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Labyrinth Exploration, Emotional Reactivity, and Conditioned Fear in Young Roman/Verh Inbred Rats

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An inbreeding program has been carried out with the Swiss sublines of Roman high- and low-avoidance rats since 1993. The present study reports the first experiments conducted with young animals of those inbred strains (RHA-I/Verh and RLA-I/Verh, respectively) from the sixth and seventh inbreeding generations. The results confirmed expected behavioral profiles. Compared to the RHA-I/Verh strain, RLA-I/Verh rats showed decreased entries into the illuminated central arena of an hexagonal tunnel maze, as well as decreased spontaneous locomotor activity and increased defecations, in two independent experiments. Young RLA-I/Verh females explored less than did their RHA-I/Verh counterparts during session 1 of a conditioned-fear experiment preceding shock administration, and in session 2 (conducted 24 h after the application of three footshocks), they showed greater conditioned behavioral inhibition (i.e., reduced amount of rearing), as well as higher defecation scores, than did RHA-I/Verh females.

KEY WORDS: Roman/Verh inbred strains; exploratory activity; emotionality; tunnel maze; conditioned fear.

INTRODUCTION

The Swiss sublines of Roman high-avoidance (RHA/Verh) and Roman low-avoidance (RLA/Verh) rats have demonstrated divergent behavioral patterns related to endogenous anxiety over many generations, the latter being the more fearful of the two lines of rats (Driscoll and Bättig, 1982; Ferré *et al.*, 1995; Steimer *et al.*, 1997b). These behavioral "hyperemotional" (RLA/Verh) and "hypoemotional" (RHA/Verh) phenotypes correspond to certain neurochemical and neuroendocrinological data concerning physiological processes underlying fear and emotional reactivity (Aubry *et al.*, 1995; Gentsch *et al.*, 1982; Giorgi *et al.*, 1994; Steimer *et al.*, 1997a,b; Walker *et al.*, 1989).

Inbred Roman strains derived from those RHA/Verh and RLA/Verh outbred lines, therefore, could be a good animal model to investigate the molecular genetic basis of anxiety. With this in mind, an inbreeding program has been carried out since 1993 in order to produce inbred Roman strains (by brother × sister mating) useful for genetic analysis and for the identification of genetic loci related to behavioral traits of anxiety. We report here the first experiments done with young Roman inbred rats from the sixth and seventh generation in two behavioral tests of emotionality/anxiety: an hexagonal tunnel maze and a fear conditioning test.

In the first publication concerned with RHA/Verh and RLA/Verh rats, Bättig *et al.* (1976)

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described a tunnel maze consisting of a central hexagonal arena and two concentric hexagonal alleys, interconnected by radial alleys, which could be used to measure the suppression of spontaneous activity by situational fear or anxiety (Bättig, 1983). In this regard, it has been shown that entries into the maze central arena (and also the total explored area) (a) are reduced when the arena is brightly illuminated (Martin *et al.*, 1982; Nil and Bättig, 1981), (b) can be increased by anxiolytic drugs or by infantile stimulation, especially in RLA/Verh rats (Fernández-Teruel *et al.*, 1991; Martin *et al.*, 1982), and (c) are negatively correlated with defecation and activity during maze testing (Fernández-Teruel *et al.*, 1994). The RHA/Verh and RLA/Verh outbred lines have consistently been shown to differ in maze activity and exploration patterns, RHA/Verh rats being those which display increased activity and entries into the illuminated center as well as reduced defecations (Bättig *et al.*, 1976; Fernández-Teruel *et al.*, 1991, 1994; Martin *et al.*, 1982; Nil and Bättig, 1981). Such between-line differences already appear in postweaning (4–5-week-old) animals (Fernández-Teruel *et al.*, 1991, 1994).

As for fear-conditioning, adult RLA/Verh rats have shown a more pronounced bradycardia in response to a conditioned emotional stressor (Roozendal *et al.*, 1992), greater shock-induced suppression of drinking, and increased context-conditioned defecation and self-grooming (Imada, 1972; Ferré *et al.*, 1995; Steimer *et al.*, 1997a,b; Castanon and Mormède, 1994) than adult RHA/Verh rats. The fear-conditioning test used in the present study with young rats was the measurement of behavioral parameters prior to and following exposure to footshocks in a shuttle box-type apparatus.

Two sets of postweaning animals from the sixth and seventh inbreeding generations were tested in a hexagonal tunnel maze (see Fernández-Teruel *et al.*, 1991) (experiments 1a and 1b) and young females from the seventh generation were also tested for fear-conditioning (experiment 2).

