Objective assessment of lower urinary tract afferents in humans using evoked potentials

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Objective assessment of lower urinary tract afferents in humans using evoked potentials

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For the degree of

Doctor of Sciences

Presented by

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Zurich, 2012
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Summary

Only a few objective methods are available to determine the function of the lower urinary tract (LUT). Although urodynamic investigation is the gold standard to assess LUT function in daily clinical practice, it is invasive, time consuming, expensive, and technically demanding with limited availability. Additionally, the reliability of this investigation is discussed controversially; some studies quote a good reliability whereas others do not. Most importantly, urodynamic investigation cannot directly assess the innervation of the LUT. However, information on nerve fibre integrity and function would be desirable to optimize appropriate treatment.

Electrophysiological measurements such as electrical stimulations of a defined nerve followed by cortical evoked potentials (EPs) are known to objectively determine nerve fibre function and its integrity. It is a very useful instrument to predict the walking ability of patients already shortly after spinal cord injury. Up to date, there is no equivalent for the LUT. Thus, the aim of this thesis was to develop an electrophysiological tool to assess LUT afferents and a setup for the application of electrical stimulation followed by an EP.

A-delta and C fibres are known to be present in the LUT but it is unclear if also A-beta fibres exist in the LUT. Our setups used aimed to activate A-delta and A-beta fibres and to determine its functionality.

This thesis contributes to the understanding of the nerve fibre afferents travelling from sacral dermatomes and the LUT to the cortex. It is known that LUT function also depends on intact afferent and efferent nerve fibres. Electrical stimulations in the LUT increase the basic knowledge about the nerve fibres present in the LUT and provide, combined with electrical and heat stimulation of sacral dermatomes, additional information on the function of the human LUT. To test these tools, the known neurophysiological methods of electrical and heat stimulation were performed on sacral dermatomes. The well-established electrical stimulation on defined nerves or dermatomes, which activates A-beta fibres, was adapted to
Summary

the LUT. Additionally, a new setup of electrical stimulation in the LUT was developed, aiming to selectively activate A-delta fibres. During all those stimulations, EPs were recorded from the cortex. The latency and amplitudes of the EPs were determined and the intraclass correlation coefficient (ICC) was calculated to test the reliability of these methods.

This thesis provides new insights into the LUT afferent function using electrical and heat stimulations on sacral dermatomes and electrical stimulation of the LUT. In addition, the literature on cortical activity during different LUT stimulations is reviewed. In the first study, electrical and heat stimulations were performed in young healthy females to test their feasibility and reliability on sacral dermatomes. With this study, first results were generated about the latency of A-beta and A-delta stimulations of the sacral level. In the second study, 3 Hz and 0.5 Hz electrical stimulation were applied to the bladder dome, trigone, proximal and distal urethra in young healthy females. After the 0.5 Hz stimulation, reliable EPs were seen with ICC values of more than 0.8 and latencies about 130 ms. The latencies obtained suggest that responsive fibres are of the A-delta type. At 3 Hz stimulation, no reliable EPs could be detected. In the third study, the 0.5 Hz setup was conducted in female subjects older than 35 years to test the influence of age. In elderly subjects, reliable EPs could be recorded from trigone and proximal urethra. The latencies after 0.5 Hz stimulation of the human LUT were shorter in the older compared to the younger healthy volunteers. This could be due to age related structural changes in the LUT.

The findings of this thesis provide a first step in the development of a new diagnostic tool assessing LUT afferents. We clearly showed that electrical stimulation of the LUT is feasible and provides reliable EPs, i.e. our newly developed objective tool has the potential to improve functional LUT assessment. Thus, further research is highly warranted, especially including patients with and without an underlying neurological disease.
Zusammenfassung

Es gibt nur wenige objektive Messmethoden zur Bestimmung der Funktion des unteren Harntrakts (UHT). Die urodynamische Untersuchung, der Goldstandard zur Bestimmung der UHT-Funktion, ist invasiv, zeitaufwändig, teuer und technisch anspruchsvoll und nur limitiert verfügbar. Dazu kommt, dass die Reliabilität dieser Untersuchung kontrovers diskutiert wird, einige Studien sprechen für eine gute Reliabilität, andere gegen sie. Vor allem kann die Innervation des UHT mittels urodynamischer Untersuchung nicht direkt bestimmt werden. Zur Optimierung der Therapie wären allerdings Kenntnisse über die Integrität und Funktion der Nervenfasern wünschenswert.


Diese Arbeit leistet einen Beitrag für das bessere Verständnis der afferenten Nervenfasern, die von sakralen Dermatomen und aus dem UHT zum Cortex verlaufen. Es ist bekannt, dass die UHT-Funktion sowohl von intakten afferenten wie efferenten Innervationen abhängt. Die elektrische Stimulation des UHT kann einerseits das Wissen über die in der Blase vorhandenen Nervenfasern erhöhen, anderseits können zusammen mit elektrischer und Wärmestimulation von sakralen Dermatomen zusätzliche Informationen über die
Zusammenfassung


Diese Arbeit liefert neue Einsichten in die afferente UHT-Funktion mittels elektrischer und Wärmestimulation sakraler Dermatome und elektrischer Stimulation des UHT. Zusätzlich wurde eine Literatur-Recherche über die kortikale Aktivität während unterschiedlicher UHT-Stimulationen durchgeführt. In der ersten Studie wurden elektrische und Wärmestimulation an sakralen Dermatomen an jungen gesunden Frauen durchgeführt, um die Durchführbarkeit und die Reliabilität dieser beiden Methoden auf diesen Dermatomen zu prüfen. Mit dieser Studie wurden erste Resultate über die Latenz von A-Beta und A-Delta Stimulationen vom sakralen Niveau generiert. In der zweiten Studie wurden eine 3 Hz und eine 0.5 Hz Elektrostimulation am Blasendach und Trigonum, sowie an der proximalen und distalen Urethra an jungen gesunden Frauen durchgeführt. Nach der 0.5 Hz Stimulation wurden reliable EPs mit ICC-Werten über 0.8 und Latenzen um 130 ms erhalten. Die Latenzen deuten auf A-Delta Fasern hin. Nach der 3 Hz Stimulation wurden keine reliablen EPs gesehen. In der dritten Studie wurde die 0.5 Hz Stimulation an Frauen über 35 Jahren angewendet, um den Einfluss des Alters zu untersuchen. In den älteren Probandinnen wurden reliable EPs vom Trigonum und von der proximalen Urethra abgeleitet. Die Latenzen nach der 0.5 Hz Stimulation waren bei den älteren Probandinnen kürzer als in der Gruppe der jungen Frauen. Dies kann durch strukturelle Änderungen des UHT mit dem Alter erklärt werden.
Zusammenfassung

1. General introduction

1.1 Bladder control in daily life

The lower urinary tract (LUT) consists of 1.) the bladder, 2.) the urethra, and 3.) the external urethral sphincter (figure 1.1). The bladder (1) is a hollow muscular organ which consists of three layers: the mucosa with the urethelium and the lamina propria, the lamina muscularis mucosae ant the adventia. The urinary bladder is located in the minor pelvis right behind the symphysis. In females, the posterior bladder wall and parts of the bladder dome border on the vagina and the uterus. The main part of the bladder is the body which also includes the fundus. The body is the expandable part of the bladder and is covered by peritoneum. Another part of the bladder is the trigone which is a small muscular triangle bordered by the two ureters and the urethra. It lies at the posterior bladder base and opens out into the bladder neck. Its function is to prevent vesico-urethral reflux.

The urethra (2) is a fibromuscular tube which consists of an outer layer (striated muscle fibres) and an inner layer (smooth muscle fibres). It is lined by mucosa, submucosal vessels, and connective tissue. In females, the mucosa is about 3.5 cm long and opens right above the vaginal introitus. External muscle layers form the urethral sphincter (3) that encloses the urethra in form of a horseshoe. In females, the proximal muscle fibres enclose the whole membranous part of the urethra.

The whole LUT is lined by the same epithelium, the urothelium. The urothelium is composed of three main layers: the basal cell layer which is attached to the basement membrane, the intermediate layer, and the superficial layer which consist of large hexagonal cells. The elasticity of the urinary bladder is maintained by subapical vesicles which are inserted in the cell membrane when the bladder expands. The barrier function between the urine and the interstitial body fluid is asserted by tight junction complexes, specialized lipid molecules, uroplakin proteins, and polysaccharide glycosaminoglycan layer in the apical layer.
General introduction

The urinary bladder has two functions: the storage of the urine where a low intravesical pressure has to be obtained [1] and the micturition which is the synergic, prompt, and complete execration of urine at a convenient and socially accepted location. These two functions are assured by a complex interaction of different structures of the LUT and the peripheral and the central nervous system.

![Illustration of the human female lower urinary tract](image)

*Figure 1.1: Illustration of the human female lower urinary tract*

The most important afferents for the micturition process are myelinated A-delta fibres and unmyelinated C-fibres. The A-delta fibres respond both to passive distension and active contraction (table 1.1). They are responsible for the physiologic sensation of fullness [2]. C fibres have a high-mechanical threshold and respond primarily to chemical irritation of the
General introduction

bladder mucosa or to cold (table 1.1) [3]. Sensation of C-afferents is followed by hyperexcitability of the micturition reflex [2]. Afferent A-delta and C fibres run in the pelvic nerve and transfer the information about the bladder filling state from the bladder wall to the spinal cord. There, they enter the spinal cord through the dorsal horn of the sacral spinal cord [4]. Information about the tension of the bladder wall is conveyed to supraspinal centres as well. It is assumed that sacral neurons mainly project to the periaqueductal gray (PAG) which sends signals to the pontine micturition centre (PMC) and other supraspinal structures like the prefrontal cortex, the insula and the anterior cingulated gyrus (ACG) [4-5].

Table 1.1: Comparison of the afferent nerve fibres

<table>
<thead>
<tr>
<th>fibre type</th>
<th>responds to:</th>
</tr>
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<tbody>
<tr>
<td>A-delta</td>
<td>passive distension</td>
</tr>
<tr>
<td></td>
<td>active contraction</td>
</tr>
<tr>
<td>C</td>
<td>-chemical irritation</td>
</tr>
<tr>
<td></td>
<td>- temperature</td>
</tr>
</tbody>
</table>

The cell bodies of A-delta fibres and C fibres are located in the dorsal root ganglia at the level of S2 – 4 and T11 – L2 spinal segments [6]. A dense nexus of sensory nerves has been identified in the suburothelial layer of the urinary bladder in humans, with some terminal fibres projecting into the urothelium. This suburothelial plexus is particularly prominent at the bladder neck, but is relatively sparse at the dome of the bladder and is thought to be critical in the sensory function of the urothelium [7].

During the bladder filling, low level afferent firing is conveyed to the spinal cord and the brainstem. In response, efferent sympathetic nerves activity originating in the lumbar cord 1.) inhibit the bladder activity via β-receptors in the bladder wall and 2.) stimulate the contraction of smooth muscles of the urethra at the bladder neck via α-receptors. The afferent nerves also excite the Onuf nucleus, which stimulates the external sphincter and the pelvic floor muscles via the pudendal nerve to contract. When the bladder fills more, high level firing
signals are sent to the supraspinal centres. These signals are processed in the cortex and other supraspinal structures and reach awareness.

The efferent innervation of the urinary tract consists of the sympathetic, parasympathetic, and the somatic part (table 1.2) [6]. The sympathetic nerves travel through the hypogastric nerves which leave the spinal cord at T11 – L2. They are responsible for the activation of the urethral sphincter and for the inhibition of the detrusor contraction. The parasympathetic nerves travel through the pelvic nerve and are responsible for the contraction of the detrusor during micturition. The somatic nerves travel through the pudendal nerve and are responsible for the contraction of the external sphincter of the urethra to prevent urine loss during the storage phase.

**Table 1.2: Comparison of the efferent nerve fibres**

<table>
<thead>
<tr>
<th>nerve type</th>
<th>nerve</th>
<th>functions</th>
<th>enters spinal cord at:</th>
</tr>
</thead>
<tbody>
<tr>
<td>sympathetic</td>
<td>hypogastric</td>
<td>activation of urethral sphincter, inhibition of detrusor contraction</td>
<td>T11 – L2</td>
</tr>
<tr>
<td>parasympathetic</td>
<td>pelvic</td>
<td>contraction of detrusor muscle</td>
<td>S2 – S4</td>
</tr>
<tr>
<td>somatic</td>
<td>pudendal</td>
<td>contraction of external sphincter</td>
<td>S2 – S4</td>
</tr>
</tbody>
</table>

When the desire to empty the bladder is achieved, the signals are conveyed to the PMC which enables a synergic micturition in two ways: 1.) through reduction of sympathetic motoneuron activity in the lumbal spinal cord and through prevention of sympathetic receptor activation of smooth bladder neck muscles and bladder wall which results in a relaxation of the bladder neck. Additionally, the somatic motorneurons of Onuf nucleus in the sacral spinal cord are inhibited. This occurs through projections from the PMC to grey commissures at S2 – S3 which results in activation of GABAergic inhibitory interneurons which reduce the activity of neurons of the Onuf nucleus and consequently relax the external urethral sphincter. 2.) through the PMC which sends excitatory signals to muscarinic
receptors of the detrusor by pelvic ganglia. This generates a detrusor contraction and micturition is enabled.

A LUT dysfunction can affect the storage and/or the micturition (voiding) phase. Storage dysfunction includes daytime frequency (individually, the normal frequency can be estimated upon seven times per day), nocturia (waking at night one or more times to void), and urgency (symptoms of a sudden compelling desire to pass urine that is difficult to defer) [8]. Reasons for an increased daytime frequency can be urinary tract infections, overactive bladder (OAB)/detrusor overactivity (involuntary detrusor contractions during filling, spontaneous or provoked)), impaired bladder compliance, excessive fluid intake, polyuria, increased bladder sensations, and urological/urogynaecological causes (i.e. bladder stones, prolapse) [8].

The LUT dysfunctions such as OAB are highly prevalent with 11.6% of the female and 9.7% of the male population affected [9]. The OAB has a high impact on quality of life including emotional, social, and hygienic factors which decrease the ability of OAB patients to live a normal life [10-11]. OAB is often caused by an underlying neurological disease such as multiple sclerosis, spinal cord injury, spina bifida, stroke, Parkinson’s disease etc. but in many patients no obvious pathology leading to OAB is found (i.e. idiopathic OAB). In healthy subjects, during the storage phase, the detrusor is inhibited from contraction and the sphincter muscles are inhibited from relaxation as described above. This negative feedback mechanism ensures that continence is obtained and no urine is lost. In humans, suprapontine lesions result in a removal of the inhibitory control from the cerebral cortex. Thus, the bladder capacity decreases and detrusor overactivity occurs [7, 12-13].

