In the present study, bilateral infusion of N-methyl-D-aspartate (0.5 μg/side) into the ventral hippocampus of Wistar rats increased open field locomotor activity and disrupted prepulse inhibition. Both effects were observed immediately after infusion but disappeared 24 h later. Injection of haloperidol (0.2 mg/kg) or clozapine (5 mg/kg), 45 min prior to N-methyl-D-aspartate infusion, totally antagonized the hyperactivity but did not affect the disruption of prepulse inhibition.

We conclude that dopaminergic mechanisms are differentially involved in the hyperactivity and disruption of prepulse inhibition induced by N-methyl-D-aspartate stimulation of the ventral hippocampus. Activation of accumbal dopamine receptors, which is blocked by clozapine and haloperidol to a comparable extent, seems to be crucial for the hyperactivity but not the disruption of prepulse inhibition. The present finding that both clozapine and haloperidol failed to antagonize the disruption of prepulse inhibition induced by N-methyl-D-aspartate stimulation of the ventral hippocampus is discussed with respect to our previous contrary finding concerning the ameliorative effect of clozapine and with respect to the disruption of prepulse inhibition in rats being considered as a model of sensorimotor gating deficits in schizophrenia. © 2001 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: locomotor activity, neuroleptics, NMDA, rat, schizophrenia, sensorimotor gating.

Abnormal hippocampal activity has been implicated in neuropsychiatric disorders, such as schizophrenia and anxiety.21,22 In particular, overactivity of the hippocampus has been associated with the symptomatology of schizophrenia.14,1925,46 This has stimulated a substantial interest in the study of the neuronal processes and behavioural effects elicited by hippocampal overactivity.

Stimulation of the ventral hippocampus by infusion of N-methyl-D-aspartate (NMDA)5,9,37,60,61 or carbachol8 increases locomotor activity in rats. Initially, Yang and Mogenson61 proposed that dopamine via D2-class receptors in the nucleus accumbens might exert a suppressive effect upon locomotor responses to stimulation of the ventral hippocampus. This proposition was based on their finding that intra-accumbens infusion of a D2-class agonist, quipiprole, antagonized hyperactivity following NMDA infusion into the ventral hippocampus. In more recent studies, however, it was found that the locomotor response to stimulation of the ventral hippocampus depends on an intact meso-accumbal dopamine projection,60 is paralleled by adopamine increase in the nucleus accumbens,9,37 and blocked by systemic application of a dopamine D2-class receptor antagonist, the typical neuroleptic haloperidol.53 These findings are all congruent with the view that increased locomotor activity in response to stimulation of the ventral hippocampus depends on increased dopamine activity and concomitant dopamine receptor stimulation within the nucleus accumbens. Glutamatergic projections from the ventral hippocampus to the nucleus accumbens acting on dopaminergic terminals in the medial shell could be responsible for the dopaminergic activation within the nucleus accumbens.7,24,45 There is also evidence that NMDA stimulation of the ventral hippocampus increases nucleus accumbens dopamine by activating dopamine cell bodies in the ventral tegmental area.36,37 However the interaction between the ventral hippocampus and nucleus accumbens

*Corresponding author. Tel.: +41-1-655-7448; fax: +41-1-655-7203.
E-mail address: feldon@toxi.biol.ethz.ch (J. Feldon).
†Present address: GlaxoSmithKline Beecham Pharmaceuticals, Department of Neuroscience Research Group, New Frontiers Science Park (North), Third Avenue, Harlow, Essex CM19 5AW, UK.

Abbreviations: ANOVA, analysis of variance; NMDA, N-methyl-D-aspartate; PPI, prepulse inhibition.
comes about, it is of crucial importance for the initiation and guidance of locomotor activity in exploratory and goal-directed behaviour.18,28 Aberrations of locomotor activity in rats have been proposed to be homologous to some changes in human cognitive function observed in acute schizophrenia.23

Another behavioural effect which can be observed in rats after stimulation of the ventral hippocampus by infusion of carbachol10,11 or NMDA,31,33,56,63 and which is more closely related to schizophrenic symptomatology, is the disruption of prepulse inhibition (PPI). PPI is the reduction of the startle response to an intense acoustic pulse by an immediately preceding weaker stimulus, or prepulse, and used as a measure of sensorimotor gating. Disruption of PPI in rats is considered to model sensorimotor gating deficits observed in, and thought to be causative for schizophrenia.20,48,49 The mechanisms underlying disruption of PPI by stimulation of the ventral hippocampus seem to be different from those underlying the hyperactivity after stimulation of the ventral hippocampus. In two studies, systemic application of the dopamine D2-class receptor antagonist haloperidol did not antagonize the PPI disruption after NMDA stimulation of the ventral hippocampus,56,63 However, the atypical neuroleptic clozapine seemed to antagonize the disruption of PPI caused by NMDA stimulation of the ventral hippocampus.56 The finding suggested that PPI disruption by NMDA stimulation of the ventral hippocampus could serve as a tool for differentiating the mechanisms of typical and atypical neuroleptics.

The present study was designed to replicate and extend previous studies on hyperactivity and PPI disruption after NMDA stimulation of the ventral hippocampus and the effects of acute pretreatment with haloperidol or clozapine. Wistar rats were bilaterally infused with 0.5 μg NMDA in 0.5 μl vehicle or 0.5 μl vehicle only. The infusion speed was 0.5 μl/min. The infusion cannulae were connected to 10-μl Hamilton microsyringes mounted on a microinfusion pump (KD scientific or WPI sp200i). The tips of the infusion cannulae were placed inside the guide cannulae to prevent occlusion. After surgery, rats were allowed to recover for five days during which the experimenters gave the rats daily health checks and gentle handling, and allowed to recover for five days during which the experimenters gave the rats daily health checks and gentle handling, and replaced missing stylets.

**Experimental Procedures**

**Animals**

A total of 87 male adult Wistar rats (Zur.Wist[Han]) were used. The animals were housed in groups of four per cage under a reversed light-dark cycle (lights on: 19:00-07:00) in a temperature (21 ± 1°C) and humidity (55 ± 5%) controlled room. All rats were allowed free access to food and water. There were 16 unoperated animals and 71 animals that received bilateral implantation of infusion guide cannulae aiming at the ventral hippocampus. After surgery, all 87 rats were individually caged. Beginning three days before surgery and throughout the studies, all rats were handled daily. Behavioural testing was carried out in the dark phase of the cycle. All experiments were conducted in accordance with Swiss regulations for animal experimentation and all efforts were made to minimize the number of animals and their suffering.

**Implantation of guide cannulae for intracerebral infusion**

Rats were anaesthetized (i.p.) with 1 ml of Nembutal (sodium pentobarbital, 50 mg/ml, Abbott Labs, North Chicago, IL) per kg body weight and their head was placed in a Kopf stereotaxic frame. After application of a local anaesthetic (lidocaine), the scalp was incised to expose the skull. Then, bregma and lambda were aligned in the same horizontal plane. A small hole (1.5 mm diameter) was drilled on each side of the skull to reveal the dura covering the cortex overlying the ventral hippocampus. Three small stainless steel screws were screwed into the skull and guide cannulae (9 mm, 26-gauge, stainless steel) were implanted bilaterally into the brain with their tips aiming at the following coordinates above the ventral hippocampus (in mm according to Paxinos and Watson57; 5.2 posterior and ±5 lateral to bregma, and 5 ventral to dura. Guide cannulae were fixed with dental cement for which the three screws served as anchors to the skull. Stainless steel stylets (34-gauge) which extended 0.5 mm beyond the tips of the guide cannulae were placed inside the guide cannulae to prevent occlusion. After surgery, rats were allowed to recover for five days during which the experimenters gave the rats daily health checks and gentle handling, and replaced missing stylets.

**Intracerebral microinfusion**

Rats were manually restrained and the stylets removed from the guide cannulae. Then, infusion cannulae (34-gauge) were inserted into the guide cannulae. The infusion cannulae were connected to 10-μl Hamilton microsyringes mounted on a microinfusion pump (KD scientific or WPI sp200i). The tips of the infusion cannulae protruded into the ventral hippocampus by 1.5 mm beyond the tip of the guide cannulae, thus aiming at a final dorsoventral coordinate of 6.5 mm below the dura. The rats were bilaterally infused with either 0.5 μg NMDA, 0.5 μl vehicle or 0.5 μl vehicle only. The infusion speed was 0.5 μl/min. After infusion, the infusion cannulae were left in the brain for 60 s to allow for absorption of the infusion bolus by the brain tissue and then replaced by the stylets. Rats were then immediately subjected to behavioural testing.

**Systemic injection**

Haloperidol (0.2 mg/kg) and clozapine (5 mg/kg) were administered i.p., 45 min before infusion. These doses, according to
experience in our laboratory, have comparable and moderate effects on locomotor activity when administered alone and are within the range of doses which have been shown to antagonize hyperactivity induced by dopamine infusion into the nucleus accumbens. For control injections, 0.9% saline was used. Injection volume was 1 ml/kg.

Drugs

All drug solutions were prepared freshly on the day of experiment. NMDA (Sigma, Switzerland) was dissolved in sterilized 0.1 M phosphate-buffered solution, pH 7.4, as vehicle at a concentration of 1 mg/ml. Haloperidol from ampoules with 5 mg/ml solution (Janssen-Cilag, Baar, Switzerland) was diluted with 0.9% saline to a concentration of 0.2 mg/ml. Clozapine (Novartis, Basel, Switzerland) was dissolved in 0.9% saline, acidified by 0.1 M HCl, at a concentration of 5 mg/ml, and the solution was adjusted to pH 5–6 by addition of Na₂CO₃.

Apparatus and procedures for behavioural testing

Open field locomotor activity. Locomotor activity was measured in four closed square arenas (76.5 X 76.5 X 49 cm) made of dark grey plastic. Behaviour in the arenas was recorded by a video camera suspended from the ceiling and relayed to a monitor and a Video Tracking, Motion Analysis & Behaviour Recognition System (EthoVision®, Noldus, Wageningen, The Netherlands). The room was dimly illuminated (0.5 Lux provided by two halogen lights) and the experimenter was out of the room during the entire experiment. The distance the rat moved in centimetres throughout the complete arena was calculated by the Noldus system for each 10-min block of testing.

Prepulse inhibition. The testing was conducted in four ventilated startle chambers (SR-LAB, San Diego Instruments, San Diego, CA), containing a transparent Plexiglas tube (diameter 8.2 cm, length 20 cm) mounted on a Plexiglas frame. Noise bursts were presented via a speaker mounted 24 cm above the tube. Motion inside the tube was detected by a piezoelectric accelerometer below the frame. The amplitude of the whole body startle to an acoustic pulse was defined as the average of 100 1-ms accelerometer readings collected from pulse onset. A background noise level of 68 dB(A) was maintained throughout the test sessions. A test session started with 5 min of acclimatization, after which four startle pulses (30 ms, 120 dB(A)) were presented. These four initial startle pulses served to achieve a relatively stable level of startle reactivity for the remainder of the test session, as most rapid habituation of the startle reflex occurs within the first few startle pulse presentations. To measure PPI, six blocks of 11 trials were presented. The 11 trials of each block included: two “pulse alone” trials, one “prepulse followed by pulse” and one “prepulse alone” trial for each of four prepulse intensities, and one “no stimulus” trial. The prepulses were broad band bursts of 20 ms duration and an intensity of either 72, 76, 80 or 84 dB(A). Between prepulse offset and pulse onset, there was a time span of 80 ms. The different trial types were presented pseudorandomly with an intertrial interval of 10–20 s (average 15 s). Altogether, a complete test session lasted for about 25 min. The percentage PPI (% PPI) induced by each prepulse intensity was calculated as: [100 – (100 x startle amplitude on “prepulse followed by pulse” trial) / (startle amplitude on “pulse alone” trial)].

Experimental design

The experimental groups consisted of unoperated rats (UNOP) and cannulated rats divided into four infusion groups. The infusion groups received bilateral infusions (NMDA or vehicle) into the ventral hippocampus immediately before behavioural testing and a systemic injection (saline, haloperidol or clozapine) 45 min before infusion. The infusion groups were: VEH: vehicle infusion preceeded by saline injection; NMDA: NMDA infusion (0.5 μg/ side) preceded by saline injection; H-NMDA: NMDA infusion (0.5 μg/side) preceded by haloperidol injection (0.2 mg/kg); C-NMDA: NMDA infusion (0.5 μg/side) preceded by clozapine injection (5 mg/kg). The rats were tested in squads of four with the four different startle and open field chambers counterbalanced across the different experimental groups as far as possible. Altogether, three experiments were conducted with two batches of rats. The first batch included 32 rats, six UNOP rats and 26 rats which were bilaterally implanted with infusion cannulae aiming at the ventral hippocampus. These 32 rats were first subjected to an open field experiment and then a PPI experiment to test the effects of haloperidol and clozapine pretreatment on hyperactivity and PPI disruption induced by NMDA stimulation of the ventral hippocampus. The effect of clozapine in the PPI experiment was incongruous with our previous study. Therefore, we conducted a second PPI experiment with a second batch of rats in order to retest the clozapine effect on PPI disruption induced by NMDA stimulation of the ventral hippocampus.

Open field experiment. The open field experiment with the 32 rats of the first batch was run on three successive days. On day 1, each rat was placed in the centre of one of the arenas and left in the open field for 30 min. After testing of baseline locomotor activity on day 1, the 26 cannulated rats were allocated to the four infusion groups so that all experimental groups had matched baseline locomotor activity. The group sizes were as follows: UNOP, VEH and NMDA: n = 6; H-NMDA and C-NMDA: n = 7. On day 2 (day of infusion), each rat was first placed in the centre of one of the arenas and left in the open field for 30 min. Then, all rats were returned to their home cages. The rats of the four infusion groups received their respective systemic injections. After the injection, the rats were replaced in the centre of the arenas and left in the open field for 45 min. Then, all rats were returned to their home cages again, and the rats of the infusion groups received their infusions. After infusion, rats were immediately replaced in the centre of the arenas and left in the open field for another 90 min. On day 3 (day after infusion), each rat was replaced in the centre of one of the arenas for 30 min to check for possible long-term effects of the infusion on locomotor activity.

Prepulse inhibition experiments. Five days after the open field experiment, the 32 rats of the first batch were used for a PPI experiment which was conducted on three successive days. The rats’ allocation to the experimental groups was the same as in the open field experiment. On day 1, all rats were subjected to a baseline PPI testing. On day 2, the effects of NMDA infusion and neuroleptic pretreatment were tested. The UNOP rats were subjected to a PPI testing as on day 1. The rats of the infusion groups received the respective systemic injections 45 min before PPI testing and the respective infusions immediately before PPI testing. On day 3, all rats were tested for PPI as on day 1 to check for possible long-term effects of the NMDA infusion. The second PPI experiment with the second batch of 55 naive rats was run identically to the first PPI experiment, except without a H-NMDA group. After matching of the 45 cannulated rats according to the measurements during the baseline PPI testing on day 1, the group sizes of the second PPI experiment were as follows: UNOP: n = 10; VEH: n = 17; NMDA and C-NMDA: n = 14.

Histology

After completion of the behavioural experiments, the cannulated rats were deeply anaesthetized with an overdose of 2.5 ml/ kg Nembutal (sodium pentobarbital, 50 mg/ml, i.p.) and transcardially perfused with 0.9% NaCl solution to rinse out the blood, followed by 250 ml of 4% formalin (4°C) to fix the brain tissue. After extraction from the skull, the brains were postfixed in 4% formalin solution and subsequently cut into 40-μm coronal sections on a freezing microtome. For the verification of the infusion sites, every fifth section through the ventral hippocampus was mounted on gelatine-treated slides and stained with Cresyl Violet. After staining, the sections were dehydrated and coverslipped. Subsequently, they were examined light
Fig. 1. Infusion sites in the ventral hippocampus. (A) Photomicrograph of a coronal brain section with the tracks of the guide cannulae and beneath them the infusion sites visible in both hemispheres. (B) Approximate location of the tips of the infusion cannulae depicted on plates of coronal sections through the rat brain. Filled circles represent sites of vehicle infusion, open circles represent sites of NMDA infusion. Values on the right represent distance from bregma. The CA1, CA2 and CA3 fields of the hippocampus (CA1, CA2, CA3), the dentate gyrus (DG), the entorhinal cortex (Ent), and the subiculum (S) are indicated.

Histology

The centres of the infusion sites, i.e. the tips of the infusion cannulae, were concentrated within or around the border of the ventral hippocampus (Fig. 1). Damage due to the implantation of the guide cannulae was restricted to the area immediately surrounding the guide cannulae. Based on the histological examinations, the behavioural data of all 71 cannulated rats were included in the statistical analysis.

Immediate effects of N-methyl-D-aspartate infusion into the ventral hippocampus

The bilateral infusion of 0.5 μg NMDA into the ventral hippocampus did not produce convulsions. In some rats, the infusion elicited a slight tremor, sniffing and whisker vibrations. However, these effects were restricted to the first few minutes following infusion.

Open field experiment

Infusion of NMDA (0.5 μg per side) into the ventral hippocampus markedly increased locomotor activity in the open field. This effect was temporary, i.e. restricted to the open field testing on the day of infusion, and completely antagonized by the injection of haloperidol (0.2 mg/kg) or clozapine (5 mg/kg), 45 min before the NMDA infusion (Fig. 2). There were no differences in the total distance travelled by the groups during the periods preceding NMDA infusion. Separate ANOVAs of the total distance moved during the 30 min of initial habituation to the open field and the 40 min of open field testing following the systemic injection of vehicle, haloperidol (0.2 mg/kg) or clozapine (5 mg/kg) yielded neither a significant group effect (F<0.8, P>0.5) nor a significant interaction of group and 10-min blocks (F<1.4, P>0.13). For both periods, there was only a significant effect of 10-min blocks (F>20, P<0.0001) reflecting within-session habituation to the open field. In addition, during the first 10-min block of the period following injection, average activity of all groups was markedly lower than during the first 10-min block of the 30 min of open field testing preceding the injection [mean distance moved (cm) ± S.E.M.: 1320 ± 144 compared to 2840 ± 152, P<0.0001], reflecting habituation to being re-exposed to the open field. For the 90 min of open field testing following the infusion of NMDA or vehicle, ANOVA yielded significant main effects of group (F4,27 =6.02, P<0.0015) and 10-min blocks (F8,216 =12.06, P<0.0001), as well as a significant interaction of group and 10-min blocks (F8,216 =3.50, P<0.0001). Post hoc comparisons revealed that the average distance travelled [mean (cm) ± S.E.M.] per 10-min block throughout the 90 min following infusion as repeated measures for the analysis of the startle data.
was significantly higher in the NMDA group (1770 ± 461) than in the UNOP (700 ± 140), VEH (620 ± 169), H-NMDA (380 ± 114) and C-NMDA (500 ± 84) groups (P < 0.0005), with the latter four groups not differing significantly (P > 0.3). The hyperactivity of the NMDA group compared to the other groups was most pronounced during the first 10-min block following infusion. A comparison of the distance [mean (cm) ± S.E.M.] moved during this period revealed that the NMDA group (5970 ± 1878) was more than three times as active as the UNOP (1620 ± 273), VEH (1630 ± 361), H-NMDA (680 ± 292) and C-NMDA (940 ± 324) groups (P < 0.0005). The latter four groups did not differ significantly from each other (P > 0.4). On day 3 of the open field experiment (day after infusion), there was no difference between the five groups in the distance travelled in the open field throughout the 30 min of open field testing (F4,27 = 1.02, P > 0.4). ANOVA of the activity data revealed only a significant effect of 10-min blocks (F2,27 = 7.15, P < 0.0001), reflecting habituation to the open field. Thus, the effect of the NMDA infusion on locomotor activity was no longer evident 24 h after infusion.

Prepulse inhibition experiments

Startle. On all three days of both PPI experiments, there was a significant main effect of the prepulse intensity on the %PPI (P < 0.0001) reflecting a gradual increase in PPI with increasing prepulse intensity.32 The average startle amplitudes (mean ± S.E.M.) in the 16 pulse alone trials for the three successive days were: 1320 ± 58, 1200 ± 84 and 1050 ± 69, in the first PPI experiment, and 1040 ± 54, 760 ± 55 and 750 ± 55, in the second PPI experiment. For both experiments, neither combined nor separate analyses of the three days revealed a significant group effect on the startle responses in the 16 pulse alone trials. In particular, it is important that the average startle amplitudes (mean ± S.E.M.) on the day of infusion did not differ between the UNOP (1120 ± 234), VEH (1320 ± 154), NMDA (1130 ± 177), H-NMDA (970 ± 200) and the C-NMDA groups (1470 ± 159) of the first PPI experiment (F4,27 = 1.13, P > 0.3), and the UNOP (870 ± 158), VEH (840 ± 96), NMDA (620 ± 105) and C-NMDA (720 ± 98) groups of the second PPI experiment (F3,51 = 1.00, P > 0.4). Thus, it can be ruled out that the PPI disruption following NMDA infusion into the ventral hippocampus (see below) is due to changes in startle amplitude during pulse alone trials.

Prepulse inhibition. On all three days of both PPI experiments (Fig. 3A, B), there was a significant main effect of the prepulse intensity on the %PPI (P < 0.0001) reflecting a gradual increase in PPI with increasing prepulse intensity.32 NMDA infusion (0.5 µg per side) into the ventral hippocampus on day 2 disrupted PPI. This PPI disruption was temporary, i.e. restricted to the sessions immediately following infusion, and not antagonized by injection of haloperidol (0.2 mg/kg) or clozapine (5 mg/kg), 45 min prior to the infusion (Fig. 3A, B). ANOVA revealed a significant main effect of days on %PPI in both experiments (P < 0.0001). In addition, the interaction of group and day was significant in the first PPI experiment involving the H-NMDA group (F8,54 = 2.27, P < 0.05) and approached significance in the second PPI experiment involving only the UNOP, VEH, NMDA and C-NMDA groups (F6,102 = 1.97, P = 0.0762). In both PPI experiments, the groups exhibited no difference in baseline %PPI measured on day 1 (F < 0.10, P > 0.75). On
DISCUSSION

In the present study, bilateral infusion of NMDA (0.5 μg/side) into the ventral hippocampus increased locomotor activity and disrupted PPI of the acoustic startle response. Both hyperactivity and PPI disruption were restricted to the behavioural testing immediately following infusion. While both haloperidol and clozapine (0.2 mg/kg and 5 mg/kg, respectively; injected i.p. 45 min prior to infusion) completely antagonized the hyperactivity following NMDA infusion into the ventral hippocampus, neither of the two neuroleptics was effective in antagonizing the PPI disruption induced by the NMDA infusion.

Hyperactivity following N-methyl-D-aspartate stimulation of the ventral hippocampus

Hyperactivity after NMDA stimulation of the ventral hippocampus is consistent with previous studies (20, 27, 60, 61) (B. Pouzet, W.-N. Zhang and J. Feldon, unpublished observations). Principally, this hyperactivity could reflect a direct activation of locomotor activity by the stimulated hippocampus or increased exploration due to a disruption of spatial learning (compare Refs 1 and 15). In the present study, the hyperactivity was most pronounced during the 10-min block immediately following the NMDA infusion and had virtually disappeared about 70–90 min after infusion. The immediate onset of the hyperactivity indicates a direct activation of locomotor activity by the NMDA-stimulated ventral hippocampus. In contrast, a spatial learning deficit should result in a delayed onset of hyperactivity, reflecting a failure of habituation to the open field. In our study, such a spatial learning deficit induced by NMDA infusion would probably not have become manifest, as the habituation largely occurred during the two periods of open field testing prior to the NMDA infusion. Thus, we can clearly conclude that NMDA stimulation of the ventral hippocampus activated locomotor activity in the open field directly and not by interfering with memory processes involved in normal habituation to the open field. This suggests that, under normal conditions, activity of the ventral hippocampus drives locomotor activity.
Effects of haloperidol and clozapine on hyperactivity induced by N-methyl-D-aspartate stimulation of the ventral hippocampus

We demonstrated that systemic haloperidol and clozapine are equally effective in blocking the increased activity following NMDA stimulation of the ventral hippocampus. As to the effect of haloperidol, our study confirms and extends the recent finding, that 0.13 mg/kg haloperidol administered 5 min before infusion antagonized hyperactivity induced by unilateral infusion of 0.2 μg NMDA into the ventral hippocampus. In vivo, haloperidol and clozapine are similarly effective in blocking limbic, including accumbal, dopamine receptors and both antagonize hyperactivity induced by dopamine infusion into the nucleus accumbens. Thus, our finding that haloperidol and clozapine equally blocked the hyperactivity following NMDA stimulation of the ventral hippocampus is in line with the notion that this hyperactivity depends on activation of accumbal dopamine receptors. However, the involvement of dopaminergic mechanisms in the regulation of locomotor activity and in the hyperactivity induced by NMDA stimulation of the ventral hippocampus is probably quite complex. This is, for example, suggested by the fact that systemic administration of the dopamine D2-class receptor blocker haloperidol (and present study) as well as the infusion of the dopamine D2-class receptor agonist quinpirole into the nucleus accumbens (mainly its core region) blocked hyperactivity following NMDA stimulation of the ventral hippocampus. One possibility is that the activation of accumbal dopamine D2-class receptors has to be impulse dependent or phasic, but not tonic, to stimulate locomotor activity. Thus, dopamine D2-class receptor agonists as well as antagonists would antagonize hyperactivity by disturbing impulse-dependent or phasic D2-class receptor activation. In addition, there is a lot of evidence that the locomotor responses mediated by dopamine receptors in the nucleus accumbens depend on a complex interaction of dopamine D1- and D2-class receptors, and that the locomotor responses to changes in dopaminergic neurotransmission in the nucleus accumbens differ between the core and shell of the nucleus accumbens, and even between different subregions within the shell. As to the hyperactivity induced by NMDA stimulation of the ventral hippocampus, the specific contributions of D1-class and D2-class receptors and the differential involvement of dopaminergic mechanisms in the different subterritories of the nucleus accumbens remain to be examined.

Disruption of prepulse inhibition following N-methyl-D-aspartate stimulation of the ventral hippocampus and effects of haloperidol and clozapine

The two PPI experiments of this study confirm and extend previous findings concerning the PPI disruption by NMDA stimulation of the ventral hippocampus and the failure of systemic administration of the D2-class receptor blocker haloperidol to antagonize this PPI disruption. First, we demonstrated that PPI in Wistar rats is significantly disrupted by bilateral infusion of NMDA into the ventral hippocampus at a dose of 0.5 μg per side. Previously, 0.7 μg per side has been reported to disrupt PPI in Wistar rats and infusion of 0.4 μg per side failed to yield a significant PPI disruption in Sprague–Dawley rats. Second, injection of 0.2 mg/kg haloperidol 45 min prior to testing failed to antagonize PPI disruption by bilateral infusion of 0.5 μg NMDA into the ventral hippocampus. This is in line with previous studies using 0.1 mg/kg, 10 min prior to 0.8 μg NMDA per side, and 0.2 mg/kg, 45 min prior to 0.7 μg NMDA per side. Thus, an ameliorative effect of acute haloperidol on PPI disruption by NMDA stimulation of the ventral hippocampus can surely be ruled out. Consequently, dopamine D2-class receptor-mediated processes clearly do not play a major role in the PPI disruption following NMDA stimulation of the ventral hippocampus.

In the present study, clozapine (5 mg/kg, i.p.) was as ineffective as haloperidol (0.2 mg/kg, i.p.) in antagonizing PPI disruption following bilateral NMDA stimulation (0.5 μg/side) of the ventral hippocampus. This is in contrast to the findings of our previous study which suggested that clozapine has ameliorative effects on PPI disruption by this manipulation. In the earlier study, the effects of clozapine (5 mg/kg, i.p.) were tested on rats that received the fourth bilateral infusion of 0.7 μg NMDA per side. Therefore in those rats a considerable part of NMDA receptor-expressing neurons surrounding the infusion sites might have been destroyed by preceding infusions of NMDA. Thus, it may be that the NMDA infusion failed to disrupt PPI in some of the rats (in fact, our own unpublished observations indicate that after several infusions of NMDA into the ventral hippocampus the behavioural effects of this manipulation are decreased). There were five rats that received clozapine before NMDA infusion and five that received saline before NMDA infusion. Because of the small group sizes, it is possible that the NMDA infusion was, by chance, ineffective in more animals of the clozapine group, thereby suggesting an ameliorative effect of clozapine. It is also worth mentioning that there are quite a few inconsistent findings concerning clozapine’s effects in the PPI model. Thus, in several studies, clozapine (1.3–13 mg/kg; i.p.) did not reverse PPI disruption induced by the non-competitive NMDA antagonist phencyclidine (2 and 5 mg/kg; s.c.) or MK-801 (0.05–0.15 mg/kg; s.c.), while one study reported that PPI disruption induced by phencyclidine (1 mg/kg; s.c.) or MK-801 (0.1 mg/kg; s.c.) was partially antagonized by clozapine (5 mg/kg; i.p.). Findings concerning clozapine’s effects on apomorphine-induced PPI disruption are also controversial. Some studies reported clozapine to antagonize apomorphine-induced PPI disruption, while in these studies, the relationship between the doses of clozapine and the restoration of PPI was inconsistent. In one study, apomorphine (0.5 mg/kg; s.c.)-induced PPI disruption was antagonized by 1–4 mg/kg (i.p.) of clozapine but not higher doses (10 and 20 mg/kg; i.p.). Another study yielded that apomorphine (0.5 mg/kg; s.c.)-induced PPI disruption was antagonized by 9–12 mg/kg (i.p.) of clozapine but not lower doses (1.5, 3, and 6 mg/kg; i.p.). Finally, the most recent study
found apomorphine (0.5 mg/kg; s.c.)-induced PPI disruption to be antagonized by 4–12 mg/kg (i.p.) clozapine. Other studies failed to demonstrate an ameliorative effect of clozapine (5 or 20 mg/kg; i.p.) on PPI disruption induced by apomorphine [0.05 (our own unpublished observations) or 0.5 mg/kg s.c.]. Moreover, it was even found in some experiments that clozapine alone, at doses of 5 mg/kg (our own unpublished observations), 10 mg/kg or 20 mg/kg, induced PPI disruption. Interestingly, this is in line with the result of our first PPI experiment in the present study (Fig. 3A) and the outcomes of some experiments of our study on MK-801-induced PPI disruption, where PPI disruption seemed to be exacerbated by clozapine. In conclusion, the effects of clozapine in the PPI model seem not to be very reliable. This might be related to clozapine’s complex neuropharmacological actions. In any case, based on the results of our present study, it can be stated that clozapine does not reliably antagonize PPI disruption by NMDA stimulation of the ventral hippocampus.

The PPI disruption induced by bilateral stimulation of the ventral hippocampus with 0.5 μg NMDA per side was restricted to the day of infusion. The intact PPI one day after NMDA infusion is congruent with the observations in our previous experiments where we used the slightly higher NMDA dose of 0.7 μg per side. In contrast, another group found the PPI impairment following NMDA infusion into the ventral hippocampus not to be normalized until 48 h after infusion. The longer lasting PPI impairment did not occur in rats with lesions of the piriform cortex, suggesting a crucial involvement of this region in the carry-over of PPI impairment after NMDA infusion into the ventral hippocampus. In the studies which reported the longer lasting PPI disruption, the ventral hippocampus was infused with 1.5 μg NMDA per side. Thus, it may be that the ventral hippocampus has to be activated above a threshold to activate those neuronal processes, apparently involving the piriform cortex, which are responsible for the longer lasting PPI deficit. However, it has to be taken into consideration that a high dose of 1.5 μg NMDA per side could cause longer lasting damage to the ventral hippocampus in addition to the temporary stimulation. Although rats with NMDA lesions of the ventral hippocampus did not exhibit a PPI deficit when tested several days after the lesion, the damage induced by infusion of a high dose of NMDA may elicit processes that could be responsible for the PPI impairment lasting for longer than 24 h after infusion. With our dose of 0.5 μg NMDA per side, not only the PPI impairment but also the hyperactivity following NMDA infusion was clearly restricted to the time following infusions. This indicates that the behavioural effects observed in the present study were due to a temporary and not to a longer lasting alteration in activity of the ventral hippocampus.

**Possible mechanisms underlying disruption of prepulse inhibition following N-methyl-D-aspartate stimulation of the ventral hippocampus**

The experiments of the present study dissociated the mechanisms underlying the hyperactivity and the PPI disruption following NMDA stimulation of the ventral hippocampus. While the hyperactivity was completely blocked by the preceding injection of haloperidol or clozapine, the PPI disruption was unaffected by both neuroleptics. Our results constitute further evidence that the hyperactivity following NMDA stimulation of the ventral hippocampus is largely due to increased dopamine receptor activation in the nucleus accumbens. Since the PPI disruption following stimulation of the ventral hippocampus by NMDA is not antagonized by systemic administration of the potent dopamine D2-class receptor antagonist haloperidol, it is clearly not mediated via D2-class receptor activation in the nucleus accumbens. There is the possibility that the increased accumbal dopamine levels following NMDA stimulation of the ventral hippocampus disrupt PPI by D1-class receptor-mediated mechanisms. However, this is very unlikely because selective D1-class agonists do not interfere with PPI and D1-class receptor antagonists do not ameliorate PPI deficits induced by dopamine agonists. Nevertheless, excitotoxic lesions of the nucleus accumbens have been found to disrupt PPI and the hippocampus-accumbens projection is very prominent. Thus, thinking about dopamine-independent processes in the accumbens that might be responsible for the PPI disruption following NMDA stimulation of the ventral hippocampus stands to reason. Hippocampal stimulation shifts accumbal neurons to an activated state. This process could be dopamine independent and responsible for the PPI disruption. It is not easy to explain how both accumbal lesions and activation of accumbal neurons by hippocampal stimulation should yield the same effect, namely PPI disruption. Findings in our laboratory (S. Kaufmann, A. L. Jongen-Rélo and J. Feldon, unpublished observations) suggest that lesions of the accumbens core rather than of the accumbens shell disrupt PPI (conflicting with the finding of Kodsi and Swerdlow that both core and shell lesions disrupt PPI). Consistent with the topography of the hippocampus-accumbens projection, ventral hippocampal stimulation could mainly lead to activation of neurons in the accumbens shell. If we assume that shell activation inhibits neural activity in core, activation of shell neurons by stimulation of hippocampus-accumbens projections could disrupt PPI in a similar way as core lesions do. It may also be that other hippocampal connections than those with the nucleus accumbens are involved in PPI disruption after NMDA stimulation of the ventral hippocampus. For example, the ventral parts of the hippocampus have strong projections to the medial prefrontal cortex and the amygdala. After local manipulations of the amygdala [Ref. 3 (but see Ref. 17) and Ref. 57], but also of the medial prefrontal cortex, PPI disruption which was proposed to be independent of increased activation of accumbal D2-class receptors has been found. Thus, stimulation of the ventral hippocampus could induce alterations in neuronal activity in the amygdala or the medial prefrontal cortex, which lead to PPI disruption independent of dopaminergic processes in the nucleus accumbens. Specifically, alterations in neuronal activity
in the amygdala were proposed to affect PPI independent of dopaminergic mechanisms via amygdaloid projections to the ventral pallidum, which has direct efferents to brainstem centres mediating PPI of the startle response. It was proposed to differentiate between reversible state- and fixed trait-linked PPI deficits in schizophrenia, whose neural substrates might be partially dissociable. It was suggested that PPI deficits susceptible to antipsychotic treatment and probably mediated by subcortical dopamine represent reversible state-linked deficits, whereas treatment-resistant PPI deficits represent the fixed trait-linked deficits that might be due to temporal lobe abnormalities. Possibly, the neuroleptic-resistant PPI deficits induced in rats by NMDA stimulation of the ventral hippocampus and systemic administration of MK-801 might be comparable to the fixed trait-linked PPI deficits of schizophrenic patients.

**Implications with respect to schizophrenia**

While D2-class receptor activation within the nucleus accumbens is without a doubt one way to impair PPI, there is clear evidence for manipulations disrupting PPI via alternative ways. NMDA stimulation of the ventral hippocampus (and present study) and systemic administration of non-competitive NMDA antagonists, like MK-801, phencyclidine and ketamine, are the best examined examples for such manipulations. Given the construct validity of PPI disruption in rats for the pathology of schizophrenia, identification of the common neuronal processes elicited by these two manipulations could provide new insights into the causes of schizophrenic symptoms which are resistant to antidopaminergic treatment. MK-801-induced PPI disruption has been found to be antagonized by compounds with strong antagonist properties at 5-HT2A receptors, in particular the 5-HT2A receptor antagonist, and it may be worthwhile to examine the effects of such compounds on PPI disruption by NMDA stimulation of the ventral hippocampus. Although clozapine has a several-fold higher affinity at the 5-HT2A than at dopamine receptors, clozapine doses, which effectively antagonized hyperactivity induced by dopamine receptor hyperstimulation (and present study), failed to restore PPI disruption induced by NMDA stimulation of the ventral hippocampus (present study) or by systemic MK-801 (but see Ref. 4). This is not necessarily incongruent with an involvement of 5-HT2A receptor stimulation in these two forms of PPI disruption. For example, it is possible that restoration of PPI requires a very strong antagonism of 5-HT2A receptor stimulation or that clozapine’s interactions with other neurotransmitter receptors counteract the ameliorative effects of the 5-HT2A receptor antagonism. This would mean that only more specific compounds with very high 5-HT2A receptor affinity, such as risperidone and ketanserin, are effective. Interestingly, based on a preliminary cross-sectional study conducted with schizophrenic patients, it was suggested that PPI deficits susceptible to antipsychotic treatment and probably mediated by subcortical dopamine represent reversible state-linked deficits, whereas treatment-resistant PPI deficits represent the fixed trait-linked deficits that might be due to temporal lobe abnormalities. Possibly, the neuroleptic-resistant PPI deficits induced in rats by NMDA stimulation of the ventral hippocampus and systemic administration of MK-801 might be comparable to the fixed trait-linked PPI deficits of schizophrenic patients.

**Conclusions**

Stimulation of the ventral hippocampus induced by bilateral infusion of NMDA (0.5 μg per side) led to the temporary increase in locomotor activity in the open field and to the temporary disruption of PPI. Obviously, the neuronal processes responsible for these two behavioural sequelae of stimulation of the ventral hippocampus are different. The present study provides clear evidence for this fact, since both haloperidol (0.2 mg/kg) and clozapine (5 mg/kg), administered i.p. 45 min before infusion, blocked the increase in locomotor activity but were without an effect on PPI disruption. Activity of the ventral hippocampus drives locomotor activity, probably via dopamine receptor-mediated mechanisms in the nucleus accumbens. In contrast, dopamine receptor activation in the nucleus accumbens does not seem to be involved in PPI disruption induced by NMDA stimulation of the ventral hippocampus. Our previous suggestion that PPI disruption by NMDA stimulation of the ventral hippocampus could serve as a tool to differentiate between the action of typical and atypical neuroleptics was not confirmed by the present study. The atypical neuroleptic clozapine was as ineffective in antagonizing the PPI disruption as the typical neuroleptic haloperidol.

**Acknowledgements**—This work was supported by the Swiss Federal Institute of Technology Zurich. Clozapine was a gift from Novartis Pharma, Basel. In addition, the authors are very grateful for the support provided by the members of the animal, technical and administrative services of the Laboratory of Behavioural Neurobiology.

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1.3.

NMDA stimulation of the ventral hippocampus


(Accepted 18 December 2000)
Hyperactivity, decreased startle reactivity, and disrupted prepulse inhibition following disinhibition of the rat ventral hippocampus by the GABA<sub>A</sub> receptor antagonist picrotoxin

Received: 4 January 2001 / Accepted: 14 March 2001 / Published online: 21 June 2001
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Abstract Rationale: Functional imaging studies have revealed overactivity of the hippocampus in schizophrenic patients. Neuropathological data indicate that hyperactivity of excitatory hippocampal afferents and decreased hippocampal GABA transmission contribute to this overactivity. In rats, excitation of the ventral hippocampus, e.g. by NMDA, results in hyperactivity and disruption of sensorimotor gating measured as prepulse inhibition (PPI) of the acoustic startle response, behavioral effects related to psychotic symptoms in humans.

Objective: The present study examined whether disinhibition of the ventral hippocampus by the GABA<sub>A</sub> antagonist picrotoxin would result in similar psychosis-related behavioral disturbances (hyperactivity, decreased PPI) as NMDA stimulation.

Methods and results: Wistar rats received bilateral infusions of subconvulsive doses of picrotoxin (100 or 150 ng/0.5 μl per side) into the ventral hippocampus and were then immediately tested for open field locomotor activity or startle reactivity and PPI. Only the higher dose induced hyperactivity and decreased PPI. Both doses decreased acoustic startle reactivity to a similar extent. The decreased PPI appeared not to result from decreased startle reactivity, but was associated with a diminished potency of the prepulses to inhibit the startle reaction to the startle pulse, indicating a sensorimotor gating deficit. All effects were temporary, i.e. disappeared when the rats were tested 24 h after infusion.

Conclusions: Decreased GABAergic inhibition in the ventral hippocampus of rats yielded psychosis-related behavioral effects, very similar to those induced by NMDA stimulation. Thus, a concurrence of decreased GABAergic inhibition and increased afferent excitation in the hippocampus of schizophrenic patients might contribute to psychotic symptoms.

Keywords: GABA · Locomotor activity · Prepulse inhibition · Schizophrenia · Sensorimotor gating · Ventral hippocampus

Introduction

Animal studies concerned with hippocampal functioning receive particular interest, as aberrant hippocampal functioning in humans has been implicated in neuropsychiatric disorders such as anxiety and schizophrenia (Gray et al. 1991; Gray 1995; Benes 2000; Grace 2000). Functional imaging studies indicate that hippocampal activity is increased in schizophrenic patients and hippocampal overactivity has been associated with the experience of positive symptoms (Friston et al. 1992; Liddle et al. 1992; Silbersweig et al. 1996; Heckers et al. 1998; Dierks et al. 1999; Frith 1999; Shergill et al. 2000).

In rats, overactivity of the ventral hippocampus, induced, for example, by infusion of NMDA to stimulate the NMDA-type receptor of the excitatory transmitter glutamate, generates behavioral effects which might be related to some positive symptoms occurring in schizophrenia. Locomotor activity in the open field is increased following NMDA stimulation of the ventral hippocampus (Yang and Mogenson 1987; Wu and Brudzynski 1995; Brudzynski and Gibson 1997; Bardgett and Henry 1999; Legault and Wise 1999; Bast et al. 2001b). Aberrant locomotor activity in the rat might be homologous to some changes in human cognitive function observed in acute schizophrenia (Gray et al. 1999). Furthermore, prepulse inhibition (PPI) of the acoustic startle response is disrupted following NMDA stimulation of the ventral hippocampus (Wan et al. 1996; Klärner et al. 1998; Koch et al. 1999; Zhang et al. 1999; Bast et al. 2001b). PPI is the reduction of the startle response to an intense acoustic pulse by an immediately preceding weaker stimulus, or prepulse. It may reflect sensorimotor gating mechanisms induced by the prepulse and preventing its processing from being inter-
ruptured (Graham 1975; Norris and Blumenthal 1996). Deficient sensorimotor gating, as reflected by disruption of PPI, has been found in several neuropsychiatric disorders, especially in schizophrenia (Braff et al. 1978; Grillon et al. 1992; Perry and Braff 1994; Karper et al. 1996; Braff et al. 1999; Perry et al. 1999; Kumari et al. 2000; Parwani et al. 2000; Weike et al. 2000). Although there is little doubt about the existence of a PPI deficit in schizophrenia, its contribution to schizophrenic symptoms is not yet clear. However, deficient PPI has been postulated to contribute to sensory overload and cognitive fragmentation, which in turn result in psychotic symptoms, and correlations between decreased PPI and the severity of psychotic symptoms, in particular positive symptoms, have been reported by some studies (Perry et al. 1999; Braff et al. 1996; Perry et al. 1999; Weike et al. 2000). Disrupted PPI in rats is used to模型 sensorimotor gating deficits observed in schizophrenia (Swerdlow et al. 1994, 2000a).

Overactivity of a brain structure can come about by increased excitatory neurotransmission and by disinhibition, i.e. a decrease of inhibitory transmission. In the hippocampus, inhibition is mainly mediated by actions of GABA, which is released by hippocampal interneurons, at the GABA_A receptor, a ligand-operated Cl^-channel (Buhl et al. 1994). Neuropathological studies indicate that increased excitatory transmission via hippocampal afferents in concert with a local decrease of GABAergic inhibition (possibly due to a loss of hippocampal GABA interneurons) might yield the hippocampal overactivity revealed by functional imaging studies in schizophrenic patients (for review see: Benes 2000). Moreover, dysfunctions of GABA transmission have been implicated in the processes leading to psychosis (Keverne 1999; Lacroix et al. 2000) and psychotic symptoms in schizophrenia have been found to be correlated with reduced GABAergic inhibition in the medial temporal region (Busatto et al. 1997). Thus, decreased inhibitory GABA transmission in the hippocampus of schizophrenic patients might contribute to psychotic symptoms in schizophrenia.

In the present study, we tested in Wistar rats if the disinhibition of the ventral hippocampus by local microinfusion of the GABA_A receptor antagonist picrotoxin would yield effects on PPI and locomotor activity in the open field that are similar to those resulting from stimulation of the ventral hippocampus by NMDA (Zhang et al. 1999; Bast et al. 2001b). In a recent study, bilateral infusion of 5 or 10 ng picrotoxin/side into the ventral hippocampus of Sprague-Dawley rats did not significantly affect startle amplitude or PPI (Japha and Koch 1999). The authors reported difficulties in testing higher doses because of convulsions occurring at higher doses. In our own pilot studies, doses up to 50 ng picrotoxin/side did not reveal any considerable behavioral effect or convulsive properties. Therefore, we used doses of 100 or 150 ng picrotoxin/side in the present study.

### Materials and methods

#### Animals

A total of 50 male adult Wistar rats (Zur:Wist[Han]br, Research Unit Schwerzenbach, Schwerzenbach, Switzerland), weighing about 250 g at the time of surgery, were used in this study. The animals were housed in groups of four per cage under a reversed light-dark cycle (lights on: 1900-0700 hours) in a temperature (21±1°C) and humidity (55±5%) controlled room and were allowed free access to food and water. All rats received bilateral implantation of infusion guide cannulae aiming at the ventral hippocampus. After surgery, they were individually caged. Beginning 3 days before surgery and throughout the studies, all rats were handled daily. Behavioral testing was carried out in the dark phase of the cycle. All experiments were conducted in accordance with Swiss regulations for animal experimentation.

#### Implantation of guide cannulae for intracerebral infusion

Rats were anesthetized with 1 ml of Nembutal (sodium pentobarbital, 50 mg/ml; Abbott Labs, North Chicago, III., USA) per kg body weight and their head was placed in a Kopf stereotaxic frame. After application of a local anesthetic (lidocaine), the scalp was incised to expose the skull. Bregma and lambda were aligned in the same horizontal plane. A small hole (1.5 mm diameter) was drilled on each side of the skull to reveal the dura covering the cortex overlying the ventral hippocampus. Three small stainless steel screws were screwed into the skull and two guide cannulae (9 mm, 26 gauge; stainless steel) were implanted bilaterally into the brain through boreholes in the skull. The tips of the guide cannulae were aiming above the ventral hippocampus (5.2 mm posterior and ±5 mm lateral to bregma, and 5 mm ventral to dura). Guide cannulae were fixed by dental cement for which the three screws served as anchors to the skull. Stainless steel stylets (34 gauge) extending 0.5 mm beyond the tips of the guide cannulae were placed inside the guide cannulae to prevent occlusion. After surgery, rats were allowed to recover for 5 days during which the experimenters gave the rats daily health checks and gentle handling, and replaced missing stylets.

#### Intracerebral microinfusion

The rats were manually restrained, the stylets were removed from the guide cannulae, and infusion cannulae (34 gauge), connected to 10-μl Hamilton microsyringes mounted on a microinfusion pump (KD scientific or WPI sp2000i), were inserted into the guide cannulae. The tips of the infusion cannulae protruded into the ventral hippocampus 1.5 mm beyond the tips of the guide cannulae, thus aiming at a final dorsoventral coordinate of 6.5 mm below the dura. The rats were bilaterally infused with 100 or 150 ng picrotoxin in 0.5 μl vehicle or 0.5 μl vehicle only. The infusion speed was 0.5 μl/min. After infusion, the infusion cannulae were left in the brain for 60 s to allow for absorption of the infusion bolus by the brain tissue and then replaced by the stylets. Rats were then immediately subjected to behavioral testing.

#### Drug preparation

The picrotoxin solutions were prepared freshly on the day of experiment. Picrotoxin (CmpH2O2, Fluka, Switzerland) was dissolved in 0.9% saline as vehicle to obtain concentrations of 200 or 300 μg/ml for infusion of 100 or 150 ng (0.17 or 0.25 nmol)/0.5 μl. The final pH was 6–7.
Apparatus and procedures for behavioral testing

Open field locomotor activity

Locomotor activity was measured in four closed square arenas (76.5 cm x 76.5 cm x 49 cm) made of dark gray plastic and placed in a dimly illuminated (20 ± 0.5 Lux provided by two halogen lights) room. Behavior in the arenas was recorded by a video camera suspended from the ceiling and relayed to a monitor and a video tracking, motion analysis and behavior recognition system (EthoVision, Noldus, Wageningen, The Netherlands).

Startle and prepulse inhibition

The testing was conducted in four ventilated startle chambers (SR-LAB, San Diego Instruments, San Diego, Calif., USA), containing a transparent Plexiglas tube (diameter 8.2 cm, length 20 cm) mounted on a Plexiglas frame. Noise bursts were presented via a speaker mounted 24 cm above the tube. Motion inside the tube was detected by a piezoelectric accelerometer below the frame. The amplitude of the whole body startle to an acoustic pulse was measured immediately following infusion to compare the acute effect of picrotoxin infusion into the ventral hippocampus and its locomotor activity (between-subjects design). On day 3 (day after infusion), each rat was placed in the center of one of the arenas and its locomotor activity was monitored for 30 min.

Prepulse inhibition experiment

The PPI experiment was conducted on 3 successive days and included three groups: VEH (n=16), PTX100 (n=7), and PTX150 (n=18). On day 1, basal startle and PPI were tested without infusion for all rats. On day 2 (day of infusion), startle and PPI were measured immediately following infusion to compare the acute effect of picrotoxin and vehicle infusion (between-subjects design). On day 3 (day after infusion), all rats were tested for startle and PPI without an infusion.

Histology

After completion of the behavioral experiments, rats were deeply anesthetized with 2.5 ml/kg Nembutal (sodium pentobarbital, 50 mg/ml, IP) and transcardially perfused with 0.9% saline solution to rinse out the blood, followed by 250 ml of 4% formalin (4°C) to fix the brain tissue. The brains were extracted from the skull, post-fixed in 4% formalin solution, and subsequently cut into 40-μm coronal sections on a freezing microtome. For the verification of the infusion sites, every fifth section through the ventral hippocampus was mounted on gelatin-treated slides and stained with cresyl violet. After staining, the sections were dehydrated and coverslipped. Subsequently, they were examined by light microscopy to verify that the tips of the infusion cannulae were placed in the ventral hippocampus and to draw their approximate locations onto plates taken from the atlas of Paxinos and Watson (1998).

Data analysis

All statistical analyses were performed with the StatView software system (Abacus Concepts, Inc., Berkeley, Calif., USA, 1992) using analysis of variance (ANOVA). Data are presented as means. In the text and in bar plots, the variability is indicated by the standard error of the mean (SEM). In line plots, for the sake of clarity, twice the standard error (SE) derived from the appropriate mean square yielded by the ANOVA was used to indicate variability. Fisher's protected least significant difference test was used for post hoc comparisons following a significant main effect revealed by ANOVA. In order to interpret significant interactions indicated by ANOVA, significant differences between the mean values of interest were estimated by using 2 SE derived from the appropriate mean square of the ANOVA (see Winer 1971, p. 195), since the StatView software only conducts post hoc comparisons relevant to main effects. Significant differences were accepted at P<0.05.

Open field experiment

The total distance the rat moved in cm throughout the complete arena was calculated for each 10-min block of testing. Groups...
were taken as between-subjects factor and the 10-min blocks as repeated measures.

Prepulse inhibition experiment

The percentage PPI (%PPI) induced by each prepulse intensity was calculated as: \(\left\{\frac{100 \times \text{startle amplitude on prepulse followed by pulse trial}}{\text{startle amplitude on pulse alone trial}}\right\} \times 100\) from the data taken during the six test blocks to measure PPI. For statistical analysis of the startle and %PPI data, groups were used as between-subjects factor and the 16 pulse alone trials (startle data) or four prepulse intensities (%PPI data) were taken as repeated measures. On the day of infusion, decreased %PPI occurred with a concomitant decrease of the average startle amplitude in the 16 pulse alone trials. As recently discussed extensively by Swerdlow et al. (2000a), decreased %PPI with concomitant changes of startle amplitude in pulse alone trials cannot unequivocally be ascribed to deficient induction of sensorimotor gating processes by the prepulses. In order to assess if the prepulses' potency to reduce the startle response was affected, PPI was also assessed by comparing the startle magnitude in the pulse alone and the prepulse followed by pulse trials of the six test blocks. For this purpose, startle data of the pulse alone trials and the four different prepulse followed by pulse (one for each prepulse intensity) trials were subjected to ANOVA using the five trial types as repeated measures.

Results

Location of infusion sites

In all 50 cannulated rats, the tips of the infusion cannulae were located within or around the border of the ventral hippocampus (Fig. 1). Damage resulting from the implantation of the guide cannulae was restricted to the area immediately surrounding the tracks of guide and infusion cannulae.

Picrotoxin infusions into the ventral hippocampus did not induce convulsions

Picrotoxin infusions (100 and 150 ng/side) into the ventral hippocampus did not induce convulsions. Also, tremor, sniffing, or whisker shaking, which sometimes occur following NMDA infusions (Bast et al. 2001b), were not observed. Some rats which received 150 ng picrotoxin/side exhibited shaking of their hind body during a period of some minutes beginning briefly after infusion.

Picrotoxin infusion into the ventral hippocampus increased locomotor activity

On day 1 of the open field experiment (day before infusion), PTX150 and VEH groups did not differ in the distance moved in the open field \(F(1,29)=0.1, P>0.7\); data not shown). ANOVA revealed only a highly significant effect of 10-min block \(F(2,58)=133.8, P<0.0001\), reflecting habituation to the open field. On day 2 (day of infusion), both groups did not differ throughout the 30 min preceding the infusion \(F(1,29)=2.8, P>0.1\); Fig. 2, left]. For these 30 min, ANOVA yielded only a highly significant effect of 10-min block \(F(2,58)=79.0, P<0.0001\), reflecting habituation. Following infusion, the PTX150 group exhibited increased activity as compared to the VEH group (Fig. 2, middle). ANOVA of the total distance moved yielded a significant effect of group \(F(1,29)=4.5, P<0.05\), a highly significant effect of 10-min block \(F(5,145)=40.2, P<0.0001\), as well as a highly significant group x 10-min block interaction \(F(5,145)=6.9, P<0.0001\). The latter reflected that the locomotor activity in the PTX150 group was significant-
Before infusion of vehicle (VEH, n=17) or 150 ng picrotoxin/side (PTX150, n=14) into the ventral hippocampus (left), following infusion, activity was further recorded for 60 min (middle). The day after infusion (right), the rats’ locomotor activity was recorded for 30 min. Locomotor activity is represented as the distance moved (mean in cm) during consecutive 10-min blocks. The bar represents 2 SE derived from the ANOVA. Asterisks denote a difference of more than 2 SE between the two groups.

Picrotoxin infusion into the ventral hippocampus decreased the startle amplitude and caused a dose-dependent disruption of prepulse inhibition.

The startle reactivity measured in the pulse alone trials was decreased by infusion of both 100 and 150 ng picrotoxin/side into the ventral hippocampus (Fig. 3A). ANOVA yielded a significant effect of group on the average startle amplitude during the 16 pulse alone trials on the day of infusion [day 2; F(2,38)=8.4, P<0.002], but not on the day before infusion [day 1; F(2,38)=0.8, P>0.4] or the day after infusion [day 3; F(2,38)=1.1, P>0.3]. Post hoc comparisons revealed that on the day of infusion the average startle amplitude (mean±SEM) was decreased in the PTX150 (460±72; P<0.0002) and PTX150 (460±72; P<0.0002) groups, which did not differ (P>0.25), as compared to the VEH group (1020±130). Short-term habituation of the startle response (Koch 1999) was not affected by picrotoxin infusion into the ventral hippocampus. This was reflected by a highly significant effect of pulse alone trial (all P<0.0001) occurring without a groupxpulse alone trial interaction (all P>0.1) on each of the 3 days.

Infusion of 150, but not 100 ng picrotoxin/side decreased the %PPI (Fig. 3B). ANOVA yielded a significant effect of group on the %PPI on the day of infusion [day 2; F(2,38)=7.0, P<0.005], but not on the day before infusion [day 1; F(2,38)=0.6, P>0.5] or the day after infusion [day 3; F(2,38)=1.1, P>0.25]. Post hoc comparisons revealed that on the day of infusion the average %PPI (mean±SEM) over all four prepulse intensities was decreased in the PTX150 (18.2±4.7) as compared to both the PTX100 (45.4±10.4; P<0.01) and the VEH group (44.1±5.8; P<0.002), which did not differ (P>0.9). A gradual increase of the %PPI with an increase in prepulse intensity (Koch 1999) was evident in all groups on each of the three days. This was reflected by a highly significant effect of prepulse intensity (all P<0.0001) occurring without a groupxprepulse intensity interaction (all P>0.1) on each day.

A comparison of the startle amplitude throughout the pulse alone trials and the prepulse followed by pulse trials (PP+P) which differed by more than 2 SE from that during the pulse alone trials (P) of the same group are denoted by asterisks.

Fig. 2. Locomotor activity in the open field after picrotoxin infusion into the ventral hippocampus. On the day of infusion, the rats’ locomotor activity in the open field was recorded for 30 min before infusion of vehicle (VEH; n=17) or 150 ng picrotoxin/side (PTX150, n=14) into the ventral hippocampus (left). Following infusion, activity was further recorded for 60 min (middle). The day after infusion (right), the rats’ locomotor activity was recorded for 30 min. Locomotor activity is represented as the distance moved (mean in cm) during consecutive 10-min blocks. The bar represents 2 SE derived from the ANOVA. Asterisks denote a difference of more than 2 SE between the two groups.

Fig. 3A–C Startle reactivity and prepulse inhibition after picrotoxin infusion into the ventral hippocampus. Rats were bilaterally infused with vehicle (VEH; n=16), 100 ng picrotoxin/side (PTX100, n=7) or 150 ng picrotoxin/side (PTX150, n=18) into the ventral hippocampus on day 2. All groups were tested for PPI without infusion 1 day before (day 1) and one day after (day 3) the infusion day (day 2). A. Average startle magnitude (mean±SEM) during the 16 pulse alone trials for all 3 days. Differences to the VEH group indicated by post hoc comparisons are denoted *(P<0.0002; #P=0.053). B. Mean percentage of prepulse inhibition averaged across all prepulse intensities for all three days. The bars represent 2 SE derived from ANOVA. C. Mean startle amplitude throughout the pulse alone (P) and the prepulse followed by pulse (PP+P) trials of the six test blocks to measure PPI on the day of infusion. The different prepulse intensities in dB(A) are indicated. The bar represents 2 SE derived from ANOVA. Startle amplitudes in the prepulse followed by pulse trials (PP+P) which differed by more than 2 SE from that during the pulse alone trials (P) of the same group are denoted by asterisks.
PTX 100 and VEH groups (Fig. 3C). ANOVA of the startle data measured throughout the six blocks to test PPI on the day of infusion yielded a significant effect of group $[F(2,38)=3.8, P<0.05]$, reflecting the decreased startle reactivity in the PTX100 and PTX150 as compared to the VEH group, a highly significant effect of trial type $[F(4,152)=31.5, P<0.0001]$, and a highly significant grouptrial type interaction $[F(8,152)=6.7, P<0.0001]$. The interaction reflected that the PTX100 and the VEH group exhibited a similar gradual decrease in startle reaction to the pulse when the pulse was preceded by increasing prepulses, whereas this effect of the prepulses was markedly decreased in the PTX150 group. In the VEH and the PTX group, the startle amplitude was significantly decreased in all prepulse followed by pulse trials as compared to the pulse alone trials, whereas in the PTX150 group only the highest, but not the three lower prepulse intensities had the potency to significantly reduce the startle reaction to the pulse (based on comparisons using the SE derived from the ANOVA). Moreover, in the pulse alone trials, startle amplitude (mean±SEM) appeared to be higher in the PTX100 (520±130) than in the PTX150 (400±71) group. This relation seemed to be reversed in the prepulse followed by pulse trials. When the pulses were preceded by prepulses of 76, 80, and 84 dB(A), the startle amplitude to the pulses appeared to be higher in the PTX100 (250±48, 220±48, 170±33) group. Thus, the decreased %PPI in the PTX150 group was not just due to a decreased startle reactivity in the pulse alone trials but associated with a diminished potency of the prepulses to reduce the startle amplitude in the prepulse followed by pulse trials.

**Discussion**

Disinhibition of the ventral hippocampus by local micro-infusion of the GABA_A antagonist picrotoxin induced hyperactivity in the open field, decreased startle reactivity, and disrupted PPI. All effects were evident only throughout the test sessions immediately following infusion but not 24 h later, indicating that they were due to temporary alterations of neuronal activity in the ventral hippocampus. Infusions of 100 or 150 ng picrotoxin/side into the ventral hippocampus of our Wistar rats did not induce convulsions. In contrast, Japha and Koch (1999) reported doses above 10 ng picrotoxin/side to be convulsive when infused into the ventral hippocampus of Sprague-Dawley rats. The different observations might be related to strain differences. In agreement with our observation, however, doses higher than 300 ng/side had to be applied to the amygdala to induce the slightest seizure grade in Wistar rats in an animal model of temporal lobe epilepsy (Turski et al. 1985).

Hyperactivity following picrotoxin infusion into the ventral hippocampus

The hyperactivity in the open field following disinhibition of the ventral hippocampus by picrotoxin adds to the evidence that activity of the ventral hippocampus drives locomotor activity. Thus, infusions of stimulatory neuroactive substances into the ventral hippocampus stimulate locomotor activity (Yang and Mogenson 1987; Wu and Brudzynski 1995; Brudzynski and Gibson 1997; Brenner and Bardgett 1998; Bardgett and Henry 1999; Legault and Wise 1999; Bast et al. 2001b), whereas increased inhibition of the ventral hippocampus by the GABA_A receptor agonist muscimol or complete inactivation by the sodium channel blocker tetrodotoxin result in hypoactivity in the open field (Bast et al. 2001a). In view of the data from infusion studies, hyperactivity observed under certain circumstances after ventral hippocampal lesions (Nadel 1968; Lipska et al. 1992; Richmond et al. 1999) does not appear to reflect a suppressive influence of the ventral hippocampus on locomotor activity. This hyperactivity might rather be related to lesion-induced damage in extra-hippocampal areas involved in the regulation of locomotor activity, like midbrain dopamine nuclei and their forebrain projection fields (Halim and Swerdlow 2000). The effects of ventral hippocampal infusions might be mediated by the meso-accumbens dopamine system, which can be influenced by the ventral hippocampus via direct projections to the nucleus accumbens (Groenewegen et al. 1987) or via a loop including projections from the ventral hippocampus to the prefrontal cortex (Verwer et al. 1997; see Legault et al. 2000). For example, the hyperactivity induced by NMDA stimulation of the ventral hippocampus seems to be due to increased dopamine transmission in the nucleus accumbens (Wu and Brudzynski 1995; Brudzynski and Gibson 1997; Bardgett and Henry 1999; Legault and Wise 1999; Bast et al. 2001b).

Decreased startle reactivity and disruption of prepulse inhibition following picrotoxin infusion into the ventral hippocampus

While infusion of both 100 and 150 ng picrotoxin/side into the ventral hippocampus decreased the startle reactivity to a similar extent, only the higher dose decreased PPI. This is in line with several findings demonstrating that the startle reflex and PPI can be modulated independently and, specifically, that alterations of the startle amplitude do not necessarily result in changes of PPI (e.g. Caine et al. 1992; Wan et al. 1996; Bast et al. 2000; Zhang et al. 2000). Nevertheless, changes in startle reactivity may in some instances result in decreased PPI. Consequently, decreased PPI cannot unequivocally be ascribed to impaired sensorimotor gating when it is paralleled by changes of the startle amplitude throughout the pulse alone trials (for a detailed discussion see...
Swerdlow et al. 2000a). In the present study, %PPI was reduced only by the higher dose of picrotoxin, whereas both doses led to a similar decrease in startle reactivity, indicating that this decrease cannot account for the PPI deficit. Moreover, the comparison of the startle amplitude in the different trial types demonstrated that the higher dose of picrotoxin specifically impaired the pre-pulses' potency to reduce the startle amplitude in pre-pulse followed by pulse trials. Thus, the decreased PPI following infusion of 150 ng picrotoxin/side into the ventral hippocampus appears to reflect a genuine impairment of sensorimotor gating.

Decreased startle reactivity and disruption of PPI following disinhibition of the ventral hippocampus by picrotoxin resemble the effects of ventral hippocampal stimulation by infusion of excitatory substances, like NMDA (Wan et al. 1996; Klarner et al. 1998; Koch et al. 1999; Zhang et al. 1999; Bast et al. 2001b) and the acetylcholine agonist carbachol (Caine et al. 1992). Thus, overactivity of the ventral hippocampus might be a critical factor to induce these effects. In contrast, ventral hippocampal lesions did not alter basal PPI (Pouzet et al. 1999), but enhanced sensitivity to PPI disruption by the dopamine agonist apomorphine (Swerdlow et al. 1995, 2000b), and one study reported increased startle reactivity following ventral hippocampal lesions (Swerdlow et al. 1995). Moreover, though the non-competitive NMDA antagonist MK-801 disrupts PPI very potently when administered systemically, ventral hippocampal MK-801 infusions did not affect PPI (Bakshi and Geyer 1998; Bast et al. 2001b). However, in view of our own results indicating decreased startle reactivity and disrupted PPI following tetrodotoxin or muscimol infusion into the ventral hippocampus (T. Bast, W.-N. Zhang, J. Feldon, unpublished observations), one has to consider that not only overactivity but also other alterations of activity of the ventral hippocampus may affect startle reactivity and PPI.

The PPI disruption following NMDA stimulation of the ventral hippocampus seems to be dopamine-independent (Wan et al. 1996; Zhang et al. 1999; Bast et al. 2001b) and might be mediated by non-dopaminergic processes in the prefrontal cortex, the nucleus accumbens, or in the amygdala, all of which receive projections from the ventral hippocampus (Groenewegen et al. 1987; Verwer et al. 1997; Pitkänen et al. 2000; see Bast et al. 2001b). The same might hold for the PPI disruption following disinhibition of the ventral hippocampus by picrotoxin. However, Japha and Koch (1999), based on the fact that haloperidol seemed to antagonize a non-significant PPI impairment after infusion of 10 ng picrotoxin/side into the ventral hippocampus of Sprague-Dawley rats, proposed the opposite. Testing the effects of haloperidol pretreatment on the PPI disruption induced by infusion of 150 ng picrotoxin/side into the ventral hippocampus of our Wistar rats could clarify this issue. The circuits mediating the decrease of startle reactivity following stimulation or disinhibition of the ventral hippocampus might involve projections from the ventral hippocampus to the amygdala or the bed nucleus of the stria terminalis (Swanson and Cowan 1977; Pitkänen et al. 2000), which have access to the brain stem startle circuit (Koch 1999).

Only the higher dose of picrotoxin disrupted PPI, whereas both doses decreased the startle reactivity markedly and to a similar extent. In contrast, infusions of NMDA doses which potently disrupted PPI were not always found to decrease startle reactivity (Wan et al. 1996; Klarner et al. 1998; Koch et al. 1999; Zhang et al. 1999; Bast et al. 2001b; W.-N. Zhang, T. Bast, J. Feldon, unpublished observations). Thus, disinhibition of the ventral hippocampus by picrotoxin has a stronger impact on the startle reactivity, whereas NMDA stimulation more strongly affects PPI. This indicates slight differences in the neuronal processes induced by these two forms of ventral hippocampal activation.

As a protective reaction to an aversive stimulus, the startle response is enhanced by aversive states, such as anxiety and fear, in rats as well as humans, and enhanced startle reactivity is a diagnostic criterion of human anxiety disorders (Lang 1995; Rodgers 1997; Filion et al. 1998; Koch 1999). Anxiety-related states, i.e. enhanced behavioral reactivity to aversive stimuli, have been associated with activity in the septo-hippocampal system (Gray 1995) and particularly with decreased GABA_A receptor-mediated inhibition in the hippocampus (see Crestani et al. 1999; Löw et al. 2000). The markedly decreased startle reactivity following infusion of the GABA_A antagonist picrotoxin into the ventral hippocampus does not support these concepts.

Previous work has indicated that motor activity may decrease startle reactivity and also PPI (Wecker and Ison 1986; Plappert et al. 1993). Whereas in particular grooming had a strong effect, gross postural changes, such as turning, did not affect startle and PPI (Wecker and Ison 1986). Picrotoxin infusion into the ventral hippocampus induced increased locomotor activity, which results from gross postural changes, while there was no evidence of increased grooming. Thus, the hyperactivity induced by the picrotoxin infusion into the ventral hippocampus cannot account for the decreased startle reactivity and PPI observed in the present study.

**Conclusion**

Blockade of GABA_A receptors in the ventral hippocampus induced hyperactivity in the open field and an impairment of sensorimotor gating. These effects are similar to those induced by direct excitation of the ventral hippocampus and related to psychotic symptoms. A concurrence of increased afferent excitation and decreased GABAergic inhibition might contribute to the hippocampal overactivity in schizophrenic patients (Benes 2000). In order to clarify the relevance of these aberrations with respect to schizophrenic symptoms, it is of interest to examine whether excitation or disinhibition of the ventral hippocampus in rats elicits further psychosis-related be-
havioral disturbances, such as disruption of latent inhibition and other attentional processes (Gray et al. 1991; Feldon and Weiner 1992). Interestingly, unpublished observations (B. Pouzet, W.-N. Zhang, and J. Feldon) indicate that NMDA stimulation, in contrast to cytotoxic lesions, of the ventral hippocampus disrupts latent inhibition. Moreover, the effects of picrotoxin in the dorsal hippocampus remain to be explored. Recent experiments, however, demonstrating NMDA stimulation of the dorsal hippocampus to hardly affect PPI and locomotor activity (W.-N. Zhang, T. Bast, J. Feldon, unpublished observations), indicate that these processes might be much less affected by dorsal than by ventral hippocampal disinhibition.

Acknowledgements This work was supported by grants from the Swiss Federal Institute of Technology Zurich. The authors thank the animal facility team for their care of the animals, Ms. Liz Weber for her histology preparations, Mr. Peter Schmid for the set-up and maintenance of the computerized systems for behavioral analysis, and Ms. Bonnie Strehler for her assistance with manuscript preparation.

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Effects of Hippocampal N-Methyl-d-Aspartate Infusion on Locomotor Activity and Prepulse Inhibition: Differences Between the Dorsal and Ventral Hippocampus

Wei-Ning Zhang, Tobias Bast, and Joram Feldon
Swiss Federal Institute of Technology Zurich

Prepulse inhibition (PPI) of the acoustic startle response and open-field locomotor activity were measured after bilateral infusion of N-methyl-d-aspartate into the ventral (0.10, 0.25, 0.50 μg/side) and dorsal (0.10, 0.25, 0.50, 0.70 μg/side) hippocampus of Wistar rats. Dose-dependent hyperactivity and disruption of PPI—behavioral effects related to psychotic symptoms—were observed after ventral infusions but were virtually absent after dorsal infusions. This functional dorsal–ventral difference might be related to the different connections of the dorsal and ventral hippocampus with the amygdala, nucleus accumbens, and prefrontal cortex, which have been implicated in the regulation of locomotor activity and PPI. Hippocampal overactivity has been associated with schizophrenia. The findings suggest that overstimulation of the ventral hippocampal projections may contribute to behavioral outcomes related to psychotic symptoms.

The hippocampal formation is, in addition to its mnemonic functions, involved in the regulation of basic non-mnemonic processes that are essential for the generation of adaptive behaviors, and hippocampal dysfunction has been associated with human neuropsychiatric disorders such as schizophrenia (Grace, 2000; Gray, 1995, 1998; Gray, Feldon, Rawlins, Hemsley, & Smith, 1991). This contributes to a substantial interest in studying the effects of hippocampal manipulations on basic behavioral processes in the rat.