MATERIALS AND METHODS

Animals

Male and/or female RHA-I/Verh and RLA-I/Verh rats were used. After weaning (postnatal day 22) animals were housed in groups of four to six same-sexed rats/cage. They were maintained with food and tap water available ad libitum, under conditions of controlled temperature (22 ± 2°C) and a 12-h light–dark cycle (lights on 0700).

Experiments 1a and 1b: Labyrinth Testing

A hexagonal tunnel maze with concentric interconnected alleys was used [for further details and a plan of the maze see Fernández-Teruel *et al.* (1991, 1994)]. A total of 42 infrared photocell units, which were interfaced to an IBM-XT personal computer, was uniformly distributed within the maze alleys. Entry into and exit from the central arena (illuminated by a 60-W incandescent bulb suspended 28 cm above the center) could also be thus measured. The ceiling and side walls (9 cm high) were fitted together to form a unit that could be lifted from the floor to permit easy removal of a subject and subsequent cleaning. The maze contained no barriers, thus permitting the rats to ambulate freely from one alley to another.

Behavioral testing, which was completely automated, was carried out between 0830 and 1700. Each rat received a single 6-min test in the maze. At the conclusion of each test the floor of the maze was thoroughly wiped clean with a slightly damp cloth. The order of testing was arranged so that approximately equal numbers of rats from each group were tested during each portion of the light cycle. Before testing the animals were familiarized with the experimental room for at least 10 min, and they were weighed immediately before starting the test.

The parameters scored during maze testing were total activity (TA; number of photobeam interruptions during a test) and entries into the central illuminated arena (EC).

Experiment 1a. Male and female 4-week-old rats from each inbred strain (sixth generation of inbreeding) were used. Each of the four experimental groups contained animals from at least six litters.

Experiment 1b. Male and female 6-week-old rats from each inbred strain (seventh generation of inbreeding) were used. Each of the four experimental groups contained animals from nine litters. Defecations (during weighing + during maze testing) were also scored in this experiment.

Table I. Mean ± SE Total Activity (TA), Entries into the Center (EC), and Defecations (DEF) in the Hexagonal Tunnel Labyrinth*

	RHA-I		RLA-I	
	Males	Females	Males	Females
Expt 1a				
TA	233.4 ± 9.7	239.6 ± 14.2	161.6 ± 12.4[a]	187.2 ± 14.7[a]
EC	7.3 ± 1.6	9.5 ± 1.2	3.8 ± 1.5[b]	2.6 ± 0.7[a]
Expt 1b				
TA	260.3 ± 11.0	274.0 ± 9.5	182.9 ± 5.7[a,c]	224.7 ± 10.7[a]
EC	5.5 ± 1.1	7.7 ± 1.4	3.5 ± 0.8	5.1 ± 1.1
DEF	0.56 ± 0.13	0.20 ± 0.07	1.97 ± 0.19[a,c]	1.03 ± 0.23[a]

* [a]$p < .01$ vs. the corresponding RHA group (same sex). [b]$p < .05$ (one tailed) vs. the corresponding RHA group (same sex). [c]$p < .01$ vs. the corresponding female group (same strain). Duncan's t tests; $n = 10$/group in experiment 1a; $n = 30$/group in experiment 1b.

Experiment 2: Conditioned Fear

Sixteen 8-week old females from each inbred strain (seventh generation of inbreeding) were used. There were animals from at least six litters in each experimental group.

The shuttle box used for exploration testing was divided into two 27 × 27 × 27-cm compartments connected by an opening of 7 × 7 cm. The ceiling was transparent, and the box was uniformly illuminated by a 60-W incandescent bulb placed 40 cm above it. After weighing an animal, behavioral testing (session 1) started by placing the rat into one of the compartments of the shuttle box and allowing it to explore the box freely for 4 min. Crossings, rearings, and defecation were scored during that period. Immediately following this 4-min testing, the animals were confined to one of the compartments by closing the opening and eight rats from each strain received three footshocks (each of 0.5 mA, 3 s) at 30-s intervals (RHA-I/S and RLA-I/S groups). The remaining eight rats per strain were also confined to one of the compartments but footshocks were not administered (RHA-I/C and RLA-I/C groups). Twenty-four hours after that procedure, all animals were again placed in the shuttle box for another 4 min, comparable to session 1 (session 2). Testing was performed during the morning in a counterbalanced manner.

