Improving Sacral Neuromodulation for Lower Urinary Tract and Bowel Dysfunction

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A Translational and Multidisciplinary Approach

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Gutta cavat lapidem non vi, sed saepe cadendo

CONTENTS

a.a.	Acetid acid
ATP	Adenosine triphosphate
BC	Bladder capacity
Botox	Botulinum toxin
CCCS	Cleveland clinical continence score
Con	Conventional
DBI	Defecatory behaviour index
DRG	Dorsal root ganglion
DGP	Dynamic graciloplasty
EPs	Evoked potentials
EAS	External anal sphinter
EOS	End of service
EUS	External urethral sphincter
FI	Faecal incontinence
FIQLS	Faecal Incontinence Quality of Life Scale
GSRS-IBS	Gastrointestinal Symptoms Rating Scale for Irritable Bowel Syndrome
H&E	Haematoxylin and eosin
HF	High frequency
IPG	Implantable pulse generator
LF	Low frequency
LUTD	Lower urinary tract dysfunction
MCC	Maximum cystometric capacity
MMA	Mixed model analysis
MoA	Mechanisms of action
NOUR	Non-obstructive urinary retention
OAB	Overactive bladder
PAC-SYM	Patient Assessment of Constipation Symptoms Questionnaire
PAC-QOL	Patient Assessment of Constipation Quality of Life Questionnaire

ABBREVIATIONS

pBOO	Partial bladder outlet obstruction
PNE	Percutaneous nerve evaluation
PNS	Pudendal nerve stimulation
PTNS	Posterior tibial nerve stimulation
p_{ura1}	Proximal urethral pressure
p_{ura2}	Mid urethral pressure
p_{ves}	Bladder pressure
RA	Replacement Adaptor 09106®
rCBF	Regional cereral blood flow
RCT	Randomized controlled trials
RoB	Risk of Bias
RQ	Research questions
RV	Residual volume
SCS	Spinal cord stimulation
SMCS	St. Mark's Continence Score
SNM	Sacral neuromodulation
S3	Third sacral nerve root
TLP	Tined lead procedure/placement
T_{mot}	Motor threshold
US	Ultrasound imaging
VD	Vaginal distention
VPI	Voiding pattern index
VSA	Voiding spot assay

General Introduction

Chapter 1

Sacral neuromodulation (SNM) is an established and validated therapy for lower urinary tract and bowel dysfunction. However this therapy is expected to bear more potential than what is currently used. Therefore, the purpose of this thesis is to further improve SNM as a treatment for these patients. This chapter will firstly describe the clinical indications for SNM. This is followed by a clinical background and evidence related to SNM as an established therapy for these indications and the use of animal models in SNM for lower urinary tract and bowel dysfunction. Lastly, the aim and research questions of this academic thesis are formulated.

Clinical indications for Sacral Neuromodulation

Lower urinary tract dysfunction

Lower urinary tract dysfunction (LUTD) includes urinary storage and evacuation symptoms. Most commonly LUTD entails storage symptoms such as urinary incontinence and overactive bladder (OAB) and evacuation symptoms such as voiding dysfunction. OAB, a symptom complex including urinary urgency, frequency and urgency incontinence, has a prevalence of approximately 12% which is increasing with age in the adult population [1, 2]. Urgency urinary incontinence occurs in half of the female patients and a quarter of the male patients that suffer from OAB [1]. The severity of symptoms is currently established using a voiding diary, containing information on urgency episodes, voiding frequency, voided volume and leaking episodes or a pad test for the incontinence part [3]. The symptoms can objectively be assessed during urodynamic investigation, which is an invasive diagnostic tool in order to measure pressure inside the bladder during filling or voiding with instantaneous measurement of urinary flow. Storage symptoms can vigorously affect quality of life and can also increase the risk of renal failure due to high bladder pressures. The latter usually occurring in patients suffering from OAB associated with neurological diseases.

Conservative treatment for storage symptoms starts with behavioural and lifestyle interventions, followed by pelvic floor physiotherapy or pharmacological interventions with anti-muscarinic or beta-adrenoceptor agonistic drugs. When these treatments work suboptimal and patients still experience symptoms or side effects of the drugs, minimally

invasive techniques are available, i.e. SNM, posterior tibial nerve stimulation (PTNS) and intravesical botulinum toxin (BOTOX) injections [4]. If storage symptoms are still refractory to treatment, more invasive procedures can be used such as bladder augmentation or urinary deviation (urostoma).

Similar symptoms as in patients with storage symptoms, such as urinary urgency and urinary frequency, can occur in patients with chronic voiding dysfunction (evacuation symptoms). In addition, patients can experience weak stream, nocturia, straining and incomplete emptying of the bladder. This may give rise to decreased voiding efficiency and urinary tract infections due to sustained urine residuals in the bladder. This array of symptoms can point into the direction of either lower urinary tract obstruction or an underactive bladder [5, 6]. Due to post-void residuals with an increased risk of urinary tract infections, chronic catheterization is often required [7]. Treatment options for acute urinary retention are catheterization and pharmacological therapy such as alpha blockers and 5-alpha-reductase inhibitors [8]. In addition or in treatment of refractory cases, chronic voiding dysfunction patients can benefit from SNM. SNM can increase voided volume, and reduce post-void residual volume and therefore reduce the need for catheterization [9].