In OAB patients, two reasons for different brain responses are possible: 1.) the afferent signals from the bladder and 2.) the processing of the information in the brain are abnormal in these individuals [14]. Bladder and/or urethral afferents could be increased or decreased [14]. The view of increased activity in those afferents is not supported by other studies,
where neither the periaqueductal grey (PAG) as receiver of the afferents nor the insula where the sensations are mapped were seen over-activated in urgency-incontinent patients [12, 14-15]. Decreased activity of bladder and urethral afferents is compatible with studies which showed a decrease in insular activity with age [15]. This finding suggests that a reduced sensory feedback from the bladder in elderly may compromise bladder control and predispose to detrusor overactivity and urgency incontinence [14]. An abnormal brain response may either be interpreted that as the cause or the consequence of the problem. Considering that several brain diseases such as stroke and Parkinson’s disease are known to affect the bladder, abnormal supraspinal processing of LUT sensations is clearly involved in the pathomechanisms. Also vesical or urethral factors, like ischemia on the bladder, may also play a role and can vary individually [14].

1.2 Stimulations

Somatosensory evoked potentials (SSEPs) are the recording of electroencephalogram (EEG) signals after electrical stimulation of fast conducting sensory nerve fibres (like A-beta). To activate these nerve fibres, a peripheral nerve or a dermatome is stimulated repetitively (e.g. with a 3 Hz bipolar square wave stimulation). Through these nerve fibres, action potentials travel from the periphery through the spinal cord to the cortex. Cortical SSEPs are recorded above the corresponding area of the sensory cortex using surface or needle electrodes. This method is used in daily clinical practice to determine the nerve conduction velocity or the functionality of afferent nerve fibres. It enables to diagnose e.g. carpal tunnel syndrome or, together with further diagnostic tools, multiple sclerosis. In patients after a spinal cord injury, the severity of their disease can be estimated in an early state. The SSEPs give information about nerve fibres travelling in the dorsal column of the spinal cord. Reduced or abolished SSEPs are correlated with a dysfunction of the proprioception [16].
Another possibility to get EPs is the stimulation of certain dermatomes with heat. Contact heat stimulation evoked potentials (CHEPS) are known to recruit mainly A-delta fibres [17-18]. Unlike the SSEPs, CHEPS are conducted through the spinothalamic tract and cross the midline of the spinal cord at the level of their entrance. Pathological CHEPS are correlated with dysfunctional pain and temperature perception [19].

Electrical stimulation was also performed in the human lower urinary tract (LUT). Some studies applied an electrical current and determined the current perception threshold (CPT) of the urethra and bladder [20-29]. It was postulated that 2'000 Hz stimulates A-beta fibres, 250 Hz A-delta fibres and 5 Hz C-fibres, which was only shown in animal experiments and without a hundred per cent selectivity [30]. Although there is currently no evidence for the presence of A-beta fibres in the LUT, whereas A-delta and C fibres were demonstrated in the animal and in the human LUT [31-32], thresholds could be determined in all studies using all three frequencies.

In other studies, electrical stimulation of the LUT was applied to record EPs. Most of these studies applied the impulses repetitively with 2 Hz at about three times the sensory threshold (=CPT) and recorded the EPs above the midline of the sensory cortex (at Cz according to the 10-20 system. Unfortunately, those previous studies used very inconsistent measurement setups (i.e. bladder volume during stimulation, the stimulation strength, filter criteria) and investigated different subject populations which hamper a meaningful comparison and resulted in rather different EP shapes and lack of reliability.

Up to date, heat stimulation cannot be performed in the LUT as the existing thermodes are too large to be introduced into the bladder and the effect of the application of heat to the bladder mucosa is unknown.

In summary, recordings of cortical EPs after electrical stimulation of the human LUT are possible. Unfortunately, wave shapes differed from subject to subject and the reliability was
not determined. An improvement to consistent wave shapes could be done with other stimulation setups. The presence of A-delta and C fibres is proven, but there is no evidence for the presence of A-beta fibres. CHEPS can be used to activate selectively A-delta fibres from different dermatomes, whereas electrical stimulation on dermatomes activates mainly A-beta fibres. The question is if the A-delta fibres in the LUT can be elicited using an electrical impulse.

1.3 Aim of the thesis

The aim of this thesis was to get more information about the afferent nerve fibre activity measured by EPs after stimulation of sacral dermatomes with electrical and heat stimuli and of the LUT with electrical stimulation.

The specific issues addressed in this thesis are the following:

- Review of the present literature on cortical activity evoked in different LUT stimulations (chapter 2)
- Assessment of latencies and amplitudes as well as of the reliabilities of their cortical EPs after 1) heat stimulation of the S2, S3, and L5 dermatome and 2) electrical stimulation of the tibial nerve, pudendal nerve, and the S3 dermatome (chapter 3)
- Testing of the feasibility of a newly designed setup for electrical stimulation in the human LUT in young healthy female subjects, comparison of latencies and amplitudes and calculation of the reliability after stimulation of various localizations of the LUT (chapter 4)
- Translation of the results found in young healthy females to an older population investigating the feasibility and reliability of the newly developed setup also in healthy female subjects older than 35 years. Comparison of latencies and amplitudes of different LUT stimulation localizations and calculation of their reliabilities (chapter 5)
2. Neuroimaging of supraspinal human lower urinary tract control – a systematic review

2.1 Abstract

An intact supraspinal neuronal control of the lower urinary tract (LUT) is essential for daily life functions such as micturition and continent storage of urine. Impairment or loss of such control inevitably results in LUT dysfunction and consequently reduces the quality of life. The aims of this paper were to systematically review the literature on human neuroimaging studies of supraspinal LUT control, to identify correlations between the patterns of supraspinal activity and specific LUT-related tasks or stimulation conditions, and to highlight questions that still need to be addressed.

System-level understanding of the supraspinal network that controls micturition in humans requires the development of a measure of its anatomical architecture, functional connectivity, and causal interactions. New and emerging neuroimaging techniques have provided with minimally invasive, yet powerful tools to study the mechanisms of human supraspinal control of the LUT function. Despite the increasing amount of literature in this field, the relation between the supraspinal structures and their interactions is still poorly understood. Multi-modal neuroimaging studies along with sophisticated analytic strategies are needed to estimate the contextually induced effective connectivity, and will provide accurate activation patterns among the LUT-related brain regions.

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1 This manuscript will be submitted to Nature Reviews Urology. The authors are Flavia Gregorini, Matthias Walter, Jens Wöllner, Behnaz Jarrahi, Clare J. Fowler, Thomas M. Kessler and Ulrich Mehnert. Flavia Gregorini and Matthias Walter conducted the literature search and the analysis of the papers. The manuscript was written by Flavia Gregorini and Matthias Walter and revised by Ulrich Mehnert and the co-authors.
2.2 Introduction

In humans, the lower urinary tract (LUT) has two functions: (1) low pressure continent storage of urine and (2) periodical, self-determined, complete release of the stored urine. For a proper execution of those functions, the LUT structures (bladder, bladder neck, urethra and urethral sphincter) rely on an intact neuronal innervation that involves different neurons, nerves and fibre types from different levels of the spinal cord (Figure 2.1 a, b). In healthy individuals, this innervation and LUT reflexes from the spinal cord are under the control of a complex supraspinal network.

In the last two decades a rising number of neuroimaging studies were published investigating the human supraspinal network involved in LUT control. There are several neuroimaging techniques available to investigate functional supraspinal properties including functional magnetic resonance imaging (fMRI), positron emission tomography (PET), single photon emission computed tomography (SPECT), and near infrared spectroscopy (NIRS). Since increased neuronal activity at a particular brain region leads to a higher metabolic turnover and change in the hemodynamics of the brain, these neuroimaging techniques are capable of detecting specific cerebral areas that are activated in response to a particular stimulus or condition. For example, fMRI measures neuronal activity by detecting a blood oxygenation level dependent (BOLD) contrast signal, while PET and SPECT take advantage of changes of regional glucose consumption, and regional cerebral blood flow, respectively. In recent years, fMRI has been preferred to SPECT and PET due to its superior spatial resolution, its non-invasive nature, and lack of a need to administer radioactive isotopes. The purpose of this review was to summarize the methods and findings of all currently available neuroimaging studies investigating human LUT function to provide an overview of the role of different supraspinal areas in LUT control.
Figure 2.1 a, b: Schematic illustration of spinal cord and brain stem regions involved in lower urinary tract (LUT) control and their most relevant neuronal connection to the LUT. The illustration summarizes the findings of neurophysiological animal studies and early functional neuroimaging studies in humans from De Groat, Holstege and Blok:

During the storage phase (a), the detrusor is relaxed and the bladder neck closed due to a certain sympathetic tone on bladder body and bladder neck. Sympathetic fibres travel along the hypogastric nerve from the sympathetic nuclei in the intermediolateral column of the lumbar spinal cord to the LUT and provide adrenergic input to beta-receptors on intramural ganglia of the bladder body (→ relaxation) and alpha-receptors at the bladder neck (→ contraction/closure). Bladder afferents traverse through the pelvic nerve and enter the dorsal horn of the sacral spinal cord. At low filling volumes, there might be only little afferent activity and weak afferent signals might reach the PAG and diencephalic structures (e.g. thalamus), but bladder sensations do usually not reach consciousness during this state.

With increasing bladder volumes, afferent activity might increase probably due to changes in intravesical pressure and at some degree of filling, bladder sensations will reach consciousness in form of a first desire to void. From the sacral dorsal horn, excitatory collaterals reach to the sympathetic nuclei in the lumbar intermediolateral column and to the sacral frontal horn, where the motor neurons of the external urethral sphincter (EUS) are located (Onuf’s nucleus), to facilitate sympathetic input to the bladder and bladder neck, and somatic input to the EUS respectively.

This supports continence during increasing bladder volumes, when voiding has to be postponed. Another region supposed to be responsible for continence is the pontine L-region (named L-region as it is lateral to the other relevant pontine structure named pontine micturition centre or M-region or Barrington’s nucleus), which has excitatory input to the EUS motor neurons in Onuf’s nucleus and thus facilitates the elevation of the EUS tone. If the decision to empty the bladder is made (in the higher brain centres), the periaqueductal gray (PAG) activates the pontine micturition centre (PMC) (b).

The switch between L-region and PMC activation is sometimes model-like simplified as moving a lever from one program to the other. Only one region can be activated at a time. From the PMC strong inhibitory inputs reach the sympathetic nuclei in the intermediolateral lumbar cord to suppress the sympathetic input to bladder body and bladder neck to enable a synergic micturition. Simultaneously, the PMC has strong excitatory projections to the parasympathetic nuclei in the sacral spinal cord that in turn activate the detrusor via muscarinic receptors. In addition to the parasympathetic activation, the PMC has excitatory collaterals to inhibitory interneurons in the sacral cord that reduce the activity of EUS motor neurons and thus facilitate EUS relaxation and synergic micturition.
2.3 In general

Flow diagram of literature searches and results according to the PRISMA statement is shown in Fig.2. After the removal of duplicates as well as screening abstract and full-text articles, 48 eligible studies reporting on functional neuroimaging of LUT function in humans were identified for further qualitative analyses, i.e. 22 fMRI studies (46 %) [15, 33-53], 13 PET studies (27 %) [54-66], 10 SPECT studies (21 %) [13, 67-75], and 3 NIRS studies (6 %) [76-78] (Table 1). Twenty two studies (46 %) investigated healthy subjects only [15, 35-36, 39-43, 46-47, 51, 53-57, 63-64, 66, 68, 75-76], 18 studies (38 %) investigated patients only who had different underlying disorders: urinary urgency incontinence (UUI) (n= 7) [13, 44, 48-50, 58, 69], Parkinson’s disease (PD) (n= 5) [60-62, 71, 74], patients with stress urinary incontinence (SUI) (n= 1) [33], Fowler’s syndrome (n= 1) [37], temporal lobe epilepsy with ictal urgency (n= 1) [67], frontotemporal lobar degeneration (FTLD) (n= 1) [65], idiopathic normal-pressure hydrocephalus (iNPH) (n= 1) [72], spinal cord injury (SCI) patients (n= 1) [52], and eight studies (17 %) investigated both, healthy subjects and patients: overactive bladder (OAB) (n= 1) [38], multiple system atrophy (MSA) (n= 1) [73], detrusor overactivity (DO) (n= 2) [34, 78], UUI (n= 2) [51, 70], DO and UUI (n= 1) [15] and Fowler’s syndrome (n= 1) [59].

Overall, 973 subjects were enrolled have been studied under different bladder conditions: storage phase, micturition phase, pelvic floor muscle contraction, neurostimulation and neuromodulation as well as ice water test: 413 healthy (42 %) and 560 patients (58 %), 541 females (56 %), 405 males (41 %), and 27 (3 %) for whom the gender was not reported, Mean ages ranged from 18 to 98 years (Table 1).

The most important areas involved in supraspinal LUT control included the prefrontal and frontal gyrii, the limbic system, the insula, the anterior cingulate cortex (ACC), basal ganglia, the thalamus, the hypothalamus, the cerebellum, the pons, and the periaqueductal grey (PAG).
Table 2.1: Selection of included studies. S = storage, M = micturition, P = passive emptying of bladder, N = not specified, 1 = detrusor overactivity, 2 = ice water test, 3 = pelvic floor muscle contraction, 4 = sacral neuromodulation, 5 = pudendal nerve stimulation, 6 = deep brain stimulation, 7 = manual pressure, + = activation (statistically significant), - = deactivation (statistically significant), and (+) or (−) = trends only (statistically not significant).

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>Year</th>
<th>No. of Subjects</th>
<th>Gender</th>
<th>Age</th>
<th>Kind of Subjects</th>
<th>Bladder Condition</th>
<th>Mode of Bladder Filling</th>
<th>Additional Intervention</th>
<th>Findings</th>
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<td>Tedeschi et al.</td>
<td>MRI</td>
<td>2012</td>
<td>20</td>
<td>M/F</td>
<td>20</td>
<td>77/73</td>
<td>S</td>
<td>urethral catheter</td>
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<tr>
<td>Nasser et al.</td>
<td>fMRI</td>
<td>2016</td>
<td>10</td>
<td>M/F</td>
<td>40</td>
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<td>S, P</td>
<td>urethral catheter</td>
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<td>Griffiths et al.</td>
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<td>2015</td>
<td>3</td>
<td>M/F</td>
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<td>healthy subjects</td>
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<td>urethral catheter</td>
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<td>patients with urinary incontinence (UI)</td>
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<td>Liu et al.</td>
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<td>2016</td>
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2.4 Results and discussion

2.4.1 Storage phase

In 40 studies (83%, 21 fMRI, 13 PET, 4 SPECT, and 3 NIRS) 685 subjects (350 healthy, 335 patients, 392 females, 266 males, and 27 for whom the gender was not reported) were measured during the storage phase.

In almost 60% (23/40) of these studies, bladder filling was performed during functional neuroimaging using a transurethral catheter [15, 34-38, 42-44, 48-52, 54, 58, 60-64, 77-78], while in 43% (17/40) urodynamic parameters were recorded additionally [15, 34, 38, 44, 50-51, 58, 60-64, 70, 73-74, 77-78].