Studies in rats, involving hippocampal lesions and hippocampal infusions, have implicated the hippocampus in the regulation of locomotor activity and of prepulse inhibition (PPI) of the acoustic startle response. Regulation of locomotor activity by the rat hippocampus is important for exploratory and goal-directed behavior and involves connections to the nucleus accumbens and possibly to the prefrontal cortex (Floresco, Seamans, & Phillips, 1997; Mogenson, Wu, Yang, & Yim, 1993). Aberrant locomotor activity in the rat may be homologous to some alterations of human cognitive function observed during acute schizophrenia (Gray, Kumari, Lawrence, & Young, 1999). PPI is the reduction of the startle response to an intense acoustic pulse caused by an immediately preceding weaker stimulus, or prepulse, and may reflect sensorimotor gating mechanisms that prevent the processing of the prepulse from being interrupted (Graham, 1975; Norris & Blumenthal, 1996). PPI is mediated via brainstem circuits but is thought to be regulated by forebrain circuits including the hippocampus, prefrontal cortex, amygdala, and nucleus accumbens (Broersen, Feldon, & Weiner, 1999; Koch, 1999; Lacroix, Broersen, Feldon, & Weiner, 2000; Swerdlow, Caine, Braff, & Geyer, 1992). Deficient sensorimotor gating, as reflected by disruption of PPI, has been found in several neuropsychiatric disorders. In particular, it has been associated with schizophrenic symptoms. Disrupted PPI in rats is used to model the sensorimotor gating deficits observed in schizophrenia (Swerdlow, Braff, & Geyer, 2000; Swerdlow, Braff, Taaid, & Geyer, 1994).

Functional imaging studies have indicated that hippocampal activity is increased in schizophrenic patients, and hippocampal overactivity has been associated with the experience of positive symptoms (Diercks et al., 1999; Friston, Liddle, Frith, Hirsch, & Frackowiak, 1992; Heckers et al., 1998; Silbersweig et al., 1995). In rats, overactivity of the ventral hippocampus can be induced by local infusion of N-methyl-d-aspartate (NMDA) to stimulate NMDA-type glutamate receptors, which are very abundant in the hippocampal formation (Petralia, Yokotani, & Wenthold, 1994). Such stimulation of the ventral hippocampus causes hyperactivity and severe disruption of PPI (Bardgett & Henry, 1999; Bast, Zhang, Heidbroder, & Feldon, 2001; Brudzynski & Gibson, 1997; Klarnert, Koch, & Schnitzler, 1998; Koch, Klarnert, & Schnitzler, 1999; Legault & Wise, 1999; Wan, Caine, & Swerdlow, 1996; Wu & Brudzynski, 1995; Yang & Mogenson, 1987; Zhang, Pouzet, Jongen-Rêlo, Weiner, & Feldon, 1999). Moreover, overactivity of the ventral hippocampus increases dopamine release within the nucleus accumbens, although which hippocampal connections are critical for this effect is still being debated (Blaha, Yang, Floresco, Barr, & Phillips, 1997; Brudzynski & Gibson, 1997; Legault,
The ventral and dorsal parts of the vertebrate hippocampal formation are connected with different sets of extrahippocampal structures (Amaral & Witter, 1995; Siegel & Tassoni, 1971; Swanson & Cowan, 1977; Verwer, Meijer, Van Uum, & Witter, 1997; Witter, 1986). This suggests functional differences between the ventral and dorsal hippocampus. In fact, spatial learning and memory, the mnemonic functions with which the hippocampus is most strongly associated, seem to depend more on neuronal processes in the dorsal rather than the ventral hippocampus (Bannerman et al., 1999; Jung, Wiener, & McNaughton, 1994; Moser & Moser, 1998; Moser, Moser, & Andersen, 1993). This was considered to be consistent with the dorsal part of the hippocampus receiving highly processed sensory information from the sensory cortices (but see Pouzet, Welzl, et al., 1999). In contrast, the ventral hippocampus in particular contains projections to structures that have been associated with the regulation of PPI and locomotor activity, namely, the prefrontal cortex, the amygdala, and the nucleus accumbens, predominantly its shell part. Among these structures, only the nucleus accumbens, predominantly its core part, receives projections from the dorsal hippocampus (Amaral & Witter, 1995; Groenewegen, Vermeulen-Van der Zee, Te Kortschot, & Witter, 1987; Kelley & Domesick, 1982; Pitzäehn, Pikkarainen, Nurminen, & Ylinen, 2000; Siegel & Tassoni, 1971; Swanson & Cowan, 1977; Verwer et al., 1997; Witter, 1986).

Thus, the dorsal and ventral hippocampus might be involved differently in the regulation of PPI and locomotor activity. Furthermore, clarification of these differences might provide clues as to the hippocampal connections that are critical for the regulation of PPI and locomotor activity in the rat and are involved in the pathophysiology of schizophrenia. PPI disruption and hyperactivity after NMDA infusion into the ventral hippocampus have been the subject of several studies. In contrast, the effects of NMDA infusion into the dorsal hippocampus are drug-specific and there are no general effects caused by the surgery or the infusion procedure (Bast, Zhang, Feldon, & White, 2000; Bast, Zhang, Heidbreder, & Feldon, 2001; Zhang, Heidbreder, & Gibson, 1997; Wan et al., 1996; Wu & Brudzynsky, 1995; Zhang et al., 1999).

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**Surgical Procedures**

Rats were anesthetized with 1 ml Nembutal (sodium pentobarbital, 50 mg/ml, Abbott Labs, North Chicago, IL) per kilogram of body weight. The rat's head was placed in a Kopf stereotaxic frame, and after application of a local anesthetic (lidocaine), its scalp was incised to expose the skull. Bregma and lambda were aligned in the same horizontal plane. A small hole (1.5 mm in diameter) was drilled on each side of the skull to reveal the dura covering the cortex overlying the hippocampus. Stainless steel guide cannulas (26 gauge, 9 mm and 7 mm for ventral or dorsal hippocampus, respectively) in a Perspex holder were implanted bilaterally into the brain, aimed at the ventral (−5.2 mm posterior and ±0.0 mm lateral to bregma, and −5.0 mm ventral to dura) or dorsal (±3.0 mm posterior and ±1.5 mm lateral to bregma, and −2.5 mm ventral to dura) hippocampus. The guide cannulas were fixed to the skull with three anchoring skull screws and dental cement. Stainless steel stylets (34 gauge) extending 0.5 mm beyond the tips of the guide cannulas were placed inside the guide cannulas to prevent occlusion. After surgery, rats were allowed to recover for 5 days before behavioral procedures began, during which time the experimenter gave the rats daily health checks and gentle handling and replaced missing stylets.

**Intracerebral Infusions**

The rats were manually restrained, the styels were carefully removed, and infusion cannulas (34 gauge, stainless steel) were inserted into the brain through the previously implanted guide cannulas. The tip of the infusion cannula protruded 1.5 mm beyond the tip of the guide cannula into the ventral or dorsal hippocampus. The infusion cannulas were connected to 10-μl Hamilton (Reno, NV) microsyringes by flexible polyetheretherketone tubing. The syringes were mounted on a KD Scientific (New Hope, PA) microinfusion pump. The infusion volume was 0.5 μl/side, delivered at a rate of 0.5 μl/min. After infusion, the infusion cannulas were kept in place for an additional 60 s to allow the injection bolus to be absorbed by the tissue. Styels were then reinserted, and rats were placed immediately into the behavioral testing chamber.

**Drugs**

NMDA (Sigma, Switzerland) was dissolved in 0.1 M phosphate-buffered saline vehicle (pH 7.4), producing solutions of different doses.
(0.10, 0.25, 0.50, and 0.70 µg/0.5 µl) for bilateral infusion into the ventral or dorsal hippocampus. The highest doses were based on previous experiments in which they caused disruption of PPI and pronounced hyperactivity without producing seizures when infused into the ventral hippocampus (Bast, Zhang, Heidbreder, & Feldon, 2001; Zhang et al., 1999).

Apparatus and Procedures for Behavioral Testing

Prepulse Inhibition

The apparatus consisted of four startle chambers (SR-LAB, San Diego Instruments, San Diego, CA), containing a transparent Plexiglas tube (diameter 8.2 cm, length 20 cm) mounted on a Plexiglas frame within a ventilated enclosure. Acoustic noise bursts were presented by means of a speaker mounted 24 cm above the tube. Motion inside the tube was detected by a piezoelectric accelerometer located below the frame. The amplitude of the whole-body startle to an acoustic pulse was defined as the average of one hundred 1-ms accelerometer readings (0–4,095 relative units) collected from pulse onset. Each rat was put into the PPI chamber for a 5-min acclimation period, with a 68-dB(A) background noise level that continued throughout the session. After the acclimation period, four 30-ms startle pulses of 120 dB(A) broadband burst were presented. These four initial startle pulses served to achieve a relatively stable level of startle reactivity for the remainder of the test session, as the most rapid habituation of the startle reflex occurs within the first few startle pulse presentations (Koch, 1999). After the four initial pulses, six blocks of 11 trials were presented to measure PPI. Each block consisted of four different trial types, presented pseudorandomly throughout the session: pulse alone (2 trials), prepulse alone (1 trial for each prepulse intensity), prepulse + pulse (1 trial for each prepulse intensity), or no stimulus (1 trial). The four different prepulses had an intensity of 72, 76, 80, or 84 dB(A) and a duration of 20 ms. The time interval between the prepulse offset and the pulse onset was 80 ms. The different trial types were presented pseudorandomly, with an intertrial interval of 10–20 s (average 15 s). Altogether, a complete test session lasted about 23 min. The percentage of PPI induced by each prepulse intensity was calculated as 100 - [(100 x startle amplitude on prepulse trial) / (startle amplitude on pulse-alone trial)].

Open-Field Locomotor Activity

Locomotor activity was measured in four closed square arenas (76.5 cm × 76.5 cm, with walls 49.0 cm high) of dark gray plastic, placed in a dimly illuminated room (200 ± 0.5 lux provided by two halogen lights). Behavior in the arenas was recorded by a video camera suspended from the ceiling and was relayed to a monitor and a motion analysis and behavior recognition system (EthoVision, Noldus, Wageningen, the Netherlands). The computer calculated the total distance the rat traveled in the complete arena for each 10-min block of testing.

Experimental Design

One week after surgery, behavioral testing began. Experiments were run with groups of 4 rats. The different testing boxes and the order of testing were counterbalanced among the experimental groups as far as possible. The dorsal hippocampus and ventral hippocampus groups were each divided into two batches. In the first series of experiments, one batch was first subjected to an open-field experiment, and the other batch was first subjected to a PPI experiment. This first series of experiments included infusion of NMDA at doses of 0.00, 0.25, and 0.50 µg/side into the ventral hippocampus and 0.00, 0.10, and 0.25 µg/side into the dorsal hippocampus. We chose 0.50 µg/side as the highest dose because we found this dose to have strong effects on open-field activity and PPI when infused into the ventral hippocampus (Bast, Zhang, Heidbreder, & Feldon, 2001). Because the dorsal hippocampus has a higher density of NMDA receptors (Martens, Capito, & Wine, 1998; Petralla et al., 1994) and because, in our initial experiments involving ventral infusions, 0.25 µg NMDA/side had a very clear effect, we decided to use the lower NMDA doses of 0.10 and 0.25 µg/side for infusion into the dorsal hippocampus. The first series of experiments, however, revealed that all doses tested had pronounced effects on locomotor activity and PPI when infused into the ventral hippocampus, but none of the doses significantly affected locomotor activity or PPI when infused into the dorsal hippocampus. Therefore, we did a second series of experiments to test the effects of low doses of NMDA (0.10 and 0.25 µg/side) into the ventral hippocampus and the effects of 0.50 µg NMDA per side into the dorsal hippocampus. Rats that had been subjected to open-field testing in the first series of experiments were subjected to PPI testing in the second series and vice versa. Moreover, rats that had previously received vehicle (0.00 µg NMDA per side) received NMDA infusion, and rats that had previously received the lower doses of NMDA received vehicle infusion. In the third series of experiments, locomotor activity and PPI were measured after infusion of 0.70 µg NMDA per side into the dorsal hippocampus. In this series, rats from the open-field experiment of the second series were tested for PPI and vice versa. Moreover, rats that had received one of the lower doses of NMDA in the first series and vehicle in the second series received 0.70 µg NMDA per side, and rats that had received vehicle in the first series and 0.50 µg NMDA per side in the second series received vehicle infusion in the third series. Thus, rats implanted in the ventral hippocampus received two infusions, only one of them with NMDA. Rats implanted in the dorsal hippocampus received three infusions, one or two of them with NMDA, with the highest dose at the end. In view of the cytotoxic potential of NMDA, the described order of infusions was chosen to minimize the amount of NMDA applied to the brain and to apply the lower NMDA doses to drug-naïve rats. Between the infusions, there was always a time span of at least 7 days. The numbers of subjects tested with the different doses (in micrograms per 0.5 µl per side) of NMDA infused into the dorsal or ventral hippocampus in the PPI and open-field experiments appear in the respective figure captions.

Open-Field Experiments

The open-field experiments were conducted on 3 successive days. On Day 1, each rat was placed in the center of one of the arenas, and its locomotor activity in the open field was monitored for 30 min. On the basis of baseline locomotor activity on Day 1, the rats were allocated to the different groups, so that all groups were matched for baseline locomotor activity as far as possible. On Day 2 (day of infusion), each rat was placed in the center of one of the arenas, and its locomotor activity in the open field was monitored for 30 min. The rats were then returned to their home cages and moved to an infusion room to receive their infusions. After the infusion, all rats were placed into the arena for an additional 90 min to check for the immediate effect of NMDA infusion into the ventral or dorsal hippocampus on locomotor activity (between-subjects design). On Day 3, each rat was placed in the center of one of the arenas, and its locomotor activity was monitored for 30 min to check for possible long-term effects of the infusion.

PPI Experiments

The PPI experiments were conducted on 3 successive days: Day 1, testing of basal PPI without infusion; Day 2 (day of infusion), PPI testing immediately after infusion; Day 3, PPI testing without infusion. On the basis of Day 1 data, rats were allocated to the different groups, so that the groups were matched for PPI and startle as far as possible. The immediate effects of NMDA infusion into the hippocampus on PPI were examined by comparing the PPI of the different groups on Day 2 (between-subjects design). The purpose of the PPI session on Day 3 was to test for long-term effects.
DORSAL AND VENTRAL HIPPOCAMPUS NMDA INFUSIONS

Histology

After completion of the behavioral experiments, rats were deeply anesthetized with an overdose of 2.5 ml/kg Nembutal (sodium pentobarbital, 50 mg/ml ip) and transcardially perfused with 0.9% (w/vol) sodium chloride solution, followed by 250 ml of 4% (w/vol) Formalin (4 °C) to fix the brain tissue. Brains were extracted from the skull, postfixed in 4% Formalin solution, and subsequently cut into 40-μm coronal sections on a freezing microtome. To verify the injection sites, every fifth section through the ventral or dorsal hippocampus was mounted on gelatin-treated slides and stained with cresyl violet. After staining, the sections were dehydrated through an alcohol series, cleared with xylene, and coverslipped with Eukitt (Kindler, Freiburg, Germany). Subsequently, the sections were examined with a light microscope to verify the appropriate location of the tips of the infusion cannulas; the locations were then traced onto plates taken from the atlas of Paxinos and Watson (1998).

Data Analysis

All statistical analyses were performed with the StatView and SuperANOVA software system (Abacus Concepts, Berkeley, CA). Data were first subjected to analysis of variance (ANOVA). Post hoc comparisons to further analyze main effects were conducted with Fisher's protected least significant difference test. Significant differences were accepted at p < .05. All values are presented as means. In the text and in bar plots, variance is indicated by the standard error (SE) derived from the appropriate mean square of the ANOVA. The SE can also be used for estimations in further analyzing significant interactions indicated by the ANOVA, as 2 SE is an approximation of the critical difference needed to consider two mean values as significantly different (cf. Winer, 1971, p. 199). As described in the Experimental Design section, several experiments were run, all involving a vehicle group but only particular NMDA doses. For the overall analysis, this results in a higher number of subjects in the vehicle groups as compared with the NMDA groups. There might be reservations concerning the results revealed by the overall analysis because of a potentially confounding impact of the differences in group sizes. Therefore, it is important to note that the statistical analysis of each single experiment, involving balanced group sizes, yielded results qualitatively identical to those of the overall analysis.

Results

Histology

In all 74 cannulated rats, the centers of the infusion sites, that is, the tips of the infusion cannulas, were located in the targeted areas within or around the border of the ventral or dorsal hippocampus, respectively (see Figure 1). Tissue damage was found in the hippocampus and the cortex overlying the hippocampus. This damage was restricted to the area immediately surrounding the location of guide and infusion cannulas. It largely resulted from the implantation of the guide cannulas, although cytotoxicity of NMDA may also have contributed to the damage around the infusion site.

Effects of Hippocampal NMDA Infusion on Locomotor Activity

Ventral Hippocampus

Infusion of NMDA (0.10–0.50 μg/side) into the ventral hippocampus markedly increased locomotor activity in the open field (see Figure 2A). This effect was dose dependent and temporary; that is, hyperactivity was restricted to the open-field testing on the day of infusion. A 4 × 3 ANOVA (Group × 10-Min Block) performed on the total distance moved by the different groups on Day 1 (data not shown) yielded only a significant main effect of 10-min block, F(2, 56) = 91.36, p < .01. This reflected habituation to the open field. On Day 2 (day of infusion), the four groups did not differ in the total distance moved in the entire open field during the 30 min before infusion (see Figure 2A, left). As on Day 1, a 4 × 3 (Group × 10-Min Block) ANOVA yielded only a significant main effect of time block, F(2, 56) = 118.66, p < .01, reflecting habituation. Infusion of NMDA into the ventral hippocampus caused a dose-dependent increase in locomotor activity (see Figure 2A, middle). A 4 × 9 ANOVA (Group × 10-Min Block) of the total distance moved in the entire open field during the 90 min after infusion yielded a significant main effect of group, F(3, 28) = 3.20, p < .05; a highly significant main effect of 10-min block, F(8, 224) = 10.50, p < .01; and a significant Group × 10-Min Block interaction, F(24, 224) = 2.10, p < .01. Post hoc comparisons revealed that the total locomotion distance (in centimeters) was increased in the groups that received 0.10 μg (17,964 ± 4,490; p = 0.06), 0.25 μg (18,597 ± 5,725; p < .05), and 0.50 μg (24,226 ± 5,723; p < .01) NMDA per side, compared with the group that received only vehicle (6,290 ± 751). The significant Group × 10-Min Block interaction reflected the fact that NMDA-induced hyperactivity was most evident during the first 60 min. In particular, during the first 10 min, the hyperactivity of the group that received 0.50 μg/side NMDA was most pronounced and exceeded the hyperactivity in those groups that received lower NMDA doses. During the following 50 min, activity in all groups that received NMDA infusion was similarly elevated compared with the group that received vehicle infusion. A comparison of the distance moved during the first 10 min revealed that the group that received 0.50 μg NMDA per side (6,930 ± 1,458) was more than 4 times as active as the group that received vehicle (1,588 ± 196), and more than 2 times as active as the groups that received 0.10 μg (3,798 ± 773) and 0.25 μg (3,292 ± 429) NMDA per side. The latter two NMDA groups, which had very similar activity levels, were also considerably more active than the vehicle group (2 SE = 2,120 cm). The day after infusion, the groups no longer differed in the total distance moved throughout the 30 min of open-field testing (see Figure 2A, right). An ANOVA on the total distance moved did not reveal a main effect of group, F(3, 28) = 2.26, p > .10, or a Group × 10-Min Block interaction, F(6, 56) = 0.42, p > .80. There was a significant effect only of 10-min block, F(2, 56) = 64.30, p < .01, reflecting habituation in all groups. Thus, 24 hr after infusion, the effect of the NMDA infusion into the ventral hippocampus on locomotor activity was no longer evident.

Dorsal Hippocampus

Infusion of NMDA (0.10–0.70 μg/side) into the dorsal hippocampus did not alter locomotor activity in the open field (see Figure 2B). A 5 × 3 ANOVA (Group × 10-Min Block) of the total distance moved by the different groups on Day 1 (data not shown) yielded a significant main effect only of 10-min block, F(2, 92) = 167.10, p < .01, reflecting habituation. On Day 2, the five groups did not differ in the total distance moved in the open field during...
the 30 min preceding infusion (see Figure 2B, left). As on Day 1, a $5 \times 3$ (Group $\times$ 10-Min Block) ANOVA yielded a significant main effect only of 10-min block, $F(2, 92) = 94.49, p < .01$, reflecting habituation. Infusion of NMDA into the dorsal hippocampus did not affect locomotor activity in the open field (see Figure 2B, middle). A $5 \times 9$ ANOVA (Group $\times$ 10-Min Block) of the total distance moved in the open field during the 90 min after infusion on Day 2 yielded a significant main effect only of 10-min block, $F(8, 368) = 39.20, p < .01$, reflecting habituation, but no group effect, $F(4, 46) = 1.00, p > .40$. Total distance moved was very similar in the groups that received vehicle infusion (7,943 ± 814), or infusion of 0.10 µg (8,528 ± 2,096), 0.25 µg (9,049 ± 1,540), 0.50 µg (5,025 ± 891), and 0.70 (7,793 ± 2,068) NMDA per side. The day after infusion (see Figure 2B, right),
DORSAL AND VENTRAL HIPPOCAMPUS NMDA INFUSIONS

A: Ventral Hippocampus

$\mu g$ NMDA/side

- 0.0 (vehicle)
- 0.10
- 0.25
- 0.50

Day of infusion

Day after infusion

Distance Moved (cm)

10 20 30 40 50 60 70 80 90 10 20 30 min

Figure 2. Effects of N-methyl-D-aspartate (NMDA) infusion into the ventral (A) and dorsal (B) hippocampus on locomotor activity in the open field. On the day of infusion, the rats' locomotor activity in the open field was recorded for 30 min before infusion of different NMDA doses (in micrograms per 0.5 µl per side) into the ventral (0.0 [vehicle, n = 11], 0.10 [n = 5], 0.25 [n = 10], 0.50 [n = 6]) or dorsal (0.0 [vehicle, n = 22], 0.10 [n = 7], 0.25 [n = 6], 0.50 [n = 8], 0.70 [n = 8]) hippocampus. After infusion, activity was recorded for an additional 90 min. The day after infusion, the rats' locomotor activity was recorded for 30 min. Locomotor activity is represented as total distance moved during consecutive 10-min blocks. The bar represents 1 SE, derived from the analysis of variance.

ANOVA on the total distance moved in the open field yielded a significant main effect only of 10-min block, $F(2, 92) = 145.33, p < .01$, reflecting habituation.

One rat infused with 0.25 $\mu g$ NMDA per side into the dorsal hippocampus was discarded from analysis on the basis of activity data for the first 10 min after infusion. During this period, the rat exhibited enormously increased activity, which was nearly 6 times as high (distance moved: 17,028 cm) as the average activity (2,953 ± 727) of the other 6 rats that received the same dose of NMDA into the dorsal hippocampus. Histological examination showed that the cannulas were properly placed in the dorsal hippocampus of this rat. Out of 29 rats, this rat was the only one that exhibited hyperactivity after NMDA infusion into the dorsal hippocampus, even though 16 of these rats received 2- or 3-fold higher doses of NMDA. Thus, we considered this rat to be an outlier and discarded it from statistical analysis. Nevertheless, the question remains as to which processes were elicited by infusion of a relatively small dose of NMDA into the dorsal hippocampus of this rat to produce this hyperactivity.

Effects of Hippocampal NMDA Infusion on PPI

Ventral Hippocampus

Startle. Infusion of NMDA into the ventral hippocampus decreased the mean startle response at the highest dose of 0.50
Separate 4 × 16 ANOVAs (Group × Pulse-Alone Trial) on each day's startle data yielded a significant main effect of pulse-alone trial for each of the 3 days, *F*(15, 360) = 5.56, *F*(15, 360) = 5.23, and *F*(15, 360) = 3.48 (ps < .01), reflecting short-term habituation of the startle response (Koch, 1999). In addition, there was a significant main effect of group on the day of infusion (Day 2), *F*(3, 24) = 4.39, *p* < .02, but not on the preceding day, *F*(3, 24) = 0.36, *p* > .78, or the following day, *F*(3, 24) = 2.04, *p* > .13. On the day of infusion, the mean startle response over the 16 pulse-alone trials was lower in the group that received 0.50 μg NMDA per side (485 ± 98) than in the three other groups (0.10 μg/side: 832 ± 227, 0.25 μg/side: 920 ± 222, vehicle: 1,217 ± 115). This difference was statistically significant only in comparison with the vehicle group (*p* < .01).

**PPI.** Infusion of NMDA into the ventral hippocampus caused a dose-dependent disruption in PPI. One day after infusion, PPI was restored (see Figure 3A, right). Separate 4 × 4 ANOVAs (Group × Prepulse Intensity) of each day's PPI data yielded a significant main effect of prepulse intensity on the percentage of PPI for each of the 3 days, *F*(3, 72) = 34.58, *F*(3, 72) = 8.56, and *F*(3, 72) = 29.89 (ps < .01), reflecting a gradual increase in PPI as a function of prepulse intensity (Koch, 1999). In addition, a highly significant main effect of group was found for the day of infusion (Day 2), *F*(3, 24) = 19.44, *p* < .01, but not for the preceding day, *F*(3, 24) = 0.06, *p* > .95, or the following day, *F*(3, 24) = 2.79, *p* > .05. Post hoc comparisons revealed that, on the day of infusion, the percentage of PPI averaged over all prepulse intensities was decreased in the groups that received 0.10 μg (29.7 ± 5.3, *p* < .01), 0.25 μg (16.9 ± 10.0, *p* < .01), and 0.5 μg (−3.9 ± 7.2, *p* < .01) NMDA per side, compared with the vehicle group (55.5 ± 4.1). Moreover, percentage of PPI was significantly lower in the group that received 0.50 μg NMDA per side compared with the groups that received 0.10 μg (*p* < .05) and 0.25 μg (*p* < .05) NMDA per side, reflecting the fact that PPI disruption by infusion of NMDA into the ventral hippocampus was dose dependent. The groups that received the two lower NMDA doses

**Figure 3.** Effects of N-methyl-D-aspartate (NMDA) infusion into the ventral (A) and dorsal (B) hippocampus on startle magnitude during the 16 pulse-alone trials (left) and on percentage of prepulse inhibition (PPI) averaged across all prepulse intensities (right). Rats were bilaterally infused with different doses (in micrograms per 0.5 μl per side) of NMDA into the ventral (0.0 [vehicle, *n* = 12], 0.10 [n = 5], 0.25 [n = 5], 0.50 [n = 6]) or dorsal (0.0 [vehicle, *n* = 25], 0.10 [n = 7], 0.25 [n = 7], 0.50 [n = 10], 0.70 [n = 8]) hippocampus on Day 2. All groups were tested for PPI without infusion 1 day before (Day 1) and 1 day after (Day 3) the infusion day. Values are presented as means (± SEM). Asterisks indicate significant differences from the group that received vehicle infusion (*p* < .05, **p** < .01). Pound signs indicate significant differences between the 0.50-μg group and those that received the lower NMDA doses (##p** < .01).
did not differ from each other (p > .20). One day after infusion, PPI appeared to be largely restored, even though an ANOVA of the percentage of PPI data yielded a nearly significant group effect, $F(3, 24) = 2.79$, $p = .062$, showing that the group that had received 0.50 ug NMDA per side 1 day before exhibited slightly reduced PPI (35.1 ± 4.8) compared with the groups that received vehicle (53.8 ± 2.7), 0.10 ug (49.1 ± 7.4) or 0.25 ug (53.9 ± 9.3) NMDA per side (cf. Klanner et al., 1998, and Koch et al., 1999, who found residual PPI deficits 1 day after bilateral ventral hippocampal stimulation with a high dose of NMDA [1.4 ug/side] and who implicated activation of the piriform cortex in this carry-over effect).

For each group, the startle amplitudes throughout the pulse-alone trials and the prepulse + pulse trials of the six blocks on the day of infusion were compared (see Figure 4A) to assess whether the reduced percentage of PPI in the NMDA groups really reflected a genuine impairment in sensorimotor gating and not just changes in basal startle responsiveness (cf. Bast, Zhang, & Feldon, 2001a). This comparison indicated that the decreased percentage of PPI in the groups that received NMDA reflected a genuine impairment of sensorimotor gating, that is, a diminished ability of theprepulses to decrease the startle amplitude to the startle pulses.

A 4 × 5 ANOVA (Group × Trial Type) of the startle data yielded a significant Group × Trial Type interaction, $F(12, 96) = 6.47$, $p < .01$, reflecting the fact that the impact of the prepulses on the startle response to the pulse differed among the groups. Additional analysis (using 2 SE = 200) indicated that the startle amplitude was decreased compared with that from the pulse-alone trials in all prepulse + pulse trials in the vehicle group, in the trials with the three higher prepulses in the 0.10 ug NMDA group, only in the trial with the highest prepulse intensity in the 0.25 ug NMDA group, and in none of the prepulse + pulse trials in the 0.50 ug NMDA group. Moreover, in the pulse-alone trials, the startle response appeared to be lower in the groups that received 0.10 and 0.25 ug NMDA per side than in the vehicle group, whereas this relation was reversed when the pulse was preceded by one of the three higher intensity prepulses. The startle response in the 0.50 ug NMDA group appeared always to be lower than or at the same level as the startle response of the vehicle group. Thus, the failure of the prepulses to decrease the startle amplitude may be related to a floor effect, that is, that the basal startle response in the pulse-alone trials was too low (340 ± 86) to be further decreased. However, in a recent study, we measured startle amplitudes as low as 170 ± 33 throughout the trial, with the highest prepulse intensity in rats that received ventral hippocampal picrotoxin infusions at a dose that decreased startle without affecting PPI (Bast et al., 2001a). Moreover, in the present study, for example, the group that received 0.25 ug NMDA per side into the dorsal hippocampus exhibited startle amplitudes of 220 ± 25 and 260 ± 38 in the trials with the two highest prepulse intensities (cf. Figure 4A and 4B).

**Dorsal Hippocampus**

**Startle.** Infusion of NMDA into the dorsal hippocampus decreased the startle response in the 16 pulse-alone trials at the three higher doses (see Figure 3B, left). Separate 5 × 16 ANOVAs (Group × Pulse-Alone Trial) of each day's startle data yielded a significant main effect of pulse-alone trials for each of the 3 days, $F(1, 52) = 7.34$, $F(1, 52) = 5.79$, and $F(1, 52) = 5.52$ (ps < .01), reflecting short-term habituation of the startle response (Koch, 1999). In addition, a significant main effect of group was revealed for the day of infusion (Day 2), $F(4, 52) = 5.03$, $p < .03$, but not for the preceding day, $F(4, 52) = 0.89$, $p > .45$, or the following day, $F(4, 52) = 1.63$, $p > .18$. On the day of infusion, the mean startle in the 16 pulse-alone trials was lower in the groups that received 0.25 ug (654 ± 107), 0.50 ug (717 ± 132), and 0.70 ug (702 ± 107) NMDA per side, compared with the groups that received 0.10 ug NMDA per side (998 ± 157) or vehicle (1,090 ± 91). The difference was significant compared with the vehicle group (ps < .03).

**PPI.** Infusion of NMDA into the dorsal hippocampus hardly appeared to affect PPI (see Figure 3B, right). Separate 5 × 4 ANOVAs (Group × Prepulse Intensity) of each day's PPI data yielded a significant main effect of prepulse intensity on the percentage of PPI for each of the 3 days, $F(3, 156) = 59.16$, $F(3, 156) = 32.39$, and $F(3, 156) = 61.32$ (ps < .01), reflecting a gradual increase in prepulse inhibition with increasing prepulse intensity in rats that received ventral hippocampal picrotoxin infusions at a dose that decreased startle without affecting PPI (Bast et al., 2001a).
intensity (Koch, 1999). There was no difference between the groups on any of the 3 days: Day 1, F(4, 52) = 0.07, p > .98; Day 2, F(4, 52) = 1.34, p > .25; Day 3, F(4, 52) = 1.09, p > .35, when data were combined from both of the PPI experiments involving infusions into the dorsal hippocampus. However, there appeared to be a tendency for the higher NMDA doses (0.50 and 0.70 μg/side) to disrupt PPI. Therefore, we conducted a separate analysis of the data obtained in the PPI experiment from the third series of experiments involving the infusion of vehicle (n = 8) and 0.70 μg NMDA per side (n = 8) into the dorsal hippocampus. Separate ANOVAs on the percentage of PPI of each group for the day of infusion, F(1, 14) = 4.90, p < .05 (vehicle: 50.2 ± 5.4; 0.70 μg/side: 32.4 ± 5.9), but not for the preceding day, F(1, 14) = 1.68, p > .20, or the following day, F(1, 14) = 0.44, p > .50. Thus, the highest dose of NMDA appeared to have slightly disruptive effects on PPI on the day of infusion.

Furthermore, for each group, the startle amplitude throughout the pulse-alone trials and the prepulse + pulse trials of the six blocks on the day of infusion were compared (see Figure 4B). In agreement with the results obtained from the analysis of the percentage of PPI data, these comparisons indicated largely intact sensorimotor gating in all groups. A 5 × 5 ANOVA (Group × Trial Type) of the startle data yielded a significant Group × Trial Type interaction, F(16, 208) = 3.13, p < .01. Additional analysis (using 2 SE = 150) indicated that only the groups that received 0.50 and 0.70 μg NMDA per side exhibited a slight decrease in the ability of the lowest prepulse intensity to induce a decrease in startle amplitude.

Discussion

In the present study, bilateral infusion of NMDA into the ventral hippocampus—at doses from 0.10–0.50 μg/side—led to markedly increased locomotor activity in the open field and strong disruption of PPI. In contrast, bilateral infusion of NMDA into the dorsal hippocampus—at doses from 0.10 to as high as 0.70 μg/side—hardly affected locomotor activity and PPI.

Hippocampal Regulation of Locomotor Activity

The pronounced hyperactivity seen after NMDA infusion into the ventral hippocampus is in line with numerous previous studies that found hyperactivity after chemical stimulation of the ventral hippocampus by NMDA or other excitatory neuromodulatory compounds (Bardgett & Henry, 1999; Bast et al., 2001a; Bast, Zhang, Heidbreder, & Feldon 2001; Brenner & Bardgett, 1998; Brudzynski & Gibson, 1997; Legault & Wise, 1999; Wu & Brudzynski, 1995; Yang & Mogenson, 1987). As in our previous study (Bast, Zhang, Heidbreder, & Feldon, 2001), the hyperactivity disappeared 24 hr after the infusion, demonstrating that hyperactivity was in fact due to a temporary change in activity of the ventral hippocampus and not to possible permanent cell damage. Moreover, the hyperactivity induced by NMDA infusion into the ventral hippocampus in the present study was dose dependent. In contrast to the pronounced hyperactivity seen after NMDA infusion into the ventral hippocampus, NMDA infusion into the dorsal hippocampus did not affect locomotor activity in the open field. Although the NMDA receptor is more strongly expressed in the dorsal than in the ventral hippocampus (Martens et al., 1998; Petralia et al., 1994), even 0.70 μg NMDA per side—a dose that was about 50% higher than that which increased locomotor activity four-fold during the first 10 min after infusion into the ventral hippocampus—did not increase activity when infused into the dorsal hippocampus. The present data are in line with our finding that tetrodotoxin inactivation or muscimol (gammaminobutyric acid-A agonist) inhibition of the ventral (Bast, Zhang, & Feldon, 2001b) but not the dorsal hippocampus (Zhang, Bast, & Feldon, 2001a) markedly decreased locomotor activity. They indicate that activity of the ventral, but not the dorsal, hippocampus drives locomotor activity. Nevertheless, microinfusions of neurotransmitter substances into the dorsal hippocampus have also been demonstrated to influence locomotor activity. For example, at the same infusion coordinates as in the present study, MK-801 (6.25 μg/ side) infusion not only into the ventral hippocampus (Zhang, Bast, & Feldon, 2001b), but also into the dorsal (Zhang et al., 2000) hippocampus, increased activity in the open field, with the effect of the ventral infusion being much more pronounced. Moreover, the acetylcholine agonist carbachol has been reported to increase locomotor activity when infused into the ventral hippocampus (Brenner & Bardgett, 1998) or the dorsal hippocampus (Flicker & Geyer, 1982; Mogenson & Nielsen, 1984).

The hyperactivity seen after NMDA stimulation of the ventral hippocampus and the hyperactivity that has been found after lesions of the ventral hippocampus (Lipska, Jaskiw, Chraput, Karoum, & Weinberger, 1992; Richmond et al., 1999) seem at first glance to be at odds. However, changes in locomotor activity after infusions into the ventral hippocampus are due to temporarily altered activity of the ventral hippocampus, whereas the hyperactivity observed after lesions of the ventral hippocampus is a long-term effect, possibly reflecting compensatory changes in brain circuits regulating locomotor activity (cf. Bast et al., 2001b).

Hippocampal Regulation of PPI and Startle

Disruption of PPI by chemical stimulation of the ventral hippocampus—by NMDA, picrotoxin, or carbachol—has consistently been found in several studies (Bast et al., 2001a; Bast, Zhang, Heidbreder, & Feldon, 2001; Caine, Geyer, & Swerdlow, 1992; Klanner et al., 1998; Koch et al., 1999; Wan et al., 1996; Zhang et al., 1999). The present finding of severe, dose-dependent PPI disruption after infusion of even low NMDA doses (0.10, 0.25, and 0.50 μg/side) into the ventral hippocampus confirms and extends previous studies and underlines the extreme susceptibility of PPI to NMDA-induced overactivity of the ventral hippocampus. Similar to our previous studies (Bast, Zhang, Heidbreder, & Feldon, 2001; Zhang et al., 1999), PPI disrupted by NMDA infusion into the ventral hippocampus was largely restored 24 hr after infusion. This indicates that the PPI disruption was due to temporarily increased activity and not to permanent cell damage in the ventral hippocampus. In the present study, the statistical analysis indicated a significant decrease of startle amplitude after infusion of 0.50 μg NMDA per side into the ventral hippocampus. In previous studies, NMDA infusion into the ventral hippocampus either decreased (0.70 or 0.80 μg/side) or did not significantly affect (0.50, 0.70, 0.80, or 1.50 μg/side) startle amplitude, sometimes even in different experiments within the same study (Bast, Zhang, Heidbreder, & Feldon, 2001; Klanner et al., 1998; Koch et
observed without a concomitant significant effect on startle amplitude, or is at least much more pronounced than the decrease in startle amplitude (Bast, Zhang, Heidbreder, & Feldon, 2001; Klarner et al., 1998; Koch et al., 1999; Wan et al., 1996; Zhang et al., 1999; present study). Infusion of NMDA into the dorsal hippocampus hardly affected PPI, even though there was a tendency for the highest dose (0.70 μg/side) to disrupt PPI. Given, however, that even 0.10 and 0.25 μg NMDA per side disrupted PPI when infused into the ventral hippocampus and, moreover, that the expression of the NMDA receptor is higher in the dorsal than in the ventral hippocampus (Martens et al., 1998; Petralia et al., 1994), one can clearly conclude that, in the ventral hippocampus, neuronal overactivity induced by NMDA has a much stronger impact on PPI than it does in the dorsal hippocampus. Some evidence to this effect has been provided by a recent study in Sprague–Dawley rats, which failed to induce PPI disruption by bilateral NMDA infusion into the dorsal hippocampal formation at a dose (0.80 μg/side) that disrupted PPI when infused into ventral parts of the hippocampal formation (Swedlow et al., 2001). This failure, however, accompanied by an abnormally low baseline PPI measured in control rats that received dorsal hippocampal saline infusions. In the present study, the statistical analysis also indicated a decrease of startle amplitude after infusion of the three higher NMDA doses (0.25, 0.5, and 0.7 μg/side) into the dorsal hippocampus. However, it has to be considered that in the experiments involving the infusions into the dorsal hippocampus, the groups differed markedly in their basal startle amplitudes (cf. Figure 3B, left panel, Day 1).

Previous studies, using manipulations other than NMDA stimulation, did not reveal clear differences between the ventral and dorsal hippocampus in the regulation of PPI. For example, similar PPI disruption has been reported after infusion of the acetylcholine agonist carbachol into the subiculum and CA1 region of the ventral hippocampus and into the dentate gyrus of the dorsal hippocampus (Caine et al., 1992; Caine, Geyer, & Swedlow, 1991). However, only carbachol infusion into the ventral subiculum disrupted PPI without significantly decreasing startle amplitude, indicating that the observed PPI deficits perhaps only partially reflected disrupted sensorimotor gating. Moreover, reports from one group that infusion of the noncompetitive NMDA antagonist MK-801 (6.25 μg/side) into the dorsal but not the ventral hippocampus slightly disrupted PPI (Bakshi & Geyer, 1998, 1999) could not be confirmed by our own findings that MK-801 infusion into the ventral hippocampus (Bast et al., 2000) or the dorsal hippocampus (Zhang et al., 2000; find also therein a discussion of possible reasons for the discrepancies between our results and those of Bakshi & Geyer, 1998, 1999) did not disrupt PPI. However, we found that muscimol or tetrodotoxin infusion into the dorsal hippocampus, at the same coordinates as in the present study, markedly disrupted PPI (Feldon, Bast, & Zhang, 2001). Thus, manipulations of this part of the hippocampus can markedly affect PPI. Different effects on PPI have been reported after excitotoxic lesions of the ventral or the dorsal hippocampus. Although neither lesion disrupted PPI, ventral, but not dorsal, lesions were found to increase the sensitivity to apomorphine-induced disruption of PPI (Pouzet, Feldon, et al., 1999; Swedlow et al., 1996; Swedlow, Taaid, et al., 2000). Moreover, one study reported that dorsal lesions even slightly enhanced PPI (Swedlow, Taaid, et al., 2000). Overall, the findings reviewed above indicate the difficulty of drawing general conclusions concerning the different impacts of the dorsal and ventral hippocampus on the regulation of PPI.

Anatomical Substrates of Dorsal and Ventral Hippocampal Modulation of Locomotor Activity, PPI, and Startle

Hyperactivity after NMDA stimulation of the ventral hippocampus is probably due to increased accumbal dopamine release (Bardgett & Henry, 1999; Bast, Zhang, Heidbreder, & Feldon, 2001; Brudzynski & Gibson, 1997; Wu & Brudzynsky, 1995), which may depend on excitatory hippocampus–accumbens projections (Blaha et al., 1997; Mitchell et al., 2000; Taepavararuk et al., 2000) or on trans-synaptic activation of dopaminergic cells projecting from the ventral tegmental area to the nucleus accumbens (Legault et al., 2000; Legault & Wise, 1999). The activation of these mesoaccumbal dopamine cells has been proposed to be mediated by a loop including excitatory projections from the ventral hippocampus to the prefrontal cortex, because stimulation of the prefrontal cortex has been demonstrated to increase activity of the mesoaccumbens dopaminergic neurons, probably by polysynaptic mechanisms (see Carr & Sesack, 2000). The different locomotor effects of ventral and dorsal hippocampal NMDA stimulation are consistent with the view that the ventral hippocampal projection to the prefrontal cortex is important for hippocampal modulation of locomotor activity. They may also reflect the different projection pattern of the ventral and dorsal hippocampus to nucleus accumbens subregions, as dopamine signaling in different accumbal subregions may differently affect locomotor activity (e.g., Canales & Iversen, 2000; Hedou, Feldon, & Heidbreder, 1999; Heidbreder & Feldon, 1998; Swanson, Heath, Stratford, & Kelley, 1997).

The disparate impact of dorsal and ventral hippocampal NMDA stimulation on PPI is consistent with current concepts implicating the prefrontal cortex and amygdala, which receive projections only from the ventral hippocampus, in the modulation of PPI (see introduction section). Moreover, increased stimulation of hippocampal efferents to the accumbens shell, which mainly originate from the ventral hippocampus, might particularly contribute to the disruption of PPI (as discussed in Bast, Zhang, Heidbreder, & Feldon, 2001).
Projections to amygdala and bed nucleus of the stria terminalis constitute the hippocampal access to the brainstem circuits that mediate the startle response (Koch, 1999) and may mediate the changes in basal startle responsiveness after dorsal and ventral hippocampal infusions seen in the present study and in previous studies (e.g., Bakshi & Geyer, 1998, 1999; Bast et al., 2001a; Caine et al., 1991, 1992; Zhang et al., 1999, 2000). These projections originate exclusively from the ventral hippocampus (Pitkänen et al., 2000; Swanson & Cowan, 1977), but neuronal activity in the dorsal hippocampus might also exert an influence on them via intrahippocampal projections (Amaral & Witter, 1989). In view of the more direct access of the ventral hippocampus to brain sites involved in the modulation of locomotor activity and PPI, such intrahippocampal projections may also be important in modulating the above-discussed effects of dorsal hippocampal manipulations on locomotor activity and PPI.

General Conclusions

The present study demonstrates that the regulation of locomotor activity and PPI is much more susceptible to disruption by NMDA-induced overactivity of the ventral versus the dorsal hippocampus. Our results are in line with the idea that the hippocampal connections with the amygdala, the prefrontal cortex, and the nucleus accumbens (possibly mainly its shell) are particularly involved in the hippocampal modulation of locomotor activity and PPI, respectively. In humans, hippocampal overactivity has been associated with positive symptoms in schizophrenia (Dierks et al., 1995).}

References


DORSAL AND VENTRAL HIPPOCAMPUS NMDA INFUSIONS

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Received January 3, 2001
Revision received May 29, 2001
Accepted June 27, 2001

Annotation:
A1 See chapter 1.7. of the thesis: Bast T, Feldon J (in preparation for submission) Hippocampal modulation of sensorimotor processes: effects of pharmacological manipulations of the rat ventral and dorsal hippocampus on locomotor activity, prepulse inhibition (PPI), and startle reflex.
Prepulse inhibition in rats with temporary inhibition/inactivation of ventral or dorsal hippocampus

W.-N. Zhang¹, T. Bast¹, J. Feldon*

Behavioral Neurobiology Laboratory, The Swiss Federal Institute of Technology Zurich, Schorenstrasse 16, CH 8003 Schwerzenbach, Switzerland

Received 27 March 2002; received in revised form 5 July 2002; accepted 5 July 2002

Abstract

Prepulse inhibition (PPI) of the acoustic startle response is a measure of sensorimotor gating and is decreased in neuropsychiatric diseases, including schizophrenia. Hippocampal involvement in PPI has been the subject of several studies, in particular, as aberrant hippocampal activity has been associated with schizophrenia. In rats, chemical stimulation of the ventral hippocampus reduced PPI, while normal PPI was found following hippocampal lesions, suggesting that ventral hippocampal overactivity is detrimental for PPI, but that normal hippocampal activity does not contribute substantially to PPI. In the present study, we investigated the importance of hippocampal activity for PPI by examining PPI in Wistar rats with temporarily decreased hippocampal activity, aiming to avoid compensatory processes that may occur with permanent lesions. Bilateral ventral or dorsal hippocampal infusions of the γ-aminobutyric acid A (GABAₐ) receptor agonist muscimol (1 µg/side) or the sodium-channel blocker tetrodotoxin (TTX, 10 ng/side) reduced PPI. This reduction is probably neuroleptic-resistant since haloperidol and clozapine did not antagonize the muscimol-induced decreases in PPI. PPI reduction by muscimol inhibition or TTX inactivation of the dorsal or ventral hippocampus indicates that hippocampal activity contributes to sensorimotor gating, suggesting intact PPI after permanent hippocampal lesions to reflect compensatory processes. The data are discussed with respect to hippocampal dysfunction in schizophrenia.

Keywords: Clozapine; Haloperidol; Muscimol; Neuroleptics; Prepulse inhibition; Schizophrenia; Sensorimotor gating; Startle; TTX; Ventral and dorsal hippocampus

1. Introduction

In rats and humans, prepulse inhibition (PPI) of the acoustic startle response, i.e. the reduction of the response by a weak prepulse preceding the startle pulse, is used to measure sensorimotor gating mechanisms protecting neural information processing (Graham, 1975; Norris and Blumenthal, 1996; Koch, 1999). Deficient sensorimotor gating, as reflected by disrupted PPI, exists in several neuropsychiatric disorders. In particular, sensorimotor gating deficits are well documented in schizophrenia and may correlate with positive symptoms, even though the relation between PPI deficits and schizophrenia remains to be clarified (Braff et al., 2001; Hamm et al., 2001). Experimentally induced disruption of PPI in rats is used to elucidate the neurological aberrations underlying deficient sensorimotor gating (Swerdlow et al., 2000a).

The hippocampal involvement in the regulation of PPI has been the subject of several studies, in particular, as aberrant hippocampal activity is widely believed to contribute to schizophrenic symptoms (Gray et al., 1991; Benes, 2000; Goethel et al., 2000; Grace, 2000). Overactivity of the ventral hippocampus, induced, for example, by infusion of N-methyl-D-aspartate (NMDA; e.g., Wan et al., 1996, Klarner et al., 1998, Zhang et al., 1999, Bast et al., 2001d) or the γ-aminobutyric acid A (GABAₐ) receptor antagonist picrotoxin (Bast et al., 2001b), clearly disrupts PPI. It is not clear, however, whether normal hippocampal activity contributes to the maintenance of PPI. Infusion of the noncompetitive NMDA antagonist MK-801 into the dorsal hippocampus (Bakshi and Geyer, 1998, 1999) or neonatal lesions of the ventral hippocampus (Lipska et al., 1995; Le Pen et al., 2000) have been reported to impair PPI,

* Corresponding author. Tel.: +41-1-655-7448; fax: +41-1-655-7203. E-mail address: feldon@behav.biol.ethz.ch (J. Feldon).
1 Contributed equally to the present study.
and rats with ventral hippocampal lesions exhibited increased sensitivity to PPI disruption induced by the dopamine agonist apomorphine (Swedlow et al., 1995, 2000b). However, MK-801 infusion into the ventral hippocampus did not affect PPI (Bakshi and Geyer, 1998; Bast et al., 2000) and we also found intact PPI following MK-801 infusion into the dorsal hippocampus (Zhang et al., 2000b). In particular, several studies that found intact basal PPI following lesions to the ventral, dorsal, or complete hippocampus (Kemble and Ison, 1971; Pouzet et al., 1999; Swedlow et al. 1995, 2000b) did not support the view that normal hippocampal activity contributes to PPI, even though slightly decreased PPI has very recently been reported after lesions to the ventral, but not dorsal, subiculum (Caine et al., 2001).

Permanent lesions may fail to reveal a structure’s normal function since, for example, other structures may rapidly compensate for the permanent loss of one structure (Bures and Buresova, 1990; Lomber, 1999). Moreover, we recently found that temporary inhibition or inactivation of the ventral hippocampus by the GABA<sub>A</sub> receptor agonist muscimol or the sodium channel blocker tetrodotoxin (TTX), respectively, decreased locomotor activity (which is in line with the hyperactivity following stimulation of the ventral hippocampus), whereas ventral hippocampal lesions tend to increase activity (see Bast et al., 2001c). In the present study, in view of the possible problems associated with permanent lesions (see Bast et al., 2001a), we temporarily inhibited or inactivated the ventral or dorsal hippocampus of Wistar rats during PPI testing to examine whether hippocampal activity is necessary to maintain normal sensorimotor gating. In addition, the effects of the neuroleptics haloperidol and clozapine on the PPI disruption induced by hippocampal inhibition were examined. The present data have previously been published in preliminary form (Feldon et al., 2001).

2. Experimental procedures

2.1. Subjects

Seventy-six male Wistar rats (Zur WIST[HanIbm], Research Unit Schwerzenbach, Schwerzenbach, Switzerland), weighing about 250 g at the time of surgery, were used in this study. They were housed in groups of four per cage under a reversed light–dark cycle (lights on: 19:00–07:00) in a temperature (21 ± 1 °C) and humidity (55 ± 5%) controlled room. All animals were allowed free access to food and water. Forty-eight rats received bilateral implantation of guide cannulae aiming at the dorsal hippocampus, and 28 rats received bilateral implantation of guide cannulae aiming at the ventral hippocampus. After surgery, all rats were individually caged. Starting one day before surgery and then throughout the studies, all rats were handled daily. Behavioral testing was carried out in the dark phase of the cycle. All experiments were conducted in accordance with Swiss regulations for animal testing.

2.2. Surgery

Rats were anesthetized with 1 ml of nembutal (sodium pentobarbital, 50 mg/ml, Abbott Labs, North Chicago, IL) per kg body weight and their head was placed in a Kopf stereotaxic frame. After application of a local anesthetic (lidocaine), the scalp was incised to expose the skull. Bregma and lambda were aligned in the same horizontal plane. A small hole (1.5 mm in diameter) was drilled on each side of the skull to reveal the dura covering the cortex overlying the hippocampus. Stainless steel guide cannulae (26 gauge, 9 or 7 mm for ventral or dorsal hippocampus, respectively) in a Perspex holder were implanted bilaterally into the brain aiming above the ventral (–5.2 mm posterior and ±5.0 mm lateral to bregma, and –5.0 mm ventral to dura) or dorsal (–3.0 mm posterior and ±1.5 mm lateral to bregma, and –2.5 mm ventral to dura) hippocampus. The guide cannulae were fixed to the skull with three anchoring skull screws and dental cement. Stainless steel stylets (34 gauge) extending 0.5 mm beyond the tips of the guide cannulae were placed inside the guide cannulae to prevent occlusion. After surgery, rats had at least 5 days of recovery before being subjected to any behavioral testing. Moreover, they received daily health checks, and missing stylets were replaced.

2.3. Intracerebral drug infusion

Muscimol [C<sub>4</sub>H<sub>6</sub>N<sub>2</sub>O<sub>2</sub>(1/2 H<sub>2</sub>O); Tocris, Bristol, UK] was dissolved in 0.9% saline at a concentration of 2 μg/μl on the day of infusion. TTX (C<sub>11</sub>H<sub>12</sub>N<sub>2</sub>O<sub>6</sub>; Tocris) was stored at –40 °C in aliquots containing 40 ng/μl in 0.9% saline. On the day of infusion, these aliquots were thawed and diluted with 0.9% saline to obtain solutions with a concentration of 20 ng/μl. For the intracerebral infusion, rats were manually restrained, the stylets removed carefully, and infusion cannulae (34 gauge, stainless steel) were inserted into the brain through the previously implanted guide cannulae. The tips of the infusion cannulae protruded 1.5 mm beyond the tip of the guide cannulae into the ventral or dorsal hippocampus. Thus, the final dorso-ventral coordinate for the ventral and dorsal hippocampus was 6.5 and 4.0 mm below the dura, respectively. The infusion cannulae were connected to 10-μl Hamilton microsyringes by flexible polyetheretherketone (PEEK) tubing. The syringes were mounted on a KDs microinfusion pump. All rats were infused bilaterally and the infusion volume was 0.5 μl/side, delivered with a speed of 0.5 μl/min. Following infusion, the infusion cannulae were kept in place for an additional 60 s to allow the injection bolus to be absorbed by the tissue and were then replaced by the stylets. As in our previous study (Bast et al., 2001c), muscimol (1 μg/0.5 μl/side) was infused immediately and TTX (10 ng/0.5 ml/side) 20 min before the
behavioral sessions. Accordingly, half of the rats infused with vehicle, i.e. 0.9% saline (0.5 µl/side), received infusion immediately before the behavioral sessions, the other half 20 min before the behavioral sessions. Rats used in the present study received at most four intracerebral infusions, each of them 1 week apart.

2.4. Systemic drug administration

Haloperidol from ampoules containing 5 mg/ml solvent (Janssen-Cilag, Baar, Switzerland) was diluted with 0.9% saline to a concentration of 0.2 mg/ml. Clozapine (Novartis, Basel, Switzerland) was dissolved in 0.9% saline, acidified by 0.1 M HCl, at a concentration of 5 or 10 mg/ml, and the solution was adjusted to pH 5–6 by addition of Na₂CO₃. Vehicle, i.e. 0.9% saline, haloperidol (0.2 mg/kg), and clozapine (5 or 10 mg/kg) were injected intraperitoneally, in a volume of 1 ml/kg, 45 min prior to the test sessions.

2.5. Apparatus and procedure for behavioral testing

Startle response and PPI were tested in four startle chambers (SR-LAB, San Diego Instruments, San Diego, CA), containing a transparent Plexiglas tube (diameter 8.2 cm, length 20 cm) mounted on a Plexiglas frame within a ventilated enclosure. Acoustic noise bursts were presented via a speaker mounted 24 cm above the tube. Motion inside the tube was detected by a piezoelectric accelerometer below the frame. The amplitude of the whole body startle response to an acoustic pulse was defined as the average of one hundred 1-ms accelerometer readings collected from pulse onset. Each rat was put into the PPI chamber for a 5-min acclimatization period with a 68-dB(A) background noise level that continued throughout the session. After the acclimatization period, four startle pulses of 120 dB(A) broad band burst for 30 ms were presented. These four initial startle pulses served to achieve a relatively stable level of startle reactivity for the remainder of the test session, as most rapid habituation of the startle reflex occurs within the first few startle pulse presentations (Koch, 1999). After the four initial pulses, six blocks of 11 trials were presented to measure PPI. Each block consisted of four different trial types, presented pseudorandomly throughout the session: pulse alone (two trials), prepulse alone (one trial for each prepulse intensity), prepulse followed by pulse (one trial for each prepulse intensity), or no stimulus (one trial). The four different prepulses had an intensity of either 72, 76, 80, or 84 dB(A) and a duration of 20 ms. The time interval between the prepulse offset and the pulse onset was 80 ms. The different trial types were presented pseudorandomly with an intertrial interval of 10–20 s (average 15 s). Altogether, a complete test session lasted about 23 min. The percentage of PPI (%PPI) induced by each prepulse intensity was calculated as: [100 − (100 × startle amplitude on prepulse trial)/(startle amplitude on pulse-alone trial)].

2.6. Experimental design and groups

2.6.1. General

The experiments were conducted on three successive days. Rats were tested in squads of four. The different testing boxes and the order of testing was counterbalanced among the experimental groups as far as possible. On Day 1, all experimental groups were subjected to a startle and PPI testing without infusion to verify that they had similar baseline values of startle and PPI. If necessary, the experimental groups were rearranged for further testing so that they were matched for their baseline values as far as possible. On Day 2 (day of infusion), rats were tested for startle response and PPI following the different intracerebral infusions and systemic injections. By comparing startle amplitude and PPI of the different groups on Day 2, acute effects of the treatments were assessed (between-subjects design). On Day 3, all rats were subjected to a further testing of startle response and PPI without a preceding treatment as on Day 1 to test for possible long-term effects of the drug treatments on Day 2.

Before being subjected to the experiments of the present study, most animals had been used in one or two previous experiments. Unless otherwise mentioned, animals were allocated to the experimental groups counterbalanced according to their previous experimental history (see below). Startle and PPI data on Day 1 of the present study's experiments were virtually identical to that measured in our Wistar rats in previous studies (Bast et al., 2000, 2001a,b,d; Zhang et al., 1999, 2000b, 2002), demonstrating that the preceding experiments to which the rats used in the present study had been subjected did not affect baseline startle and PPI values. Moreover, in the data analysis (see below), the experimental history of the animals was considered in order to exclude interactions between the treatments in the present and the preceding experiments.

2.6.2. Effects of TTX or muscimol infusion into the dorsal or ventral hippocampus on the startle response and its PPI

In the first two experiments, the effects of dorsal or ventral hippocampal muscimol and TTX infusion on startle response and PPI were examined. The rats were allocated to three different experimental groups to receive either vehicle (VEH), muscimol (MUS), or TTX infusion before testing on Day 2. Forty-two rats with cannulae aiming at the dorsal hippocampus were used to test the effects of dorsal hippocampal TTX and muscimol infusion (VEH, n = 16; MUS and TTX, n = 13). These rats had received the same infusion as in the present study in an open field experiment 1 week before, and another bilateral infusion (0.5 µl saline/side, or 0.5 µg NMDA or 6.25 µg MK-801 in 0.5 µl saline/side) in a fear conditioning experiment (with a tone being used as conditioned stimulus and freezing being used as measure of conditioned fear) 2 weeks before. Twenty-eight rats with cannulae aiming at the ventral hippocampus were used to test the effects of ventral hippocampal TTX and muscimol
pus do not induce considerable additional neuronal damage
histological examinations using cresyl violet staining and
or received saline infusion in the previous fear conditioning
them were naive, the other 14 had received one bilateral
transmission, in particular in the nucleus accumbens, is
hippocampus resulted in decreased PPI Increased dopamine
muscimol inhibition of both the ventral and dorsal hip¬
2 6 3 Effects of neuroleptic pretreatment on PPI disruption
induced by muscimol infusion into the ventral or dorsal
hippocampus
In the first two experiments, TTX inactivation as well as
muscimol inhibition of both the ventral and dorsal hippo¬
campus resulted in decreased PPI. Increased dopamine
transmission, in particular in the nucleus accumbens, is
widely held to be one important cause of decreased PPI,
and some forms of experimentally induced PPI disruption
are antagonized by neuroleptics (Koch, 1999; Zhang et al.,
2000a; Geyer et al., 2001; Swerdlow et al., 2001a). Thus,
even though there is evidence for stimulation rather than
deactivation of (ventral) hippocampal activity to increase
accumbal dopamine transmission (e.g., Brudzynski and
Gibson, 1997; Mitchell et al., 2000; Taepavarapruk et al.,
2000; Legault and Wise, 2001), we conducted two further
experiments to examine the effects of pretreatment with the
typical neuroleptic haloperidol and the atypical neuroleptic
clozapine on PPI disruption induced by muscimol infusion
into the dorsal or ventral hippocampus. We chose muscimol
infusions since the first two experiments yielded that the
decrease in PPI was stronger after hippocampal muscimol
infusions than after TTX infusions. Moreover, this decrease
was not accompanied by significant alterations in startle
reactivity and clearly reflected a genuine impairment of
sensorimotor gating (see Results). The experimental groups
received the following treatments on Day 2: systemic
injection of saline followed by infusion of vehicle (Sal¬
VEH) or systemic injection of saline, 0.2 mg/kg haloperidol,
or 5 mg/kg clozapine followed by infusion of muscimol
(Sal-MUS, Hal-MUS, Clo5-MUS). In the experiment testing
neuroleptics’ effects on PPI disruption by dorsal hippo¬
campal muscimol infusion, we also included a group
receiving 10 mg/kg clozapine before infusion of muscimol
(Clo10-MUS). Thus, the experiment to test the effects of
neuroleptic pretreatment on PPI disruption induced by
dorsal hippocampal muscimol infusion included five groups
(Sal-VEH, n = 9; Sal-MUS, n = 10; Hal-MUS, n = 9; Clo5-
MUS and Clo10-MUS, n = 10), and the experiment to test
neuroleptics’ effects on PPI disruption by ventral hippo¬
campal muscimol infusion included four groups (Sal-VEH,
Sal-MUS, Hal-MUS, and Clo5-MUS, n = 7). In the experi¬
ments involving neuroleptic pretreatment and muscimol
infusion, the rats that had been used in the first two PPI
experiments 1 week before, as well as six additional rats
with cannulae aiming at the dorsal hippocampus, were used.
The six additional rats had previously been subjected to the
same fear conditioning and open field experiments as the
other 42 rats with cannulae aiming at the dorsal hippocam¬
pus but had then, 1 week before the present experiment,
been used in a pilot experiment involving dorsal hippo¬
campal TTX infusion (10 ng/side) and PPI testing imme¬
diately (instead of 20 min as in the present study) thereafter.

2.6.4. Histology
After completion of the behavioral experiments, rats
were deeply anesthetized with an overdose of 2.5 ml/kg
nembutal (sodium pentobarbital, 50 mg/ml, ip) and trans¬
cardially perfused with 0.9% NaCl solution, followed by
120 ml of 4% formalin (4 °C) to fix the brain tissue.
Brains were extracted from the skull, postfixed in 4% formalin
solution, and subsequently cut into 40-μm coronal
sections on a freezing microtome. To verify the injection
sites, every fifth section through the ventral or dorsal
hippocampus was mounted on gelatin-treated slides and
stained with cresyl violet. After staining, the sections were
dehydrated through an alcohol series, cleared with xylene,
and coverslipped with Eukitt (Kindler, Freiburg, Germany).
Subsequently, the sections were examined with a light
microscope to verify the appropriate location of the tips
of the infusion cannulae and draw it onto plates taken from
the atlas of Paxinos and Watson (1998).

2.6.5. Data analysis
Statistical analyses were performed with the StatView
and SuperANOVA software system (Abacus Concepts,
Berkeley, CA, 1992). Data were first subjected to ANOVA.
Groups were used as between-subjects factor. For the %PPI
data, the four different prepulse intensities were used as
repeated-measures factor. For the startle data, repeated¬
measures factors were the 16 repeated pulse-alone trials as
well as the pulse-alone and prepulse followed by pulse trials
throughout the six blocks to measure PPI. Post hoc compar¬
isons were conducted using Fisher’s protected least signifi¬
cant difference test. Significant differences were accepted at
P < .05. All values are presented as means. In the text and in
bar plots, variance is indicated by the standard error of the
mean (S.E.M.). In line plots, for the sake of clarity, variance
is indicated by the standard error (S.E.) derived from the
appropriate mean square of the ANOVA. Since the data of
the rats infused with vehicle did not differ significantly
whether the infusions were given immediately or 20 min
prior to PPI testing, they were collapsed for the analysis
presented in the Results section. Finally, data were subjected
to statistical analyses using the different experimental his¬
Infused into the dorsal hippocampus, TTX but not muscimol tended to increase the startle response in the 16 pulse-alone trials, even though neither drug induced significant alterations as compared to the VEH group (Fig. 2A, left). Separate ANOVA of the startle data of each day yielded a significant main effect of pulse-alone trials for each of the 3 days \( F(15,585) = 6.47, F(15,585) = 3.32, \) and \( F(15,585) = 3.70, \) all \( P < .0001 \), reflecting short-term habituation of the startle response (Koch, 1999). In addition, there was a significant main effect of groups on the day of infusion [Day 2; \( F(2,39) = 6.25, P < .005 \)] but not on the day preceding [Day 1; \( F(2,39) = 0.87, P > .09 \)] or following [Day 3; \( F(2,39) = 0.78, P > .45 \)] infusion. Post hoc comparisons indicated that on the day of infusion, the mean startle response over the 16 pulse-alone trials was increased in the TTX group (1413 ± 61) as compared to the MUS (1065 ± 77, \( P < .002 \)). Compared to the VEH group (1232 ± 63), the startle amplitude tended to be increased in the TTX (\( P = .06 \)) and decreased in the MUS (\( P < .09 \)) group. Infused into the ventral hippocampus, TTX decreased the startle response in the 16 pulse-alone trials while muscimol had no significant effect (Fig. 2A, right).

Fig. 1. Infusion sites in the dorsal (n = 48, left) and ventral (n = 28, right) hippocampus: photomicrograph of a coronal brain section with the tracks of the guide cannulae and beneath them the infusion sites visible in both hemispheres (top) and approximate location of the tips of the infusion cannulae depicted on plates of coronal sections through the rat brain (Paxinos and Watson, 1998) (bottom). Values represent distance from bregma. CA1, CA2, and CA3: CA1, CA2, and CA3 field of the hippocampus; DG: dentate gyrus; Ent: entorhinal cortex; S: subiculum.
3.2.2. Prepulse inhibition

Both TTX and muscimol infusion into the dorsal (Fig. 2B, left) as well as ventral (Fig. 2B, right) hippocampus decreased the %PPI. One day after infusion, PPI was completely restored. Separate ANOVA of the PPI data of each day yielded a significant main effect of prepulse intensities on the %PPI for each of the 3 days [dorsal infusions: F(3,117) = 59.9, F(3,117) = 57.7, and F(3, 117) = 68.9; all F<.0001; ventral infusions: F(3,75) = 48.21, F(3,75) = 24.81, and F(3,75) = 54.31, all F<.0001], reflecting a gradual increase in PPI as a function of the intensity of the prepulse (Koch, 1999). In addition, a significant main effect of groups was found for the day of infusion [Day 2; dorsal infusions: F(2,39) = 10.97, P<.0002; ventral infusions: F(2,25) = 4.30, P<.03], but not for the day preceding [Day 1; dorsal infusions: F(2,25) = 1.46, P>.25] or following [Day 3; F(2,25) = 1.06, P>.35] infusion. This reflected that on the day of infusion, the mean startle amplitude over the 16 pulse-alone trials was decreased in the TTX (504±109; P<.006) as compared to the VEH (1186±158) group. Startle amplitude in the MUS group (851 ±199) fell between that of the TTX (P>.15) and the VEH (P>.17) group.
infusion. On the day of infusion, the %PPI averaged over all prepulse intensities was significantly decreased in the MUS (dorsal infusions: 22.0±3.6; P<.0001; ventral infusions: 20.6±11.8; P<.008) groups as compared to the VEH groups (dorsal infusions: 49.1±4.4; ventral infusions: 49.7±4.3). The decrease of PPI in the TTX group only reached statistical significance for the dorsal infusions (dorsal infusions: 30.7±4.6; P<.005; ventral infusions: 34.9±5.4, P=.15) although, for ventral (P>.2) as well as for dorsal (P>.17) hippocampal infusions, TTX and MUS groups did not differ significantly.

Reduced %PPI accompanied by alterations in startle amplitude may not completely reflect a genuine deficit in sensorimotor gating, but may be partly due to altered startle reactivity (Swerdlow et al., 2000a). Although only the ventral hippocampal TTX infusions induced a statistically significant alteration in startle amplitude throughout the 16 pulse-alone trials, we addressed the issue of whether alterations in startle amplitude may have contributed to the reduced %PPI. For that purpose, similar to a previous study (Bast et al., 2001b), we scrutinized the average startle amplitudes in the pulse alone and the prepulse followed by pulse trials of the six test blocks to measure PPI on the day of infusion (Fig. 2C). With respect to TTX and muscimol infusion into the dorsal hippocampus (Fig. 2C, left), this analysis indicated a genuine impairment of sensorimotor gating. ANOVA yielded a significant effect of group [F(2,39)=4.9, P<.02], reflecting the increased startle reactivity in the TTX group. Moreover, ANOVA indicated a significant effect of trial type [F(4,156)=95.7, P<.0001], and a highly significant Group x Trial type interaction [F(8,156)=5.0, P<.0001]. This reflected that the prepulse-induced inhibition of the startle response to the pulse and the gradual increase in inhibition with increasing prepulse intensity was stronger in the VEH than in the MUS and TTX group. Thus, the startle amplitude in the MUS group was slightly lower than that of the VEH group during the pulse-alone trials, whereas this relation was reversed throughout the trials with the three higher pulses. Furthermore, the increase in startle magnitude in the TTX group as compared to the VEH group was much more pronounced in the prepulse followed by pulse trials than in the pulse-alone trials. With respect to the infusions of muscimol and TTX into the ventral hippocampus, analysis of the startle amplitudes in the pulse alone and the prepulse followed by pulse trials of the six test blocks to measure PPI on the day of infusion yielded a slightly different picture (Fig. 2C, right). ANOVA indicated a nearly significant effect of group [F(2,25)=3.1, P<.066], mainly reflecting the decreased startle reactivity in the TTX group, a significant effect of trial type [F(4,100)=23.09, P<.0001], and a significant Group x Trial type interaction [F(8,100)=3.41, P<.002]. However, in contrast to the situation for the dorsal infusions, in no trial type were startle amplitudes higher in the TTX and MUS groups than in the VEH group. Therefore, in particular for the TTX group, the decreased potency of the prepulses to inhibit the startle amplitude may have been related to a floor effect, i.e. the lower basal startle response of the TTX (440±119) group in the pulse-alone trials was more resistant to reduction than the higher startle response of the VEH group (1180±165). However, in a recent study (Bast et al., 2001b), we observed intact PPI in spite of markedly reduced startle amplitudes in pulse-alone trials (520±130) in our Wistar rats following ventral hippocampal infusion of 100 ng picrotoxin/side. In these rats, the highest prepulse intensity lowered the startle amplitude to the pulse to 170±33. Altogether, it can be stated that muscimol infusions into the dorsal or ventral hippocampus unequivocally induced a genuine impairment in sensorimotor gating. Although less clear, this appears to apply also to the TTX infusions. In the following experiments to test the effects of neuroleptics on PPI impairments that result from decreased hippocampal activity, we used hippocampal muscimol infusions so that decreased %PPI can unequivocally be attributed to impaired sensorimotor gating.