Bowel dysfunction

In the gastrointestinal tract, bowel dysfunction includes storage and evacuation symptoms and covers faecal incontinence (FI) and constipation, respectively. The prevalence of FI in adults is approximately 9% and increasing with age from 3% in young adults to 15% in elderly over 70 years old [10]. Often, the severity of symptoms is determined using validated questionnaires, a bowel habit diary, containing information on defecation frequency, faecal incontinence episodes and ability to delay defecation, diagnostic testing such as defecography, manometry and ultrasound imaging of the sphincter complex [11-15]. Treatments for FI start with dietary adjustments, administration of fibre/bulking agents and constipating agents and pelvic floor physiotherapy, followed by transanal irrigation and minimally invasive procedures such as SNM and PTNS, or invasive neosphincter procedures and colostomy [14, 15].

Sacral Neuromodulation

SNM, or electrical stimulation of the sacral nerves (S2-S4), is an established minimally invasive treatment option for patients with lower urinary tract and bowel dysfunction (storage and evacuation complaints), and has provided patients with a substantial higher quality of life since its introduction in the early 1980's [16, 17]. Currently, approximately 80% or 53% of patients with lower urinary tract or bowel dysfunction, respectively, experience (at least partial) improvements in storage and evacuation during SNM [18, 19]. SNM treatment is considered successful when at least 50% of symptoms based on the patient's voiding/bowel habit diary improve. A reduction of these symptoms typically leads to a better control of micturition and/or defecation, as well as a decrease in the amount of incontinence pads required per day. Nevertheless, despite the 50% improvement in symptoms, most patients still have some storage or evacuation symptoms even with SNM treatment. New developments in the field of SNM not only aim to improve the used hardware (i.e. implantable pulse generator (IPG), leads), but also introduce new stimulation paradigms that could overcome these limitations.

Over the years, many hardware changes have been introduced to the field of SNM, including, but not limited to, the use of the tined lead procedure (TLP), MRI compatible devices, and smaller IPGs, all of which have decreased the occurrence of side effects and patient burden [20-22]. Besides improvements related to SNM hardware also the use of new stimulation paradigms can be helpful to further improve the outcome of this therapy. Interestingly, SNM stimulation parameters have not changed significantly over time. Originally, SNM stimulation frequency of 14 Hz and a pulse duration of 210 µs were used to treat lower urinary tract disorders and this was also adopted for bowel dysfunction [23]. First investigations on the clinical impact of altering stimulation settings for bowel dysfunction is focussed on varying either stimulation frequency or pulse duration [24]. It was reported that clinical efficacy of SNM could be improved by using a shorter pulse width or a higher stimulation frequency, as a decrease in the number of episodes of incontinence, soiling, and faecal urgency was achieved [24]. Hence, new emerging stimulation paradigms, like those based on high

frequency (up to 10kHz) and burst stimulation, used in neuromodulation treatment for other indications and diseases, like chronic pain [25, 26], may potentially be very interesting for application in SNM.

It needs to be stressed that in view of using and implementing new SNM stimulation paradigms for the treatment of lower urinary tract and bowel dysfunction only the effect of frequencies lower than 50Hz have been investigated in clinical research up till now. Although these SNM paradigms might improve the effect, ambiguous results have been noted [24, 27-30]. The use of new SNM paradigms based on high frequency and burst stimulation in the treatment of lower urinary tract and bowel dysfunction is still in its infancy and needs further investigation. In this respect it is important to note that the local spinal networks involved in pain and lower urinary tract and bowel dysfunction are very similar, and thus the use of these new stimulation paradigms as currently used in pain research may also be beneficial in SNM for lower urinary tract and bowel dysfunction [31]. Hence, further fundamental insight into the mechanisms of action underlying these new SNM paradigms for lower urinary tract and bowel dysfunction is essential and may result in optimization of this therapy. In this thesis, new SNM stimulation paradigms and a novel adaptation to the existing SNM hardware are investigated to eventually improve SNM therapy in a clinical setting.

Animal models for FI and LUTD

Over the years, several preclinical models have been developed to mimic lower urinary tract and bowel dysfunction. A recent systematic review by Evers and colleagues summarized all models for FI [32]. In general, animal models could be divided into two groups; models that directly cause specific nerve- or sphincter damage and thereby closely mimic FI, such as nerve crush or external anal sphincter disruption [33-36] and models that indirectly cause nerve and sphincter damage by mimicking an event, that caused FI, such as intrapelvic balloon models [33, 34, 36, 37]. The intrapelvic inflation of the balloons for several hours simulate prolonged and complicated natural delivery, one of the major causes of FI in humans.

Animal models for LUTD can roughly be divided into either models mimicking peripheral causes, i.e. direct damage to the bladder and its innervation, or models mimicking central

causes, i.e. damage to the central nervous system, as noted in neurodegenerative diseases like Alzheimer's disease, Multiple sclerosis and Parkinson's disease. A commonly used animal model for LUTD is the partial bladder outlet obstruction model (pBOO) [38, 39]. This type of obstruction causes spontaneous and frequent detrusor contractions during the filling phase and imperative voiding resembling OAB in humans in the first phase. In a later phase compensatory high pressure voiding and further down the line incomplete emptying consistently in combination with overflow incontinence is noted [40].

In this thesis, an intrapelvic balloon model and the pBOO model in rats are further investigated to examine the behavioural effects for FI and LUTD respectively. Moreover, these models then can be used to investigate the underlying mechanisms of action of SNM in relation to the use of a various range of new stimulation paradigms in future research.

Aims and Research Questions

The primary aim of this academic thesis is to establish two experimental models for either FI or LUTD and to study the effect and use of new SNM stimulation paradigms in the treatment of lower urinary tract or bowel dysfunction. Related to this aim the following Research Questions (RQ) were formulated:

1. What are the recent developments concerning the use and effectivity of SNM stimulation parameters for the treatment of experimental lower urinary tract and bowel dysfunction?
2. Can we establish translational and reproducible animal models for faecal incontinence and lower urinary tract dysfunction?
3. What are the recent developments of SNM stimulation parameters for the treatment of lower urinary tract and bowel dysfunction in a clinical setting?
4. What is the effect of Burst SNM on the urodynamic responses in patients suffering from overactive bladder dysfunction or non-obstructive urinary retention?
5. Is a novel adaptation to the existing SNM hardware safe and feasible for use in patients with bowel dysfunction?