Statistical Analysis

Factorial analysis of variance (ANOVA), followed by Duncan's multiple-range test for comparisons between groups, were applied to data from experiments 1a and 1b. Crossings, rearings, and defecation (log transformed) from experiment 2 were analyzed by two-way ANOVA with repeated measures. Student's t tests were also used for comparisons between pairs of groups.

RESULTS

The results from experiments 1a and 1b are shown in Table I. Both experiments showed higher exploratory activity [TA; $F(1,39) = 12.4, p < .001$, and $F(1,119) = 44.7, p < .001$, experiments 1a and 1b, respectively] and more entries into the illuminated center [EC; $F(1,39) = 15.4, p < .001$, and $F(1,119) = 4.33, p < .04$, experiments 1a and 1b, respectively] in RHA-I than in RLA-I rats. There was also a tendency for females to be more active, although it was significant only in experiment 1b [Sex effect on TA, $F(1,119) = 8.6, p < .01$]. The analysis of defecations in experiment 1b showed Strain [$F(1,119) = 44.2, p < .001$] as well as Sex effects [$F(1,119) = 15.0, p < .001$], with RLA-I rats defecating more than RHA-I rats and females defecating less than males (see Table I and Duncan's tests). No Strain × Sex interactions were found for any of the parameters studied.

Results from experiment 2 are shown in Fig. 1. Since there were no within-strain differences in any of the measures studied in session 1 (i.e., before shock administration), results from this session were analyzed pooling the rats within each strain (thus $n = 16$/group). It was observed that RHA-I rats displayed more rearing behavior than RLA-I animals [$t(30) = 4.35, p < .001$] (Fig. 1B) in session 1, whereas there were no significant differ-

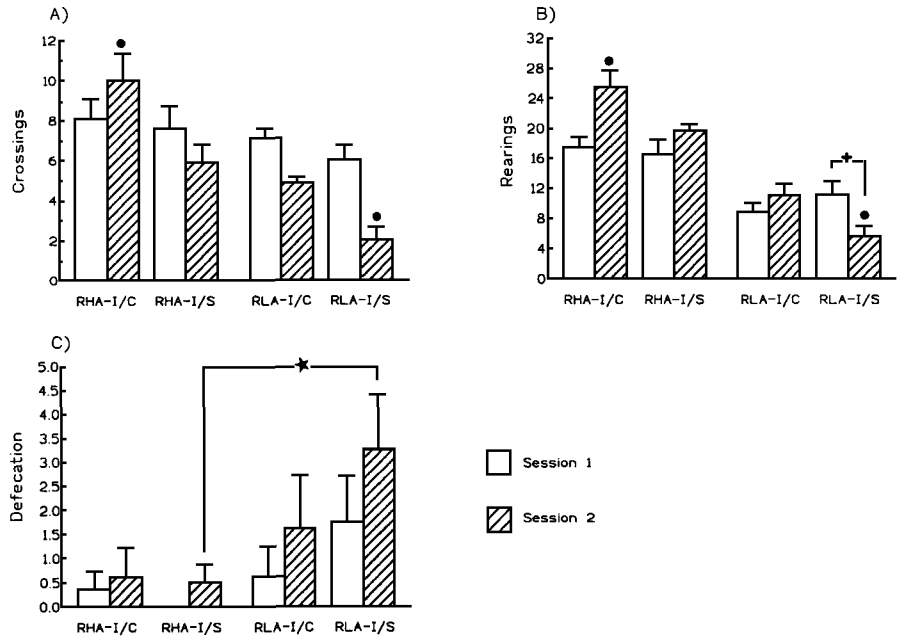

Fig. 1. Mean ± SE crossings (A), rearings (B), and defecations (C) in a fear-conditioning test. RHA-S and RLA-S are the groups of the corresponding strain receiving footshock immediately following session 1; RHA-C and RLA-C are the control nonshocked groups. (●) $p < .05$ vs. all groups; (★) $p < .05$ between the groups indicated (Duncan's test); (+) $< .05$ between the groups indicated (paired t test).

ences in crossings or in defecations ($p = .1$) between the two strains in the same session.