3.3. Effects of neuroleptic pretreatment on PPI disruption induced by muscimol infusion into the ventral or dorsal hippocampus

3.3.1. Startle

Consistent with the results from the first two experiments, the startle response in the 16 pulse-alone trials was not significantly affected by any treatment combination in the two experiments involving neuroleptic pretreatment and dorsal (Fig. 3A, left) or ventral (Fig. 3A, right) hippocampal muscimol infusions. Separate ANOVA of the startle data of each day yielded only a significant main effect of pulse-alone trials for each of the 3 days [dorsal infusions: F(15,645)=5.94, F(15,645)=13.58, and F(15,645)=5.90, all P<.0001; ventral infusions: F(15,360)=3.92, F(15,360)=11.76, and F(15,360)=5.86, all P<.0001], but no main effect of groups [dorsal infusions: F(4,43)=0.73, P>.5; F(4,43)=1.67, P>.17; F(4,43)=1.39, P>.25; ventral infusions: F(3,24)=0.22, P>.8; F(3,24)=0.62, P>.6; F(3,24)=1.11, P>.36].

3.3.2. Prepulse inhibition

PPI disruption induced by dorsal (Fig. 3B, left) or ventral (Fig. 3B, right) hippocampal muscimol inhibition was not antagonized by a preceding injection of haloperidol or clozapine. Separate ANOVA of the PPI data of each day yielded a significant main effect of prepulse intensities on the %PPI for each of the 3 days [dorsal infusions: F(3,129)=137.6, F(3,129)=36.7, and F(3,129)=103.6; all P<.0001; ventral infusions: F(3,72)=70.5, F(3,72)=16.0, and F(3,72)=37.9; all P<.0001]. Although inspection of the data (Fig. 3B) indicated that muscimol infusions into the dorsal or ventral hippocampus on Day 2 decreased %PPI, the effect of groups on the %PPI over all four prepulse intensities did not reach statistical significance for any of the 3 days [dorsal infusions: Day 1, F(4,43)=0.033, P>.99; Day 2,
Fig. 3. Effects of haloperidol and clozapine pretreatment on disruption of PPI by muscimol infusion into the dorsal (left) and ventral (right) hippocampus: startle magnitude during the 16 pulse-alone trials (A), %PPI induced by the four prepulse intensities (B). Rats received pretreatment with saline (Sal), 0.2 mg/kg haloperidol (Hal), 5 or 10 mg/kg clozapine (Clo5, Clo10), 45 min, and bilateral infusion of vehicle (VEH) or muscimol (MUS), immediately, before testing on Day 2. All groups were tested for PPI without injection and infusion 1 day before (Day 1) and 1 day after (Day 3) the infusion day. Values are presented as mean ± S.E.M. Bars represent 1 S.E. derived from ANOVA. Group sizes: see Experimental procedures, Experimental design and groups.

Finally, in view of the results of the first two experiments, one-tailed t tests based on the S.E. derived from the ANOVA were conducted to confirm that PPI was decreased in the groups that received ventral hippocampal muscimol infusions (since our statistics software does not conduct these comparisons, they were calculated according to Winer, 1971, p. 199). The t tests indicated that %PPI in the groups that received muscimol infusions was significantly (all F<.05) decreased as compared to the VEH group at each of the three highest (Sal-MUS, Hal-MUS) or even at all four prepulses (Clo-MUS). In the experiments involving hippocampal muscimol infusion and neuroleptic pretreatment, statistical power to reveal differences between groups was decreased as compared to the first two experiments without the neuroleptic pretreatment (four to five groups with n=7–10 as compared to three groups with n=8–16). Considering this, the results of the experiments involving neuroleptic pretreatment and muscimol infusions confirmed that dorsal and ventral hippocampal inhibition by muscimol decreases PPI, even though some of the relevant effects only approached significance. Moreover, they clearly indicated that pretreatment with haloperidol or clozapine does not antagonize this decrease of PPI. If anything, clozapine pretreatment slightly intensified the decrease in PPI following ventral hippocampal MUS infusion.
4. Discussion

In the present study, temporary inactivation by TTX (10 ng/side) or inhibition by muscimol (1 μg/side) of the dorsal or ventral hippocampus impaired PPI. Muscimol inhibits local neuronal activity by acting on the GABA_A receptor while TTX is also inactivating signal conduction by fibers of passage. Given that both compounds similarly decreased PPI, one can conclude that neuronal activity within the dorsal as well as ventral hippocampus is necessary for normal PPI. Effects on startle amplitude were less cut-off. TTX significantly increased startle reactivity when infused into the dorsal hippocampus, but tended to decrease it when infused into the ventral hippocampus. Muscimol had no significant effects on startle reactivity, but tended to decrease it when infused into the ventral hippocampus. Effects on startle reactivity have been observed after different manipulations of the ventral or dorsal hippocampus (e.g. Caine et al., 1991, 1992; Wan et al., 1996; Bakshi and Geyer, 1998, 1999; Zhang et al., 1999, 2000b, 2002; Bast et al., 2001b; Swerdlow et al., 2000b) and may be mediated by hippocampal projections to the amygdala or the bed nucleus of the stria terminalis, which have access to the brain stem startle circuit (Koch, 1999). Similar to the hippocampal manipulations in the present study, ventral hippocampal NMDA infusions, for example, affected startle reactivity less markedly and less reliably than PPI (Wan et al., 1996; Zhang et al., 1999, 2002; Bast et al., 2001d). Importantly, in the present study, in-depth analysis of the startle data yielded that the alterations in startle reactivity are unlikely to account for the observed reductions of PPI. This indicates that the PPI impairment following TTX inactivation and muscimol inhibition of the dorsal or ventral hippocampus reflects a genuine impairment of sensorimotor gating. Moreover, this PPI impairment is probably neuroleptically-resistant and, thus, not caused by excess dopamine transmission (as a result of the hippocampal manipulation) since both clozapine (5 and 10 mg/kg or 5 mg/kg, respectively) and haloperidol (0.2 mg/kg) did not antagonize the PPI impairment induced by dorsal or ventral hippocampal inhibition by muscimol.

4.1. Permanent lesions versus temporary deactivation

Previous studies using permanent lesions (Kemble and Ison, 1971; Pouzet et al., 1999; Swerdlow et al., 1995, 2000b), in contrast to the present study, did not indicate that activity of the ventral, as well as dorsal, hippocampus is important for the maintenance of PPI, even though a very recent study reported slight impairments of PPI after ventral, but not dorsal, subiculum lesions (Caine et al., 2001). Based on previous studies and our infusion parameters, considerable effects of TTX and muscimol during testing in the present study should have been restricted to a spherical region of less than 1 mm radius surrounding the injection sites within the dorsal or ventral hippocampus (Martin, 1991; Zhuravin and Bures, 1991). Thus, our results are unlikely to reflect drug diffusion to extrahippocampal sites. Permanent lesions of the hippocampus or its subparts may result in changes in other brain areas during the period between surgery and testing (Stein, 1979; Halim and Swerdlow, 2000). Thus, the effects of permanent hippocampal lesions may actually reflect massive neuronal reorganization throughout the brain rather than only the loss of hippocampal function. In particular, compensatory changes are likely to occur and possible recovery of function is considered a problem for the interpretation of studies using permanent lesions (Bures and Buresova, 1990; Lomber, 1999). Given that lesion-induced changes and compensatory mechanisms are likely to need some time after surgery to occur (e.g. Halim and Swerdlow, 2000), PPI may be found to be decreased when measured during the first few days following hippocampal lesions. In any case, the present results, as well as our recent study (Bast et al., 2001c), where we observed locomotor effects of ventral hippocampal inhibition or inactivation that markedly differed from those reported after lesions, indicate that the application of temporary deactivation techniques may lead to concepts of a brain structure's normal function that substantially differ from those based on studies using permanent lesions. Thus, some issues that have previously only been examined by means of permanent lesions may deserve re-examination by means of acute manipulations (see also Bast et al., 2001a).

4.2. Hippocampal modulation of PPI

While hippocampal lesions (Kemble and Ison, 1971; Pouzet et al., 1999; Swerdlow et al., 1995, 2000b; but see Caine et al., 2001) and ventral hippocampal infusions of the psychotomimetic MK-801 (Bakshi and Geyer, 1998; Bast et al., 2000) did not result in decreased basal PPI, chemical stimulation of the ventral hippocampus has consistently been found to disrupt PPI (e.g., Caine et al., 1992; Wan et al., 1996; Klärner et al., 1998; Zhang et al., 1999; Bast et al., 2001b,d). As to the dorsal hippocampus, the picture is less clear. Stimulation of the ventral and dorsal hippocampus by the acetylcholine agonist carbachol was reported to yield similar reductions of PPI (Caine et al., 1991, 1992). In contrast, dorsal hippocampal NMDA infusion virtually did not affect PPI (Swerdlow et al., 2001b; Zhang et al., 2002). Slight PPI disruption was reported following dorsal hippocampal MK-801 infusion in Sprague-Dawley rats (Bakshi and Geyer, 1998, 1999), but was not confirmed in Wistar rats (Zhang et al., 2000b). Altered hippocampal activity may decrease PPI by changing neuronal activity in the amygdala, the prefrontal cortex, or the nucleus accumbens. These forebrain areas receive strong hippocampal projections (Amaral and Witter, 1995) and have—directly (Sesack et al., 1989) or via the ventral pallidum—access to the brainstem circuits thought to mediate PPI. Experimental manipulations of these fore-
Brain regions have been reported to disrupt PPI (Koch, 1999; Swerdlow et al., 2001a). The different neurophysiological and neurochemical changes in these target areas that result from different alterations of dorsal and ventral hippocampal activity largely remain to be examined, but it is likely that different manipulations of the ventral and dorsal hippocampus induce PPI reduction by different pathways and different mechanisms. Thus, according to the topography of hippocampal projections, ventral hippocampal manipulations may directly alter neural transmission mainly in the accumbens shell, the prefrontal cortex, and the amygdala, whereas alterations of dorsal hippocampal activity may directly affect mainly processes in the accumbens core (Swanson and Cowan, 1977; Kelley and Domesick, 1982; Groenewegen et al., 1987; Amaral and Witter, 1995; Verwer et al., 1997; Pitkänen et al., 2000). Moreover, at least some of the hippocampal manipulations decreasing PPI differ in their effects on other behavioral processes, indicating different neurochemical and neurophysiological changes in hippocampal target areas. For example, ventral hippocampal stimulation induces marked hyperactivity (e.g. Bradzynski and Gibson, 1997; Bast et al., 2001b,d) while decreasing ventral hippocampal activity by TTX and muscimol resulted in hypoactivity (Bast et al., 2001c). Increased dopamine transmission, one prominent mechanism of PPI disruption (Koch, 1999; Zhang et al., 2000a; Swerdlow et al., 2001a), can virtually be ruled out to account for the reductions in PPI following acute hippocampal manipulations since dopamine receptor antagonists did not antagonize these reductions (Caine et al., 1991; Wan et al., 1996; Zhang et al., 1999; Bast et al., 2001d; present study). Among the reductions in PPI induced by manipulations in hippocampal projection areas, only that following lesions of the amygdala has also been demonstrated to be resistant against systemic dopamine receptor antagonists (Wan and Swerdlow, 1997). Given that increased dopamine transmission is thought to disrupt PPI largely by alterations of shell or core activity (Koch, 1999; Swerdlow et al., 2001a), manipulations directly inducing such alterations may also disrupt PPI independent of increased dopamine transmission. Dorsal hippocampal manipulations may directly modulate core activity, whereas alterations of ventral hippocampal activity may modulate shell activity, and, via feedback-forward processing (Zham, 2000), also activity in the core (see also Bast et al., 2001d). Interestingly, decreased dopamine transmission in the prefrontal cortex has been suggested to impair PPI (Ellenbroek et al., 1996; Zavitsanou et al., 1999) and, since electrical stimulation of the ventral hippocampus has been indicated to increase extracellular dopamine in the prefrontal cortex (Gurden et al., 2000), ventral hippocampal deactivation may decrease prefrontal dopamine transmission. However, findings from our laboratory do not support the view that PPI is impaired by decreased prefrontal dopamine transmission (Lacroix et al., 2000; Pezze et al., 2001). Thus, at present, we know several specific alterations of hippocampal activity disrupting PPI, but the underlying mechanisms and pathways remain to be clarified.

4.3. Relevance with respect to schizophrenia

Pathological changes of the hippocampus have received a lot of attention with respect to the etiology of schizophrenia. Although more recent functional imaging studies have quite consistently indicated that hippocampal activity is increased in schizophrenia and that hippocampal overactivity is associated with the experience of positive symptoms (reviewed by Heckers, 2001), there is also evidence that the hippocampus in schizophrenic patients may be hypofunctional in some respects (Harrison, 1999; Harrison and Eastwood, 2001; Weinberger, 1999; Gothelf et al., 2000). In this context, it has to be considered that different etiologies might underlie the clinical syndrome recognized as schizophrenia (Lewis and Lieberman, 2000). The present and previous results from animal experiments (see above) indicate that hippocampal pathology of different kinds may contribute to PPI deficits observed in schizophrenia. Regardless of whether deficient sensorimotor gating, as reflected by decreased PPI, may contribute to positive symptoms or just reflect pathologically altered brain function in schizophrenia, the underlying mechanisms are of interest concerning a treatment of schizophrenia. Since PPI deficits induced by alterations of hippocampal activity in the rat appear to be resistant against treatment with conventional neuroleptics, their understanding might help to guide the development of new strategies to cure treatment-resistant schizophrenia (see also Bast et al., 2001d).

Acknowledgements

This work was supported by grants from the Swiss Federal Institute of Technology Zurich. Clozapine was a gift from Novartis Pharma, Basel, Switzerland. Thanks are due to the animal facility team for care of the animals, Ms. Liz Weber for histology preparations, Mr. Peter Schmid for setup and maintenance of the computerized systems for behavioral analysis, and Ms. Jane Fotheringham for assistance with manuscript preparation.

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Annotation:
A1 See also Bast T, Pezze MA, Feldon J (in press) Effects of dopamine receptor blockade or stimulation in the rat medial prefrontal cortex on regulation of prepulse inhibition and locomotor activity. Behav Pharmacol.
Hippocampal modulation of sensorimotor processes: Effects of pharmacological manipulations of the rat ventral and dorsal hippocampus on locomotor activity, prepulse inhibition (PPI), and startle reflex

Tobias Bast, Joram Feldon
In preparation for submission
Abstract

While the hippocampus makes unique contributions to memory, it has also long been associated with sensorimotor processes, i.e. innate processes involving control of motor responses to sensory stimuli. Moreover, hippocampal dysfunction has been implicated in neuropsychiatric diseases, such as schizophrenia and anxiety disorders, primarily characterized by non-mnemonic deficits in the processing of and responding to sensory information. This review is concerned with the hippocampal modulation of three sensorimotor processes in rats – locomotor activity, prepulse inhibition (PPI) of the startle reflex, and the startle reflex itself –, whose alterations are related to human psychosis or anxiety disorders. Its main purpose is to present and discuss the picture emerging from studies examining the effects of pharmacological manipulations of the dorsal and ventral hippocampus by local drug microinfusions. While a role of the hippocampus in regulating locomotor activity, PPI, and startle reactivity has also been suggested based on the effects of hippocampal lesions, the microinfusion studies have revealed additional important details of this role and suggest modifications of notions based on lesion studies. In summary, the microinfusion studies corroborate that hippocampal mechanisms can directly influence locomotor activity, PPI, and startle reactivity, and that aberrant hippocampal function may contribute to neuropsychiatric diseases, in particular psychosis. The relation between different sensorimotor processes and hippocampal neurotransmission, the role of ventral and dorsal hippocampus, and the extrahippocampal mechanisms mediating the hippocampal modulation of different sensorimotor processes can partly be dissociated. Thus, the hippocampal modulation of these sensorimotor processes appears to reflect multiple operations, rather than one unitary operation.
Contents
1. Introduction

2. Background
   2.1. Hippocampal modulation of sensorimotor processes: relevance for normal behavior and neuropsychiatric disorders
      2.1.1. Locomotor activity and PPI
      2.1.2. Startle reflex
   2.2. Ventral and dorsal hippocampus
   2.3. Effects of permanent hippocampal lesions on locomotor activity, PPI, and startle reflex
      2.3.1. Locomotor activity
      2.3.2. PPI
      2.3.3. Startle reflex
   2.4. Relevance of the meso-accumbens dopamine system
   2.5. Inhibitory control: a unitary function accounting for the hippocampal modulation of sensorimotor processes?
   2.6. Sensorimotor effects of hippocampal manipulations: primary effects or secondary to memory deficits?

3. Use of pharmacological manipulations of the hippocampus by local drug microinfusions to examine the hippocampal modulation of sensorimotor processes
   3.1. Hippocampal deactivation
      3.1.1. Comparison with permanent hippocampal lesions
   3.2. Hippocampal stimulation
   3.3. Other pharmacological manipulations of the hippocampus

4. Effects of hippocampal drug microinfusions on locomotor activity, PPI, and startle reflex
   4.1. Locomotor activity
      4.1.1. Ventral hippocampus
      4.1.2. Dorsal hippocampus
   4.2. PPI
      4.2.1. Ventral hippocampus
      4.2.2. Dorsal hippocampus
   4.3. Startle reflex: ventral and dorsal hippocampus
4.4. Dissociations
   4.4.1. Stimulation and deactivation
   4.4.2. PPI and startle reflex
   4.4.3. PPI and locomotor activity
   4.4.4. Ventral and dorsal hippocampus

4.5. Comparison with the effects of permanent hippocampal lesions

5. Pathways and extrahippocampal mechanisms mediating the sensorimotor effects of hippocampal drug infusions
   5.1. Relevant anatomical connections: ventral-dorsal differentiation
   5.2. Dopamine mechanisms: dissociation between the hippocampal modulation of PPI and locomotor activity
      5.2.1. Dopamine in the nucleus accumbens
      5.2.2. Dopamine in the medial prefrontal cortex
      5.2.3. Dopamine in the hippocampus
   5.3. Glutamate mechanisms in the nucleus accumbens: a further dissociation between the hippocampal modulation of PPI and locomotor activity

6. Theoretical implications
   6.1. Hippocampal modulation of sensorimotor processes
   6.2. Integrating hippocampal functions

7. Methodological implications

8. Clinical implications
   8.1. Anxiety disorders
   8.2. Schizophrenia

9. Future directions

Acknowledgements
References
1. Introduction

The present article is concerned with the hippocampal modulation of three particular sensorimotor processes, i.e. innate processes essentially involving control of motor responses to sensory input: locomotor activity, prepulse inhibition (PPI) of the acoustic startle reflex, and the startle reflex itself. We will present and discuss a picture of the hippocampal modulation of these processes basically emerging from experiments examining the effects of pharmacological manipulations of the rat dorsal and ventral hippocampus by local drug microinfusions. The paper will start with a description of the background for these experiments (2.). In this part, we will describe locomotor activity, PPI, and startle, as well as the reasons for an interest in the hippocampal modulation of these processes (2.1.), present a brief summary of the relevant effects of hippocampal lesions (2.3.), and introduce several issues relevant with respect to the relation between the hippocampus and sensorimotor processes (2.2., 2.4.-2.6.). After this, we will depict the possibilities pharmacological manipulations of the hippocampus by local drug microinfusions offer to examine the relation between hippocampal neurotransmission and sensorimotor processes (3.). This more methodological part also gives an overview of pharmacological hippocampal manipulations, makes an attempt to categorize them, and exposes the rationales for studying these manipulations’ effects on sensorimotor processes (3.1.-3.3.). The bulk of the article will then be devoted to the sensorimotor effects of hippocampal drug microinfusions (4.) and the possible mechanisms mediating these effects (5.). We will conclude by deriving some theoretical (6.), methodological (7.), and clinical (8.) implications suggested by the review of the microinfusion studies and by identifying directions for future research (9.). In this article, the terms hippocampus or hippocampal are used to refer to the hippocampal subfields CA1 to CA3, the dentate gyrus, and the subiculum.

2. Background

2.1. Hippocampal modulation of sensorimotor processes: relevance for normal behavior and neuropsychiatric disorders

A lot of research and concepts concerning hippocampal function center around the hippocampus-dependence of more or less specific memory processes (e.g., O'Keefe and Nadel, 1979, Olton et al., 1979; Rawlins, 1985; Rudy and Sutherland, 1995; Morris and Frey, 1997; Eichenbaum, 2000; Fanselow, 2000; Aggleton and Pearce, 2001; Anagnostaras et al., 2001; Bast et al., 2001b). However, hippocampal mechanisms have also long been related to the modulation of sensorimotor processes (e.g., Altman et al., 1973; Gray and McNaughton,
Sensorimotor processes constitute the basis of behavior and their disruption may also affect the formation and expression of memory. Clarifying the hippocampal modulation of sensorimotor processes is, thus, of prominent importance for understanding the significance of the hippocampus for behavior generally and for interpreting the effects of hippocampal manipulations on learning and memory (Vanderwolf and Cain, 1994; Cahill et al., 2001). It has also to be considered, though, that alterations in sensorimotor processes may occur secondary to memory deficits (2.6.).

Hippocampal dysfunction has been associated with neuropsychiatric disorders, such as schizophrenia and anxiety disorders, which are primarily characterized by non-mnemonic deficits related to impaired processing of and responding to sensory stimuli (Gray et al., 1991; Gray, 1982, 1995, 1998; Weinberger, 1999; Benes, 2000; Grace, 2000). Neuroimaging and neuropathological studies have suggested hippocampal overactivity, possibly due to a concurrence of decreased GABA (γ-aminobutyric acid)-mediated inhibition and increased afferent excitation, in schizophrenic patients, and this overactivity has been associated with the experience of positive symptoms (Liddle et al., 1992; Busatti et al., 1995; Benes, 2000; Heckers, 2001; Heckers and Konradi, 2002). In addition, there is evidence that the hippocampus of schizophrenics may be hypofunctional in some respects (Harrison, 1999; Weinberger, 1999; Benes, 2000; Gotthelf, 2000; Harrison and Eastwood, 2001; Heckers, 2001; Heckers and Konradi, 2002). In this context, it has to be considered that different etiologies may underlie the clinical syndrome recognized as schizophrenia (Lewis and Lieberman, 2000). Based on the anxiolytic-like effects of manipulations directly impairing hippocampal function, the hippocampus has been implicated in controlling the strength of anxiety-related protective and defensive responses to aversive and threatening stimuli (Gray, 1982, 1995; File et al., 2000; Deacon et al., 2002; Kjelstrup et al., 2002). Hyperfunction of some hippocampal processes, possibly due to deficient inhibitory GABA transmission within the hippocampus, has been suggested to contribute significantly to clinical symptoms of anxiety disorders (Gray, 1982, 1995; Crestani et al., 1999; Löw et al., 2000). As will be explained (2.1.1., 2.1.2.), alterations of locomotor activity, PPI, or the startle reaction in rats may be related to schizophrenia or anxiety disorders, respectively, in humans. Understanding the hippocampal modulation of these sensorimotor processes in rats is, therefore, relevant for clarifying the contribution of hippocampal dysfunction to these human neuropsychiatric diseases.

1.7.
2.1.1. Locomotor activity and PPI

The main focus of the microinfusion studies reviewed in the present article (4., 5.) was the hippocampal modulation of locomotor activity and/or PPI of the acoustic startle reflex. Locomotor activity serves the animal's translocation and is a fundamental component of several adaptive behaviors, including exploration, approach to goals, and escape from aversive situations (Mogenson et al., 1980). PPI refers to the reduction of the acoustic startle reflex (2.1.2.) by a weak stimulus, or prepulse, briefly (30-500 ms) preceding the startle stimulus. It is usually expressed as percentage of PPI (% PPI). That is, the percentage by which the magnitude of the startle response to the startle pulse is reduced in trials in which the animals are presented with prepulse plus startle pulse as compared to trials in which the animals are presented with the startle pulse alone. PPI is thought to reflect sensorimotor-gating mechanisms protecting the early pre-attentive processing of the prepulse by attenuating motor responses to other sensory stimuli. Sensorimotor-gating mechanisms are considered a fundamental component of information processing in the brain, necessary for stimulus recognition and sequential organization of behavior (Graham, 1975; Norris and Blumenthal, 1996; Swerdlow et al., 2000a; Fendt et al., 2001). Both locomotor activity (Mogenson et al., 1980; Holstege, 1991; Mogenson and Yang, 1991; Alheid and Heimer, 1996) and PPI (Koch, 1999; Fendt, 2001; Swerdlow et al., 2000a, 2001a) are mediated by brainstem circuits, but modulated by forebrain circuits. In particular, a critical role of the meso-accumbens dopamine system is well established, with increased accumbal dopamine inducing hyperactivity (Le Moal and Simon, 1991; Mogenson and Yang, 1991; Ikemoto, 2002), as well as PPI disruption (Koch, 1999; Swerdlow et al., 2000a, 2001a).

Increased locomotor activity and reduced PPI in rats are related to psychosis in humans. A deficit in sensorimotor gating, reflected by reduced PPI, is well documented in schizophrenia, and this deficit has been proposed to contribute to sensory overload or flooding and, finally, cognitive fragmentation, thought disorder, and other psychotic symptoms (Braff and Geyer, 1990; Geyer et al., 1990; Braff et al., 2001). Locomotor hyperactivity may be homologous to some cognitive abnormalities in acute schizophrenia (Gray et al., 1999). For example, a disrupted "integration of past regularities of experience with current stimulus recognition, learning, and action" has been proposed as a core deficit contributing to acute psychosis (Gray et al., 1991; Gray, 1998) and may as well be the reason for increased exploratory locomotor activity in rats. Finally, reduced PPI and increased locomotor activity in rats, similar to positive psychotic symptoms, have been associated with increased subcortical dopamine transmission (Beninger, 1983; Swerdlow et al., 1990, 2001a; Gray et al., 1991;
1.7.

LeMoal and Simon, 1991; Koch, 1999; Grace, 2000; Lipska and Weinberger, 2000; Seeman and Kapur, 2000; Carlsson et al., 2001; Ikemoto, 2002). Thus, neural substrates modulating locomotor activity and PPI in the rat may correspond to brain mechanisms abnormal in schizophrenia.

Regulation of accumbal dopamine by the direct hippocampus-accumbens projection (Kelley and Domesick, 1982; Groenewegen et al., 1987), has been proposed as a mechanism by which the hippocampus modulates PPI and locomotor activity in the rat and that may be disturbed in schizophrenia (Gray et al., 1991; Mittleman et al., 1993; Bardgett et al., 1995; Gray, 1995, 1998). However, there are several other ways for the hippocampus to influence PPI and locomotor activity. For example, the amygdala has been implicated in the modulation of PPI and the medial prefrontal cortex has been implicated in the modulation of PPI and locomotor activity (e.g., Beninger et al., 1990; Alheid and Heimer, 1996; Lacroix et al., 1998, 2000; Koch, 1999; Pezze et al., 2001; Swerdlow et al., 2001; Tzschentke, 2001; Bast et al., in press a), and both amygdala (Pitkänen et al., 2000) and prefrontal cortex (Jay and Witter, 1991; Jay et al., 1992; Carr and Sesack, 1996; Verwer et al., 1997) receive strong projections from the ventral hippocampus.

2.1.2. Startle reflex

The acoustic startle reflex is a rapid contraction of the skeletal muscles elicited by a sudden auditory stimulus and assumed to be a protective reaction. It is commonly quantified by measuring dislocation of a cage floor in rats or by measuring the eyeblink component using electromyography of the orbicularis oculi muscle in humans. The startle reflex is mediated by a brain-stem circuit (Davis et al., 1982; Yeomans and Frankland, 1996), but modulated by forebrain sites (Koch, 1999). For example, the bed nucleus of the stria terminalis and the amygdala have access to the startle circuit and exert control over it (Davis et al., 1997; Koch, 1999). The hippocampus may modulate the startle reflex via projections from the ventral hippocampus to the amygdala and the bed nucleus of the stria terminalis (Swanson and Cowan, 1977; Pitkänen et al., 2000).

The effects of hippocampal manipulations on the startle reflex have basically been reported by studies primarily interested in the hippocampal modulation of PPI of the startle reflex (2.1.1.), rather than of the startle reflex itself. In these studies, alterations of startle reactivity itself may constitute a confounding factor, since changes of PPI accompanied by changes in startle reactivity cannot unequivocally be attributed to a genuine alteration in sensorimotor gating (Swerdlow et al., 2000a). For the interpretation of these studies it is, therefore, critical
whether the effects on PPI and startle reactivity can be dissociated (see also 4.4.2.). It is important to note, however, that emotional and attentional states influence startle reactivity (Lang, 1995; Koch, 1999). According to the concept of emotional priming, the startle response as a protective reaction to an aversive and threatening stimulus may be enhanced by negative and attenuated by positive emotional states (Lang, 1995). For example, enhanced startle reactivity is a diagnostic criterion for anxiety disorders in humans and used as an animal model of anxiety (Davis et al., 1997; Rodgers, 1997). Moreover, startle reactivity partly reflects attention or responsiveness to salient stimuli (Anthony and Graham, 1983; Acri et al., 1991). Theoretically, a failure in the allocation of information processing resources as a consequence of deficient sensorimotor gating, as reflected by reduced PPI, may result in reduced responsiveness to the most salient stimuli in a stimulus-laden environment (Braff and Geyer, 1990; Braff et al., 2001). Reduced startle reactivity in rats may, thus, reflect impairments related to the attentional and sensorimotor-gating deficits in schizophrenic patients (McGhie and Chapman, 1961; Braff and Geyer, 1990; Geyer et al., 1990; Barr, 2001; Braff et al., 2001). In schizophrenic patients, while considerable numerical reductions of startle reactivity have been reported (Braff et al., 1992; Ludewig et al., 2002), significant reductions may be difficult to detect due to the high interindividual variability of the startle measure (see also 4.3.) or they may only occur during a severe breakdown of information processing in psychotic episodes. Habituation of the startle reflex, i.e. its decrease with repeated startle-pulse presentation, however, has been found to be reduced in schizophrenics, even though this phenomenon is not very robust (see Ludewig et al., 2002), and this habituation deficit has been proposed to reflect an impairment in reducing attention to familiar stimuli (Geyer et al., 1990; Braff et al., 1992). Thus, alterations in startle reactivity observed in studies examining the hippocampal modulation of PPI may not just be considered as a confounding factor, but deserve some more regard (4.3.).

2.2. Ventral and dorsal hippocampus

The ventral and dorsal parts of the vertebrate hippocampus are connected with different sets of extra-hippocampal structures (Swanson and Cowan, 1977; Witter, 1986; Amaral and Witter, 1995). This suggests that the functions of the ventral and dorsal hippocampus, as well as the effects of ventral and dorsal hippocampal manipulations, may differ, even though gradually, rather than absolutely, because of strong intrahippocampal connections (Amaral and Witter, 1989). Spatial learning and memory has been demonstrated to be more susceptible to dorsal than to ventral hippocampal lesions (Moser et al., 1993; Moser and Moser, 1998a, b;
Bannerman et al., 1999; Richmond et al., 1999; Ferbinteanu and McDonald, 2000; Kjelstrup et al., 2002). This has been suggested to be consistent with highly processed information from the sensory cortices entering the hippocampus mainly in its dorsal parts (Moser and Moser, 1998b). In contrast, connections with structures that have been associated with the regulation of locomotor activity, PPI, and startle reflex, namely the prefrontal cortex, the amygdala, and the nucleus accumbens (2.1.1, 2.1.2.), predominantly its shell part, are mainly found in the ventral parts of the hippocampus. A projection to the nucleus accumbens, predominantly its core part, is the only direct dorsal hippocampal connection with these structures (Swanson and Cowan, 1977; Kelley and Domesick, 1982; Witter, 1986; Groenewegen et al., 1987; Jay and Witter, 1991; Jay et al., 1992; Amaral and Witter, 1995; Carr and Sesack, 1996; Verwer et al., 1997; Pitkänen et al., 2000). This neuroanatomical differentiation within the hippocampus suggests that locomotor activity, PPI, and startle reactivity may depend more on the ventral than on the dorsal hippocampus.

2.3. Effects of permanent hippocampal lesions on locomotor activity, PPI, and startle reactivity

2.3.1. Locomotor activity

Many studies have reported effects of hippocampal lesions on locomotor activity. Consistent differences between the effects of ventral and dorsal hippocampal lesions have not emerged from these studies. Even though there are some variations in the reported effects, the notion has emerged that complete or partial hippocampal lesions render rats hyperactive in familiar and nonfamiliar environments, as well as more susceptible to the locomotor stimulating effects of dopamine agonists (e.g., Gray and McNaughton, 1983; Lipska et al., 1991, 1992; Cassel et al., 1998; Pouzet et al., 1999; Coutureau et al., 2000; Caine et al., 2001).

2.3.2. PPI

The majority of evidence suggests that basal PPI is quite resistant against ventral, dorsal, or complete hippocampal lesions in adult rats (Kemble and Ison, 1971; Swerdlow et al., 1995, 2000; Pouzet et al., 1999; Finamore et al., 2001), but a slight reduction of basal PPI after lesions to the ventral, but not dorsal, subiculum, vanishing with repeated testing, has been reported (Caine et al., 2001). Lesions of the ventral hippocampus in neonate rats, however, have been found to result in decreased basal PPI in the adult rat (Lipska et al., 1995b; Le Pen et al., 2000; Le Pen and Moreau, 2002). This indicates an interference with the development
of circuits mediating or regulating PPI and, thus, points to a close connection of these circuits with the ventral hippocampus. Moreover, ventral hippocampal lesions in adult (Swerdlow et al., 1995, 2000b; but see Pouzet et al., 1999), as well as neonatal (Lipska et al., 1995b), rats have been reported to induce increased sensitivity to dopamine agonist-induced PPI disruption, while this effect was not observed following dorsal hippocampal lesions in adult rats (Swerdlow et al., 2000b).

2.3.3. Startle reflex

Increased basal acoustic startle reactivity has been reported after complete (Coover and Levine, 1972) and ventral hippocampal lesions (Swerdlow et al., 1995), as well as after dorsal subiculum lesions (Caine et al., 2001). However, several studies failed to observe a significant effect of partial or complete hippocampal lesions on startle reactivity (Kemble and Ison, 1971; Leaton, 1981; Lipska et al., 1995b; Lee and Davis, 1997a,b; Pouzet et al., 1999; Le Pen et al., 2000; Swerdlow et al., 2000b; Le Pen and Moreau, 2002). No deficits in the habituation of the startle response have been reported after any hippocampal lesions. Given the relatively high variability of basal startle reactivity, which may cover possible treatment effects (4.3.), existing data suggest that partial and complete hippocampal lesions, though not strongly affecting basal startle reactivity, may render the rat slightly hyperreactive to an auditory startle pulse.

2.4. Relevance of the meso-accumbens dopamine system

As described above (2.1.1.), a control of accumbal dopamine transmission by the hippocampus has been proposed as a possible mechanism mediating the hippocampal modulation of PPI and locomotor activity. Consistent with this notion, dysregulation of the meso-accumbens dopamine system has been implicated in the effects of ventral and complete hippocampal lesions on the regulation of PPI and locomotor activity (Isaacson, 1984; Mittleman et al., 1993; Wilkinson et al., 1993; Bardgett et al., 1995; Lipska et al., 1995a, b; Swerdlow et al., 1995, 2000b). Hippocampal lesions remove the prominent hippocampal projections to the nucleus accumbens (Kelley and Domesick, 1982; Groenewegen et al., 1987), and it has been demonstrated that cytotoxic ventral hippocampal lesions reduced dopaminergic innervation of forebrain sites, including the nucleus accumbens (Halim and Swerdlow, 2000). Such accumbal denervation may result in supersensitivity of accumbal neurons to dopamine receptor stimulation (Swerdlow et al. 2000b; Bannermann et al., 2001). However, direct evidence for such a denervation supersensitivity, for example a proliferation
of postynaptic dopamine receptors in the nucleus accumbens, remains to be provided (see Halim and Swerdlow, 2000). In addition, it was reported that hippocampal lesions, while not affecting basal dopamine concentrations, resulted in increased amphetamine-induced dopamine release in the nucleus accumbens (Wilkinson et al., 1993). In any event, the contribution of dysregulation of the meso-accumbens system to the effects of ventral and complete hippocampal lesions on the regulation of locomotor activity and PPI has so far not been demonstrated directly. Interestingly, the disruption of basal PPI resulting from neonatal ventral hippocampal lesions has been demonstrated to be resistant against the typical neuroleptic haloperidol, which primarily blocks dopamine receptors, while some atypical neuroleptics, acting on additional receptors, antagonized this effect (LePen and Moreau, 2002). This points at a connection between the ventral hippocampus and circuits that influence PPI without the involvement of the meso-accumbens dopamine system.

2.5. Inhibitory control: a unitary hippocampal function accounting for the hippocampal modulation of sensorimotor processes?

There have been numerous attempts to propose one unitary hippocampal function that completely describes the role of the hippocampus in behavior (Schmajuk, 1984). Alterations in sensorimotor processes after hippocampal lesions have often been suggested to be consistent with theories associating the hippocampus with some kind of inhibitory control on physiological or behavioral processes (Douglas, 1967; Kimble, 1968; Altman, 1973; Gray, 1982, 1995; Chan et al., 2001). For example, both hippocampal involvement in PPI (Swerdlow et al., 2001a) and increased startle reactivity following hippocampal lesions (Coover and Levine, 1972) have been related to a hippocampal contribution to inhibitory processes without further specifying these processes. The hyperactivity following hippocampal lesions has been proposed to reflect the loss of inhibitory control over the dopaminergic tonus in the nucleus accumbens (Coutureau, 2000), the disruption of Pavlovian internal inhibition (Kimble, 1969; Douglas, 1967), the disruption of mechanisms to hold still (Blanchard et al., 1977) or to brake response emissions (Altman et al., 1973), or the disruption of a behavioral inhibition system that inhibits ongoing behavior and increases arousal and attention when the animal is confronted with novel or aversive stimuli (Gray, 1982, 1995). Thus, many authors have considered the effects of hippocampal lesions on sensorimotor processes as consistent with the hippocampus exerting some kind of inhibition, but a commonly accepted concept concerning the nature of the inhibitory mechanism does not exist.
2.6. Sensorimotor effects of hippocampal manipulations: primary effects or secondary to memory deficits?

While research concerned with the substrates of mnemonic processes needs to consider the so-called “performance account” of memory deficits, i.e. that a memory deficit is secondary to alterations in sensorimotor processes (Vanderwolf and Cain, 1994; Cahill et al., 2001; Bast et al., in press b), there may as well be a “mnemonic account” for some manipulations’ sensorimotor effects. Increased locomotor activity in an open field following hippocampal manipulations may, for example, reflect a deficit in the animal’s habituation to the environment due to the disruption of hippocampal processes serving spatial or contextual memory (Anagnostaras et al., 1999; Fanselow, 2000). Such a deficit in habituation to the environment may also result in increased startle reactivity according to the emotional priming concept (2.1.2), given that an unfamiliar environment is more aversive than a familiar one. Therefore, the possibility of a “mnemonic account” has to be considered when interpreting the effects of hippocampal drug infusions on locomotor activity and startle reactivity (4.).

3. Use of pharmacological manipulations of the hippocampus by local drug microinfusions to examine the hippocampal modulation of sensorimotor processes

Intracerebral microinfusions, i.e. infusion of small volumes of drug solution into specific brain sites by means of small infusion cannulae that are inserted into stereotaxically implanted guide cannulae, allow temporary site-specific pharmacological manipulation of the brain. Provided sufficiently fine guide and infusion cannulae (e.g., 26-gauge guide cannulae and 34-gauge infusion cannulae), the spatial specificity of intracerebral microinfusions is critically dependent on the infused volume. Even though drugs may differ in their diffusion properties, one can roughly estimate that the drug spread from the infusion center, occurring primarily along the external wall of the infusion cannula, is below 1 mm with the commonly applied infusion volume of 0.5 μl (Myers, 1966; Myers et al., 1971; Routtenberg, 1972; Sandkühler et al., 1987; Zhuravin and Bures, 1991; Martin and Ghez, 1999). The dorsal and ventral horn of the hippocampus are hose-like structures with a dorso-ventral extent of about 2-3 mm, a medio-lateral extent of about 3-5 mm, and an antero-posterior extent of about 3-4 mm (Paxinos and Watson, 1998). In addition, the densely packed alvear, fimbria, and callosal fiberbundles surrounding the hippocampal surfaces appear to prevent diffusion out of the hippocampus (Morris et al., 1989). Thus, the use of microinfusions permits to examine the
behavioral effects of temporary pharmacological manipulations specific to the ventral or dorsal hippocampus (Fig. 1). The possibility to separately manipulate ventral and dorsal hippocampal neurotransmission is important since the functions of the ventral and dorsal hippocampus may differ in view of their different connections (2.2.).

In addition to intracerebral microinfusions, reverse microdialysis could be used to induce temporary pharmacological manipulations of hippocampal neurotransmission by local drug application. Probably for practical reasons, reverse microdialysis has hardly been used to examine the effects of temporary pharmacological manipulations of the hippocampus on sensorimotor processes, with the exception of a few studies (Takahashi et al., 2000, 2001, 2002). In these studies, the large dimensions of the probes, however, did not allow manipulating the ventral or dorsal hippocampus separately.

Local drug microinfusions offer the possibility to induce various alterations in hippocampal neurotransmission and to thoroughly characterize the causal relation between mechanisms in the ventral and dorsal hippocampus and the three sensorimotor processes of interest, with the effects of different drugs yielding complementary information. Before describing the examined effects of several pharmacological manipulations of the hippocampus on locomotor activity, PPI, and startle (4., Table 1), we will briefly provide an overview and categorization of these manipulations and describe the rationale for studying their effects on sensorimotor processes. Even though all commonly used drugs have specific targets (neurotransmitter receptors, ion channels), microinfusion of some drugs may alter overall neuronal activity in the infused hippocampal area and result in conditions that may be referred to as either hippocampal deactivation (3.1.) or stimulation (3.2.). Nevertheless, different forms of such stimulation or deactivation may differ in their behavioral effects. Furthermore, there are drugs, which have more subtle effects on hippocampal neurotransmission and whose effects on overall hippocampal activity can neither be described as deactivating nor as stimulating (3.3.).

3.1. Hippocampal deactivation

Hippocampal deactivation can be achieved by local microinfusion of drugs, such as the Na⁺ channel blockers tetrodotoxin (TTX) or lidocaine, preventing action potential initiation and propagation, or of GABAergic drugs, such as the GABA_A-receptor agonist muscimol, which increases inhibitory transmission. Inhibition of the hippocampal excitatory network is basically mediated by GABA_A-receptors (Buhl et al., 1994). While the Na⁺ channel blockers antagonize or completely block neurotransmission by both local neurons and fibers passing
Fig. 1 Line drawing showing the three-dimensional organization of the rat hippocampus with its dorsal and ventral horn (adapted from Amaral and Witter, 1995) and cutouts of Nissl-stained coronal brain sections showing typical infusion sites as used for bilateral pharmacological manipulations of the dorsal (top) or ventral (bottom) hippocampus in the laboratory of the authors. The rectangles indicate the approximate location of the cutouts. The cutouts show the wide tracks of the permanently indwelling guide cannulae (26 gauge) and beneath some finer tissue damage resulting from the temporary insertion of the infusion cannulae (34 gauge) protruding 1.5 mm from the guide cannulae. Hippocampal subfields are indicated on the right in the cutouts. CA1, CA2, CA3: CA1, CA2, and CA3 fields of the hippocampus; DG, dentate gyrus; S, subiculum.
through the infusion site, muscimol selectively inhibits local neurons bearing GABA$_A$-receptors (Sandkühler et al., 1987; Zhuravin and Bures, 1991; Martin and Ghez, 1999). The information yielded by the effects of pharmacological hippocampal deactivation on a given behavioral process is whether this process depends on hippocampal activity at all.

3.1.1. Comparison with permanent hippocampal lesions

It is important to briefly consider the relation between the effects of a temporary deactivation and a permanent lesion. At first glance, hippocampal microinfusion of Na$^+$ channel blockers or muscimol appears to temporarily induce conditions similar to those resulting from conventional (electrolytic, radiofrequency, aspiration) or fiber-sparing excitotoxic hippocampal lesions (Jarrard, 2001), respectively, and, thus, similar behavioral effects might be expected. It has, however, long been recognized that permanent focal brain lesions may induce secondary changes throughout the entire brain (Schoenfeld and Hamilton, 1977; Stein, 1979). Indeed, widespread changes in extrahippocampal brain sites have been demonstrated following both hippocampal aspiration lesions (Bär et al., 1981) and ventral hippocampal lesions by the excitotoxin ibotenate (Lipska et al., 1992; Halim and Swerdlow, 2000). On the one hand, secondary changes may cause additional behavioral symptoms, not reflecting the absence of the primarily lesioned structure, while, on the other hand, they may also lead to the recovery of behavioral functions by compensating for the initial focal damage (Schoenfeld and Hamilton, 1977). Moreover, the requirement for the animal to permanently cope with the absence of a part of brain tissue has been suggested to evoke compensatory mechanisms leading to recovery of behavioral function, often observed within a few days after the lesion (Lomber, 1999). Thus, while both permanent hippocampal lesions and temporary hippocampal deactivation induce a loss of hippocampal function, the behavioral symptoms of the permanent lesions may not only reflect this loss of hippocampal function, but also secondary changes and compensatory mechanisms.

3.2. Hippocampal stimulation

Hippocampal stimulation can be induced by compounds, such as NMDA (N-methyl-D-aspartate), other excitatory amino acids, or carbachol, which directly excite hippocampal principal neurons by stimulating NMDA-type or ionotropic glutamate receptors or muscarinic acetylcholine receptors, respectively. Another way to stimulate hippocampal activity is microinfusion of drugs, such as the GABA$_A$-receptor antagonist picrotoxin, inducing disinhibition by antagonizing hippocampal inhibitory transmission. Hippocampal stimulation
may be used to study the behavioral effects of increased transmission via hippocampal efferents and to characterize the pathways and mechanisms mediating hippocampal modulation of behavioral processes (e.g., Yang and Mogenson, 1987; Floresco et al., 2001). It has to be considered, however, that the behavioral effects of hippocampal stimulation may also reflect the disruption of particular hippocampal functions because the excess local stimulation may interfere with normal hippocampal processing (e.g., Berke and Eichenbaum, 2001; Bast et al., in press b). One characteristic of drugs genuinely stimulating hippocampal activity is that they, if applied to the hippocampus at high doses, induce epileptic seizures (Flicker and Geyer, 1982c; Hallak et al., 1993; Alam and Starr, 1996; Bardgett and Henry, 1999). Indeed, with many excitatory drugs, including glutamate, kainate, and AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionate), it is difficult to induce behaviorally effective hippocampal stimulation without inducing seizures (Bardgett and Henry, 1999). If hippocampal stimulation is used to characterize hippocampal modulation of sensorimotor processes, it is important to use sufficiently low doses in order to prevent seizures, since the accompanying convulsions themselves are likely to interfere with most behavioral processes.

One important reason for the interest in the effects of hippocampal stimulation on sensorimotor processes in rats is that hippocampal overactivity, possibly due to a concurrence of increased afferent excitation and a local decrease of GABAergic inhibition, has been indicated in schizophrenics and associated with psychotic symptoms (Liddle et al., 1992; Busatti et al., 1995; Benes, 2000; Heckers, 2001; Heckers and Konradi, 2002). Studies examining whether and by which mechanisms microinfusions stimulating hippocampal activity in rats result in psychosis-related alterations of sensorimotor processes (2.1.) may help to clarify the relation between hippocampal overactivity and psychotic symptoms.

3.3. Other pharmacological manipulations of the hippocampus

Hippocampal microinfusion of drugs, such as the non-competitive NMDA receptor antagonist MK-801 (dizocilpine) or dopamine agonists or antagonists, induce alterations in hippocampal neurotransmission that cannot be characterized as a stimulation or deactivation. For example, MK-801 antagonizes the effects of glutamate at the NMDA receptor by blocking the channel pore and, thus, reduces further excitation of already activated neurons (Collingridge and Lester, 1989). However, although MK-801 antagonizes neuronal excitation on a cellular level it may induce disinhibition of particular hippocampal circuits by antagonizing NMDA-receptor-mediated tonic activation of GABAergic, i.e. inhibitory, hippocampal interneurons (Olney et al., 1999). Similarly, the effects of dopamine agonists or
antagonists on the overall neuronal activity in the hippocampus cannot simply be characterized, since activation of dopamine receptors facilitates some, but inhibits other aspects of hippocampal neurotransmission (Lisman and Otmakhova, 2001).

NMDA receptor hypofunction (Olney et al., 1999) and increased dopamine transmission, as well as aberrant hippocampal function have been related to psychotic symptoms (2.1.), and all drugs used to treat psychosis antagonize dopamine transmission in some way (Grace, 2000; Seeman and Kapur, 2000; Carlsson et al., 2001). Moreover, it has been suggested that PPI disruption induced by noncompetitive NMDA receptor antagonists, such as MK-801 or phencyclidine, is selectively ameliorated by atypical antipsychotic drugs, such as clozapine (Bakshi et al., 1994), which are characterized by an increased therapeutic efficacy as compared to typical neuroleptics (Kinon and Lieberman, 1996). Even though many experiments failed to substantiate this suggestion (Hoffman et al., 1993; Wiley et al., 1994; Varty and Higgins, 1995; Bast et al., 2000, 2001a), it stimulated a strong interest in the neural substrates mediating this PPI disruption. Therefore, studies examined the effects of hippocampal microinfusions of MK-801 or dopaminergic drugs on sensorimotor processes in order to clarify whether hippocampal NMDA receptor hypofunction or dopamine receptor stimulation results in psychosis-related alterations of sensorimotor processes in rats or whether actions of dopamine receptor antagonists in the hippocampus might antagonize such alterations.

4. Effects of hippocampal drug microinfusions on locomotor activity, PPI, and startle reflex

In the following, the effects of ventral and dorsal hippocampal drug microinfusions on locomotor activity, PPI, and startle reflex will be described and discussed. Previously unpublished results of experiments in our laboratory, which examined some effects of dorsal hippocampal stimulation by picrotoxin and deactivation by tetrodotoxin and muscimol, will also be considered. These results complement the data basis and are presented in Fig. 2 and 3. In order to give an overview and to facilitate reading of the paragraphs concerned with dissociations between different manipulations and processes, as well as between ventral and dorsal hippocampus, a summary of all effects is given in Table 1. The legend of Table 1 also includes information about the doses at which the sensorimotor effects of hippocampal drug microinfusions were reported.
Table 1 Summary of the effects of pharmacological manipulations of the rat ventral and dorsal hippocampus by local drug microinfusions on locomotor activity, PPI, and startle reflex.

<table>
<thead>
<tr>
<th>PHARMACOLOGICAL MANIPULATION</th>
<th>DRUG</th>
<th>PART OF THE HIPPOCAMPUS</th>
<th>LOCOMOTOR ACTIVITY</th>
<th>PPI</th>
<th>STARTLE REFLEX</th>
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<td><strong>STIMULATION</strong></td>
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<td>Direct excitation^A</td>
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<td>Stimulation of NMDA-type glutamate receptors</td>
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<td>Disinhibition</td>
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<td>GABA&lt;sub&gt;A&lt;/sub&gt;-receptor antagonist</td>
<td>Picrotoxin</td>
<td>Ventral</td>
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<td><strong>DEACTIVATION</strong></td>
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<td>Neurons</td>
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<td>GABA&lt;sub&gt;A&lt;/sub&gt;-receptor stimulation</td>
<td>Muscimol</td>
<td>Ventral</td>
<td>↓15</td>
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<td>Neurons and fibers</td>
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<td>Na&lt;sup&gt;-&lt;/sup&gt;-channel blockade^B</td>
<td>TTX</td>
<td>Ventral</td>
<td>↓15</td>
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<td>Others^C</td>
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<td>Non-competitive blockade of NMDA receptors</td>
<td>MK-801 (dizocilpine)</td>
<td>Ventral</td>
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<td>Dopamine-receptor stimulation</td>
<td>diverse (see references)</td>
<td>Ventral</td>
<td>↑22</td>
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^A In addition, the effects of direct ventral hippocampal excitation by glutamate or AMPA on locomotor activity have been examined, with doses below seizure threshold not significantly affecting locomotor activity (Bardgett and Henry, 1999).

^B In addition, the effects of ventral hippocampal Na<sup>-</sup>-channel blockade by lidocaine on locomotor activity have been examined, with the dose used (20 µg/side) yielding no significant effect (Bardgett and Henry, 1999; Zhang, Bast, and Feldon, unpublished observations). Moreover, locomotor activity in a holeboard apparatus was decreased during continuous 40-min dorsal hippocampal infusion of lidocaine (Flicker and Geyer, 1982c), with this effect probably reflecting an interference with response to novelty (see 4.1.2.).

^C In addition, increased locomotor activity in a holeboard apparatus has been reported during continuous 40-min infusion of norepinephrine into the dorsal hippocampus (3 or 10 µg/1 µl/side; Flicker and Geyer, 1982d) and ventral hippocampal infusion of the metabotropic glutamate receptor agonist ACPD (trans-(±)-l-amino-l,3-cyclopentanedicarboxylic acid; 0.09-9 µg/0.5 µl) has been demonstrated not to affect locomotor activity (Bardgett and Henry, 1999).

Legend is continued on the following page.
The symbols describing the effects of the different manipulations on locomotor activity, PPI, and startle have the following meanings:

† increase, ‡ decrease, — no significant alteration, ? not examined;

ventral hippocampal infusion of D1- and D2-family-receptor antagonists did not affect basal locomotor activity, but prevented the induction of hyperactivity by a simultaneous ventral hippocampal NMDA infusion;

ventral hippocampal infusion of haloperidol did not affect basal PPI, but partly antagonized the reduction of PPI by systemic injection of apomorphine.

The superscript numbers indicate the references for the presented effects. Information about the used drug doses is provided in brackets; infusions were bilateral, unless mentioned otherwise:

1. Yang and Mogenson, 1987 (0.5 μg/0.2 μl); Wu and Brudzynski, 1995, Brudzynski and Gibson, 1997 (unilateral infusions, 2 μg/0.2 μl); Bardgett and Henry, 1999 (bilateral infusion: 0.07, 0.7 μg/0.5 μl, ineffective doses: 0.007, 0.0007 μg/0.5 μl; unilateral infusion: 0.2 μg/0.2 μl); Legault and Wise, 1999 (unilateral infusion, 0.74 μg/0.5 μl, ineffective doses: 0.074 and 0.28 μg/0.5 μl); Bast et al., 2001e (0.5 μg/0.5 μl); Giménez-Llort et al., 2002 (0.1, 0.5, and 1 μg/0.5 μl, ineffective dose: 0.05 μg/0.5 μl); Zhang et al., 2002a (0.1, 0.25, 0.5 μg/0.5 μl).

2. Wan et al., 1996a (0.8 μg/0.5 μl, ineffective dose: 0.4 μg/0.5 μl); Klarner et al., 1998, Koch et al., 1999 (1 μg/0.5 μl); Zhang et al., 1999 (0.7 μg/0.7 μl); Bast et al., 2001e (0.5 μg/0.5 μl); Swerdlow et al., 2001b (0.8 μg/0.5 μl, ineffective dose: 0.4 μg/0.5 μl); Zhang et al., 2002a (0.1, 0.25, 0.5 μg/0.5 μl).

3. Wan et al., 1996a (0.8 μg/0.5 μl); Klarner et al., 1998 (1 μg/0.5 μl); Swerdlow et al., 2001b (0.8 μg/0.5 μl, ineffective dose: 0.4 μg/0.5 μl); Zhang et al., 1999 (0.7 μg/0.5 μl), 2002a (0.5 μg/0.5 μl, ineffective doses: 0.1, 0.25 μg/0.5 μl).

4. Wan et al., 1996a (0.4, 0.8 μg/0.5 μl); Koch et al., 1999 (1 μg/0.5 μl); Bast et al., 2001e (0.5 μg/0.5 μl).

5. Zhang et al., 2002a (0.1, 0.25, 0.5, 0.7 μg/0.5 μl).

6. Swerdlow et al., 2001b (0.4, 0.8 μg/0.5 μl); Zhang et al., 2002a (0.1, 0.25, 0.5, 0.75 μg/0.5 μl).

7. Swerdlow et al., 2001b (0.8 μg/0.5 μl, ineffective dose: 0.4 μg/0.5 μl); Zhang et al., 2002a (0.25, 0.5, 0.7 μg/0.5 μl, ineffective dose: 0.1 μg/0.5 μl).

8. Brenner and Bardgett, 1998 (0.4 μg/0.2 μl).

9. Caine et al., 1992 (0.2, 0.4 μg/0.5 μl, ineffective dose 0.1 μg/0.5 μl).

10. Mogenzon and Nielsen, 1984 (0.2, 0.4 μg/0.2 μl, 0.8 μg/0.4 μl, unilateral infusions: 0.1, 0.2 μg/0.1 μl, 0.4 μg/0.2 μl); Flicker and Geyer, 1982b (40-min continuous infusion: 0.1, 0.3 μg/1 μl).

11. Caine et al., 1991 (0.4, 0.8, 1.6 μg/0.5 μl, ineffective dose 0.2 μg/0.5 μl), 1992 (0.8 μg/0.5 μl, ineffective doses 0.2, 0.4 μg/0.5 μl).

12. Bast et al., 2001c (locomotor activity: 150 ng/0.5 μl; PPI: 150 ng/0.5 μl, ineffective dose: 100 ng/0.5 μl; startle reflex: 100, 150 ng/0.5 μl).

13. Flicker and Geyer, 1982c (40-min continuous infusion: 0.1, 1 μg/1 μl); Bast, Zhang, and Feldon, previously unpublished observations, see Fig. 2A.

14. Bast, Zhang, and Feldon, previously unpublished observations, see Fig. 2B.

15. Bast et al., 2001d (muscimol: 0.5, 1 μg/0.5 μl; TTX: 5, 10 ng/0.5 μl).

16. Zhang et al., 2002b (muscimol: 1 μg/0.5 μl; TTX: 10 ng/0.5 μl).

17. Zhang, Bast, and Feldon, previously unpublished observations, see Fig. 3.

18. Zhang et al., 2001 (6.25 μg/0.5 μl).

19. Bakshi and Geyer, 1998 (0.25, 1.25, 6.25 μg/0.5 μl); Bast et al., 2000 (6.25 μg/0.5 μl).

20. Zhang et al., 2000 (6.25 μg/0.5 μl).

21. Bakshi and Geyer, 1998 (6.25 μg/0.5 μl, ineffective doses: 0.25, 1.25 μg/0.5 μl), 1999 (6.25 μg/0.5 μl).

22. Smialowski and Maj, 1985 [apomorphine (direct dopamine receptor agonist), 12 μg/1 μl, ineffective dose: 6 μg/1 μl].

23. Ellenbroek et al., 2002 [amphetamine (indirect dopamine receptor agonist), 10 μg/0.5 μl, ineffective doses: 1, 3 μg/0.5 μl; SKF81297 (D1-family receptor agonist), 3, 10 μg/0.5 μl, ineffective dose: 1 μg/0.5 μl; quinpirole (D2-family receptor agonist with particularly strong affinity for the D3 subtype), 3, 10 μg/0.5 μl, ineffective dose: 10 μg/0.5 μl].

24. Giménez-Llort et al., 2002 [0.1, 0.5 μg NMDA + 1 μg SCH23390 (D1-family-receptor antagonist) or 5 μg raclopride (D2-family-receptor agonist) / 0.5 μl: ineffective dose of raclopride: 1 μg/0.5 μl].

25. Hart et al., 1998 (systemic apomorphine, 0.5 mg/kg, subcutaneous; haloperidol: 5 μg/1 μl, ineffective doses: 0.05, 0.5 μg/1 μl).
4.1. Locomotor activity

4.1.1. Ventral hippocampus

The effects of ventral hippocampal drug microinfusions demonstrate that the ventral hippocampus plays an important role in the regulation of locomotor activity. Pharmacological stimulation of the ventral hippocampus by NMDA (Yang and Mogenson, 1987; Wu and Brudzynski, 1995; Brudzynski and Gibson, 1997; Bardgett and Henry, 1999; Legault and Wise, 1999; Bast et al., 2001d; Giménez-Llort et al., 2002; Zhang et al., 2002a), carbachol (Brenner and Bardgett, 1998), or picrotoxin (Bast et al., 2001c) results in marked locomotor hyperactivity, while pharmacological deactivation of the ventral hippocampus by TTX or muscimol decreases locomotor activity (Bast et al., 2001c). Hypoactivity was also observed during TTX perfusion through a 4-mm microdialysis probe implanted into the caudal hippocampus and stretching over a substantial portion of the ventral hippocampus (Takahashi et al., 2000). Ventral hippocampal deactivation by the Na⁺ channel blocker lidocaine, however, did not yield significant effects on locomotion (Bardgett et al., 1999; Zhang, Bast, Feldon, unpublished observations). This probably reflects that the ventral hippocampal deactivation by lidocaine at the dose applied (20 μg/side) was not sufficient to affect locomotor activity. In contrast, the use of novel spatial information required to navigate in a novel situation has been suggested to be impaired by ventral hippocampal deactivation with the same dose of lidocaine (Floresco et al., 1996, 1997), indicating that the ventral hippocampal mechanisms serving navigation and locomotion are partly dissociable. Moreover, ventral hippocampal infusion of the non-competitive NMDA receptor antagonist MK-801 increased locomotor activity (Zhang et al., 2001), while local infusions of dopamine receptor antagonists did not affect basal locomotor activity, but antagonized hyperactivity induced by simultaneous NMDA infusion, with the D1-class receptor antagonist SCH-23390 being more effective than the D2-class receptor antagonist raclopride (Giménez-Llort et al., 2002). The effects of MK-801 may be due to blockade of NMDA receptors on inhibitory hippocampal interneurons (Olney et al., 1999), consistent with the hyperactivity following direct blockade of inhibitory transmission by picrotoxin (Bast et al., 2001c). A reduction of the dopamine-mediated enhancement of NMDA receptor-mediated excitation of hippocampal pyramidal neurons may account for the effects of the dopamine receptor antagonists (Yang, 2000). Finally, it is important to note that a habituation deficit (2.6.) can virtually be excluded as an account for the hyperactivity observed following ventral hippocampal stimulation or MK-801 infusion. While these pharmacological manipulations resulted in an immediate hyperactivity (Bast et al., 2001c, e; Zhang et al., 2001, 2002a), a habituation deficit would be
reflected by a delayed onset of the hyperactivity (see Anagnostaras et al., 1999; Bast et al., 2001e).

Taken together the effects of ventral hippocampal drug infusions, in particular the opposite effects of pharmacological stimulation and deactivation (see also 4.4.1.), suggest that activity of the ventral hippocampus directly drives locomotor activity. The pattern of neuronal activation appears to be important, though, in order for ventral hippocampal stimulation to increase locomotion. For example, the activation may need to be sustained or affect particular populations of neurons. This has been suggested based on the finding that several agonists of excitatory-amino-acid-transmitter receptors, other than NMDA, failed to induce a marked hyperactivity after ventral hippocampal infusions at doses below the seizure threshold (Bardgett and Henry, 1999).

4.1.2. Dorsal hippocampus

Alterations in locomotor activity have also been observed after drug microinfusions into the dorsal hippocampus. Pharmacological stimulation of the dorsal hippocampus by carbachol (Flicker and Geyer, 1982; Mogenson and Nielsen, 1984) or picrotoxin (Flicker and Geyer, 1982; Fig. 2A) induced locomotor hyperactivity, similar to the effect of these manipulations in the ventral hippocampus (4.1.1.), with the results of one study suggesting that carbachol infusions into the dentate gyrus were much more effective than into other sites of the dorsal hippocampus (Mogenson and Nielsen, 1984). Stimulation of the dorsal hippocampus by NMDA, however, did not affect locomotor activity, in contrast to the marked hyperactivity induced by ventral hippocampal NMDA stimulation (Zhang et al., 2002). Dorsal hippocampal infusion of MK-801 increased activity (Zhang et al., 2000), though less than MK-801 infusion into the ventral hippocampus (Zhang et al., 2001). This effect of MK-801 may partly reflect decreased excitation of inhibitory interneurons due to NMDA-receptor blockade (Olney et al., 1999), given that direct blockade of inhibitory transmission in the dorsal hippocampus by picrotoxin also induced hyperactivity. However, the patterns of hyperactivity induced by MK-801 and picrotoxin differed, and, therefore, different mechanisms may account for the locomotor effects following dorsal hippocampal infusion of these two drugs (see below). Locomotor hyperactivity has also been observed during 15 min following dorsal hippocampal infusion of the direct dopamine-receptor agonist apomorphine (Smialowski and Maj, 1985) and during a 40-min continuous infusion of norepinephrine into the dorsal hippocampus (Flicker and Geyer, 1982d). Moreover, we recently found that deactivation of the dorsal hippocampus by TTX or muscimol infusion slightly increased locomotor activity in an open
Fig. 2 Effects of picrotoxin infusion into the dorsal hippocampus on (A) locomotor activity, as well as on (B) PPI, and startle reflex in Wistar rats (Bast, Zhang, Feldon, previously unpublished results). There were three infusion groups to receive one of the following bilateral dorsal hippocampal infusions: vehicle (0.5 µl saline/side; VEH), 150 ng picrotoxin/side (PTX150), or 250 ng picrotoxin/side (PTX250); the picrotoxin infusions did not induce behavioral signs of seizures. Procedures for cannulae implantation and infusion into the dorsal hippocampus, as well as husbandry and general handling of the animals were the same as described in Zhang et al. (2000, 2002a,b) and Bast et al. (in press b). All values are given as mean, variation is indicated by 1 standard error of the differences of the means (1 SE). A Activity during three 10-min blocks immediately preceeding infusion (left), the six 10-min blocks immediately following infusion (middle), as well as during three 10-min blocks one day after infusion (right). In addition to the test sessions whose results are presented all rats were subjected to a 30-min session to test baseline activity one day prior to infusion. The three groups were matched according to this baseline activity. Activity was measured in dark cubicles by an automated system comparing pixel changes between adjacent 1-s video images as used in Bast et al. (in press a) and Pezze et al. (in press). While all groups’ activity was very similar during the period preceeding infusion, as well as on the day after infusion, dorsal hippocampal picrotoxin infusion induced a marked hyperactivity. ANOVA revealed a strong tendency for an overall increased locomotor activity in the PTX150 and PTX250 groups ($F_{2,33} = 2.923, P = 0.0678$), as well as a highly significant interaction of infusion group x 10-min block ($F_{10,165} = 6.071, P < 0.0001$), for the six 10-min blocks immediately following infusion. The interaction reflected that activity of the PTX150 and PTX250 groups was about twice as high as that of the VEH group during the first two 10-min blocks following infusion, but was similar for all three groups during the rest of the 60 min following infusion. B PPI and startle reactivity during a 23-min test session immediately following infusion. The figures show % PPI (see 2.1.1.) during trials in which a loud startle pulse [52 dB(A) above background, 30 ms] was presented 80 ms after the offset of a weak prepulse (20 ms) of one of four different intensities (left), as well as startle amplitude during 16 trials in which the animals were presented with the startle pulse alone (right). In addition to the test session whose data are presented all rats were subjected to a session to measure their baseline startle reactivity and PPI one day prior to the infusion. The three groups were matched according to these baseline values. Measurement of startle and PPI was conducted in an identical way as described in Bast et al. (2000, 2001c,e, in press a) and Zhang et al. (1999; 2000, 2002a,b). ANOVA of PPI and startle data revealed only a significant effect of prepulse intensities ($F_{3,45} = 39.165, P < 0.0001$), reflecting an increase in PPI with increasing prepulse intensity, or of pulse alone trials ($F_{15,495} = 5.129, P < 0.0001$), reflecting habituation of the startle reflex (see Koch, 1999); there were no significant main effects or interactions involving the infusion group (all $P > 0.2$).
field (Fig. 3), which is in marked contrast to the pronounced decrease in locomotor activity following pharmacological deactivation of the ventral hippocampus (Bast et al., 2001d). The effects of TTX and muscimol are somewhat at odds with the reduced locomotor activity in a holeboard box during a 40-min continuous infusion of lidocaine into the dorsal hippocampus (Flicker and Geyer, 1982c). However, the locomotor activity in the holeboard box depended on arousal induced by novel objects placed into the holes before the test session. Thus, the fact that hippocampal lidocaine infusion markedly reduced the response to novel objects (Flicker and Geyer, 1982c) may explain the decreased locomotor activity in the holeboard box.

While the locomotor effects of ventral hippocampal drug microinfusions clearly indicate that ventral hippocampal activity directly drives locomotion (4.1.1.), a direct and linear relation between dorsal hippocampal activity and locomotor activity cannot account for the reported locomotor effects of pharmacological manipulations of the dorsal hippocampus. Thus, both pharmacological deactivation and stimulation of the dorsal hippocampus have been found to increase locomotor activity. Importantly, the patterns of hyperactivity resulting from different pharmacological manipulations of the dorsal hippocampus are different. Activity in rats that received dorsal hippocampal infusion of TTX, muscimol (Fig. 3), or MK-801 (Zhang et al., 2000) is comparable to control rats within the first few minutes following infusion, but then does not decrease to the same extent, resulting in hyperactivity during later periods. A similar pattern of hyperactivity was also found during continuous 40-min infusions of carbachol into the dorsal hippocampus (Flicker and Geyer, 1982b), while another study only reported that average activity was increased during 20 min following dorsal hippocampal carbachol infusion (Mogenson and Nielsen, 1984). The described pattern of hyperactivity may reflect at least partly a deficit in habituation to the environment (Anagnostaras et al., 1999; 2.6.), consistent with the detrimental effects of dorsal hippocampal pharmacological deactivation and NMDA-receptor blockade on spatial and contextual memory (Morris et al., 1989; Young et al., 1994; Fenton et al., 1995; Packard and McGaugh, 1996; Moser and Moser, 1998a; Steele and Morris, 1999; Lee and Kesner, 2002; Bast et al., in press b). In contrast, the hyperactivity induced by dorsal hippocampal stimulation with picrotoxin is most pronounced within the period immediately following infusion (Fig. 2A), most consistent with the view that the dorsal hippocampal activation induced by this manipulation stimulates locomotor activity directly. Dorsal hippocampal NMDA stimulation may not affect locomotor activity, because it may not induce the appropriate pattern of dorsal hippocampal activity to stimulate locomotion directly, and, even though disrupting auditory fear conditioning (Bast et
Fig. 3 Effects of TTX or muscimol infusion into the dorsal hippocampus on locomotor activity in Wistar rats (Zhang, Bast, Feldon, previously unpublished results). There were three infusion groups to receive one of the following bilateral dorsal hippocampal infusions: vehicle (0.5 μl saline/side; VEH), 1 μg muscimol/side (MUS), or 10 ng TTX/side (TTX). Procedures for surgery, infusion, as well as husbandry and general handling of the animals were the same as described in Zhang et al. (2000, 2002a,b) and Bast et al. (in press b). All values are given as mean, variation is indicated by 1 standard error of the differences of the means (1 SE). The figure presents activity as total distance moved in cm for three 10-min blocks preceding infusion (left) and six 10-min blocks following infusion (middle), as well as during three 10-min blocks one day after infusion (right). The test sessions preceding and following infusion on the day of infusion are 20 min apart. The TTX group, as well as half of the VEH group, were infused immediately after the pre-infusion session, i.e. 20 min before the post-infusion session; the MUS group and the other half of the VEH group were infused 20 min after the pre-infusion session, i.e. immediately before the post-infusion session. The time points for the infusions were based on the literature and previous experiments (see Bast et al., 2001d; Zhang et al., 2002b). In addition to the test sessions whose results are presented all rats were subjected to a 30-min session to test baseline activity one day prior to infusion. The three groups were matched according to this baseline activity. Activity was measured in dimly lit open-square arenas by a Noldus tracking system as described in Bast et al. (2001c-e) and Zhang et al. (2000, 2001, 2002a). While all groups' activity was very similar during the period preceeding infusion, as well as on the day after infusion, dorsal hippocampal deactivation by muscimol or TTX induced a slight hyperactivity. ANOVA did not indicate a main effect of infusion group ($F_{2,37} = 1.450, P > 0.2$), but a significant interaction of infusion group x 10-min block ($F_{10,185} = 1.895, P < 0.05$) for the six 10-min blocks immediately following infusion. The interaction reflected that activity of all three groups was similar during the first two 10-min test blocks after infusion, but that afterwards MUS and TTX groups exhibited slightly increased locomotor activity as compared to the VEH group, indicating a habituation deficit (see 4.1.2.).
al., in press b), it may not interfere with dorsal hippocampal mechanisms of spatial and contextual memory essential for habituation to an environment. Thus, altogether, the locomotor effects of dorsal hippocampal drug infusions may reflect the contribution of dorsal hippocampal mechanisms to spatial and contextual memory, as well as the fact that a particular kind of dorsal hippocampal activation can stimulate locomotor activity directly.