Outline of the thesis

Research Question 1 is addressed in *Chapter 2* and provides an overview of the current literature (systematic review) on SNM stimulation parameters and its effect on lower urinary tract and bowel dysfunction in preclinical research.

The development of translational and reproducible animal models for either FI or LUTD (*RQ2*) is addressed in *Chapters 3* and *4*. *Chapter 3* presents a rat model for FI and focusses on a non-invasive vaginal balloon inflation model and its effect on defecation behaviour, an outcome parameter for FI. We hypothesized that the vaginal balloon inflation model will result in changed defecation behaviour and it was aimed to cause FI in at least 50% of the animals. In *Chapter 4 (RQ2)*, we further investigated a rat model for LUTD, and analysed the effect of a partial bladder outlet obstruction on voiding behaviour, ultrasound imaging and cystometry outcome parameters in adult rats.

Chapter 5 addresses *Research Question 3* and provides an overview of the current literature on SNM stimulation parameters and its effect on lower urinary tract and bowel dysfunction in clinical research.

Chapter 6 presents data related to *Research Question 4*. The effect of Burst SNM on bladder and urethral pressure is explored in patients suffering from LUTD. By the use of urodynamic investigations in patients under anaesthesia, we aimed to evaluate bladder and urethral pressure using Burst SNM.

Chapter 7 addresses *Research Question 5* and explores the safety and feasibility of the Interstim II with replacement adaptor 09106® in patients with bowel dysfunction. We hypothesized that the replacement adaptor 09106® is a safe and feasible solution for patients that could not be offered IPG replacement with the Interstim II solely.

The main findings of this thesis are discussed in the context of current knowledge and future perspectives in *Chapter 8* and summarized in *Chapter 9*.

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Sacral Neuromodulation for Lower Urinary Tract and Bowel Dysfunction in Animal Models: A Systematic Review with Focus on Stimulation Parameter Selection

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Chapter 2

Abstract

Objective: Conventional SNM has shown to be an effective treatment for lower urinary tract and bowel dysfunction, but improvements of clinical outcome are still feasible. Currently, in preclinical research new stimulation parameters are being investigated to achieve better and longer effects. This systematic review summarizes the status of SNM stimulation parameters and its effect on urinary tract and bowel dysfunction in preclinical research.

Methods: The literature search was conducted using three databases: Ovid (Medline, Embase) and PubMed. Articles were included if they reported on stimulation parameters in animal studies for lower urinary tract or bowel dysfunction as a primary outcome. Methodological quality assessment was performed using the SYRCLE Risk of Bias (RoB) tool for animal studies.

Results: Twenty-two articles were eligible for this systematic review and various aspects of stimulation parameters were included: frequency, intensity, pulse width, stimulation signal, timing of stimulation, and unilateral vs bilateral stimulation. In general, all experimental studies reported an acute effect of SNM on urinary tract or bowel dysfunction, whereas at the same time various stimulation settings were used.

Conclusions: The results of this systematic review indicate that SNM has a positive therapeutic effect on lower urinary tract and bowel dysfunction. Using LF-SNM, HF-SNM, bilateral SNM and higher pulse widths showed beneficial effects on storage and evacuation dysfunction in animal studies. An increased variability of stimulation parameters may serve as a basis for future improvement of the effect of SNM in patients suffering from urinary tract or bowel dysfunction.

Keywords: Sacral nerve stimulation, voiding dysfunction, faecal, incontinence, stimulation paradigms

Introduction

Since the introduction in the 1980's sacral neuromodulation (SNM) has been used in patients with lower urinary tract dysfunction [1, 2]. Patients with urinary tract dysfunction reported not only a positive effect of SNM on their lower urinary tract symptoms but also on defecatory complaints. The latter resulted in the first electrical stimulation of sacral nerves for the treatment of faecal incontinence [3].

Nowadays, SNM is an established surgical intervention for urinary tract and bowel dysfunction, more specifically storage and evacuation disorders, intended to treat patients unresponsive to conservative treatment. In urinary tract dysfunction, conservative treatment includes physiotherapy or medication, and in bowel dysfunction, diet and fluid advice, medication, biofeedback therapy, and colonic irrigation. Storage disorders refer to an overactive bladder, a hyposensitive rectum, an underactive urethral or anal sphincter causing incontinence. Evacuation disorders refer to an underactive bladder, slow transit colon, and an overactive urethral or anal sphincter causing retention or constipation.

SNM is a minimally invasive therapy involving chronic electrical stimulation of the third sacral nerve root (S3). Successful SNM for both urinary tract and bowel dysfunction is often defined as an improvement in symptomatology of 50% or more compared to baseline. In an accompanying paper, we systematically reviewed the effect on stimulation parameters for sacral neuromodulation on lower urinary tract and bowel dysfunction related to clinical outcome [4]. These results showed that the efficacy of SNM on both lower urinary tract and bowel dysfunction might be improved by changing stimulation parameters.