The storage phase takes more than 90% of the time. During this period, it is important that the detrusor is relaxed and that the internal and external sphincters are closed, so that the urine remains in the bladder. As the bladder volume increases during bladder filling, the PAG becomes activated by afferent input which originates inside the bladder wall, and runs through the pelvic nerves and the lateral dorsal horn of the sacral spinal cord to the brain [43]. Based on the information it receives about the bladder, the PAG decides which brain area should be activated at a specific time. The PAG represents a central relay station for the storage and micturition process. It is the place which activates the PMC when it is necessary to start the micturition, this decision is influences not only by the degree of the bladder wall distension but also by cognitive, social, and emotional factors provided by other brain regions associated with the limbic system and prefrontal cortex [57]. Therefore, activity in the prefrontal cortex was seen during the storage phase [35].

During bladder filling, also hypothalamus showed activity [15, 51, 56] to suppress the PAG in order to inhibit micturition. The hypothalamic activity decreased with increasing bladder volume [54].

After the transmission of the sensory information from the bladder wall via the spinothalamic tract to the midbrain PAG, the ACC, the insula, and the prefrontal cortex get the information
via the thalamus which is the major relay station for sensory afferents. The thalamus was thus activated during bladder filling [63].

The ACC is activated in parallel with the increasing bladder volume [34, 54] with an even exaggerated raise in activity when strong desire to void was reported. This observation can be justified in light on an increasing inhibitory influence to the PMC to attempt micturition [34].

The filling bladder can give a feeling of unpleasantness, emotional stress involved with urgency or fear of impending incontinence, activation can be seen in more emotional brain regions like the insula and the limbic system. As some of the studies were performed using a catheter to fill the bladder, the insula and the limbic system could also be activated by this uncomfortable feeling of the catheter. Activity in the insula could also be related to voluntary control of voiding and to suppress urgency [40, 51]. Activation of the limbic system during bladder filling was also explained to be due to the growing inhibitory activity of this system [53, 59].

Also the cerebellar activity was observed during the storage phase, there seems to be a subconscious cerebellar control of the pelvic floor muscle, bladder neck, and external sphincter. Towards desire to void, cerebellar activity was reported for FDV and increased urge, but not for normal desire to void.

When the bladder is full, the PMC in the pons controls the initiation of the micturition, i.e. a contraction of the detrusor muscle of the bladder, and simultaneous relaxation of the striated bladder sphincter (Figure 2.1). Another part of the pons, the L-region, is involved in the contraction of the external urethral sphincter and the pelvic floor muscles to inhibit involuntary urine loss [79]. The L-region was seen activated during FDV sensations [66] and in other studies with filed bladder condition [44, 51]. But there was also one study which did report no activation of the L-region.

In patients, some activation patterns differed from the ones in healthy subjects. In patients with a poor bladder control, no pontine activation was seen during detrusor overactivity [15,
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62], where normally the switch between the storage and the micturition phase takes place. The PAG and the insula were activated during DO in PD patients [62]. In women with OAB, urgency correlated with activation of the insula during low bladder filling, while control subjects had less activation [38]. This finding could be related to the fear of urine loss. Also the limbic system activation was enhanced in patients with UUI [58-59] and OAB [38] compared to healthy subjects. This activation likely represents abnormal processing of sensory input in brain regions associated with emotional response to discomfort [38]. In patients with UUI, the ACC showed an abnormally strong activation during bladder filling, either due to the fear of leakage as already postulated in the limbic system section [51] or as a result of the pelvic floor contractions in an attempt to decrease the urge to void (UV). This pattern of enhanced activation correlated to urgency in high bladder volume in patients with OAB [38]. It was also mentioned that UUI with DO had stronger activation (dACC) then those without DO, while both showed only little deactivation (pregenual ACC) [50]. On the other hand, older subjects had a diminished ACC response to bladder filling [35], most likely because of their decreased UV sensation and the ensuing lack of reaction [59]. This reduced activation of the ACC was also reported in elderly women with frontotemporal lobar degeneration with urinary incontinence [65]. The thalamus was reported to become activated in older continent older women [35], but showed deactivation during bladder filling in UUI patients [48]. As the thalamus is the relay station for afferents, deactivation could be a sign for decreasing incoming information about the bladder filling.

2.4.2 Micturition phase

In contrast to the storage phase, only 110 subjects (11%, 93 healthy, 17 patients, 60 females, and 50 males) were evaluated in 9 studies (19%, 3 fMRI, 3 PET, 2 SPECT, and 1 NIRS).
The micturition takes only a little part of the time and only takes place when not only the bladder is full, but also the environment is right. For the micturition, the detrusor has to contract and the urethral sphincters as well as the pelvic floor muscles have to relax (Figure 2.1). To initiate the micturition, a switch is performed in the PMC.

During the micturition, the PMC was activated and PAG activation was increased further compared to the storage phase, consistent with the observation of detrusor contraction and sphincter relaxation due to stimulation of efferent neuronal pathways under PMC control.

The PAG is a central relay station between afferent sensory information of the bladder, the supraspinal network that controls micturition, and the efferent pathway that controls the voiding reflex. Its main role is to activate the PMC premotor neurons when the bladder volume is increased to the micturition threshold. The effect is the detrusor contraction and the relaxation of the sphincters and the pelvic floor muscles.

Comparing micturition to withholding of urine has revealed higher PAG activity during micturition [55, 64], which may be responsible for the disinhibition of the PMC [64].

During micturition, insula activation was seen [64] or not [55-56], whereas the limbic system was never reported to be activated (Table 2.1). Activation of the prefrontal cortex was seen during micturition [57, 64, 68]. In the bladder control mechanism, the prefrontal cortex might play an important role in deciding whether or not to empty the bladder [57].

In addition it is possible that the ACC activity was undetectable during micturition in this study because of its involvement in motor control [55-56, 63-64]. This hypothesis is supported by the finding that ACC was either activated [46, 57] or showed at least a trend towards activation [40] during pelvic floor muscle contractions [33]. The activation of the ACC during micturition may have been due to the perception of bladder volume alteration rather than the process of micturition itself.
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The hypothalamus showed activation during micturition [64]. As it was shown to project directly to the PMC [34, 55], possibly for indicating to PMC when the setting was ideal for initiating the micturition. The thalamus was active during micturition in men [64], while women showed more thalamic activation during attempted micturition [39]. About cerebellar activation, controversial findings are present in literature. One study found cerebellar activity [64], whereas two others did not [56-57]. Activity was expected as it is an unconscious centre for coordination of motor movements, which could be the simultaneous relaxation of the sphincters and the pelvic floor and the contraction of the detrusor muscle.

2.4.3 Pelvic floor muscle contractions

PFMC were the object of interest in 8 studies (17%, fMRI n=7, PET n=1) [33, 40-41, 45-47, 53, 55] with 120 subjects (12%, 110 healthy, 10 patients, 56 male, and 64 female). They were compared to or within different conditions: rest or relaxed state, toe movement, abdominal straining, empty or filled bladder, healthy vs. SUI, and male vs. female.

PFMC enable healthy subjects to avoid urinary leakage while having a full bladder, either until the person is socially comfortable to void or when there is no possibility given to empty the bladder. It is also possible, yet difficult, to interrupt micturition by it.

PFMC are performed voluntary and include the external urethral and anal sphincter, which is controlled by the Onuf’s nucleus through the pudendal nerves [80] in contrast to other pelvic muscles, which are innervated by axons that are supplied by a medially placed motor nucleus at level S2-S4 [80]. Localization of PFMC at the motor cortex has not yet been specified, unlike other muscles, e.g. legs or arms, which have a more distinct area at the classic homunculus by Penfield and Rasmussen.

The overall picture of brain activities during PFMC is heterogeneous as were the outline algorythms of the different tasks.
Two studies [45, 55] compared brain activities of muscle contraction (abdominal or toe) with PFMC. There was a wide overlap of brain activation during PFMC versus toe movement, especially in the SMA, ACC, PFC, thalamus, basal ganglia, and cerebellum, assuming a close anatomic relationship with each other [45]. Only few differences were detected with the left PFC, insula and brainstem tended to be more active during PFMC, while toe movements resulted in stronger activation of precentral and central areas [45].

In contrast to this, there was less overlap comparing PFMC and abdominal straining, only primary motor cortex (M1), PFC, and thalamus activation. That still makes sense, since these brain areas are involved during voluntary muscle contractions [55].

Interestingly, M1 activation was lacking in other studies, but showed activity in the SMA [41, 53] and additionally in the SMC [46-47].

Overall, activation was seen during PFMC in the pons [41, 45, 47], PAG [47], insula [33, 41, 45], limbic system [41, 47], ACC [33, 45, 47, 55], basal ganglia [33, 45-47, 53], thalamus [45, 47, 55], cerebellum [33, 45-47, 53, 55], and PFC [33, 40-41, 45, 47], but never in the hypothalamus.

Activation of emotion related brain areas (insula, ACC and PFC) as well as the SMA and cerebellum, even as a trend inside the thalamus, PAG and pons, was related to an increasing urge to void in healthy young women [40]. These activations were reduced during PFMC, while urgency to void decreased [40].

Training with PFMC is very effective. After 3 months, a significant reduction of activity of the insula, PFC, and ACC was seen, while the SMC became more centred during active contractions in patients with SUI as a sign for neuroplastic changes [33]. Because SUI patients felt less emotional stress and were consecutively in a more convenient situation, this reduction is very plausible, since it was mentioned earlier in healthy young women [40].
During PMFC in full bladder condition, activation inside the cerebellum and the basal ganglia arise, which are meant to have inhibitory bladder control, while in empty bladder state non-significant activity was found in healthy volunteers [53]. When comparing the gender to PFMC and relax condition [46-47], similar patterns in both showed strong activations in the pons, PAG, cerebellum, thalamus, basal ganglia, SMC, SMA, PFC, insula and ACC. Seseke et al. indicated that the small differences possibly are a result of the different micturition habits with men having a higher bladder capacity and void less often, and the reason of stronger activity could due to the higher bladder outlet resistance [47].

2.4.4 Neurostimulation and neuromodulation

Altogether 78 subjects (8%, 16 healthy, 62 patients, 16 male, and 62 female) in 7 studies (15%, fMRI n=3, PET n=4) were evaluated with a variety of conditions (stimulation or modulation on vs. off, empty or filled bladder, healthy vs. SUI, and male vs. female) while using different kinds of neuromodulation, in detail: sacral neuromodulation (SNM) (n= 3) [37, 58-59], deep brain stimulation of the subthalamic nucleus (STN-DBS) (n= 2) [60-61], and dorsal clitoral (DCNS) or pudental nerve stimulation (PNS) (n= 2) [42, 52].

Neurostimulation and neuromodulation are often used synonymously, but there are important differences to bear in mind. The function of a neuron is affected directly with neurostimulation, i.e. it results in a direct effect at the target organ, e.g. contraction of the detrusor muscle using a sacral anterior root stimulator (SARS). With neuromodulation the function of the downstream neuron is affected by stimulation of a neuron, i.e. modulated. Therefore, a indirect effect at the target organ is established.

Even though the precise mechanism is not fully understood, these diverse techniques show promising results for patient with LUTD.
Herzog et al. showed, that DBS in stimulation on condition in patients with PD [60], lead to improvements of UDI parameters due to changes perception of urgency and desire to void, with increased bladder volume. These clinical findings were coherent to an increased activity inside the basal ganglia. This could be interpreted as a sign of restored working force of the basal ganglia. As mentioned earlier, given the hypothesis of inhibitory influence of the basal ganglia, consequently reduced ACC and PFC activity seemed to be the result of a better bladder control under running DBS, while ACC and PFC were both more activated during off-condition as a compensatory mechanism to restore continence with their inhibitory influence to the PMC [60]. In the following study, Herzog et al. [61] could again demonstrate improvements of UDI parameters compared to off-condition, while changed brain activity were demonstrated. This time PAG, insula and thalamus were significantly activated, which is coherent to findings during storage phase [54, 63], but surprisingly not the ACC.

Kavia et al. [37] evaluated the effect of SNM in patients with FS and outlined an increased bladder volume with SNM on vs. off with simultaneous activity inside the PAG, which as mentioned earlier is regarded as a central relay centre and can now initiate the micturition process via PMC. This mirrors as a relief to FS patients, who suffer from impaired voiding.

Dasgupta et al. [59] were also able to demonstrate that SNM re-establish an impaired voiding in FS patients. Compared to healthy subjects, who had midbrain activity in full bladder condition, FS patients only showed an activated ACC as a correlation to the stressful situation of not being able to empty the bladder. With SNM, this activity pattern changed and was similar to the healthy subjects showing midbrain activity, while ACC was extenuated.

Blok et al. [58] could even distinguish activity pattern for acute and chronic SNM in patients with UUI. This was the first time to demonstrate the central effect of SNM. Initially activation was seen during acute SNM areas for sensorimotor learning, bladder filling and sensation. These results are coherent to the previous group [59]. In chronic SNM less activation of these areas was present [58].
In patients with LUTD, PNS is used directly as an alternative to SNM or when SNM has failed. Zempleni et al. [52] evaluated the effect of acute versus short-term PNS in a small group of heterogeneous SCI patients. Bladder filling with acute PNS versus rest showed stronger activation inside the cerebellum, insula, and PFC, than without. These activations were even stronger in short-term PNS (after two weeks of stimulation) with corresponding improvement of UDI findings, e.g. bladder sensation, and voluntary voiding, indicating a neuromodulative effect within the supraspinal network regarding bladder control.

In healthy women similar effects of stronger activations in the cerebellum, insula and PFC were present during bladder filling with DCNS versus bladder filling alone. [42] DCNS affects the pudendal nerve and therefore, the results can be compared to the PNS data [52].

### 2.4.5 Ice water test

Only 2 studies (4%) with 23 healthy subjects only (<1%, 6 male, and 17 female) concentrated on bladder cooling using intravesical cold saline infusion (n= 2) [43, 63].

IWT is a diagnostic tool within the urodynamic investigation producing early involuntary contractions of the detrusor muscles in patients with DO.

It has been hypothesized, that recruitment of C fibres, which normally are inactive, leading to uncomfortable sensations up to pain, with can be triggered by ice water, is the pathophysiological reason of bladder overactivity [81].

Mehnert et al. [43], using fMRI, demonstrated only deactivation partly on the left side of the PFC (i.e. BA 10) and the cerebellum bilaterally during infusion. When the bladder was drained, the insula and the right posterior part of the cerebellum were activated. Interestingly, PAG, pons, and thalamus showed significant activation in 75% (9/12), while the ACC only in 50% (6/12) in the single subject analysis but none of them in group analysis.
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In a PET study by Matsuura et al. [63], who directly compared the bladder infusion with cold to body warm water (“bladder distension”), showed no overlap of activation between both groups. While bladder infusion showed active brain areas similar to those findings described in the storage phase, ice water instillation showed strong activity in areas more involved in processing of emotion, e.g. ACC, PFC, basal ganglia.

The results of both studies reflect a different pattern of activation and deactivation when cold water is compared to body warm water to fill the bladder. This could emphasize the fact that different neuronal pathways (fibre quality) must be stimulated.

2.5 Conclusion

Recent advances in neuroimaging techniques have provided us with minimally invasive methods to investigate supraspinal activation patterns in relation to human LUT function. Neuroimaging studies from the last two decades have consistently pointed to a complex supraspinal network that controls LUT function. These studies tremendously increased our understanding of how the human LUT function is coordinated and how it can be affected by neurological disorders but also by general conditions such as aging. Neuroimaging studies of LUT function are highly valuable for the evaluation of therapies for LUT dysfunction, especially when it comes to the still incompletely understood mechanisms of action of neuromodulatory therapies.