4.2. PPI

4.2.1. Ventral hippocampus

The alterations of PPI induced by ventral hippocampal drug infusions demonstrate that the ventral hippocampus is important for normal PPI. Pharmacological stimulation of the ventral hippocampus – by local microinfusion of NMDA (Wan et al., 1996; Klarner et al., 1998; Koch et al., 1999; Zhang et al., 1999, 2002a; Bast et al., 2001e; Swerdlow et al., 2001b), carbachol (Caine et al., 1992), or picrotoxin (Bast et al., 2001c) – has consistently been found to reduce PPI. These effects may reflect interference with extrahippocampal processes due to stimulation of ventral hippocampal efferents. However, a reduction of PPI results also from pharmacological deactivation of the ventral hippocampus by TTX or muscimol (Zhang et al., 2002b). This reduction is less pronounced than that resulting from ventral hippocampal stimulation, but it clearly indicates that neuronal mechanisms within the ventral hippocampus are required to maintain normal PPI.

While systemic administration of NMDA-receptor antagonists markedly disrupts PPI, PPI is unaffected by ventral hippocampal MK-801 infusion (Bast et al., 2000; Bakshi and Geyer, 1998). Thus, the NMDA receptor-mediated mechanisms required for normal PPI are not primarily located in the ventral hippocampus. Ventral hippocampal mechanisms may, however, essentially contribute to the PPI disruption induced by systemic administration of dopamine agonists, given that infusion of the dopamine-receptor antagonist haloperidol into the ventral subiculum partly antagonized the reduction of PPI by systemic administration of the dopamine-receptor agonist apomorphine (Hart et al., 1998). Thus, ventral hippocampal dopamine transmission may exert a negative control over PPI, even though it remains to be examined whether ventral hippocampal infusion of dopamine-receptor agonists can reduce PPI.

4.2.2. Dorsal hippocampus

PPI appears to be much less susceptible to dorsal than to ventral hippocampal stimulation. Thus, dorsal hippocampal infusion of NMDA (Swerdlow et al., 2001b; Zhang et al., 2002) or
picrotoxin (Fig. 2B), at doses which resulted in pronounced PPI disruption when infused into the ventral hippocampus, did virtually not affect PPI. Dorsal hippocampal stimulation by carbachol, however, has been reported to reduce PPI, even though it was not demonstrated that this effect was independent of the concomitant reduction of the startle reaction (Caine et al., 1991, 1992; see 4.4.2.). While PPI is more susceptible to ventral than to dorsal hippocampal stimulation, it is similarly disrupted following dorsal and ventral hippocampal deactivation by local infusion of TTX or muscimol (Zhang et al., 2002b). Moreover, findings of one group indicated that, in Sprague-Dawley rats, dorsal, in contrast to ventral, hippocampal MK-801 infusion slightly reduced PPI to about 80-90% of the control values (Bakshi and Geyer, 1998, 1999). Given that in parallel the startle amplitude was increased to nearly 200% of the control values, this rather marginal reduction of PPI may, however, have reflected a ceiling effect rather than a genuine disruption of sensorimotor gating (see discussion by Bakshi and Geyer, 1998). In Wistar rats, neither dorsal (Zhang et al., 2000) nor ventral (Bast et al., 2000) hippocampal MK-801 infusions reduced PPI, suggesting that NMDA receptor-mediated mechanisms in both ventral and dorsal hippocampus are not essential for normal PPI. Finally, experiments examining PPI following dorsal hippocampal infusion of dopaminergic drugs suggested that stimulation of both D1- and D2-class dopamine receptors in the dorsal hippocampus reduced PPI (Ellenbroeck et al., 2002). Thus, as has indirectly been indicated also for the ventral hippocampus (Hart et al., 1998; 4.2.1.), dopamine transmission in the dorsal hippocampus appears to exert a negative control over PPI.

4.3. Startle: Ventral and dorsal hippocampus

The studies examining the effects of hippocampal drug microinfusions on PPI (4.2.) also yielded that several pharmacological manipulations of both ventral and dorsal hippocampus influence startle reactivity, with the effects of ventral and dorsal hippocampal drug infusion being quite similar. Dorsal hippocampal MK-801 infusion is the only pharmacological hippocampal manipulation found so far to increase startle reactivity (Bakshi and Geyer, 1998, 1999; Zhang et al., 2000). Any habituation deficit cannot account for this increase. The increase was evident from the first until the last presentation of the startle pulse alone (altogether 16 presentations), ruling out a deficit in the habituation to the environment (2.6.), and occurred in parallel with an intact habituation of the startle response (Zhang et al., 2000). Decreases of the startle magnitude have been found after pharmacological stimulation of the ventral and dorsal hippocampus by NMDA (Wan et al., 1996; Klarner et al., 1998; Zhang et
al., 1999, 2002a; Bast et al., 2001e) or carbachol (Caine et al., 1991, 1992), and of the ventral hippocampus by picrotoxin (Bast et al., 2001c), as well as after pharmacological deactivation of the ventral hippocampus by TTX (Zhang et al., 2002b). Some of these hippocampal manipulations have not yielded a significant reduction in startle amplitude in each experiment. For example, TTX infusion into the ventral hippocampus (Zhang et al., 2002b), as well as ventral hippocampal NMDA stimulation (Wan et al., 1996; Klarner et al., 1998; Koch et al., 1999; Zhang et al., 1999, 2002a; Bast et al., 2001e) have been found to decrease the startle amplitude or to have no significant effect on it, even in different experiments within the same studies. These variances may be related to the considerable interindividual variations of the startle amplitude, as well as to its strong plasticity (e.g., habituation, fear potentiation, pleasure attenuation; Koch, 1999). Moreover, the main focus of the reviewed studies was PPI and, therefore, parameters were not deliberately adjusted in order to obtain reliable treatment effects on startle amplitude, for example by avoiding floor or ceiling effects. Thus, caution is expedient before definitely excluding an influence on startle reactivity for those pharmacological hippocampal manipulations, which have hitherto only been found not to alter startle reactivity significantly. These manipulations include ventral hippocampal infusion of muscimol (Zhang et al., 2002b), haloperidol (Hart et al., 1998) or MK-801 (Bakshi and Geyer, 1998; Bast et al., 2000), dorsal hippocampal infusion of muscimol, TTX (Zhang et al., 2002b), amphetamine, specific D1- and D2-class-receptor agonists (Ellenbroeck et al., 2002), and picrotoxin (Fig. 2B). Indeed, for some of these infusions strong tendencies have been found to affect startle reactivity (Bakshi and Geyer, 1998; Zhang et al., 2002b). Finally, none of the reviewed studies reported an impairment of startle habituation following any hippocampal drug microinfusion.

4.4. Dissociations

4.4.1. Stimulation and deactivation

The locomotor effects of pharmacological stimulation and deactivation of the ventral hippocampus could be clearly dissociated, with stimulation increasing and deactivation decreasing locomotor activity (4.1.1.). This indicates that the increased locomotor activity resulting from ventral hippocampal stimulation actually reflects increased efferent transmission from the ventral hippocampus and not disruption of ordered transmission within the hippocampus due to excess stimulation. Otherwise, the sensorimotor effects of stimulation and deactivation of the ventral or dorsal hippocampus were often similar (4.1.-4.3.). In these cases, it is possible that not increased excitation of hippocampal efferents accounts for the
effects of the stimulation, but that the effects of both stimulation and deactivation may have reflected a disruption of normal hippocampal function (e.g., Berke and Eichenbaum, 2001; Bast et al., in press b; 3.1.).

4.4.2. PPI and startle reflex

While PPI is used as a measure of sensorimotor gating, it has been recognized that experimental manipulations may decrease PPI by merely inducing changes in startle reactivity that result in floor or ceiling effects (see Swerdlow et al., 2000a for a detailed discussion). This possibility can be excluded when the manipulations’ effects on the startle reaction to a startle pulse alone and on the reaction to a startle pulse preceded by a weaker prepulse can be dissociated. Except for hippocampal carbachol stimulation (Caine et al., 1991, 1992), such a dissociation has been demonstrated for all hippocampal pharmacological manipulations the effects of which on PPI and startle have been examined (4.2.-4.3., Table 1). This indicates that these manipulations induce a genuine disruption of sensorimotor gating, even though the possibility exists that a given manipulation’s effect on startle reactivity does not reach statistical significance, but nevertheless contributes to significant changes in PPI. However, as has been discussed (2.1.2.), a sensorimotor-gating deficit may, theoretically, result in decreased startle reactivity, and this may account for the situation that reduced PPI sometimes cannot clearly be dissociated from reduced startle reactivity, as it is the case for hippocampal carbachol stimulation.

4.4.3. PPI and locomotor activity

Modulation of dopamine activity in the nucleus accumbens has been proposed as a mechanism by which the hippocampus might control locomotor activity and PPI, and aberrations of this modulation may result in psychosis-related alterations of PPI and locomotor activity in rats, as well as in psychotic symptoms in humans (2.1.1., 2.4.). However, the different effects of hippocampal drug infusions on locomotor activity and PPI partly dissociate the hippocampal mechanisms regulating locomotor activity and PPI (4.1., 4.2., Table 1), clearly demonstrating that one single mechanisms cannot account for the hippocampal control of these sensorimotor functions. First, the effect of a hippocampal manipulation on locomotor activity does not predict its effect on PPI. For example, activity is decreased by pharmacological deactivation of the ventral hippocampus and increased by ventral hippocampal stimulation, but both manipulations disrupt PPI. Second, while normal locomotor activity can be increased and decreased by pharmacological manipulations of the
ventral hippocampus, it appears that normal PPI can only be reduced, but not enhanced, by alterations in hippocampal neurotransmission. Finally, as will be described below (5.2.), dopaminergic mechanisms seem to be of different importance for the hippocampal modulation of locomotor activity and PPI.

4.4.4. Ventral and dorsal hippocampus

The reviewed microinfusion studies partly dissociated ventral and dorsal hippocampal mechanisms relevant for locomotor activity or PPI, while startle reactivity appears to be affected rather similarly by ventral and dorsal hippocampal drug infusions (4.1.-4.3., Table 1). Overall, the different alterations of locomotor activity and PPI resulting from ventral as compared to dorsal hippocampal drug infusions suggest that ventral hippocampal mechanisms are more closely and more directly related to these two sensorimotor processes than dorsal hippocampal mechanisms. This functional difference between ventral and dorsal hippocampus is consistent with the neuroanatomical ventral-dorsal differentiation of the hippocampus with respect to connections with extrahippocampal structures implicated in the regulation of locomotor activity and PPI (5.1.).

4.5. Comparison with the effects of permanent hippocampal lesions

Complete and partial hippocampal lesions have been found to affect the regulation of locomotor activity, PPI, and startle reflex (2.3.). Thus, the results of lesion and microinfusion studies are consistent in suggesting that the hippocampus plays a role in the regulation of these processes. The microinfusion studies, however, yielded a more detailed picture of this role and suggest modifications of some previous notions based on lesion studies.

First, the hyperactivity following ventral, dorsal, and complete hippocampal lesions would be consistent with the notion that hippocampal activity is important to inhibit locomotor activity and that ventral and dorsal hippocampus are equally involved in the regulation of locomotor activity (2.3., 2.5.). In contrast, the effects of hippocampal drug infusions clearly suggest that locomotion is driven by ventral hippocampal activity (4.1.1.), while dorsal hippocampal activity is less directly related to the regulation of locomotion, but may also rather have a facilitating influence on locomotor activity (4.1.2.). Thus, the hyperactivity following ventral and complete hippocampal lesions may reflect lesion-induced secondary changes or compensatory adjustments in locomotor circuits evoked by the permanent loss of locomotor drive normally provided by the ventral hippocampus (3.1.1.). In addition, it has been demonstrated that a habituation deficit, as discussed above as possible reason for the
1.7.

hyperactivity following dorsal hippocampal TTX, muscimol, carbachol, and MK-801 infusion (4.1.2.), may contribute to the hyperactivity after dorsal hippocampal lesions (Anagnostaras et al, 1999).

Second, even though pointing at interactions between the ventral, but not dorsal, hippocampus and circuits mediating or regulating PPI, lesion studies failed to provide clear evidence that hippocampal activity is critical for normal PPI (2.3.2.). In contrast, the PPI disruption induced by pharmacological deactivation of the ventral or dorsal hippocampus (Zhang et al., 2002b) clearly demonstrates that activity of both ventral and dorsal hippocampus is important to maintain normal PPI. The failure of permanent hippocampal lesions to clearly reveal this may be related to compensatory processes. Interestingly, the alterations in the regulation of PPI induced by either pharmacological stimulation (4.2.) or permanent lesions of the ventral or dorsal hippocampus (2.3.2.) revealed that the ventral hippocampus is more closely related to the regulation of PPI than the dorsal hippocampus. The effects of the lesions may be due to anterograde damage (Halim and Swerdlow, 2000) and those of the stimulation may reflect excitation of hippocampal projections. Thus, the effects of both lesions and stimulation may be mediated via hippocampal efferents and reflect the differential projection of ventral and dorsal hippocampus to structures involved in the regulation of PPI (see 5.1.).

Third, while partial or complete hippocampal lesions have been found either not to affect or to increase startle reactivity (2.3.3.), dorsal hippocampal MK-801 infusion is the only pharmacological hippocampal manipulation reported to significantly increase the startle magnitude, while most pharmacological hippocampal manipulations, including both stimulation and deactivation, rather decrease startle reactivity (4.3.). Again, the difference between the effects of pharmacological deactivation and permanent lesions indicates that secondary changes or compensatory mechanisms may contribute to the lesion effects.

5. Pathways and extrahippocampal mechanisms mediating the sensorimotor effects of hippocampal drug infusions

5.1. Relevant anatomical connections: ventral-dorsal differentiation

Hippocampal projections to the amygdala, nucleus accumbens shell and core, and prefrontal cortex may mediate the hippocampal modulation of locomotor activity, PPI, and startle reactivity. All these structures have direct or indirect access to the brain stem centers mediating locomotion, PPI, and startle reactivity, and direct manipulations of these structures have been demonstrated to result in alterations of these sensorimotor processes (Holstege,
The picture emerging from the microinfusion (4.4.4.) and partly also the lesion studies (2.3.2., 4.5) that the regulation of locomotor activity and PPI is more dependent on ventral than on dorsal hippocampal mechanisms mirrors the ventral-dorsal differentiation of hippocampal projections to amygdala, nucleus accumbens, and prefrontal cortex. Thus, the ventral hippocampus projects directly to amygdala, prefrontal cortex, and to the nucleus accumbens, predominantly to its shell, while efferents to the nucleus accumbens, predominantly its core, are the only direct connection of the dorsal hippocampus with these structures (Siegel and Tassoni, 1971; Swanson and Cowan, 1977; Kelly and Domesick, 1982; Witter, 1986; Groenwegen et al., 1987; Jay and Witter, 1991; Jay et al., 1992; Amaral and Witter, 1995; Carr and Sesack, 1996; Verwer et al., 1997; Pitkänen et al., 2000). Moreover, the projection targets of the ventral hippocampus project to midbrain dopamine neurons (see Lisman and Otmakhova, 2001, p. 564, for review), while the nucleus accumbens core does not (Usada et al., 1998). Thus, the ventral hippocampus may transsynaptically activate forebrain dopamine transmission, which is likely to be of great functional relevance (5.2.). However, there are intrahippocampal projections connecting dorsal and ventral hippocampus (Amaral and Witter, 1989). These intrahippocampal connections may account for the differences between the ventral and dorsal hippocampus in the modulation of locomotor activity and PPI being rather gradual than absolute, as well as for similar effects of ventral and dorsal hippocampal manipulations on startle reactivity. Pharmacological manipulations of the dorsal hippocampus may alter ventral hippocampal activity via intrahippocampal connections and this may underly many of these manipulations’ effects on locomotor activity, PPI, and startle (4.1-4.3.).

Much research is still needed in order to characterize the neurophysiological and neurochemical alterations in hippocampal projection areas induced by the different pharmacological manipulations of the hippocampus, as well as to clarify the possible involvement of these alterations in the sensorimotor effects resulting from the respective hippocampal drug infusions (9.). Nevertheless, the possible contribution of some extrahippocampal mechanisms to the hippocampal modulation of locomotor activity and PPI has already been examined directly. The notions that have emerged from this research will be presented in the following (5.2.-5.3.).
5.2. Dopamine mechanisms: dissociation between the hippocampal modulation of PPI and locomotor activity

The modulation of locomotor activity and PPI by alterations in dopamine transmission is well documented. Dopaminergic stimulation results in hyperactivity and reduced PPI, while decreased dopamine transmission reduces locomotor activity and may enhance PPI. Moreover, it has been demonstrated that mechanisms in the nucleus accumbens partly mediate these effects (Beninger, 1983; LeMoal and Simon, 1991; Koch, 1999; Geyer et al., 2001; Swerdlow et al., 1990, 2001a). Because of the established role of dopamine in the regulation of locomotion and PPI, the strong hippocampal projections to the nucleus accumbens (Kelley and Domesick, 1982; Groenewegen et al., 1987), and the relevance of dopaminergic mechanisms with respect to schizophrenia (Gray et al., 1991; Grace, 2000; Seeman and Kapur, 2000; Carlsson et al., 2001), the effects of dopamine-receptor blockade by antipsychotics on hyperactivity and PPI disruption resulting from hippocampal drug infusions have been studied intensively. The results of these experiments unequivocally dissociated the roles of dopamine-receptor mediated processes in the hippocampal modulation of locomotion and PPI. The disruption of PPI resulting from ventral hippocampal NMDA (Wan et al., 1996; Bast et al., 2001e) or dorsal hippocampal carbachol stimulation (Caine et al., 1991), as well as from ventral or dorsal hippocampal deactivation (Zhang et al., 2002b), was unaffected by dopamine-receptor blockade with the typical antipsychotics haloperidol or spiperone or the atypical antipsychotic clozapine. In contrast, the hyperactivity resulting from ventral hippocampal NMDA stimulation can be blocked by the typical antipsychotic haloperidol, the atypical antipsychotic clozapine, or the specific D1-class receptor antagonist SCH-23390 (Bardgett and Henry, 1999; Bast et al., 2001e); haloperidol has also been demonstrated to block the hyperlocomotion induced by ventral hippocampal carbachol stimulation (Brenner and Bardgett, 1998). Furthermore, destruction of dopaminergic forebrain projections from the ventral tegmental area prevented ventral hippocampal NMDA stimulation from increasing locomotor activity (Wu and Brudzynski, 1995). These, data suggest that dopamine transmission is critical for the hippocampal modulation of locomotor activity, but not PPI. The finding that hyperactivity resulting from electrical stimulation of the ventral hippocampus could be completely blocked by the D1-class receptor antagonist SCH-23390, but was only slightly attenuated by the specific D2-class receptor antagonist sulpiride, suggests that D1-class receptor-mediated mechanisms may be particularly relevant for the hippocampal modulation of locomotion (Taepavaranuk et al., 2000).
It is noteworthy that, in contrast to the now widely accepted notion that dopamine supports the hyperactivity induced by ventral hippocampal stimulation, early work by Yang and Mogenson (1987) demonstrated that infusion of the dopamine D2-class receptor agonist quinpirole into the nucleus accumbens reduced hyperactivity induced by ventral hippocampal NMDA stimulation. In light of evidence that specific D3-receptor stimulation in the nucleus accumbens inhibits locomotor activity (Ouagazzal and Creese, 2000), this effect appears to reflect the high affinity of quinpirole to D3 receptors (Sokoloff et al., 1991).

5.2.1. Dopamine in the nucleus accumbens

With respect to the dopamine mechanisms relevant for the hyperactivity induced by ventral hippocampal stimulation the nucleus accumbens received a lot of attention. Numerous independent studies demonstrated that stimulation of the ventral hippocampus – induced pharmacologically, by NMDA or the GABA_A-receptor antagonist bicuculline, or electrically – increases accumbal dopamine release in awake or anesthetized rats (Blaha et al., 1997; Brudzynski and Gibson, 1997; Legault and Wise, 1999; Legault et al., 2000; Mitchell et al., 2000; Taevarapruk et al., 2000). There is evidence that the increased accumbal dopamine release may be mediated by direct interactions of ventral hippocampal and dopaminergic terminals, which are in direct apposition to one another in the nucleus accumbens (Sesack and Pickel, 1990; Blaha et al., 1997; Taevarapruk et al., 2000), as well as by transsynaptic activation of dopamine neurons in the ventral tegmentum (Legault et al., 2000; Floresco et al., 2001). Given that locomotor activity can be increased and decreased by direct stimulation (Ikemoto, 2002) or inhibition (Ahlenius et al., 1987), respectively, of dopamine transmission in the nucleus accumbens, the positive modulation of accumbal dopamine transmission by activity of the ventral hippocampus may account for the hippocampal modulation of locomotor activity.

5.2.1. Dopamine in the medial prefrontal cortex

The fact that ventral hippocampal stimulation activates dopamine neurons in the ventral tegmentum (Legault et al., 2000; Floresco et al., 2001) suggests that ventral hippocampal activity positively modulates dopamine transmission not only in the nucleus accumbens, but in all forebrain sites receiving dopaminergic projections from the ventral tegmentum. Indeed, a microdialysis study found increased dopamine release in the medial prefrontal cortex after electrical stimulation of the ventral hippocampus (Gurden et al., 2000), and post mortem studies yielded evidence that dopamine activity in the medial prefrontal cortex is increased by
ventral hippocampal NMDA stimulation (Bast, Zhang, Ferger, Feldon, submitted) and decreased 28 days after ventral hippocampal lesions (Lipska et al., 1992). Furthermore, the activation of dopamine transmission in the medial prefrontal cortex by ventral hippocampal NMDA stimulation is confirmed by preliminary results of microdialysis experiments conducted in our laboratory (Bast, Pezze, Peleg, Ferger, Feldon, unpublished observations). In addition to transynaptic activation of the ventral tegmentum, the facilitating effects of ventral hippocampal activity on dopamine activity in the medial prefrontal cortex may involve direct interactions of ventral hippocampal and dopamine terminals, which are in direct apposition to one another in the medial prefrontal cortex (Carr and Sesack, 1996). Interestingly, there is evidence that prefrontal dopamine, similar to accumbal dopamine, may facilitate locomotor activity (Beninger et al., 1990; Pezze et al., 2001; Bast et al., in press a). Thus, a facilitation of dopamine activity in the medial prefrontal cortex by ventral hippocampal activity may also play an important role in the drive of locomotor activity by the ventral hippocampus.

5.2.1. Dopamine in the hippocampus

The hippocampus receives dopaminergic projections from the ventral tegmentum (Gasbarri et al., 1997). Stimulation of the ventral hippocampus, which activates dopamine neurons in the ventral tegmentum (Legault et al., 2000; Floresco et al., 2001), may therefore result in increased dopamine transmission within the hippocampus itself. Dopaminergic stimulation of the dorsal hippocampus has been suggested to increases locomotor activity (Smialowski and Maj, 1985) and the induction of hyperactivity by ventral hippocampal NMDA stimulation could be blocked by simultaneous ventral hippocampal infusion of dopamine-receptor antagonists (Giménez-Llort et al., 2002). The latter may reflect removal of a tonic facilitating effect of dopamine transmission on NMDA receptor-mediated processes in the ventral hippocampus (Yang, 2000). Nevertheless, the existing evidence is also consistent with the possibility that the positive modulation of locomotion by ventral hippocampal activity may depend on transsynaptic activation of dopamine transmission from the ventral tegmentum to the hippocampus.

5.3. Glutamate mechanisms in the nucleus accumbens: a further dissociation between the hippocampal modulation of PPI and locomotor activity

Hippocampal projections appear to be mainly glutamatergic (Walaas and Fonnum, 1980; Ottersen and Storm-Mathisen, 1985; Jay et al., 1992), suggesting that the primary effects of hippocampal drug infusions are mediated by alterations in glutamate release and, thus, in...
glutamate-receptor stimulation, in the hippocampal projection targets. For example, hippocampal stimulation may increase glutamate release in hippocampal projection targets. The possible contribution of increased stimulation of ionotropic glutamate receptors in the nucleus accumbens to the reduction of PPI and the hyperactivity resulting from NMDA or electrical stimulation of the ventral hippocampus has been examined. Similar to ventral hippocampal NMDA stimulation (Wan et al., 1996; Bast et al., 2001e), stimulation of ionotropic glutamate receptors in the nucleus accumbens shell by AMPA reduced PPI in a dopamine-independent way (Wan and Swerdlow, 1996). However, stimulation of ionotropic glutamate receptors in the nucleus accumbens shell does not appear to play a major role in the disruption of PPI by ventral hippocampal NMDA, since a further investigation found this disruption not affected by infusion of CNQX, an antagonist of ionotropic glutamate receptors, into the nucleus accumbens shell or core (Wan et al., 1996). In contrast, blockade of ionotropic glutamate receptors in the nucleus accumbens by DNQX antagonized the hyperactivity induced by electrical stimulation of the ventral hippocampus (Taepavarapruk et al., 2000), suggesting an additional dissociation between the substrates involved in the hippocampal modulation of PPI and locomotor activity.

6. Theoretical implications

6.1. Hippocampal modulation of sensorimotor processes

The reviewed sensorimotor effects of hippocampal drug microinfusions (4., Table 1) corroborate that the hippocampus is involved in the regulation of sensorimotor processes. Even though particular forms of hyperactivity induced by some dorsal hippocampal drug infusions may partially reflect mnemonic effects (4.1.2.), it is clear that hippocampal mechanisms can directly influence locomotor activity, PPI, and startle reactivity.

The hippocampus does not appear to regulate these sensorimotor processes in a unitary fashion. First, different sensorimotor processes differ in their relation to hippocampal neurotransmission (4.4.2., 4.4.3.). Alterations in hippocampal neurotransmission can decrease and increase locomotor activity (4.1.). The level of ventral hippocampal activity appears to be linearly related to locomotor activity, suggesting that the ventral hippocampus may function as a kind of switch turning locomotion on and off. Some modes of dorsal hippocampal activation appear also to increase locomotion directly. In contrast, PPI can only be reduced by alterations in hippocampal neurotransmission, including pharmacological deactivation and stimulation, suggesting that normal hippocampal activity is important to maintain normal PPI (4.2.). It is conceivable, for example, that all these pharmacological manipulations interfere
with the hippocampal theta-modulation which has been implicated in mechanisms protecting stimulus processing (Vinogradova, 2001). The relation between hippocampal neurotransmission and startle reactivity is most difficult to characterize, given that most alterations of hippocampal neurotransmission, including both pharmacological stimulation and deactivation, decrease startle reactivity, while a specific blockade of dorsal hippocampal NMDA receptor-mediated processes increased startle reactivity (4.3.). Second, ventral hippocampal mechanisms appear to be more relevant than dorsal hippocampal processes for the regulation of locomotor activity and PPI, while no clear differences between ventral and dorsal hippocampus have been indicated concerning the regulation of startle reactivity (4.4.4.). The differences between ventral and dorsal hippocampus may mainly reflect the ventral-dorsal differentiation of hippocampal projections to amygdala, nucleus accumbens, and prefrontal cortex (5.1.). Third, even though the pathways and extrahippocampal mechanisms mediating the hippocampal modulation of locomotion, PPI, and startle reactivity largely remain to be delineated (9.), existing data already demonstrate dissociations between the hippocampal modulation of PPI and locomotion. Dopaminergic mechanisms (5.2.), as well as the direct glutamatergic projection from the ventral hippocampus to the nucleus accumbens (5.3.), have been demonstrated to be critical for the hippocampal modulation of locomotor activity, but not PPI.

6.2. Integrating hippocampal functions

It has been recognized that the hippocampus does not have one unitary function (Schmajuk, 1984). Rather, it appears that in the hippocampus several functions are integrated and this may particularly enable this structure to play a central role in the organization of behavior. While the hippocampus processes sensory input in order to create memory, probably making some unique contributions to spatial and other forms of so-called relational memory (O'Keefe and Nadel, 1979; Morris and Frey, 1997; Eichenbaum, 2000; Aggleton and Pearce, 2001), this structure can also directly modulate the translation of sensory input into motor responses. This integration of mnemonic and sensorimotor functions within the hippocampus may serve to set memory or experience into action. For example, normal exploratory behavior requires the interaction of processes underlying spatial memory with processes regulating locomotion. The anatomical substrates for this integration of function within the hippocampus is the highly processed input provided by the sensory association cortices predominantly to the dorsal hippocampus (Moser and Moser, 1998b) and the strong projections to structures with access to brain stem centers mainly originating from the ventral
hippocampus, as well as strong intrahippocampal projections between dorsal and ventral hippocampus (2.4., 5.1.). Consistent with this anatomical arrangement spatial learning appears to be more closely linked to the dorsal hippocampus (Moser et al., 1993; Moser and Moser, 1998a, b; Bannerman et al., 1999; Richmond et al., 1999; Ferbinteanu and McDonald, 2000; Kjelstrup et al., 2002), while the role of the ventral hippocampus appears to be dominant as to the influence on sensorimotor process (4.4.4., 6.1.). As suggested by the strong intrahippocampal connections (Amaral and Witter, 1995) and as required for the integration of functions, this functional differentiation, however, is not absolute (Moser and Moser, 1998a; Ferbinteanu et al., 2000; 4., Table 1).

7. Methodological implications

The findings of studies using temporary pharmacological hippocampal manipulations suggested substantial complementations and modifications of notions concerning the relation of hippocampal activity and sensorimotor processes that were based mainly on lesion studies (4.5.). This reflects partly the higher number of possibilities pharmacological manipulations offer for the alteration of hippocampal activity. Moreover, as has recently also been recognized in the context of research concerned with the role of the hippocampus in classical fear conditioning (Anagnostaras et al., 2001, 2002; Bast et al. 2001b; Corcoran and Maren, 2001), problems like secondary changes and recovery of function may confound the interpretation of lesion studies (3.1.1., 4.4.4.). Thus, intracerebral drug microinfusions should be increasingly used in order to further our understanding of structure-function relationships.

8. Clinical implications

8.1. Anxiety disorders

The hippocampus has been associated with the protective and defensive reactions to threatening and aversive stimuli and hyperfunction of some hippocampal processes has been proposed to contribute to anxiety disorders (2.1.). Most pharmacological manipulations of the hippocampus, including stimulation and deactivation, decreased the startle reaction (4.3.), a protective response to a loud noise (2.1.2.). While not directly confirming that hyperfunction of hippocampal processes leads to anxiety-related exaggerations of protective responses (Gray et al., 1982, 1995; Crestani et al., 1999; Löw et al., 2000), these effects are consistent with the suggestion that the hippocampus supports such protective responses (Gray et al., 1982, 1995; File, 2000; Deacon et al., 2002; Kjelstrup et al., 2002). The similar effects of pharmacological stimulation and deactivation of the hippocampus may have reflected that both manipulations
have interfered with the relevant hippocampal mechanisms (3.2., 4.4.1). The most specific hypothesis concerning the substrate of anxiety links anxiety to decreased GABA_A receptor-mediated inhibition in the hippocampus (Crestani et al., 1999; Löw et al., 2000). The markedly decreased startle reactivity following ventral hippocampal infusion of the GABA_A receptor antagonist picrotoxin (Bast et al., 2001c) fails to support this concept. This may, however, reflect that picrotoxin infusion did not induce a sufficiently specific disinhibition of the relevant processes, but may rather have interfered with them by inducing excess ventral hippocampal activity (3.2., 4.4.1.). Interestingly, dorsal hippocampal MK-801 infusion, which may decrease local GABA_A receptor-mediated inhibition (Olney et al., 1999), increased startle reactivity markedly (Bakshi and Geyer, 1998; Zhang et al., 2000).

8.2. Schizophrenia

Neuropathological and neuroimaging studies suggest that the hippocampus in schizophrenics may be overactive due to a concurrence of increased afferent excitation and decreased GABAergic inhibition, while in some respects the hippocampus may also be hypofunctional in schizophrenia (2.1.). The psychosis-related alterations of locomotor activity and PPI (2.1.1.) by different pharmacological manipulations of the hippocampus, including direct excitation, disinhibition by picrotoxin, and deactivation by TTX or muscimol (4.1., 4.2.), suggest that several kinds of hippocampal dysfunction may actually contribute to human psychosis. Whether the reductions in startle reactivity resulting from many hippocampal manipulations (4.3.) partly reflect attentional or information-processing deficits related to psychosis (2.1.2.) remains to be clarified (9.). Altogether, the hippocampus may be an important target structure for antipsychotic treatments. Interestingly, dopaminergic stimulation of the dorsal hippocampus resulted in psychosis-related effects, hyperactivity (Smialowsky and Maj, 1985) and reduced PPI (Ellenbroek et al., 2002), and dopamine-receptor blockade in the ventral hippocampus was demonstrated to antagonize dopamine-dependent forms of hyperactivity (Giménez-Llort et al., 2002) and PPI disruption (Hart et al., 1998). This indicates that even the current antidopaminergic treatments of schizophrenia may partly exert their beneficial effects in the hippocampus.

Research further examining the extrahippocampal mechanisms mediating the psychosis-related alterations of locomotor activity and PPI following hippocampal drug infusions (9.) may yield additional targets for new antipsychotic treatments. Even though much remains to be done in this respect, available evidence (5.) already indicates that aberrant hippocampal function may evoke psychotic symptoms by several dissociable mechanisms, partly
independent of increased dopamine transmission in the ventral striatum, the main target of current antipsychotic treatment strategies (Grace, 2000; Seeman and Kapur, 2000; Carlsson et al., 2001). Interestingly, the psychosis-related effects of these manipulations respond differently to treatment with current antipsychotics. While the hyperactivity induced by ventral hippocampal stimulation can be completely blocked by typical and atypical antipsychotics, PPI reductions resulting from hippocampal drug microinfusions, similar to PPI reductions in some groups of schizophrenic patients (Braff et al., 2001; Hamm et al., 2001; Kumari and Sharma, 2002), were resistant against antipsychotic treatment (5.2.). Thus, the mechanisms by which hippocampal drug infusions affect locomotor activity and PPI may be relevant for schizophrenia responding to current treatment, as well as for treatment-resistant psychosis.

The higher susceptibility of PPI and locomotor activity to ventral than to dorsal hippocampal drug microinfusions in rats (4.4.4., 6.1.) indicates that human psychosis may particularly be related to aberrant function of the anterior hippocampus, which corresponds to the rat ventral hippocampus (Witter, 1987). This corroborates the recent suggestion that it may be important to differentiate between the anterior and posterior hippocampus with respect to the role of hippocampal dysfunction in schizophrenia (Heckers and Konradi, 2002). The high-resolution neuroimaging techniques to directly examine this issue have just begun to become available. Some data support a preferential dysfunction of the anterior hippocampus in schizophrenia, but others do not (see Csernansky et al., 1998; Velakoulis et al., 2001; Szeszko et al., 2002; and references therein).

9. Future directions

First, it would certainly be useful to further characterize the behavioral effects of the various pharmacological manipulations of the hippocampus. The effects of pharmacological manipulations of the hippocampus on startle reactivity may be further examined in experiments specifically designed to robustly reveal alterations in startle reactivity. The question whether the changes in startle reactivity following hippocampal drug infusions reflect impaired attention or a generally altered disposition to anxiety-related responses may be clarified using additional behavioral tests designed to assess attentional performance (Robbins et al., 1993) and anxiety (Davis et al., 1997; Rodgers, 1997). In order to further corroborate the relation between aberrant hippocampal function and psychosis one future line of research may test whether the pharmacological hippocampal manipulations resulting in
reduced PPI or hyperactivity yield additional psychosis-related disturbances, such as the
disruption of latent inhibition (Gray et al., 1991; Robbins et al., 1993; Moser et al., 2000).

Second, several experimental approaches should be pursued in order to clarify the
pathways and mechanisms mediating the reviewed sensorimotor effects of pharmacological
hippocampal manipulations (4.), in particular in light of the clinical relevance of this issue
(8.2.). Even though previous studies yielded some concrete notions, the pathways and
mechanisms mediating the hippocampal modulation of locomotor activity still need to be
further characterized, and direct evidence for extrahippocampal substrates mediating the
hippocampal modulation of PPI and startle reflex has not at all emerged from previous
experiments (5.). It will be useful: first, to further characterize the neurochemical and
neurophysiological alterations induced by the different pharmacological hippocampal
manipulations in hippocampal projection sites; second, to examine which alterations in
hippocampal projection sites yield similar sensorimotor effects as the hippocampal
manipulations; third, which systemic treatments or, finally, local manipulations of
hippocampal projections sites antagonize the effects of the hippocampal manipulations.

Acknowledgements The authors’ work relevant for the preparation of this article was
supported by the Swiss Federal Institute of Technology Zurich (ETHZ) and the Swiss
National Science Foundation. The authors thank the staff of the technical services of the
Research Unit Schwerzenbach for their excellent support, in particular Peter Schmid and Liz
Weber, and acknowledge the collaboration of Boris Ferger, Daria Peleg, Marie Pezze, Ilsun
White, and Wei-Ning Zhang in studies relevant for this review. Particular thanks are due to
Jane Fotheringham for help with the preparation of the manuscript and to Marie Pezze for
stimulating and helpful discussions and critically reading the manuscript.
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PART II:

Hippocampus and simple associative or relational memory: The effects of pharmacological manipulations of the ventral and dorsal hippocampus on classical fear conditioning to discrete and contextual cues
The ventral hippocampus and fear conditioning in rats

Different anterograde amnesias of fear after tetrodotoxin inactivation and infusion of the GABA\(_A\) agonist muscimol

Received: 27 October 2000 / Accepted: 8 March 2001 / Published online: 16 May 2001
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Abstract Studies on the involvement of the rat hippocampus in classical fear conditioning have focused mainly on the dorsal hippocampus and conditioning to a context. However, the ventral hippocampus has intimate connections with the amygdala and the nucleus accumbens, which are involved in classical fear conditioning to explicit and contextual cues. Consistently, a few recent lesion studies have indicated a role for the ventral hippocampus in classical fear conditioning to explicit and contextual cues. The present study examined whether neuronal activity within the ventral hippocampus is important for the formation of fear memory to explicit and contextual cues. Tetrodotoxin (TTX; 10 ng/side), which completely blocks neuronal activity, or muscimol (1 \(\mu\)g/side), which increases GABA\(_A\) receptor-mediated inhibition, were bilaterally infused into the ventral hippocampus of Wistar rats before the conditioning session of a classical fear-conditioning experiment. Conditioning to a tone and the context were assessed using freezing as a measure of conditioned fear. TTX blocked fear conditioning to both tone and context. Muscimol only blocked fear conditioning to the context. The data of the present study indicate that activity of neurons in the ventral hippocampus is necessary for the formation of fear memory to both explicit and contextual cues and that neurons in the ventral hippocampus that bear the GABA\(_A\) receptor are important for the formation of fear conditioning to a context. Conditioning to a tone and the context were assessed using freezing as a measure of conditioned fear. TTX blocked fear conditioning to both tone and context. Muscimol only blocked fear conditioning to the context. The data of the present study indicate that activity of neurons in the ventral hippocampus is necessary for the formation of fear memory to both explicit and contextual cues and that neurons in the ventral hippocampus that bear the GABA\(_A\) receptor are important for the formation of fear conditioning to a context.

Keywords Classical fear conditioning • Locomotor activity • Muscimol • Tetrodotoxin • Ventral hippocampus • Rat

Introduction

In rats as well as primates, the hippocampus is widely believed to play an important role in learning and memory, when complex representations, assembled from elemental stimuli and their spatial and/or temporal relations, are involved (Eichenbaum et al. 1999; Jarrard 1993; O'Keefe and Nadel 1978; Rudy and Sutherland 1995; Wallenstein et al. 1998). The hippocampus is thought to be involved in classical conditioning mainly when contextual information is utilized or when the conditioned stimulus (CS) is a contextual one (Fanselow 2000; Holland and Bouton 1999; Jarrard 1993; Maren and Holt 2000).

In classical fear conditioning, a neutral CS such as a tone (explicit CS) or the environment (contextual CS) is paired with an aversive unconditioned stimulus (US), such as an electrical foot shock. After a few pairings, the CS elicits conditioned fear responses, including freezing (Fanselow 1984). There is a consensus that neuronal processing in the amygdala is important for classical fear conditioning to contextual as well as explicit CS. Whereas some consider the amygdala to be the site where the fear memory is formed and stored (Fanselow and LeDoux 1999; Fendt and Fanselow 1999), others hold the view that amygdaloid processes modulate the formation and storage of the memory in other brain regions (Cahill et al. 1999). In addition, the nucleus accumbens, in particular its dopaminergic innervation from the midbrain, seems to be involved in classical aversive conditioning, including fear conditioning (Haralambous and Westbrook 1999; Murphy et al. 2000; Parkinson et al. 1999; Pezze et al. 2000; Riedel et al. 1997; Schwartz and Carey 1985; Westbrook et al. 1997; Wilkinson et al. 1998; Young et al. 1993; A.L. Jongen-Rêlo, S. Kaufmann and J. Feldon, unpublished findings). As to the
hippocampal involvement in classical fear conditioning, studies examining the conditioned freezing response after hippocampal lesions have led to the view that the dorsal hippocampus is specifically involved in fear conditioning to a contextual CS. Consistent with the widely held notions of hippocampus-dependent learning, this is attributed to the dorsal hippocampus being essential for contextual learning (Fanselow 2000; Fendt and Fanselow 1999; Maren et al. 1998). However, it is also suggested that hippocampal lesions might impair the expression of conditioned fear in the form of freezing, rather than contextual memory (Gewirtz et al. 2000; Richmond et al. 1999).

The ventral hippocampus possesses connections linking it with the basic neuronal processes underlying classical fear conditioning. It has intimate reciprocal connections with the amygdala and strong projections to the nucleus accumbens (Groenewegen et al. 1987; Pitkänen et al. 2000; Swanson and Cowan 1977). Moreover, activity of the ventral hippocampus regulates synaptic plasticity in the basolateral amygdala (Maren and Fanselow 1995) and extracellular dopamine levels in the nucleus accumbens (Blaha et al. 1997; Brudzynski and Gibson 1997; Legault and Wise 1999; Legault et al. 2000; Mitchell et al. 2000). Thus, in addition to the involvement of the dorsal hippocampus in classical fear conditioning to contextual CS, classical fear conditioning might depend on activity of the ventral hippocampus.

Indeed, some behavioral studies have suggested that normal function of the ventral hippocampus is necessary for classical aversive conditioning, including fear conditioning. Formation and retrieval of memory in the passive-avoidance paradigm are impaired following temporary inactivation of the ventral hippocampus by tetrodotoxin (TTX; Ambrogi Lorenzini et al. 1997). As for classical fear conditioning, electrolytic or excitotoxic lesions of the ventral hippocampus, prior to or 1 day after conditioning, impair conditioned freezing to both explicit and contextual CS (Maren 1999; Maren and Fanselow 1995; Richmond et al. 1999). Thus, the ventral hippocampus could be important for the formation (acquisition and consolidation), retrieval, or expression of conditioned fear.

The present study examined whether normal activity of the ventral hippocampus is necessary for the formation of classical fear conditioning to explicit and contextual CS. For that purpose, 10 ng TTX/0.5 μl per side or 1 μg muscimol/0.5 μl per side were bilaterally infused into the ventral hippocampus of Wistar rats before the conditioning session of a classical fear-conditioning experiment, with freezing being utilized to assess conditioned fear. TTX, blocking voltage-dependent sodium channels, should temporarily induce the condition existing after electrolytic lesions, i.e., inactivation of the ventral hippocampus as well as fibers of passage (Ambrogi Lorenzini et al. 1999). Muscimol should temporarily inhibit neuronal activity within the ventral hippocampus, because muscimol is an agonist at the GABA_A receptor, the Cl^- channel receptor mediating inhibition of the hippocampal excitatory network by GABA-releasing interneurons (Buhl et al. 1994). Activity of the ventral hippocampus is important for the regulation of locomotor activity (Bardgett and Henry 1999; Bast et al. 2001, 2001; Brudzynski and Gibson 1997; Legault and Wise 1999; Yang and Mogenson 1987). Moreover, observations during the fear-conditioning experiment indicated that infusion of TTX and muscimol into the ventral hippocampus markedly decreased activity of the rats. Therefore, in an open-field experiment, we measured locomotor activity following infusion of TTX (10 ng/0.5 μl per side or 5 ng/0.5 μl per side) or muscimol (1 μg/0.5 μl per side or 0.5 μg/0.5 μl per side) into the ventral hippocampus.

Materials and methods

Animals

Sixty-six male, adult Wistar rats [Zur:Wist(Hanlbm); Research Unit Schwerzenbach, Schwerzenbach, Switzerland], weighing about 250–300 g at the time of surgery, were used in this study. The animals were housed in groups of four per cage under a reversed light-dark cycle (lights on 19:00–07:00) in a temperature (21±1°C) and humidity (55±5%)-controlled room. All rats were allowed free access to food and water. Twelve rats were left unoperated (UNOP) and 54 rats received bilateral implantation of infusion guide cannulae aimed at the ventral hippocampus. After surgery, all 66 rats were individually caged. Beginning 3 days before surgery and throughout the studies, all rats were handled daily. All experimental procedures were carried out in the dark phase of the cycle. Principles of laboratory animal care (NIH publication no. 86-23, revised 1985) and Swiss regulations for animal experimentation were followed.

Implantation of guide cannulae for intracerebral infusion

Rats were anesthetized with 1 ml of Nembutal (50 mg pentobarbital sodium/ml; Abbott Labs, North Chicago, Ill.) per kilogram body weight and their head was placed in a stereotaxic frame (Kopf Instruments, Tujunga, Calif.). After application of a local anesthetic (lidocaine), the scalp was incised to expose the skull, and bregma and lambda were aligned in the same horizontal plane. Guide cannulae (9 mm, 26-gauge stainless steel) were implanted bilaterally through small holes (diameter 1.5 mm) drilled on each side of the skull. The tips of the guide cannulae aimed at the following coordinates above the ventral hippocampus: 5.2 mm posterior and ±5 mm lateral to bregma, and 5 mm ventral to dura. The guide cannulae were fixed with dental cement for which three small, stainless steel screws, previously screwed into the skull, served as anchors. Stainless steel stylets (34-gauge), which extended 0.5 mm beyond the tips of the guide cannulae, were placed inside the guide cannulae to prevent occlusion. After surgery, the experimenters gave the rats daily health checks and gentle handling, and replaced missing stylets. The behavioral experiments commenced 5 days after surgery.

Intracerebral microinfusion and drugs

For microinfusions into the ventral hippocampus, rats were manually restrained and the stylets removed from the guide cannulae. Then, infusion cannulae (34-gauge) were inserted into the guide cannulae. The infusion cannulae were connected to 10-μl Hamilton microsyringes mounted on a microinfusion pump (KD scientific or WPI sp200i). The tips of the infusion cannulae protruded into the ventral hippocampus 1.6 mm beyond the tip of the guide cannulae, thus aiming at a final dorsoventral coordinate of 6.6 mm
below the dura. The rats were bilaterally infused with TTX (5 ng or 10 ng) or muscimol (0.5 μg or 1 μg) in 0.5 μl vehicle (0.9% saline) or with 0.5 μl vehicle only. The infusion speed was 0.5 μl/min. To allow for absorption of the infusion bolus by the brain tissue, the infusion cannulae were left in the brain for 60 s after infusion before being replaced by the styllets. Muscimol [CH3N2O (1/2 H2O); Tocris, Bristol, UK] was dissolved in 0.9% saline at a concentration of 2 μg/μl or 1 μg/μl on the day of infusion. TTX (C15H17N308; Tocris, Bristol, UK) was stored at -40°C in aliquots containing 40 ng/μl in 0.9% saline. On the day of infusion, these aliquots were thawed and diluted with 0.9% saline to obtain solutions with a concentration of 20 ng/μl or 10 ng/μl. Muscimol was infused immediately and TTX 20 min before the behavioural sessions. Accordingly, half of the rats infused with vehicle received infusion immediately before the behavioral sessions and the other half, 20 min before the behavioral sessions. Doses and time points for the infusion of TTX and muscimol were chosen on the basis of the literature (Ambrogi Lorenzini et al. 1997; 1999; Givens and Olton 1990; Mao and Robinson 1998; Martin 1991; Zhuravin and Bures 1991). Since, to our knowledge, no adverse long-term effects have been reported after intracerebral infusion of these two drugs, we used comparatively high doses to ensure a drug effect.

Apparatus for behavioral testing

Fear conditioning

Eight operant test boxes (Habitest; Coulboum Instruments, Allen-town, Pa.) were used. Conditioning and the context test sessions took place in four shock boxes fitted with a parallel-grid shock floor (16 parallel bars; E10–10RF; Coulboum Instruments), through which scrambled shocks could be delivered. The shock boxes were placed in light- and sound-attenuating chambers measuring 55 cm × 40 cm × 55 cm. These chambers had two side walls of aluminum and a rear and front wall of clear Perspex. A white waste tray was situated below the grid floor. The tone test sessions were conducted in four no-shock boxes fitted with a lattice grid (E10-18NS; Coulboum Instruments) and placed in light- and sound-attenuating chambers measuring 72 cm × 45 cm × 45 cm. The no-shock boxes had three black walls and a front wall of clear Perspex. A brown waste tray was situated below the lattice grid. The four shock and the four no-shock boxes were placed in two different rooms. Presentation of the auditory CS and delivery of electric foot shock were controlled by a PC with dedicated software (S. Frank, Psychology Department, University of Tel Aviv, Israel) connected to a Coulboum Universal Environment Interface (E91–12) with Coulboum Universal Environment Port (L91–12). The auditory CS [85 dB(A)] was produced by a 2.9-KHz tone module (E12–02) fixed at one wall of the operant chamber. Shocks were delivered with a Coulbourn precision animal shocker (E13–12), which generated bipolar, rectangular 10-ms current pulses with a frequency of 10 Hz. Background noise was provided by a ventilation fan affixed to the light- and sound-attenuating chambers during all sessions. A monochrome minivideocamera with a wide-angle (160°) 2.5-mm lens (VPC-46SB; CES, Zurich, Switzerland) was attached to the center of the ceiling of each operant chamber. Four infrared (875 nm) light-emitting diodes (HS1D-4220; Hewlett Packard) positioned in the ceiling of each operant chamber provided light sufficient for camera function. Throughout all sessions, images from each of the four shock or no-shock boxes, respectively, were provided by these cameras, integrated into a four-quarter single image by a multiplexer (DX216CE; Sony), and recorded by a videotape recorder (SV1700; Sony). The images were transferred to a computer (7600/120 Power Macintosh) equipped with an analysis program (Image; http://rsb.info.nih.gov/nih-image) and a macroprogram (P. Schmid, Behavioral Neurobiology Laboratory, Swiss Federal Institute of Technology, Zurich). The proportion of time that was spent in total immobility except for respiratory movements by rats placed in the boxes was measured by this system comparing adjacent 1-s frames of the recorded images. These measures of immobility correspond to measures of freezing behavior as obtained by use of visual criteria commonly employed. The validation and principle of the automated analysis of freezing behavior have been described in detail in previous publications (Murphy et al. 2000; Pryce et al. 1999; Richmond et al. 1998, 1999).

Open-field locomotor activity

Locomotor activity was measured in four closed square arenas (76.5 cm × 76.5 cm × 49 cm) made of dark gray plastic and placed in a room dimly illuminated (50lux provided by two halogen lights). Behavior in the arenas was recorded by a video camera suspended from the ceiling and relayed to a monitor and a video tracking, motion analysis and behavior recognition system (Etho; Noldus, Wageningen, The Netherlands). For testing of open-field locomotor activity, the rats were placed in the center of an arena and the total distance the rat moved in centimeters throughout the complete arena was calculated by the Noldus system for each 10-min block of testing.

Experimental design and procedures for behavioral testing

Fear-conditioning experiments

All 66 rats were used in one of two fear-conditioning experiments (I and II) to test the effects of muscimol or TTX infusion into the ventral hippocampus on the formation of fear conditioning to an explicit CS (auditory CS) and a contextual CS (environment). Freezing, i.e., total immobility except for respiratory movements, was used to assess conditioned fear and measured by the automated system described above. Originally, fear-conditioning experiment I, which was conducted with 32 rats (26 cannulated, six UNOP), was planned to assess the effects of muscimol and TTX infusion on conditioning to both an explicit and a contextual CS. However, the procedure of experiment I did not result in significant conditioning to the contextual CS in the control groups. For that reason, a second fear-conditioning experiment with another 34 naive rats (28 cannulated, six UNOP) was conducted using a procedure to yield robust conditioning to the contextual CS. The timing of treatments for the different groups, and the conditioning and test procedures in fear-conditioning experiments I and II are summarized in Table 1.

In both experiments, cannulated rats were allocated to one of three infusion groups to receive bilateral infusions into the ventral hippocampus before conditioning on day 1: TTX10 (n=7), 10 ng TTX/0.5 μl per side 20 min before the conditioning session; MUSI (n=7), 1 μg muscimol/0.5 μl per side, immediately before the conditioning session; VEH (n=12 in experiment I and n=14 in experiment II), 0.5 μl vehicle per side 20 min before (n=6 or 7) or immediately (n=6 or 7) before the conditioning session. In addition, in each fear-conditioning experiment, UNOP rats (n=6) served to test for unspecific effects of cannulae implantation and infusion procedure. UNOP rats were handled the same way as the cannulated rats but were not subjected to the surgery procedures before the fear-conditioning experiment. Furthermore, they received no treatment instead of the infusion before conditioning. The conditioning and test procedures during fear-conditioning experiments I and II were the same for all groups.

Fear-conditioning experiment I

For conditioning, rats were put in the shock boxes for a total of 27 min and were exposed to ten pairs of a 30-s auditory CS [85 dB(A), 2.9 KHz] and a 1-s foot shock (current pulse amplitude 0.5 mA) separated by 2-min blocks between an initial and a final 2-min block. The 1-s foot shock was contiguous with the final second of the CS. The proportion of time spent immobile was calculated for the eleven 2-min blocks preceding and following the 30-s CS and for the duration of each 30-s CS. One day after conditioning, in the context test session, rats were tested for freezing to the contextual CS, i.e., the environment.
## Table 1 Summary of the procedures in fear-conditioning experiments I and II

<table>
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<th>Day 1</th>
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<td>Infusion</td>
<td>Conditioning</td>
<td>Context test</td>
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<tr>
<td><strong>Fear-conditioning experiment I</strong></td>
<td>TTX10 group: 10 ng tetrodotoxin/side 20 min before conditioning</td>
<td>All groups: 27 min in shock box; after 2 min, ten tone (30 s)-shock (1 s, 0.5 mA) pairings separated by 2-min blocks; last 1 s of tone and foot shock contiguous</td>
<td>All groups: 8 min in shock box to test for freezing in the context of the foot shocks</td>
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<td>MUSI group: 1 μg muscimol/side, immediately before conditioning</td>
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<td>VEH group: vehicle infusion, either 20 min before or immediately before conditioning</td>
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<td>UNOP group: no treatment</td>
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<tr>
<td><strong>Fear-conditioning experiment II</strong></td>
<td>TTX10 group: 20 min 10 ng tetrodotoxin/side before conditioning</td>
<td>All groups: 30 min and in shock box; after 5 min, 5 s five 1-s foot shocks (0.5 mA) separated by 5-min blocks</td>
<td>All groups: 8 min in shock box to test for freezing in the context of the foot shocks</td>
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in which they had received the foot shocks. For that purpose, rats were placed in the shock box for 8 min without being exposed to the auditory CS or the foot shock. Two days after conditioning, in the tone test session, rats were tested for freezing to the auditory CS. For that purpose, they were put in the no-shock box for a total of 11 min. After 3 min, the auditory CS was presented for the remaining 8 min. During context and tone test sessions, the proportion of time spent immobile was calculated for each 1-min block.

**Fear-conditioning experiment II.** The conditioning procedure of fear-conditioning experiment I did not result in marked freezing during the context test session. Therefore, we conducted a second fear-conditioning experiment to test the effects of TTX and muscimol infusion into the ventral hippocampus on the formation of fear conditioning to a contextual CS. In the second fear-conditioning experiment, conditioning was conducted with an unsignaled shock, i.e., without an auditory CS, to achieve a strong association between the shock and the contextual CS, i.e., the environment in which the rats received the foot shock (compare Olding-Smee 1978). For conditioning, rats were put in the shock boxes for a total of 30 min and 5 s and were exposed to five 1-s foot shocks (current pulse amplitude 0.5 mA) separated by 5-min blocks between an initial and a final 5-min block. Five-minute blocks were chosen to provide sufficient time — in particular before the first shock — for the rats to build up a context representation that could be associated with the shock (compare Fanselow 1986, 2000). The proportion of time spent immobile was calculated for the six 5-min blocks preceding and following the 1-s foot shock. One day after conditioning, in the context test session, rats were tested for freezing to the contextual CS, i.e., the shock boxes. For that purpose, rats were placed in the shock box for 8 min, and the proportion of time spent immobile was calculated for each 1-min block.

**Verification of intact shock reaction.** TTX and muscimol infusion induced hypoactivity, and the TTX10 and MUSI rats exhibited quite high levels of immobility before the first shock of the conditioning session, especially in fear-conditioning experiment II (see Results). This indicated that the immobility in the TTX10 and MUSI rats measured during the conditioning sessions of the two fear-conditioning experiments was not only reflective of a conditioned postshock response but partly due to hypoactivity induced by the infusions preceding conditioning. Therefore, we verified that all rats were really exhibiting an intact postshock response. For that purpose, we compared the proportion of immobility during the 30-s periods preceding and the 30-s periods immediately following all 1-s foot shocks (pre-S and post-S period) delivered after the first foot shock. The intact reaction of a rat to a foot shock includes an immediate postshock activity burst of several seconds, which is an unconditioned shock response. With repeated shock presentations in an enclosure, this unconditioned shock response is followed by the conditioned component of freezing. Thus, an intact shock response should be revealed by reduced immobility during the post-S periods as compared to the pre-S periods (Fanselow 1982).

**Open-field experiment**

Twenty-five cannulated rats from fear-conditioning experiment I were subjected to an open-field experiment 7 days after the fear-
conditioning experiment. The open-field experiment was designed to test the effects of muscimol or TTX infusion into the ventral hippocampus on locomotor activity in the open field. It was based on observations during the fear-conditioning experiment indicating that bilateral infusion of both muscimol (1 μg/0.5 μl per side) and TTX (10 ng/0.5 μl per side) into the ventral hippocampus reduced locomotor activity. To get an impression of dose-response relations, we split the TTX10 group of the fear-conditioning experiment into a TTX10 (n=4) and a TTX5 (n=3) group to receive 10 ng or 5 ng TTX/0.5 μl per side, respectively, into the ventral hippocampus and the MUS1 group into a MUS1 (n=4) and a MUS0.5(n=3) group to receive 1 μg or 0.5 μg muscimol/0.5 μl per side, respectively, into the ventral hippocampus. In addition, there was a VEH group (n=1) to receive vehicle infusion into the ventral hippocampus. We did not include unoperated animals, since in previous experiments we did not find a difference between unoperated rats and rats infused into the hippocampus with vehicle concerning activity in the open field (Bast et al. 2001; Zhang et al. 2000). On day 1 of the open-field experiment, animals were tested for baseline activity in a 30-min session. Based on baseline activity of day 1, the rats were allocated to the subgroups of the TTX, MUS, and VEH groups so that all groups had matched baseline activity. On day 2, rats received the following bilateral infusions into the ventral hippocampus before being subjected to an open-field testing of 60 min: TTX10 and TTX5, 10 ng or 5 ng TTX/0.5 μl per side 20 min before open-field testing; MUS1 and MUS0.5, 1 μg or 0.5 μg/0.5 μl per side immediately before open-field testing; VEH, 0.5 μl vehicle/side 20 min (n=5) or immediately (n=6) before open-field testing. We did not include a habituation phase before infusion, because we expected a decrease in locomotor activity by our drug infusions. This could have been masked by a floor effect if the animals had already had a low level of activity due to habituation. On day 3 of the open-field experiment, rats were subjected to 30 min of open-field testing to test for possible long-term effects of the drug infusions.

Histology

After completion of the behavioral experiments, the cannulated rats were deeply anesthetized with an overdose of 2.5 ml/kg Nembutal (50 mg pentobarbital sodium/ml i.p.) and transcardially perfused with 0.9% NaCl solution to rinse out the blood, followed by 250 ml of 4% formalin (4°C) to fix the brain tissue. After extraction from the skull, the brains were postfixed in 4% formalin solution and subsequently cut into 40-μm coronal sections on a freezing microtome. For the verification of the infusion sites, every fifth section through the ventral hippocampus was mounted on a gelatin-treated slide and stained with cresyl violet. After staining, the sections were dehydrated and coverslipped. Subsequently, they were examined light-microscopically to verify that the tips of the infusion cannulae were placed in the ventral hippocampus and to draw their approximate locations onto plates taken from the atlas of Paxinos and Watson (1998).

Data analysis

Statistical analysis was conducted with the Statview software system. Data were first subjected to analysis of variance (ANOVA). Groups were taken as between-subjects factor and the different time blocks of testing as repeated measures. If indicated by a significant outcome of the ANOVA, post hoc comparisons were conducted using Fisher’s protected least significant difference test. Significant differences were accepted at P<0.05. In the fear-conditioning experiments, the proportion of time spent immobile was analyzed. In the open-field experiment, the total distance moved was analyzed. Since the data of the VEH rats did not differ significantly whether the infusions were given immediately or 20 min prior to conditioning or open-field testing, they were collapsed for the analysis presented in the Results section. All data are presented as mean values. Throughout the text and in bar plots, the variability is indicated by the standard error of the mean (SEM). In line plots, for the sake of clarity, the standard error (SE) derived from the appropriate mean square yielded by the ANOVA was used to indicate variability. Total distance moved is given in centimeters. The proportion of time spent immobile is given as a percentage.

Results

Histology

The centers of the infusion sites, i.e., the tips of the infusion cannulae, were located within or around the borders of the ventral hippocampus in all cannulated rats (Fig. 1). Damage due to the implantation of the guide cannulae was restricted to the area immediately surrounding the guide cannulae. Based on the histological examinations, the behavioral data of all 54 cannulated rats were included in the statistical analysis.

Fear-conditioning experiment I: effects of muscimol or TTX infusion into the ventral hippocampus on fear conditioning to a tone

During the conditioning session, the groups did not differ in the percentage of time spent freezing during the ten 30-s CS presentations (F3,28=1.36, P>0.25) or during the eleven 2-min blocks preceding and following the CS presentations (F3,28=2.18, P<0.1; Fig. 2A, B). ANOVA of the percentage of time spent immobile yielded only a significant effect of the ten CS presentations (F9,252=8.36, P<0.0001) and the eleven 2-min blocks (F10,280=13.65, P<0.0001). This reflected that in all groups freezing developed throughout the first two applications of the CS foot-shock pairings. Nevertheless, it is important to note that the proportion of immobility in the TTX10 rats was already comparatively high during the 2-min block preceding the first foot shock (Fig. 2B). ANOVA of the proportion of time spent immobile during the first 2-min block yielded a significant effect of group (F3,28=4.36, P<0.02). Post hoc comparisons revealed that the percentage of time spent immobile during that period was significantly higher in the TTX10 (18.8±8.6%) than in the UNOP (2.1±0.5%; P<0.01), VEH (0.7±0.3%; P<0.002), and MUS1 (5±2.4%; P<0.03) group. The latter three groups did not differ from each other (P>0.4). The immobility of the TTX10 rats before the first foot shock could not reflect a conditioned fear response. Rather, it seemed to be due to hypoactivity induced by the infusion of TTX 20 min before conditioning. In fact, by mere visual inspection, markedly decreased activity was evident in the TTX rats, beginning about 10 min following infusion and lasting beyond the conditioning session. Although immobility was not
increased in the MUSI rats during the first 2-min block preceding the first foot shock in experiment I, visual observation during and after conditioning revealed also decreased activity in this group. These observations prompted us to conduct the open-field experiment to examine the effects of TTX and muscimol infusion into the ventral hippocampus on locomotor activity.

Fig. 1A, B Infusion sites in the ventral hippocampus. A Photomicrograph of a coronal brain section with the tracks of the guide cannulae and beneath them the infusion sites visible in both hemispheres. B Approximate location of the tips of the infusion cannulae depicted on plates of coronal sections through the rat brain (Paxinos and Watson 1998). Open circles represent sites of vehicle infusion (26 rats), open squares represent sites of muscimol infusion (14 rats), and black circles represent sites of TTX infusion (14 rats). Values on the right represent distance from bregma. (CA1, CA2, and CA3 CA1, CA2, and CA3 field of the hippocampus, DG dentate gyrus, Eni entorhinal cortex, S subiculum)

Fig. 2A–D Freezing during the three sessions of fear-conditioning experiment I. There were four experimental groups: unoperated rats (UNOP, n=6); rats which received bilateral infusion of vehicle (VEH, n=12) 20 min (n=6) or immediately (n=6) before the conditioning session; rats which received bilateral infusion of 1 ng muscimol/side, immediately before the conditioning session (MUSI, n=1); rats which received bilateral infusion of 10 ng tetrodotoxin/side 20 min before the conditioning session (TTX10, n=1). The values are means. The inset bar represents 1 SE as derived from ANOVA. A Freezing during the ten 30-s blocks of conditioned stimulus (CS) presentation in the conditioning session. B Freezing during the eleven 2-min blocks preceding and following the CS presentations during the conditioning session. C Freezing during the eight 1-min blocks of the context test. D Freezing during the three 1-min blocks preceding the CS presentation and the subsequent eight 1-min blocks of CS presentation during the tone test.
Context test

No group exhibited marked freezing throughout the eight 1-min blocks of the context test session (Fig. 2C). The percentage of time spent freezing, averaged over 8 min, varied from 2% in the TTX10 to 13% in the UNOP group. ANOVA of the percentage of time spent immobile yielded neither an effect of group \( (F_{3, 28}=1.94, P>0.1) \) or the eight 1-min blocks \( (F_{7, 196}=1.75, P>0.1) \) nor an interaction of group and 1-min blocks \( (F_{21, 196}=1.29, P>0.1) \), reflecting that all groups exhibited a similar low level of freezing throughout the whole context test session.

Tone test

The TTX10 rats showed virtually no conditioned fear throughout the whole tone test session, whereas UNOP, VEH, and MUSI rats exhibited marked conditioned freezing to the tone CS (Fig. 2D). During the 3 min preceding the CS presentation, the proportion of time spent immobile was very low (less than 5%) and did not differ between the groups \( (F_{3, 28}=1.50, P>0.2) \). However, during the 8 min of CS presentation, all groups except the TTX10 group exhibited marked freezing, i.e., conditioned fear. ANOVA of the percentage of time spent immobile during these 8 min yielded a main effect of group \( (F_{3, 28}=3.23, P<0.05) \). Post hoc comparisons confirmed that the percentage of time spent immobile during the 8 min of CS presentation was lower in the TTX10 \( (6.6\pm0.9\%) \) as compared to the UNOP \( (43.9\pm4.7\%; P<0.01) \), VEH \( (31.02\pm2.9\%; P<0.03) \), and MUSI \( (28.3\pm3.6\%; P<0.08) \) groups. The latter three groups did not differ from each other \( (P>0.2) \).

Fear-conditioning experiment II: effects of muscimol or TTX infusion into the ventral hippocampus on fear conditioning to a context

Conditioning session

The TTX10 group exhibited a higher level of immobility during the six 5-min blocks preceding and following the five 1-s foot shocks (Fig. 3A). ANOVA of the percentage of time spent immobile during the six 5-min blocks yielded a significant effect of groups \( (F_{3, 30}=3.91, P<0.02) \) and post hoc comparisons revealed that the percentage of time spent immobile during the six 5-min blocks was significantly higher in the TTX10 \( (60.2\pm3.6\%) \) than in the UNOP \( (37.1\pm4.2\%; P<0.01) \), VEH \( (40.2\pm2.7\%; P<0.01) \), and MUSI \( (37.6\pm4.3\%; P<0.01) \) groups. The latter three groups did not differ from each other \( (P>0.6) \). Furthermore, ANOVA yielded an effect of the 5-min blocks \( (F_{3, 30}=18.57, P<0.0001) \), as well as a significant interaction of groups and 5-min blocks \( (F_{15, 150}=3.46, P<0.0001) \). This reflected that immobility increased as a consequence of the first two shock administrations in the UNOP, the VEH, and – to a lesser extent – also in the MUSI group, whereas the TTX10 rats exhibited a constant, high level of immobility throughout all of the six 5-min blocks. Moreover, the TTX10 and also the MUSI rats already spent a high proportion of time immobile during the 5-min block preceding the first shock. ANOVA of the time spent immobile during this period yielded a significant effect of group \( (F_{3, 30}=16.34, P<0.0001) \). Post hoc comparisons indicated that the percentage of time spent immobile was higher in the TTX10 \( (51.6\pm11.9\%) \) and also the MUSI \( (19.7\pm6.9\%) \) group than in the UNOP \( (20.4\pm0.8\%; P<0.001 and 0.07) \) and VEH \( (1.6\pm0.4\%; P<0.0001 and 0.02) \) group, which did not differ from each other \( (P>0.9) \). In addition, the immobility was significantly higher in the TTX10 than in the MUSI group \( (P<0.001) \).

Similar to the situation for the TTX10 group during the time block preceding the first shock in the conditioning session of experiment I, the high levels of immobility during the 5-min block preceding the first shock could not reflect conditioned fear but were probably due to hypoactivity induced by the TTX and muscimol infusions before conditioning. It is important to note that the proportions of immobility in time block 1 preceding the first shock in the conditioning sessions of experiments 1
and 2, as depicted in Figs. 2B and 3A, cannot be compared directly, since the shock was delivered after 2 min in the first and after 5 min in the second experiment. However, separate analysis of the first 2 min of the conditioning session of experiment 2 yielded a similar picture to that of the analysis of the first 2-min block of experiment 1, with immobility levels of only the TTX10 but not the MUS1 group being increased. For this period, ANOVA yielded a significant group effect ($F_{3,30}=14.0, P<0.0001$). Post hoc comparisons revealed that the percentage of time spent immobile during the first 2 min of the conditioning session was significantly higher in the TTX10 (38.8±11.5%) than in the UNOP (0.3±0.2%; $P<0.0001$), VEH (1.0±0.6%; $P<0.0001$), and MUS1 (2.0±0.9%; $P<0.0001$) group. The latter three groups did not differ from each other ($P>0.8$). Although the immobility levels of the TTX10 group during the first 2 min of the conditioning session were higher in experiment II (38.8±11.5%) than in experiment I (18.8±8.6%), combined ANOVA of the immobility values measured during the first 2 min of the conditioning sessions in experiment I and II yielded only a significant effect of group ($F_{3,38}=17.52, P<0.0001$) - due to higher immobility values in the TTX10 group - but neither a significant effect of experiment ($F_{1,38}=1.45, P>0.2$) nor a significant interaction of experiment and group ($F_{3,38}=2.7, P>0.05$).

**Context test**

One day after conditioning, both MUS1 and TTX10 rats showed virtually no conditioned fear to the conditioning context, whereas UNOP and VEH rats exhibited marked conditioned freezing (Fig. 3B). ANOVA of the percentage of time spent immobile revealed a significant effect of group ($F_{3,36}=6.08, P<0.0025$). Post hoc comparisons revealed that, throughout the 8 min of the context test session, UNOP (21.2±3.6%) and VEH (23.2±2.2%) rats spent a higher percentage of time immobile than MUS1 (4.9±1.0%; $P<0.025$) and TTX10 rats (4.0±0.8%; $P<0.005$) and VEH rats did not differ from each other ($P>0.7$), nor did MUS1 and TTX10 rats ($P>0.8$). Furthermore, ANOVA of the percentage of time spent immobile yielded a significant effect of the eight 1-min blocks ($F_{7,210}=4.16, P<0.0005$) and a significant interaction of groups and 1-min blocks ($F_{21,210}=2.26, P<0.002$). This reflected a gradual increase in conditioned fear to a maximum throughout the whole session.

**Verification of intact shock reaction**

All groups exhibited an intact shock reaction, as revealed by reduced immobility in the post-S as compared to the pre-S periods during the conditioning session of both fear-conditioning experiments (Fig. 4). Mainly due to the overall higher level of immobility in the TTX10 rats during the conditioning session, ANOVA yielded an effect of group on the percentage of time spent freezing throughout the pre-S and post-S periods (experiment I: $F_{3,28}=3.32, P<0.05$; experiment II: $F_{3,36}=6.68, P<0.0025$). Nevertheless, there was a highly significant effect of the periods (experiment I: $F_{1,28}=39.13, P<0.0001$; experiment II: $F_{1,36}=89.81, P<0.0001$), which was independent of the group (interaction of period and group: experiment I: $F_{3,28}=25.65, P<0.0001$; experiment II: $F_{3,30}=25.65, P<0.0001$), as derived from ANOVA. See legends of Figs. 2 (fear-conditioning experiment I) and 3 (fear-conditioning experiment II) regarding groups and numbers of animals.