From the point of human ethical concerns, in addition to further understanding of the mechanisms of action, the effects and fine-tuning of SNM in the treatment of urinary tract and bowel dysfunctions is studied in animals. The reason to use preclinical animal studies on SNM is three-fold: 1; finding the optimal balance between a positive effect of SNM and potential harmful side effects. 2; fully standardized research in patients with regard to the effect of SNM stimulation paradigms is limited due to ethical concerns and large clinical

variability. 3; implantable pulse generators (IPGs) currently used in clinics are restricted to only a limited range of SNM stimulation settings. In contrast, preclinical studies allow the use of SNM settings beyond conventionally used clinical stimulation paradigms and have previously provided valuable insights into the efficacy and working mechanisms of SNM. To date, no comprehensive overview of the effects of individual SNM parameters for treatment of urinary tract or bowel dysfunction in animal models is available. This systematic review of preclinical literature on SNM and its effect on urinary tract and bowel dysfunction with focus on stimulation parameters fills this gap in literature. The combination of this systematic review on animal studies and a systematic review of the clinical studies of SNM in urinary tract and bowel dysfunction [4] serves as a basis for future improvement towards the effects of SNM in patients suffering from urinary tract or bowel dysfunction.

Methods

Search strategy

A systematic literature search was conducted using three databases: Medline (PubMed), Ovid (Embase) and PubMed. Search terms used for all databases are included in Appendix 1. Results were uploaded to EndNote to assess for relevance. Reviewers were not blinded to author names, institution, or study title. One reviewer (PD) collected the following characteristics of the included studies: the first author, year of publication, species, sex and number of species, model of disorder, control condition, anaesthesia used, stimulation settings, and results of the studies.

Study selection and inclusion criteria

Two reviewers (PD, RA) performed extensive searches of available literature up until January 14th, 2020. This search was a shared search for the present preclinical review and a clinical review (see accompanying paper [4]) and results were allocated to each systematic review after the final study inclusion. Studies were eligible for inclusion when in line with the following inclusion criteria: 1) preclinical or clinical study; 2) intervention of temporary or permanent SNM; 3) comparison of various SNM stimulation parameters. In addition, no language limitations were used and both reviews and meta-analyses were excluded.

Quality of the literature included was assessed by two reviewers (PD, RA) using the SYRCLE Risk of Bias (RoB) tool for animal studies [5]. The items in the RoB tool relate to performance bias, selection bias, attrition bias, detection bias, reporting bias, and other biases. Papers were marked as low risk, high risk, or unclear risk. RoB was rated 'high' if there were expectations of bias; 'unclear' if information was missing, incomplete, or not clear; and 'low' if all items were explained clearly and no bias was found.

Results

Based on the online search 5659 papers were identified and one additional paper was included through hand searching (see Figure 1 for Flow-Chart). Of which 1534 were excluded because of duplications. Due to irrelevance by title and abstract screening, 4042 papers were excluded and 45 more papers were excluded after full text screening. Finally, 39 studies were relevant for inclusion, of which 22 were preclinical studies.

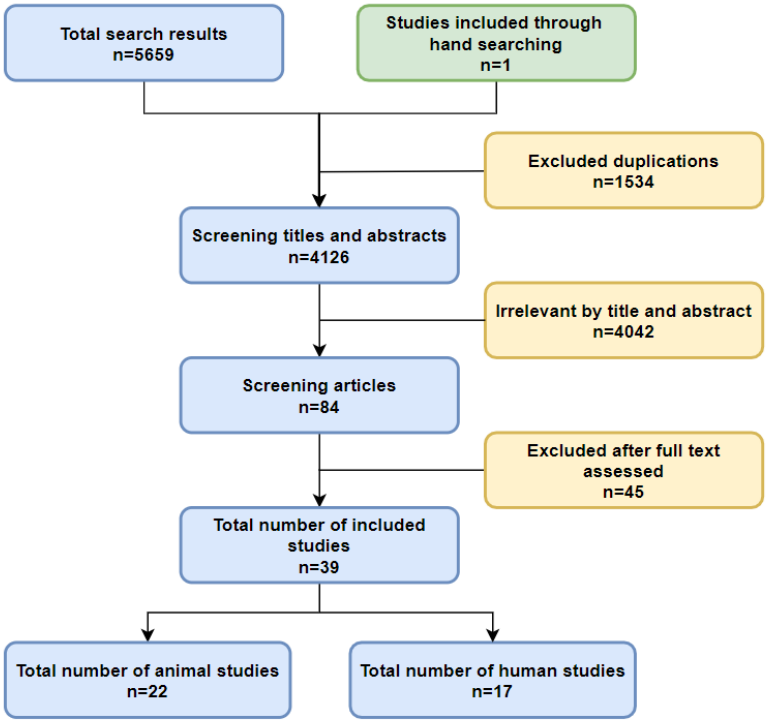

Figure 1. Flowchart of the included studies.

Characteristics of included studies

Papers included and relevant characteristics are shown in Table 1. Within these, a large variability was noted with respect to both approach and use of specific stimulation parameters and outcome parameters as well as the animal species. To analyse the outcome, papers were sub-grouped based on type of SNM stimulation parameters: frequency, intensity, pulse width, stimulation signal, timing of stimulation, and unilateral vs bilateral stimulation. Intensity was often expressed as a percentage of the motor threshold (T_{mot}). T_{mot} is defined as the lowest intensity to evoke pelvic floor muscle contractions, hind toe twitches, or tail twitches. Thereafter, a distinction was made based on outcome parameters: for urinary tract dysfunction: bladder activity, bladder capacity, external urethral sphincter (EUS) activity, and for bowel dysfunction: anal canal cortical evoked potentials (EPs) and rectal volume. Study design and primary outcome of the literature selected are shown in Table 2 (urinary tract dysfunction) and Table 3 (bowel dysfunction).