Currently the prefrontal and frontal cortices, limbic system, insula, ACC, BG, thalamus, hypothalamus, cerebellum, pons, and PAG have been indicated as the most prominent supraspinal areas involved in the processing of the LUT signals and regulation of urinary continence and micturition. However, only a few neuroimaging studies have attempted to examine the structural connectivity of this network with some inference of their interactions. Understanding a complex supraspinal network requires the development of a measure of its anatomical architecture, functional connectivity, and causal interactions. With current
techniques, we are still not able to distinguish the inhibitory or excitatory nature of supraspinal activation and deactivation patterns. Another current drawback is the very heterogeneous body of study designs and protocols used impeding meaningful comparisons.

Nevertheless, based on the previous work, we now have an excellent basis to continue exploring. The current experience with the neuroimaging techniques in relation to LUT investigation is consistently growing and future combination of methods (i.e. multi-modal neuroimaging studies such as EEG-fMRI) or implementation of additional measurement and analysis methods (e.g. functional connectivity analysis) will enhance accuracy and information content of neuroimaging measurements by enabling us to better understand connectivity and distinguish between the directionality of supraspinal area interactions.
3. Tibial, pudendal and sacral evoked potentials after electrical and contact heat stimulation

3.1 Abstract

Objective: To evaluate the test-retest reliability and compare latencies of 1) contact heat evoked potential stimulation (CHEPS) of the L5, S5, and S3 dermatomes and 2) somatosensory evoked potentials (SSEPs) of the tibial nerve, pudendal nerve, and S3 dermatome.

Methods: Healthy volunteers (n=10) were investigated using 3Hz electrical and 52°C heat stimulation at the above mentioned localizations. All evoked potentials (EPs) were recorded at Cz. All subjects were investigated three times separated by four weeks.

Results: Mean N2 latencies using CHEPS were 331.7 ± 45.1ms, 293.0 ± 51.0ms, and 309.5 ± 28.0ms at L5, S5, and S3, respectively. Mean latencies of SSEPs were 39.1 ± 1.8ms, 39.7 ± 3.5ms, and 38.4 ± 4.1ms at the tibial nerve, pudendal nerve, and S3 dermatome, respectively.

Conclusions: Both methods provide high EP return rates (80-97%) with good reliabilities for SSEPs and fair reliabilities for CHEPs. Latency differences between CHEPS and SSEP are potentially related to the stimulated fibre type (A-delta vs A-beta).

Significance: The results obtained provide the basis for a multimodal neurophysiological workup of the segmental innervation relevant for pelvic organ function. They warrant further investigation of pelvic and sacral nerve function and relative changes within different involved nerve fibre populations in relation to pelvic organ dysfunction.

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2 This manuscript will be submitted to PlosOne. Flavia Gregorini conducted the study together with Jens Wöllner. She scheduled the measurements, did the data analysis, and the statistics. The manuscript was written by Flavia Gregorini and Jens Wöllner.
3.2 Introduction

Visual, auditory, or somatosensory stimuli are known to elicit evoked potentials (EPs) which can be recorded with the electroencephalogram (EEG). Latency and amplitude of the averaged EPs are known as objective and reliable markers for the nerve fibre integrity and function and are therefore often used in clinical practice and for longitudinal clinical studies in neuropathological conditions (i.e. spinal cord injury) [82-84]. Due to the non-invasive character and easy application, this method is widely used and accepted. It can be focused on specific anatomic body regions and their particular function and physiology e.g. fibre composition.

By introduction of a variety of stimulation modalities, e.g. by using contact heat evoked potential stimulation (CHEPS) and somatosensory evoked potentials (SSEPs) of peripheral major nerves; it is assumed to allow for a differentiation between different fibre types. CHEPS preferentially stimulate A-delta fibres [17-18], whereas electrical stimulation activates A-beta fibres [85]. Unfortunately, there are little to no comparative data available of CHEPS and SSEPs from sacral/pelvic sites. However, neurophysiological assessment of sacral/pelvic sites using CHEPS and SSEPs has relevance for future investigations of pelvic organ (dys-)functions of bowel, sexual and lower urinary tract. Prior to studies in patients, knowledge of the normal variability and reliability of the EPs from sacral/pelvic sites using CHEPS and SSEPs in healthy subjects is mandatory to identify relevant changes in neuropathological conditions and to assess their validity as a clinical tool.

Thus, the aims of this study were 1) to evaluate the test-retest reliability of latencies from CHEPS at L5, S2, and S3 dermatomes and of SSEPs from stimulation at the tibial and pudendal nerve and S3 dermatome and 2) to compare these latencies from CHEP and SSEP stimulation to better understand the nerve fibre physiology of these body regions.
Tibial, pudendal and sacral evoked potentials after electrical and contact heat stimulation

Our hypothesis is that CHEPS and SSEPs can be reliably recorded from all sites. Due to the slower conduction velocity of A-delta fibres compared to A-beta fibres, CHEPS are supposed to show longer latencies.

3.3 Methods

3.3.1 Subjects

A group of volunteers was recruited. Prior to inclusion, all subjects provided written informed consent. Inclusion criteria were healthy female subjects and age of at least 18 years. Exclusion criteria were any neurological or urological diseases, pregnancy or lactation, and any regular medication intake (except contraceptive medication). The study was approved by the local Ethics Committee (Kantonale Ethikkommission Zürich, KEK Nr. 25/2009) and was conducted in accordance with the Declaration of Helsinki. Clinical registration number is NCT01389921 (www.clinicaltrials.gov).

3.3.2 Experimental setting

All measurements were repeated three times with an interval of approximately four weeks. During each measurement, subjects were investigated in a comfortable supine position at stable room temperature of 22°C by the same investigators (FG, JW). All stimulations were applied on the right side of the body, whereas stimulation type (i.e. heat and electrical) and localization (i.e. L5 (= foot), S2 (= pubic region), S3 (= buttocks)) were applied in a randomized order.

3.3.3 CHEPS

CHEPS was applied at the L5, S2, and S3 dermatome using a contact heat stimulator (Medoc, Ramat Yishai, Israel). For the L5 stimulation, the thermode was placed on the dorsum of the foot. The S3 dermatome was stimulated placing the thermode on the buttocks. For CHEPS at the S2 dermatome, the thermode was placed on the pubic region about 3 – 4 cm above the clitoris. Sensory threshold was defined as the first sensed stimulus when
increasing the temperature. Pain threshold was defined as the first perceived painful stimulus when increasing the temperature. Both thresholds were determined twice and then averaged. Twenty stimuli of 52 °C were applied at each dermatome to elicit an evoked potential. They were applied every 8 – 10 s (random delay) at different places within the same dermatome [86]. All CHEPS measurements were performed with eyes open. Blinks were allowed after an auditory signal (i.e. beep) that followed about 4 s after each stimulus. Baseline temperature, heating rate, and cooling down were previously described [86]. EEG segments from 100 ms pre- to 1000 ms post-stimuli were extracted for analysis. Only artefact free-segments were analysed and averaged. Based on international convention, the first prominent negative peak was labelled as N2 and the first prominent positive peak as P2. Latencies of the N2 and P2 peak were evaluated semi-automatically using Brain Vision Analyzer2 (BrainProducts, Munich, Germany).

3.3.4 SSEPs

SSEPs were evoked by electrical stimulation at the tibial and pudendal nerve and at the S3 dermatome. For the tibial nerve stimulation, the electrodes were attached behind the ankle, separated by 1 cm. For pudendal stimulation, the cathode was placed on the clitoris, the anode at the inside of the right labia. For the S3 stimulation, two electrodes were placed on the middle of the buttocks, separated by 1 cm. SSEPs were evoked at 3 Hz and 0.2 ms pulse width as previously described [87].

The stimuli were applied with the Dantec Keypoint® 4m (Neurolite AG, Belp, Switzerland) while subjects had their eyes closed.

The sensory threshold was determined by the staircase method as previously described [88]. In order to elicit SSEPs, 400 stimuli were applied with at least two times sensory threshold current for pudendal nerve and S3 dermatome stimulation and with motor response for tibial nerve stimulation. The EEG recordings were segmented from 50 ms pre- to 200 ms post-
Tibial, pudendal and sacral evoked potentials after electrical and contact heat stimulation stimulus. Artefact-free segments were averaged and the P40 latency was evaluated semi-automatically using BrainVision Analyzer2 (BrainProducts, Munich, Germany).

### 3.3.5 EEG

The EEG was recorded continuously at 5000 Hz for SSEPs and at 500 Hz for CHEPS. 62 recording electrodes were used. Evoked potentials were determined at the Cz as active electrode. Reference for SSEP was the Fz electrode [82-84] and for CHEPS the average of all electrodes [86, 89]. The ground electrode was set at AFz position for both measurements. All data were filtered offline (SSEP from 0 - 70 Hz, CHEPS from 0.5 - 30 Hz plus 50 Hz Notch filter for both stimulations). Impedance of electrodes was continuously monitored and kept below 20 kΩ for all electrodes.

Recordings were performed using BrainVision Recorder (BrainProducts, Munich, Germany). Analysis of EEG data was performed using BrainVision Analyzer2 software (BrainProducts, Munich, Germany).

### 3.3.6 Statistics

Statistical analysis was performed using IBM® SPSS® Statistics 19.0 for Windows (IBM, Chicago, Illinois, U.S.A.). Intraclass correlation (ICC) was used to evaluate re-test reliability of latencies and amplitudes. ICCs were categorized as follows: >0.75 excellent reliability, 0.6 – 0.75 good reliability, 0.4 – 0.59 fair reliability and <0.4 poor reliability [90].

Bland and Altman plots are presented to visualize the reliability of the measurements whereas the mean and the 1.96 times standard deviation of the differences was calculated to set the limits of agreement as previously described [91]. Significant differences between localizations were analysed with paired Student’s t-tests. The alpha level used as a significance criterion for the statistical tests was 0.05. For comparison of latencies between CHEPS and SSEPs, the coefficient of variation (CV) was calculated.
3.4 Results

3.4.1 Subjects

Ten healthy female volunteers with a mean age of 23 ± 4 years, a mean body height of 168 ± 6 cm, and a mean body weight of 57 ± 5 kg were included. Both stimulation techniques were well tolerated at all three localizations. No adverse events occurred or have been reported.

3.4.2 Thresholds

Mean perception threshold for heat stimulation was 40.3 ± 2.3 °C, 39.7 ± 2.4 °C and 40.8 ± 1.9 °C at the L5, S2 and S3 dermatome, respectively. Mean pain threshold for heat stimulation was 49.5 ± 2.4 °C, 49.0 ± 3.1 °C and 50.3 ± 2.6 °C at the L5, S2 and S3 dermatome, respectively (Table 3.1). No significant differences in perception or pain threshold were detected between the three dermatomes.

Mean perception threshold for electrical stimulation was 3.2 ± 1.3 mA, 3.6 ± 1.3 mA and 2.1 ± 0.6 mA at the tibial nerve, pudendal nerve and S3 dermatome, respectively (Table 3.2). S3 dermatome perception thresholds were significantly lower than tibial and pudendal nerve thresholds.

3.4.3 Latencies

Mean latencies following heat stimulation at L5 were 331.7 ± 45.1 ms for N2 and 440.7 ± 44.6 ms for P2; at S2 293.0 ± 51.0 ms for N2 and 398.6 ± 47.6 ms for P2, and at S3 309.5 ± 28.0 ms for N2 and 428.5 ± 80.1 ms for P2 (Table 1, Figure 3.4). N2 latencies from L5 were significantly longer than from S3 (p = 0.013) and S2 (p < 0.001). P2 latencies from L5 were significantly longer than from S2 (p < 0.001) (Figure 3.1).
The mean coefficient of variation (CV) was 0.14, 0.18, and 0.09 for CHEP latencies from L5, S2, and S3 respectively (Tables 3.1 and 3.2).

There was no correlation between the N2 latencies and the subjects' heights ($R^2 < 0.001$) in all localizations.
Table 3.1: Mean perception and pain thresholds, N2 and P2 latencies ± standard deviation (SD) for all three measurements of contact heat evoked potential stimulation (CHEPS) at L5, S2, and S3 dermatomes. Overall means and intraclass correlation (ICC) values of perception and pain thresholds, N2 and P2 latencies are provided for each stimulation site. The right column shows the coefficient of variation (CV) of the N2 and P2 latencies for all three measurements.

<table>
<thead>
<tr>
<th>Dermatome</th>
<th>Measurement</th>
<th>Perception threshold [°C] (mean ± SD)</th>
<th>Pain threshold [°C] (mean ± SD)</th>
<th>N2 latency [ms] (mean ± SD)</th>
<th>P2 latency [ms] (mean ± SD)</th>
<th>CV of latencies</th>
<th>N2</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>L5</td>
<td>1</td>
<td>40.1 ± 2.7</td>
<td>49.2 ± 3.1</td>
<td>331.6 ± 47.6</td>
<td>436.6 ± 53.6</td>
<td>0.14</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>39.9 ± 1.3</td>
<td>49.1 ± 2.0</td>
<td>336.6 ± 52.7</td>
<td>447.0 ± 49.6</td>
<td>0.18</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (Overall)</td>
<td>41.0 ± 2.6</td>
<td>50.2 ± 2.1</td>
<td>326.4 ± 37.4</td>
<td>438.2 ± 37.4</td>
<td>0.11</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>ICC</td>
<td></td>
<td>0.26</td>
<td>0.24</td>
<td>0.55</td>
<td>0.52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>1</td>
<td>39.6 ± 3.1</td>
<td>48.1 ± 2.8</td>
<td>287.6 ± 57.3</td>
<td>338.6 ± 66.6</td>
<td>0.20</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>40.0 ± 2.1</td>
<td>48.9 ± 3.5</td>
<td>293.1 ± 57.6</td>
<td>408.9 ± 30.2</td>
<td>0.20</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (Overall)</td>
<td>39.5 ± 2.2</td>
<td>49.9 ± 2.9</td>
<td>298.4 ± 42.4</td>
<td>399.2 ± 39.9</td>
<td>0.14</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>ICC</td>
<td></td>
<td>0.46</td>
<td>0.72</td>
<td>0.51</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td>1</td>
<td>39.9 ± 1.6</td>
<td>49.4 ± 3.1</td>
<td>310.6 ± 41.0</td>
<td>417.7 ± 33.8</td>
<td>0.13</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>41.1 ± 1.6</td>
<td>50.4 ± 2.3</td>
<td>307.1 ± 30.2</td>
<td>418.7 ± 60.0</td>
<td>0.10</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (Overall)</td>
<td>41.4 ± 1.9</td>
<td>51.1 ± 2.4</td>
<td>311.3 ± 8.6</td>
<td>449.1 ± 123.8</td>
<td>0.03</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>ICC</td>
<td></td>
<td>0.15</td>
<td>0.50</td>
<td>0.48</td>
<td>0.57</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean latencies following the electrical stimulation were 39.1 ± 1.8 ms for the tibial nerve, 39.7 ± 3.5 ms for the pudendal nerve and 38.4 ± 4.1 ms for S3 dermatome (Table 3.2). There were no significant differences of SSEP latencies between the stimulation localizations (Figure 3.2). The mean CV was 0.05, 0.09, and 0.11 for SSEP latencies from the tibial nerve, pudendal nerve, and S3 dermatome, respectively (Tables 3.1 and 3.2).
Tibial, pudendal and sacral evoked potentials after electrical and contact heat stimulation

Figure 3.2: Box plots of the latencies of the cortical evoked potentials after electrical stimulation of the tibial nerve, pudendal nerve, and S3 dermatome.