**Open-field experiment: dose dependent decrease in locomotor activity by infusion of muscimol or TTX into the ventral hippocampus**

After matching, ANOVA carried out on the total distance moved by the UNOP, VEH, MUS0.5, MUS1, TTX5, TTX10 group on day 1 (data not shown) yielded only a significant main effect of the three 10-min blocks of testing ($F_{2,35}=75.56, P<0.0001$). This reflected the gradual reduction in activity, i.e., habituation, over time.

Infusion of TTX and muscimol before the 60 min of open-field testing on day 2 decreased locomotor activity dose dependently (Fig. 5). ANOVA of the total distance moved during the six 10-min blocks of open-field testing (Fig. 5A) yielded a significant effect of groups ($F_{4,20}=11.19, P<0.0001$) and 10-min blocks ($F_{4,100}=25.65, P<0.0001$), as well as an interaction of groups and 10-min blocks ($F_{20,100}=6.81, P<0.0001$). This reflected that activity was decreased dose-dependently by TTX and muscimol as compared to the VEH rats during the first 30 min; whereas, during the second
One day following infusion, the groups no longer differed in the total distance moved throughout the 30-min open-field testing (data not shown). ANOVA of the total distance moved did not reveal a main effect of groups ($F_{4,20}=1.15$, $P>0.3$) or an interaction of groups and 10-min blocks ($F_{8,40}=1.15$, $P>0.3$). There was just a significant effect of 10-min blocks ($F_{2,40}=47.45$, $P<0.0001$), reflecting habituation in all groups throughout the 30 min of testing.

**Discussion**

In the present study, temporary inactivation of the ventral hippocampus by TTX (10 ng/side) infusion prevented the formation of fear memory to an explicit and a contextual cue. Increasing inhibitory transmission in the ventral hippocampus by infusion of the GABA$_A$ agonist muscimol (1 µg/side) only disabled fear conditioning to a contextual CS. In addition, both TTX (5 ng/side and 10 ng/side) and muscimol (0.5 µg/side and 1 µg/side) infusion into the ventral hippocampus dose-dependently decreased locomotor activity in the open field.

Hypoactivity in the open field following total inactivation or increased inhibition of neuronal activity in the ventral hippocampus is consistent with the view that activity of the ventral hippocampus drives locomotor activity. This view has been suggested by previous findings of increased open-field activity following stimulation of the ventral hippocampus (Bardgett and Henry 1999; Bast et al. 2001, 2001; Brudzynski and Gibson 1997; Legault and Wise 1999; Wu and Brudzynski 1995; Yang and Mogenson 1987). Infusion of lidocaine—which produces a similar, but shorter, blockade of sodium channels than TTX (Ambrogi Lorenzini et al. 1999)—into the ventral hippocampus at a dose of 20 µg/side did not affect open-field activity in previous experiments (Bardgett and Henry 1999; W.-N. Zhang, T. Bast, and J. Feldon, unpublished findings). We suggest that this is due to the ventral hippocampus not being sufficiently inactivated by this dose of lidocaine. Hyperactivity, which has been found after lesions of the ventral hippocampus (Lipska et al. 1992; Richmond et al. 1999), seems at first glance at odds with our present findings. However, the hypoactivity observed in the present study is an acute effect of temporarily decreased activity of the ventral hippocam-
The results of our two fear-conditioning experiments are in line with recent studies that suggest a role for the ventral hippocampus in classical fear conditioning to contextual and explicit cues. These studies have demonstrated reduced freezing to aversively conditioned explicit and contextual cues following lesions of the ventral hippocampus before and after conditioning (Maren 1999; Maren and Fanselow 1995; Richmond et al. 1999). Richmond et al. (1999) have proposed that these freezing deficits might result from lesions of the ventral hippocampus interfering with the expression of conditioned fear in the form of freezing, as they induce hyperactivity. In contrast to lesions of the ventral hippocampus, muscimol and TTX infusion into the ventral hippocampus induced hypoactivity (for possible explanations of this difference, see previous paragraph) facilitating the expression of freezing behavior. This explains why freezing during the conditioning sessions tended to be increased following infusion of muscimol and TTX into the ventral hippocampus, whereas freezing was decreased during conditioning following lesions of the ventral hippocampus (Richmond et al. 1999). In contrast to lesions, manipulations of the ventral hippocampus induced by muscimol and TTX applied before conditioning were temporary and did not affect basal activity of the ventral hippocampus on the test days of the fear-conditioning experiment. Thus, an involvement – of whatever kind – of the ventral hippocampus in the expression of freezing cannot account for the decreased freezing observed on the test days in the present studies. Our results indicate that the specific associative processes underlying the formation of classical fear conditioning are dependent on normal activity of the ventral hippocampus. However, one has also to consider alternative interpretations of our results. TTX and muscimol infusion into the ventral hippocampus might interfere with fear conditioning by disrupting the detection of the aversive unconditioned stimulus, i.e., the foot shock. This can virtually be excluded for the muscimol infusion, because its effect was restricted to conditioning to the contextual cue. In addition, decreased freezing during the 30-s periods immediately following the shocks (post-S periods) relative to the 30-s periods preceding the shocks (pre-S periods), which is reflective of the postshock activity burst (Fanselow 1982), was observed in all experimental groups. This indicates an intact shock reaction in all groups. In the MUS1 and the TTX10 rats, the ventral hippocampus was not in the same drug state during conditioning and test sessions. Therefore, the virtual absence of conditioned fear in these groups during the test sessions might reflect state dependency (Overton 1964) of fear learning. Using freezing to assess conditioned fear, it is difficult to test for this, as the appropriate control groups would have to receive TTX or muscimol infusion into the ventral hippocampus before the test sessions. Similar to the situation during the conditioning sessions of the two fear-conditioning experiments in the present study, this would result in high levels of freezing due to the hypoactivity induced by these treatments and therefore make the detection of reduced conditioned fear difficult. Studies on the involvement of the amygdala in fear conditioning (Garraci et al. 2000; Helmstetter and Bellgowan 1994; Muller et al. 1997), however, suggest that local microinfusions into single brain areas do not interfere with learning of fear merely on the basis of state dependency. Finally, decreased exploratory locomotor activity during the conditioning session due to the muscimol and TTX infusions might interfere with building up a neuronal representation of the conditioning context and thereby be responsible for the anterograde deficits in contextual fear conditioning observed in the MUS1 and TTX10 rats. However, it is not very likely that the decreased exploratory locomotor activity following muscimol and TTX infusion into the ventral hippocampus can effectively prevent the registration of those components that are most characteristic of the conditioning context, i.e., the grid floor and the dark enclosure. Thus, we assume that the results of our fear-conditioning experiments reflect that activity of the ventral hippocampus is important for the specific associative processes underlying the formation of fear memory to explicit and contextual stimuli.

Formation of long-lasting memory comprises the initial acquisition, a short-term consolidation, believed to be completed within seconds to tens of minutes, and the subsequent long-term consolidation of an association (McGaugh 2000; Nadel and Moscovitch 1997). In our fear-conditioning experiments, the infusions of TTX and muscimol occurred before conditioning, i.e., acquisition and short-term consolidation, but the drugs might have been active in the ventral hippocampus beyond the conditioning sessions, i.e., throughout the early phase of the long-term consolidation. Our open-field data demonstrate that the muscimol infusion affected behavior for at
least 60 min after the beginning of the test sessions. Although the effects of TTX on locomotor activity were evident no longer than 30 min following the start of the open-field session, this does not necessarily mean that the drug’s effect in the ventral hippocampus did not extend beyond this period. Thus, principally, both muscimol and TTX might have disrupted activity of the ventral hippocampus necessary for the acquisition and short-term consolidation as well as the early long-term consolidation of fear conditioning. It is important to note that the unimpaired freezing following TTX or muscimol infusion during the conditioning session does not mean that the acquisition and short-term consolidation of conditioned fear were unimpaired. Since both TTX and muscimol infusion facilitated freezing by inducing hypoactivity, freezing throughout the conditioning may have been high despite decreased conditioned fear. An involvement of the ventral hippocampus in acquisition and short-term consolidation as well as early long-term consolidation of classical aversive conditioning has been suggested by a previous study, reporting TTX infusion into the ventral hippocampus (10 ng/1 μl per side) 1 h before, immediately after, and 15 min after conditioning to interfere with memory formation in the passive avoidance paradigm (Ambrogi Lorenzini et al. 1997). Thus, in our fear-conditioning experiments, infusion of TTX and muscimol into the ventral hippocampus may have impaired the formation of fear memory by interfering with acquisition, short-term consolidation, early long-term consolidation, or all of these processes.

The effects of the TTX and muscimol infusions on fear conditioning were different. While TTX (10 ng/side) infusion into the ventral hippocampus caused complete anterograde amnesia of fear, muscimol (1 μg/side) infusion into the ventral hippocampus only blocked formation of fear memory to a contextual CS. The specific anterograde amnesia for contextual fear following muscimol infusion might merely be due to the fact that the dose used was only sufficient to block the weaker conditioning to the context but not the stronger conditioning to the tone. However, one has to consider that the mechanisms by which TTX and muscimol take effect are different. TTX leads to temporary functional inactivation of all neurons and traversing fibers in the infusion area (Ambrogi Lorenzini et al. 1999), whereas muscimol only increases inhibition of neurons bearing the GABA_A receptor. Thus, even given that both substances are applied at doses to induce their maximal physiological effect, the behavioral effects of TTX and muscimol may differ. In fact, the patterns of behavioral effects observed in the present study after infusion of muscimol and TTX into the ventral hippocampus differ. While the hypoactivity in the open field was rather more pronounced following infusion of muscimol (1 μg/side) than following infusion of TTX (10 ng/side; Fig. 5), and disruption of contextual conditioning was similar following the infusion of both substances (Fig. 3B), only the infusion of TTX led to a disruption of fear conditioning to a tone (Fig. 2D). Thus, with respect to fear conditioning, the effects of muscimol in the ventral hippocampus might in particular disturb conditioning to a context, whereas the effects of TTX affect conditioning to a tone and to a context to a similar extent. The fibers and cell bodies in the ventral hippocampus that are most likely to be involved in classical fear conditioning are those connecting neurons of the ventral hippocampus with the amygdala and the nucleus accumbens (Groenewegen et al. 1987; Pitkänen et al. 2000; Swanson and Cowan 1977). The general anterograde amnesia of fear after TTX infusion might be due to the fact that these connections are completely inactivated by TTX. Since muscimol only increases inhibition of neurons in the ventral hippocampus that are subject to inhibition via GABA_A receptors, it may have more specific effects on fear conditioning than TTX. Nevertheless, formation of fear conditioning to a context was disrupted by muscimol infusion into the ventral hippocampus in the present study, indicating that neurons within the ventral hippocampus bearing the GABA_A receptor are at least important for fear conditioning to a context. There is evidence for the hippocampal network of GABA-releasing inhibitory interneurons being important for memory formation (Buzsáki and Chrobak 1995). The GABA_A agonist muscimol interferes with this processing. Therefore, it is possible that the impaired formation of fear memory to a context induced by muscimol in the ventral hippocampus does not primarily result from an inhibition of neuronal activity per se. Rather, the anterograde amnesia of fear to a context caused by muscimol in the ventral hippocampus might be due to the fact that undisturbed signaling in the local network of GABA-releasing interneurons is necessary for the specific processing of contextual information which is required for forming a memory of fear to a context.

A prominent notion of the hippocampus’s role in classical conditioning, and in particular in fear conditioning, is that the hippocampus processes contextual information necessary to form or utilize particular CS-US associations which are formed and stored elsewhere in the brain (Fanselow 2000; Fendt and Fanselow 1999; Holland and Bouton 1999; Jarrard 1993; Maren and Fanselow 1995; Maren and Holt 2000). In fact, disrupting activity of the dorsal hippocampus by lesions or infusions has mainly been reported to impair fear conditioning to a contextual CS but not to a tone (for review, see Fanselow 2000). Our present results and previous lesion studies (Maren 1999; Richmond et al. 1999), however, suggest that normal activity of the ventral hippocampus is important for the formation of fear conditioning not only to a context but also to a tone.

TTX and muscimol infusion into the ventral hippocampus might disrupt the specific associative processes underlying the formation of fear conditioning by interfering with the sending or receiving of neuronal signals by the ventral hippocampus. For example, deficient activity of the ventral hippocampus during fear conditioning might prevent memory formation by disrupting signaling to the amygdala or the nucleus accumbens, or by disrupting the reception of inputs from the amygdala, or
by disrupting reciprocal signaling between dorsal and ventral hippocampus. At the moment, it is not possible to decide which interactions are important. Disconnection lesions (Floresco et al. 1997) might help to identify the critical connections. Moreover, to understand the interactions in this network, future studies are necessary which further characterize or start to examine the neurochemical and neurophysiological changes in the nucleus accumbens (Murphy et al. 2000; Pezze et al. 2000; Wilkinson et al. 1998; Young et al. 1993), the amygdala, and the hippocampus (Nail-Boucherie et al. 2000) during fear conditioning, and which clarify how neurochemical and neurophysiological changes in these structures are related to each other. Experiments to address these issues are currently being run or are in preparation in our laboratory.

Finally, the present study did not address the issue of whether the importance of the ventral hippocampus for fear memory in a classical conditioning paradigm is temporary, i.e., restricted to the formation (acquisition and consolidation) of the memory, or whether the ventral hippocampus is permanently necessary for the retrieval of the memory. Currently, there is a debate as to the question of whether the hippocampus's relevance for different forms of memory is time limited or not (Knowlton and Fasselov 1998; Nadel and Moscovitch 1997; Nadel et al. 2000). The role of the dorsal hippocampus in classical fear conditioning to a contextual CS has recently been suggested to be time limited (Anagnostaras et al. 1999). In the ventral hippocampus this issue is still unexplored. TTX infusion into the ventral hippocampus has been reported to impair retrieval of the passive avoidance response memory, with the impairment being more pronounced after TTX infusion into the dorsal hippocampus (Ambrogi Lorenzini et al. 1996, 1997). Using freezing as a measure of conditioned fear, the effects of inactivation of the ventral hippocampus by lesions or inactivating infusions on memory retrieval in a classical fear-conditioning paradigm will be difficult to examine, since both manipulations influence activity and thus affect freezing.

In conclusion, the results of the present study indicate that neuronal activity within the ventral hippocampus is necessary for the formation of fear memory to explicit and contextual cues by classical conditioning. The complete anterograde amnesia of fear after TTX inactivation of the ventral hippocampus suggests that the ventral hippocampus contains neurons that are important for the formation of fear memory to contextual as well as to explicit cues. The anterograde amnesia of fear to a context after infusion of muscimol into the ventral hippocampus indicates that neurons bearing the GABAA receptor are among those neurons in the ventral hippocampus which are involved in the formation of fear conditioning to a context. In addition, the marked hyperactivity following muscimol and TTX infusion into the ventral hippocampus further corroborates that neuronal activity within the ventral hippocampus drives locomotor activity.
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Annotation:
Research report

The ventral hippocampus and fear conditioning in rats: different anterograde amnesias of fear after infusion of N-methyl-D-aspartate or its noncompetitive antagonist MK-801 into the ventral hippocampus

Wei-Ning Zhang, Tobias Bast, Joram Feldon *

Behavioral Neurobiology Laboratory, Swiss Federal Institute of Technology Zurich, Schorenstrasse 16, Postfach, CH 8603 Schwerzenbach, Switzerland

Received 10 October 2000; received in revised form 7 May 2001; accepted 7 May 2001

Abstract

Previous studies on hippocampal involvement in classical fear conditioning mainly focused on the dorsal hippocampus and conditioning to a context. However, in line with the strong interconnectivity of the ventral hippocampus with amygdala and nucleus accumbens, more recent studies indicated an even more global role for the ventral hippocampus in fear conditioning. The present study examined the formation of classical fear conditioning to explicit and contextual cues following stimulation or blockade of N-methyl-D-aspartate (NMDA) receptors in the ventral hippocampus. NMDA (0.5 μg/side) or the noncompetitive NMDA antagonist MK-801 (dizocilpine; 6.25 μg/side) were bilaterally infused into the ventral hippocampus of Wistar rats before fear conditioning to explicit and contextual cues. Conditioned fear was assessed using an automated measurement of freezing. NMDA stimulation of the ventral hippocampus blocked fear conditioning to both the tone and the context. MK-801 selectively blocked fear conditioning to the context. Our results support that the ventral hippocampus plays a role in the formation of classical fear conditioning. The specific anterograde amnesia for fear to a context after MK-801 infusion into the ventral hippocampus indicates that formation of classical fear conditioning to a context but not to a tone requires activation of NMDA receptor-mediated processes in the ventral hippocampus. Given that NMDA stimulation of the ventral hippocampus disrupts also processes not mediated by NMDA receptors, the complete anterograde amnesia following NMDA infusion into the ventral hippocampus might be due to the concurrent severe disruption of normal ventral hippocampal activity. However, strong stimulation of the ventral hippocampus might also disrupt fear conditioning by interfering with processes in the projection areas of the ventral hippocampus, such as the amygdala or the nucleus accumbens. In addition, we report that MK-801 (6.25 μg/side) infusion into the ventral hippocampus increased locomotor activity in the open field. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: NMDA receptor; Ventral hippocampus; Intracerebral infusion; Fear conditioning; Emotions; Learning and memory; Locomotor activity

1. Introduction

Classical fear conditioning is a prominent model to investigate the neuronal processes underlying learning and memory and the generation of emotions in health and disease [19,28]. In fear conditioning, a rat is trained to associate a neutral stimulus, such as a tone (explicit stimulus) or the environment (contextual stimulus),
with an aversive unconditioned stimulus (US), such as an electric foot shock. The neutral stimulus becomes an aversive conditioned stimulus (CS) predicting the aversive US, and thus eliciting a state of conditioned fear which is reflected by specific behavioral responses, such as freezing [14].

There is a consensus that neuronal processes in the amygdala are crucially involved in fear conditioning to explicit as well as contextual CS. Whereas some consider the amygdala to be the site where the association of CS and US is formed and permanently stored [18,19,28], others hold the view that amygdaloid processes modulate the formation and storage of this association in other brain areas [13]. The nucleus accumbens, together with its dopaminergic innervation from the midbrain, also appears to play an important role in fear conditioning and classical aversive conditioning. For example, it has been found that lesions or temporal inactivation of the nucleus accumbens affect formation of classical aversive conditioning, including fear conditioning [23,47,54,55,58], and that extracellular dopamine is increased in the nucleus accumbens during the conditioning phase of a fear conditioning experiment and afterwards in response to the aversive CS [41,49,59,60].

The ventral hippocampus has intimate reciprocal connections with the amygdala and strong projections to the nucleus accumbens [21,50,57]. Electrical stimulation of the ventral hippocampus has been shown to induce synaptic plasticity in the basolateral amygdala [36], and electrical stimulation as well as chemical stimulation by N-methyl-D-aspartate (NMDA) leads to an increase of extracellular dopamine in the nucleus accumbens [9,12,29,30,39]. Given the strong anatomical and functional interconnectivity of the ventral hippocampus with the amygdala and the nucleus accumbens, both of which seem to be critical for fear conditioning, manipulations of the ventral hippocampus should affect classical fear conditioning.

Hitherto, studies on the involvement of the hippocampus in fear conditioning mainly focused on the dorsal hippocampus and its specific involvement in fear conditioning to a contextual CS. Studies which examined the effect of lesions to the dorsal hippocampus on fear conditioning to explicit and contextual CS indicated a specific role of the dorsal hippocampus in fear conditioning to a contextual CS when freezing was measured as the conditioned response ([16,54], but see [53]) but not when potentiated startle was taken as the measure of conditioned fear [20]. Some studies, however, suggested also an involvement of the ventral hippocampus in fear conditioning. Thus, deficits in freezing to a contextual as well as an explicit CS were found in rats whose ventral hippocampus has been permanently lesioned [33,36,53]. The freezing deficits in these experiments have been proposed to be due to lesion-induced increases in activity rather than to a genuine impairment of learning and memory [53]. However, our recent study involving temporary inactivation or inhibition of ventral hippocampal activity before fear conditioning suggested that the ventral hippocampus is actually involved in the formation of fear memory to a contextual as well as an explicit CS [5].

The present study tested the effects of NMDA receptor stimulation or blockade in the ventral hippocampus of rats on the formation of fear memory to an explicit and a contextual cue. Stimulation of the ventral hippocampus has a strong impact on dopamine transmission in the nucleus accumbens and on synaptic plasticity in the amygdala, both of which seem to be involved in fear conditioning (see above). Moreover, recent results indicated that ventral hippocampal processing is essential for fear conditioning to contextual and explicit cues [5], and NMDA receptor stimulation should strongly interfere with this processing. Therefore, we hypothesized that stimulation of NMDA receptors in the ventral hippocampus would impair the formation of fear conditioning to both contextual and explicit cues. We tested this hypothesis by examining the effects of bilateral NMDA infusion into the ventral hippocampus (0.5 μg/0.5 μl/side) before the conditioning phase of a classical fear conditioning experiment on subsequent freezing in response to explicit and contextual CS. NMDA receptor-mediated processes in the hippocampus have been implicated in learning and memory [10]. There is evidence that such processes are also involved in the learning and memory of fear, in particular of fear to a contextual CS [17,35,56,61]. To test if NMDA receptor-mediated processes in the ventral hippocampus are necessary for the formation of fear memory, we examined how infusion of the non-competitive NMDA antagonist MK-801 (dizocilpine; 6.25 μg/0.5 μl/side) into the ventral hippocampus would affect fear conditioning to an explicit and a contextual CS. Principally, freezing deficits following a particular manipulation could be due to this manipulation simply enhancing activity. This has been put forward in order to account for freezing deficits in fear conditioning experiments following hippocampal lesions [20,53]. Thus, when freezing is used to assess the effects of a manipulation on the learning and memory of fear, it is important to examine the manipulation's effect on activity. We have reported elsewhere that bilateral infusion of NMDA at the concentration used in this study (0.5 μg/0.5 μl/side) temporarily increases locomotor activity in the open field but does not affect locomotor activity on the day following infusion [7]. The effects of MK-801 infusion into the ventral hippocampus (6.25 μg/0.5 μl/side) on locomotor activity in the open field are reported in the present study. The doses of MK-801 and NMDA used in the present study are based on our experience from previous experiments involving intrahippocampal infusions in our Wistar rats [7,62,63].
2. Materials and methods

2.1. Subjects

A total of 120 male Wistar rats (Zur:WIST[HanIbm], Research Unit Schwerzenbach, Switzerland), aged about 3 months and weighing approximately 300 g at the beginning of the experiment served as subjects. They were housed in groups of four per cage under a reversed light–dark cycle (lights on: 19:00–07:00 h) in a temperature (21 ± 1 °C) and humidity (55 ± 5%) controlled room. All animals were allowed free access to food and water. Thirty-three rats were left unoperated and 87 received bilateral implantation of infusion cannulae aiming at the ventral hippocampus. After surgery, all 120 rats were individually caged. Starting 3 days before surgery and then throughout the studies, all rats were handled gently by the experimenter every day to minimize the stress associated with the handling of the animals throughout the experiments. Behavioral testing was carried out in the dark phase of the cycle. All experiments were conducted in accordance with Swiss regulations for animal testing.

2.2. Surgery

Rats were anesthetized with 1 ml/kg body weight of sodium pentobarbital (50 mg/ml, Abbott Labs, North Chicago, IL) and their head placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA). After application of a local anesthetic (lidocaine), an incision was made into the scalp to expose the skull. The vertical coordinates of bregma and lambda were measured in order to align them in the same horizontal plane. A small hole (1.5 mm diameter) was drilled on two sides to reveal the dura covering the cortex overlying the hippocampus. Guide cannulae (26 gauge, 9 mm, stainless steel) were implanted bilaterally into the brain aiming at the following coordinates above the ventral hippocampus (in mm according to [48]): AP = −5.2 posterior, ML = ±5.0 lateral to bregma, and DV = −5.0 ventral to dura. The guide cannulae were fixed to the skull with three anchoring screws and dental cement. Stainless steel stylets which extended 0.5 mm beyond the guide tips were inserted into the guide cannulae to prevent occlusion and removed only for infusions. After surgery, rats were allowed to recover for 5 days during which the experimenters gave the rats daily health checks and gentle handling, and replaced missing stylets.

2.3. Intracerebral infusion

Rats were manually restrained and the stylets removed carefully. Infusion cannulae (34 gauge, stainless steel) were inserted into the guide cannulae so that they protruded 1.6 mm beyond the tip of the guide cannulae to reach the ventral hippocampus. Thus, the final DV coordinate for the ventral hippocampus was 6.6 mm below the dura. The infusion cannulae were connected to 10-µl Hamilton microsyringes by flexible PEEK tubing. The syringes were mounted on a Kds pump. The infusion volume was 0.5 µl/side delivered with a speed of 0.5 µl/min. Following infusion, the infusion cannulae were kept in place for an additional 60 s to allow the injection bolus to be absorbed by the tissue. Stylets were reinserted and rats were placed immediately into the behavioral testing chamber.

2.4. Drugs

NMDA and MK-801 solutions for infusion were prepared freshly on the day of infusion. NMDA (SIGMA, Switzerland) was dissolved in 0.1 M phosphate-buffered saline (pH 7.4), to obtain a solution containing 1 mg/ml for infusion of 0.5 µg NMDA/0.5 µl into each side of the ventral hippocampus. The dose of 0.5 µg NMDA/0.5 µl/side was based on previous experiments in which this dose caused pronounced hyperactivity and disruption of prepulse inhibition without producing seizures [7]. MK-801 (dizocilpine; Merck, Sharp, and Dohme, UK) was dissolved in isotonic 0.9% saline (pH 6) at a concentration of 12.5 mg/ml by slight sonification. A total of 6.25 µg MK-801/0.5 µl were infused into each side of the ventral hippocampus. We have previously shown that this dose of MK-801 increases both startle amplitude and locomotor activity when infused into the dorsal hippocampus of Wistar rats [63].

2.5. Apparatus for behavioral testing

2.5.1. Open field locomotor activity

Locomotor activity was measured in four closed square arenas (76.5 × 76.5 × 49 cm) made of dark gray plastic and placed in a dimly illuminated room (20 ± 0.5 Lux provided by two halogen lights). Behavior in the arenas was recorded by a video-camera suspended from the ceiling and relayed to a monitor and a Video Tracking, Motion Analysis & Behavior recognition system (EthoVision®, Noldus, Wageningen, The Netherlands). The total distance traveled by the rat in the complete arena was calculated by the computer for each 5-min block of testing.

2.5.2. Fear conditioning

Eight operant test boxes (Habitest; Coulbourn Instruments, Allentown, PA) were used. Conditioning and the context test sessions took place in four shock boxes fitted with a parallel grid shock floor (16 parallel bars; E10-10RF; Coulbourn Instruments) through which scrambled shocks could be delivered. The shock
II.2. Experimental design

One week after surgery, behavioral testing began. In all experiments, rats were placed into the behavioral testing chambers immediately after the intracerebral infusions. Experiments were run in groups of four rats. The different testing boxes and the order of testing was counterbalanced among the experimental groups as far as possible. The experimental groups included unoperated rats (UNOP) and cannulated rats to receive infusion of 0.5 μl saline vehicle/side (VEH), 0.5 μg NMDA/0.5 μl/side (NMDA), or 6.25 μg MK-801/0.5 μl/side (MK-801) into the ventral hippocampus. UNOP rats were included to check for unspecific effects of cannulae implantation or infusion procedures on the behaviors under study. NMDA is neurotoxic and, though at much higher doses than that used in the present study, utilized to induce excitotoxic lesions (e.g. [53]). The neurotoxic potential of MK-801 is also well known [44]. Therefore, we included tests to assure that the observed effects of drug infusions were due to acute drug effects and not due to long-term damage to the ventral hippocampus.

2.6.1. Open field experiment

Twenty-four rats, eight UNOP rats and 16 cannulated rats, were used to test the effect of MK-801 infusion (6.25 μg/0.5 ml/side) into the ventral hippocampus on locomotor activity in the open field. The open field experiment comprised 3 successive days. On day 1, each rat was placed in the center of one of the arenas and allowed to habituate to the open field box for 30 min. After matching for baseline locomotor activity on day 1, the 16 cannulated animals were allocated to either the MK-801 (n = 8) or the VEH group (n = 8). On day 2, animals were placed individually into the open field box for 30 min of free exploration. All four rats were then returned to their home cages, infused bilaterally with MK-801 or vehicle, and then replaced into the arena for an additional 30 min of free exploration to check for the immediate effect of MK-801 infusion into the ventral hippocampus on locomotor activity. On day 3, each rat was replaced in the center of one of the arenas for 30 min of free exploration to check for possible long-term effects of the MK-801 infusion. Our open field experiment, including thorough habituation of the rats to the open field prior to infusion to avoid high control levels of exploratory activity following infusion, was designed to be particularly sensitive in revealing an infusion-induced hyperactivity, which could contribute to freezing deficits during the fear conditioning experiments.

2.6.2. Fear conditioning experiments

Three experiments with a total of 108 rats were conducted to test the effects of NMDA (0.5 μg/0.5 μl/side) or MK-801 (6.25 μg/0.5 μl/side) infusion into the ventral hippocampus on the formation of fear conditioning to an explicit CS (auditory stimulus) and a contextual CS (environment).

2.6.2.1. Fear conditioning experiment I. The first fear conditioning experiment was conducted with 13 UNOP, 18 VEH, 13 NMDA, and 15 MK-801 rats. Five VEH and 7 MK-801 rats used in this fear conditioning
experiment had previously been used as VEH or MK-801 rats, respectively, in the open field experiment. For conditioning, rats were put in the shock boxes for a total of 27 min and were exposed to ten pairings of a 30-s auditory CS (85 dB[A]) and a 1-s foot shock (current pulse amplitude: 0.5 mA) at 2-min intervals between an initial and a final 2-min interval. The 1-s foot shock was contiguous with the final second of the CS. The proportion of time spent freezing was calculated for the 11 2-min blocks preceding and following the 30-s CSs and for the duration of each 30-s CS. One day after conditioning, in the context test session, rats were tested for freezing to the contextual CS, i.e. the environment in which they received the foot shocks. For that purpose, rats were placed in the shock boxes for 8 min without being exposed to the auditory CS or the foot shock. Two days after conditioning, in the tone test session, rats were tested for freezing to the auditory CS. For that purpose, they were put in the no-shock boxes for a total of 11 min. After 3 min, the auditory CS was presented for the remaining 8 min. During the context and tone test sessions, the proportion of time spent freezing was calculated for each 1-min block.

To exclude that long-term damage to the hippocampus could account for freezing deficits observed after infusion of MK-801 and NMDA into the ventral hippocampus, we ran the following control experiment. Six VEH, seven MK-801, and seven NMDA rats were reconditioned and retested without receiving infusions before conditioning and with the two test sessions run on the same day, 1 week after the first fear conditioning experiment. Similar conditioned freezing in the VEH as compared with the MK-801 and NMDA groups would verify that long-term damage induced by the drug infusions cannot account for the freezing deficits following the drug infusions before conditioning.

2.6.2.2. Fear conditioning experiment II. The conditioning procedure used in fear conditioning experiment I did not result in marked freezing during the context test session. Therefore we conducted a second fear conditioning experiment to test the effects of NMDA and MK-801 infusion into the ventral hippocampus on the formation of fear conditioning to a contextual CS. In the second fear conditioning experiment, conditioning was conducted with an unsignaled shock, i.e. without an auditory CS, to achieve a strong association between the electrical foot shock and the contextual CS, i.e. the environment in which the rats received the foot shock (compare [43]). In this experiment, 29 rats were used: six UNOP, seven VEH, eight MK-801, and eight NMDA rats. For conditioning, rats were put in the shock boxes for a total of 30 min and 5 s and were exposed to five 1-s foot shocks (current pulse amplitude: 0.5 mA) at 5-min intervals in between an initial and a final 5-min interval. Five-min blocks were chosen to provide sufficient time—in particular before the first block—for the rats to build up a context representation that could be associated with the foot shock (compare [15]). The proportion of time spent freezing was calculated for the six 5-min blocks preceding and following the 1-s foot shock. One day after conditioning, in the context test session, rats were tested for freezing to the contextual CS, i.e. the shock boxes. For that purpose, rats were placed in the shock boxes for 8 min and the proportion of time spent freezing was calculated for each 1-min block.

2.6.2.3. Fear conditioning experiment III. The results of the fear conditioning experiments I and II suggested that MK-801 infusion into the ventral hippocampus blocked formation of fear conditioning to a context (experiment II) but not to a tone (experiment I). In order to corroborate this conclusion, fear conditioning experiment III was conducted to allow a comparison of the effects of MK-801 infusion into the ventral hippocampus on fear conditioning to a context and to a tone within one experiment. Based on the experience from experiments I and II, conditioning parameters were chosen to obtain a similar level of fear conditioning to the context and to the tone. Twenty rats were used for fear conditioning experiment III: six UNOP, seven VEH, and seven MK-801 rats. The conditioning session consisted of five presentations of an unsignaled shock (similar to experiment II) followed by five tone-shock pairings (similar to experiment I). The rats were put in the shock boxes for a total of 35 min. Throughout the first 20 min, the rats were exposed to five unsignaled 1-s foot shocks (0.5 mA) at 2.5-min intervals in between an initial 5-min interval and a final 2.5-min interval. During the remaining 15 min, the rats were exposed to five pairings of a 30-s auditory CS (85 dB[A]) and a 1-s foot shock (current pulse amplitude: 0.5 mA) at 2.5-min intervals between an initial and a final 2.5-min interval. The 1-s foot shock was contiguous with the final second of the CS. The proportion of time spent freezing was calculated for the 2.5-min blocks preceding and following the 1-s foot shock and the 30-s CS, as well as for the duration of each 30-s CS. One day or 2 days, respectively, after conditioning the rats were subjected to context and tone test sessions as in fear conditioning experiment I.

2.7. Histology

After completion of the behavioral experiments, all cannulated rats were deeply anaesthetized with an overdose of Nembutal (150 mg/kg, i.p.) and perfused with 0.9% NaCl solution, followed by 250 ml of 4% formalin (4 °C) to fix the brain tissue. The brains were removed, stored in 4% formalin, and subsequently cut into 40-μm coronal sections on a freezing microtome. For the
verification of the infusion sites, every fifth section through the ventral hippocampus was mounted on gelatin-treated slides and stained with cresyl violet. After staining, the sections were dehydrated through an alcohol series, cleared with xylene, and coverslipped with Eukitt (Kindler, Freiburg, Germany). Subsequently, the sections were examined with a Zeiss Axiosphot light microscope to verify and draw the locations of injector tip placement onto plates taken from the atlas of Paxinos and Watson [48].

2.8. Data analysis

Data were first subjected to analysis of variance (ANOVA), calculated with the StatView and SuperANOVA software system (Abacus Concepts, Berkeley, CA, 1992). Treatment group was taken as between-subjects factor and the different time blocks of testing as repeated measures. For the open field experiment, total distance moved was analyzed. For the fear conditioning experiments, the proportion of time spent freezing was analyzed. Post hoc comparisons were conducted using Fisher's protected least significant difference test. Significant differences were accepted at P<0.05. Total distance moved is given in cm. The proportion of time spent freezing is given as percentage. Values are presented as means. In the text, variability is indicated by the standard error of the mean (S.E.M.). In the figures, for the sake of clarity, the standard error (S.E.) derived from the appropriate mean square of the ANOVA indicates variability.

3. Results

3.1. Histology

The centers of the infusion sites, i.e. the tips of the infusion cannulae, were concentrated within or around the border of the ventral hippocampus (Fig. 1). Damage due to the implantation of the guide cannulae was restricted to the area immediately surrounding the guide cannulae. Based on the histological examinations, the behavioural data of all 87 cannulated rats were included in the statistical analysis.

3.2. Open field experiment: effect of MK-801 infusion into the ventral hippocampus on locomotor activity

After matching, ANOVA carried out on the total distance moved by the UNOP, VEH, and MK-801 groups on day 1 yielded only a significant main effect of time block \( F_{5, 105} = 31.32, P < 0.001 \). This reflects the gradual reduction in activity, i.e. habituation, over time (data not shown).

![Fig. 1. Location of infusion sites in the ventral hippocampus. (A) Photomicrograph of a cresyl violet-stained coronal section from the brain of a rat with representative placement of cannulae in the ventral hippocampus. (B) Reconstructions of coronal cross-sections through the rat brain depicting the approximate locations of the cannulae tips in the ventral hippocampus. ○ represent sites of vehicle infusion (35 rats), □ represent sites of MK-801 infusion (31 rats), and ● represent sites of NMDA infusion (21 rats). Values represent distance in mm from bregma. Sketches are derived from the atlas of Paxinos and Watson [48]. Abbreviations: CA1 field of the hippocampus (CA1); CA3 field of the hippocampus (CA3); entorhinal cortex (Ent); subiculum (S).]
II.2.

W.-N. Zhang et al. / Behavioural Brain Research 126 (2001) 159-174

Fig. 2. Effects of MK-801 infusion into the ventral hippocampus on open field locomotor activity. On the day of infusion, locomotor activity was monitored in the open field 30 min before (left) and following (middle) the infusion of MK-801 (6.25 μg/0.5 μl/site) or vehicle into the ventral hippocampus. On the day after infusion, locomotor activity was monitored in the open field for 30 min (right). There were three groups: unoperated (UNOP; n = 8), vehicle infused (VEH; n = 8) and MK-801 infused (MK-801; n = 8). Locomotor activity is represented as the total distance moved in cm during the consecutive 5-min blocks of the testing periods. The bar represents 1 standard error (S.E.) derived from the ANOVA.

On day 2, total distance moved in the entire open field arena did not differ (F(2, 21) = 0.35, P > 0.7) between the three groups over the 30 min preceding the MK-801 or vehicle infusion (Fig. 2, left panel). ANOVA yielded only a significant main effect of time block (F(5, 105) = 43.12, P < 0.001), reflecting habituation. After infusion of MK-801 and vehicle, MK-801 rats exhibited hyperactivity as compared with the UNOP and VEH groups (Fig. 2, middle panel). ANOVA of the total distance moved in the entire open field arena yielded a significant main effect of treatment (F(2, 21) = 4.91, P < 0.02), again a highly significant main effect of time block (F(5, 105) = 38.82, P < 0.001), reflecting habituation, and a significant interaction of group x time block (F(10, 105) = 1.94, P < 0.05). Post hoc comparisons revealed increased locomotor activity (total distance moved during 30 min following infusion) in the MK-801 group (8126 ± 975) relative to both the VEH (5537 ± 748, P < 0.03) and the UNOP (4893 ± 531, P < 0.01) groups, which did not differ (P > 0.5). The significant interaction of treatment and time block reflects that the hyperactivity in the MK-801 as compared with the UNOP and VEH groups was most pronounced during the first 20 min following infusion.

During the 30 min of open field testing on day 3, the groups did not differ in the total distance moved (F(2, 21) = 0.59, P > 0.5; Fig. 2, right panel). ANOVA yielded only a significant main effect of time block (F(5, 105) = 24.92, P < 0.001), reflecting habituation. Thus, hyperactivity induced by MK-801 infusion into the ventral hippocampus was restricted to about 20 min of testing following infusion.

3.3 Fear conditioning experiment I: effects of NMDA and MK-801 infusion into the ventral hippocampus on fear conditioning to a tone

3.3.1 Conditioning session

During the conditioning session NMDA and MK-801 rats exhibited less freezing than the UNOP and VEH rats. ANOVA of the percentage of time spent freezing during the 11 2-min blocks preceding and following the 30-s CS revealed highly significant main effects of treatment (F(3, 55) = 10.09, P < 0.0001) and interval (F(10, 550) = 14.82, P < 0.0001), as well as a significant interaction of treatment and interval (F(30, 550) = 2.40, P < 0.0001; Fig. 3A). Post hoc comparisons revealed that the percentage of time spent freezing during the 11 2-min blocks was significantly higher in the UNOP (36.1 ± 2.5) and VEH groups (37.7 ± 3.2) than in the NMDA (21.3 ± 3.8, P < 0.01) and MK-801 groups (18.6 ± 2.8, P < 0.01). There was no difference between the UNOP and VEH rats (P > 0.7) or between the NMDA and MK-801 rats (P > 0.5). ANOVA of the percentage of time spent freezing during the ten 30-s periods of CS presentation revealed a highly significant main effect of treatment (F(3, 55) = 14.82, P < 0.0001) and CS presentation (F(9, 495) = 8.84, P < 0.0001), as well as a...
II.2.

Fig. 3. Freezing during the three sessions of fear conditioning experiment I. The four groups were: unoperated (UNOP; n = 13), vehicle infused (VEH; n = 18), NMDA infused (NMDA; n = 13) and MK-801 infused (MK-801; n = 15). Rats were bilaterally infused with MK-801 (6.25 μg/0.5 μl/side), NMDA (0.5 μg/0.5 μl/side) or vehicle into the ventral hippocampus before conditioning. The bar represents 1 standard error (S.E.) derived from the ANOVA. (A) Proportion of time spent freezing during the 11 2-min blocks preceding and following the CS-shock pairings in the conditioning session. (B) Proportion of time spent freezing during the ten 30-s periods of CS presentation in the conditioning session. (C) Proportion of time spent freezing during the eight 1-min blocks of the context test session. (D) Proportion of time spent freezing during the three 1-min blocks preceding CS presentation (B1–3) and the subsequent eight 1-min blocks of CS presentation (B4–11) of the tone test session.
significant interaction of treatment and CS presentation ($F_{27, 495} = 1.81, P < 0.01$; Fig. 3B). Post hoc comparisons revealed that the percentage of time spent freezing during the ten CS presentations was significantly higher in the UNOP (41.1 ± 4.0) and the VEH group (35.2 ± 3.5) than in the NMDA (20.6 ± 3.6, $P < 0.01$) and the MK-801 group (12.2 ± 2.2, $P < 0.0001$). There was no significant difference between the UNOP and VEH rats ($P > 0.2$) or between the NMDA and MK-801 rats ($P > 0.09$).

3.3.2. Context test

Only the UNOP rats exhibited freezing during the context test session of the first fear conditioning experiment (Fig. 3C). ANOVA of the percentage of time spent freezing during the 8 min of the context test session revealed significant main effects of treatment ($F_{3, 55} = 4.52, P < 0.01$) and 1-min block ($F_{7, 385} = 2.70, P < 0.01$), as well as a significant interaction of treatment and block ($F_{21, 385} = 1.86, P < 0.02$). The results of the ANOVA reflected that only the UNOP rats exhibited conditioned fear to the context about 1–2 min after being put into the shock chambers in which conditioning took place. Post hoc tests revealed a significantly higher percentage of time spent freezing in the UNOP group (12.9 ± 2.8) as compared with the NMDA group (3.5 ± 0.6, $P < 0.01$), the MK-801 group (3.7 ± 0.7, $P < 0.01$), and the VEH group (6.5 ± 2.2, $P < 0.05$). The three infusion groups did not differ from each other ($P > 0.2$). The effect of block reflected that conditioned fear in the UNOP rats gradually developed to a maximum at min 3–5, after which extinction of the conditioned fear set in.

3.3.3. Tone test

NMDA rats exhibited markedly reduced conditioned fear to the tone CS in comparison with the UNOP, VEH, and MK-801 groups (Fig. 3D). During the 3 min preceding the tone presentation no group exhibited a considerable amount of freezing and UNOP (1.3 ± 0.5), VEH (0.6 ± 0.2), MK-801 (0.9 ± 0.3) and NMDA (1.7 ± 0.9) rats did not differ in the percentage of time spent freezing during these 3 min ($F_{3, 55} = 1.12, P > 0.3$; Fig. 3D, B1–3). For the percentage of time spent freezing during the 8 min of tone presentation, ANOVA revealed a significant main effect of treatment ($F_{3, 55} = 3.84, P < 0.02$), a highly significant main effect of 1-min block ($F_{7, 385} = 11.30, P < 0.0001$), as well as a significant interaction of treatment and block ($F_{21, 385} = 2.08, P < 0.005$; Fig. 3D, B1–3). The effect of group reflects that freezing to the tone was most pronounced in the UNOP rats, very marked in the VEH and MK-801 rats, but very low in the NMDA group. Moreover, as reflected by the effect of block and the interaction of group and block, a gradual increase of conditioned fear to a maximum at min 4–6 and a subsequent extinction occurred in the UNOP, VEH, and MK-801 rats, whereas in the NMDA group freezing was at a very low level throughout the total 8-min period of CS presentation. Therefore, the differences between the groups in the level of freezing were most pronounced during the three 1-min blocks immediately after onset of the tone. Post hoc comparisons revealed that during these 3 min the NMDA rats (6.9 ± 2.4) spent a significantly lower percentage of time freezing than the UNOP (48.5 ± 8.5; $P < 0.0001$), VEH (26.7 ± 5.9; $P < 0.05$), and MK-801 (31.1 ± 7.1; $P < 0.02$) groups. In addition, conditioned fear, as reflected by the percentage of time spent freezing, was or tended to be higher in the UNOP group as compared with the MK-801 ($P < 0.07$) and the VEH rats ($P < 0.02$), which did not differ ($P > 0.6$). It is noteworthy that the procedure used in fear conditioning experiment I resulted in pronounced conditioned fear to the tone but not to the context. During the 8 min of CS presentation in the tone test session the overall proportion of time spent freezing was 21.5%, whereas it was only 6.6% during the 8 min of the context test.

3.3.4. Control experiment

In the control experiment, the amount of freezing during conditioning and test sessions did not differ between the VEH, MK-801 and NMDA rats (data not shown). Thus, decreased levels of freezing observed after MK-801 and NMDA infusions before conditioning were due to acute drug effects but not to long-term damage to the ventral hippocampus.

3.4. Fear conditioning experiment II: effects of NMDA and MK-801 infusion into the ventral hippocampus on fear conditioning to a context

3.4.1. Conditioning session

During the conditioning session NMDA and MK-801 rats exhibited less freezing than the UNOP and VEH rats (Fig. 4A). ANOVA of the percentage of time spent freezing during the six 5-min blocks preceding and following the 1-s footshocks revealed a highly significant main effect of treatment ($F_{3, 25} = 11.0, P < 0.0001$) and block ($F_{5, 125} = 13.99, P < 0.0001$), as well as a highly significant interaction of treatment and block ($F_{15, 125} = 3.84, P < 0.0001$). Post hoc comparisons revealed that the percentage of time spent freezing during the six 5-min blocks was significantly higher in the VEH group (41.3 ± 4.0) than in the NMDA group (27.2 ± 4.1, $P < 0.05$) and the MK-801 group (10.2 ± 3.6, $P < 0.001$). Although the amount of freezing in the UNOP group (37.1 ± 5.4) was similar to that in the VEH group, the difference as compared with the NMDA and MK-801 groups reached significance only for the latter ($P < 0.001$). Furthermore, MK-801 rats spent a significantly lower percentage of time freezing
than the NMDA group ($P < 0.01$). This was due to the fact that NMDA rats spent a considerable proportion of time (32%) freezing during the first 5-min interval of the conditioning session, whereas all other rats exhibited no freezing. This high amount of freezing did not reflect conditioned fear but rather immobility as an immediate, short-lasting effect of the NMDA infusion in some NMDA rats.

### 3.4.2. Context test

Both NMDA and MK-801 rats exhibited less conditioned fear to the context than the UNOP and the VEH rats (Fig. 4B). ANOVA of the percentage of time spent freezing during the total 8 min of the context session yielded a significant interaction of treatment and 1-min block ($F_{1,175} = 1.97$, $P < 0.01$) and a highly significant main effect of block ($F_{7,175} = 4.13$, $P < 0.001$). In addition, the main effect of treatment approached significance ($F_{3,25} = 2.33$, $P = 0.09$). The significant effect of 1-min block and the significant interaction of treatment and block reflect that the proportion of time spent freezing strongly increased during the first 2 min of the context test session in the VEH group and during the first 4–6 min in the UNOP groups and strongly decreased from the sixth to seventh minute in the UNOP group, whereas the MK-801 and NMDA rats exhibited a rather stable low level of freezing ($< 15\%$) throughout the total 8 min. Both UNOP and VEH rats spent a markedly higher percentage of time freezing during the first 6 min of the context test session than the MK-801 and NMDA rats. Indeed, ANOVA of the percentage of time spent freezing during the first 6 min revealed a significant main effect of treatment ($F_{3,25} = 3.01$, $P < 0.05$), in addition to the significant main effect of block ($F_{5,125} = 6.54$, $P < 0.0001$) and the significant interaction of treatment and block ($F_{15,125} = 1.93$, $P < 0.05$). Post hoc comparisons further confirmed that the percentage of time spent freezing during the first 6 min was lower in the MK-801 group (5.8 ± 2.9; $P < 0.05$) and in the NMDA group (8.3 ± 4.4; $P < 0.06$ and $P < 0.07$, respectively) than in the UNOP group (24.6 ± 7.9) and the VEH group (23.1 ± 7.3).

### 3.5. Fear conditioning experiment III: different effects of MK-801 infusion into the ventral hippocampus on fear conditioning to a context and tone—a within-experiment comparison

#### 3.5.1. Conditioning session

Following presentation of the first shock, MK-801 rats exhibited less freezing than the UNOP and VEH rats (Fig. 5A, B). ANOVA of the percentage of time spent freezing during the 13 2.5-min blocks preceding and following the foot shocks or the tone-shock pairings (Fig. 5A) revealed a highly significant main effect of treatment ($F_{2,7} = 9.66$, $P < 0.002$) and block ($F_{12,204} = 21.95$, $P < 0.0001$), as well as a significant interaction of treatment and block ($F_{24,204} = 1.73$, $P < 0.05$). The main effect of block and the interaction of treatment and block reflected a gradual increase of freezing throughout the first two shock presentations that was stronger in the UNOP and VEH groups than in the MK-801 group. Post hoc comparisons revealed that the percentage of time spent freezing during the 13 2.5-min blocks was significantly higher in the UNOP group (49.4 ± 3.2) and the VEH group (45.9 ± 5.0) than in the MK-801 group (22.3 ± 5.4, all $P < 0.003$). ANOVA of the percentage of time spent freezing during the five 30-s periods of auditory CS presentation (Fig. 5B) revealed a highly significant main effect of treatment ($F_{2,17} = 13.17$, $P < 0.001$) and CS presentation ($F_{5,68} = 3.76$, $P < 0.01$). The significant effect of CS presentation, which was not affected by treatment (interac-
II.2.


169

tion: $F_{8,68} = 0.98, P > 0.45$), reflected a slight, gradual increase of the freezing response to the CS with repeated presentations of the CS-shock pairings in all groups. Nevertheless, post hoc comparisons revealed that the percentage of time spent freezing during the five CS presentations was significantly higher in the UNOP (48.7 ± 6.5) and the VEH group (37.4 ± 6.7) than in the MK-801 group (8.0 ± 3.8, all $P < 0.002$). There was no significant difference between the UNOP and VEH rats ($P > 0.19$).

3.5.2. Context test

MK-801 rats exhibited less conditioned fear to the context than the UNOP and VEH rats (Fig. 5C). ANOVA of the percentage of time spent freezing during the 8 min of the context test session revealed significant main effects of treatment ($F_{2,17} = 8.63, P < 0.005$) and 1-min block ($F_{7,119} = 4.56, P < 0.001$), as well as a significant interaction of treatment and block ($F_{14, 119} = 1.79, P < 0.05$). The significant effect of 1-min block and the significant interaction of treatment and block reflected that, in the UNOP and VEH rats, conditioned freezing gradually increased during the first 3–4 min of the context test session and then gradually decreased from the fifth minute—reflecting extinction—whereas the MK-801 rats exhibited a rather stable low level of freezing (< 10%) throughout the total 8 min. Post hoc comparisons revealed a significantly higher percentage of time spent freezing in the UNOP group (51.4 ± 10.7, $P < 0.001$) and the VEH group (34.4 ± 8.3, $P < 0.02$) as compared to the MK-801 group (6.3 ± 3.0). The UNOP group and the VEH group did not differ significantly from each other ($P > 0.14$).

3.5.3. Tone test

MK-801, UNOP, and VEH rats all exhibited similar conditioned fear to the tone CS (Fig. 5D). During the 3 min preceding the tone presentation, no group exhibited a considerable amount of freezing, and the UNOP (10.5 ± 4.7), VEH (7.2 ± 2.4) and MK-801 (2.7 ± 1.3) rats did not differ in the percentage of time spent freezing during this period ($F_{2,17} = 1.78, P > 0.19$; Fig.

![Fig. 5. Freezing during the three sessions of fear conditioning experiment III. The three groups were: unoperated (UNOP; $n = 6$), vehicle infused (VEH; $n = 7$) and MK-801 infused (MK-801; $n = 7$). Rats were bilaterally infused with MK-801 (6.25 µg/0.5 µl/side) or vehicle into the ventral hippocampus before conditioning. The bar represents 1 standard error (S.E.) derived from the ANOVA. (A) Proportion of time spent freezing during the two 2.5-min blocks preceding the shocks (B1–2), the 2.5-min blocks following each of the five unsignalad shocks (B3–7), and the 2.5-min blocks preceding and following each of the five tone-shock pairings (B8–13) in the conditioning session. (B) Proportion of time spent freezing during the five 30-s periods of CS presentation in the tone conditioning period of the conditioning session. (C) Proportion of time spent freezing during the eight 1-min blocks of the context test session. (D) Proportion of time spent freezing during the three 1-min blocks preceding CS presentation (B4–11) in the tone test.]
II.2.

W.-N. Zhang et al. / Behavioural Brain Research 126 (2001) 159-174

5D, B1–3). For the percentage of time spent freezing during the 8 min of tone presentation (Fig. 5D, B4–11), ANOVA revealed only a significant interaction of treatment and block ($F_{16, 119} = 2.03, P < 0.05$), but neither a significant effect of treatment ($F_{2, 17} = 1.42, P > 0.25$) nor of 1-min block ($F_{2, 119} = 1.20, P > 0.3$). The interaction of treatment and block reflected that during the last 4 min of testing the UNOP rats, in contrast to the MK-801 and VEH rats, did not exhibit extinction of conditioned fear to the continuous CS presentation. Nevertheless, in agreement with the outcome of the ANOVA, post hoc comparisons did not reveal a significant difference in freezing levels during the 8 min of tone presentation between the UNOP (58.8 ± 12.0), MK-801 (35.7 ± 9.8), and VEH (35.8 ± 10.9) rats (all $P > 0.15$).

4. Discussion

The present study indicates that stimulation or blockade of NMDA receptors in the ventral hippocampus impairs the formation of fear memory by classical fear conditioning. NMDA infusion (0.5 μg/0.5 μl/side) into the ventral hippocampus before fear conditioning resulted in reduced freezing to a contextual and a tone CS, 1 or 2 days, respectively, later. In contrast, infusion of the noncompetitive NMDA antagonist MK-801 (6.25 μg/0.5 μl/side) into the ventral hippocampus prior to conditioning specifically decreased freezing to a contextual CS. The results of our control experiment demonstrated that the anterograde amnesia of fear observed after NMDA and MK-801 infusion into the ventral hippocampus was temporary and due to the acute drug effect but not to lesions induced by the drug infusions. In addition, we found that MK-801 infusion into the ventral hippocampus increased locomotor activity in the open field. This hyperactivity was only evident for about 20–25 min following infusion but not 1 day later.

The locomotor hyperactivity following MK-801 infusion into the ventral hippocampus is in line with the hyperactivity observed after systemic administration of MK-801 (e.g. [32,45]). The present finding and previous studies indicate that hyperactivity induced by systemic MK-801 is mediated by blockade of NMDA receptors in the ventral hippocampus (present study), the dorsal hippocampus [63], the nucleus accumbens, and the prefrontal cortex [38].

Increased locomotor activity following infusion of the NMDA antagonist MK-801 into the ventral hippocampus seems at first glance to be incongruous with the same effect being observed after NMDA infusion into the ventral hippocampus (e.g. [7]). However, locomotor activity is not only increased by NMDA stimulation of the ventral hippocampus, but also by stimulating the ventral hippocampus by excitatory amino acids [3], by the acetylcholine agonist carbachol [11], and by the GABA$_A$ antagonist picrotoxin [4]. The NMDA antagonist MK-801 might indirectly cause a stimulation of the ventral hippocampus by blocking NMDA receptors of inhibitory GABA-interneurons (for review see [44]). Stimulation of the ventral hippocampus induced electrically and by NMDA leads to increased extracellular dopamine in the nucleus accumbens [9,12,29,30,39]. In agreement with the general view of the function of accumbal dopamine [31], the concomitant increase of dopamine receptor stimulation within the nucleus accumbens is thought to be responsible for the increase in locomotor activity by NMDA stimulation of the ventral hippocampus [3,7,12]. Thus, even though no increase of extracellular dopamine was found in the nucleus accumbens following infusion of 0.35 and 3.4 μg into one ventral hippocampus of anesthetized rats [39], the increased locomotor activity observed in the present study following MK-801 infusion (6.25 μg/side) into the ventral hippocampus might indicate that this manipulation stimulates meso-accumbal dopamine transmission.

The results of our fear conditioning experiments provide further evidence for an involvement of the ventral hippocampus in fear conditioning to explicit and contextual CS, which has been suggested by recent lesion studies [33,36,53] and, in particular, by our finding of different anterograde amnesias of fear following the infusion of deactivating substances (tetrodotoxin and muscimol) into the ventral hippocampus [5]. Decreased freezing during the time blocks surrounding the CS and US presentations in the conditioning session, as observed after NMDA and MK-801 infusion in the present study, is often taken to reflect a deficit in contextual memory (e.g. [36]). The locomotor hyperactivity induced by infusion of MK-801 (present study) and NMDA [7] might account at least for a part of the freezing deficits in the conditioning session. This hyperactivity, however, cannot account for the freezing deficits in the MK-801 and NMDA rats during the test sessions 1 or 2 days after infusion, because it disappeared 1 day following infusion (present study; [7]). Another factor that might account for the reduced conditioned fear in the MK-801 and NMDA rats is state dependency [46], i.e. that the ventral hippocampus was in a different drug state during conditioning and testing. With drug infusions that affect activity and freezing as a measure of conditioned fear, it is very difficult to control for this factor (compare also [5]). The appropriate control groups would have to receive MK-801 and NMDA infusions before the test session and this would probably result in decreased freezing due to hyperactivity induced by the drug infusion. However, several studies showed that state-dependent learning does not account for the effects of intraamyg-
daloid microinfusions on fear conditioning [22,24,40]. Altogether, the freezing deficits during the test sessions observed in the MK-801 and the NMDA group seem to reflect an actual deficit in the specific associative processes underlying the formation of fear memory due to the infusion of MK-801 and NMDA before the conditioning session. Formation of long-lasting memory comprises the initial acquisition, a short-term consolidation, believed to be completed within seconds to tens of minutes, and the subsequent long-term consolidation of an association [37,42]. Although we infused the rats immediately before conditioning, we cannot exclude that the action of NMDA and MK-801 in the ventral hippocampus extended beyond the 30 min of the conditioning session. Thus, it is possible that the deficits in fear memory observed in the test sessions 1 or 2 days later are due to the fact that NMDA and MK-801 disturbed the long-term consolidation of the fear memory acquired during conditioning. The importance of NMDA receptor-mediated processes in the hippocampus for the consolidation of emotional memory, especially when contextual information is involved, has been indicated by previous studies. Systemic administration of the NMDA antagonist agmatine up to 6 h after conditioning produced retrograde amnesia of fear to a context [56], and immediate, but not delayed (30–180 min), infusion of the competitive NMDA antagonist aminophosphopentaneic acid (AP5) into the dorsal hippocampus after training led to retrograde amnesia of an inhibitory avoidance task [25,26]. However, the effects of MK-801 and NMDA infusion into the ventral hippocampus on open field activity were most evident during the first 20–30 min following infusion (present study; [7]). Thus, it is likely that the deficits in fear memory were mainly due to drug actions within about 30 min following infusion, i.e., during the conditioning session when acquisition and short-term consolidation of fear memory took place. However, as long as an impact on long-term consolidation cannot be excluded, we can state that blockade and stimulation of NMDA receptors in the ventral hippocampus interfere with the formation, i.e., acquisition, short-term, and/or long-term consolidation, of fear memory.

While NMDA receptor blockade in the ventral hippocampus only impaired the formation of fear memory to a context, NMDA receptor stimulation in the ventral hippocampus impaired fear memory to both context and tone. Thus, NMDA receptor stimulation in the ventral hippocampus might have an impact on basic processes which are common to fear conditioning to an explicit and a contextual CS. Interestingly, increasing the activity of the ventral (but not dorsal) hippocampus by electrical kindling has been found to result in impairments in active avoidance learning, another form of aversive conditioning [8]. The connections of the ventral hippocampus to the amygdala or to the nucleus accumbens [21,30,57] might constitute the access of the ventral hippocampus to the basic processes of fear conditioning. Plasticity in the amygdala is known to be critical for fear conditioning in general [13,18,19,28] and stimulation of the ventral hippocampus has been demonstrated to have an impact on amygdaloid plasticity [36]. Moreover, changes of extracellular dopamine in the nucleus accumbens seem to be involved in fear conditioning [41,49,59,60], and NMDA stimulation of the ventral hippocampus has been shown to increase extracellular dopamine in the nucleus accumbens [12,29,30,39]. Thus, NMDA stimulation of the ventral hippocampus might disrupt the formation of fear memory by interfering with basic processes in the amygdala and/or the nucleus accumbens. This interpretation is in line with a modulatory role of the hippocampus in the formation of fear memory [18,19,28,36]. It has also to be considered that stimulation of NMDA receptors in the ventral hippocampus strongly interferes with regular processing in the ventral hippocampus. Our recent study indicated that neuronal processes in the ventral hippocampus might be essential for the formation of fear conditioning to contextual and explicit cues [5]. Thus, the mere disruption of regular processing in the ventral hippocampus might also contribute to the general anterograde amnesia of fear conditioning following NMDA stimulation of the ventral hippocampus.

As an alternative to a modulatory role of the hippocampus in fear conditioning [18,19,28,36], it might be that the hippocampus is the place where fear memory or parts of it are stored. Abrerrant local NMDA receptor activation might then lead to anterograde amnesia by interfering with long-term potentiation, which is believed to be involved in hippocampal memory storage [10]. If appropriate NMDA receptor activation in the ventral hippocampus were important for the storage of fear memory in general, local blockade of NMDA receptors should lead to the same general anterograde amnesia of fear as local NMDA receptor stimulation. MK-801 infusion into the ventral hippocampus, however, resulted in anterograde amnesia selective for fear to a context. Thus, the selective effects of the MK-801 infusion, in addition to the evidence for complete amnesia of fear after amygdala lesions ([18,19,28], but see [13]), rule out that fear memory is completely stored by means of NMDA receptor-mediated mechanisms within the ventral hippocampus. However, our results are compatible with the view that fear memory with a contextual component is stored in the ventral hippocampus by means of NMDA receptor-mediated processes. Based on a review of data from studies which involved different learning tasks, Nadel and Moscovich [42] concluded that extensive lesions of the hippocampus, involving the ventral hippocampus, can induce extended retrograde amnesia.
This led them to propose that, against the standard model viewing the hippocampus as a temporary memory system, the hippocampus can have a permanent role in the storage of memory traces. However, this proposition has been disputed [27] and only a time-limited retrograde amnesia of fear to a context was reported after lesions of the dorsal hippocampus [2]. The role of the ventral hippocampus in the storage of fear memory to a context remains to be examined in studies involving lesions of the ventral hippocampus at several time points following conditioning. In view of the fact that ventral hippocampal lesions may induce hyperactivity [53], measures of conditioned fear that are less influenced by activity changes than is freezing might be used in these studies.

One prominent notion of the hippocampus's role in fear conditioning is that the hippocampus processes contextual information and conveys it to the amygdala where it is associated with the US [19,28,36,61]. Our results are consistent with the view that, in order to elicit conditioned fear in a later stage, contextual information has to be processed by NMDA receptor-mediated mechanisms in the ventral hippocampus to become associated with an aversive CS [61].

As mentioned above, MK-801 might indirectly lead to a stimulation of the ventral hippocampus [44]. Nevertheless, MK-801 infusion into the ventral hippocampus impaired only fear conditioning to a context but not to a tone, whereas direct ventral hippocampal stimulation by NMDA resulted in a general anterograde amnesia of fear. Moreover, in previous studies we found that other processes (startle reactivity and prepulse inhibition of the startle response) were also affected by NMDA but not MK-801 infusion into the ventral hippocampus [6,7]. The different effects of MK-801 and NMDA infusion may nonetheless be congruent with the assumption that both substances induce a stimulation of the ventral hippocampus. The putative indirect stimulation by MK-801 is probably much milder than the direct stimulation by NMDA. Moreover, the stimulation induced by MK-801 surely differs also qualitatively from that induced by NMDA. In any case, it would be worthwhile to examine the different neurochemical and neurophysiological alterations in the ventral hippocampus and its projection areas induced by infusion of MK-801 and NMDA into the ventral hippocampus. Relating the different neurochemical and physiological changes to the different behavioral effects promises interesting insights into the neuronal basis of the respective behavioral processes, including the formation of fear memory.

A last issue to be addressed is the observed tendency of the unoperated rats to exhibit increased conditioned freezing as compared to the infusion groups. We do not think that this is related to lesions induced by the cannulae implantations or the intracerebral infusions. Rather, it fits with the experience from our lab that unoperated rats generally tend to exhibit a stronger behavioral suppression after aversive conditioning than rats which had previously experienced implantation and infusion procedures. Thus, unoperated rats often show stronger behavioral suppression than the infusion groups in the test sessions of the conditioned emotional response task (e.g. [63]). In addition, in fear conditioning experiments, unoperated rats tend to exhibit higher levels of freezing than rats that are implanted with microdialysis probes to take samples from the nucleus accumbens (M. Pezze, personal communication).

In conclusion, the present data support the notion emerging from recent studies [5,33,36,53] that classical fear conditioning depends on activity not only of the dorsal but also of the ventral hippocampus, and that the hippocampal involvement in classical fear conditioning is not restricted to processing of contextual cues. The selective amnesia of fear to a context after infusion of the noncompetitive NMDA antagonist MK-801 into the ventral hippocampus indicates that NMDA receptor-mediated mechanisms in the ventral hippocampus are necessary for learning fear to a context. These mechanisms might be required to store memory of fear to a context in the ventral hippocampus or to process contextual information required to form the association of a contextual CS and an aversive US which is stored elsewhere in the brain. Since normal neuronal activity of the ventral hippocampus has been suggested to be required for the formation of fear conditioning to contextual as well as to explicit cues [5], the general anterograde amnesia of fear after NMDA stimulation of the ventral hippocampus might be due to the severe disruption of normal neuronal activity in the ventral hippocampus concurrent with this manipulation. Stimulation of the ventral hippocampus might also prevent the formation of conditioned fear by interfering with processes in the projection areas of the ventral hippocampus, such as the amygdala or the nucleus accumbens. Such a mechanism might be relevant under normal conditions since all kinds of sensory and emotional information from subcortical and cortical areas converge in the hippocampus [1], and therefore the ventral hippocampus might provide a pathway via which cognitive processes and experiences could exert an impact on basic mechanisms of fear conditioning.

Acknowledgements

This research was supported by a grant from the Swiss Federal Institute of Technology, Zurich. The authors thank the animal facility team for their care of the animals, Ms Liz Weber for her histology preparations, Mr Peter Schmid for the setup and maintenance
of the computerized systems for behavioral analysis, and Ms Bonnie Strehler for her assistance with manuscript preparation.

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II.2.


Annotations:
Dorsal hippocampus and classical fear conditioning to tone and context in rats: The
effects of local NMDA-receptor blockade and stimulation

Tobias Bast, Wei-Ning Zhang, Joram Feldon

Hippocampus, in press
Abstract

Consistent with the hippocampus being important for learning more complex stimulus relations, but not for simple associative learning, the dorsal hippocampus has commonly been implicated in classical fear conditioning to context, but not to discrete stimuli, such as a tone. Particularly, a specific and central role in contextual fear conditioning has been attributed to mechanisms mediated by dorsal hippocampal NMDA-type glutamate receptors. The present study characterized the effects of blockade or tonic stimulation of dorsal hippocampal NMDA receptors by bilateral local infusion of the noncompetitive NMDA receptor antagonist MK-801 (dizocilpine maleate; 6.25µg/side) or of NMDA (0.7µg/side), respectively, on classical fear conditioning to tone and context in Wistar rats. Freezing was used to measure conditioned fear. Regardless of whether conditioning was conducted with tone-shock pairings or unsignaled footshocks (back- or foreground contextual conditioning), both NMDA and MK-801 infusion before conditioning resulted in reduced freezing during subsequent exposure to the conditioning context. Freezing during subsequent tone presentation in a new context, normally resulting from conditioning with tone-shock pairings, was not impaired by MK-801, but strongly reduced by NMDA infusion before conditioning; such freezing was also reduced by NMDA infusion before tone presentation (in an experiment involving NMDA infusions before conditioning and subsequent tone presentation to assess the role of state dependent learning). It was assessed whether unspecific infusion effects (altered sensorimotor functions, state dependency) or infusion-induced dorsal hippocampal damage contributed to the observed reductions in conditioned freezing. Our data suggest that formation of fear conditioning to context, but not tone, requires NMDA receptor-mediated mechanisms in the dorsal hippocampus. As indicated by the effects of NMDA, some dorsal hippocampal processes may also contribute to fear conditioning to tone. The role of the dorsal hippocampus and local NMDA receptor-mediated processes in fear conditioning to tone and context was discussed in comparison with ventral hippocampal processes.

Key words: freezing; glutamate; intracerebral microinfusion; learning and memory; MK-801
II.3.

Introduction

In classical fear conditioning in rats, a conditioned stimulus (CS) is paired with an inescapable aversive unconditioned stimulus (US), such as an electrical foot-shock, so that it elicits conditioned fear responses, such as freezing. A discrete stimulus, such as a tone, as well as the environmental context can serve as CS. With a discrete CS, fear conditioning is simple associative learning. With a contextual CS, it may additionally involve relational learning to form a unified representation of environmental stimuli and their mutual relations. Such relational learning has been linked to processes underlying the formation of spatial and human declarative, in particular episodic, memory and, in contrast to simple associative learning, has commonly been associated with the hippocampus (Nadel and Willner, 1980; Eichenbaum, 1996; Fanselow, 2000; Anagnostaras et al., 2001; Kandel, 2001; Morris, 2001).

Recent studies yielded strong evidence that the ventral hippocampus is not only involved in contextual fear conditioning, but also in simple fear conditioning to tone (Maren, 1999; Richmond et al., 1999; Bast et al., 2001b,d; Zhang et al., 2001). It is still the common view, however, that the dorsal hippocampus, which indeed may differ functionally from the ventral hippocampus (Moser and Moser, 1998; Zhang et al., 2002), contributes to contextual fear conditioning by supporting a unified context representation, but is not required for fear conditioning to tone (Fanselow, 2000; Anagnostaras et al., 2001; Gale et al., 2001; Rudy and O'Reilly, 2001; Wallenstein and Vago, 2001; but see Maren et al., 1997). In particular, fear conditioning to context, similar to spatial and episodic-like learning (Morris et al., 1989; Steele and Morris, 1999; Lee and Kesner, 2002; but see Cain, 1997), has been suggested to require processes mediated by NMDA-type glutamate receptors in the dorsal hippocampus, and prevalent concepts of fear conditioning to context imply a central contribution of NMDA receptor-mediated synaptic plasticity in the dorsal hippocampus to context representation (Fanselow et al., 1994; Young et al., 1994; Anagnostaras et al., 2001; Stiedl et al., 2000; Gale et al., 2001). However, while we recently provided respective data for the ventral hippocampus (Zhang et al., 2001), it remains to be demonstrated directly that blockade of NMDA receptors in the rat dorsal hippocampus impairs only fear conditioning to context, but not to tone.

The four experiments (Exp. 1-4) of the present study further examined the role of the dorsal hippocampus and local NMDA receptor-mediated processes in classical fear conditioning to tone and context in Wistar rats. Freezing was used as a measure of conditioned fear. NMDA receptor-mediated signaling in the dorsal hippocampus was manipulated by local microinfusion of the non-competitive NMDA receptor antagonist MK-
801 (dizocilpine), which blocks the pore of the receptor channel, or the prototypic and selective NMDA receptor agonist NMDA (Collingridge and Lester, 1989). MK-801 impairs any kind of NMDA receptor-mediated signaling. NMDA tonically stimulates NMDA receptor-mediated transmission and thereby disrupts processes depending on the temporal and synaptic order of NMDA receptor activation, such as NMDA receptor-mediated synaptic plasticity (Martin et al., 2000). Moreover, by inducing strong excitation of the local neuronal network, NMDA in the dorsal hippocampus may also interfere with coordinated dorsal hippocampal neurotransmission not primarily mediated by NMDA receptors. Prevalent concepts of the role of the dorsal hippocampus and local NMDA receptor-mediated signaling in classical fear conditioning (see above) would predict that both MK-801 and NMDA in the dorsal hippocampus impair fear conditioning to context, but not to tone. Part of the presented results has previously been published in a preliminary form (Bast et al., 2001a).