Table 1. Study characteristics

Study ID	Species	Sex	Amount	Model	Anaesthesia	Procedure
Boger 2012 [14]	Cat	m	6	Detrusor sphincter dyssynergia; induced by S1/S2 stimulation	α-chloralose and isoflurane	Extradural cuff electrode
Braun 2003 [21] **Seif 2003 [22]**	Minipig	?	12	Detrusor hyperactivity induced by 0.25% formalin solution	Anaesthetized, without specification	Transforaminally
Brouillard 2019 [17]	Rat	f	13	Healthy	Urethane and conscious	Transforaminally
Cong 2019 [20]	Pig	both	7	Bladder overactivity induced by 2.5% acetic acid (control saline)	α-chloralose	Transforaminally
Evers 2014 [25]	Rat	f	72	Healthy	Urethane	Transforaminally
Evers 2016 [26]	Rat	f	32	Intra-pelvic balloon inflation	Urethane	Transforaminally
Huang 2019 [27]	Rat	m	39	Constipation induced by 2mg/kg ip loperamide (control saline)	Conscious	Transforaminally, extradural
Kaufman 2009 [23]	Minipig	?	8	Detrusor hyperactivity induced by 0.25% formalin solution	α-chloralose	Laminectomy of segments, intradural
Li 2017 [6]	Pig	both	13	Bladder overactivity induced by 5% acetic acid (control saline)	α-chloralose	Transforaminally, by localizing needle
Li 2018 [7]	Cat	both	7	Pudendal nerve stimulation to mimic bladder underactivity	α-chloralose	Laminectomy of segments, intradural
Potts 2019 [24]	Rat	f	24	Healthy	Urethane	Transforaminally
Shaker 1998 [15]	Dog	m	11	Detrusor sphincter dyssynergia; induced by spinal cord section at T10 level	Isoflurane	Laminectomy of segments, extradural cuff electrode
Sievert 2002 [16]	Dog	m	20	Healthy	Atropine and pentobarbital	Laminectomy of segments, intra- and extradural
Snellings 2012 [8]	Cat	m	14	Healthy	α-chloralose	Extradural cuff electrode
Su 2012 [9]	Rat	f	164	Healthy	Urethane	Removed S1 processes
Su 2013 [10]	Rat	f	159	Healthy	Urethane	Not specified
Su 2016 [11]	Rat	m	31	Bladder overactivity induced by 0.3% acetic acid (control saline)	Urethane	Removed S1 processes
Su 2017 [12]	Rat	f	46	Healthy	Urethane	Removed S1 processes
Su 2017 [13]	Rat	f	126	Healthy	Urethane	Removed S1 processes
Zhang 2013 [18]	Cat	both	19	Healthy	α-chloralose	Dorsal laminectomy, intradural
Zhang 2017 [19]	Cat	both	6	Bladder overactivity induced by 0.5% acetic acid (control saline)	α-chloralose	Dorsal laminectomy, intradural

f, female; ip, intraperitoneal; m, male;

Table 2. Study design and outcome for urinary tract dysfunction

Study ID	Location	Duration	Frequency (Hz)	Pulse width (µs)	Intensity	Extra information	Outcome
Boger 2012 [14]	S2 S1 S1	60 or 90 sec	20 20 12500	100	7 ± 3 Vpp 3 ± 1 Vpp 15 ± 3 Vpp	Intermittent bilateral Continuous Continuous	HF-SNM (12.5 kHz) can prevent EUS activation and allow complete bladder voiding.
Braun 2003 [21], Seif 2003 [22]	S3	10 min interval	15	1000 210	2.0 V	Quasi-trapezoidal 2/40 signal Biphasic rectangular	Quasi-trapezoidal SNM inhibited unstable detrusor contractions more than rectangular SNM.
Brouillard 2019 [17]	S1 left	60 sec, started at the onset of the steep rise in bladder pressure signaling an imminent void.	1000 3000		1 mA	Sinusoidal waveform	HF-SNM can suppress imminent voiding for 35 to 262 sec.
Cong 2019 [20]	S3 left	During bladder filling	14	64 204 624	5.64 ± 0.76 V (T_{mot}) 3.11 ± 0.48 V 2.52 ± 0.49 V		All pulse widths inhibited bladder overactivity compared to acetic acid levels. Motor threshold for pulse width of 64 µs was significantly higher than the other two thresholds.
Kaufman 2009 [23]	S3 DRT	Every 5 min	15	210	2.0 V	Unilateral left Unilateral right Bilateral	Bilateral SNM reduced overactive detrusor contractions better compared to unilateral SNM.
Li 2017 [6]	S3		5, 15, 30, 50	210	4 V		SNM at 15, 30 and 50 Hz increased bladder capacity. Frequencies higher than 15 Hz did not lead to better outcomes.
Li 2018 [7]	S1/S2 DRT	During bladder filling	15 30	200	1-1.5x, 1.5-2x T_{mot} 1x, 1-1.5x, 1.5-2x T_{mot}		S1 SNM (15-30 Hz; 1-1.5x T_{mot} - 1.5-2x T_{mot}) blocked pudendal inhibition.
Potts 2019 [24]	L6/S1	During bladder filling	10	100	Just below T_{mot}	Bilateral, biphasic	SNM between 50-100% and 75-100% of bladder filling cycle increases bladder capacity over control fills.
Shaker 1998 [15]	S2 and S1 left and right	20 sec	30 600	180 60 175 150 60 20 - 500	1.8 mA 0.9 mA 0.3 - 1.5 mA 0.7 - 1.5 mA 0.7 mA		HF-SNM reduced urethral pressure. Optimal blocking parameters are 600 Hz, 60 µs, 1,3 mA. Bilateral SNM did not increase the blocking effect.