There was no correlation between the SSEP latencies and the subjects' heights ($R^2 < 0.001$ in all localizations).
Tibial, pudendal and sacral evoked potentials after electrical and contact heat stimulation

Table 3.2: Mean perception thresholds, current intensities, and P40 latencies ± standard deviation (SD) for all three measurements of somatosensory evoked potentials (SSEPs) at the tibial nerve, pudendal nerve, and S3 dermatome. Overall means and intraclass correlation (ICC) values of perception thresholds, current intensities, and P40 latencies are provided for each stimulation site. The right column shows the coefficient of variation (CV) of the P40 latencies for all three measurements.

<table>
<thead>
<tr>
<th>Nerve/Dermatome</th>
<th>Measurement</th>
<th>Perception threshold [mA]</th>
<th>Current intensity [mA]</th>
<th>P40 latency [ms]</th>
<th>CV of P40 latencies</th>
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</thead>
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<td></td>
<td></td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
<td></td>
</tr>
<tr>
<td>tibial</td>
<td>1</td>
<td>2.66 ± 1.06</td>
<td>8.0 ± 1.4</td>
<td>39.03 ± 1.78</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.67 ± 1.45</td>
<td>8.8 ± 2.4</td>
<td>38.93 ± 1.91</td>
<td>0.05</td>
</tr>
<tr>
<td>Overall mean</td>
<td>3</td>
<td>3.22 ± 1.38</td>
<td>8.4 ± 2.7</td>
<td>39.09 ± 2.01</td>
<td>0.05</td>
</tr>
<tr>
<td>ICC</td>
<td></td>
<td>-0.15</td>
<td>-0.18</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>pudendal</td>
<td>1</td>
<td>3.61 ± 1.62</td>
<td>9.2 ± 3.3</td>
<td>39.85 ± 3.66</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.49 ± 1.13</td>
<td>8.7 ± 3.4</td>
<td>38.30 ± 4.24</td>
<td>0.11</td>
</tr>
<tr>
<td>Overall mean</td>
<td>3</td>
<td>3.78 ± 1.08</td>
<td>8.8 ± 1.6</td>
<td>40.80 ± 2.57</td>
<td>0.06</td>
</tr>
<tr>
<td>ICC</td>
<td></td>
<td>0.53</td>
<td>0.23</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td>1</td>
<td>2.02 ± 0.74</td>
<td>10.9 ± 3.2</td>
<td>37.70 ± 4.54</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.13 ± 0.59</td>
<td>9.6 ± 2.6</td>
<td>39.04 ± 4.34</td>
<td>0.11</td>
</tr>
<tr>
<td>Overall mean</td>
<td>3</td>
<td>2.08 ± 0.55</td>
<td>10.9 ± 3.2</td>
<td>38.40 ± 3.73</td>
<td>0.10</td>
</tr>
<tr>
<td>ICC</td>
<td></td>
<td>0.59</td>
<td>0.08</td>
<td>0.85</td>
<td></td>
</tr>
</tbody>
</table>

3.4.4 Reliability

The ICC values for heat perception thresholds were 0.26, 0.46, and 0.15 at the L5, S2, and S3 dermatome, respectively. The ICC values for heat pain thresholds were 0.24, 0.72, and 0.50 at the L5, S2, and S3 dermatome, respectively (Table 3.1).

Following heat stimulation at L5 and S2, 29 out of 30 (97%) measurements resulted in evaluable EPs and latencies. Following heat stimulation at the S3 dermatome, 24 out of 30 (80%) measurements resulted in evaluable EPs and latencies.
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Latencies of CHEPS at L5, S2 and S3 showed ICC values for N2 and P2 of 0.55, 0.51, 0.48 and 0.52, 0.25 and 0.57, respectively (Table 3.1). The Bland and Altman plots illustrate that two values are outside the limits of agreement for all three stimulation sites and that the limits of agreement were narrower in all N2 peaks compared to the P2 peaks (Figure 3.3). For the N2 peak, CHEPS from S3 showed the narrowest limits of agreement and from S2 the widest one. For the P2 peak, CHEPS from L5 had the narrowest limits of agreement and from S3 rather wide limits of agreement.

Mean electrical perception thresholds resulted in ICC values of -0.15, 0.53, and 0.59 at tibial nerve, pudendal nerve and S3 dermatome, respectively (table 3.2).

Following tibial nerve and the S3 dermatome stimulation, 28 out of 30 (93%) measurements resulted in evaluable EPs and latencies. Following pudendal nerve stimulation, 24 out of 30 (80%) measurements resulted in evaluable EPs and latencies.

Tibial, pudendal and S3 electrical stimulation showed EP latency ICC values of 0.73, 0.51 and 0.85, respectively (Table 3.2). In general, the limits of agreement were narrow (Figure 3.3).
Tibial, pudendal and sacral evoked potentials after electrical and contact heat stimulation

Figure 3.3: Bland and Altman plots for the latencies after heat stimulation (rows A and B) and electrical stimulation (row C). The left column shows the Bland and Altman plots after tibial nerve (electrical) and L5 dermatome (heat) stimulation, the middle column the pudendal nerve (electrical) and S2 dermatome (heat) stimulation and the right column the S3 dermatome (electrical and heat) stimulation. The solid line shows the mean of all differences, the dashed line 1.96 times standard deviation for the limits of agreement. Note the different scale of the y-axis on the P2 EP of the S3 dermatome.
Figure 3.4: Averages of the cortical evoked potentials at the Cz electrode after heat (upper row) and electrical stimulation (lower row). First column: Grand averages with tibial nerve/ L5 dermatome in black, pudendal nerve/ S2 dermatome in grey and S3 dermatome stimulation as dashed line. Averages from a representative single subject of the first, second and third measurement after tibial nerve/ L5 dermatome stimulation, pudendal nerve/ S2 dermatome stimulation, and S3 dermatome stimulation are displayed in the second, third and fourth column, respectively.
3.5 Discussion

In the present study, we demonstrated the following new findings: 1) Latency values for CHEPS from the L5, S2, and S3 dermatome, and for SSEPs from the S3 dermatome 2) Good to excellent reliability of latencies from SSEPs at the tibial nerve and the S3 dermatome, fair reliabilities of latencies from CHEPS at all three localizations and from SSEPs at the pudendal nerve. 3) When stimulated at the same locations, CHEPS revealed significantly longer latencies than SSEP presumably due to different fibre properties of A-delta and A-beta fibres primarily stimulated by CHEPS and SSEPs, respectively.

3.5.1 CHEPS

CHEPS is the application of heat stimuli with a thermode at a certain dermatome [86, 89]. This type of heat stimulation primarily activates A-delta fibres [17-18] and is processed in the spinothalamic tract of the spinal cord [86]. According to the known conduction velocity of this fibre type, N2 latencies were expected around 300 - 400 ms [86, 89].

However, N2 latencies from L5 were significantly longer compared to S2 and S3. Longer latencies from the foot could be expected due to longer distance to the cortex (Figure 3.1). In contrast, nerve fibres from the S2 and from the S3 dermatome enter the spinal cord close to each other, which explains the similar latencies of those two dermatomes.

Regarding CHEPS latencies from the lower extremity, Wydenkeller et al. observed P2 latencies of 596.6 ms following L4 stimulation at the ankle which is longer than the L5 latencies observed in this present study (441 ms) (Table 3.1) [86]. As a possible explanation we consider different stimulation conditions (baseline, slope, stimulation temperature) that changes the duration of stimulation and consequently results in different latencies. In contrast to our constant stimulation temperature of 52°C, Wydenkeller et al. stimulated with pain thresholds of about 48-56°C. Another study also reported longer latencies (N2 = 440.5 ±
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23.7 ms and the P2 peak = 569.1 ± 23.3 ms) after L5 stimulation [17]. However, comparison remains difficult as stimulation parameters were different in the study by Chen et al.[17], who used a lower baseline stimulation temperature (32°C).

Because there are no studies providing CHEPS data from L5, S2, and S3, other dermatomes have to serve as references. According to a similar distance of certain cervical and sacral dermatomes to the cortex, sacral latencies are comparable to the ones generated from the C6, C7, and C8 dermatomes at the dorsum of the hand. However, data from the present study reveal shorter latencies for the S2 dermatome (N2: 293 ms, P2: 398 ms) and for the S3 dermatome (N2: 310 ms, P2: 429 ms) than demonstrated for the C6 (N2: 409.2 ms, P2: 541.0 ms) [92] C7 dermatome (N2: 375 ms, P2: 517 ms) [17], and C8 (N2: 414.1 ms, P2: 544.2 ms) [92]. A potential explanation for this finding is the different ratio of the peripheral to central distances from S2 / S3 as compared to C6-8. For cervical dermatomes, the peripheral part is longer than the central part, whereas for the sacral dermatomes it is vice versa with a short peripheral part and a long part in the central nervous system. This indicates slower conduction velocity in the periphery, further contributing to the observation of longer latencies for L5 CHEPS as compared to S2/3 CHEPS in the present data (see above).

With 97%, 97%, and 80% of heat stimulations resulting in evaluable EPs for L5, S2, and S3 respectively, return rate was high in this experiment compared to the study by Chen et al. reporting rates of 36.8% - 62.5% [17]. However, the P2 latency reliability of CHEPS was only fair to poor (Figure 3.3). A reason for that might be the difficult marker placement in CHEPS as the configuration of the potentials made it frequently difficult to determine P2.

Intervals between repeated measurements were four weeks and although measurements were performed following a standardized procedure in the same setting at the same day time, differences in attention, stimulus tolerance, and variations of local aspects at the stimulation site, (i.e. skin condition, sweating) potentially contribute to N2 and P2 variability.
This variability may also be reflected by the low reliability of the perception and pain thresholds (Table 3.1).

Compared to the current literature, the reliability of our N2 and P2 CHEP latencies is lower. A recent study by Kramer et al. presented ICCs of 0.57 to 0.73 for N2 latencies and between 0.47 and 0.92 for P2 latencies of cervical dermatomes [92]. A potential reason for this discrepancy might lie in the fact that the A-delta receptor density decreases with distance to the brain [93], hence the evocation of potentials is expected to be more difficult from sacral than from cervical dermatomes.

Pain and perception thresholds from sacral dermatomes using CHEPS have not been reported previously. For the foot a mean pain threshold of 48.9° C has been reported by Wydenkeller et al. [86], which was similar to pain threshold at L5 in the present study (49.6° C).

3.5.2 SSEPs

SSEP following electrical stimulation of the tibial nerve is an established method, which allows detecting functional deficits within the dorsal columns of the spinal cord or within peripheral nerves. Electrical stimulations activate A-delta fibres and the evoked signals travel through the dorsal column of the spinal cord [85]. Latencies of SSEPs seem to be more reliable than amplitudes, as amplitudes also depend on the stimulation technique and strength [83, 94].

The latencies of the tibial SSEPs recorded in our current study correspond well with values previously reported in literature [83, 85, 95-96]. The observation that there was no correlation with body size may deem unexpected but is likely owed to low size variability of included subjects.

Our latency values from the pudendal SSEPs were in the range (39-41 ms) of what has been previously reported [97-99].
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SSEPs from both, tibial and pudendal nerve, and also the S3 dermatome resulted in similar latencies (table 3.2) which correspond well with the results of studies by Fitzpatrick et al. and Choi et al. [100-101]. Although the peripheral pathway is shorter for pudendal nerve and S3 dermatome compared to tibial nerve SSEPs, the central conduction time of the pudendal SSEP has been demonstrated to be 9-10ms longer than for the tibial SSEP, potentially due to a different conducting pathway with more synaptic interconnections or a different fibre population [100]. This central conduction delay is assumed to be responsible for the observation of comparable SSEP latencies following pudendal and tibial SSEPs. The same explanation probably applies for S3 dermatome SSEPs.

Regarding the reliability, ICC values of the SSEP latencies showed fair to excellent values for tibial (ICC = 0.73), pudendal nerve (ICC = 0.51), and S3 dermatome (ICC = 0.85) stimulation (table 2).

3.5.3 Physiology and pathophysiology

Electrical stimulation predominantly elicits A-beta fibres which transmit faster than the thinly myelinated A-delta fibres stimulated by CHEPS, resulting in the significantly shorter SSEP latencies compared to the longer CHEP latencies [85-86].

Another reason for the different findings might be the character of the stimulation. For SSEPs an exact electrical stimulation that lasts for only 0.2 ms has been applied, whereas CHEPS result from an increasing heat stimulus that reaches its maximum after 242 ms [102]. Thus, the stimulus characteristics will likely be responsible for the result of less synchronized responses and a relevant extra delay due to an onset delay of the pain stimulus.

For SSEPs, the primary cortical complex is clinically used (for tibial SSEP, it is at about 40 ms after the stimulus), whereas the secondary complex is not used. It is developed during the processing of the afferent information in the association cortex [103]. For CHEPS, it is
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unclear, if a primary cortical complex exists. Because of its characteristics (habituation, distractibility etc.), it is likely that the N2/P2 responses observed after heat stimulation is a secondary complex, which might explain the lower reliability compared to SSEPs. Although the reliability of CHEP latencies was lower than of SSEP latencies, the CVs for latencies from S3 are similar with both techniques, whereas CHEP latencies of the L5 and S2 dermatome are less precise than SSEP latencies (Tables 3.1 and 3.2).

In conclusion, data of CHEPS at the S3 and S2 dermatome as well as electrical stimulation of the S3 dermatome were compared. As expected from the different sensory systems presumably stimulated by CHEPS and SSEPs, CHEPS latencies were significantly longer as compared to SSEPs. Direct comparison of the two methods is difficult due to different stimulation setups. CHEPS and SSEPs can reliably be recorded from L5, S2 and S3 dermatomes, tibial and pudenal nerves, and S3 dermatome, whereas CHEPS revealed a lower reliability than SSEP. As the CVs of the latencies are similar with both techniques, they can be used as markers for the nerve fibre integrity. These data are relevant for further investigations of pelvic and sacral nerve fibres in relation to pelvic organ function. Further investigations in patients with LUT dysfunction are needed.

A potential limitation of the study is the subject sample size, which does not allow conclusions for the general population. However, reliability was calculated using three measurements, each separated by four weeks. Critical appraisal of methodological stimulus effects like the long duration from baseline to the maximal temperature in CHEPS compared to the electrical stimulation is mandatory.
4 Sensory evoked potentials of the human lower urinary tract

4.1 Abstract

**Purpose:** To investigate feasibility and reliability of recording sensory evoked potentials (SEPs) following lower urinary tract (LUT) electrical stimulation. SEPs might reveal improved insights in afferent processing within specific locations of the human LUT.