Methods

Subjects

One hundred and forty male adult Wistar rats (Zur:Wist[HanIbm]; Research Unit Schwerzenbach, Schwerzenbach, Switzerland), weighing about 250-300 g and about 10 weeks old at the time of surgery, were used for the four experiments of the present study (Exp. 1, 2, and 4: 40 rats each; Exp. 3: 20 rats). Animals were housed under a reversed light-dark cycle (lights on: 19:00-07:00) in a temperature (21 ± 1°C) and humidity (55 ± 5%) controlled room and allowed free access to food and water. All rats received bilateral implantation of guide cannulae aiming at the dorsal hippocampus. Before surgery, rats were housed in groups of four per cage; after surgery, they were individually caged. Beginning three days before surgery and thereafter until the beginning of the behavioral experiments, all rats were handled daily. All experimental procedures were carried out in the dark phase of the cycle. The Principles of Laboratory Animal Care (NIH publication no. 86-23, revised 1985) and Swiss regulations for animal experimentation were followed.

Implantation of guide cannulae for intracerebral infusions

Rats were anesthetized with 1 ml of Nembutal (sodium pentobarbital, 50 mg/ml, Abbott Labs, North Chicago, IL) per kg body weight and their head was placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA). After application of a local anesthetic (Lidocaine), the scalp was incised to expose the skull, and bregma and lambda were aligned in the same horizontal plane. A pair of guide cannulae (9 mm, 26 gauge, stainless steel) in a Perspex
II.3.

holder was implanted through small holes (1.5 mm diameter) drilled on each side of the skull. The tips of the guide cannulae aimed at the following coordinates above the dorsal hippocampus (in mm): 3.0 posterior and ±1.5 lateral to bregma, and 2.5 ventral to dura. These coordinates have been used in previous studies examining the behavioral effects of dorsal hippocampal infusions (Zhang et al., 2000, 2002). The guide cannulae were fixed with dental cement for which three small stainless screws, previously screwed into the skull, served as anchors. Stainless steel stylets (34 gauge) which extended 0.5 mm beyond the tips of the guide cannulae were placed inside the guide cannulae to prevent occlusion. After surgery, the health of the rats was checked daily and lost stylets were replaced. The behavioral experiments commenced seven days after surgery.

**Intracerebral microinfusions and drugs**

For microinfusions into the dorsal hippocampus, rats were manually restrained and the stylets removed from the guide cannulae. Infusion cannulae (34 gauge, stainless steel), connected via flexible polyetheretherketone (PEEK) tubing to 10-μl Hamilton microsyringes mounted on a microinfusion pump (KD scientific or WPI sp200i), were then inserted into the guide cannulae. The tips of the infusion cannulae protruded 1.5 mm from the guide cannulae into the dorsal hippocampus, thus aiming at a final dorsoventral coordinate of 4 mm below dura. The rats were bilaterally infused with NMDA (0.7 μg; N-methyl-D-aspartate; C₅H₉NO₄; Sigma, Switzerland) or MK-801 (6.25 μg; dizocilpine maleate; C₁₆H₁₅NC₄H₄O₄; Merck, Sharp & Dohme, UK) in 0.5 μl vehicle (0.9% saline), or with 0.5 μl vehicle (VEH) only per side. The infusion rate was 0.5 μl/min. To allow for absorption of the infusion bolus by the brain tissue, infusion cannulae were left in the brain for 60 s after infusion before being replaced by the stylets. Immediately (Exp. 1 to 3) or 4 min (Exp. 4) after that, rats were subjected to the experimental sessions. Drug solutions for infusions were freshly prepared on the day of infusion. Solution of MK-801 in isotonic 0.9% saline at the required concentration was facilitated by slight sonification. Doses and time points for the drug infusions were chosen on the basis of our previous experiments (Zhang et al., 2000, 2002). Dorsal hippocampal NMDA infusion at the same dose as used in the present study resulted in a slight decrease of startle reactivity, which disappeared within 24 h after infusion, while not affecting prepulse inhibition of the startle reflex or locomotor activity in the open field (Zhang et al., 2002). Importantly, the NMDA dose used in the present study does not induce convulsions and, in a study developing a rat model of hippocampal seizures, much higher doses of NMDA (5-30 μg/side) have been infused into the dorsal hippocampus in order to induce convulsions.
II.3.

(Hallak et al., 1993). Dorsal hippocampal MK-801 infusion at the dose used in the present study increased startle reactivity as well as locomotor activity in the open field (Zhang et al., 2000). Both effects disappeared within 24 h after infusion.

**Histological examination of cannula placements and infusion-induced neuronal damage**

After completion of the behavioral experiments, all brains were examined histologically in order to verify correct cannula placement. In view of the neurotoxic potential of NMDA (Hajos et al., 1986) and MK-801 (Olney et al., 1989), some brains were subjected to a closer histological analysis in order to examine whether NMDA and MK-801 caused any additional neuronal damage as compared to VEH infusions. For that purpose, we used immunohistochemical staining of the neuronal marker protein NeuN in order to selectively and unequivocally visualize neuronal cells (Wolf et al., 1996; Jongen-Rêlo et al., 2002).

For verification of cannulae placements, rats were deeply anesthetized with an overdose of 2.5 ml/kg Nembutal (sodium pentobarbital, 50 mg/ml, i.p.) and transcardially perfused with 0.9% saline at room temperature to rinse out the blood, followed by about 100 ml of 4% formalin (4°C) to fix the brain tissue. During perfusion, the aorta was clamped. After extraction from the skull, the brains were post-fixed in 4% formalin solution and subsequently cut into 40-μm coronal sections on a freezing microtome. Every fifth section through the dorsal hippocampus was mounted on a gelatine-treated slide and stained with cresyl violet. After staining, the sections were dehydrated and coverslipped. Subsequently, they were examined with a light microscope to verify that the tips of the infusion cannulae were placed in the dorsal hippocampus, and their approximate locations were noted onto corresponding plates taken from the rat brain atlas of Paxinos and Watson (1998).

For comparison of neuronal damage induced by one single infusion of VEH (n=2), NMDA (n=2), or MK-801 (n=4), rats were transcardially perfused three to four weeks after the infusion. Perfusion was conducted as above. However, the 4% formalin solution was prepared freshly in 0.1 M phosphate-buffered solution (pH 7.2; PBS). Further, it was ensured that the flow rate (14 ml/min) as well as the volume of saline (28 ml) and formalin (140 ml) were the same for each rat in order to allow comparison of histological results. After extraction from the skull, the brains were post-fixed in 4% formalin for at least one week and then transferred into a cryoprotectant solution of 30% sucrose in PBS. Serial 40-μm coronal sections were prepared with a freezing microtome and collected in PBS. One series was mounted on gelatine-treated slides, stained with cresyl violet, dehydrated and coverslipped. An adjacent series was subjected to immuno-histochemical staining of the neuronal marker protein NeuN.
according to the immuno-ABC technique, using a primary monoclonal mouse antibody against NeuN (mNeuN, Chemicon, Switzerland) and a biotinylated secondary antibody (biotinylated horse and mouse IgG, Vector, Switzerland), before being mounted on slides and coverslipped (see Jongen-Rêlo et al., 2002 for further details). For assessment of neuronal damage in the hippocampus, the brain sections were examined with a light microscope. A digital camera controlled by Neurolucida software was used to prepare photomicrographs of immuno-stained tissue for documentation.

Apparatus for behavioral testing

Eight operant test boxes, four shock boxes and four no-shock boxes, (Habitest; Coulborn Instruments, Allentown, PA) were used. Shock boxes were used for conditioning and context-test sessions, while the no-shock boxes were used to assess fear to the tone CS in an environment distinct from that during conditioning (see Basic experimental design). Shock boxes were fitted with a parallel grid shock floor (16 parallel bars; E10-10RF; Coulborn Instruments), through which scrambled shocks could be delivered, and placed in light- and sound-attenuating chambers measuring 55 cm x 40 cm x 55 cm. These chambers had two side walls of aluminium and a rear and front wall of clear Perspex. A white waste tray was situated below the grid floor. The four no-shock boxes were fitted with a lattice grid (E10-18NS; Coulborn Instruments), were each placed in a light- and sound-attenuating chamber measuring 72 cm x 45 cm x 45 cm, and had three black walls and a front wall of clear Perspex. A brown waste tray was situated below the lattice grid. The four shock and the four no-shock boxes were placed in two different rooms. Presentation of the tone CS and delivery of electric foot shock were controlled by a PC with dedicated software (S Frank, Psychology Department, University of Tel Aviv, Israel) connected to a Coulborn Universal Environment Interface (E91-12) with Coulborn Universal Environment Port (L91-12). The tone CS [85 dB(A)] was produced by a 2.9 kHz tone module (E12-02) fixed on one wall of the operant chamber. Shocks were delivered with a Coulborn Precision Animal Shocker (E13-12) which generated bipolar rectangular 10-ms current pulses with a frequency of 10 Hz. Background noise was provided by a ventilation fan affixed to the light- and sound-attenuating chambers during all sessions. A monochrome minivideo camera with a wide angle (100°) 2.5-mm lens (VPC-465B; CES AG, Zurich, Switzerland) was attached to the center of the ceiling of each operant chamber. Four infrared (875 nm) light-emitting diodes (HSDL-4220; Hewlett Packard) positioned in the ceiling of each operant chamber provided light sufficient for camera function. Throughout all sessions, images from the test boxes were provided by these
cameras, integrated into a four-quarter single image (100000 pixels) by a multiplexer (DX216CE, Sony), and recorded by a video-recorder (SVT1000; Sony).

**Automated measurement of activity and freezing.** The video images were transferred to a computer (7600/120 Power Macintosh) equipped with an analysis program (Image; [http://rsb.info.nih.gov/nih-image](http://rsb.info.nih.gov/nih-image)) and a macroprogram (P Schmid, Behavioral Neurobiology Laboratory, Swiss Federal Institute of Technology Zurich). The percentage of changed pixels between two adjacent 1-s quarter images recorded from a box was used as a measure of activity (see Richmond et al., 1998 for further details). Freezing is commonly identified as cessation of any movement except for respiratory movements (e.g., Fanselow et al., 1994; Young et al., 1994). If the percentage of changed pixels between two adjacent 1-s images was less than 0.05 %, this corresponded well to such movement cessation, and the behavior of the rat was scored as “freezing” for the respective second. Validation and principle of the automated analysis of freezing behavior have been described in detail in previous publications (Richmond et al., 1998; Pryce et al., 1999).

**Basic experimental design, behavioral measures, and data analysis**

Altogether, four fear-conditioning experiments (Exp. 1 to 4) were conducted in the present study. Rats were always subjected to the behavioral sessions in squads of four, with the experimental boxes and testing order being counterbalanced between the different groups as far as possible. The groups differed only with respect to the infusions they received into the dorsal hippocampus and were otherwise treated identically. Infusions of VEH, MK-801, or NMDA (see Intracerebral microinfusions and drugs) were applied only before conditioning in Exp. 1 to 3, and before conditioning, as well as before the first tone test, in Exp. 4. The different conditioning and test sessions were at least 24 h apart. Conditioning sessions consisted of presentations of different combinations of unsignaled 1-s foot shocks (0.5-mA current-pulse amplitude) or tone-shock pairings (30-s tone coterminating with a 1-s footshock) in the shock boxes and had different duration depending on the experiment. Context-test sessions were conducted to assess long-term conditioned fear (i.e. conditioned fear persisting beyond the conditioning session) to the context in which the foot shocks were experienced. For that purpose, the rats were put in the shock boxes and left undisturbed for 8 min. Tone-test sessions were conducted in Exp. 2 to 4, in which rats received tone-shock pairings during conditioning in order to assess long-term conditioned fear to the tone. Tone tests were conducted in the no-shock boxes, i.e. an environment distinct from the conditioning context, and consisted of a continuous 8-min tone presentation, preceded by 2 or 3 min
II.3.

without stimulation. The infusion groups as well as the conditioning and testing procedures applied in Exp. 1-4 are described in detail below and summarized in Table 1.

| Table 1 Summary of procedures in the four experiments (Exp. 1 to 4) of the present study |
|-----------------------------------------|-----------------------------------------|----------------------------------------|----------------------------------------|
| **INFUSION**                            | **CONDITIONING**                        | **CONTEXT TEST**                       | **TONE TEST**                          |
| Immediate before conditioning:         | Five unsignaled shocks each preceded   | One day after conditioning             | No tone test                           |
| VEH(16), MK-801(8), NMDA(16)           | and followed by 5-min blocks           |                                        |                                        |
| As in Exp. 1                           | 10 tone-shock pairings each preceded   | One day after conditioning             | Two days after conditioning            |
| Immediately before conditioning:       | and followed by 2-min blocks           | One day after conditioning             | Three days after conditioning          |
| VEH, MK-801 (10 each)                  | Five unsignaled shocks, then five      | One day after conditioning + additional extinction two days after conditioning |
| 4 min before conditioning and tone test 1: VEH-VEH, VEH-NMDA, NMDA-VEH, NMDA-NMDA (10 each) | tone-shock pairings, each shock and pairing preceded and followed by 2.5-min blocks |                                        |                                        |
| 8 min before conditioning and tone test 1: VEH-VEH, VEH-NMDA, NMDA-VEH, NMDA-NMDA (10 each) | Eight tone-shock pairings each preceded and followed by 1-min blocks | Six, 10, and 13 days after conditioning | Seven, 11, and 14 days after conditioning |

Tone: 30 s, 2.9 kHz, 85 dB(A). Shock: 1-s, 0.5-mA foot shock. During pairings, last tone second was congruent with the shock.

Infusion parameters and concentrations: 6.25 µg/side MK-801, 0.7 µg/side NMDA, 0.5 µl/side saline as vehicle (VEH) infused within 1 min.

Conditioned fear during conditioning and testing was automatically measured in the form of freezing.

During all sessions, freezing was assessed by the automated analysis system as a measure of conditioned fear. Freezing during conditioning of Exp. 2-4, in which rats were conditioned with tone-shock pairings, was analyzed separately for the time blocks surrounding the tone-shock pairings and the 30-s tone presentations. Such a separate analysis may yield some hints as to whether a treatment differently affects the development of freezing to the context and to the discrete CS during conditioning, even though freezing during the tone presentations is certainly “contaminated” by freezing to the conditioning context, as is freezing between the tone presentations by freezing to the tone. During conditioning sessions some unconditioned behaviors were also assessed. The video images were watched in order to assess whether all infusion groups reacted to the shock by similar vigorous twitching or jumping, marked components of the unconditioned immediate shock response (Anagnostaras et al., 1999b). Activity before the first tone or shock was assessed using the percentage of changed pixels between adjacent 1-s video images that was given by the automated system. Finally, in Exp. 2 and 4, the unconditioned activity response to the first tone presentation was analyzed by comparing the activity scores during the 30 s immediately before the tone with those during the 30-s tone presentation. In these experiments, the tone was the first salient stimulus
presented during conditioning. Under such conditions, a tone may induce a pattern of behavioral arousal, including increased activity (Inglis and Fibiger, 1995).

Statistical analysis was conducted using the Statview software system (SAS Institute Inc., NC). From the freezing scores obtained for each second ("freezing" or "not freezing"), the percentage of time spent freezing in a given time block was calculated. By averaging the percentage of pixels changed between adjacent 1-s video images in a given time block, a relative activity measure for this time block was calculated. Data were analyzed by ANOVA, using the different infusions as between-subjects factor and the different time blocks of testing as repeated-measures factor. When there were more than two infusion groups, Fisher's protected least significant difference post hoc comparisons were used to further analyze main effects of infusion. Level of significance was set at $P < 0.05$.

**Exp. 1: Foreground contextual fear conditioning after dorsal hippocampal NMDA or MK-801 infusion**

Foreground contextual fear conditioning, i.e. fear conditioning to a context in which unsignaled shocks were presented, has been found to be impaired by dorsal hippocampal lesions in rats (Kim et al., 1993; Young et al., 1994), even though another study did not confirm this effect (Phillips and LeDoux, 1994), and by dorsal hippocampal infusion of the competitive NMDA-receptor antagonist D,L-2-amino-5-phosphonovalerate (APV) in both rats (Young et al., 1994) and mice (Stiedl et al., 2000). Exp. 1 was intended to confirm the requirement of dorsal hippocampal NMDA receptors, as well as to examine the effect of tonic stimulation of these receptors, in foreground contextual fear conditioning. There were three infusion groups to receive VEH (n=16), NMDA (n=16), or MK-801 (n=8) into the dorsal hippocampus before conditioning. The rats were experimentally naive, except for eight rats each of the VEH as well as the NMDA group, which received three infusions, one or two of them with NMDA (0.1, 0.25, or 0.7 µg/side), in prepulse-inhibition and open-field experiments finished one week before. Conditioning sessions lasted a total of 30 min and 5 s and consisted of presentation of five unsignaled 1-s foot shocks separated by 5-min blocks between an initial and a final 5-min block. Context tests were conducted one day after conditioning. In our laboratory, the above conditioning and testing parameters yield reliable and marked fear conditioning to the context in cannulated rats, which have a tendency to be less fearful than unoperated rats (Bast et al, 2001d; Zhang et al., 2001). As to the analysis of Exp. 1, it is important to note that, before conducting an overall analysis, the test data of the
NMDA and VEH rats were analyzed in order to ensure that the different experimental history of the rats (naive vs. used in previous experiments) did not interact with the infusion ($P>0.69$).

**Exp. 2: Fear conditioning to a tone after dorsal hippocampal NMDA or MK-801 infusion**

A specific involvement of dorsal hippocampal NMDA-receptor signaling in fear conditioning to context, but not tone, is a central assumption in current views on the hippocampal role in fear conditioning (Young et al., 1994; Anagnostaras et al., 2001; Gale et al., 2001). However, only in mice has intact fear conditioning to a tone actually been demonstrated after blockade of dorsal hippocampal NMDA receptors by local APV infusion (Stiedl et al., 2000). Exp. 2 further examined the effects of altered NMDA receptor-mediated signaling in the dorsal hippocampus on simple fear conditioning to tone. There were three infusion groups to receive VEH ($n=16$), NMDA ($n=16$), or MK-801 ($n=8$) into the dorsal hippocampus before conditioning. As in Exp. 1, the rats were experimentally naive, except for eight rats each of the VEH as well as the NMDA group, which received three infusions, one or two of them with NMDA (0.1, 0.25, or 0.7 μg/side), in prepulse-inhibition and open-field experiments finished one week before. Conditioning sessions lasted a total of 27 min and consisted of 10 tone-shock pairings separated by 2-min blocks between an initial and a final 2-min block. The context-test sessions, to test for freezing to the background context, were conducted one day after conditioning. Two days after conditioning, rats were subjected to tone-test sessions. Tone-test sessions lasted a total of 11 min. After 3 min, the tone was presented for the remaining 8 min. In our laboratory, these conditioning and testing parameters yield reliable and marked fear conditioning to the tone, but sometimes, in particular in cannulated rats, only a little or no freezing to the background context (Richmond et al., 1998; Bast et al, 2001d; Murphy et al., 2001; Zhang et al., 2001). As in Exp. 1, the test data of the NMDA and VEH rats in Exp. 2 were analyzed before the overall analysis in order to verify that the different experimental history of the rats (naive vs. used in previous experiments) did not interact with the infusion ($P>0.37$).

**Exp. 3: Different effects of dorsal hippocampal MK-801 infusion on fear conditioning to tone and context – a further within-subject comparison**

Exp. 1 and 2 indicated that MK-801 infusion into the dorsal hippocampus specifically impaired fear conditioning to context, but not tone. Exp. 3 aimed to corroborate this by demonstrating a dissociation of MK-801’s effects on fear conditioning to context and tone within the same animals. Exp. 2 had already yielded some evidence for such a dissociation,
but the overall level of freezing to the background context in the context test was very low and differences between infusion groups were not very pronounced. Two infusion groups to receive either VEH (n=10) or MK-801 (n=10) into the dorsal hippocampus before conditioning, and all consisting of experimentally naive animals, were used in Exp. 3. Conditioning sessions lasted a total of 35 min. The first 20 min consisted of five unsignaled 1-s footshocks at 2.5-min intervals in between two initial and one final 2.5-min intervals. The last 15 min consisted of five tone-shock pairings at 2.5-min intervals between an initial and a final 2.5-min interval. One day and two days after conditioning, the rats were subjected to 8-min context-test sessions. Three days after conditioning, tone-test sessions of 11 min were conducted, with the tone being presented after 3-min for the remaining 8 min as in Exp. 2. Except for a second context test being conducted after the first one, the conditioning and testing parameters in Exp. 3 were the same as applied in a previous experiment (Zhang et al., 2001), where we obtained marked fear conditioning to both context and tone. The additional context test was included in the present experiment to further support extinction of contextual fear in order to minimize a possible contribution of contextual fear, which might have generalized from the shock to the no-shock boxes, to freezing during the tone test.

Exp. 4: Impairment of fear conditioning by dorsal hippocampal NMDA infusion – the role of state dependency

Exp. 1 and 2 indicated that fear conditioning to both context and tone was impaired by dorsal hippocampal NMDA infusion. Exp. 4 aimed to confirm and further examine this impairment. In particular, we tested whether the reduced conditioned freezing during testing might merely have reflected state dependency, i.e. that associations formed in an altered brain-state may subsequently be retrieved only in a similar brain-state (Overton, 1964), rather than an impairment of the specific processes underlying the formation of conditioned fear. Using freezing as measure of conditioned fear, a test for state dependency, involving drug infusions before conditioning and testing, is difficult when the infusions affect activity and thereby may affect the performance of the conditioned fear response (see Bast et al., 2001d; Zhang et al., 2001). NMDA infusions into the dorsal hippocampus, in contrast to dorsal hippocampal MK-801 infusion (Zhang et al., 2000) and ventral hippocampal drug infusions, whose effects on fear conditioning we studied previously (Bast et al., 2001d; Zhang et al., 2001), did not cause marked alteration of activity in previous open-field experiments (Zhang et al., 2002). Thus, while the contribution of state dependency to impaired fear conditioning after hippocampal manipulations is in many cases difficult to examine, it was possible to
address this important issue in the case of dorsal hippocampal NMDA infusion. Given that Exp. 4 involved infusions before testing, the results are also relevant with respect to the role of the dorsal hippocampus during retrieval/expression and extinction of conditioned fear. Forty experimentally naive rats were used in Exp. 4. Before conditioning, rats received either VEH or NMDA into the dorsal hippocampus (each n=20), resulting in two groups during conditioning and first context test. Before the first tone test, all rats received a second infusion of VEH or NMDA. Half of the rats received the same infusion as before conditioning, and one half received a different infusion, resulting in four groups differing with respect to the combinations of infusions (before conditioning-before first tone test): VEH-VEH, VEH-NMDA, NMDA-VEH, NMDA-NMDA (each group n=10). The four groups were matched with respect to the behavioral measurements taken before the first tone test. Based on the observation in Exp. 1 and 2 that dorsal hippocampal NMDA infusion may result in movement inhibition lasting a few minutes, infusions in Exp. 4 were applied 4 min before the experimental sessions. Conditioning sessions lasted a total of 13 min and consisted of eight tone-shock pairings separated by 1-min blocks between an initial and a final 1-min block. The first context-test sessions were conducted six days after conditioning. Seven days after conditioning, rats were subjected to the first tone-test sessions. Tone-test sessions lasted a total of 10 min. After 2 min, the tone was presented for the remaining 8 min. Additional context- and tone-test sessions without preceding infusions were conducted 10 and 13 days or 11 and 14 days, respectively, after conditioning to examine possible effects of the infusions on the extinction of conditioned fear, and to further corroborate that the effects of NMDA in the dorsal hippocampus are temporary. The sessions following the infusions were planned to be as short as possible because the observation in Exp. 1 and 2, that freezing during conditioning was reduced in the NMDA rats only until about the fifth shock, indicated that action of the drug might fade within about 15 min. The time span between conditioning and first test sessions was chosen so that repeated infusions were one week apart, in order to allow for recovery of the brain tissue from possible disturbances resulting from the first intracerebral infusion (Routtenberg, 1972). In order to reduce the number of repeated infusions to a minimum, infusions were only applied before conditioning and the first tone test, but not before the other tests.

**Results**

*Histology*
In all rats used in Exp. 1-4, the tips of the infusion cannulae were located within or around the borders of the dorsal hippocampus (Fig. 1). Therefore, the behavioral data of all rats were included in the analysis. Damage to hippocampal neuron layers and interspersed neurons, indicated by interruption in NeuN staining, was mainly restricted to the tracks of the infusion cannulae and the immediately surrounding areas, and may have been slightly more expanded in rats infused with NMDA. Overall, however, neuronal damage in the dorsal hippocampus did not differ markedly between animals infused with VEH, MK-801, or NMDA (Fig. 2).

**Effects of MK-801 and NMDA infusions on unconditioned behavior**

While dorsal hippocampal MK-801 infusion did not induce any behavioral abnormalities that were detectable by mere visual inspection of the rat, dorsal hippocampal NMDA infusion induced an easily visible short-lasting movement inhibition in some rats. These rats, when put into their cages or the test boxes after infusion and left untouched, stood rigidly on their slightly outwards-set four paws and stared in one direction. A few minutes (about 5 min) after infusion, behavioral abnormalities were no longer detectable by mere visual inspection of the rats infused with NMDA.

Fig. 3 depicts unconditioned activity before the first tone or shock in Exp. 1-4 (Fig. 3A), and the alterations of unconditioned activity in response to the first tone in Exp. 2 and 4 (Fig. 3B) for the different infusion groups.

Due to the short-lasting movement inhibition induced by dorsal hippocampal NMDA infusion in some rats (see above) average activity throughout the periods preceding the first tone or shock was decreased in the NMDA group as compared to the other groups in Exp. 1 and 2, where infusions were given immediately before conditioning. Rats that received MK-801 infusions (Exp. 1 to 3) or rats that received NMDA infusions 4 min before conditioning (Exp. 4) exhibited similar activity levels as VEH rats (Fig. 3A). In Exp. 1 and 2, ANOVA yielded a significant effect of infusion on the average activity throughout the 5 min preceding the first shock ($F_{2,37}=3.40, P<0.05$) or the 2 min preceding the first tone ($F_{2,37}=5.33, P<0.01$), respectively. Post hoc comparisons indicated significant differences between the NMDA and MK-801 groups in Exp. 1 ($P<0.05$), and between the NMDA and VEH group in Exp. 2 ($P<0.0025$). Moreover, the difference between VEH and NMDA rats in Exp. 1 closely approached significance ($P=0.050$). VEH and MK-801 rats did not differ in Exp. 1 ($P>0.50$) or 2 ($P>0.18$). ANOVA of activity during the 5 min preceding the first shock in Exp. 3 ($F_{1,18}=0.00, P>0.97$) or the 1 min preceding the first tone in Exp. 4 ($F_{1,38}=1.46, P>0.23$) did not yield a significant effect of the infusion given before conditioning. Analysis of activity
Fig. 1 Approximate locations of the tips of the infusion cannulae in the different infusions groups of Exp. 1 to 4, depicted on coronal sections drawn after Paxinos and Watson (1998). Numbers of animals per infusion group are indicated in parentheses. Values on the left represent distance from bregma.
Fig. 2 Photomicrographs of coronal sections through the dorsal hippocampus immediately around the infusion sites from rats that received one infusion of VEH (0.5 µl saline), MK-801 (6.25 µg/0.5 µl), or NMDA (0.7 µg/0.5 µl). Neurons are visualized by immuno-staining of the neuronal marker protein NeuN. Arrowheads indicate the approximate location of the tips of the infusion cannulae. The scale bar represents 500 µm.
Fig. 3 Effects of the different infusions on A: unconditioned activity before the first tone or shock in Exp. 1 to 4, and B: on the increase of unconditioned activity in response to the first tone in Exp. 2 and 4. In Exp. 1 to 3, infusions were applied immediately, in Exp. 4, 4 min before behavioral testing. Numbers of animals per infusion group are indicated in parentheses. Presented values are means. Bars represent 1 standard error (SE) derived from the appropriate mean square of the ANOVA and provide an estimate of population variance.
levels in the different 1-min blocks of the periods preceding the first tone or shock (data not shown) did not reveal a decrease of activity over time, which would have reflected habituation of exploratory activity, in any group. In Exp. 2 and 4, where the tone was the first salient stimulus presented, all infusion groups were more active during the tone presentation than during the immediately preceding period (Fig. 3B), indicating that the tone induced similar behavioral arousal in all groups. For both experiments, ANOVA of the average activity in the 30-s periods preceding and during the tone yielded an effect of period (Exp. 2: $F_{1,37}=20.19$, $P<0.0001$; Exp. 4: $F_{1,38}=9.39$, $P<0.005$) without an interaction of infusion group and period (Exp. 2: $F_{2,37}=2.60$, $P>0.08$; Exp. 4: $F_{2,38}=1.56$, $P>0.21$). Moreover, in both experiments there was a significant main effect of group, reflecting movement inhibition in some of the NMDA rats (Exp. 2: $F_{2,37}=4.09$, $P<0.025$; Exp. 4: $F_{1,38}=4.58$, $P<0.05$).

Inspection of the video images from the conditioning sessions of all four experiments yielded that all infusion groups exhibited similar vigorous twitching or jumping as immediate response to the foot shock, indicating that NMDA or MK-801 infusions did not affect shock sensitivity.

**Effects of MK-801 and NMDA infusions on conditioned freezing**

*Exp. 1: Foreground contextual fear conditioning after dorsal hippocampal NMDA or MK-801 infusion*

The freezing data of the conditioning and the context-test session of Exp. 1 are depicted in Fig. 4.

During conditioning, freezing resulting from the inescapable foot shocks was decreased in MK-801 as compared to VEH rats until the end of the session. NMDA rats exhibited similarly low freezing as MK-801 rats during the 5-min blocks following the first three shocks, but showed freezing comparable to the VEH rats in the last two 5-min blocks. Moreover, as compared to the other two groups, NMDA rats spent more time immobile in the 5-min block before the first shock, reflecting the short-lasting movement inhibition induced by the dorsal hippocampal NMDA infusion. ANOVA of freezing throughout all six 5-min blocks preceding and following the shocks in the conditioning session yielded an effect of infusion ($F_{2,37}=4.39$, $P<0.025$) and 5-min block ($F_{5,185}=23.21$, $P<0.0001$) as well as an interaction of these two factors ($F_{10,185}=3.23$, $P<0.001$), reflecting that differences between the groups changed throughout the session. During the first 5-min block ($F_{2,37}=5.32$, $P<0.01$), NMDA rats exhibited increased immobility as compared to the VEH ($P<0.001$) and MK-801 groups ($P<0.025$), which did not differ ($P>0.99$). In the following three 5-min blocks ($F_{2,37}=5.81$,}
Fig. 4 Freezing during conditioning and context-test sessions of Exp. 1. VEH (n=16), NMDA (n=16), or MK-801 (n=8) was infused into the dorsal hippocampus immediately before conditioning with five unsignaled footshocks. Mean percentage of time spent freezing is depicted for the six 5-min blocks preceding and following the five unsignaled footshocks during conditioning and for the eight 1-min blocks of the context test. Bars represent 1 standard error (SE) derived from the appropriate mean square of the ANOVA.
II.3.

Average freezing levels were lower in MK-801 (P<0.05) and NMDA (P<0.005) rats, which did not differ (P>0.76), than in the VEH group. Finally, in the last two 5-min blocks (F_{2,37}=5.65, P<0.01), freezing was decreased in the MK-801 group as compared to VEH (P<0.0025) as well as NMDA (P<0.05) rats, which no longer differed (P>0.13).

During the context test, both NMDA and MK-801 rats exhibited lower conditioned freezing than the VEH group. ANOVA of freezing values throughout the eight 1-min blocks of the context test yielded a significant effect of 1-min block (F_{7,259}=5.61, P<0.0001), reflecting a gradual increase of freezing throughout the first three 1-min blocks, as well as an effect of infusion (F_{2,37}=5.12, P<0.025). Post hoc comparisons revealed that average freezing levels throughout the eight 1-min blocks were lower in NMDA than in VEH rats (P<0.005). There was a strong tendency (P=0.068) for MK-801 rats, which exhibited similarly low freezing levels as the NMDA rats (P>0.50), to also show less freezing than the VEH group.

Exp. 2: Fear conditioning to a tone after dorsal hippocampal NMDA or MK-801 infusion

During conditioning, freezing in response to the foot shocks was decreased in MK-801 as compared to VEH rats until the end of the session. NMDA rats exhibited similarly low freezing as MK-801 rats during the first half of the session, but showed freezing comparable to the VEH rats throughout the second half. Before the first shock, NMDA rats spent more time immobile than the two other groups, reflecting movement inhibition induced by the dorsal hippocampal NMDA infusion. ANOVA of freezing throughout the 11 2-min blocks preceding and following the tone-shock pairings, as well as of freezing during the 10 30-s blocks of tone presentation, yielded an effect of infusion (F_{2,37}=4.64, P<0.025; F_{2,37}=12.32, P<0.0001) and time block (F_{10,370}=9.85, P<0.0001; F_{9,333}=4.84, P<0.0001) as well as an interaction of these two factors (F_{20,370}=2.90, P<0.001; F_{18,333}=2.46, P<0.001), reflecting that differences between the groups changed throughout the session. During the first 2-min block (F_{2,37}=5.32, P<0.01), NMDA rats spent or tended to spend, respectively, more time immobile than the VEH (P<0.005) or MK-801 group (P=0.070), which did not differ (P>0.4). Similar differences appeared to exist throughout the subsequent 30-s tone presentation of the first tone-shock pairing, although ANOVA only yielded a strong tendency towards an effect of infusion on the proportion of time spent immobile during this period (F_{2,37}=2.99, P=0.063). During the following five 2-min blocks (F_{2,37}=8.08, P<0.0025), as well as the adjacent 30-s tone presentations (F_{2,37}=12.82, P<0.0001), of the second to sixth tone-shock pairings, average freezing levels were lower in MK-801 (P<0.0025; P<0.0001) and NMDA (P<0.0025;
Fig. 5 Freezing during conditioning, context-, and tone-test sessions of Exp. 2. VEH (n=16), NMDA (n=16), or MK-801 (n=8) was infused into the dorsal hippocampus immediately before conditioning with 10 tone-shock pairings. Mean percentage of time spent freezing during conditioning is depicted for the 11 2-min blocks preceding and following the tone-shock pairings as well as for the 10 30-s tone presentations of the pairings. Mean percentage of time spent freezing during context- and tone test is depicted for each of the eight or 11, respectively, 1-min blocks of the sessions. Bars represent 1 standard error (SE) derived from the appropriate mean square of the ANOVA.
II.3.
P<0.001) rats, which did not differ (P>0.43; P>0.08), than in VEH rats. Finally, the effect of infusion on average freezing levels during the last five 2-min blocks (F2,37=3.20, P=0.052) and the last four tone presentations (F2,37=7.96, P<0.0025) closely approached significance or was significant, respectively. Post hoc comparisons indicated that average freezing was decreased in the MK-801 group as compared to VEH (P<0.05; P<0.001) and NMDA (P<0.025; P<0.001) rats, which did not differ anymore (P>0.93; P>0.89).

During the context test, all groups exhibited relatively low levels of freezing (<14%) throughout all eight 1-min blocks, indicating that the conditioning procedure did not result in marked conditioned fear to the context. Nevertheless, ANOVA yielded an effect of infusion on freezing levels (F2,37=3.1, P<0.05), and post hoc comparisons indicated that, as compared to the VEH group, freezing was or tended to be reduced, respectively, in the NMDA (P<0.025) and the MK-801 (P=0.090) group, which exhibited very similar freezing (P>0.77).

During the tone test, conditioned freezing to the tone appeared to be decreased in the NMDA group as compared to both VEH and MK-801 rats. During the three 1-min blocks before tone onset, rats virtually did not exhibit conditioned fear, as evidenced by low levels of immobility (<4%), which did not differ between the infusion groups (main effect of infusion: F2,37=1.31, P>0.28; interaction infusion x 1-min block: F4,74=4.51, P>0.60). ANOVA of freezing levels throughout the eight 1-min blocks after tone onset yielded a trend towards an effect of infusion (F2,37=2.67, P=0.082), indicating the higher freezing levels in VEH and MK-801 rats as compared to the NMDA group, a significant main effect of 1-min block (F10,370=18.82, P<0.0001), and an interaction of infusion and 1-min block (F10,370=2.15, P<0.005). The interaction reflected that, throughout the first two to three 1-min blocks after tone onset, VEH and MK-801 rats exhibited similarly marked freezing (about 30 %), which then appeared to decrease faster in the VEH than in the MK-801 group towards the end of the session, while NMDA rats exhibited little freezing (about 10%) throughout all eight 1-min blocks of tone presentation.

Exp. 3: Different effects of dorsal hippocampal MK-801 infusion on fear conditioning to tone and context – a further within-subject comparison

Fig. 6 depicts the freezing data for the different sessions of Exp. 3.

During conditioning, freezing in response to the foot shocks was decreased in MK-801 as compared to VEH rats. ANOVA of freezing during the twelve 2.5-min blocks preceding and following the unsignaled shocks and tone-shock pairings yielded an effect of infusion (F1,18=28.10, P<0.0001), 2.5-min block (F11,198=25.53, P<0.0001), as well as an interaction of
Fig. 6 Freezing during conditioning, context-, and tone-test sessions of Exp. 3. VEH (n=10), or MK-801 (n=10) was infused into the dorsal hippocampus immediately before conditioning with five unsignaled footshocks and five tone-shock pairings. Mean percentage of time spent freezing during conditioning is depicted for the two 2.5-min blocks preceding the shocks, for the five 2.5-min blocks following the unsignaled shocks and the tone-shock pairings, as well as for the five 30-s tone presentations of the pairings. Mean percentage of time spent freezing during the context and tone tests is depicted for each of the eight or 11, respectively, 1-min blocks of the sessions. Bars represent 1 standard error (SE) derived from the appropriate mean square of the ANOVA.
these two factors ($F_{11.198}=6.16, P<0.0001$). The interaction reflected that, while both infusion groups exhibited virtually no freezing during the two 2.5-min blocks preceding the first shock, freezing levels increased to a level of about 60% in the VEH but only to about 20% in the MK-801 group within the 2.5-min blocks following the first two shocks. ANOVA of freezing levels during the 30-s tone presentations of the five tone-shock pairings presented in the second half of the conditioning session revealed only an effect of infusion ($F_{1.18}=41.13, P<0.0001$), but not an effect of tone presentation ($F_{4.72}=0.25, P>0.91$) or an interaction of infusion and tone presentation ($F_{4.72}=0.33, P>0.86$). Thus, during conditioning, MK-801 rats exhibited less freezing than VEH rats, in absence as well as in presence of the tone.

During the first context test, VEH, but not MK-801 rats, exhibited marked conditioned freezing. ANOVA of freezing levels throughout the eight 1-min blocks yielded an effect of infusion ($F_{1.18}=6.23, P<0.025$). Although it appeared that conditioned freezing in the VEH rats developed gradually throughout the first three 1-min blocks, and that towards the end of the session VEH rats exhibited slight extinction of conditioned freezing, ANOVA did not reveal an effect of 1-min block ($F_{7.126}=1.74, P>0.10$) or an interaction of infusion and 1-min block ($F_{7.126}=1.07, P>0.38$). During the second context test, both VEH and MK-801 rats exhibited similarly low levels of immobility ($F_{1.18}=0.11, P>0.74$). ANOVA on the time spent immobile throughout the eight 1-min blocks only yielded an effect of 1-min block ($F_{7.126}=4.10, P<0.0005$) as immobility levels slightly increased towards the end of the session, probably reflecting a decrease in activity due to habituation to the context.

In the tone test, MK-801 and VEH rats exhibited similar marked conditioned freezing to the tone. During the three 1-min blocks before tone onset, rats exhibited virtually no conditioned fear, as evidenced by low levels of immobility (<6%) which did not differ between groups. ANOVA on freezing levels during the eight 1-min blocks of tone presentation did not reveal an effect of infusion ($F_{1.18}=0.68, P>0.42$) or an interaction of infusion and 1-min block ($F_{7.126}=0.87, P>0.53$). There was only a significant effect of 1-min block ($F_{7.126}=2.79, P<0.01$), reflecting that freezing levels, which had increased steeply throughout the first 1-min block of tone presentation, reached peak levels in the second 1-min block of tone presentation and then gradually decreased towards the end of the session.

Exp. 4: Impairment of fear conditioning by dorsal hippocampal NMDA infusion – the role of state dependency

The freezing data for Exp. 4, in which rats received infusions before conditioning as well as before the first tone test, are depicted in Fig. 7.
Fig. 7 Freezing during conditioning, context-, and tone-test sessions of Exp. 4. Conditioning was conducted with eight tone-shock pairings. A first infusion of VEH or NMDA was given 4 min before conditioning, resulting in two infusion groups (each n = 20) for conditioning and context test 1. A second infusion of VEH or NMDA was given 4 min before tone test 1, so that four infusion groups (each n = 10), differing with respect to the combination of infusions received before conditioning and tone test 1, resulted for tone test 1 and the following test sessions. Mean percentage of time spent freezing during conditioning is depicted for the nine 1.5-min blocks preceding and following the tone-shock pairings as well as for eight 30-s tone presentations of the pairings. Mean percentage of time spent freezing during the context and tone tests is depicted for each of the eight or 10, respectively, 1-min blocks of the sessions. Bars represent 1 standard error (SE) derived from the appropriate mean square of the ANOVA.
During the conditioning session, the development of conditioned freezing in response to the inescapable foot shocks was retarded in the rats that received NMDA infusion before conditioning, as compared to the rats that received VEH infusion. Throughout the first part of the session, NMDA rats exhibited less freezing than VEH rats, while towards the end of the session both groups exhibited similar levels of conditioned freezing. ANOVA of freezing levels during the nine 1.5-min blocks preceding and following the foot shocks yielded no effect of infusion ($F_{1,38}=2.26, P>0.14$), but an effect of time block ($F_{8,304}=22.96, P<0.0001$) as well as an interaction of infusion and time block ($F_{8,304}=9.99, P<0.0001$). ANOVA of freezing levels during the 30-s tone presentations of the eight tone-shock pairings yielded an effect of infusion ($F_{1,38}=15.08, P<0.0005$) and time block ($F_{7,266}=38.28, P<0.0001$), as well as an interaction of tone presentation and infusion ($F_{7,266}=10.30, P<0.0001$). The interaction between time block or tone presentation and infusion reflected that conditioned freezing was lower in the NMDA than in the VEH group until administration of about the fifth shock, i.e. during the second to fifth 1.5 min block ($F_{1,38}=25.10, P<0.0001$) and the second to fifth tone presentation ($F_{1,38}=45.10, P<0.0001$), while afterwards, i.e. during the last four 1.5-min blocks ($F_{1,38}=2.59, P>0.11$) and the last three tone presentations ($F_{1,38}=0.10, P>0.75$), NMDA and VEH rats showed similar freezing levels. Moreover, levels of immobility before the first shock, i.e. during the first 1.5-min block ($F_{1,38}=5.05, P<0.05$) and the 30-s tone of the first tone-shock pairing ($F_{1,38}=4.34, P<0.05$), were slightly higher in NMDA than in VEH rats, reflecting that some of the rats that received NMDA 4 min before conditioning still exhibited a slight movement inhibition at the beginning of the session.

During the first context test, VEH, but not NMDA, rats exhibited marked conditioned freezing. ANOVA of freezing levels during the eight 1-min blocks yielded an effect of group ($F_{1,38}=9.77, P<0.005$) and of 1-min block ($F_{7,266}=2.83, P<0.01$), as well as interaction of these two factors ($F_{7,266}=2.90, P<0.01$). The interaction reflected that conditioned freezing in the VEH group increased from about 10% in the first 1-min block to about 25% in the third and fourth 1-min blocks, and then decreased toward the end of the session, reflecting extinction of conditioned fear to the context, while NMDA rats exhibited little freezing (<5%) throughout the whole session.

During the first tone test, all three groups that received NMDA before conditioning or the first tone test, or at both occasions, exhibited markedly reduced conditioned freezing to the tone as compared to the group that had received VEH infusion at both occasions. ANOVA of freezing levels during the two 1-min blocks before tone onset yielded an effect of the infusion ($F_{1,36}=6.01, P<0.025$) given 4 min before the tone test, reflecting movement inhibition in
II.3.

some rats that received NMDA before the tone test session. Moreover, there was also an effect of the infusion received before conditioning ($F_{1,36}=4.13$, $P<0.05$), but no interaction between the two infusions ($F_{1,36}=1.87$, $P>0.18$). This reflected that average levels of immobility were higher in rats that had received VEH before conditioning than in those that had received NMDA before conditioning. First, VEH-VEH rats exhibited higher freezing than the NMDA-VEH rats in the second 1-min block, possibly reflecting that in the rats that had received VEH before conditioning there was still some fear to the conditioning context that generalized to the test context. Second, immobility levels, reflecting movement inhibition induced by the NMDA infusion before conditioning, were higher in VEH-NMDA than in NMDA-NMDA rats. This might reflect tolerance to the effects of NMDA due to the previous NMDA infusion. However, NMDA-induced movement inhibition was only observed in some rats, and it is striking that NMDA-NMDA rats exhibited twice as much immobility before tone onset in the first tone test than rats which received NMDA before conditioning, during the period preceding the first shock during conditioning. Thus, the higher immobility levels in VEH-NMDA as compared to NMDA-NMDA rats before tone onset in the first tone test are most likely reflecting a sampling error. For the eight 1-min blocks of tone presentation, an effect of time ($F_{7,252}=10.52$, $P<0.0001$) was the only simple effect revealed by ANOVA of freezing. However, all possible interactions of between-subjects (infusion before conditioning, infusion before first tone test) and repeated-measures (1-min block) factors were significant. Most importantly, there was a three-way interaction of infusion before conditioning, infusion before first tone test, and time block ($F_{7,252}=3.48$, $P<0.0025$). This reflected that freezing in the rats receiving two VEH infusions was higher as compared to all other groups during the beginning of the tone presentation, and that this difference was gradually decreasing, indicating extinction of conditioned fear.

Freezing levels throughout the context test 2 and 3 were relatively low, indicating extinction of conditioned fear to the context, and did not differ between the groups (all main effects and interactions involving infusion before conditioning or first tone test: $F<3.53$, $P>0.06$). During tone test 2, however, considerable conditioned freezing was still exhibited by the groups that received VEH before conditioning. ANOVA of freezing during the eight 1-min blocks of tone presentation yielded an interaction of infusion before conditioning and 1-min block ($F_{7,252}=3.92$, $P<0.0005$). This reflected that freezing in the rats which had received VEH before conditioning was higher as compared to the other groups only during the first four 1-min blocks of tone presentation ($F_{1,36}=7.36$, $P<0.025$). Although the VEH-NMDA group appeared to exhibit higher levels of conditioned freezing than the VEH-VEH group,
indicating that NMDA infusion before tone test 1 may have impaired extinction of conditioned fear to the tone, ANOVA on freezing during the eight 1-min blocks of tone presentation did not yield significant interactions involving both infusions (P>0.11). During tone test 3, the tone onset still appeared to induce weak conditioned freezing in the two groups that received VEH infusions before conditioning. ANOVA on freezing during the eight 1-min blocks of tone presentation yielded an interaction of infusion before conditioning and 1-min block (F7,252=2.86, P<0.01). In the first 1-min block after tone onset, rats that had received VEH before conditioning exhibited higher conditioned freezing than rats that had received NMDA (F1,36=4.28, P<0.05).

Discussion

Altogether, the four experiments of the present study yielded two major findings. Dorsal hippocampal MK-801 infusion before conditioning to context or tone resulted in reduced freezing to the context, but not the tone, in subsequent test sessions (Exp. 1-3). Freezing to both context and tone, however, was reduced by dorsal hippocampal NMDA infusion before conditioning (Exp. 1, 2, and 4).

Dorsal hippocampal infusion of MK-801 and NMDA

In view of the neurotoxic potential of MK-801 (Olney et al., 1989) and NMDA (Hajos et al., 1986), we examined infusion-induced neuronal damage using selective visualization of neurons by immunostaining of the neuronal marker protein NeuN (Wolf et al., 1996). This examination yielded that most of the neuronal damage in the dorsal hippocampus was restricted to the cannulae tracks and the immediately surrounding areas. Even though NMDA may have induced slight additional damage, overall neuronal damage in the dorsal hippocampus did not differ markedly between rats infused with VEH, MK-801, or NMDA. That NMDA in the dorsal hippocampus exerts mainly temporary effects is further corroborated by the results of Exp. 4, where rats infused with NMDA only before tone test 1 exhibited reduced freezing as compared to VEH rats during tone test 1, but not 2. Moreover, with the small infusion volume (0.5 µl/side) and fine infusion cannulae (34 gauge) used in the present study, the estimated spread of the infused substances, occurring preferentially dorsally along the external wall of the infusion cannula, is less than 1 mm (Myers, 1966; Myers et al., 1971; Routtenberg, 1972). Thus, it can be assumed that differences observed between rats infused with VEH as compared to those infused with MK-801 or NMDA reflected a
II.3.

temporary alteration of neuronal activity by blockade or stimulation of dorsal hippocampal NMDA receptors.

MK-801 inhibits or shuts off any NMDA receptor-mediated signaling. NMDA tonically stimulates NMDA receptor-mediated mechanisms, disrupting the time and synapse specificity of NMDA receptor-mediated signaling. Thus, disruption of the same behavioral process by both MK-801 and NMDA in the dorsal hippocampus indicates that this process depends on time and synapse specific local NMDA receptor-mediated mechanisms, such as synaptic plasticity (Martin et al., 2000). Although MK-801 disrupts NMDA receptor-mediated signaling generally, whereas NMDA only interferes with the specificity of this signaling, it is possible that some processes, like fear conditioning to tone in the present study, are spared by dorsal hippocampal MK-801 infusion, but disrupted by local NMDA infusion. NMDA induces strong local neuronal excitation and thereby likely interferes also with local coordinated signaling not primarily mediated by NMDA receptors. Furthermore, NMDA may induce aberrant stimulation of dorsal hippocampal efferents and thereby disrupt normal processing in projection sites of dorsal hippocampal neurons. Which of the two possibilities accounts for the effects of NMDA in the dorsal hippocampus on fear conditioning will be discussed below.

Conditioned freezing resulting from the different conditioning procedures

Freezing levels observed during conditioning and test sessions of Exp. 1-3 in the VEH rats are comparable to those obtained under very similar experimental conditions in previous studies (Bast et al., 2001d; Zhang et al., 2001). Freezing during test sessions, though marked, reached peak levels of at most 45%. Such levels are low enough to largely rule out the possibility of ceiling effects occluding treatment-induced reductions in conditioned fear. Thus, the specific reduction of freezing during context-, but not tone-test, sessions in MK-801 rats cannot result from contextual conditioning being weaker than conditioning to the tone. This is also supported by the fact that MK-801 rats showed reduced freezing in the context test of Exp. 1, but not in the tone test of Exp. 2, despite the VEH rats exhibiting similar peak levels (about 30%) of freezing in both cases.

Interestingly, freezing during the tone test in Exp. 4 (Fig. 7) was stronger than freezing during the tone test in Exp. 2 (Fig. 4), although rats received more tone shock pairings in Exp. 2. The different levels of conditioned freezing were possibly due to the longer time span between conditioning and testing in Exp. 4 (7 days) as compared to Exp. 2 (1 day), suggesting an enhancement of fear memory over periods of several days. While it is well accepted that
memory enhances, i.e. consolidates, over time (McGaugh, 2000), only a few studies have reported proceeding enhancement in the expression of memory over periods longer than 24 h (compare Marti et al., 2001). It may therefore be of general interest for the concept of memory consolidation to further examine the possibility that consolidation of fear memory is proceeding over several days.

**Unspecific infusion effects**

*Alterations in sensorimotor functions*

Observations in the present study did not indicate effects of the infusions on US or CS processing, since infusion groups did not considerably differ in the unconditioned immediate shock response or the unconditioned activity response to the first tone presentation. Altered startle reactivity found after dorsal hippocampal infusion of NMDA (Zhang et al., 2002) and MK-801 (Zhang et al., 2000) may indicate that these infusions affect the perception or evaluation of US and CS, since alterations in startle reactivity have been related to changes in attentional or emotional states (Koch, 1999). Given, however, that startle reactivity was decreased by NMDA, but increased by MK-801, in the dorsal hippocampus, the reduced conditioned freezing observed in the present study appears not to be linked to the infusion-induced alterations in startle reactivity.

The measure of unconditioned activity prior to the first tone or shock at the beginning of conditioning was not altered by MK-801, while NMDA induced short-lasting movement inhibition in some rats. In our previous studies, MK-801 in the dorsal hippocampus markedly increased activity for about 30 min (Zhang et al., 2000), while NMDA did not alter activity (Zhang et al., 2002) in rats habituated to their environment. This suggests that, during conditioning in the present study, unconditioned activity was increased in the MK-801, but not altered in the NMDA group, except for the first few minutes of the session. High levels of unconditioned activity at the beginning of the conditioning sessions probably masked the increase in activity induced by the dorsal hippocampal MK-801 infusion while favoring detection of the short-lasting movement inhibition induced by the NMDA infusion. Short-lasting movement inhibition may occur after subconvulsive stimulation of the dorsal as well as ventral hippocampus (Hallak et al., 1993; Zhang et al., 2001; present study), even though, overall, the latter induces hyperactivity (Bast et al., 2001e; Zhang et al., 2002). This may reflect an initial strong disruption of coordinated hippocampal electrical activity related to movement initiation (Leung et al., 2000). The short-lasting movement inhibition, or the underlying mechanisms, were not linked to the freezing deficits observed in the present study.
II.3.

Movement inhibition only occurred in some of the rats infused with NMDA, and inspection of the data revealed freezing deficits were not related to whether or not a rat exhibited movement inhibition. Reduced freezing has been proposed to reflect, in some cases, hyperactivity interfering with the performance of the freezing response, for example, in rats with hippocampal lesions (e.g., Richmond et al., 1999; Gewirtz et al., 2000). In view of the hyperactivity induced by MK-801 in the dorsal hippocampus (Zhang et al., 2000), a performance deficit is likely to account for reduced freezing observed in the presence of MK-801 in the dorsal hippocampus during conditioning. This also explains why, during conditioning, MK-801 rats exhibited reduced freezing also during the tone although exhibiting unimpaired conditioned fear to the tone during later testing without drug. Furthermore, this account is consistent with the finding that freezing during conditioning was not reduced by the NMDA receptor antagonist APV in the dorsal hippocampus (Young et al., 1994) at a dose not affecting activity (Kawabe et al., 1998). Rats with NMDA in the dorsal hippocampus are not hyperactive (Zhang et al., 2002) and performed a normal freezing response after about four to six shock administrations during conditioning. The latter effect was not due to fading of drug activity given that it was observed regardless of conditioning lasting about 30 min (Exp. 1 and 2) or only 10 min (Exp. 4). Thus, NMDA in the dorsal hippocampus does not interfere with performance of the freezing response.

**State dependency**

Learning can be state-dependent, i.e. information learned in a particular brain state induced by systemic treatment (Overton, 1964) or specific manipulations of single brain sites, such as electrical stimulation of the dorsal hippocampus (McIntyre et al., 1985), can in some cases only be retrieved with the same brain state prevailing. If reduced conditioned fear is demonstrated during testing in rats conditioned and tested with drugs, the reduction in conditioned fear cannot merely be due to state dependency. With respect to dorsal hippocampal MK-801 infusion, which induces hyperactivity and thus is likely to disrupt the performance of freezing (see above), such a demonstration is not possible using freezing as a measure of conditioned fear (see Bast et al., 2001d; Zhang et al., 2001). However, when tested without drug, rats that received MK-801 infusion before conditioning, only exhibited reduced conditioned freezing to context, but normal freezing in response to the tone. This specific reduction in fear to context argues against state dependency, unless fear conditioning to tone and context are differently state dependent (compare Gale et al., 2001). Moreover, it was reported that infusions of NMDA receptor antagonists into the amygdala (Kim and
II.3.

McGaugh, 1992) and even systemic injection of MK-801 (Nakagawa et al., 1996) did not induce state dependency of inhibitory avoidance learning, another form of classical aversive conditioning. The virtual absence of conditioned fear during tone test in rats conditioned and tested with NMDA in the dorsal hippocampus (Exp. 4) is a direct demonstration that the effects of NMDA in the dorsal hippocampus on fear conditioning do not merely reflect state dependency. A similar pattern of results has been obtained with muscimol infusion into the amygdala (Helmstetter and Bellgowan, 1994; Muller et al., 1997). Finally, normal conditioned fear during testing was found in rats that received behaviorally effective infusions of a protein kinase inhibitor or a dopamine antagonist before testing, but not conditioning, into the amygdala (Goosens et al., 2000; Guarraci et al., 2000); of the local anesthetic bupivacaine before conditioning, but not testing, into the nucleus accumbens (Haralambous and Westbrook, 1999); or of a dopamine agonist or antagonist before conditioning, but not testing, into the medial prefrontal cortex (Pezze et al., 2002a, in press). Thus, even though generalization over different drugs (Castellano and McGaugh, 1990) and brain regions (Phillips and LePiane, 1981) has to be made with caution, there is no clear evidence of drug infusions into single brain sites inducing state dependency of conditioned fear, while several findings argue against this possibility.

Specific infusion effects on fear conditioning

Freezing emerging after the first shock during conditioning is widely considered to reflect at least partly, a conditioned fear response and, thus, short-term memory of fear (e.g., Kim et al., 1992, 1993; Young et al., 1994; Fanselow, 2000). As discussed above, reduced freezing observed in MK-801 rats during conditioning probably resulted from infusion-induced hyperactivity, and the data of the present study do not therefore allow one to decide whether MK-801 in the dorsal hippocampus impairs formation of short-term conditioned fear. The retarded development of freezing during conditioning in the NMDA rats, however, probably reflected an interference with the formation of short-term fear memory.

The reduced freezing during test sessions in rats that had received dorsal hippocampal MK-801 or NMDA infusions before conditioning indicated a genuine impairment of specific memory processes involved in the formation of long-term conditioned fear to tone and/or context. Formation of long-term memory comprises the initial acquisition, short-term consolidation, completed within seconds or tens of minutes, and the subsequent long-term consolidation (Nadel and Moscovitch, 1997; McGaugh, 2000). Our data do not allow one to decide which of the several stages contributing to long-term memory formation the drug
infusions actually affected. The drug infusions may have interfered with initial acquisition and short-term consolidation, which is believed to occur within seconds or tens of minutes after the acquisition, and, given that the drugs may have been active in the dorsal hippocampus beyond the conditioning sessions, the early phase of the subsequent long-term consolidation. Finally, reduced freezing during the tone test in rats that received dorsal hippocampal NMDA infusion only before the tone test (Exp. 4) indicates a disruption of retrieval/expression of long-term conditioned fear to tone by NMDA in the dorsal hippocampus. In Exp. 4, rats that had NMDA in the dorsal hippocampus only during tone test 1 appeared to exhibit increased freezing during tone test 2, indicating reduced extinction of conditioned fear. Effects of dorsal hippocampal manipulations on extinction of conditioned fear may be the subject of future studies.

At the single doses used in the present study, MK-801 (6.25µg/0.5 µl/side) infusion into the dorsal hippocampus only impaired fear conditioning to context, while NMDA (0.7µg/0.5µl/side) infusion impaired fear conditioning to both tone and context. It is possible that other drug doses would have had other effects. For example, while the observed effects of MK-801 indicate that fear conditioning to context is more susceptible to NMDA receptor blockade in the dorsal hippocampus than fear conditioning to tone, higher doses of MK-801 may also have affected fear conditioning to tone. It is important to note, however, that the MK-801 solution used in the present study was nearly saturated and, thus, MK-801 can hardly be infused into the dorsal hippocampus at doses higher than that used in the present study. On the other hand, while the effects of NMDA observed in the present study demonstrate that NMDA in the dorsal hippocampus can strongly impair fear conditioning to both tone and context, lower doses of NMDA could have preferentially interfered with fear conditioning to context. Altogether, the infusion effects on fear conditioning observed in the present study are consistent with the notion that fear conditioning to context is more susceptible to dorsal hippocampal manipulations than simple fear conditioning to tone (Anagnostaras et al., 2001), but that, nevertheless, dorsal hippocampal manipulations may also affect fear conditioning to tone (Maren et al., 1997).

**Role of the dorsal hippocampus and local NMDA receptor-mediated processes in fear conditioning: comparison with the ventral hippocampus**

**Fear conditioning to context**

Several studies indicated fear conditioning to context to be more sensitive to lesions of the rat dorsal hippocampus than fear conditioning to tone (Selden et al., 1991; Kim and Fanselow,
1992; Phillips and LeDoux, 1992, 1994; Anagnostaras et al., 1999), even though impaired fear conditioning to a tone (Maren et al., 1997) and intact contextual fear conditioning (Phillips and LeDoux, 1994; Maren et al., 1997; Richmond et al., 1999) were also found after such lesions. Moreover, infusion of the competitive NMDA receptor antagonist APV into the rat dorsal hippocampus resulted in anterograde amnesia of foreground contextual fear conditioning, while this manipulation’s effects on fear conditioning to a tone have not been examined (Young et al., 1994). Based on the above data, NMDA receptor-mediated processes in the rat dorsal hippocampus have been suggested to be required for fear conditioning to context, but not tone (Young et al., 1994; Anagnostaras et al., 2001; Gale et al., 2001). This suggestion is confirmed directly by the present finding that blockade of NMDA receptors in the rat dorsal hippocampus by local infusion of the noncompetitive antagonist MK-801 impaired the formation of fore- and background contextual fear conditioning, while leaving fear conditioning to a tone intact. Similar results were obtained in a recent study after infusion of APV into the dorsal hippocampus of mice (Stiedl et al., 2000). In our previous study, MK-801 infusion into the ventral hippocampus of rats also induced such selective effects on fear conditioning to a context (Zhang et al., 2001). Thus, in the dorsal as well as ventral hippocampus, NMDA receptor signaling is only required for fear conditioning to context, but not tone. This is consistent with a specific role of NMDA receptor-mediated synaptic plasticity (Martin et al., 2000) in the ventral as well as dorsal hippocampus in contextual fear conditioning, even though NMDA receptors may contribute to several aspects of hippocampal synaptic transmission (e.g., Rosenblum et al., 1999). In addition to NMDA receptors, acetylcholine receptors in the rat dorsal hippocampus have recently been indicated to be involved in fear conditioning to context, but not tone (Gale et al., 2001; Wallenstein and Vago, 2001). Interestingly, this has been related to a possible modulation of NMDA receptor-mediated synaptic plasticity in the dorsal hippocampus by cholinergic transmission (Gale et al., 2001).

NMDA receptor activation in the dorsal and ventral hippocampus may solely be required to form a unified representation of the single elements making up a context or also for the association between context and US. Dorsal hippocampal mechanisms have been suggested to solely support the formation of a unified context representation, which afterwards becomes independent of the dorsal hippocampus and is possibly stored in the neocortex. This suggestion is supported by the finding that exposure to the conditioning context several weeks before lesioning the dorsal hippocampus protected rats from antero- and retrograde amnesia of contextual fear conditioning (Young et al., 1994; Anagnostaras et al., 2001). Moreover, it
II.3.

is consistent with the dependence of spatial learning upon the dorsal hippocampus (Moser and Moser, 1998) and, in particular, local NMDA receptor-mediated processes (Morris et al., 1989; Steele and Morris, 1999; Lee and Kesner, 2002), given that spatial learning involves the formation of a unified representation of the environment (Nadel and Willner, 1980; Anagnostaras et al., 2001). The ventral hippocampus is commonly believed to be less important for spatial learning than the dorsal hippocampus (Moser and Moser, 1998), and thus it may also be less important in forming a context representation. Rather, in contextual fear conditioning, ventral hippocampal processes may complement the role of the dorsal hippocampus by supporting formation and, possibly, also further processing and expression of the context-US association. This would also be plausible based on the anatomy of the hippocampus (Amaral and Witter, 1995). While the dorsal hippocampus is well equipped with projections from the sensory cortices, providing contextual information, only the ventral hippocampus has direct connections with extrahippocampal structures implicated in formation, processing, and expression of conditioned fear, such as amygdala (e.g., Cahill et al., 1999; Fanselow and LeDoux, 1999), nucleus accumbens (e.g., Riedel et al., 1997; Haralambus and Westbrook, 1999; Murphy et al., 2000; Pezze et al., 2001b, 2002b), and prefrontal cortex (e.g., Feenstra et al., 2001; Lacroix et al., 2001; Pezze et al., 2001a, 2002a, in press). For example, it has been proposed that context and US are associated via synaptic plasticity in the projection from the ventral hippocampus to the basolateral amygdala (Maren and Fanselow, 1995; Anagnostaras et al., 2001). Information about the contextual representation formed with participation of dorsal hippocampal mechanisms can reach the ventral hippocampus via direct intrahippocampal connections between dorsal and ventral hippocampus or via the parahippocampal cortices, in particular the entorhinal cortex.

Fear conditioning to tone

In contrast to the specific impairments in contextual fear conditioning after blockade of dorsal hippocampal NMDA receptors, tonic stimulation of these receptors by NMDA disrupted formation of short- and long-term fear to both tone and context, and also the retrieval/expression of long-term fear to the tone. This disruption of basic mechanisms of fear conditioning may reflect interference with ordered neuronal processing within the dorsal hippocampus or tonic stimulation of dorsal hippocampal projections resulting in interference of neuronal processing outside the dorsal hippocampus. For example, ventral hippocampal NMDA stimulation results in complete anterograde amnesia of fear (Zhang et al., 2001), and the effects of dorsal hippocampal NMDA stimulation may reflect concomitant stimulation of
the ventral hippocampus via intrahippocampal connections. This is, however, unlikely, given that dorsal hippocampal NMDA stimulation hardly affects behavioral processes other than fear conditioning (sensorimotor gating, locomotor activity) that are markedly altered by ventral hippocampal stimulation (Bast et al., 2001c,e; Zhang et al., 2002). Moreover, the nucleus accumbens core, which receives direct dorsal hippocampal projections (Groenewegen et al., 1987), has been implicated in fear conditioning. However, processing in the nucleus accumbens core may mainly contribute to contextual fear conditioning (see Pezze et al., 2001b). Finally, examination of Fos-protein production following electrical stimulation of the dorsal hippocampus suggested that subconvulsive dorsal hippocampal stimulation does not propagate out of the dorsal hippocampus (Sato et al., 1998). Thus, the complete anterograde amnesia of fear induced by NMDA in the dorsal hippocampus likely reflects disturbed neuronal processing within the dorsal hippocampus. This interpretation is in contrast to prevalent concepts, emphasizing the specific role of the dorsal hippocampus in contextual fear conditioning (Fanselow, 2000; Anagnostaras et al., 2001; Rudy and O'Reilly, 2001).

Evidence supporting this interpretation was, however, provided by two experiments, in which electrolytic as well as cytotoxic lesions of the dorsal hippocampus impaired fear conditioning to a tone (Maren et al., 1997), and by preliminary results indicating deficits in fear conditioning to tone following temporary inhibition of dorsal hippocampal neurons by the GABA_A agonist muscimol (Bellgowan and Helmstetter, 1995). Connections between dorsal and ventral hippocampus may link dorsal hippocampal processes to other structures contributing to formation, processing, and expression of conditioned fear to a tone, such as amygdala, nucleus accumbens, and prefrontal cortex (see above). A contribution of the ventral hippocampus to formation of conditioned fear to tone has clearly been suggested by several recent studies (Maren, 1999; Richmond et al., 1999; Bast et al., 2001b,d; Zhang et al., 2001).

Intact fear conditioning after dorsal hippocampal lesions

There is an apparent discrepancy between the suggested involvement of the dorsal hippocampus in fear conditioning to context and, possibly also tone, and intact fear conditioning found after permanent dorsal hippocampal lesions by several studies (see above). It has long been recognized that the loss of function induced by permanent lesions of a brain structure may be compensated for by other structures due to redundancy of neural structures and connections. Consequently, a behavioral function may be performed even after permanent lesion of a structure that normally serves this function (Bures and Buresova, 1990; Lomber, 1999). Functional compensation has recently been proposed to account for intact fear
conditioning after permanent dorsal hippocampal lesions (Anagnostaras et al., 2001; Bast et al., 2001b). However, it is not clear why compensation after dorsal hippocampal lesions occurs in some studies, but not in others. Thus, a review of the literature does not yield a clear relation between a particular experimental parameter (time between lesion and conditioning/testing, time between conditioning and testing, conditioning and testing procedures) and the effect of dorsal hippocampal lesions on fear conditioning.

**Conclusion**

The present study demonstrated that NMDA receptor-mediated processes in the rat dorsal hippocampus are required for formation of fear to a context, possibly to form a context representation, but not for fear conditioning to tone. Given that NMDA receptor-mediated processes in the dorsal hippocampus are also required for formation of spatial and episodic-like memory (Morris et al., 1989; Steele and Morris, 1999; Lee and Kesner, 2002), these processes may generally contribute to form representations of more complex relationships among stimuli, and, thus, also participate in the formation of human declarative memory (Eichenbaum, 1996). The impairments in formation and retrieval/expression of fear conditioning to tone by NMDA in the dorsal hippocampus indicated that some processes in the dorsal hippocampus, as in the ventral hippocampus (Maren, 1999; Richmond et al., 1999; Bast et al., 2001b,d; Zhang et al., 2001), may be involved in simple fear conditioning to a tone. This suggestion may be substantiated by future studies examining the effects of temporary dorsal hippocampal inactivation (e.g., by local tetrodotoxin infusion) on classical fear conditioning. Altogether, the present data are in line with the view that some hippocampal mechanisms selectively contribute to particular learning and memory functions while collectively the role of the hippocampus in learning and memory may be more general than previously assumed (see Bast et al., 2001b).

**Acknowledgements** This work was supported by grants from the Swiss Federal Institute of Technology Zurich. The support of the technical and administrative staff at the ETH research station Schwerzenbach is gratefully acknowledged, with particular acknowledgements being due to Jacqueline Kupper, Pascal Guela, Oliver Asprion, and Sepp Torlucci for animal care; Liz Weber for histological preparations; Peter Schmid for set-up and maintenance of the computerized systems for behavioral analysis; Jane Fotheringham for assistance with manuscript preparation; Christian Schlatter for help with preparation of guide cannulae. Thanks are also due to Ben Yee for critically reading the manuscript and to Marie Pezze, Ana
II.3.

Jongen-Rêlo, Carmen Sandi, Axel Becker, and Matti Mintz for comments, discussions, and advice that were helpful to accomplish the present study.
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209
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II.3.


214
II.3.


LETTER TO THE EDITOR

Hippocampus and Classical Fear Conditioning

Tobias Bast, Wei-Ning Zhang, and Joram Feldon

Behavioral Neurobiology Laboratory, Swiss Federal Institute of Technology Zurich, Schwerzenbach, Switzerland

To the Editors:

In a recent contribution to this journal, Anagnostaras et al. (2001) reviewed data regarding the involvement of the hippocampus in classical fear conditioning. The authors focused essentially on the role of the dorsal hippocampus and concluded that, in accordance with prevalent views that hippocampus-dependent memory involves the encoding of complex relationships among stimuli, the data support a specific mnemonic role for the dorsal hippocampus in contextual fear conditioning: the acquisition and consolidation of contextual representations. In this letter, we would like to point out that experimental data, including recent findings of our own, indicate that the role of the hippocampus in fear conditioning may extend beyond the specific involvement of the dorsal hippocampus in contextual fear conditioning and not simply accord with prevalent theories of hippocampus-dependent memory.