				100	1.1 - 1.5 mA		
Sievert 2002 [16]	S1/S2/S3		1 - 1000	220	0.08 - 10 V	Unilateral Bilateral	Bilateral S3 stimulation increased bladder pressure sign better than unilateral SNM.
Snellings 2012 [8]	S1	for 30s after absolute bladder pressure	2, 5, 7.5, 10, 15, 20, 30	100	0.8, 1, 2x T_{mot}	Unilateral	S1 SNM at 7.5 or 10 Hz and 2x T_{mot} showed maximum inhibition of the normal number of bladder contractions.
Su 2012 [9]	L6	10 min	10 0.01, 0.1, 1, 20, 50, 100	100	2, 3, 4, 6x T_{mot} 6x T_{mot} (=0.6 mA)	Bilateral	SNM at 10 Hz and high intensity (2 - 6x T_{mot}) showed maximum inhibition of the number of bladder reflex contractions.
Su 2013 [10]	L6 left	10 min	0.5 10	100	0.6 mA (supra T) 0.6 mA, 1x T_{mot} 0.8, 1x T_{mot}	 Bilateral, pulse match/mismatch	Bilateral L6 SNM was more effective than unilateral SNM using a bladder rhythmic contraction model.
Su 2016 [11]	L6	On every other void	1 10 50	100	1x T_{mot} 0.5, 1, 2x T_{mot} 1x T_{mot}		High intensity SNM in most effective for altering bladder activities.
Su 2017 [12]	L6	10 min	10	30 60 90 120 210	0.11 ± 0.02 mA (T_{mot}) 0.12 ± 0.02 mA (T_{mot}) 0.19 ± 0.03 mA (T_{mot}) 0.12 ± 0.03 mA (T_{mot}) 0.16 ± 0.03 mA (T_{mot})		All pulse widths showed inhibition of bladder activity, and no significant differences were found between pulse widths tested. Optimal pulse width was 40 µs.
Su 2017 [13]	L6	15 min	0.01, 0.1, 1, 4, 10, 40, 100 Interburst 0.01 - intraburst 0.1, 1, 10, 40, 1000 Interburst 0.1 - intraburst 1, 10, 40, 100, 1000 Interburst 1 - intraburst 10, 40, 100, 1000	100	1 T_{mot}	Bilateral biphasic	SNM in burst patterns reduced the number of bladder contractions with an optimum of a four-pulse burst interburst of 1 Hz and intraburst of 40 Hz, but this burst pattern did exceed a continuous stimulation of 10 Hz.
Zhang 2013 [18]	S1/S2/S3 DRT or VRT		5 15 30	200	0.25, 0.5, 1, 2x T_{mot} 1x T_{mot} 1x T_{mot}		SNM at 5 Hz was optimal for increasing bladder capacity. S1 DRT SNM was more effective than S2 DRT.
Zhang 2017 [19]	S1/S2/S3 DRT or VRT		5 15 30	200	0.25, 0.5, 1x T_{mot} 1x T_{mot} 1x T_{mot}		SNM at 5 Hz (S1/S2; 1x T_{mot}) inhibited bladder overactivity and increased bladder capacity.

DRT, dorsal root; EUS, external urethral sphincter; HF-SNM, high frequency sacral neuromodulation; SNM, sacral neuromodulation; T_{mot}, motor threshold; VRT, ventral root.

Table 3. Study design and outcome for bowel dysfunction

Study ID	Location	Duration	Freq (Hz)	Pulse width (µs)	Intensity	Extra info	Outcome
Evers 2014 [25]	S1 left	30min, 3min, 18 s, 1.8 s 3 min	0.1, 1, 10, 100 0.1, 1, 10, 25, 100 2	1000	15 V 0.25, 0.5, 0.75x T_{mot}	No of pulses is 180 No of pulses is 18, 180, 1800, 4500, 18000 Optimal frequency	Optimal frequency for anal canal cortical EPs is 2 Hz. SNM at 0.5x, 0.75x and 1x T_{mot} increased anal EPs compared to 0x and 0.25x times T_{mot} in rats.
Evers 2016 [26]	S1 left	10 min	2 14	1000	1x T_{mot}		Optimal frequency for anal canal cortical Eps is 2 Hz.
Huang 2019 [27]	S3 right		5 15 30	100 210 100 210 500 210	90% of T_{mot}		SNM inhibited constipation best using 5 Hz, 100 µs and 90% of T_{mot}.

EPs, evoked potentials; SNM, sacral neuromodulation; T_{mot}, motor threshold.

Risk of bias assessment/methodological quality

A methodological quality assessment was performed on all papers included (Table 4). In general, randomization (item 1, 4, 6), concealment (item 3), blinding (item 5, 7), and missing data (item 8) were poorly reported. Conversely, papers were free of selective reporting (item 9) and mentioned baseline characteristics (item 2). Other potential bias that could have led to high risk (item 10) regarded anaesthesia used during the experiment and missing data about materials and stimulation parameters.