**Subjects and Methods:** Electrical stimulation with 0.5Hz and 3Hz was applied to the bladder dome, trigone, proximal, and distal urethra using a transurethral catheter. SEPs were recorded cortical at the Cz electrode, referenced to the Fz electrode. All measurements were repeated three times for reliability assessment using intraclass correlation coefficients (ICCs).

**Results:** Ten healthy female subjects with mean heights of 168±6cm and mean age of 23±4 years were included. The most prominent and consistent SEPs landmark across different locations was the first negativity peak (N1). Following 0.5Hz stimulation N1 was reliably recorded at 128.8±23.8ms (ICC=0.88), 141.9±51.5ms (ICC=0.83), 133.1±32.1ms (ICC=0.90), and 132.5±33.6ms (ICC=0.81) at the bladder dome, trigone, proximal and distal urethra, respectively in all subjects.

Following 3Hz stimulation, no reliable SEPs could be recorded.

**Conclusions:** SEPs can be reliably recorded from different locations of the LUT following 0.5Hz stimulation with a characteristic N1 response at about 130ms. These latencies are compatible with a conduction velocity in the range of 3-10m/s which corresponds to transmission by A-delta fibres. The inability to retrieve reliable responses at 3Hz stimulation might potentially be related to less involvement of fast conduction fibres (i.e. A-beta) in afferent sensation along the human LUT. The value of a more distinct diagnosis of sensory sensation in LUT disorders needs to be evaluated in further studies.

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3 This manuscript is accepted at the Journal of Urology. Flavia Gregorini conducted the study together with Jens Wöllner. She scheduled the measurements, did the data analysis, and the statistics. The manuscript was written by Flavia Gregorini and Jens Wöllner.
4.2 Introduction

LUTS have great impact on the health related quality of life, including impairments in sexuality, emotional well-being and productivity at home and at work [10, 104]. The estimated worldwide prevalence of LUTS is high with 45.2% having at least one LUTS [9]. A large proportion of LUTS (i.e. OAB symptoms) affects the storage phase and is attributed to aberrant sensory function of the LUT [9-10, 105-106]. However, in many cases the exact cause and pathologic mechanism responsible for LUTS is still unknown, which might be partly due to a lack of accurate and specific diagnostic tools. There is currently no objective and reliable clinical assessment tool of human bladder and urethral afferent nerve function and integrity available. Such an assessment tool would enable a greater understanding of the role of sensory nerves in LUTS (i.e. OAB).

Recording of SEPs is an established and recommended diagnostic instrument to assess the functionality of afferent nerve fibres [85, 107-108]. During repetitive stimulation of a nerve or a dermatome SEPs can be recorded at certain electrodes placed above the sensory cortex. The clinical utility of SEPs is based on their ability to reveal abnormal sensory function, when findings from previous investigations (i.e. history, neurologic examination, urodynamics) are equivocal [108].

Few older case series reported about the possibility of SEP recording from the LUT following electrical stimulation of the VUJ and the PU in healthy subjects [109-111] and patients [112-115]. These studies presented first feasibility results, mainly discussing methodological aspects. Unfortunately, this approach was not systematically followed up until now. Consequently, there is a lack of information on the reliability of this technique and data from other LUT sites (i.e. trigone, bladder dome). Knowledge of the reliability (i.e. percentage of obtained responses) and variability of SEP parameters is essential to allow distinction of possible pathological changes in a patient population. Thus, it was the aim of the current study to investigate feasibility and reliability of SEPs recorded following electrical stimulation
Sensory evoked potentials of the human lower urinary tract

at different localizations of the LUT. Using two different stimulation setups, we additionally aimed at a better understanding of nerve fibre composition of the human LUT.

4.3 Patients and Methods

4.3.1 Subjects

Female healthy volunteers were recruited. Inclusion criteria were good mental and physical health, written informed consent, and age of at least 18 years. Healthy was defined as the absence of any health troubles assessed by a complete medical history and physical examination. Exclusion criteria were any neurological or urological pathology, any previous LUT surgery or treatment, LUTS, pregnancy or lactation, UTI, and any regular medication intake (except contraceptive medication). The absence of LUTS was assessed using a 3-day bladder diary with the following cut-off values: daytime frequency ≤ 8, nocturia ≤ 1, and no UI.

The experiment was approved by the local Ethics Committee and was conducted in accordance with the Declaration of Helsinki. This study was registered at www.clinicaltrials.gov (No. NCT01389921).

4.3.2 Electrical stimulation

Using a custom made 8Ch transurethral catheter (Figure 4.1, Unisensor AG, Attikon, Switzerland), bipolar stimulation was randomly applied at four different LUT sites, bladder dome, trigone, proximal and distal urethra with a frequency of 0.5Hz (1ms pulse width, 200 repetitions) and 3Hz (0.2ms pulse width, 400 repetitions [87]). The stimulation intensity was increased as far as tolerable without being painful, but at least 2-3x sensory thresholds. All measurements were performed in supine position.
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Figure 4.1: Schematic figure of the custom made 8 Ch catheter (Unisensor AG, Attikon, Switzerland) in position for trigonal stimulation in the female LUT. The catheter has three platinum stimulation electrodes (E1-3, each 1 mm width) and a radiopaque marker (7 mm width) for precise positioning under fluoroscopic control. The orange connecting pieces between the electrodes have a width of 3 mm each. Bipolar stimulation was applied at the bladder dome using E1 and E2, and at the trigone, proximal and distal urethra using E2 and E3.

To warrant a constant bladder volume of 60mL during measurements, an additional 10Ch catheter (Speedicath, Coloplast, Humlebaek, Denmark) was placed transurethrally through which the bladder was completely drained and refilled with 60mL of contrast medium (Ultravist 150, Bayer Schweiz AG, Switzerland) after stimulation at each localization. To ensure exact and reproducible catheter position at each stimulation site, the catheter placement was performed under fluoroscopic control (Figure 4.2a-d). At the bladder dome, the stimulation was performed between electrode 1 and 2 (Figure 4.1) with direct contact of
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the catheter tip to the mucosa of the bladder dome (Figure 4.2a). At all other sites, stimulation was performed between electrode 2 and 3 (Figure 4.1).

All measurements were performed three times with an interval of four weeks (= three separate measurement sessions for each subject).

Figure 4.2: Fluorsoscopic control of catheter positioning in bladder dome (a), trigone (b), proximal (c) and distal urethra (d). Stimulations were always performed at a bladder volume of 60 mL.

4.3.3 Sensory evoked potentials

Three cortical electrodes were placed using an EEG cap (EasyCap, EasyCap GmbH, Germany) with the active electrode at Cz (according to the 10-20 system [116]) and reference at Fz. The ground electrode was placed at AFz position. Impedance was kept
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below 20kΩ for all electrodes. EP signals were continuously recorded during stimulation at each LUT site using BrainVision Recorder (BrainProducts, Munich, Germany) with a sampling frequency of 500Hz and 5000Hz for the 0.5Hz and 3Hz stimulation, respectively.

All data were filtered (band pass 0.5-30Hz and 0-70Hz for 0.5Hz and 3Hz stimulation respectively plus Notch filter for both stimulations) and segmented offline using BrainVision Analyzer2 (BrainProducts, Munich, Germany). All segments were generated from -100 to +1000ms and from -50 to +200ms in reference to the stimulus time points of the 0.5Hz and 3Hz stimulation, respectively. The odd and even segments of each stimulation were averaged and the peaks of the SEPs were determined semiautomatically. According to the direction of the detected SEP deflections, the peaks were labelled first and second positivity (P1 and P2) and first negativity (N1). The latency of each peak (i.e. P1, N1, and P2) was analysed and peak-to-peak amplitudes (i.e. P1N1 and N1P2) were calculated.

A SEP was regarded as stable when the odd and the even run of the SEP were in parallel and the peaks (P1, N1, P2) were visually clearly identifiable. Regarding to this, the responder rate reflects the percentage of measurements per stimulated LUT site that results in a stable SEP.

4.3.4 Statistics

IBM® SPSS® Statistics 19 for Windows (IBM Chicago, Illinois, U.S.A.) was used for all statistical analyses. Normal distribution of data was tested using Q-Q plots and the Kolmogorov-Smirnov test. Differences of P1, N1, and P2 latencies from different LUT stimulation sites were analysed using post-hoc ANOVA. An α-level of 0.016 (Bonferroni corrected) was regarded as significant. The reliability of latencies and amplitudes was analysed with the ICC. All values are given as mean and SD.
4.4 Results

4.4.1 Subjects

Ten healthy female volunteers with a mean age of 23 ±4 years and a mean height of 168 ±6 cm were included. All subjects completed all three measurement sessions and reported good tolerability of stimulations. Following seven of overall 30 measurement sessions, five subjects reported self-limited dysuria. No UTI or other adverse effects were observed or reported.

The data were normally distributed.

4.4.2 0.5 Hz stimulation

Stable SEPs following 0.5 Hz stimulation at the bladder dome, trigone, proximal urethra, and distal urethra were recorded in 24/30 (80%), 23/30 (77%), 19/30 (63%), and 21/30 (70%) measurements, respectively (Table 4.1).
Table 4.1: Responder rates, mean values and standard deviation of the current intensity, the P1, N1 and P2 latencies, and P1N1 and N1P2 amplitudes of sensory evoked potentials following 0.5Hz stimulation at the bladder dome, trigone, proximal and distal urethra of 10 healthy female subjects. The values are presented for each of the three measurement sessions and as overall sum (responder rate) and overall mean with standard deviation (latencies and amplitudes). The intraclass correlation coefficient (ICC) is indicated as measure of reliability. # p = 0.046
Figure 4.3: Grand averages of sensory evoked potentials recorded at Cz during 0.5 Hz (left column) and 3 Hz (right column) electrical stimulation in the LUT of 10 healthy female subjects from the first (upper row), second (middle row) and third measurement session (lower row). Black lines = bladder dome, dashed lines with square dots = trigone, simple dashed lines = proximal urethra, and dashed lines with long dash dots = distal urethra.

All SEPs showed a constant W-shape throughout all three measurement sessions at all LUT stimulation sites (Figure 4.3, left column and Fig.4) with two positive (P1 and P2) and one negative (N1) peak, whereas the N1 peak was the most prominent deflection (Figure 4.3, left column and Figure 4.4).
Figure 4.4: Averages (n=200 stimuli in each) of sensory evoked potentials recorded at Cz during 0.5 Hz stimulation of the bladder dome, trigone, proximal and distal urethra of a representative subject with good (left
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column) and poor (right column) responses. The diagrams show first (black line), second (dark grey line) and third (light grey) measurement session.

The latencies of all four stimulation sites were similar without significant difference (Table 4.1, Figure 4.5a).

Although amplitudes of SEPs from the bladder dome showed the highest values, no significant difference of amplitudes was detected (Figure 4.5b, Table 4.1).

Data on latencies, amplitudes, and ICC values of all four LUT stimulation sites are summarized in Table 4.1.

Figure 4.5: Comparison of P1, N1 and P2 latencies (a) and P1N1 and N1P2 amplitudes (b) of bladder dome, trigone, proximal and distal urethra. Presented are overall means and standard deviation of all three measurement sessions. # p = 0.046.
4.4.3 3 Hz stimulation

Applying the 3Hz stimulation, only one positive (P1) and one negative (N1) peak were detected (Figure 4.3, right column). SEP shapes were less consistent and SEP peaks less clearly detectable than using 0.5Hz stimulation. Stable SEPs following 3Hz stimulation at the bladder dome, trigone, proximal, and distal urethra were recorded in 19/30 (63%), 17/30 (56%), 10/30 (33%), and 9/30 (30%) measurements, respectively (Table 4.2). Due to the low responder rates, no ICC calculation was performed.

Data on latencies and amplitudes of all four LUT stimulation sites are summarized in Table 4.2.

Table 4.2: Responder rates, mean values and standard deviation of the current intensity, the P1, N1 and P2 latencies, and P1N1 and N1P2 amplitudes of sensory evoked potentials following 3Hz stimulation at the bladder dome, trigone, proximal and distal urethra of 10 healthy female subjects. The values are presented for each of the three measurement sessions and as overall sum (responder rate) and overall mean with standard deviation (latencies and amplitudes).
4.5 Discussion

The main findings of the present study are: 1) Recording of cortical SEPs following electrical stimulation at the bladder dome, trigone, proximal, and distal urethra is feasible in healthy female subjects. 2) Stimulation using 0.5Hz but not 3Hz resulted in stable and reliable SEPs with the N1 peak as most prominent and reliable landmark. 3) No significant differences of N1 latencies were observed between stimulated LUT sites. Overall mean N1 latency following 0.5Hz stimulation was 130ms.

Previous studies reported about the feasibility of SEP recording following LUT stimulation at the VUJ [109, 111-112], PU [110, 115] and the dorsal bladder wall [113]. Stimulations were mainly performed with 2Hz and SEPs were recorded at the Cz or Cpz electrode referenced to Fz or Fpz. Although positive and negative deflections of SEPs were named differently, the equivalent to our N1 potential was the most consistently reported outcome parameter. 

healthy subjects, mean N1 latencies following VUJ and PU stimulation were 94.6ms (range: 76-112ms) [109] and 92.4ms (range: 74-134ms) [110], respectively. However, similar N1 latencies were reported following VUJ or trigonal stimulation also in patients with SUI/DO (96.3ms, range: 70-110ms) [114], peripheral diabetic polyneuropathy (94.8ms, range: 68-123ms)[112], and erectile dysfunction (88.7ms) [111].

Although most stimulations were performed with 2Hz at similar sites, the heterogeneous study populations and inconsistencies of some important measurement settings (i.e. bladder volume, stimulation strength, filtering) hamper a meaningful comparison of those studies and resulted in varying SEP shapes and latencies. Especially bladder volume and stimulation strength have been demonstrated to significantly influence SEP shape and latencies [110] which brought us to measure at constant bladder volumes (60mL) and a minimal stimulation strength (2-3x sensory threshold). Even more important regarding SEP shape and latency are filter settings which have been very different in previous studies ranging from 5-16Hz (high pass) to 250-3200Hz (low pass) [109, 111, 113-115]. To enable detection of SEPs conducted by slower fibres like A-Delta (conduction velocity from the LUT: 3-10 m/s [117]) which are supposed to be most relevant for the human LUT [6], a slow frequency of 0.5Hz with an according band pass filter of 0.5-30Hz was selected. As reference in accordance to standard neurophysiological SEP measurements and recently published data on LUT SEPs, we also included the 3Hz stimulation [85, 118].

4.5.1 0.5 Hz stimulation

Similar to previous studies using 2Hz stimulation [110-111], the N1 peak was the best landmark for our LUT SEPs during 0.5Hz stimulation due to better detectability and reliability compared to P1 and P2 (Table 4.1). A reason for that might be the difficult marker placement as the configuration of the potentials made it frequently difficult to determine P1 and P2. Our mean N1 latencies range between 129ms (bladder dome) and 142ms (trigone), which is longer compared to the data from healthy subjects of previous studies [109-110]. This may
be owed to a more synchronous and selective excitation of slower nerve fibres due to less refractoriness using the lower stimulation frequency. Nerve conduction velocity based on a mean N1 latency of 130ms would result in an estimated 5.4m/s, which correlates well with the conduction velocity of A-Delta fibres [117]. To avoid refractoriness, it would be favourable to stimulate with even lower frequencies (i.e. 0.2Hz, 0.1Hz). However, due to very low repetition rates (every 5–10sec) it is not practical for stimulation in the LUT due to changes of bladder volume over time.