The two-way ANOVA analysis (Strain factor—RHA-I, RLA-I; and shock factor—control, shocked) with repeated measures (sessions 1 and 2) revealed overall significant Strain × Session effects on crossings [$F(1,28) = 16.51, p < .001$] and rearings [$F(1,28) = 13.61, p < .001$], thus indicating that the reduction in those measures in session 2 was greater in the RLA-I strain. Overall significant Shock × Session effects on crossings and rearings were also found [$F(1,28) = 11.73, p < .01$, and $F(1,28) = 10.12, p < .01$, respectively]. A more detailed analysis suggested that the strongest behavioral suppression in session 2 (i.e., conditioned-fear as a consequence of receiving shocks immediately after session 1) occurred in RLA-I rats. This was especially evident in the rearing measure, as the RLA-I/S was the only group showing a clear and significant between-session decrease in rearing behavior (paired t test, $t = 3.27, p = .014$), whereas the remaining groups increased rearing from session 1 to session 2 (Fig. 1B). Finally, RLA-I animals defecated more than RHA-I rats [overall significant Strain effect: $F(1,28) = 4.14, p = .05$], and group comparisons in session 2 showed that the only significant difference in that measure appeared between RLA-I/S and RHA-I/S groups ($p < .05$, Duncan's test), with RLA-I/S rats showing the highest defecation score.

It is worth mentioning here that the time spent self-grooming was different [as expected (see Ferré *et al.*, 1995; Steimer *et al.*, 1997b)] between both strains in session 1 (means ± SE: RHA-I, 12.9 ± 1.9; RLA-I, 31.4 ± 3.9; $p < .05$), but it was not differentially affected by shock, as observed by the scores obtained in session 2 (RHA-I/C, 15.4 ± 3.3; RHA-I/S, 15.1 ± 4.0; RLA-I/C, 29.0 ± 5.0; RLA-I/S, 27.4 ± 11.1).

DISCUSSION

The results from experiments 1a and 1b regarding total activity, as well as emotionality (i.e., entries into the illuminated center and defecation) in the hexagonal tunnel maze closely resemble those obtained in previous experiments performed with RHA/Verh and RLA/Verh outbred rats of a similar age (Fernández-Teruel *et al.*, 1991, 1994). It can also be observed (Table I) that females of

both strains tended to be more active and to defecate less than males, results which are also in agreement with those found in the Roman/Verh outbred lines (Fernández-Teruel *et al.*, 1991, 1994). Thus, concerning labyrinth behavior, young Roman/Verh inbred rats show behavioral profiles very similar to what has typically been observed in the outbred parental lines.

In experiment 2, RHA-I/Verh rats reared much more than did RLA-I/Verh rats, whereas no differences in crossings between the two strains were found in session 1 (see Fig. 1B). It appears that RHA-I/Verh animals preferred to explore in vertical directions (rearing behavior) instead of moving from one side to the other of the apparatus (crossings), an effect that has already been observed several times when using shuttle boxes for measuring exploratory behavior (unpublished). With regard to defecation, the two strains did not differ in session 1 since only 6% (1/16) of RHA-I/Verh and 37% (6/16) of RLA-I/Verh animals defecated during that 4-min testing period. The possibility exists that a longer testing time would have revealed differences in defecation similar to those observed in other studies (e.g., Ferré *et al.*, 1995).

The exploratory pattern changed in session 2. Control (nonshocked) animals from both strains (RHA-I/C and RLA-I/C groups) differed in both rearings and crossings due to the fact that the RHA-I/C group showed increased exploratory behavior (i.e., both parameters) from session 1 to session 2, whereas RLA-I/C animals did not (see Figs. 1A and B). As habituation of exploratory behavior may be dependent upon spatial information storage, such differences in exploratory habituation patterns (i.e., between sessions) could be in line with previous studies indicating that RHA/Verh rats are less efficient than RLA/Verh animals in several learning tasks (see Fernández-Teruel *et al.*, 1997; Nil and Bättig, 1981). In session 2, those two nonshocked groups still presented no significant defecation scores.

After shock administration, however, young RHA-I/S rats decreased crossings and rearings compared to RHA-I/C rats ($p < .05$, Duncan's test), and the same was observed when comparing RLA-I/C and RLA-I/S groups (Figs. 1A and B). In addition to showing the lowest crossing and rearing scores, the RLA-I/S group showed the highest defecation scores in session 2, leading to significant differences with respect to RHA-I/S animals. It appears that the specific decrease in rearing behavior (Fig. 1B) and increase in defecation (Fig. 1C) shown by young RLA-I/S rats, from session 1 to session 2, is most likely attributable to a higher sensitivity to fear-conditioning in that strain. This is consistent with previous results reported with adult RLA/Verh outbred rats which indicated a higher context-conditioned fear (compared to the RHA/Verh line) in situations involving shock administration (Ferré *et al.*, 1995; Roozendal *et al.*, 1992).

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