Table 4. Quality assessment SYRCLE's Risk of Bias

SYRCLE's RoB		1	2	3	4	5	6	7	8	9	10
		Selection bias 1	Selection bias 2	Selection bias 3	Performance bias 1	Performance bias 2	Detection bias 1	Detection bias 2	Attrition bias	Reporting bias	Other potential bias
Boger et al. [14]	2012	✗	−	−	✗	−	−	−	✓	✓	✗
Braun et al. [21]	2003	✗	✓	−	✗	−	−	−	−	✓	✗
Brouillard et al. [17]	2019	✗	✓	−	✗	−	−	−	✓	✓	−
Cong et al. [20]	2019	✗	✓	−	✗	−	−	−	✓	✓	✗
Evers et al. [25]	2014	✗	✓	−	−	−	−	−	✓	✓	−
Evers et al. [26]	2016	✗	✓	−	−	−	✓	✓	✗	✓	−
Huang et al. [27]	2019	−	✓	−	−	−	−	−	✓	✓	−
Kaufmann et al. [23]	2009	✓	✓	−	✗	−	−	−	✓	✓	✗
Li et al. [6]	2017	✗	✓	−	✗	−	✓	−	✓	✓	✗
Li et al. [7]	2018	✗	✓	−	✗	−	−	−	✗	✓	✗
Potts et al. [24]	2019	−	✓	−	−	−	✓	−	✓	✓	−
Seif et al. [22]	2003	✗	✓	−	✗	−	−	−	−	✓	✗
Shaker et al. [15]	1998	✗	✓	−	✗	−	−	−	−	✓	✗
Sievert et al. [16]	2002	✗	✓	−	✗	−	✓	−	−	✓	✗
Snellings et al. [8]	2012	✓	−	−	✗	−	−	−	✗	✓	✗
Su et al. [9]	2012	✗	✓	−	✗	−	−	−	−	✓	✗
Su et al. [10]	2013	−	✓	−	✗	−	−	−	−	✓	−
Su et al. [11]	2016	✓	✓	−	✗	−	−	−	−	✓	✗
Su et al. [12]	2017	✗	✓	−	✗	−	−	−	−	✓	−
Su et al. [13]	2017	−	✓	−	✗	−	−	−	−	✓	−
Zhang et al. [18]	2013	✓	✓	−	✗	−	−	−	−	✓	✗
Zhang et al. [19]	2017	✗	✓	−	✗	−	−	−	✓	✓	✗

1: ✓ = adequate randomization; − = randomization but no details; ✗ = no evidence of randomization; **2**: ✓ = all baseline characteristics given; − = not all baseline characteristics given; ✗ = baseline characteristics not given; **3**: ✓ = evidence of allocation concealed; − = no information on concealment of allocation; ✗ = evidence of inadequate concealment of allocation; **4**: ✓ = evidence of random housing of animals; ✗ = no information on housing agreement at all; **5**: ✓ = evidence of caregivers/investigators were blinded to intervention; − = no information on blinding to intervention; ✗ = evidence of non-blinding to intervention; **6**: ✓ = evidence of random selection for assessment; − = no information on random selection for assessment; ✗ =evidence of non-random selection for assessment; **7**: ✓ = evidence of assessor blinded; − = no information on assessor blinding; ✗ = evidence of non-blinded assessor; **8**: ✓ = explanation of animal missing data; − = no information if all animals were included in final analysis; ✗ = no explanation of missing animal data; **9**: ✓ = free of selective reporting based on methods/results; ✗ = selective reporting; **10**: ✓ = free of other high bias risk; − = insufficient data to determine risk of other bias; ✗ = existence of problems with potential for high risk of bias

SNM and Urinary tract dysfunction

Effect of Frequency

Significant improvement of urinary tract dysfunction in animals has been found in several studies investigating SNM frequencies below 100 Hz (Table 5). Although not all studies on the effects of SNM frequency were performed with similar stimulation intensity or pulse width, stimulation seems to be optimal within a frequency range of 7.5-15 Hz [6-12]. The use of SNM at various frequencies (0.01-100 Hz) was shown to significantly reduce the number of bladder contractions per minute [9, 10, 13] or bladder pressure [8] using frequencies from 0.05-50 Hz, with the best results applying 4, 7.5, and 10 Hz stimulation. SNM in burst patterns (4-6 pulse burst; interburst 0.01-1 Hz, intraburst 0.1-1000 Hz) reduced the number of bladder contractions per minute with an optimum of a four-pulse burst 1 Hz interburst and 40 Hz intraburst [13], although this burst pattern did not exceed a continuous stimulation of 10 Hz.

Table 5. Frequency and outcome in urinary tract dysfunction

Frequency (Hz)	Bladder activity	Bladder capacity	EUS activity	Reference
0.01 - 5	0	?		[6, 8-11, 13, 18, 19]
7.5 - 15	−	+	+	[6-11, 13, 16, 18, 19]
20 - 40	?	?	+	[6-11, 13-16, 18, 19]
50	−	?		[6, 9, 11]
100 - 200	0		−	[9, 13, 16]
600 - 12500	−		−	[14, 15, 17]

− = inhibition or decrease; 0 = no changes; + = excitation or increase; ? = ambiguous outcome

Contradictory effects were reported [14-16] as low frequency (LF)-SNM (10-30 Hz) was shown to affect voiding in dyssynergic reflexive EUS activity and showed elevated bladder and EUS pressure. The best SNM frequencies that inhibit EUS for voiding were 20 and 100 Hz, which resulted in the most optimal bladder response and the lowest EUS pressure and the fastest EUS fatigue, respectively [16]. Stimulation at 30 Hz was applied to evoke maximal bladder pressure. Nonetheless, this stimulation also evoked EUS pressure, since the intensity threshold for EUS pressure is lower than the intensity threshold for bladder pressure [15].

With focus on uncoordinated contractions of bladder and EUS, detrusor sphincter dyssynergia was induced by providing intermittent bilateral S2 SNM at 20 Hz to evoke bladder pressure and continuous S1 SNM at 20 Hz to evoke EUS pressure [14]. The use of high frequency (HF)-SNM (12.5 kHz, 600 Hz or 200 Hz) allowed voiding, caused by a possible EUS blockade [14, 15] or stimulation related EUS fatigue, which seemed closest to normal voiding [16]. The optimal blocking parameters (600 Hz; 60 µs; 1.3 mA) caused a maximum blocking of EUS pressure and a minimal blocking of bladder pressure [15]. HF-SNM at 1 or 3 kHz was reported to suppress imminent voids for 35-262 sec when SNM was applied for 60 sec after the onset of an imminent void. Bladder pressure continued to rise steeply after the SNM onset and reduced rapidly to a lower level for the remaining 60 sec SNM until a void occurred [17].