Although the stimulation sites are innervated by different nerves (i.e: pudendal, sacral, and hypogastric nerve) [6], this seems not to affect 0.5Hz latencies. This might be related to an overlap of bladder and urethral afferents in the lateral dorsal horn and dorsal commissure indicating a similar afferent pathway [119]. In addition, the difference in distance from the stimulation localizations to the cortex may be too small to produce a measurable latency difference.

In contrast to the N1 latencies, amplitudes showed only fair to poor reliability, which can be related to several factors such as habituation and stimulus tolerance next to changes in impedance on the recording site and changes in the relaxation state of the subject during the recording. Despite fluoroscopic control of catheter position and stimulation at constant bladder volumes, it remains difficult to precisely control contact intensity of the stimulation electrodes to the LUT mucosa as compared to stimulation on the skin.

### 4.5.2 3 Hz stimulation

Although, responder rates of 57% and 63% for the trigone and bladder dome, respectively, appeared to be good, they were still lower than those for the 0.5Hz stimulation. Furthermore, using 3Hz stimulation only three subjects had an SEP during each of the three measurement sessions so that no reasonable ICC could be calculated (Table 4.2).
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The low responder rate and reliability of SEPs after 3Hz stimulation might potentially be related to a lesser involvement of A-beta fibres in healthy LUT afferents which would also explain the difficulty to obtain reliable SEPs in earlier studies using 2Hz stimulation. In addition, SEPs following 3Hz stimulation resulted in latencies of approximately 120ms (Fig.4), which would rather correspond to the conduction velocity spectrum of A-delta fibres. However, a contribution of A-beta fibres to LUT afferent function cannot be finally excluded or demonstrated with this study.

As the sensory innervation of the female distal urethra is primarily via the pudendal nerve, the lack of a typical cortical response at 40ms as it is usually obtained with clitoral or external penile 3Hz stimulation [120-121] was somewhat unexpected. Possible explanations include [122]:

a) Different distribution and density of primary afferent endings within the urethral wall compared to the epidermis.

b) Light touch and two-point discrimination are typically A-beta fibre transmitted sensations. However, the capability of two-point discrimination and perception of light touch is different comparing the urethral mucosa and skin areas although both are innervated by the pudendal nerve, suggesting differences in afferent fibre composition of the urethra and the genital/perineal skin.

c) The lack of 40ms cortical potentials does not preclude that fast pudendal afferents are completely missing. It might rather indicate that they may not be sufficiently stimulated under the current conditions due to different activation thresholds or a fibre density that is too low to allow for a reliable cortical response.

The present study advances the development of an objective measurement tool for investigation of human LUT afferent nerve fibre function. Such tool has great potential to
improve the diagnosis of LUT dysfunctions (e.g. OAB) and to objectively evaluate related therapies. It might complement urodynamic investigations and add the possibility to distinguish between bladder and urethral afferent function.

Potential limitations of this study are: 1) Small subject sample size which is however adequate for this proof-of-concept study. 2) Inclusion of only female subjects, which does not allow conclusions for the general population. However, we aimed at a homogeneous group for the proof-of-concept and later comparability to other groups.

4.6 Conclusions

Following 0.5Hz stimulation of the LUT, cortical SEPs can reliably be obtained from different sites at latencies suggesting that transmitting fibres are mainly A-delta. The lack of reliable SEPs using 3Hz stimulation could be explained by refractoriness or a lack of susceptibility of these slow fibres and/or absence of faster conducting fibre populations such as A-beta which is compatible with findings from animal studies. In the future, these findings can provide a means of assessment of the therapeutic potential of novel drugs or neuromodulation which target the bladder sensory pathway.

4.7 Acknowledgement

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5. Age-related differences in somatosensory evoked potentials of the lower urinary tract

5.1 Abstract

**Purpose:** To investigate potential age related changes on the assessment of human lower urinary tract (LUT) afferents using sensory evoked potentials (SEPs) and current perception thresholds (CPT).

**Materials and Methods:** A group of healthy female volunteers with a minimum age of 35 years was recruited. Electrical stimulation was applied with a transurethral stimulation catheter (8 F) to the bladder dome, trigone, proximal and distal urethra. Measurements were performed three times with an interval of four weeks. The CPTs were determined using the method of limits after 0.5 Hz and 3 Hz electrical stimulation. Latencies and amplitudes were analyzed from cortical SEPs recorded at Cz referenced to Fz during LUT stimulation with 0.5 Hz and 3 Hz. Outcomes of this study group were compared to a previously investigated group of younger subjects.

**Results:** Ten healthy subjects (42.5 ±5.5 years, 62.4 ±11.1 kg, and 165 ±5 cm) were included. Compared to younger subjects (n = 10, 23 ±3.7 years), the older subjects demonstrated significantly shorter N1 latencies. Only EP latencies from the distal urethra were longer in the older subjects (not significant). No correlation between N1 latency and age was observed. Reliability of latencies was higher in the younger subjects. No significant differences in N1P2 amplitudes at the trigone, proximal and distal urethra were observed between both groups. Following stimulation at the bladder dome, older subjects presented significantly higher amplitudes. The younger subject group revealed a significant higher CPT in the bladder dome compared to the older subjects group.

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4 This manuscript will be submitted in 2013. Flavia Gregorini conducted the study together with Jens Wöllner. She scheduled the measurements, did the data analysis, and the statistics. The manuscript was written by Flavia Gregorini and Jens Wöllner.
Age-related differences in somatosensory evoked potentials of the lower urinary tract

**Conclusions:** Cortical EPs after electrical 0.5 Hz stimulation of the LUT can be obtained in subjects older than 35 years. In the older subjects, latencies after 0.5 Hz stimulation were less reliable and shorter than in younger subjects. This could be due to age related structural changes in the LUT.
5.2 Introduction

About 11.6% of the female population worldwide suffers from an overactive bladder (OAB) [9]. However, the exact pathomechanism is still unknown and there is no objective diagnostic marker for OAB. The OAB becomes more prevalent with age [11]. With a population continuously with increasing in age such disease becomes more prevalent which makes a better understanding of the underlying pathomechanism even more important.

Evoked potentials (EPs) after electrical, visual or auditory stimulation are a well-established and daily used method to determine the afferent nerve fibre function in humans. Stimulation of a certain nerve or dermatome with electric current is followed by an EP which can be measured along the spinal cord or on the cortex. Latency and amplitude of those EPs give information about the afferent nerve fibre function and is part of the neurophysiological armamentarium in the diagnosis of various neurological diseases.

A few studies were conducted with electrical stimulation of the LUT urethra in healthy subjects using 0.5 Hz, 2 Hz [109-112, 114, 118], and 3 Hz stimulation, respectively. The 0.5 Hz setup showed as only one an excellent reliability of the N1 peak latency after stimulation of the bladder dome, trigone, proximal and distal urethra. Another possibility to determine the afferent nerve function is the determination of the current perception threshold (CPT). CPTs were showed to increase with age after stimulation of the bladder and the urethra [23-24].

Although, OAB is known to increase with age, the influence of age on LUT EPs is currently unknown. However, to use LUT EP as a diagnostic tool for LUT symptoms as OAB it is mandatory to understand the influence of age on measurement outcomes of LUT EPs. Thus, it was the aim of the present study to evaluate if age has an influence on the latencies and amplitudes of LUT EPs. Additionally, CPTs were performed on four different stimulation sites in the LUT and the influence of stimulation frequency, age, catheter localization was examined.
Age-related differences in somatosensory evoked potentials of the lower urinary tract

5.3 Patients and Methods

5.3.1 Subjects
A group of healthy female volunteers was recruited. Inclusion criteria were good mental and physical health, written informed consent, and age of at least 35 years. Exclusion criteria were any neurological or urological diseases, no gynecological and urological operations (except cesarean) pregnancy or lactation, urinary tract infection, and any regular medication intake (except contraceptive medication). To extract a potential influence of the menstrual cycle on the signal in pre-menopausal females, the subjects were measured three times with an interval of four weeks. All measurements were performed in supine position.

The experiment was approved by the local Ethics Committee and was conducted in accordance with the Declaration of Helsinki. This study was registered at clinicaltrials.gov (No. NCT01389921).

5.3.2 Electrical stimulation
A bipolar square wave electrical stimulation was applied with a special transurethral catheter (Unisensor AG, Attikon, Switzerland) to the bladder dome, trigone, distal and proximal urethra as previously described. Measurements were performed three times with an interval of 4 weeks. The impulses were applied with 0.5 Hz using intensities as high as bearable for the subjects but at least two to three times sensory threshold. The sequence of stimulation sites was randomized between either bladder dome – trigone – proximal urethra – distal urethra or distal urethra – proximal urethra – trigone – bladder dome. The stimuli were applied with the Dantec Keypoint® 4m (Neurolite AG, Belp, Switzerland) while subjects had their eyes closed. The CPT was identified using the method of limits.
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5.3.3 EEG

Electroencephalogram (EEG) was recorded continuously with a sampling frequency of 500 Hz continuously using BrainVision Recorder (BrainProducts, Munich, Germany). Three electrodes were set, whereas Cz electrode (according to the 10-20 system) was the active electrode, reference was the Fz electrode, the ground electrode was set at AFz position.

Impedance was kept below 20 kΩ for all electrodes. All data were filtered offline from: 0.5Hz to 30 Hz for the 0.5 Hz stimulation and to 70 Hz for the 3 Hz stimulation plus Notch filter using BrainVision Analyzer2 (BrainProducts, Munich, Germany). The signal was segmented from -100 to +1000 ms for the 0.5 Hz stimulation and -50 to +200 ms for the 3 Hz stimulation. The segments of every measurement were averaged and the N1 latencies of the EPs and the N1P2 peak-to-peak amplitude were determined semiautomatically.

5.3.4 Statistics

IBM® SPSS® Statistics 19 for Windows (IBM Chicago, Illinois, U.S.A.) was used to perform the following analysis: 1.) Intraclass correlation (ICC) to test the reliability of latencies, amplitudes and the CPT 2.) Comparison to a younger subject group as performed using t-tests, and 3.) Post-hoc ANOVA (Bonferroni corrected) to compare the latencies and amplitudes of the four stimulation sites of the LUT.

The alpha level used as a significance criterion for the statistical tests was 0.05. All values are given as mean and standard deviation (SD).

5.4 Results

5.4.1 Subjects

Ten younger subjects (23 ±3.7 years, 57 ±5.1 kg, 167 ±4.5 cm) and ten older subjects (42.5 ±5.5 years, 62.4 ±11.1 kg, 165 ±5 cm) were included. All subjects tolerated the stimulation
well and expect a burning during micturition no adverse effects were registered None of the younger subjects ever beard a child or had a cesarean, three of the older subjects beard child, none had a cesarean and one got an abortion.

The older subject group was stimulated with significantly higher stimulation intensity in the trigone and distal urethra (Table 5.1).

<table>
<thead>
<tr>
<th>frequency</th>
<th>group</th>
<th>mean ±SD [mA]</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>bladder dome</td>
<td>0.5 Hz</td>
<td>young: 14.9 ±10.3</td>
<td>old: 14.7 ±8.4</td>
</tr>
<tr>
<td>trigone</td>
<td>0.5 Hz</td>
<td>young: 5.5 ±4.2</td>
<td>old: 10.3 ±6.9</td>
</tr>
<tr>
<td>proximal urethra</td>
<td>0.5 Hz</td>
<td>young: 7.6 ±7.3</td>
<td>old: 9.5 ±5.8</td>
</tr>
<tr>
<td>distal urethra</td>
<td>0.5 Hz</td>
<td>young: 5.6 ±2.</td>
<td>old: 10.6 ±7.8</td>
</tr>
</tbody>
</table>

5.4.2 Electrical stimulation

The N1 latency after the bladder dome stimulation was 108.9 ±7.8 ms, after trigonal stimulation 116.2 ±10.7 ms, after the stimulation of the proximal urethra 113.2 ±13.4 ms and 131.3 ±35.6 ms after stimulation of the distal urethra (Table 5.2).

<table>
<thead>
<tr>
<th>N1 latency [ms]</th>
<th>N1P2 amplitude [μV]</th>
</tr>
</thead>
<tbody>
<tr>
<td>younger</td>
<td>older</td>
</tr>
<tr>
<td>bladder dome</td>
<td>122.0 ±13.6</td>
</tr>
<tr>
<td>trigone</td>
<td>124.5 ±19.7</td>
</tr>
<tr>
<td>proximal urethra</td>
<td>123.3 ±23.6</td>
</tr>
<tr>
<td>distal urethra</td>
<td>122.3 ±20.5</td>
</tr>
</tbody>
</table>

The N1 latencies of the bladder dome, trigone, and of the proximal urethra were significantly shorter compared to the younger group. In the older subjects group, the N1 latency of the distal urethra was significantly longer (p = 0.014) than of the bladder dome (Figure 5.1). No significant differences were seen in the younger subject group.
Age-related differences in somatosensory evoked potentials of the lower urinary tract

Figure 5.1: Comparison of the evoked potentials after 0.5 Hz electrical stimulation of the bladder dome (A), trigone (B), proximal urethra (C) and distal urethra (D). Group of older subjects with bold line, group of younger subjects with dashed line

The N1P2 amplitude in the bladder dome was 8.8 ±4.2 μV, in the trigone 4.1 ±1.2 μV, in the proximal urethra 4.4 ±1.7 μV and in the distal urethra 4.1 ±1.1 μV (Table 5.2). Significant higher amplitudes were seen in the bladder dome compared to the younger subject group. In the older subjects group, the N1P2 amplitude of the bladder dome was significantly higher compared to the three other localizations. No significant differences were seen in the younger subject group.