DORSAL HIPPOCAMPUS AND FEAR CONDITIONING TO A TONE

We agree with Anagnostaras et al. (2001) that most available data can be reconciled with the suggested specific role of the dorsal hippocampus in contextual fear conditioning. However, our own recent data, while indicating that blockade of dorsal hippocampal N-methyl-D-aspartate (NMDA) receptors by MK-801 resulted primarily in anterograde amnesia of contextual fear (consistent with the same effect reported by Young et al. (1994), after dorsal hippocampal infusion of the NMDA receptor antagonist D,L-2-amino-5-phosphonovalerate), suggested that dorsal hippocampal infusion of NMDA may not only impair the formation of fear conditioning to a context, but also to a tone (Bast et al., 2001a). It is possible that dorsal hippocampal NMDA stimulation interferes with fear conditioning either by disrupting basic processes in the nucleus accumbens, which receives dorsal hippocampal projections (Groenewegen et al., 1987), or by inducing ventral hippocampal stimulation, which may result in complete anterograde amnesia of fear (Zhang et al., 2001b; see below). An argument against the second possibility is that the effects of dorsal and ventral hippocampal NMDA stimulation on behavioral processes (sensorimotor gating, locomotor activity) other than fear conditioning are different (Zhang et al., 2001a). Given that NMDA infusion should severely disrupt normal neuronal activity, the complete anterograde amnesia of fear after dorsal hippocampal NMDA infusion may also reflect that simple classical fear conditioning to a tone requires processing in the dorsal hippocampus. This proposition is somewhat at odds with the absence of anterograde amnesia of fear to a tone after dorsal hippocampal lesions (e.g., Richmond et al., 1999). However, permanent lesions of a brain structure may fail to impair behavioral functions that are disrupted by temporary manipulations of the same structure, possibly because of compensatory processes leading to a recovery of the respective function (Bures and Buresova, 1990). The use of alternate cognitive solutions, which Anagnostaras et al. (2001) suggested to account for dorsal hippocampal lesions sometimes failing to induce anterograde amnesia of contextual fear, may be considered one special form of compensatory processes leading to functional recovery. Moreover, although sensorimotor gating is intact after hippocampal lesions, we found it severely impaired after dorsal or ventral hippocampal infusion of muscimol (which increases GABAA receptor-mediated neuronal inhibition) or tetrodotoxin (which leads to complete inactivation of local neurons, as well as fibers of passage) (see Feldon et al., 2001). Interestingly, Bellgowan and Helmstetter (1995) reported some evidence of muscimol infusion into the dorsal hippocampus impairing classical fear conditioning to a tone. Future studies using temporary manipulations of dorsal hippocampal processing should, therefore, further investigate the involvement of the dorsal hippocampus in fear conditioning to a tone.

VENTRAL HIPPOCAMPUS AND FEAR CONDITIONING TO CONTEXT AND TONE

Discussing the temporally stable, nonspecific loss of fear memory after complete hippocampal lesions that has been reported by Weisend et al. (1996), Anagnostaras et al. (2001) mentioned "the possibility...that ventral hippocampal neurons, perhaps because of their connections with the amygdala, play a more general role in the pro-
of the fear response than dorsal hippocampal neurons” (p. 12). However, Anagnostaras et al. (2001) did not further pursue the role of the ventral hippocampus. Connections to amygdala and nucleus accumbens link the ventral hippocampus with basic neuronal processes that are thought to underlie classical fear conditioning (see Bast et al., 2001c). Remarkably, the dorsal hippocampus is not directly connected to the amygdala, but only via the ventral hippocampus (Pitkänen et al., 2000). Thus, the view that in contextual fear conditioning the dorsal hippocampus forms a context representation associated with the shock via hippocampal-amygdala long-term potentiation (see Anagnostaras et al., 2001) already implies an involvement of the ventral hippocampus.

Anagnostaras et al. (2001) referred to anterograde amnesia of contextual fear after permanent lesions, as well as several temporary manipulations of the dorsal hippocampus, as important evidence that the dorsal hippocampus is essential for the formation of contextual fear. Available data indicate that permanent lesions and temporary manipulations of the ventral hippocampus also result in anterograde amnesia of fear. Three studies consistently found anterograde impairments of freezing to a context as well as to a tone after excitotoxic or electrolytic lesions of the ventral hippocampus (Maren and Fanselow, 1995; Maren, 1999; Richmond et al., 1999). Richmond et al. (1999) proposed that these anterograde freezing impairments may reflect lesion-induced locomotor hyperactivity, while Maren (1999) gave some good reasons for these impairments reflecting actual memory impairments. Our own recent results supported the mnemonic account of anterograde freezing deficits after ventral hippocampal lesions. We found anterograde amnesia of fear to a context as well as to a tone after ventral hippocampal retrodotoxin infusion and anterograde amnesia of fear to a context after ventral hippocampal muscimol infusion (Bast et al., 2001c). These data indicated that neurons in the ventral hippocampus are important for the formation of fear conditioning to both context and tone. Moreover, blockade of ventral hippocampal NMDA receptors by MK-801 resulted in anterograde amnesia of fear to a context, while ventral hippocampal stimulation by NMDA resulted in anterograde amnesia of fear to both context and tone (Zhang et al., 2001b). While the former indicated that formation of fear memory to a context requires NMDA receptor-mediated processes in the ventral hippocampus, the latter may have reflected disruption of normal ventral hippocampal processing or disruption of, for example, amygdaloïd processing by excess excitation via ventral hippocampal projections. Altogether the available experimental data suggest that, in addition to the dorsal hippocampal mechanisms proposed by Anagnostaras et al. (2001), some mechanisms in the ventral hippocampus are specifically involved in the formation of contextual fear conditioning. Moreover, some processes in the ventral hippocampus appear to be also important for the formation of simple classical fear conditioning to a tone.

Several issues concerning the role of the ventral hippocampus in fear conditioning still remain to be examined (cf. Bast et al., 2001c). For example, one interesting issue is whether the ventral hippocampus may be permanently important for the storage and/or retrieval of fear memory. Complete hippocampal lesions have been reported to induce temporally stable retrograde amnesia of fear (Weisdorf et al., 1996), whereas dorsal hippocampal lesions induce temporally graded retrograde amnesia of fear (see Anagnostaras et al., 2001). The indicated difference between the effects of complete and dorsal hippocampal lesions may be attributable to permanent involvement of the ventral hippocampus in the storage and/or retrieval of fear memory, suggesting an interesting difference between dorsal and ventral hippocampal mechanisms in fear conditioning. We agree with Anagnostaras et al. (2001) that the behavioral effects of ventral hippocampal lesions may not merely reflect the absence of normal ventral hippocampal activity (see discussion of locomotor effects of permanent ventral hippocampal lesions as compared to those of temporary ventral hippocampal inhibition or inactivation in Bast et al., 2001c). Indeed, excitotoxic ventral hippocampal lesions by ibotenate were recently demonstrated to induce widespread extrahippocampal damage (Hamil and Swerdlow, 2000), and the problem that focal lesions, regardless of the lesion technique used, may induce widespread changes throughout the entire brain has long been recognized (see Stein, 1979). Thus, future studies to clarify the ventral hippocampal involvement in storage and retrieval of fear memory may preferentially use temporary manipulations of ventral hippocampal processing. When freezing or fear potentiated startle are used as measures of conditioned fear in such studies, which involve ventral hippocampal manipulations before testing of conditioned fear, it is important to consider that alterations of ventral hippocampal activity may influence both locomotor activity and startle reactivity (e.g., Bast et al., 2001b–d; Zhang et al., 2001a,b). So, performance and mnemonic accounts (see Anagnostaras et al., 2001) for possible deficits in the expression of conditioned fear have to be carefully separated. Indeed, as also proposed by Anagnostaras et al. (2001), alternative indices of conditioned fear (see Antoniadis and McDonald, 2000), whose expression may be less dependent on hippocampal activity, would be useful for future studies on the mnemonic role of the ventral and also dorsal hippocampus in classical fear conditioning.

**IMPLICATIONS FOR VIEWS ON HIPPOCAMPAL FUNCTION**

The specific role Anagnostaras et al. (2001) proposed for the dorsal hippocampus in contextual fear conditioning was appealing because it fit well with prevalent views on hippocampus-dependent memory that were strongly stimulated by the specific memory impairments reported from human amnesics with damage to the temporal lobe, including the hippocampus. According to these views, hippocampal mechanisms underlie the formation of complex representations of stimuli and their spatial and/or temporal relations, but are not essential for simple associative learning. With respect to classical delay conditioning, these views imply that the hippocampus is only important when contextual information has to be used, assuming that spatial and contextual learning depend
on similar hippocampal mechanisms (see Anagnostaras et al., 2001; Bast et al., 2001c).

Considering the evidence discussed in this letter, in particular that concerning the role of the ventral hippocampus in fear conditioning, prevalent concepts of hippocampal-dependent memory processes may need some modifications. First, while the role of the dorsal hippocampus in fear conditioning to a tone deserves further investigation, the available data strongly suggest that processes in the ventral hippocampus are important for the formation of simple fear conditioning to a tone. Thus, hippocampal mechanisms appear to be involved in a form of simple classical delay conditioning that does not depend on the use of contextual information. Second, although some processes in the dorsal (see Anagnostaras et al., 2001) as well as in the ventral hippocampus (Bast et al., 2001c; Zhang et al., 2001b) appear to be specifically important for fear conditioning to a context but not to a tone, only dorsal and not ventral hippocampal lesions affected spatial learning in the watermaze (Moser et al., 1995; Richmond et al., 1999). So, different hippocampal mechanisms may underlie spatial and contextual learning (see also Richmond et al., 1999). However, given that lesions may fail to impair functions that are impaired by temporary interference with neuronal activity (Bures and Buresova, 1990; Feldon et al., 2001), the effects of temporary ventral hippocampal manipulations on spatial learning should be examined. Furthermore, the dissociation of contextual and spatial learning in the hippocampus could be substantiated by demonstrating that the same manipulation of the ventral hippocampus specifically impairs fear conditioning to a context, but not to a tone, while not affecting spatial learning.

Assuming a similar functional significance of the hippocampus in rats and humans, the involvement of the rat (ventral) hippocampus in simple classical fear conditioning appears to be at odds with the specific memory impairments in human temporal lobe amnesia. However, in amnestic, parts of the functions normally sustained by the hippocampus may still be intact because of incomplete hippocampal damage or recovery of function due to compensatory mechanisms. Interestingly, aberrant hippocampal function also appears to exist in schizophrenic patients (e.g., Silbersweig et al., 1995; Harris, 1999; Weinberger, 1999; see also Bast et al., 2001b,d; Zhang et al., 2001a). Indeed, deficits in simple classical conditioning have been reported from schizophrenics (Ax, 1990; Martins Serra et al., 2001). Although these deficits cannot unequivocally be ascribed to hippocampal dysfunction, given that several brain abnormalities exist in schizophrenia (e.g., Silbersweig et al., 1995; Harris, 1999), they are at least consistent with an involvement of the human hippocampus in simple classical fear conditioning.

REFERENCES


II.4.


Annotations:
A1 This study has been written up as a full-length report: Bast T, Zhang W-N, Feldon J (in press) Dorsal hippocampus and classical fear conditioning to tone and context in rats: the effects of local NMDA-receptor blockade and stimulation. Hippocampus.
PART III:
Extrahippocampal mechanisms mediating the hippocampal control of sensorimotor processes and fear conditioning: the role of dopamine transmission in the medial prefrontal cortex
Dopamine receptor blockade in the rat medial prefrontal cortex reduces spontaneous and amphetamine-induced activity and does not affect prepulse inhibition

T. Bast*, M.A. Pezze*, J. Feldon

Behav Pharmacol, in press

*T.B. and M.A.P. contributed equally to the present study.
Abstract

The functions and interactions of cortical and subcortical dopamine systems are of outstanding interest because alterations in these systems have been implicated in neuropsychiatric diseases, such as schizophrenia. It has been proposed that prefrontal dopamine transmission may oppose dopamine transmission in subcortical sites, such as the nucleus accumbens. Accordingly, reduced prefrontal dopamine transmission would be expected to enhance or induce behavioral effects that have been associated with stimulation of accumbal dopamine receptors. In rats, spontaneous and amphetamine-induced activity is supported by dopamine receptor stimulation in the nucleus accumbens, while prepulse inhibition (PPI) of the acoustic startle response, which is used to measure sensorimotor gating and disrupted in schizophrenia, is reduced by increased accumbal dopamine receptor stimulation. In the present experiments, we found that infusion of the dopamine D1/D2 receptor antagonist cis-flupenthixol dihydrochloride into the medial prefrontal cortex of Wistar rats (25 μg/side) reduced spontaneous activity and completely blocked induction of hyperactivity by systemic administration of D-amphetamine sulfate (1 mg/kg), while not affecting PPI. These findings do not support an antagonism between prefrontal and accumbal dopamine in the control of behavior. Rather, our data demonstrate that prefrontal dopamine transmission may modulate some behavioral processes in a similar way to accumbal dopamine.

Keywords: activity; amphetamine; cis-flupenthixol; dopamine; medial prefrontal cortex; prepulse inhibition; sensorimotor gating; schizophrenia
Introduction

Antagonism of prefrontal and subcortical dopamine transmission was first suggested based on findings that prefrontal dopamine depletion may induce or enhance behavioral alterations related to subcortical dopamine stimulation and increase indices of subcortical dopamine utilization (Pycock et al., 1980). This possibility is interesting with respect to the pathophysiology of schizophrenia, where reduced dopamine transmission in the prefrontal cortex (PFC) may be concurrent with increased subcortical dopamine transmission (Deutch, 1992; Weinberger et al., 2001). While some neurochemical and behavioral evidence has accumulated supporting that prefrontal may oppose subcortical dopamine transmission, other findings have indicated that this may not generally hold (Tzschentke, 2001). For example, D1, but not D2, receptor blockade in the medial PFC (mPFC) enhanced hyperactivity induced by accumbal dopamine stimulation (Vezina et al., 1991), while D2 receptor-mediated transmission in the mPFC has been suggested to facilitate motor activity (Beninger et al., 1990). Moreover, mPFC infusion of specific D1 or D2 receptor antagonists reduced prepulse inhibition (PPI) of the acoustic startle response (Ellenbroek et al., 1996), while mPFC infusion of haloperidol did not affect basal PPI, but partially restored PPI reduced by systemic apomorphine (Hart et al., 1998). PPI, the reduction of the startle reaction to a loud pulse by a briefly preceding weaker prepulse, is used to measure sensorimotor gating in rats and humans, and is reduced in neuropsychiatric diseases, such as schizophrenia, as well as by accumbal dopamine stimulation in rats (Swerdlow et al., 1990).

The present paper reports the effects of the D1/D2 receptor antagonist cis-flupenthixol in the mPFC on spontaneous and amphetamine-induced activity, as well as on PPI in Wistar rats. Assuming opposing roles of prefrontal and accumbal dopamine, spontaneous and amphetamine-induced activity, which are facilitated by accumbal dopamine (Ahlenius et al., 1987; Boye et al., 2001), should be increased, and PPI, which is reduced by accumbal dopamine stimulation (Swerdlow et al., 1990), should be decreased by blockade of mPFC dopamine transmission.

Methods

Thirty-six male Wistar rats (Zur:Wist[HanBm]; Research Unit Schwerzenbach, Schwerzenbach, Switzerland; 280 to 380 g) were used. All rats were implanted with guide cannulae (26 gauge) allowing insertion of infusion cannulae (34 gauge) to
infuse cis-flupenthixol dihydrochloride (FLU; SIGMA, Switzerland; 25 μg / 0.5 μl 0.9% saline / side) or infusion vehicle (VEH) bilaterally into the prelimbic mPFC. The infusion cannulae aimed at the following coordinates (in mm): 3.0 anterior and ±0.5 lateral to bregma, and 4.6 ventral to skull surface. Housing and handling of the rats, as well as general procedures for surgery, microinfusions, and verification of cannulae placements were identical to previous studies (Bast et al., 2001a-c).

Activity was measured in sixteen cubicles (25 cm wide x 40 cm long x 40 cm high), each contained within a sound-attenuating wooden cabinet. Six infrared (875 nm) light-emitting diodes in the ceiling of each cabinet provided the only lighting, sufficient for function of video cameras mounted on the cabinets' ceiling. As a measure of activity, the percentage of changed pixels between two adjacent 1-s video images from the test boxes was determined automatically (further details in Pezze et al., in press).

The whole-body acoustic startle and PPI were measured in four startle chambers (SR-LAB, San Diego Instruments, San Diego, CA) as detailed elsewhere (Bast et al., 2001a,c). In brief, a 5-min acclimatization was followed by four startle pulses [30-ms broadband burst, 120 dB(A)] and six blocks of 11 trials, presented pseudorandomly with an average intertrial interval of 15 s, to measure PPI: two pulse alone trials, one prepulse followed by pulse (80 ms between prepulse offset and pulse onset) and one prepulse alone trial for each of four prepulses [20-ms broadband burst of 72, 76, 80, or 84 dB(A)], and one no stimulus trial. Background noise was 68 dB(A). PPI (in percent) induced by each prepulse intensity was calculated as: 100 - (100 x startle amplitude on prepulse followed by pulse trial) / (startle amplitude on pulse alone trial).

In Experiment 1, examining the effects of mPFC FLU infusion on spontaneous and amphetamine-induced activity, 24 rats were used. On day 1, baseline activity of all rats was measured for 30 min. On day 2, rats received VEH or FLU infusion. Immediately after the infusion, rats were injected with D-amphetamine sulfate (Amph; SIGMA, Switzerland; 1 mg / 1 ml saline / kg, i.p.) or injection vehicle (Veh) and then subjected to 2.5-h activity testing. Thus, there were four groups: VEH-Veh, VEH-Amph, FLU-Veh, FLU-Amph. One rat was excluded because of problems with the infusion, resulting in five rats in the FLU-Amph, and six rats in the other three groups.
In Experiment 2, examining the effects of mPFC FLU infusion on startle and PPI, 12 rats were used. On day 1, baseline startle and PPI of all rats were measured. On day 2, rats received VEH or FLU infusion (six rats each) immediately before startle and PPI testing.

Data were analyzed by ANOVA using infusions and injections as between-subjects factors. Ten-min blocks of testing (activity measurements), pulse alone trials (startle measurements), and the four prepulse intensities (PPI measurements) were used as repeated-measures factors. Level of significance was $P < 0.05$.

**Results**

In Experiment 1, activity measured one day before the infusions and injections did not differ between the four groups [$F(3,19)=0.090, P=0.965$; data not shown]. FLU in the mPFC reduced spontaneous activity, revealed by comparison of the FLU-Veh and the VEH-Veh group [$F(1,10)=7.961, P<0.02$], and completely blocked induction of hyperactivity by Amph injection (Fig. 1). The latter was demonstrated by effects of infusion [$F(1,19)=24.060, P<0.0001$], and injection [$F(1,19)=17.065, P<0.001$], and an interaction of both factors [$F(1,19)=5.219, P<0.05$] in the overall ANOVA, reflecting that the VEH-Amph group was markedly more active than the other three groups (Fisher's LSD post hoc tests, $P<0.01$).

In Experiment 2, startle throughout the 16 pulse alone trials [$F(1,10)=1.247, P=0.290$] and PPI values [$F(1,10)=0.038, P=0.849$] measured one day before the infusions did not differ between the two groups (data not shown). FLU in the mPFC did not affect startle or PPI (Fig. 2). ANOVA of startle and PPI data did not yield an effect of infusion [$F(1,10)=0.499, P=0.496$; $F(1,10)=0.234, P=0.639$] or an interaction of infusion x pulse alone trial [$F(1,10)=1.490, P=0.116$] or of infusion x prepulse intensity [$F(1,10)=1.428, P=0.254$]. There were only effects of pulse alone trial [$F(15,150)=6.458, P<0.0001$], reflecting startle habituation, and of prepulse intensity [$F(3,30)=15.460, P<0.0001$], reflecting an increase in PPI with increasing prepulse intensity.
Fig. 1 Effects of the dopamine D1/D2 receptor antagonist cis-flupenthixol in the medial prefrontal cortex on spontaneous activity and amphetamine-induced hyperactivity. Rats received a bilateral infusion of cis-flupenthixol dihydrochloride (FLU; 25μg/side) or infusion vehicle (VEH) into the medial prefrontal cortex, immediately thereafter a systemic injection of D-amphetamine sulfate (Amph; 1.5 mg/kg) or injection vehicle (Veh), and activity was then monitored for 150 min. (a) Mean activity, averaged for each single 10-min block (main graph) and over the complete 150-min period (inset graph); error bars represent 1 standard error of the means in the main graph and 1 standard error of the means in the inset graph; ** more active than all other groups (Fisher’s LSD post hoc tests, P<0.01), * less active than the VEH-veh group (separate ANOVA, P<0.02). (b) Approximate location of the tips of the infusion cannulae depicted on plates of coronal sections through the rat brain (Paxinos and Watson, 1998). Values on the right indicate distance from bregma in mm.
Fig. 2 Effects of the dopamine D1/D2 receptor antagonist cis-flupenthixol in the medial prefrontal cortex on startle and prepulse inhibition. Rats received a bilateral infusion of cis-flupenthixol dihydrochloride (FLU; 25μg/side) or infusion vehicle (VEH) into the medial prefrontal cortex immediately before testing of startle reactivity and prepulse inhibition. (a) Mean startle amplitude for each single of the 16 pulse alone trials (left, main graph) and averaged over all 16 pulse alone trials (left, inset graph), as well as mean prepulse inhibition for each of the four different prepulse intensities (right, main graph) or averaged over all four prepulse intensities (right, inset graph); error bars represent 1 standard error of the differences of the means in the main graphs and 1 standard error of the means in the inset graphs. (b) Approximate location of the tips of the infusion cannulae depicted on plates of coronal sections through the rat brain (Paxinos and Watson, 1998). Values on the right indicate distance from bregma in mm.
III.1.

Discussion

Dopamine D1/D2 receptor blockade in the mPFC by FLU reduced spontaneous activity [replicating preliminary experiments with 20 µg FLU/side (Pezze et al., 2001, in press] and completely blocked the induction of hyperactivity by systemic amphetamine. This corroborates that mPFC dopamine transmission, perhaps primarily via D2 receptors, facilitates activity (Beninger et al., 1990). D1 receptor-mediated transmission in the mPFC, on the other hand, was suggested not to affect spontaneous activity, but to inhibit hyperactivity induced by amphetamine in the nucleus accumbens (Vezina et al., 1991). Duvauchelle et al. (1992) reported that activity in rats injected with a quite high dose of systemic Amph (1.5 mg/kg) was increased by mPFC infusion of relatively low FLU doses [5 or 10 µg / side; in our laboratory even 12.5 µg / side did not have any effect on spontaneous or amphetamine-induced activity (Lacroix et al., 2000)]. Different doses and/or activity measures may account for the discrepancies between their results and ours. For example, the high dose of systemic Amph may have induced stereotypies, thereby decreasing photocell interruptions which served as activity counts (although this is difficult to judge since no activity counts were reported for rats with systemic control injections), and these stereotypies may have been antagonized by FLU in the mPFC. Since the effects of mPFC FLU infusion on spontaneous and amphetamine-induced activity in the present study were similar to those expected for dopamine receptor blockade in the ventral striatum, it is important that drug spread into ventral striatal regions, located about 2 mm ventral to the infusion sites in the mPFC, can virtually be excluded. First, drug spread mainly occurs dorsally along the guide cannula and can be assumed to be less than 1 mm with the small infusion volume and the fine infusion cannulae used in the present study (Myers, 1966; Routtenberg, 1972). Second, infusion of 40 µg FLU in 1 µl saline into ventral striatal regions, but not into striatal regions located about 2 mm more dorsally, decreased activity (Ahlenius et al., 1987), clearly demonstrating the anatomical specificity of intracerebral FLU infusion.

Like haloperidol (Hart et al., 1998), FLU in the mPFC did not affect basal PPI [as in preliminary experiments with 20 µg FLU/side (Pezze et al., 2001a)], demonstrating that reduced mPFC dopamine transmission does not necessarily yield the same effect on PPI as increased accumbal dopamine transmission. The latter was suggested by findings that both D1 and D2 receptor antagonists in the mPFC reduced PPI (Ellenbroek et al., 1996). Possibly, it is relevant whether D1 and D2 receptor blockade
occurs simultaneously or separately. Interestingly, consistent with the notion that prefrontal and accumbal dopamine may modulate PPI similarly, rather than in opposite ways, PPI reduced by systemic apomorphine was similarly restored by haloperidol infusion in the mPFC or nucleus accumbens (Hart et al., 1998).

Permanent depletion of mPFC dopamine by 6-hydroxydopamine decreased spontaneous and amphetamine-induced activity, as well as PPI, in some studies (Tzschentke, 2001). These effects, different from those of temporary dopamine receptor blockade in the present study, may be related to the additional depletion of mPFC norepinephrine and serotonin and possible compensatory changes induced by the permanent manipulation.

Altogether, the present results corroborate that prefrontal dopamine may modulate locomotor activity and PPI in a way similar, rather than opposed, to accumbal dopamine. In line with this, dopamine may have similar effects on prefrontal and accumbal neurons (Yang et al., 1999; Floresco et al., 2001), and both nucleus accumbens and mPFC project directly, as well as via the pallidum, to brain stem centers mediating locomotor activity and PPI (Alheid and Heimer, 1996; Koch, 1999). Furthermore, facilitation of locomotor activity by both prefrontal and accumbal dopamine is consistent with evidence that the ventral hippocampus, which drives locomotor activity in a dopamine-dependent way (Wu and Brudzynski, 1995; Bast et al., 2001a-c), positively modulates dopamine transmission in both mPFC (Gurden et al., 2000; Bast, Zhang, Ferger, Feldon, submitted) and nucleus accumbens (Legault and Wise, 1999; Mitchell et al., 2000).

Acknowledgements This research was supported by grants from the Swiss National Science Foundation Zurich (grants N° 3160-051657 and N° 3100-042009.94) and the Swiss Federal Institute of Technology Zurich. The authors thank L. Lacroix for helpful advice to start these experiments; J. Kupper, P. Guela, O. Asprion, and J. Torlucci for animal care; P. Schmid for set-up and maintenance of the computerized systems for behavioral analysis; L. Weber for histological preparations; C. Schlatter for preparation of the guide cannulae; and J. Fotheringham for her editorial help. W. White and P. Schmid set up the system for measurement of activity.
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Significance of dopamine transmission in the rat medial prefrontal cortex for conditioned fear

Marie A. Pezze*, Tobias Bast*, Joram Feldon

Cereb Cortex, in press

*M.A.P. and T.B. contributed equally to the present study.
Abstract

Previous studies have demonstrated activation of dopamine transmission in the medial prefrontal cortex (mPFC) by conditioned fear stimuli. Therefore, the present study investigated the functional significance of mPFC dopamine for a conditioned fear response to a tone. We examined the effects of inhibition or stimulation of mPFC dopamine transmission by local microinfusion of the D1/D2 receptor antagonist cis-flupenthixol or the indirect dopamine receptor agonist D-amphetamine, respectively, in a classical-fear-conditioning paradigm in Wistar rats. Rats received tone-shock pairings and were later exposed to the tone alone. Freezing was used as measure of conditioned fear. Presence of the drugs in the mPFC during the tone-shock pairings did not affect freezing during subsequent presentation of the tone alone. However, when cis-flupenthixol and D-amphetamine were present in the mPFC during presentation of the tone alone, freezing to the tone was reduced. We demonstrated that the decreased freezing could neither be explained by state dependency nor infusion-induced alterations in activity. Our data indicate that mPFC dopamine transmission is important for the retrieval/expression, but not the formation, of conditioned fear. The reduction of conditioned fear by prefrontal infusion of both cis-flupenthixol and D-amphetamine may reflect normal expression of conditioned fear to require an optimal level of mPFC dopamine activity.
III.2.

Introduction

In rats and humans, the medial prefrontal cortex (mPFC) is characterized by similar connections and has been suggested to play a pivotal role in emotional responses (Nauta, 1971; Davidson and Irwin, 1999; Bechara et al., 2000; Öngür and Price, 2000; Simpson et al., 2000). Research on emotion in laboratory rodents has focused on fear, whose neural substrates can readily be studied in classical-fear-conditioning experiments (LeDoux, 2000). In classical fear conditioning, an emotionally neutral conditioned stimulus (CS), such as a tone, is paired with an aversive unconditioned stimulus (US), usually a footshock, so that subsequent presentation of the CS elicits conditioned fear responses.

Conditioned fear is accompanied by marked alterations in mPFC neurotransmission. In particular, neurochemical studies in rats have consistently suggested activation of mPFC dopamine transmission by presentation of a CS previously paired with footshocks (e.g., Goldstein et al., 1994; 1996; Inoue et al., 1994; Yoshioka et al., 1996; Morrow et al., 1999b,c; Feenstra et al., 1999, 2001; Feenstra, 2000; but see Wilkinson et al., 1998). This activation was proposed to be necessary for the normal expression (Morrow et al., 1999c) or extinction of fear responses to a CS (Morrow et al., 1999b; Feenstra et al., 2001). Moreover, specific patterns of electrical activity (Garcia et al., 1999; Baeg et al., 2001) and increased extracellular norepinephrine (Feenstra et al., 1999, 2001) were evoked in the mPFC by fear CS.

The functional significance of the reported alterations in mPFC neurotransmission for a normal conditioned fear response is not clear. Currently, a popular view is that processes in the mPFC are important for inhibitory control of conditioned fear (Maren, 2001). This view is based on the findings that lesions to the dorsal (mainly anterior cingulate) mPFC enhanced acquisition of conditioned fear (Morgan and LeDoux, 1995; Vouimba et al., 2000) and lesions to the ventral mPFC impaired fear extinction (Morgan et al., 1993; Quirk et al., 2000). Importantly, destruction of the most caudal and ventral infralimbic mPFC was demonstrated to be critical for the extinction deficit (Quirk et al., 2000). Based on the finding that 6-hydroxydopamine lesions of the mPFC, depleting the local catecholamine, in particular dopamine, innervation, reduced extinction of very strong, but not moderate, conditioned fear, mPFC dopamine was proposed to be important for fear extinction under certain conditions (Morrow et al., 1999b). There is also strong evidence that some subregions of the mPFC are important for the normal expression of conditioned fear. Thus, in line with Nauta’s (1971) earlier proposition that frontal cortex lesions result in an “interoceptive agnosia” reflected by an absence of normal emotional responses, lesions destroying the central mPFC, including
prelimbic and infralimbic parts, but sparing the most caudal and ventral infralimbic portions, reduced the expression of conditioned fear (Frysztak and Neafsey, 1991, 1994; Lacroix et al., 2000b; McLaughlin et al., 2001). Finally, there are several studies in which mPFC lesions, destroying considerable parts of the prelimbic and infralimbic subregions, did not affect (Holson, 1986; Rosen et al., 1992; Gewirtz et al., 1997) conditioned fear responses. This may be related to compensatory processes induced by permanent lesions (see Bast et al., 2001a). Altogether, the specific functional significance of prefrontal processes accompanying conditioned fear, the best documented of which is activation of dopamine transmission, awaits clarification.

In contrast to permanent lesions, local microinfusions of neuroactive substances allow the role of different transmitter systems in the mPFC during different stages of a fear-conditioning experiment to be addressed specifically. In order to clarify the functional significance of alterations in mPFC dopamine transmission for aversively conditioned emotional responses, the present study examined the effects of specifically manipulating mPFC dopamine transmission by local microinfusion of the dopamine D1/D2 receptor antagonist cis-flupenthixol or the indirect dopamine agonist D-amphetamine in a classical-fear-conditioning paradigm in Wistar rats. Freezing, an immobile posture in response to an inescapable aversive CS (Fanselow, 1984), was used as measure of conditioned fear.

Materials and methods

Animals

One hundred male adult Wistar rats (Zur:Wist[Hanlbm]; Research Unit Schwerzenbach, Schwerzenbach, Switzerland), about two months old and weighing about 250-300 g at the time of surgery, were used for the experiments of the present study (Exp. I: 24 rats; Exp. II: 39; Exp. III: 37). The animals were housed under a reversed light-dark cycle (lights on: 19:00-07:00) in a temperature (21 ± 1°C)- and humidity (55 ± 5%)-controlled room, and were provided with food and water ad libitum. All rats received bilateral implantation of guide cannulae aiming at the mPFC. Before surgery, rats were housed in groups of four per cage; after surgery, they were individually caged. Beginning three days before surgery and thereafter until the beginning of the behavioral experiments, all rats were handled daily. All experimental procedures were carried out in the dark phase of the cycle. Principles of laboratory animal care (NIH publication no. 86-23, revised 1985) and Swiss regulations for animal experimentation were followed.

236
Implantation of guide cannulae for intracerebral infusion

Rats were anesthetized with 1 ml of Nembutal (sodium pentobarbital, 50 mg/ml, Abbott Labs, North Chicago, IL) per kg body weight and their head was placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA). After application of a local anesthetic (Lidocaine), the scalp was incised to expose the skull, and bregma and lambda were aligned in the same horizontal plane. A pair of guide cannulae (9 mm, 26 gauge, stainless steel) in a Perspex holder (custom made) was implanted through small holes (1.5 mm diameter) drilled on each side of the skull. The tips of the guide cannulae aimed at the following coordinates (in mm): 3.0 anterior and ±0.5 lateral to bregma, and 3.1 ventral to skull surface. The guide cannulae were fixed with dental cement for which three small stainless screws, previously screwed into the skull, served as anchors. Stainless steel stylets (34 gauge) which extended 0.5 mm beyond the tips of the guide cannulae were placed inside the guide cannulae to prevent occlusion. After surgery, the experimenters gave the rats daily health checks and gentle handling, and replaced missing stylets. The behavioral experiments commenced five days after surgery.

Intracerebral microinfusion and drugs

For microinfusions into the mPFC, rats were manually restrained and the stylets removed from the guide cannulae. Infusion cannulae (34 gauge, stainless steel; custom made) were then inserted into the guide cannulae. The infusion cannulae were fixed to 26-gauge cuffs at one end to prevent them from slipping through the guide cannulae and connected to 10-µl Hamilton microsyringes mounted on a microinfusion pump (KD scientific or WPI sp200i). The tips of the infusion cannulae protruded into the mPFC 1.5 mm beyond the tip of the guide cannulae, thus aiming at a final coordinate of 4.6 mm below the skull surface. The rats were bilaterally infused with cis-flupenthixol dihydrochloride (FLU; C_{23}H_{25}F_{3}N_{2}OS x 2HCl, SIGMA, Switzerland, 20 or 25 µg) or D-amphetamine sulfate [AMPH; C_{18}H_{26}N_{2} x H_{2}SO_{4}, SIGMA, Switzerland, 13.6 µg (10 µg free base)] in 0.5 µl vehicle (0.9% saline) or with 0.5 µl vehicle (VEH) only. Drug solutions were freshly prepared on the day of infusion. The infusion speed was 0.5 µl/min. After infusion, the infusion cannulae were left in the brain for 60 s to allow for absorption of the infusion bolus by the brain tissue and then replaced by the stylets. Rats were then immediately subjected to the behavioral testing.

AMPH and FLU were chosen for this first examination of the functional significance of mPFC dopamine transmission for conditioned fear because these compounds affect both D1 and D2 receptor-mediated processes. Furthermore, we could draw on the experience from previous experiments performed in our laboratory (Lacroix et al., 2000a) in which these drugs
had been infused into the mPFC. While the indirect dopamine agonist AMPH may also stimulate the release of other monoamines (Azzaro and Rutledge, 1973), the direct nonselective dopamine agonist apomorphine also affects transmission by other monoamines; for example, apomorphine has similar affinities to dopamine and alpha1-adrenoceptors (Baldessarini et al., 1994). AMPH was infused into the mPFC at a dose of 13.6 μg (corresponding to 10 μg of the free base)/0.5 μl/side in the present experiments because this dose produced a behavioral effect in a previous study from our laboratory (Lacroix et al., 2000a). With regard to FLU, 12 μg/0.5 μl/side infused into the mPFC did not yield any behavioral effect in the aforementioned study (Lacroix et al., 2000a), and so a dose of 20 μg/0.5 μl/side was used in the first fear conditioning experiment (Exp. 1). Since this dose yielded a behavioral effect without any adverse side effect (e.g., disrupted motor coordination, brain damage), a slightly higher dose of 25 μg/0.5 μl/side was used in the final fear conditioning experiment (Exp. 3) in order to obtain the behavioral effect reliably.

Two experiments were conducted to control for the low pH (pH 2-3) of the FLU solutions. Subjects were tested for the expression of conditioned fear and for activity following infusion of acidified (adjusted to pH 2 by concentrated hydrochloric acid, n = 5), as detailed below (Exp. 1, Activity Experiment). No significant behavioral effects were observed relative to rats infused into the mPFC with normal saline (pH 6-7, n = 5) (data not shown). Therefore, subsequent comparisons were made between rats infused into the mPFC with FLU solutions (pH 2-3) and subjects receiving mPFC infusion of normal saline (pH 6-7).

**Apparatus for behavioral testing**

Eight operant test boxes, four shock boxes and four no-shock boxes, (Habitest; Coulborn Instruments, Allentown, PA) were used for the fear-conditioning experiments (Exp. I to III). Shock boxes were used for conditioning and context-test sessions, while the no-shock boxes were used to assess fear to the tone CS in an environment distinct from that during conditioning. Shock boxes were fitted with a parallel grid shock floor (16 parallel bars; E10-10RF; Coulborn Instruments), through which scrambled shocks could be delivered, and placed in light- and sound-attenuating chambers measuring 55 cm x 40 cm x 55 cm (wide x long x high). These chambers had two side walls of aluminium and a rear and front wall of clear Perspex. A white waste tray was situated below the grid floor. The four no-shock boxes were fitted with a lattice grid (E10-18NS; Coulborn Instruments) and placed in light- and sound-attenuating chambers measuring 72 cm x 45 cm x 45 cm. They had three black walls and a front wall of clear Perspex. A brown waste tray was situated below the lattice grid. The
III.2.

four shock and the four no-shock boxes were placed in two different rooms. Presentation of the tone CS and delivery of electric foot shock were controlled by a PC with dedicated software (S Frank, Psychology Department, University of Tel Aviv, Israel) connected to a Coulborn Universal Environment Interface (E91-12) with Coulborn Universal Environment Port (L91-12). The tone CS [85 dB(A)] was produced by a 2.9 kHz tone module (E12-02) fixed at one wall of the operant chamber. Shocks were delivered with a Coulborn Precision Animal Shocker (E13-12) which generated bipolar rectangular 10-ms current pulses with a frequency of 10 Hz. Background noise was provided by a ventilation fan affixed to the light- and sound-attenuating chambers during all sessions. A monochrome minivideo camera with a wide angle (100°) 2.5-mm lens (VPC-465B; CES AG, Zurich, Switzerland) was attached to the center of the ceiling of each operant chamber. Four infrared (875 nm) light-emitting diodes (HSDL-4220; Hewlett Packard) positioned in the ceiling of each operant chamber provided light sufficient for camera function. Throughout all sessions, images from the test boxes were provided by these cameras, integrated into a four-quarter single image (100000 pixels) by a multiplexer (DX216CE, Sony), and recorded by a video-recorder (SVT1000; Sony).

Sixteen cubicles (25 cm x 40 cm x 40 cm), each contained within an individual sound-attenuating wooden cabinet, were used for the Activity Experiment examining the effects of FLU and AMPH infusion into the mPFC on general activity. The cubicles had three walls of clear plastic and one side wall of wooden panels. The floor of each cubicle was a black removable pan holding a thin layer of dark, absorbent, autoclaved earth. The ceiling was open. A fan mounted on the wall of each cabinet provided ventilation. A monochrome minivideo camera with a wide angle (100°) 2.5-mm lens (VPC-465B; CES AG, Zurich, Switzerland), centered approximately 49 cm above the compartment floor, was mounted in the ceiling of each cabinet. Six infrared (875 nm) light-emitting diodes (HSDL-4220; Hewlett Packard) positioned in the ceiling of each cabinet provided light sufficient for camera function. Images provided from each of the 16 cubicles by these cameras were integrated into a sixteen-part single image (400000 pixels) by a 16-channel multiplexer (Sony model YS-DX216CE), and the image was recorded by a video-recorder (SVT1000; Sony).

Automated measurement of activity and freezing

The video images were transferred to a computer (fear-conditioning experiments: 7600/120 Power Macintosh; control experiment: Dell OptiPlex GXpro with a Pentium Pro Processor) equipped with an analysis program (Image; http://rsb.info.nih.gov/nih-image) and a
III.2.

macroprogram (P. Schmid, Behavioral Neurobiology Laboratory, Swiss Federal Institute of Technology Zurich). The percentage of changed pixels between two adjacent 1-s images from one of the test boxes was used as a measure of activity (see Richmond et al., 1998 for further details). Freezing is commonly identified as cessation of any movement except for respiratory movements (e.g., Anagnostaras et al., 1999). If the percentage of changed pixels between two adjacent 1-s images was less than 0.05 % this corresponded well to such immobility, and the behavior of the rat was scored as "freezing" for the respective second. Validation and principle of the automated analysis of freezing behavior have been described in detail in previous publications (Richmond et al., 1998; Pryce et al., 1999).

**Fear-conditioning experiments**

Three fear-conditioning experiments (Exp. I, II, and III) were conducted testing the effects of FLU or AMPH infusion into the mPFC on the formation, retrieval/expression, and extinction of fear conditioning to a tone. In all three experiments there were three types of sessions: conditioning, context-test and tone-test sessions. Conditioning sessions consisted of two (Exp. I) or six (Exp. II and III) tone-shock pairings (30-s tone coterminating with a 1-s, 0.5-mA footshock) preceded and followed by 2-min (Exp. I) or 1.5-min blocks (Exp. II and III) in the shock boxes. Context-test sessions consisted of 8 min during which the rats were placed in the shock boxes without receiving any stimulation. Context-test sessions were conducted to assess long-term conditioned fear (i.e. conditioned fear persisting beyond the conditioning session) to the context in which the foot shocks were experienced, as well as for extinction of fear to the context, in order to obtain a pure measure of fear to the tone during the subsequent tone test. Tone tests, to assess long-term conditioned fear to the tone, were conducted in the no-shock boxes, i.e. an environment distinct from the conditioning context, and consisted of a continuous 8-min tone presentation, preceded by 2 min without stimulation. During all sessions, freezing was assessed by the automated analysis system as a measure of conditioned fear. In those experiments involving drug infusions before conditioning (Exp. II and III), the effects of the drug infusions on unconditioned behaviors were assessed by observing some unconditioned behaviors in the different infusion groups during the conditioning session. The video images taken during conditioning were observed in order to assess whether all infusion groups reacted to the shock by similar vigorous twitching and jumping, marked components of the unconditioned immediate shock response (Anagnostaras et al., 1999). Activity before the first tone or shock, was assessed using the percentage of changed pixels between adjacent 1-s video images given by the automated

240
system. The different infusion groups, as well as conditioning and test procedures, in Exp. I, II, and III are described in detail below and summarized in Table 1.

**Table 1 Summary of the procedures in the three fear-conditioning experiments (Exp. I-III)**

<table>
<thead>
<tr>
<th>INFUSION</th>
<th>CONDITIONING</th>
<th>CONTEXT TEST</th>
<th>TONE TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(shock box)</td>
<td>(shock box, 8 min)</td>
<td>(no-shock box, 10 min, tone on after 2 min)</td>
</tr>
<tr>
<td><strong>Exp. I</strong></td>
<td>Immediately before tone test: VEH, FLU (20 µg/side), AMPH (13.6 µg/side)</td>
<td>Two tone-shock pairings each preceded and followed by 2-min blocks (7 min)</td>
<td>One day after conditioning</td>
</tr>
<tr>
<td><strong>Exp. II</strong></td>
<td>Immediately before conditioning and tone test: VEH, FLU (25 µg/side)</td>
<td>Six tone-shock pairings each preceded and followed by 1.5-min blocks (13.5 min)</td>
<td>Six days after conditioning</td>
</tr>
<tr>
<td><strong>Exp. III</strong></td>
<td>Immediately before conditioning and tone test: VEH, AMPH (13.6 µg/side)</td>
<td>As in Exp. II</td>
<td>As in Exp. II</td>
</tr>
</tbody>
</table>

AMPH, D-amphetamine sulfate; FLU, cis-flupenthixol dihydrochloride; VEH, vehicle.
Tone: 30 s, 2.9 kHz, 85 dB(A). Shock: 1-s, 0.5-mA foot shock, concurrent with the last second of the tone.
Conditioned fear during conditioning and testing was automatically measured in the form of freezing.

**Exp. I**

In Exp I, rats received bilateral infusions of VEH (n=8), AMPH (13.6 µg; n=8), or FLU (20 µg; n=8) into the mPFC immediately before the tone-test session. The three infusion groups were matched for freezing levels during conditioning and context test. The conditioning session consisted of two tone-shock pairings. Using a low number of pairings it was aimed at achieving a relatively low level of conditioned freezing to the tone in order to facilitate detection of an extinction deficit. Context-test sessions were conducted one day after conditioning, tone-test sessions two days after conditioning.

**Exp. II and III**

Exp I indicated that infusions of FLU as well as AMPH before tone test resulted in reduced conditioned freezing to the tone, suggesting that both treatments interfere with the retrieval/expression of conditioned fear. Exp II and III were aimed to confirm this suggestion. In particular, we tested whether the reduced conditioned freezing during tone test might merely have reflected state dependency, i.e. that associations formed in an altered brain-state may subsequently be retrieved only in a similar brain-state (Overton, 1964), rather than an
impairment of the specific processes underlying retrieval/expression of conditioned fear. For that purpose, FLU (Exp. II) and AMPH (Exp. III) were infused not only immediately before tone-test, but also before conditioning sessions. Thus, the results of Exp. II and III are also relevant regarding the role of mPFC dopamine transmission in the formation of conditioned fear. In both experiments all rats received a first infusion of either VEH or drug immediately before conditioning and a second infusion immediately before the tone test. In Exp. II, all rats received either bilateral infusions of VEH (n=19) or FLU (25 μg; n=20) into the mPFC before conditioning. Immediately before the tone test all rats received a second infusion of VEH or FLU. Half of the rats received the same infusion as before conditioning, and one half received a different infusion, resulting in four groups differing with respect to the combinations of infusions (before conditioning-before tone test): VEH-VEH (n=9), VEH-FLU (n=10), FLU-VEH (n=10), FLU-FLU (n=10). In Exp. III, all rats received either bilateral infusions of VEH (n=18) or AMPH (13.6 μg; n=19) into the mPFC before conditioning. Immediately before the tone test, all rats received a second infusion of VEH or AMPH. Half of the rats received the same infusion as before conditioning, and one half received a different infusion, resulting in four groups differing with respects to the combinations of infusions (before conditioning-before tone test): VEH-VEH (n=8), VEH-AMPH (n=10), AMPH-VEH (n=9), AMPH-AMPH (n=10). In both Exp. II and III, the groups that received the same infusion before conditioning, but different infusions before the tone test, were matched for freezing levels during the conditioning and context-test sessions.

For conditioning, rats were put in the shock boxes for a total of 13.5 min and received six tone-shock pairings. A higher number of pairings than in Exp. I was used in order to facilitate the detection of infusion-induced reductions in conditioned freezing. Six days after conditioning, a context test was conducted to minimize residual contextual freezing during the subsequent tone test. The tone test was conducted seven days after conditioning. The time span between conditioning and test sessions was chosen so that repeated infusions were one week apart in order to allow for a recovery of the brain tissue from disturbances that may result from one intracerebral infusion before applying the next one.

Activity experiment

Twenty-three rats used in Exp. I were subjected to an additional experiment, two weeks after termination of Exp. I, in order to test whether FLU or AMPH in the mPFC induce alterations in general activity that might have influenced the freezing response (see Bast et al., 2001b). (In Exp. II and III, unconditioned activity was monitored before the first shock during
the conditioning sessions, i.e. during the first 1.5 min following the infusions, but possible infusion-induced alterations in unconditioned general activity after these first 1.5 min could not be assessed.) On day 1, basal activity was measured for 30 min. The animals were then allocated to one of three infusion groups, counterbalanced with respect to the infusions received during Exp. 1 and matched according to their baseline activity. On day 2, rats received infusions of VEH (n=9), FLU (20 µg/side; n=7), or AMPH (13.6 µg/side; n=7) immediately before their activity was monitored for 2 h. On day 3, activity was measured for 30 min without a preceding infusion.

**Histology**

After completion of the behavioral experiments, the cannulated rats were deeply anesthetized with an overdose of 2.5 ml/kg Nembutal (sodium pentobarbital, 50 mg/ml, i.p.) and transcardially perfused with 0.9% NaCl solution to rinse out the blood, followed by 250 ml of 4% formalin (4°C) to fix the brain tissue. After extraction from the skull, the brains were post-fixed in 4% formalin solution and subsequently cut into 40-µm coronal sections on a freezing microtome. For the verification of the infusion sites, every fifth section through the mPFC was mounted on a gelatine-treated slide and stained with cresyl violet. After staining, the sections were dehydrated and coverslipped. Subsequently, they were examined with a light microscope under tenfold magnification. It was verified that the tips of the infusion cannulae were placed in the mPFC and their approximate locations were drawn onto plates from the atlas of Paxinos and Watson (1998). Moreover, brain damage around the infusion sites was compared between the infusion groups.

**Data analysis**

Statistical analysis was conducted using the Statview software system. From the freezing scores obtained for each second ("freezing" or "not freezing"), the percentage of time spent freezing in a given time block was calculated. By averaging the percentage of pixels changed between adjacent 1-s video images in a given time block, a relative activity measure for this time block was calculated. Data were first subjected to ANOVA, using the different infusions as between-subjects factor and the different time blocks of testing as repeated-measures factors. Post hoc comparisons were conducted using Fisher’s protected least significant difference test. Level of significance was set at $P < 0.05$. Freezing during the context-test sessions is not reported, since it was negligible in all three experiments (on average less than 15%), reflecting that the conditioning procedures used in the present study did not result in
considerable conditioned fear to the conditioning context. Data during the first four 1-min blocks of tone presentation served as a reliable measure of tone freezing that was not confounded by within-session habituation (see Corcoran and Maren, 2001).

Results

Histology

In all rats used in Experiment I-III, the tips of the infusion cannulae were located within or around the borders of the mPFC (Fig. 1). Therefore, behavioral data of all rats were included in the analysis. Damage was restricted to the area immediately surrounding the guide and infusion cannulae and did not differ between the infusion groups.

Exp. I: FLU and AMPH in the mPFC during tone test

In Exp. 1, in which rats received only one infusion of FLU, AMPH, or VEH immediately before the tone test, the three groups did not differ in their freezing levels during conditioning and context test reflecting successful matching (data not shown). Freezing exhibited by the three different infusion groups during the tone test of Exp. I is depicted in Fig. 2.

Conditioned freezing to the tone previously paired with footshocks was lower in the AMPH and FLU groups than in the VEH rats. During the two 1-min blocks before tone onset, rats exhibited virtually no conditioned fear as evidenced by low levels of immobility (<15%). ANOVA of freezing levels during the first four 1-min blocks of the tone presentation revealed a significant effect of infusion \( F(2,22) = 5.19, \ P < 0.02 \). Post hoc comparisons indicated that average freezing levels were lower in the AMPH \( (P < 0.01) \) and FLU \( (P < 0.02) \) rats than in the VEH group. AMPH and FLU rats did not differ \( (P > 0.96) \). Thus, the results of Exp. 1 indicated that both AMPH and FLU in the mPFC reduced expression of conditioned fear. To corroborate this suggestion, we conducted Exp. II and III.
Fig. 1 Infusion sites in the medial prefrontal cortex. (A) Photomicrograph of a coronal brain section with the tracks of the guide cannulae and beneath them the infusion sites visible in both hemispheres. (B) Approximate location of the tips of the infusion cannulae depicted on plates of coronal sections through the rat brain (Paxinos and Watson, 1998); numbers on the right indicate distance from Bregma in mm.
Fig. 2 Freezing during tone-test sessions of Exp. I. Infusions of VEH, FLU and AMPH were given immediately before the tone test. Mean percentage of time spent freezing during the tone test is depicted for the two 1-min blocks preceding the tone as well as for the first four 1-min blocks of tone presentation. Bars represent 2 standard errors of the differences of means (2 SED), derived from the appropriate mean square of the ANOVA according to the formula given by Cochran and Cox (1950, p. 91 ff.). The 2 SED provide an estimate of population variance for between-groups comparisons and are used in line graphs for the sake of clarity.
Experiment II & III: FLU and AMPH in the mPFC during conditioning and tone test

Unconditioned behaviors during conditioning session

Fig. 3 depicts unconditioned activity during the 1.5-min block preceding the first tone-shock pairing for the different infusion groups in Exp. II and III.

FLU (Exp. II) and AMPH (Exp. III) rats did not differ significantly from VEH rats in the unconditioned activity exhibited throughout the 1.5-min block preceding the first tone-shock pairing at the beginning of the conditioning session. ANOVA of average activity throughout the 1.5 min preceding the first tone-shock pairing did not yield significant differences between FLU and VEH rats in Exp. II \(F(1,37) = 1.75, P > 0.19\) or AMPH and VEH rats in Exp. III \(F(1,35) = 0.65, P > 0.43\).

Inspection of the video images from the conditioning sessions of Exp. II and III yielded that all infusion groups exhibited similar vigorous twitching and marked jumps as immediate response to the foot shock, indicating that FLU or AMPH infusions did not affect shock sensitivity.

Conditioned freezing during conditioning and tone test

The freezing data for conditioning and tone test of Experiment II, in which rats received infusions of either FLU or VEH before conditioning, as well as before the tone test, are depicted in Fig. 4.

During the conditioning session, the development of conditioned freezing in response to the inescapable foot shocks did not differ between rats that received FLU or VEH infusion before conditioning. ANOVA of average freezing levels during the seven 1.5-min blocks preceding and following the tone-shock pairings, as well as of the six 30-s blocks of tone presentations, did not yield an effect of the infusion received before conditioning \(F(1,37) = 0.84, P > 0.36; F(1,37) = 2.29, P > 0.13\) or an interaction of infusion x time block \(F(6,222) = 0.47, P > 0.83; F(5,185) = 1.84, P > 0.10\). There was only a significant effect of 1.5-min block \(F(6,222) = 56.31, P < 0.0001\) and 30-s tone presentation \(F(5,185) = 44.53, P < 0.0001\), reflecting the gradual increase of freezing with repeated shock administration.

During the tone test, the two groups that received FLU immediately before the tone test (VEH-FLU, FLU-FLU) exhibited less conditioned freezing to the tone than the groups that received VEH immediately before the tone test (VEH-VEH, FLU-VEH), while the infusion received before conditioning had no effect. Rats did not exhibit conditioned fear before tone onset, as indicated by the negligible freezing levels during the two 1-min blocks preceding tone onset (less than 10%). Freezing strongly increased with tone onset. However, peak levels
Fig. 3 Effects of the different infusions on unconditioned activity during the 1.5 min before the first tone shock pairing in Exp. II and III. Infusions of VEH, FLU and AMPH were applied immediately before behavioral testing. Presented values are means ± SEM.
Fig. 4 Freezing during conditioning and tone-test sessions of Exp. II. A first infusion of VEH or FLU was given immediately before conditioning, resulting in two infusion groups for conditioning. A second infusion of VEH or FLU was given immediately before the tone test, resulting in four infusion groups (FLU-FLU; FLU-VEH; VEH-FLU; VEH-VEH), differing with respect to the combination of infusions received before conditioning and tone test. Mean percentage of time spent freezing during conditioning is depicted for the seven 1.5-min blocks preceding and following the six tone-shock pairings (A) as well as for the six 30-s tone presentations of the pairings (B). Mean percentage of time spent freezing during the tone test is depicted for the two 1-min blocks preceding the tone as well as for the first four 1-min blocks of tone presentation (C). Bars represent 2 standard errors of the differences of means (2 SED).
of freezing were considerably higher in the rats that received VEH before the tone test than in those that received FLU. This was reflected by the results of a three-way ANOVA (infusion before conditioning x infusion before tone test x 1-min block) of freezing during the first four 1-min blocks of tone presentation. Thus, there was an interaction of infusion before tone test x 1-min block \( [F(3,105) = 2.79, P < 0.05] \) along with an effect of 1-min block \( [F(3.105) = 8.22, P < 0.0001] \), but neither a main effect of infusion before conditioning \( [F(1,35) = 0.33, P > 0.56] \) or of infusion before tone test \( [F(1,35) = 2.57, P > 0.11] \), nor interactions of infusion before conditioning x 1-min block \( [F(3,105) = 2.18, P > 0.09] \) or of infusion before conditioning x infusion before tone test x 1-min block \( [F(3,105) = 0.73, P > 0.53] \). Further analysis, based on the significant interaction of infusion before tone test x 1-min block, yielded that groups receiving FLU before the tone test (VEH-FLU, FLU-FLU) exhibited less freezing during the second and third 1-min blocks of tone presentation than rats receiving VEH before the tone test (VEH-VEH, FLU-VEH). Thus, separate ANOVAs of freezing levels for each single 1-min block yielded a significant main effect of infusion before tone test for the second 1-min block \( [F(1,35) = 5.05, P < 0.04] \) and a strong tendency towards an effect for the third 1-min block \( [F(1,35) = 3.67, P = 0.06] \), while not indicating differences for the first \( [F(1,35) = 0.06, P > 0.81] \) and the fourth \( [F(1,35) = 0.10, P > 0.75] \) 1-min block.

The freezing data for conditioning and tone test of Experiment III, in which rats received infusions of either AMPH or VEH before conditioning as well as before the tone test, are depicted in Fig. 5.

During conditioning, freezing that developed in response to the foot shocks was lower in rats that received AMPH before conditioning than in those that received VEH. ANOVA of freezing during the seven 1.5-min blocks preceding and following the tone-shock pairings, as well as during the six 30-s blocks of tone presentations, yielded a main effect of infusion before conditioning \( [F(1,35) = 6.09, P < 0.02; F(1,35) = 5.63, P < 0.03] \) and 1.5-min block \( [F(6,210) = 47.96, P < 0.0001] \) or 30-s block \( [F(5,175) = 37.77, P < 0.0001] \), respectively. There was also a strong tendency towards an interaction of infusion before conditioning x 30-s block of tone presentation \( [F(5,175) = 2.24, P = 0.0523] \).

During the tone test, the two groups that received AMPH immediately before the tone test (VEH-AMPH, AMPH-AMPH) exhibited less conditioned freezing to the tone than the groups that received VEH immediately before the tone test (VEH-VEH, AMPH-VEH). The infusion received before conditioning had virtually no effect on conditioned freezing during the tone test. Rats did not exhibit conditioned fear before tone onset, as indicated by the negligible
Fig. 5 Freezing during conditioning and tone-test sessions of Exp. III. A first infusion of VEH or AMPH was given immediately before conditioning, resulting in two infusion groups for conditioning. A second infusion of VEH or AMPH was given immediately before the tone test, resulting in four infusion groups (AMPH-AMPH; AMPH-VEH; VEH-AMPH; VEH-VEH), differing with respect to the combination of infusions received before conditioning and tone test. Mean percentage of time spent freezing during conditioning is depicted for the seven 1.5-min blocks preceding and following the six tone-shock pairings (A) as well as for the six 30-s tone presentations of the pairings (B). Mean percentage of time spent freezing during the tone test is depicted for the two 1-min blocks preceding the tone as well as for the first four 1-min blocks of tone presentation (C). Bars represent 2 standard errors of the differences of means (2 SED).
III.2.

freezing levels during the two 1-min blocks preceding tone onset (less than 5%). In all groups, freezing increased with tone onset. However, freezing reached considerably higher peak levels in the rats that received VEH before the tone test than in those that received AMPH. Three-way ANOVA (infusion before conditioning x infusion before tone test x 1-min block) of freezing during the first four 1-min blocks of tone presentation yielded a main effect of infusion before tone test \( [F(1,33) = 4.75, P < 0.04] \) and a significant main effect of 1-min block \( [F(3,99) = 6.00, P < 0.001] \) without an interaction of infusion before tone test x 1-min block \( [F(3,99) = 2.28, P > 0.08] \). Although it appeared as if the AMPH-AMPH group exhibited slightly higher levels of freezing than the VEH-AMPH rats, ANOVA did not yield a main effect of infusion before conditioning \( [F(1,33) = 0.01, P > 0.94] \) or any significant interaction involving infusion before conditioning [infusion before conditioning x 1-min block: \( F(3,99) = 0.50, P > 0.68 \); infusion before conditioning x infusion before tone test: \( F(1,33) = 1.58, P > 0.21 \); infusion before conditioning x infusion before tone test x 1-min block: \( F(3,99) = 0.75, P > 0.52 \)]. Thus, only the infusion received immediately before the tone test significantly affected levels of freezing to the tone.

**Activity experiment: effects of FLU or AMPH in the mPFC on general activity**

The effects of FLU or AMPH infusion into the mPFC on general activity during 2 h following infusion are depicted in Fig. 6.

FLU decreased the general activity as compared to VEH infusions, while AMPH did not have any effect. ANOVA of the average activity during the 2 h of testing yielded a significant group effect \( [F(2,20) = 14.23, P < 0.0001] \), and post hoc comparisons indicated that activity was lower in the FLU group than in both the AMPH \( (P < 0.0015) \) and VEH \( (P < 0.0001) \) groups, which did not differ \( (P > 0.2) \). A more detailed analysis was done for the activity data collected during the first 15 1-min blocks following infusion, which corresponded to the period of conditioning and tone-test sessions in the fear conditioning experiments. ANOVA yielded a significant effect of group \( [F(2,20) = 8.37, P < 0.003] \) and 1-min blocks \( [F(14,580) = 22.71, P < 0.0001] \), with the latter reflecting habituation. Post hoc comparisons indicated that average activity throughout the 15 min following infusion was lower in the FLU group than in both the AMPH \( (P < 0.0015) \) and VEH \( (P < 0.003) \) groups, which did not differ \( (P > 0.58) \). Although ANOVA did not yield an interaction of group x time block \( [F(28,580) = 1.25, P > 0.18] \), it can be seen from Fig. 6 that, consistent with the measurement of unconditioned activity during the 1.5 min preceding the first tone-shock pairing in Exp. II (Fig. 3), activity in the FLU group was not considerably lower than in the VEH and AMPH.
Fig. 6 General activity after VEH, FLU, and AMPH infusion in the Activity Experiment. Mean activity + SEM is depicted averaged over the complete 2 h (inset bar graph) as well as for each single of the first 15 1-min blocks (main line graph) following infusion. The bar in the main graph represents 2 standard errors of the differences of means (2 SED).
rats during the first 1-min block. Indeed, a separate ANOVA on activity during the first 1-min block did not yield an effect of group \[ F(2,20) = 1.40, P > 0.27 \]. On day 3, groups did not differ in their activity any more (data not shown).

Discussion

The main finding of the present study is that both the dopamine receptor antagonist cis-flupenthixol (Exp. 1 and 2) and the dopamine receptor agonist D-amphetamine (Exp. 1 and 3) in the mPFC reduced the expression of conditioned fear, measured as freezing, to a tone previously paired with footshocks. While D-amphetamine also reduced immediate conditioned freezing during conditioning, presence of neither drug during the tone-shock pairings affected conditioned fear during subsequent presentation of the tone alone. These data indicate that dopamine receptor-mediated processes within the mPFC are important for the retrieval/expression, but not the formation, of conditioned fear.

Specificity of the infusion effects

Infusion sites were located in the central mPFC, mainly in the prelimbic and the dorsal infralimbic subdivision. With the small infusion volume (0.5 µl/side) and fine infusion cannulae (34 gauge) used in the present study, the estimated spread of the infused substances is less than 1mm (Myers, 1966; Myers et al., 1971, Routtenberg, 1972). It can therefore be assumed that differences between rats infused with vehicle compared to rats infused with cis-flupenthixol or D-amphetamine reflected a temporary alteration of neuronal activity by blockade of dopamine receptors or increased release of dopamine, and possibly other monoamines, respectively, within the central mPFC.

The similar immediate shock responses observed in all infusion groups in the present study, as well as previous findings that neither cis-flupenthixol nor D-amphetamine in the mPFC, at the doses used in the present study, affected the acoustic startle reaction or its prepulse inhibition (Lacroix et al., 2000a; Pezze et al., 2001a; Bast et al., in press a), indicate that these infusions do not considerably affect sensory processing.

The Activity Experiment demonstrated that, at the doses used in the present study, cis-flupenthixol in the mPFC decreased activity (see also Beninger et al., 1990; Pezze et al., 2001a; Bast et al., in press a), while D-amphetamine did not alter activity. Consistent with the similar unconditioned activity of VEH and FLU rats during the 1.5-min block preceding the first tone-shock pairing in Exp. II, the decrease in activity induced by cis-flupenthixol in the control experiment was not evident before the second 1-min block. Infusion-induced
III.2.

hypoactivity can, however, not account for reduced freezing. Rather, hypoactivity facilitates freezing (see Bast et al., 2001b) and may have somewhat concealed reduced conditioned fear in rats infused with cis-flupenthixol.

Finally, the reduced conditioned freezing observed during the tone-test sessions in rats that received drug infusions immediately before the sessions cannot be explained by state dependency, i.e. that information learned in a particular brain state can in some cases only be retrieved with the same brain state prevailing (Overton, 1964). This was demonstrated by the results of Exp. II and III, in which the drug-vehicle groups expressed normal freezing, while the drug-drug groups demonstrated a pronounced deficit.

**Role of the mPFC and local dopamine transmission in fear conditioning**

Previous neurochemical studies indicated mPFC dopamine activation by a fear CS (e.g., Goldstein et al., 1994; 1996; Inoue et al., 1994; Yoshioka et al., 1996; Morrow et al., 1999b,c; Feenstra, 2000; Feenstra et al., 2001; but see Wilkinson et al., 1998) without clarifying the functional relation between dopamine in the mPFC and conditioned fear. The present finding that both the dopamine receptor antagonist cis-flupenthixol and the dopamine receptor agonist D-amphetamine in the mPFC reduced freezing to a tone previously paired with footshocks suggests that retrieval/expression of conditioned fear depends on normal mPFC dopamine transmission. The fact that the expression of conditioned fear was not completely blocked, but only reduced, by interference with prefrontal dopamine transmission is consistent with the widely held notion that the mPFC has a modulatory, rather than primary, role in the expression of fear (see Morrow et al., 1999a). Moreover, D-amphetamine, but not cis-flupenthixol, in the mPFC reduced the expression of conditioned freezing during conditioning. This may reflect that dopamine receptor-mediated processes important for the retrieval/expression of immediate conditioned fear during conditioning differ from those relevant for the retrieval/expression of long-term conditioned fear assessed in later test sessions. Finally, manipulating mPFC dopamine transmission during the tone-shock pairings did not affect conditioned freezing expressed during a subsequent presentation of the aversive CS, suggesting that mPFC dopamine transmission does not play a role in the formation of a stable fear memory.

As described in the introduction, the functional relation between neural activity in the mPFC and conditioned fear may depend on the subregion within the mPFC. In several previous studies, lesions centering on the prelimbic and dorsal infralimbic mPFC resulted in decreased conditioned fear suggesting that processes in these regions may be essential for
III.2.

normal conditioned fear (Frysztak and Neafsey, 1991, 1994; Lacroix et al., 2000b; McLaughlin et al., 2001). Consistent with this suggestion, the results of the present study indicate that dopamine transmission in the prelimbic and dorsal infralimbic mPFC, which was the center of the infusion sites, is critical for the normal expression of conditioned fear. Dopamine transmission in other mPFC subregions may play a different role, given that lesions affecting the anterior cingulate (Morgan and LeDoux, 1995; Vouimba et al., 2000) and the most caudal and ventral infralimbic mPFC (Morgan et al., 1993; Quirk et al., 2000) were reported to have facilitating effects on conditioned fear. This issue may be addressed in future studies.

In some studies, lesions destroying considerable parts of the prelimbic and dorsal infralimbic mPFC did not alter conditioned fear (Holson, 1986; Rosen et al., 1992; Gewirtz et al., 1997; Quirk et al., 2000). Moreover, 6-hydroxydopamine lesions to the mPFC, depleting dopamine (by 86%), norepinephrine (by 51%), which has been implicated in extinction of conditioned responses (see Dalley et al., 2001), and serotonin (by 14%) in the rostral cingulate, prelimbic, and rostral infralimbic mPFC, did not affect moderate conditioned freezing to a tone (peak levels about 40-50%), while extinction of strong conditioned freezing (peak levels about 80%) was strongly reduced (Morrow et al., 1999b). The latter suggests that the significance of catecholamine transmission in the mPFC for the extinction of conditioned fear may depend on the intensity of fear (Morrow et al., 1999b; Quirk et al., 2000). The fact that permanent damage to the prelimbic and dorsal infralimbic mPFC, in contrast to our temporary manipulation of local dopamine transmission, sometimes failed to reduce conditioned fear may reflect compensatory processes induced by permanent lesions (see Bast et al., 2001a).

Interactions of mPFC dopamine and fear circuits

While efferents to the nucleus accumbens, amygdala, periaqueductal gray, or lateral hypothalamus (Sesack et al., 1989) link the mPFC to structures that have been implicated in the expression of conditioned fear (Fendt and Fanselow, 1999; LeDoux, 2000; Murphy et al., 2000; Pezze et al., 2001b, 2002), the mPFC may receive information about CS-US associations through afferents from the amygdala (McDonald, 1991) and ventral hippocampus (Jay and Witter, 1991). Both the ventral hippocampus (Bast et al., 2001a,b, in press b; Zhang et al., 2001) and amygdala (Cahill et al., 1999; LeDoux and Fanselow, 1999; LeDoux, 2000) have been implicated in the associative processes underlying formation of conditioned fear, and the amygdala may even be the site where the CS-US association is permanently stored.
(Fanselow and LeDoux, 1999; Fendt and Fanselow, 1999; LeDoux, 2000; but see Cahill et al., 1999). Interestingly, electrical stimulation of the amygdala (Jackson and Moghaddam, 2001) or the ventral hippocampus (Gurden et al., 2000) was demonstrated to result in measurable effects on neural processes in the mPFC, including an increase of extracellular dopamine. Thus, mPFC dopamine transmission may contain information about CS-US associations transmitted from the amygdala or the ventral hippocampus, and the reduced expression of conditioned fear observed after interference with mPFC dopamine transmission may reflect that the transfer of this information was disrupted.

**Relation between mPFC dopamine and conditioned fear**

In the present study, both the dopamine receptor antagonist cis-flupenthixol and the agonist D-amphetamine reduced expression of conditioned fear when infused into the mPFC. This may reflect that normal retrieval/expression of conditioned fear requires time and synapse specific dopamine-receptor stimulation in the mPFC, which is unlikely in view of the evidence for dopaminergic volume transmission in the mPFC (see Bast et al., 2002). Alternatively, it may reflect an inverted U-shaped function relating the retrieval/expression of conditioned fear to dopamine-receptor stimulation in the mPFC, i.e. that normal retrieval/expression of conditioned fear requires an optimal level of dopamine activity in the mPFC. Similarly, working memory has been suggested to require an optimal level of prefrontal dopamine-receptor stimulation because PFC infusions of both dopamine-receptor antagonists and agonists impaired working memory (Sawaguchi and Goldman-Rakic, 1991; Williams and Goldman-Rakic, 1995; Zahrt et al. 1997, Arnsten, 1998; Seamans et al., 1998; Goldman-Rakic et al., 2000; Robbins, 2000). These effects have been found with D1 receptor-specific agents and some studies directly demonstrated that D2 receptor antagonists are without an effect (Sawaguchi and Goldman-Rakic, 1991; Williams and Goldman-Rakic, 1995; Seamans et al., 1998; but see Arnsten et al., 1995; Druzin et al., 2000). Attentional performance also appears to depend on an optimal level of mPFC D1-receptor stimulation (Granon et al., 2000; Robbins et al., 2000). Electrophysiological findings indicate that behavioral processes involving dopamine-mediated mechanisms in the mPFC may require an optimal level of local dopamine activity because an optimal level of local D1 receptor stimulation is essential for the “sharpening” of prefrontal signal transmission (Yang and Seamans, 1996). A final consideration is that D-amphetamine may not only directly stimulate dopamine, but also norepinephrine and serotonin transmission (Azzaro and Rutledge, 1973), and that this might contribute to the reduction of conditioned fear by D-
amphetamine in the mPFC. Thus, future studies should examine the effects of mPFC infusion of specific D1 or D2 receptor antagonists or agonists on conditioned fear in order to clarify whether local D1 and D2 receptors are differently involved in conditioned fear and to substantiate that excess prefrontal dopamine receptor stimulation may reduce conditioned fear.

**Conclusion**

The present study suggested that inhibition as well as stimulation of dopamine receptors in the (prelimbic and dorsal infralimbic) mPFC reduced conditioned fear to a tone previously paired with a footshock. Our findings corroborate the view that the mPFC and local dopamine transmission are essential for normal emotional responses. Furthermore, they suggest the retrieval/expression of conditioned fear, like working memory and attentional performance, may be related to mPFC dopamine transmission by an inverted U-shaped function, indicating an intriguing relation between the substrates of emotion, working memory, and attention.

_Acknowledgements_ This research was supported by grants from the Swiss National Science Foundation Zurich (grants N° 3160-051657 and N° 3100-042009.94) and the Swiss Federal Institute of Technology Zurich. The authors thank L. Lacroix for helpful advice to start this study; J. Kupper, P. Guela, O. Asprion, and J. Torlucci for animal care; P. Schmid for set-up and maintenance of the computerized systems for behavioral analysis; L. Weber for histological preparations; C. Schlatter for preparation of the guide cannulae; and J. Fotheringham for her editorial help. W. White and P. Schmid set up the system for measurement of general activity. Part of the presented results has previously been published in a preliminary form [Pezze et al., 2001a; Pezze, Bast, Feldon (2002) FENS Abstr Vol. 1, A042.21].
III.2.