The 3 Hz stimulation revealed no reliable EPs (Figure 5.2).
Age-related differences in somatosensory evoked potentials of the lower urinary tract

![Graphs showing somatosensory evoked potentials](image)

**Figure 4.2:** Comparison of the evoked potentials after 3 Hz electrical stimulation of the bladder dome (A), trigone (B), proximal urethra (C) and distal urethra (D). Group of older subjects with bold line, group of younger subjects with dashed line

### 5.4.3 Current perception threshold

The CPTs after 0.5 Hz and 3 Hz electrical stimulation are listed in Table 3. The younger subject group revealed a significant higher CPT in the bladder dome compared to the older subjects group. In the remaining stimulation localizations, no significant age related difference was observed (Table 5.3).
Age-related differences in somatosensory evoked potentials of the lower urinary tract

Table 5.3: Comparison of current perception thresholds mean ± SD and ICC-values of the younger and older subject groups at the four localizations

<table>
<thead>
<tr>
<th>frequency</th>
<th>group</th>
<th>mean CPT ±SD [mA]</th>
<th>t-test</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 Hz</td>
<td>young</td>
<td>8.47 ±4.42</td>
<td>0.000</td>
<td>0.414</td>
</tr>
<tr>
<td></td>
<td>old</td>
<td>5.12 ±1.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Hz</td>
<td>young</td>
<td>12.40 ±8.04</td>
<td>0.015</td>
<td>0.080</td>
</tr>
<tr>
<td></td>
<td>old</td>
<td>8.37 ±3.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 Hz</td>
<td>young</td>
<td>2.24 ±3.01</td>
<td>0.553</td>
<td>0.511</td>
</tr>
<tr>
<td></td>
<td>old</td>
<td>2.62 ±1.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Hz</td>
<td>young</td>
<td>3.76 ±3.57</td>
<td>0.297</td>
<td>0.094</td>
</tr>
<tr>
<td></td>
<td>old</td>
<td>4.93 ±5.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 Hz</td>
<td>young</td>
<td>2.65 ±3.46</td>
<td>0.385</td>
<td>-0.002</td>
</tr>
<tr>
<td></td>
<td>old</td>
<td>2.09 ±0.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Hz</td>
<td>young</td>
<td>4.34 ±3.78</td>
<td>0.407</td>
<td>-0.073</td>
</tr>
<tr>
<td></td>
<td>old</td>
<td>3.71 ±1.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 Hz</td>
<td>young</td>
<td>2.22 ±1.63</td>
<td>0.187</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>old</td>
<td>2.92 ±2.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Hz</td>
<td>young</td>
<td>3.96 ±2.71</td>
<td>0.187</td>
<td>0.230</td>
</tr>
<tr>
<td></td>
<td>old</td>
<td>4.96 ±3.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.4.4 Reliability

The N1 latency reliability of the bladder dome, trigone, proximal and distal urethra was 0.88, 0.83, 0.90, and 0.81, respectively in the younger volunteers and 0.27, 0.54, 0.97, and 0.27, respectively in the older volunteers.

The N1P2 amplitude reliability of the bladder dome, trigone, proximal and distal urethra was 0.55, 0.37, 0.19, and 0.55, respectively in the younger subjects and 0.59, 0.22, 0.18, and 0.08, respectively in the older subjects group.

Stable EPs were recorded following the stimulation of the bladder dome, trigone, proximal urethra, and distal urethra in 24/30 (80%), 23/30 (77%), 19/30 (63%), and 21/30 (70%), respectively in the younger subjects group and 26/30 (87%), 26/30 (87%), 27/30 (90%), and 25/30 (83%), respectively in the older subjects group.

The CPT reliability of the bladder dome, trigone, proximal and distal urethra for the 0.5 Hz stimulation was 0.41, 0.51, -0.002, and 0.12, respectively and for the 3 Hz stimulation 0.08, 0.09, -0.07, and 0.23, respectively (Table 5.3).
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5.4 Discussion

The main findings of the present study are: 1.) cortical EPs after electrical 0.5 Hz stimulation of the LUT can also be obtained in subjects from 35 to 50 years of age, 2.) the latencies from bladder dome, trigone, and proximal urethra were significantly shorter in the older subject group compared to the younger subject group, and 3.) the bladder dome showed significantly higher amplitudes compared to the younger subject group.

Latencies of EPs recorded from the cortex give information about the integrity of both peripheral and central afferent nerve fibres. As previously described, EPs are not only recordable after stimulation of a certain nerve or dermatome, but also after stimulation of the human LUT [109-115, 118]. It was also seen that a slower stimulation frequency of 0.5 Hz results in reliable latencies and wave forms compared to faster stimulations with 2 Hz and 3 Hz.

5.4.1 0.5 Hz stimulation

Stimulating subjects aged 35 years and more also results in recordable cortical EPs. The responder rate in the older subjects group was higher with values more than 80% compared to the younger subjects group with responder rates of 63 – 80%. A possible reason for this observation is that the older subjects were stimulated with higher current intensities (Table 5.1) expect in the bladder dome. But the bladder dome CPT was significantly lower in the older subjects group. Taking together the CPT and the stimulation intensity, the older subjects were stimulated with a higher intensity. The amplitudes were also higher in the older subjects group in the bladder dome (Table 5.2), which could also be due to the relatively higher stimulation intensity compared to the younger subjects group.

Comparing the EP latencies of the two groups, the latencies of the EPs after stimulation of the bladder dome and proximal urethra were significantly shorter in the older subjects group.
Age-related differences in somatosensory evoked potentials of the lower urinary tract

This result is in contradiction to other neurophysiological studies which showed longer latencies at the tibial and median nerve in older compared to younger subjects [123].

The bladder dome has a different afferent innervation than the urethra. This fact could have a great impact on the behaviour of the dome. A previous study showed larger amplitudes and lower frequencies of spontaneous bladder contractions in spinal cord transection mice evoking afferent nerve activity, which may contribute to incontinence [124]. Thus, the bladder dome can be regarded as the site where the detrusor overactivity begins. The prevalence of OAB with or without detrusor overactivity increases with age [11]. In the present study, the bladder dome was the localization which showed most differences between the two age groups. The N1 latency and the N1P2 amplitude higher after the 0.5 Hz stimulation was shorter, and the CPT was lower in both stimulation frequencies in the older subject group. It seems that the bladder dome is the localization, in which age-related changes can be detected at first.

One has to keep in mind that the bladder is innervated by the hypogastric and the pelvic nerve, whereas the trigone and the urethra are innervated by the pudendal and the sacral nerve. The bladder dome is also the localization with the highest variability of the catheter positioning. The control of the position was done visually in X-rays, but a proof of the contact of the catheter to the urothelium cannot be performed yet.

Shorter latencies of the bladder dome in the older subjects group could also be due to higher stimulation intensity in relation to the CPT. Previous studies showed that higher stimulation intensity is followed by a shorter EP latency [125-126]. After stimulation of the bladder dome, higher amplitudes were obtained in the older subjects group (Table 5.2). The stimulation intensity was the same in both groups, but it was higher in relation to the CPT. The lower CPT in the older subject group lets the question arise if bladder dome afferents are more sensible in older subjects.
Age-related differences in somatosensory evoked potentials of the lower urinary tract

The N1 latency reliability was better in the younger subjects group. This finding could be due to age-related changes of LUT afferents. It is questionable if the afferents respond sometimes better and sometimes worse to the stimulation. The stimulation intensity varies in a similar range in both groups (Table 5.1), though that this fact cannot be the reason for changing latencies in the older subject group.

5.4.2 3 Hz stimulation

No reliable EP latencies and wave forms were seen after the 3 Hz stimulation. It can be assumed that this frequency is too fast for the slow conducting nerve fibres of the LUT in the way that there was an overlap of the EPs and no clear wave form could be established. Also previous studies reported varying wave forms after 2 Hz stimulation [109], which could be due to the same reason.

5.4.3 CPT

In the bladder dome, the CPT was significantly lower in the older subject group in both frequencies. In all other stimulation localizations, no significant difference was seen. In another study which determined the CPT using 5 Hz, 250 Hz and 2000 Hz reported an increasing threshold at all frequencies with the subjects’ age in urethra and bladder [24]. This study used sine waves in contrary to the square waves in the present study. Another study compared CPTs of sine and square waves and found higher CPTs after the 2.5 Hz square wave stimulation [127]. The results were similar in the posterior urethra (4.34 mA in our study versus 4.28 mA) [127]. In the bladder dome and in the distal urethra our CPTs were higher in the present study (bladder dome: 12.4 mA in our study versus 8.29 mA; distal urethra: 3.96 mA in our study versus 1.26 mA). The age range and the stimulation were similar in both study samples.
Age-related differences in somatosensory evoked potentials of the lower urinary tract

5.5 Conclusions

Cortical EPs after electrical 0.5 Hz stimulation of the LUT can be obtained in subjects older than 35 years. The latencies after 0.5 Hz stimulation of the human LUT were shorter in the older compared to the younger healthy volunteers. This could be due to age related structural changes in the LUT.
6. General discussion

In the first study, reliable latencies after electrical stimulation of the tibial and pudendal nerve and the S3 dermatome were obtained. The reliabilities of the CHEPS latencies and amplitudes were only fair to good. As a possible reason we assume the distal decreasing receptor density. Also other researchers stated that CHEPS is difficult to be performed on the foot [86]. As nobody applied CHEPS to sacral dermatomes, comparisons remain difficult. Nevertheless, latencies from dermatomes of the hand (C6 and C8 dermatome), which are in a comparable distance to the cortex, are similar to our latencies from the S2 and S3 dermatome [92]. The generated data are relevant for further investigation of pelvic and sacral nerve fibres in relation to pelvic organ function.

In the second study, we showed that reliable latencies of cortical EPs are feasible after electrical stimulation with a 0.5 Hz impulse of the bladder dome, trigone, proximal urethra, and distal urethra in young healthy female subjects. The obtained latencies of about 130 ms let conclude that the stimulated nerve fibres are of a slow-conduction velocity type like A-delta. No significant differences were seen between the latencies of the four stimulation sites in the LUT. No reliable wave forms and latencies were obtained after the electrical stimulation of the LUT with 3 Hz. The lack of reliable cortical EPs after the 3 Hz stimulation can be interpreted as an absence of a faster fibre type like A-beta.

As CHEPS rather elicits A-delta fibres (first study), it can be compared to the 0.5 Hz stimulation where latencies of about 130 ms were seen (second study). The application or CHEPS on sacral dermatomes was followed by an EP with a latency of about 300 ms. Comparison remains difficult as CHEPS results from an increasing heat stimulus that reaches its maximum after 242 ms whereas the electrical stimulation only lasts for 0.2 ms. The latency after 0.5 Hz stimulation of the LUT is too long for A-beta fibres as pudendal nerve and S3 dermatome stimulation are followed by an EP already after 40 ms. On the
other hand, 130 ms are too fast compared to the 300 ms for CHEPS. But one has to keep in mind that the true CHEPS latency is not known because it remains unclear at which temperature an EP is elicited. Though, the true latency would be the measured latency minus the time from the eliciting temperature to the maximal temperature of CHEPS of 52 °C.

Comparison of the 3 Hz LUT stimulation with the pudendal nerve and the S3 dermatome stimulation is also difficult because no reliable latencies were seen after the 3 Hz LUT stimulation. In the grand averages, negativity was seen after about 60 ms which is longer than the latency of about 40 ms after the pudendal nerve and S3 dermatome stimulation. The cause of this remains elusive but possible reasons include refractoriness or lack of fast conducting fibre populations such as A-beta in the LUT or asynchronous excitation of slow fibre populations of the LUT.

In the third study, the 0.5 Hz electrical stimulation was applied to the same localizations of the LUT in healthy female subjects older than 35 years. The bladder dome latency was shorter and the amplitude higher than in younger subjects. Changes in the sensory innervations of the LUT with age could also be an influencing factor. The reliabilities of the latencies were poorer in the older subjects group compared to the younger subjects group. Maybe the older LUT is less able to conduct the information with the always same speed.

In the future, the recorded EEG will be analysed further to get information about where and when the EPs develop on the cortex using topographies. Further on, using sLORETA (standardized low resolution brain electromagnetic tomography) [128], source localization will be performed and these results can be compared to fMRI studies about brain activity due to different LUT stimulations (review in chapter 2).
In conclusion, in this thesis a new setup was developed and tested to get more information about the afferent innervations of the human LUT. The 0.5 Hz setup was newly developed aiming to elicit A-delta fibres. From CHEPS which selectively elicits A-delta fibres, we expected a latency of about 100 – 150 ms after A-delta stimulation in the LUT. Based on this knowledge, we decided to stimulate every two seconds, giving the fibres enough time to go back to baseline.

During the thesis, the setup was applied only in female subjects and it is not yet known if our findings in women can be translated to men. However, we already started a follow-on study investigating LUT afferents in men using the same protocol. In addition, it would be of great interest to understand the LUT afferent system in different types of LUT dysfunctions aiming to optimize appropriate treatment. A few patients with neurogenic OAB have already been measured with an adapted version of our newly developed tool to investigate LUT dysfunctions. The next step will be to further optimize the setup and introduce it into the daily clinical routine as additional tool to the urodynamic investigation. This new tool will possibly integrate in urodynamic measurement systems and, if it proves itself in practice, it will be placed on the market. The advantage of the application of LUT EPs is a fast determination of the functionality and integrity of the LUT afferents and therefore a better diagnosis of LUT dysfunctions.

It would also be interesting to examine the efferent innervations of the LUT. Normally, the efferent nerve fibres are tested with the recording of an EP in a certain muscle after transcranial magnetic stimulation. Up to date, such a project has not yet started.

Another exciting project to improve our knowledge on nerve fibre types present in the human LUT is the investigation of the bladder tissue after biopsies. Indeed, we are currently preparing a complementary tissue biopsy study and are looking forward to combine neurophysiology and structural findings.


7. References

References

References


## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>CHEPS</td>
<td>Contact heat evoked potential stimulation</td>
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<td>EEG</td>
<td>Electroencephalography</td>
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<td>EP</td>
<td>Evoked potential</td>
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<td>fMRI</td>
<td>functional magnet resonance imaging</td>
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<td>LUT</td>
<td>Lower urinary tract</td>
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<tr>
<td>ICC</td>
<td>Intraclass correlation coefficient</td>
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<tr>
<td>mA</td>
<td>Milliampère</td>
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<tr>
<td>ms</td>
<td>Millisecond</td>
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<tr>
<td>μV</td>
<td>Microvolt</td>
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<tr>
<td>OAB</td>
<td>overactive bladder</td>
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<tr>
<td>sLORETA</td>
<td>Standardized low resolution brain electromagnetic tomography</td>
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<td>SEP</td>
<td>Sensory evoked potential</td>
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<tr>
<td>SSEP</td>
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<td>ZNZ</td>
<td>Neuroscience Centre Zürich</td>
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Curriculum vitae

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Curriculum vitae

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- French, school knowledge

Poster presentations

2012  
*EAU, Paris:*
Cortical response after electrical stimulation of the bladder – a primary feasibility study

*ZNZ Symposium, Zürich:*
Cortical response after electrical stimulation of the bladder – a primary feasibility study
Age-related differences in somatosensory evoked potentials of the lower urinary tract

*SGU, Interlaken:*
Cortical response after electrical stimulation of the bladder – a primary feasibility study
Age-related differences in somatosensory evoked potentials of the lower urinary tract
Current perception threshold of the lower urinary tract are depending on age, stimulation frequency, and localization

2011

*ZNZ Symposium, Zürich:*
Cortical response after electrical stimulation of the bladder – a primary feasibility study

*Tibial, pudendal and sacral evoked potentials after electrical and contact heat stimulation*

*EFAS, Bern:*
Cortical response after electrical stimulation of the bladder – a primary feasibility study

2010

*ZNZ Symposium, Zürich:*
Is EEG a feasible method to investigate cortical activity due to intravesical electric and S3 dermatome heat stimulation?
Publications

2009  
*NCCR Meeting, Berlingen:*  
Cortical activity during filling cystometry measured by EEG - first results

Oral presentations

2012  
*Forum Urodynamicum, Zürich*  
Somatosensibel evozierte Potentiale nach elektrischer Stimulation des unteren Harntrakts

Publications

2012  


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