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Post mortem study of changes in forebrain dopamine and serotonin transmission induced by ventral hippocampal N-methyl-D-aspartate stimulation in rats: evidence for an activation of dopamine transmission in the medial prefrontal cortex

Tobias Bast, Wei-Ning Zhang, Boris Ferger, Joram Feldon
Submitted
Abstract

Hippocampal mechanisms may influence behavior by modulation of monoamine transmission in extra-hippocampal forebrain sites. In the present study, we used post mortem neurochemistry to simultaneously examine changes in dopamine (DA) and serotonin (5-HT) transmission in several forebrain sites resulting from subconvulsive ventral hippocampal N-methyl-D-aspartate (NMDA; 0.5 μg / 0.5 μl / side) stimulation in Wistar rats. This manipulation induces, for example, psychosis-related effects, including a marked hyperactivity. Rats were decapitated 20 min after infusion and brains were extracted. During the 20 min between infusion and decapitation, locomotor activity was measured. Several forebrain areas (amygdala, nucleus accumbens shell/core, medial/lateral prefrontal cortex, caudate putamen) were sampled by micropunching. Regional tissue concentrations of DA, 5-HT, the DA metabolites homovanillic and dihydroxyphenylacetic acid (HVA, DOPAC), and the 5-HT metabolite 5-hydroxyindolacetic acid (5-HIAA) were determined by high-performance liquid chromatography with electrochemical detection. Metabolite/transmitter ratios were calculated as a measure of DA or 5-HT activity. Ventral hippocampal NMDA infusion induced a marked hyperactivity; the only significant post mortem neurochemical alteration was an elevated HVA/DA ratio in the medial prefrontal cortex (mPFC). While it is possible that additional, more subtle, alterations in forebrain monoamine transmission may only be revealed by in vivo microdialysis studies, the present post mortem results suggest that subconvulsive ventral hippocampal NMDA stimulation activates DA transmission in the mPFC. As will be discussed, the suggested positive modulation of prefrontal DA transmission by ventral hippocampal activity may play an important role for the hippocampal regulation of sensorimotor and memory processes.

Keywords: HPLC; locomotor activity; metabolites; monoamines; NMDA; PFC; ventral hippocampus
Introduction

The behavioral effects observed after pharmacological manipulations of the hippocampus by local drug microinfusions in previous studies may partly be mediated by alterations in monoamine transmission in extrahippocampal forebrain sites (Zhang et al., 2000, 2001, 2002a,b; Bast et al., 2001a-d, in press b). The present study focused on pharmacological stimulation of the ventral hippocampus (VH) by NMDA because it induces particularly marked behavioral effects that have been well documented (e.g., Yang and Mogenson, 1987; Wan et al., 1996; Klarner et al., 1998; Bardgett and Henry, 1999; Bast et al., 2001d; Zhang et al., 2001, 2002a). Post mortem neurochemistry was used to assess possible alterations in forebrain dopamine (DA) and serotonin (5-HT) transmission induced by this manipulation because it allows simultaneous examination of several forebrain regions.

NMDA stimulation of the VH has received particular interest, mainly due to the association between hippocampal overactivity and psychosis in humans (Heckers, 2001), and results, among other things, in psychosis-related effects, including a marked hyperactivity (Bast et al., 2001d). Interactions of the VH with several forebrain areas, including the prefrontal cortex, the nucleus accumbens (NAC), and the amygdala (AMYG), have been suggested to mediate these behavioral effects (see Klarner et al., 1998; Bast et al., 2001d; Zhang et al., 2002a). More specifically, the hyperactivity induced by NMDA stimulation of the VH is dopamine-dependent (Yang and Mogenson, 1987; Wu and Brudzynski, 1995; Bardgett and Henry, 1999; Bast et al., 2001d) and, using electrophysiology and in vivo microdialysis, it has been demonstrated that ventral hippocampal NMDA stimulation activates DA neurons in the ventral tegmental area (VTA; Legault et al., 2000; Floresco et al., 2001) and NAC DA release (Brudzynski and Gibson, 1997; Legault and Wise, 1999; Legault et al., 2000; Mitchell et al., 2000). Moreover, activation of forebrain 5-HT transmission may be involved in the reduction of prepulse inhibition (PPI), a DA-independent psychosis-related effect of ventral hippocampal NMDA stimulation (Bast et al., 2001d).

In the present study, Wistar rats received bilateral ventral hippocampal infusion of NMDA at a subconvulsive dose (0.5 μg / 0.5 μl / side), which induces marked behavioral effects within about 20-30 min after infusion, including a DA-dependent locomotor hyperactivity and a DA-independent disruption of PPI (Bast et al., 2001d). In order to examine post mortem neurochemical correlates of these effects, rats were decapitated 20 min after infusion. During the 20 min between infusion and decapitation the rats' activity was measured as a control for the infusion being effective. The micropunch method was used to dissect medial and lateral prefrontal cortex (mPFC, lPFC), the NAC core and shell, the AMYG, and the caudate...
putamen (CP). Regional concentrations of DA, 5-HT, the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), as well as the 5-HT metabolite 5-hydroxyindolacetic acid (5-HIAA), were determined by high-performance liquid chromatography (HPLC) coupled with electrochemical detection. Elevated metabolite/transmitter-ratios may reflect increased neuronal release of DA or 5-HT, respectively, particularly after manipulations of brain sites like the hippocampus which are remote from DA terminals or neurons (Commissiong, 1985, Kilpatrick and Phillipson, 1986). Thus, possible regional activation of forebrain DA and 5-HT transmission by NMDA stimulation of the VH may be revealed by regionally elevated metabolite/transmitter ratios.

Materials and methods

**Subjects, implantation of guide cannulae, and intracerebral infusion**

Eighteen male Wistar rats (Zur:WIST[HanIbm], Research Unit Schwerzenbach, Schwerzenbach, Switzerland), weighing about 250 g at the time of surgery, were implanted with guide cannulae (26 gauge) allowing insertion of infusion cannulae (34 gauge) to infuse NMDA (SIGMA, Switzerland; 0.5 µg / 0.5 µl 0.9% saline / side) or vehicle (VEH), i.e. 0.5 µl 0.9% saline, bilaterally into the VH. The infusion cannulae aimed at the following coordinates (in mm): -5.2 posterior and ±5.0 lateral to bregma, and 6.5 mm ventral from dura. Housing and handling of the rats, as well as procedures for surgery and microinfusions were identical to previous studies (e.g., Bast et al., 2001d).

**Open-field test to measure locomotor activity**

The rat was put into a closed square arena (76.5 cm wide x 76.5 cm long x 49 cm high) of dark gray plastic in a dimly illuminated (20 ± 0.5 Lux provided by two halogen lights) room. The experimenter was out of the room during testing and the behavior of the rat was recorded by a video camera suspended from the ceiling and relayed to a monitor and a Video Tracking, Motion Analysis & Behaviour Recognition System (EthoVision®, Noldus, Wageningen, The Netherlands). As a measure of locomotor activity, the Noldus system calculated the distance the rat moved in cm throughout the complete arena for each 10-min block of testing.

**Post mortem neurochemistry**

*Choice of examined forebrain regions*

The mPFC, IPFC, the NAC, and the AMYG were chosen because they receive substantial ventral hippocampal (Amaral and Witter, 1995), as well as DA and 5-HT projections (LeMoal
and Simon, 1991; Steinbusch, 1981), and have been implicated in the behavioral effects of ventral hippocampal NMDA stimulation (Klarner et al., 1998; Bast et al., 2001d; Zhang et al., 2002a). NAC core and shell were dissected separately as the VH projects predominantly to the shell (Kelley and Domesick, 1982; Groenewegen et al., 1987). Tissue from the CP, whose monoamine tissue concentrations are well characterized (e.g., Schwarting and Huston, 1987; Puumula and Sirviö, 1998) and which does not receive substantial ventral hippocampal projections (Kelley and Domesick, 1982; Groenewegen et al., 1987), was dissected as control. DA transmission in the VTA and the VH, which has been implicated in the hyperactivity induced by ventral hippocampal NMDA stimulation (Legault and Wise, 1999; Legault et al., 2000; Giménez-Llort et al., 2002), was not assessed. The VTA is too small to be dissected by our method, and sampling of the VH was difficult because of the cannulae tracks.

**Brain extraction**

After decapitation, brains were extracted from the skull within 1 min, and chilled in ice-cold saline for about 30 s before being frozen at \(-20^\circ C\) on a piece of aluminum foil. 20 min later, when the brains were sufficiently hard, they were tightly wrapped in aluminum foil, put into an airtight plastic envelope, and stored at \(-20^\circ C\) until dissection of the brain 5 to 6 days later.

**Brain dissection and sample preparation**

For dissection (Fig. 1A), the frozen brains were placed ventral side up in an ice-chilled rat brain matrix (ASI, Warren MI, USA) with slits spaced at 1 mm. First, the brain was fixed in the matrix by one ice-chilled razor blade placed in the slit about 2 mm anterior to the end of the olfactory tract, which can easily be identified on the brain’s ventral surface and whose location corresponds to a coordinate of about 3 mm anterior to Bregma in the rat brain atlas of Paxinos and Watson (1998), and by another blade placed in the slit next to the beginning of the cerebellum. Four additional ice-chilled razor blades, spaced at 2 mm intervals from the first, most anterior, razor blade, were then placed to obtain four 2-mm coronal brain sections from which the forebrain regions of interest could be punched and whose posterior sides corresponded to approximately 3, 1, \(-1\), and \(-3\) mm from Bregma according to the atlas of Paxinos and Watson (1998). The posterior part of the brain containing the VH was removed for the histological verification of cannulae placements in the VH (see below). The four 2-mm coronal sections were put with their posterior side up onto an ice-chilled plate covered with filter paper. In order to obtain the desired tissue samples, punchneedles of 2 or 1 mm
diameter, connected to a pressure system (ASI, Warren MI, USA), were pushed into the appropriate locations on the posterior side of the sections (Fig. 1A), withdrawn, and the tissue samples were ejected by a slight pressure increase into pre-weighed and ice-chilled 1.5 ml Eppendorf tubes. The mPFC, IPFC, AMYG, and CP were punched with needles of 2-mm diameter. To separate NAC shell and core, tissue containing the core and a part of the anterior commissure was first extracted using a 1-mm punch needle, and then the tissue of the shell, surrounding the remaining hole, was removed with a 2-mm needle. The wet weight of the tissue samples was determined by weighing the tube containing the sample and then subtracting the weight of the empty tube. Precision scales (± 0.01 mg) were used. The mean wet weights (in mg±SEM) of the tissue samples taken from the different regions were as follows: mPFC, 3.86 ± 0.08; IPFC, 3.64 ± 0.11; NAC core, 0.45 ± 0.02; NAC shell, 3.36 ± 0.13; CP, 4.19 ± 0.10; AMYG, 4.57 ± 0.18. After weighing, 200 μl of ice-cold 0.05 M HCl were added to each tissue sample, the samples were homogenized by slight sonification for about 10 s, centrifuged at 15000 g, and 4 °C for 20 min and then stored at −20°C for about one to three weeks until HPLC analysis.

**HPLC determination of DA, 5-HT, and their metabolites**

An HPLC system coupled with an amperometric electrochemical detector (Decade; Antec, Leyden, The Netherlands) was used to determine concentrations of DA, 5-HT, DOPAC, HVA, and 5-HIAA in the tissue samples. Samples were manually injected via a seven-port injection valve (Model 7725i, Rheodyne, Berkeley CA, USA) and a 100-μl injection loop, and separated on a reversed-phase column (100 x 3 mm glass column, Chromsphere 5B, Varian, Palo Alto CA, USA) maintained at 30°C by a column oven. The mobile phase was prepared by making up 200 ml of HPLC-grade methanol to 5 l with an aqueous buffer containing 34.88 mM citric acid (C₆H₈O₇), 54.37 mM sodium acetate (C₂H₃O₂Na x 3H₂O), 0.67 mM sodium ethylenediammoniumtetraacetate (C₁₀H₁₄N₂O₈Na₂ x 2H₂O), 0.46 mM octanesulphonic acid (C₈H₁₇O₃SNa) as ion pair reagent, and whose pH was adjusted to 4.1 by addition of concentrated hydrochloric acid. The mobile phase was pumped through the system via a degasser at 1 ml/min by an HPLC pump (Waters 515, Milford MA, USA) connected to a pulse damper. The working potential of the electrochemical glassy carbon flow cell (VT-03; Antec, Leyden, Netherlands) was + 0.75 V vs an Ag/AgCl reference electrode. Chromatograms were recorded and analyzed with a PC equipped with dedicated software (Millenium; Millipore Corp., Bedford MA, USA). An external standard containing DA, 5-HT, HVA, DOPAC, and 5-HIAA in a concentration of 10⁻⁷ or 10⁻⁸ M in 0.05 M HCl was used for
Fig. 1 Forebrain regions examined for post mortem measures of dopamine and serotonin transmission (A) as well as infusion sites in the ventral hippocampus (B). A: The locations of the razor blades used to cut four adjacent coronal slices are indicated in a sagittal view of the rat brain with the ventral surface up (top); forebrain regions of interest were dissected by pushing micropunch needles of 1 or 2 mm diameter into the posterior face of the coronal slices as indicated (bottom); AMYG amygdala, CP caudate putamen, l/mPFC lateral/medial prefrontal cortex, NAC nucleus accumbens. B: Locations of the tips of the infusion cannulae are depicted on coronal sections of the rat brain for rats infused with VEH (n=8) and NMDA (n=9). Coronal and sagittal sections are adapted from Paxinos and Watson (1998); numbers indicate distance from bregma in mm.
III.3.

calibration. Detection limits, yielding a signal corresponding to three times noise level, were about 10 to 30 fmol of transmitter or metabolite per injection.

For HPLC analysis, the tissue samples were thawed, again centrifuged at 15000 g at 4°C for 20 min, and the supernatant was filtered through cellulose acetate filters with a pore size of 0.2 μm. 50-μl aliquots were injected onto the columns, except for NAC-shell and CP samples. The latter were injected in volumes of 20 and 10 μl, respectively, because otherwise the analytical signal for DA would have exceeded the working range of our system. Since 10-μl aliquots of the CP samples did not yield a reliable 5-HT signal, an additional 30-μl aliquot of the CP samples was injected to determine 5-HT.

**Histological verification of cannulae placements in the ventral hippocampus**

The posterior part of the brain was fixed in 4% formalin solution for several days and subsequently cut into 40-μm coronal sections on a freezing microtome. For the verification of the infusion sites, every fifth section through the ventral hippocampus was mounted on gelatine-treated slides and stained with cresyl violet. After staining, the sections were dehydrated and coverslipped. They were subsequently examined by light microscopy with tenfold magnification to verify that the tips of the infusion cannulae were placed in the ventral hippocampus and to draw their approximate locations onto plates taken from the atlas of Paxinos and Watson (1998).

**Experimental design and groups**

All 18 rats were first subjected to a 30-min habituation to the open field, 9-10 days after surgery, and then divided in two groups matched according to their baseline open-field activity measured during these 30 min. Two days later, the rats were put in the open field for 30 min, they then received ventral hippocampal infusions of VEH or NMDA (each n=9), and were returned to the open field for an additional 20 min before decapitation. One rat infused with VEH was excluded from all analyses since its brain was destroyed during extraction, resulting in eight VEH and nine NMDA rats.

**Statistical analysis**

Statistical analyses were performed with the StatView software system (Abacus Concepts, Inc., Berkeley, CA, 1992) using ANOVA. Infusion was used as between-subjects factor. Open-field activity was analyzed separately for the 30 min preceding infusion and the 20 min following infusion using the 10-min blocks of testing as a repeated-measures factor and
adopting $p < 0.05$ as the level of significance. For the analysis of neurochemical data, the tissue concentrations of transmitters and metabolites were calculated in ng/mg wet tissue weight for both sides of each region. From these values, metabolite/transmitter ratios (DOPAC/DA, HVA/DA, 5-HIAA/5-HT) were calculated for each individual rat. Neurochemical data were analyzed by separate ANOVAs of tissue concentrations and ratios for each region, using the brain side (left, right) as within-subjects factor. In view of the highly increased type I-error probability resulting from the high number of comparisons (30), a significance level of $P < 0.01$ was adopted for the analysis of the neurochemical data (Matthews et al., 2001).

**Results**

**Cannulae placements in the ventral hippocampus**

The tips of the infusion cannulae were located in the ventral portion of the hippocampus in all rats (Fig. 1B) and damage was restricted to the area immediately surrounding the guide and infusion cannulae.

**Open-field locomotor activity**

While locomotor activity did not differ between infusion groups during the 30 min preceding infusion ($F_{(1,15)} = 0.879, P > 0.36$), it was markedly increased in the NMDA as compared to the VEH group during the 20 min following infusion ($F_{(1,15)} = 4.809, P < 0.05$; Fig. 2).

**Neurochemistry**

The tissue concentrations of DA, 5-HT, their metabolites, and the metabolite/transmitter-ratios in the left and right side of the different forebrain regions of the VEH and NMDA groups are summarized in Tables 1 and 2. Due to technical problems during sample dissection or analysis, some values could not be determined for all VEH or NMDA rats. Therefore, the number $n$ of determinations contributing to a given mean value for the VEH or NMDA group may in some cases be lower than the group size.

As to infusion effects, the only statistically reliable difference between VEH and NMDA rats was an elevated HVA/DA ratio in the mPFC of rats infused with NMDA ($F_{(1,15)} = 20.660, P < 0.0004$), regardless of the hemisphere (interaction infusion x side $F_{(1,15)} = 1.106, P > 0.3$). Additionally the HVA/DA ratio in the CP tended to be higher in NMDA than in VEH rats ($F_{(1,15)} = 7.941, P = 0.013$). There were no further differences between infusion groups that
Fig. 2 Effects of ventral hippocampal NMDA infusion on locomotor activity in the open field. Rats were infused with either vehicle (VEH, n=8) or NMDA (n=9). Locomotor activity is presented as total distance moved per 10-min block (mean ± SEM) and depicted for the three 10-min blocks immediately preceding infusion and the two 10-min blocks between infusion and decapitation.
Table 1: Post mortem measures of dopamine (DA) transmission in the examined forebrain regions of both hemispheres after ventral hippocampal infusion of vehicle (VEH) or N-methyl-D-aspartate (NMDA)

<table>
<thead>
<tr>
<th>Region</th>
<th>DA</th>
<th>DOPAC</th>
<th>HVA</th>
<th>DOPAC/DA</th>
<th>HVA/DA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VEH</td>
<td>NMDA</td>
<td>VEH</td>
<td>NMDA</td>
<td>VEH</td>
</tr>
<tr>
<td>mPFC left</td>
<td>0.144±0.032</td>
<td>0.098±0.016</td>
<td>0.162±0.031</td>
<td>0.126±0.020</td>
<td>0.194±0.025</td>
</tr>
<tr>
<td>mPFC right</td>
<td>0.145±0.028</td>
<td>0.125±0.021</td>
<td>0.172±0.034</td>
<td>0.163±0.023</td>
<td>0.171±0.013</td>
</tr>
<tr>
<td>IPFC left</td>
<td>0.034±0.005</td>
<td>0.034±0.004</td>
<td>0.070±0.012</td>
<td>0.054±0.011</td>
<td>0.145±0.029</td>
</tr>
<tr>
<td>IPFC right</td>
<td>0.032±0.008</td>
<td>0.045±0.010</td>
<td>0.068±0.012</td>
<td>0.074±0.013</td>
<td>0.096±0.008</td>
</tr>
<tr>
<td>NACshell left</td>
<td>4.764±0.446</td>
<td>4.126±0.477</td>
<td>2.814±0.258</td>
<td>2.778±0.192</td>
<td>1.129±0.107</td>
</tr>
<tr>
<td>NACshell right</td>
<td>4.011±0.530</td>
<td>4.832±0.482</td>
<td>2.190±0.218</td>
<td>2.581±0.211</td>
<td>0.944±0.062</td>
</tr>
<tr>
<td>NACcore left</td>
<td>4.897±0.601</td>
<td>4.245±0.644</td>
<td>3.513±0.203</td>
<td>3.230±0.351</td>
<td>1.582±0.182</td>
</tr>
<tr>
<td>NACcore right</td>
<td>4.959±0.664</td>
<td>3.903±0.512</td>
<td>3.221±0.270</td>
<td>2.891±0.336</td>
<td>1.415±0.160</td>
</tr>
<tr>
<td>CP left</td>
<td>10.637±0.741</td>
<td>9.897±0.935</td>
<td>2.156±0.070</td>
<td>2.372±0.220</td>
<td>1.324±0.050</td>
</tr>
<tr>
<td>CP right</td>
<td>10.778±0.843</td>
<td>10.111±0.476</td>
<td>2.117±0.138</td>
<td>2.526±0.270</td>
<td>1.401±0.074</td>
</tr>
<tr>
<td>AMYG left</td>
<td>0.257±0.033</td>
<td>0.301±0.062</td>
<td>0.220±0.025</td>
<td>0.230±0.034</td>
<td>0.129±0.015</td>
</tr>
<tr>
<td>AMYG right</td>
<td>0.326±0.051</td>
<td>0.371±0.055</td>
<td>0.242±0.027</td>
<td>0.235±0.034</td>
<td>0.153±0.008</td>
</tr>
</tbody>
</table>

Tissue concentrations of DA, dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) are given in ng/mg, the metabolite/transmitter ratios are without unit. All data are presented as mean±SEM, and n indicates how many animals' individual values contributed to a given mean value. The HVA/DA values (bold print and underlined) differed significantly (P < 0.0004) between VEH and NMDA rats, regardless of the brain side. For abbreviations of examined forebrain regions, see legend of Fig. 1A.
Table 2: Post mortem measures of serotonin (5-HT) transmission in the examined forebrain regions of both hemispheres after ventral hippocampal infusion of vehicle (VEH) or N-methyl-D-aspartate (NMDA)

<table>
<thead>
<tr>
<th>Region</th>
<th>VEH 5-HT</th>
<th>VEH 5-HIAA</th>
<th>VEH 5-HIAA/5-HT</th>
<th>NMDA 5-HT</th>
<th>NMDA 5-HIAA</th>
<th>NMDA 5-HIAA/5-HT</th>
</tr>
</thead>
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<tr>
<td>mPFC</td>
<td></td>
<td></td>
<td></td>
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<td>left</td>
<td>0.042±0.006</td>
<td>0.039±0.009</td>
<td>0.214±0.018</td>
<td>0.222±0.013</td>
<td>5.615±0.699</td>
<td>10.635±3.414</td>
</tr>
<tr>
<td>right</td>
<td>0.039±0.004</td>
<td>0.034±0.005</td>
<td>0.229±0.018</td>
<td>0.229±0.013</td>
<td>6.286±0.717</td>
<td>8.752±1.832</td>
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<tr>
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<td>0.061±0.010</td>
<td>0.206±0.025</td>
<td>0.252±0.027</td>
<td>3.768±0.608</td>
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<td>0.220±0.016</td>
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<td>0.430±0.061</td>
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<td>1.430±0.167</td>
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Tissue concentrations of 5-HT and 5-hydroxyindolacetic acid (5-HIAA) are given in ng/mg, the 5-HIAA/5-HT ratio is without unit. All data are presented as mean±SEM, and n indicates how many animals' individual values contributed to a given mean value. For abbreviations of examined forebrain regions, see legend of Fig. 1A.
reached or approached the accepted level of significance (all $P > 0.05$).

With respect to neurochemical asymmetries, the only statistically reliable difference between left and right hemisphere was an elevated DA concentration in the right as compared to the left AMYG ($F_{(1,15)} = 9.434, P < 0.008$) of both VEH and NMDA rats (interaction side x infusion: $F < 1.0$). In addition, there was an interaction of side x infusion for DA concentrations in the NAC shell ($F_{(1,15)} = 11.274, P < 0.005$), but separate ANOVAs of DA concentrations in the right and the left shell did not indicate an effect of infusion for either side ($F_{(1,15)} < 1.319, P > 0.25$). There were no further differences between brain sides or interactions side x infusion that reached or approached the accepted level of significance (all $P > 0.017$).

**Discussion**

Consistent with previous studies (Yang and Mogenson, 1987; Wu and Brudzynski, 1995; Brudzynski and Gibson, 1997; Bardgett and Henry, 1999; Legault and Wise, 1999; Bast et al., 2001d; Zhang et al., 2002a), NMDA infusion into the VH caused a marked locomotor hyperactivity. This hyperactivity was accompanied by an elevated HVA/DA ratio in the mPFC, but no other statistically reliable alterations in post mortem measures of DA and 5-HT transmission were found in the examined forebrain areas.

**Tissue concentrations of DA, 5-HT, and metabolites**

The regional tissue concentrations of DA, 5-HT, and their metabolites measured in the present study are consistent with the results of previous studies (e.g., Schwarting and Huston, 1987; Jones et al., 1992; Brenner and Bardgett, 1998; Puumala and Sirviö, 1998; Matthews et al., 2001; Dalley et al., 2002). There were very marked regional differences in DA concentrations and DA metabolite/DA ratios, with the highest concentrations in the CP, followed by the NAC, and lowest levels in the PFC, whereas DA metabolite/DA ratios were highest in the PFC. Regional differences in 5-HT concentrations were less marked. In agreement with previous findings (e.g., Soghomonian et al., 1987; Matthews et al., 2001), the present study indicated that the NAC has the highest 5-HT content of the forebrain regions examined. While the 5-HT concentrations in the present study are comparatively low, and consequently 5-HIAA/5-HT ratios are relatively high, values similar to ours have also reported (Puumala and Sirviö, 1998). These discrepancies between studies may be related to differences in tissue sampling and processing. The only neurochemical asymmetry found in the present study was an elevated DA concentration in the right as compared to the left
AMYG. While previous studies did not report an asymmetry of DA concentrations in the amygdala (Sullivan et al., 1998; Thiel and Schwarting, 2001), asymmetries in regional concentrations of monoamines and their metabolites have been found by many studies, but are often not consistent (e.g., compare Jones et al., 1992; Puumala and Sirviö, 1998; Sullivan et al., 1998; Matthews et al., 2001; Thiel and Schwarting, 2001; Dalley et al., 2002). This may reflect that neurochemical asymmetries are individual, rather than population, asymmetries (see Denenberg, 1983) and, thus, may differ between different random samples of rats.

**Effects of ventral hippocampal NMDA stimulation on post mortem measures of DA and 5-HT transmission**

During increased neuronal release of DA and 5-HT, terminal transmitter levels remain fairly constant due to homeostatic mechanisms upregulating synthesis, while metabolite levels increase due to increased transmitter turnover (Commissiong, 1985). Accordingly, regionally enhanced DA and 5-HT release may be reflected by increased metabolite tissue concentrations concomitant with constant transmitter concentrations and hence increased metabolite/transmitter ratios. Such increases have been reported in forebrain areas after several acute experimental manipulations, including pharmacological and electrical stimulation of remote brain sites (e.g., Kilpatrick and Phillipson, 1986; Jones et al., 1988; Kalivas et al., 1983; Schwarting and Huston, 1987; Jones et al., 1992; Morrow et al., 1996, 2000a,b; Matthews et al., 2001).

The hyperactivity induced by ventral hippocampal NMDA infusion is DA dependent (Wu and Brudzynski, 1995; Bardgett and Henry, 1999; Bast et al., 2001d), and ventral hippocampal NMDA stimulation has been demonstrated to increase activity of VTA DA neurons (Legault et al., 2000; Floresco et al., 2001) and DA release in the NAC (Brudzynski and Gibson, 1997; Legault and Wise, 1999; Legault et al., 2000; Mitchell et al., 2000). The only alteration in post mortem measures of forebrain DA transmission found in the present study, however, was an elevated HVA/DA ratio in the mPFC. A previous post mortem neurochemical study examining the effects of ventral hippocampal stimulation by the acetylcholine agonist carbachol, at a dose inducing DA dependent hyperactivity, yielded a similar pattern of results (Brenner and Bardgett, 1998). This study did not find a statistically significant alteration of DOPAC/DA or HVA/DA ratios in any of six examined forebrain sites (NAC shell and core, mediodorsal and laterodorsal CP, infralimbic and prelimbic mPFC). Interestingly, however, the HVA/DA ratio in the infralimbic mPFC was numerically nearly twice as high in rats which received ventral hippocampal carbachol stimulation as in control
rats (0.923 ± 0.282 vs 0.495 ± 0.142; mean ± SEM). Finally, while there is reason to presume that some of the behavioral effects of ventral hippocampal NMDA infusion may be mediated by activation of forebrain 5-HT transmission (Bast et al., 2001d), the present study failed to provide evidence for that.

Two issues need to be considered. First, additional alterations in post mortem measures of DA and 5-HT transmission might have been found if the rats had been decapitated either earlier or later than 20 min following infusion. While, in the microdialysis studies, extracellular peak concentrations of DA and its metabolites in the NAC were measured within 15-30 min following infusion (Brudzynski and Gibson, 1997; Legault and Wise, 1999; Mitchell et al., 2000; but see Legault et al., 2000), additional time may be necessary for metabolite accumulation so that changes in metabolite tissue concentrations or metabolite/transmitter ratios become detectable by post mortem analysis. However, specific increases in forebrain DA and 5-HT metabolism have, for example, been found when rats were decapitated after 5, 10, 15 or 25 min of conditioned turning (Schwarting and Huston, 1987), after a 20-min exposure to an odor (Morrow et al., 2000a,b), or 15 min after pharmacological disinhibition of the mediodorsal thalamus (Jones et al., 1988). Thus, post mortem measures can respond very quickly to behavioral and pharmacological manipulations. Second, ventral hippocampal stimulation by NMDA at the low dose used in the present study may induce alterations in forebrain DA and 5-HT transmission that are too subtle to result in significant effects on post mortem measures. In the microdialysis studies, NMDA was infused into the VH unilaterally and microdialysis measurements were conducted in the ipsilateral NAC. Ventral hippocampal projections partly cross from one hemisphere to the other (Amaral and Witter, 1995). Therefore, a bilateral infusion of 0.5 μg NMDA/0.5μl, as used in the present study, may have similar effects on NAC DA transmission in one hemisphere as an ipsilateral infusion of 0.74 μg NMDA/0.5 μl. The latter was the lowest NMDA dose whose unilateral infusion into the VH yielded significant alterations of extracellular DA and metabolite concentrations in the ipsilateral NAC, with the increase of DA being about 25 % and that of DOPAC being about 10% (Legault and Wise, 1999; Legault et al., 2000). Changes in extracellular DA and DOPAC of that magnitude might only be accompanied by very subtle alterations of post mortem measures difficult to detect (compare Horger et al., 1995 and McCullough and Salamone, 1992). Finally, it has to be emphasized that the present study was aimed at finding possible neurochemical correlates of behavioral alterations resulting from NMDA infusion at doses of 0.5 μg/side, or lower, and occurring during about 20 min following infusion (Bast et al., 2001d; Zhang et al., 2001, 2002a). Thus, neurochemical
alterations possibly detectable at higher NMDA doses or if intervals between infusion and decapitation were extended beyond 20 min would be of minor interest.

Elevated HVA/DA ratio in the mPFC after ventral hippocampal NMDA stimulation

The elevated HVA/DA ratio in the mPFC indicates that ventral hippocampal NMDA stimulation activates mPFC DA transmission, even though no alterations in HVA and DOPAC concentrations or the DOPAC/DA ratio were found in the mPFC. The HVA/DA ratio is a more sensitive measure than absolute HVA concentrations because determination of the latter is flawed by a higher variance resulting from inaccuracies in weighing that are cancelled down by forming the HVA/DA ratio. Moreover, the HVA/DA ratio may be a more sensitive measure of neuronal DA release than the DOPAC/DA ratio, particularly in the mPFC, given that HVA stems to a considerable part from extraneuronal DA metabolism (Kilpatrick and Phillipson, 1986) and is the main DA metabolite in the PFC (see above).

An activation of mPFC DA transmission by pharmacological stimulation of the VH is consistent with an increase of extracellular DA in the mPFC observed after electrical stimulation of the VH in anesthetized rats (Gurden et al., 2000). Moreover, the suggested positive modulation of mPFC DA transmission by activity of the VH is supported by evidence for decreased DA turnover in the mPFC found 28 days after cytotoxic ventral hippocampal lesions (Lipska et al., 1992). Ventral hippocampal stimulation may enhance mPFC DA release via a direct interaction of ventral hippocampal and DA terminals, which are in direct apposition to one another in the mPFC (Carr and Sesack, 1996), or via an indirect activation of DA neurons in the VTA (Legault et al., 2000; Floresco et al., 2001). The latter may be mediated by the AMYG, the NAC shell, and the mPFC, which receive ventral hippocampal projections (Kelly and Domesick, 1982; Groenewegen et al., 1987; Carr and Sesack, 1996; Pitkänen et al., 2000) and send projections to the VTA (Phillipson, 1979; Usuda et al., 1998; Carr and Sesack, 2000), with recent work indicating a particularly important role for the NAC shell (Floresco et al., 2001). The accumulating evidence for a modulation of mPFC DA release by the VH complements data indicating that a blockade of mPFC DA receptors disrupts hippocampal-prefrontal interactions relevant for the integration of spatial memory with executive function (Seamans et al., 1998). Furthermore, recent studies have indicated that mPFC DA transmission may have a facilitating effect on locomotor activity (Bast et al., in press a; Pezze et al., 2001) and is critical for the expression of conditioned fear (Pezze et al., 2001, 2002, in press). Thus, modulation of mPFC DA transmission may be a mechanism contributing to the positive control the VH exerts on locomotor activity (Bast et al., 2001b-d;
Zhang et al., 2002a) and may also be relevant for the expression of conditioned fear, which is formed with the contribution of the hippocampus (Bast et al., 2001a,c, in press b; Zhang et al., 2001).

**Conclusion**

The present post mortem neurochemical study failed to detect an activation of NAC DA transmission by ventral hippocampal NMDA stimulation that was indicated by previous in vivo microdialysis studies. This suggests that post mortem neurochemistry, while allowing for convenient simultaneous examination of many discrete brain regions, may not be sensitive enough to clearly reveal all alterations of forebrain monoamine release involved in the behavioral effects of ventral hippocampal NMDA stimulation, or even “milder” hippocampal manipulations. In vivo microdialysis, enabling direct within-subjects examination of the effects of a given hippocampal manipulation on extracellular monoamine levels in the forebrain, may be more useful for further study of such alterations. Nevertheless, the present post mortem study indicated that ventral hippocampal NMDA stimulation activates mPFC DA transmission. Modulation of mPFC DA transmission by ventral hippocampal activity may be an important mechanism for the hippocampal regulation of sensorimotor functions, as well as for the expression of hippocampus-dependent memory.

*Acknowledgements* This work has been published in preliminary form (Bast T, Zhang W-N, Ferger B, Feldon. 2002. FENS Abstr Vol 1:175.3). The support of the technical and animal-care staff of the Lab of Behavioral Neurobiology is gratefully acknowledged. Special thanks are due to Liz Weber, for histological preparations, to Jane Fotheringham, for editorial assistance, to Andreas Leng, for frequent help and advice concerning HPLC methods, to Gael Hedou, for an initial introduction into micropunching, and particularly to Marie Pezze, for helpful suggestions on an earlier version of the manuscript.
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III.3.


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III.3.


285
III.3.


The effects of ventral hippocampal stimulation by N-methyl-D-aspartate on dopamine transmission in the medial prefrontal cortex: preliminary results of an in vivo microdialysis study in freely moving rats

Tobias Bast, Marie A. Pezze, Daria Peleg, Boris Ferger, Joram Feldon
Introduction

Our previous post mortem neurochemical study (Bast et al., submitted) suggested that N-methyl-D-aspartate (NMDA) stimulation of the ventral hippocampus (VH) activates dopamine (DA) transmission in the medial prefrontal cortex (mPFC) in rats. Additional evidence supporting that the VH exerts a positive control over mPFC DA transmission is the increase of extracellular DA in the mPFC by tetanic stimulation of the VH in anesthetized rats (Gurden et al., 2000), as well as decreased post mortem measures of mPFC DA turnover 28 days after cytotoxic lesion of the VH (Lipska et al., 1992). Possible neuroanatomical substrates for the modulation of mPFC DA activity by the VH are direct interactions of ventral hippocampal and dopaminergic terminals, which are in direct apposition to one another in the mPFC (Carr and Sesack, 1996), or ventral hippocampal projections to amygdala, mPFC, or nucleus accumbens shell (Swanson and Cowan, 1977; Kelly and Domesick, 1982; Groenewegen et al., 1987; Jay and Witter, 1991; Carr and Sesack, 1996; Verwer et al., 1997; Pitkänen et al., 2000) which send efferents to the ventral tegmental area (VTA; Phillipson, 1979; Usuda et al., 1998; Carr and Sesack, 2000) where the dopaminergic innervation of the mPFC originates (Fuxe et al., 1974). Indeed, NMDA stimulation of the VH activates DA neurons in the VTA (Legault et al., 2000; Floresco et al., 2001).

The behavioral effects resulting from combined and separate pharmacological manipulations of the VH and manipulations of mPFC DA transmission suggest that positive modulation of mPFC DA transmission by ventral hippocampal activity may be an important mechanism for the hippocampal regulation of locomotor activity, as well as for the expression and use of hippocampus-dependent memories. For example, ventral hippocampal activity appears to drive locomotor activity (for review see Bast et al., in preparation for submission) and it has been suggested that prefrontal DA transmission may facilitate locomotor activity (Beninger et al., 1990; Pezze et al., 2001, in press; Bast et al., in press). Thus, the VH may partly drive locomotion by enhancing DA release in the mPFC. Moreover, the VH is important for the formation of conditioned fear (for review see Bast et al., 2001a), while prefrontal DA transmission is essential for expressing, but not forming, conditioned fear (Pezze et al., in press). Thus, the modulation of prefrontal DA by the VH may play a role in transferring information essential for the expression of fear memory following its formation. Finally, there is direct evidence that the interaction of ventral hippocampal processes with prefrontal DA transmission is essential for the integration of a particular hippocampus-dependent memory with executive functions of the mPFC. Thus, disconnecting ventral hippocampal transmission and prefrontal DA transmission, by unilaterally blocking DA D1
receptors in the mPFC simultaneous to a transient deactivation of the contralateral VH by lidocaine, resulted in a failure of prospective search behavior based on trial-unique spatial short-term memory (Seamans et al., 1998).

In view of the particular functional relevance of this issue, we decided to conduct an in vivo microdialysis study in order to corroborate that NMDA stimulation of the VH increases DA activity in the mPFC of freely moving rats. Given that the projections from the VH to the mPFC are glutamatergic (Jay et al., 1992), we also tried to examine the effects of ventral hippocampal NMDA stimulation on extracellular glutamate (GLU) in the mPFC. This chapter presents a preliminary report of these in vivo microdialysis experiments, even though the collection of data has not yet completely been finished before submission of the present thesis.

**Experimental procedures**

**Subjects**

Male Wistar rats (Zur:WIST[HanIbm], Research Unit Schwerzenbach, Schwerzenbach, Switzerland), weighing about 250-300 g and about 8-10 weeks old at the time of surgery, were used in the present study. Animals were housed under a reversed light-dark cycle (lights on: 19:00-07:00) in a temperature (21 ± 1°C) and humidity (55 ± 5%) controlled room and allowed free access to food and water. All rats were unilaterally implanted with two guide cannulae for insertion of an infusion cannula into the VH and of a microdialysis probe into the mPFC. Before surgery, rats were housed in groups of four per cage; after surgery, they were individually caged. Beginning one days before surgery and thereafter until the beginning of the behavioral experiments, all rats were handled daily. All experimental procedures were carried out in the dark phase of the cycle. The Principles of Laboratory Animal Care (NIH publication no. 86-23, revised 1985) and Swiss regulations for animal experimentation were followed.

**Surgery**

Rats were anesthetized with 1 ml of Nembutal (sodium pentobarbital, 50 mg/ml; Abbot Labs, North Chicago, Ill., USA) per kg body weight. After application of a local anesthetic (lidocaine), the scalp was incised and two guide cannulae were stereotaxically implanted into the left hemisphere. One cannula was a custom-made infusion guide cannula (26 gauge) in a Perspex holder and aiming at the following coordinates above the VH to allow insertion of an infusion cannula (in mm): 5.2 posterior and 5.0 lateral to bregma, and 5 ventral from dura.
The second cannula was a microdialysis guide cannula (MAB 4.9; TSE, Stockholm, Sweden) and aimed at the following coordinates above the mPFC in order to allow insertion of a microdialysis probe into the mPFC (in mm): 3.0 anterior and 0.6 lateral to bregma, and 3 (Experiment 1) or 2 (Experiment 2) ventral to skull surface. The guide cannulae and a screw to attach the swivel for the microdialysis measurements were fixed by dental cement. Until the insertion of the infusion cannulae or microdialysis probes, respectively, the guide cannulae were closed with stylets. Rats were allowed 6 to 7 days of recovery before being subjected to the microdialysis experiment.

Microinfusion into the VH

After removal of the stylets, custom-made infusion cannulae (34 gauge), connected to 10-μl Hamilton microsyringes mounted on a microinfusion pump (KD scientific or WPI sp200i), were inserted into the guide cannulae. The tips of the infusion cannulae protruded into the VH 1.5 mm beyond the tips of the guide cannulae, thus aiming at a final dorsoventral coordinate of 6.5 mm below the dura. The rats were infused into the left VH with NMDA (SIGMA, Switzerland; 0.5 μg / 0.5 μl 0.9% saline) or vehicle (VEH), i.e. 0.5 μl 0.9% saline, at an infusion speed of 0.5 μl/min. After infusion, the infusion cannulae were left in the brain for 60 s to allow for absorption of the infusion bolus by the brain tissue and then replaced by the stylets.

Microdialysis procedure

Microdialysis measurements in freely moving rats were conducted in dimly lit (6-7 lux) buckets whose floors were lightly covered with sawdust and which were placed in a sound attenuated room. The rats were attached to a two-channel liquid swivel (Instech Lab. Inc., Plymouth Meeting, PA, USA) fixed to the bucket and guiding the in- and outlet tubing (dead volume of about 23 μl each) of a concentric microdialysis probe (MABCU4.9; TSE, Stockholm, Sweden) which was inserted into the mPFC via the previously implanted guide cannula. The probes were perfused with phosphate-buffered (pH 7.2-7.4) artificial cerebrospinal fluid (ASCF), containing 147 mM NaCl, 4 mM KCl, 1 mM MgCl₂, 2 mM Na₂HPO₄, and 1.3 mM CaCl₂ (normal-Ca²⁺ ACSF) or 2.3 mM CaCl₂ (high-Ca²⁺ ACSF), at 2 μl/min. The active membrane (cuprophan; outer diameter 0.24 mm) protruding into the mPFC from the end of the guide cannulae was 2 mm (Experiment 1) or 4 mm (Experiment 2). Thus, the sampled area included large portions of the prelimbic mPFC. This area receives the strongest dopaminergic innervation among all cortical areas (Van Eden et al., 1987) and,
additionally, receives the most substantial glutamatergic input from the VH (Jay and Witter, 1991; Jay et al., 1992). After a stabilization period (see Experimental Design), dialysates were collected each 15 or 20 min (30 or 40 µl) in vials containing 3 or 4 µl of 0.1 M hydrochloric acid (HCl) to reduce oxidation of monoamines.

In vitro recovery of the probes was determined on the day before the microdialysis experiment at room temperature (23 °C) in order to control for probe performance. Recovery for GLU was only determined for the 2-mm probes and was about 3-5 %. Recovery for DA and monoamine metabolites was about 5-7 % for the 2-mm probes and about 10-13 % for the 4-mm probes. Probes were used one to three times.

**High-performance-liquid-chromatography analysis of dialysates**

**DA and metabolites**

A high-performance-liquid-chromatography (HPLC) system coupled with an amperometric electrochemical detector (Decade; Antec, Leyden, The Netherlands) was used to determine dialysate concentrations of DA and its metabolites homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC). Samples were manually injected via a seven-port injection valve (Model 7725i; Rheodyne, Berkeley CA, USA) and a 50-µl injection loop, and separated on a reversed-phase column (100 x 3 mm glass column, Chromsphere 5B; Varian, Palo Alto CA, USA) maintained at 30 °C by a column oven. The working potential of the electrochemical glassy carbon flow cell (VT-03; Antec, Leyden, Netherlands) was + 0.75 V vs an Ag/AgCl reference electrode. An HPLC pump (Model 515; Waters, Milford MA, USA) connected to a pulse damper and a degasser was used to transport the mobile phase (see below) through the system. Chromatograms were recorded and analyzed with a personal computer equipped with dedicated software (Millenium; Millipore Corp., Bedford MA, USA; Chromeleon; Dionex, Olten, Switzerland). External standards containing DA, HVA, and DOPAC in a concentration of $10^{-10}$ to $10^{-8}$ M in 0.1 M HCl diluted 1:11 (to obtain the same pH as in the dialysates) were used for calibration.

In Experiment 1, 25-µl or 30-µl aliquots of the dialysates, depending on whether GLU was also to be assessed (see Experimental Design), were injected on the column and separation was conducted with a methanol mobile phase. The mobile phase was prepared by making up 200 ml of HPLC-grade methanol to 5 l with an aqueous buffer containing 34.88 mM citric acid ($C_6H_8O_7$), 54.37 mM sodium acetate ($C_2H_3O_2Na \times 3H_2O$), 0.67 mM sodium ethylendiammoniumtetraacetate ($C_{16}H_{14}N_2O_8Na_2 \times 2H_2O$), 0.46 mM octanesulphonic acid ($C_8H_{17}O_5SNa$) as ion pair reagent, and whose pH was adjusted to 4.1 by addition of
concentrated HCl. This mobile phase was pumped through the system at a flow rate of 1 ml/min. Separation of DA, serotonine, and their metabolites was achieved in a single run of about 8 min. The detection limit (detection signal corresponding to three times noise level) for DA was about 10-20 fmol per injection corresponding to a dialysate concentration of about 0.5 to 1 nM.

We were not able to determine dialysate concentrations of DA in Experiment 1. This prompted us to change several experimental parameters (see Experimental Design), including an increase of the sampling time from 15 to 20 min, allowing us to inject 40-μl aliquots of each dialysate. Moreover, we used an acetonitrile mobile phase. This mobile phase was prepared by making up 250 ml of HPLC-grade acetonitrile to 5 l with an aqueous solution containing 0.27 mM sodium ethylenediammoniumtetraacetate (C₁₀H₁₄N₂O₈Na₂ x 2H₂O), 0.43 mM triethylamine (C₆H₁₅N), 0.74 mM octanesulphonic acid (C₈H₁₇O₃SNa) as ion pair reagent, and whose pH was adjusted to 2.9 by addition of concentrated phosphoric acid. The acetonitrile mobile phase was pumped through the system at a flow rate of 0.7 ml/min and DA, serotonine, and their metabolites could be separated in a single run of about 12 min. Importantly, the DA peak in the chromatogram was symmetric and very well isolated, while with the methanol phase the DA signal was obtained on a slight slope resulting from the front peaks and very close to the DOPAC peak. This analytical improvement resulted in a lower and more stable detection limit of 5-10 fmol, corresponding to dialysate concentrations of about 0.1-0.5 nM with an injection volume of 40 μl.

GLU

In the first few animals used in Experiment 1, dialysates from the mPFC were also analyzed for GLU (see Experimental Design). An HPLC system consisting of a high pressure gradient HPLC pump (P 580, Dionex, Olten, Switzerland), a reversed-phase column (60 mm x 4 mm) with pre-column (5 mm x 4 mm), both filled with 5-μm Nucleosil120 C18 particles (Knauer, Berlin, Germany), a column oven (STH 585; Dionex), a fluorescence detector (RF 2000; Dionex), and an autosampler with cooling module (ASI 100T; Dionex), was used to analyze dialysate concentrations of GLU. The system was controlled by a chromatography workstation (Chromeleon, Dionex). Using the autosampler, which was set at 4 °C, 5-μl aliquots of the dialysates were automatically derivatized with 20- μl of orthophthalaldialdehyde/2-mercaptoethanol reagent (OPA complete reagent, Sigma) and 15 μl of the mix were injected on the column. The fluorescent isoindole derivatization product was quantified with the fluorescent detector applying an excitation wavelength of 340 nm and an
emission wavelength of 450 nm according to the method of Lindroth and Mopper (1979). The eluents were pumped through the system at a flow rate of 1 ml/min and the column was maintained at 30 °C. Isolation of the GLU peak was achieved by a mobile phase consisting of 0.1 mM sodium acetate dissolved in a methanol:water (1:10) mix adjusted to a pH of 6.95 by acetic acid. After 3 min, the column was flushed with methanol for 2 min. Before injection of the next sample, the column was re-equilibrated with the first mobile phase for 5 min. External standards containing GLU in a concentration of 0.5 or 1 µM in 0.1 M HCl diluted 1:11 were used for calibration. The detection limit was about 0.05 µM with 5-µl sample aliquots.

**Verification of probe and cannulae placements**

After completion of the experiments, rats were deeply anesthetized with an overdose of 2.5 ml/kg Nembutal (sodium pentobarbital, 50 mg/ml, i.p.) and transcardially perfused with 0.9% saline at room temperature to rinse out the blood, followed by about 100 ml of 4% formalin (4°C) to fix the brain tissue. During perfusion, the aorta was clamped. After extraction from the skull, the brains were post-fixed in 4% formalin solution and subsequently cut into 40-µm coronal sections on a freezing microtome. Every fifth section through the VH and mPFC was mounted on a gelatine-treated slide and stained with cresyl violet. After staining, the sections were dehydrated and coverslipped. Subsequently, they were examined with a light microscope under tenfold magnification to verify that the tips of the infusion cannulae and the microdialysis probes were placed correctly in the VH or mPFC, respectively.

**Experimental design**

Rats were subjected to all procedures in pairs, usually one rat to receive VEH and one rat to receive NMDA infusion into the VH. One day before the microdialysis measurements, the rats were habituated to the apparatus and to the handling necessary for the infusion. In the morning and in the afternoon, the rats were attached to the swivels (without the probes being inserted) and left in the buckets for 30 min, taken out for 1 min of handling similar to the handling during the infusion, and returned into the bucket for an additional 30 min. The purpose of these habituation sessions was to minimize stress during the infusion on the subsequent microdialysis day. This is important because stress has been demonstrated to increase DA concentrations (Feenstra, 2000), as well as concentrations of GLU, which were assessed in the first animals of Experiment 1, in mPFC dialysates (Moghaddam, 1993; Timmerman et al., 1999). On the microdialysis day, the rats were attached to the swivel and
the probes were inserted in the morning. Then, they were left quietly for about 2 h to allow stabilization of dialysate concentrations of DA and metabolites (Ungerstedt, 1991). When dialysate concentrations of GLU were also analyzed, measurements were not started before 3-4 h after probe insertion, because stabilization of GLU concentrations in mPFC dialysates has been reported to take particularly long (Moghaddam, 1993). After the stabilization period, two baseline samples were collected before the rats were infused into the left VH with VEH or NMDA. While, in our previous studies examining the effects of ventral hippocampal NMDA stimulation (Bast et al., 2001b; Zhang et al., 2002; Bast et al., submitted), the rats were always infused bilaterally, infusions were given unilaterally in the present study. This results in a reduction of the behavioral activation induced by the ventral hippocampal NMDA stimulation (Zhang, Bast, Feldon, unpublished observations), and, thus, makes the microdialysis measurements easier. In contrast, the neurochemical effects in the left mPFC should be similar following unilateral NMDA infusion into the left VH or after bilateral infusion, since the projections of the VH, including those to the mPFC are predominantly ipsilateral (Amaral and Witter, 1995; Verwer et al., 1997). When the infusion was completed, sample collection was resumed for additional three hours in order to assess the effects of the infusion on neurotransmitter and metabolite values in mPFC dialysates.

Experiment 1

Fourteen rats were used in Experiment 1. The probes had an active membrane of 2 mm length and the sampling intervals were 15 min yielding 30 µl of dialysate plus 3 µl of 0.1 M HCl. Twenty five µl of these samples were used for analysis of DA and metabolites and 5 µl were used in order to analyze GLU. GLU was measured because, in view of the fact that the direct projections from the VH to the mPFC are glutamatergic (Jay et al., 1992), ventral hippocampal NMDA stimulation may be expected to increase synaptic release of GLU in the mPFC. For the first eight animals of Experiment 1, normal-Ca²⁺ ACSF (1.3 mM Ca²⁺) was used to perfuse the probes. Under these conditions, we were not able to detect DA in the dialysates. We therefore started perfusing the probes with high-Ca²⁺ ACSF (2.3 mM Ca²⁺). This is a common way to increase basal dialysate values of DA in order to facilitate detection, because DA concentrations are linearly related to the Ca²⁺ concentrations of the perfusion medium (Westerink et al., 1988; Di Chiara, 1991). Despite this change, we did not succeed to measure DA in mPFC dialysates in the first experiment. Second, because GLU was not reliably measurable anyway (see Results), we stopped analyzing GLU, so that a volume of 30 µl, instead of 25 µl, was available for DA analysis.
In Experiment 2, we introduced several changes as compared to Experiment 1 in order to achieve measuring DA concentrations in dialysates from the mPFC. First, we changed the mobile phase for HPLC determination of DA, resulting in a decreased limit of detection (see High-performance-liquid-chromatography analysis of dialysates, DA and metabolites). Second, we used 4-mm probes, whose in vitro recovery was about twice as high as that of the 2-mm probes used in Experiment 1 (see Microdialysis procedures). Third, we increased the sampling interval from 15 to 20 min and, because the GLU measurements were not very reliable anyway (see Results), we stopped analyzing GLU, so that a volume of 40 µl, instead of 25 µl as in Experiment 1, was available for DA analysis. Thus, the amount of DA injected onto the column could be increased by 37.5 % as compared to Experiment 1. With these changes, we were able to detect DA in dialysates from the mPFC. Data collection for Experiment 2 could not be completed before submission of the thesis, but preliminary results obtained from six VEH and five NMDA rats will already be presented.

Data analysis
Basal concentrations of neurotransmitters and metabolites in mPFC dialysates are presented in molar concentrations not corrected for in vitro recovery. One-way ANOVA was used to compare basal values between the different treatment groups. In order to determine the effects of ventral hippocampal NMDA and VEH infusions, all dialysate concentrations were transformed in a percentage of the baseline value, i.e. the average concentration measured in the two baseline samples, for each rat. These values were then analyzed by repeated-measures ANOVA using infusion as between-subjects factor and sample as within-subjects factor. If this analysis indicated differences between the infusion groups, separate repeated-measures ANOVA were conducted for each group. In case the separate ANOVA indicated a significant repeated-measures effect, Fisher’s honest lowest significant difference test was used to determine significant differences between the samples collected after infusion and the baseline samples.

Results and discussion
All infusion cannulae were correctly placed within the VH and all microdialysis probes within the prelimbic mPFC. In Experiment 1, the 2-mm microdialysis probes were placed in
the central prelimbic mPFC, while the 4-mm probes used in Experiment 2 spanned the complete prelimbic mPFC including also more ventral and dorsal portions (Fig. 1).

Experiment 1

GLU

In only six (three NMDA and three VEH rats) out of 14 rats, it was possible to detect GLU in dialysates of the mPFC. The basal values in the two infusion groups did not differ \( (F < 1) \) and were comparable to GLU concentrations reported for dialysates from the mPFC by other studies (e.g., Moghaddam, 1993; Timmerman et al., 1999; Adams and Moghaddam, 2001) (Table 1). The \( Ca^{2+} \) content of the perfusion medium had no considerable effect on the basal GLU concentrations. The dialysates collected with normal-\( Ca^{2+} \) ASCF \( (0.80 \pm 0.40 \mu M; n = 2) \) did not differ \( (F < 1) \) from those collected with high-\( Ca^{2+} \) ASCF \( (0.71 \pm 0.36 \mu M; n = 4) \), justifying pooling of these data. While the number of measurements was very low in the present study, other studies have also indicated that GLU values in brain dialysates from several brain regions, including the mPFC, do not depend on the \( Ca^{2+} \) content of the perfusion medium (see Timmerman and Westerink, 1997). Analysis of the few mPFC dialysates, in which GLU was detectable, did not yield evidence for an effect of ventral hippocampal NMDA infusion on GLU concentrations (data not shown). In view of the low number of rats that could be included in this analysis, this result has certainly to be considered with caution. It remains to be clarified by future studies whether ventral hippocampal NMDA stimulation increases GLU concentrations in mPFC dialysates. These studies should completely focus on measurement in GLU, in order to determine GLU values reliably and in order to reduce sampling times. It has been suggested that increases in neuronal GLU release are only detectable by microdialysis when very short sampling intervals \( (< 5 \text{ min}) \) are used (Timmerman and Westerink, 1997).

DA

DA was not detectable in dialysates from the mPFC in Experiment 1 neither under basal conditions nor after ventral hippocampal NMDA stimulation and regardless of whether the 2-mm probes were perfused with normal-\( Ca^{2+} \) or high-\( Ca^{2+} \) ASCF.

DA metabolites

Basal values of DOPAC and HVA in dialysates from the mPFC were similar in the VEH and NMDA rats \( (F < 1) \) and comparable to values reported by previous studies (e.g., Feenstra...
Fig. 1 Schematic representation of the infusion sites in the VH (left) and the locations of the 2-mm probe (Experiment 1, right top) and the 4-mm probes (Experiment 2, right bottom) in the mPFC in line drawings of coronal brain sections [adapted from the atlas of Paxinos and Watson (1998)]. Numbers indicated distance from bregma in mm. The spread of the infusion sites around the indicated locations was about 0.5 mm in the anterior direction and 1 mm in the posterior direction and ± 0.5 mm horizontally and vertically. The spread of the probe locations around the indicated locations was about 1 mm in the anterior direction and 0.5 mm in the posterior direction and ± 0.5 mm horizontally and vertically. CA1, CA2, CA3: hippocampal fields CA1-3; CG: anterior dorsal cingulate cortex; DG: dentate gyrus; IL: infralimbic mPFC; PL: prelimbic mPFC; S: subiculum.
et al., 1998, 2002) (Table 1). As is characteristic for dialysates from the mPFC, HVA concentrations were higher than DOPAC concentrations (see Bast et al., 2002). The Ca\(^{2+}\) content of the perfusion medium had no considerable effect on the basal metabolite concentrations. The dialysates collected with normal-Ca\(^{2+}\) ASCF (DOPAC, 18.73 ± 5.99 nM; HVA, 35.49 ± 7.48 μM; n = 8) did not differ (F < 1) from those collected with high-Ca\(^{2+}\) ASCF (DOPAC, 18.10 ± 4.46 nM; HVA, 29.80 ± 6.22 μM; n = 6). This is consistent with previous studies demonstrating that dialysate concentrations of HVA and DOPAC hardly depend on the Ca\(^{2+}\) concentration of the perfusion medium (Westerink et al., 1986) and justifies pooling of the metabolite data from animals for which different perfusion media were used.

**Table 1** Basal concentrations (mean±SEM) of neurotransmitters and metabolites in dialysates from the mPFC.

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1</th>
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<th>Experiment 2</th>
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<tr>
<td></td>
<td>VEH</td>
<td>NMDA</td>
<td>Average</td>
<td>VEH</td>
</tr>
<tr>
<td>GLU (μM)</td>
<td>1.00±0.52</td>
<td>0.54±0.23</td>
<td>0.77±0.27</td>
<td>—</td>
</tr>
<tr>
<td>(n=3)</td>
<td></td>
<td>(n=3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA (nM)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.27±0.09</td>
</tr>
<tr>
<td>(n.d)</td>
<td></td>
<td></td>
<td></td>
<td>(n=6)</td>
</tr>
<tr>
<td>DOPAC (nM)</td>
<td>19.43±5.96</td>
<td>17.17±4.46</td>
<td>18.46±3.78</td>
<td>40.70±14.38</td>
</tr>
<tr>
<td>(n=6)</td>
<td>(n=8)</td>
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<td></td>
<td>(n=6)</td>
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<tr>
<td>HVA (nM)</td>
<td>33.08±6.72</td>
<td>33.03±7.38</td>
<td>33.05±4.92</td>
<td>61.95±17.16</td>
</tr>
<tr>
<td>(n=6)</td>
<td>(n=8)</td>
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<td>(n=6)</td>
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</table>

ANOVA of DOPAC concentrations measured in the two basal samples and the 12 samples following infusion yielded a significant main effect of sample (F\(_{13,156}\) = 2.00, P < 0.025), reflecting that in mPFC dialysates of both VEH and NMDA rats DOPAC concentrations increased following infusion and then decreased again (Fig. 2, left, middle). Even though the increase in DOPAC concentrations appeared to be much more pronounced following NMDA infusion than VEH infusion, ANOVA did not yield a main effect of infusion (F\(_{1,12}\) = 1.25, P > 0.2) or a significant interaction infusion x sample (F < 1). The slight increase of DOPAC concentrations in dialysates from the VEH group may be due to the handling during infusion (Feenstra et al., 1998).

ANOVA of HVA concentrations measured in the two basal samples and the 12 samples following infusion yielded a significant main effect of sample (F\(_{13,156}\) = 2.59, P < 0.005), as well as an interaction of infusion x sample (F\(_{13,156}\) = 1.98, P < 0.05) (Fig. 2, left, bottom). The
**Fig. 2** The effects of unilateral infusion of NMDA (0.5 µg/ 0.5 µl) or VEH on concentrations of DA and its metabolites in dialysates collected from the ipsilateral mPFC. In Experiment 1, 2-mm microdialysis probes were used and dialysates were collected every 15 min. In Experiment 2, 4-mm probes were used and dialysates were collected every 20 min. Dialysate concentrations are expressed as percentage of the average concentration determined for the two baseline samples collected before infusion (% basal value). All values are presented as mean, error bars indicate the SEM.
latter reflected that HVA concentrations in dialysates from the mPFC of NMDA rats increased to a peak value of 160% basal value in the fifth sample following infusion and then decreased again until the end of sampling ($F_{13,91} = 3.16, P < 0.001$), while HVA concentrations in the VEH group remained fairly stable ($F_{13,65} = 1.09, P > 0.3$). The fact that HVA concentrations in the VEH group were hardly affected by the handling for infusion, while DOPAC concentrations showed a slight increase, corresponds with the finding that HVA concentrations are less responsive to handling than DOPAC concentrations (Feenstra et al., 1998). Concentrations of HVA were significantly higher than the first baseline sample in the fourth sample after infusion collected from the mPFC after infusion of NMDA into the VH and significantly elevated as compared to both first and second baseline sample in the fifth to twelfth dialysate after infusion (all $P < 0.05$). Considering the dead volume of the outlet tubing, this reflects that extracellular HVA levels in the mPFC were increased from about 1 hour following NMDA stimulation of the VH. Given that HVA stems to a considerable part from metabolization of extracellular DA and from DA reuptaken into the terminal after neuronal release (Elsworth and Roth, 1997), this increase of extracellular HVA may result from an increased neuronal release of DA in the mPFC during the first hour following ventral hippocampal NMDA stimulation.

**Experiment 2**

**DA**

In Experiment 2, DA was detectable in dialysates collected from the mPFC in all animals. Dialysate concentrations of DA were similar in the VEH and NMDA rats ($F < 1$) and comparable to values reported by previous studies (e.g., Bassareo and Di Chiara, 1997; Feenstra et al., 1998, 2002) (Table 1).

ANOVA of DA concentrations measured in the two basal samples and the nine samples following infusion yielded a significant main effect of sample ($F_{10,90} = 2.41, P < 0.02$), reflecting that in mPFC dialysates of both VEH and NMDA rats DA concentrations increased rapidly following infusion (Fig. 2, right, top). Concentrations were elevated by 60% in the first sample following infusion. Afterwards they declined to baseline levels within one to two samples in the VEH groups, while they remained elevated and did not reach baseline before the ninth sample in the NMDA group. While there was no interaction infusion x sample ($F < 1$), this difference between the NMDA and the VEH group was supported by a tendency for a main effect of infusion revealed by the ANOVA ($F_{1,9} = 3.48, P = 0.095$). The rapid increase of DA in mPFC dialysates of VEH rats demonstrates that the infusion procedure
III.4.

itself increased extracellular DA concentrations in the mPFC. This is in line with the observation that DA was elevated by nearly 200% in dialysates collected from the mPFC during a 16-min period of gentle handling (Feenstra et al., 1998). The lower increase of about 60% observed in our study probably reflected the thorough habituation of the animals (see Experimental Design) and the shorter handling period (the infusion took about 2-3 min). Importantly, the elevation of DA following infusion was more pronounced in the group of rats that received ventral hippocampal NMDA infusion than in the VEH group. Thus, even though this difference only approached statistical significance, our preliminary data support that ventral hippocampal NMDA stimulation increases DA release in the mPFC.

**DA metabolites**

Basal values of DOPAC and HVA in dialysates from the mPFC were similar in the VEH and NMDA rats ($F < 1$) (Table 1). The values in Experiment 2 were about twice as high as in Experiment 1, reflecting that the recovery of the 4-mm probes used in Experiment 2 was about twice as high as the recovery of the 2-mm probes used in Experiment 1.

ANOVA of DOPAC concentrations measured in the two basal samples and the nine samples following infusion yielded a significant main effect of sample ($F_{10,90} = 2.58, P < 0.01$) and an interaction infusion x sample ($F_{10,90} = 2.67, P < 0.01$) (Fig. 2, right, middle). The interaction reflected that the increase of DOPAC in the dialysates collected after infusion was much more pronounced in the NMDA than in the VEH group. Indeed, separate ANOVA yielded a significant effect of sample only in the NMDA ($F_{10,40} = 2.29, P < 0.05$), but not in the VEH ($F_{10,40} = 1.22, P > 0.3$), group. Concentrations of DOPAC were significantly elevated, as compared to the two baseline samples, from the seventh to ninth dialysate collected after infusion of NMDA into the VH ($P < 0.05$).

ANOVA of HVA concentrations measured in the two basal samples and the nine samples following infusion yielded a significant main effect of sample ($F_{10,90} = 3.04, P < 0.005$), a tendency towards a main effect of infusion ($F_{1,9} = 3.86, P = 0.081$), as well as an interaction of infusion x sample ($F_{10,90} = 3.15, P < 0.005$) (Fig. 2, right, bottom). The latter two results reflected that HVA concentrations in dialysates from the mPFC of NMDA rats significantly increased after infusion ($F_{10,40} = 2.66, P < 0.02$), reaching a peak value of about 250% basal value in the seventh sample following infusion, while HVA concentrations in the VEH group did not show significant changes ($F < 1$). Concentrations of HVA in the fourth, and the sixth to ninth dialysate collected from the mPFC after infusion of NMDA into the VH were significantly elevated as compared to the two baseline samples (all $P < 0.05$).
III.4.

Overall, the metabolite data from Experiment 2 are similar to the findings of Experiment 1. The increased concentrations of DA metabolites in mPFC dialysates from the NMDA group, as compared to the VEH group, support that DA activity in the mPFC is increased by ventral hippocampal NMDA stimulation.

Conclusion

Altogether, the present preliminary in vivo microdialysis data obtained from freely moving rats confirm the results of our previous post mortem study (Bast et al., submitted) and demonstrate that NMDA stimulation of the VH increases DA release in the mPFC. One may already think about future experiments combining manipulations of ventral hippocampal activity and prefrontal DA transmission, similar to the study of Seamans et al. (1998), to directly characterize the functional significance of the suggested positive modulation of prefrontal DA transmission by the VH.

Acknowledgements This work was financially supported by grants from The Swiss Federal Institute of Technology Zurich (ETHZ). The support of the technical and animal-care staff of the Lab of Behavioral Neurobiology is gratefully acknowledged. Special thanks are due to Liz Weber, for histological preparations, to Barbara Krummenacher, for technical assistance, and to Andreas Leng, for frequent help and advice concerning HPLC methods.
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III.4.


305
III.4.


MAIN CONCLUSIONS
Main conclusions

The general implications of the studies presented in Part I and II of the thesis have already been discussed in detail in the two review articles (I.7., II.4.), and the results of the studies constituting Part III have been discussed in light of the other outcomes of the thesis (see also I.7.). In short, the main conclusions from the studies presented in this thesis are as follows:

First, the studies presented in Part I of the thesis underline that the contributions of the hippocampus to behavior are not restricted to memory processes. Thus, the results demonstrate that hippocampal mechanisms directly influence the three examined sensorimotor processes – locomotor activity, prepulse inhibition (PPI), and startle reflex. Given that alterations of these processes are related to neuropsychiatric diseases, in particular schizophrenia, these results also corroborate that aberrant hippocampal function may play a role in the pathophysiology of schizophrenia. Moreover, by identifying a dominant role of the ventral, as compared to the dorsal, hippocampus in the regulation of two of the examined sensorimotor processes (locomotor activity and PPI), the results presented in Part I complement previous data indicating that the dorsal hippocampus is more important than the ventral hippocampus for a particular form of hippocampus-dependent memory, spatial memory.

Second, in line with prevalent concepts emphasizing the eminent role of the hippocampus for so-called relational memory, the results presented in Part II indicate that particular mechanisms in both the ventral and dorsal hippocampus are involved specifically in fear conditioning to context. Nevertheless, some mechanisms in both the ventral and the dorsal hippocampus appear also to be important for simple associative learning, as strongly suggested by the effects of some pharmacological manipulations of the ventral and dorsal hippocampus on fear conditioning to tone.

Third, the microinfusion studies presented and discussed in Part I and II suggest modifications of prevalent notions on hippocampal function that have emerged from studies examining the effects of hippocampal lesions. Thus, one important methodological conclusion from the work presented in the thesis is that intracerebral drug microinfusions should be increasingly applied to further our understanding of structure-function relationships.

Finally, the studies presented in Part III indicate that ventral hippocampal activity can enhance dopamine transmission in the medial prefrontal cortex. Furthermore, the outcome of the studies examining the behavioral effects of manipulations of prefrontal dopamine transmission suggests that the positive control of prefrontal dopamine transmission by the ventral hippocampus may partly mediate the hippocampal modulation of locomotor activity.
Main conclusions

and that this control may also be important for the expression of conditioned fear formed with participation of the hippocampus.
CURRICULUM VITAE

Tobias Bast

Date and place of birth: 28.3.1974, Dinslaken/Niederrhein, Germany

Citizenship: German

Studies and degrees:

September 1999-October 2002: Ph.D. studies at the Swiss Federal Institute of Technology Zurich; Ph.D. program in neuroscience, Neuroscience Center Zurich

August 1999: "Diplom" (M.S.) in biochemistry, optional subject: biopsychology, subsidiary subject: philosophy, Ruhr-University Bochum, Germany

October 1996: "Vordiplom" (B.S.) in biochemistry, Ruhr-University Bochum, Germany

March 1996: "Zwischenprüfung" (intermediate examination) in philosophy, Ruhr-University Bochum, Germany

October 1994-August 1999: Studies of biochemistry and philosophy, Ruhr-University Bochum, Germany

Diploma thesis:
"Die monoaminerge Innervation des Telencephalons der Taube (Columba livia): Eine Untersuchung mittels in vivo Mikrodialyse" (The monoaminergic innervation of the telencephalon of the pigeon (Columba livia): an in vivo microdialysis study).
Supervisors: Dr. Bettina Diekamp, Prof. Dr. Onur Güntürkün; Biopsychology Group, Ruhr-University Bochum, Germany; in collaboration with PD Dr. Rainer Schwarting, Institute for Physiological Psychology, Heinrich-Heine-University Düsseldorf, Germany (October 1998-August 1999)

Positions and employments:

Academia

From January 2003: Research assistant, Department of Neuroscience, University of Edinburgh, Scotland, UK

November 2002-January 2002: Research assistant, Behavioral Neurobiology, Swiss Federal Institute of Technology Zurich

September 1999-November 2002: Doctoral research assistant, Behavioral Neurobiology, Swiss Federal Institute of Technology Zurich

June 1998-September 1999: "Studentische Hilfskraft" (undergraduate assistant), Biopsychology Group, Ruhr-University Bochum, Germany

Others

October 1994-September 1999: Nursing service, St. Vincentius-Hospital, Dinslaken, Germany

July 1993-August 1994: "Zivildienst" (alternative civilian service) in the geriatric care, Wilhelm-Lantermann-Seniorenzentrum, Dinslaken, Germany
LIST OF PUBLICATIONS

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1999

Invited presentations:
Microdialysis in the striatum and the ‘prefrontal cortex’ of the pigeon: extracellular levels of dopamine and monoamine metabolites and effects of d-amphetamine. 2nd Dopamine Workshop of the German-Science-Foundation Program of Emphasis 1001 Sensorimotor Integration. Ruhr-University Bochum, Germany, 9 December 1999
Mechanisms in the ventral and dorsal hippocampus and classical fear conditioning to discrete and contextual stimuli in rats: implications for views on hippocampal function in learning. Short talk presented at the European Research Conference on Neural Mechanisms of Learning and Memory. Evian, France, 10-15 May 2002

312