The effect of different frequencies on bladder capacity in healthy and acetic acid (a.a.) induced overactive bladder (OAB) animals was investigated [6, 7, 11, 18, 19]. SNM at 5 Hz increased bladder capacity (defined as inhibition of isovolumetric bladder contractions) in healthy and a.a. induced OAB in cats, whereas SNM at 15 and 30 Hz did not change bladder capacity [18, 19]. Contrarily, SNM at 15, 30, and 50 Hz was shown to increase bladder capacity (defined as infused volume) in a.a. induced OAB pigs equally effective while SNM at 5 Hz did not change bladder capacity [6, 7]. Conversely, the use of SNM at 10 Hz increased bladder function significantly as compared to sham, while at the same time, 1 Hz and 50 Hz SNM were ineffective [11].

Effect of Intensity

Stimulation intensities ranging from 0x to 6x T_{mot} (Table 6) were studied in animals with an overall optimal intensity of at least 1x T_{mot}. Higher intensity (2x T_{mot}) SNM caused significantly greater reductions in bladder activity than 0.8x or 1x T_{mot} [8]. Stimulation at T_{mot} caused a delayed inhibition of the number of bladder reflex contractions per minute and stimulation at high intensity (2x - 6x T_{mot}) resulted in a prolonged inhibition in rats [9]. Furthermore, the intensity required to cause significant inhibition of bladder activity was higher for unilateral

SNM (2x T_{mot}) compared to bilateral SNM (0.8x T_{mot}), suggesting that bilateral SNM at the same intensity results in more/prolonged inhibition [10].

SNM increased bladder capacity at 1x and 2x T_{mot} as compared to 0x, 0.25x, and 0.5x T_{mot} [18, 19]. Similar results were reported and showed that bladder capacity during 1x T_{mot} was significantly larger than sham, while bladder capacity during 2x T_{mot} was significantly larger than sham, 0.5x, and 1x T_{mot} [11]. SNM at S1 (1-1.5x T_{mot}) applied together with pudendal nerve stimulation (PNS), used as a model to partly mimic bladder underactivity, blocked PNS inhibition and decreased bladder capacity to control levels, whereas SNM alone did not increase bladder capacity. SNM applied at 1.5-2x T_{mot} increased bladder capacity compared to control, and together with PNS, SNM blocked PNS inhibition. These results were not seen for SNM at S2, which only showed a significant increase when SNM alone at 1.5-2x T_{mot} was applied [7].

Table 6. Intensity and outcome in urinary tract dysfunction

Intensity (x T_{mot})	Bladder activity	Bladder capacity	Reference
0.25 - 0.4		0	[18, 19]
0.5 – 0.9	0	0	[8, 10, 11, 18, 19]
1 – 1.9	?	+	[7-11, 18]
2 - 6	−	+	[8-11, 18]

− = inhibition or decrease; 0 = no changes; + = excitation or increase; ? = ambiguous outcome

Effect of Pulse Width

SNM pulse widths significantly affected bladder capacity and inhibited bladder activity in animals. In order to detect the optimal pulse widths that achieve the best clinical effects, pulse widths of 64 µs, 204 µs, and 624 µs (F=14Hz) were analysed and shown to significantly increase bladder capacity as compared to the a.a. control level [20]. Pulse widths between 30 µs and 210 µs (F=10Hz) were shown to significantly inhibited bladder activity [12]. In neither of the studies, significant differences were noted between the pulse widths tested. The latter may be related to the various stimulation intensities used, which were provided at T_{mot}. An inverse exponential correlation was found between pulse width and corresponding

T_{mot} (intensity) and the optimal pulse width determined by T_{mot} was 40 µs in one study [12]. The other study concluded that for a clinical approach, a pulse width of 204 µs might be more appropriate for SNM in patients to optimize battery life and maintain patient comfort during stimulation [20].

Effect of Stimulation signal

Several stimulation signals can be delivered to modulate the effect of SNM in animals. Sinusoidal SNM almost doubled the EUS pressure when compared to rectangular SNM using similar amplitudes [16]. Using rectangular SNM, evoked bladder pressure decreased with an increased amplitude (higher than 6 V) which did not occur using sinusoidal SNM. It was concluded that rectangular SNM has many harmonics that stimulate other nerve fibres and that 'cleaner' sinusoidal SNM may result in a more organ-specific stimulation [16, 21, 22]. Further analysis of the effect of stimulation signal revealed that quasi-trapezoidal SNM inhibited induced detrusor overactivity significantly more than the conventional rectangular SNM [21, 22].

Effect of Unilateral versus bilateral SNM

Bilateral SNM in animals was shown to significantly increase bladder pressure [16] and decreased the number of hyperactive detrusor contractions more than unilateral SNM [10, 23]. Interestingly, the intensity threshold for bilateral SNM was 0.8x T_{mot}, and for unilateral SNM 2x T_{mot}. No significant differences were found in bilateral stimulation using balanced (time-matched pulses) and unbalanced (time-mismatched pulses) current intensities [10].

Effect of timing

One study focused on the timing of SNM in relation to a specific phase of the bladder filling cycle. SNM delivered between 50-100% and 75-100% of the bladder filling cycle increased bladder capacity with 32% and 43%, respectively, over control fills. SNM delivered in the first 50% of the bladder filling cycle had no effect on bladder capacity [24].

SNM and Bowel dysfunction

Effect of Frequency

A few studies focussed on analysis of SNM parameters for bowel dysfunction [25-27]. It was noted that frequency of SNM on amplitude of anal canal EPs is highly significant in healthy rats [25]. Various frequencies (0.1 - 100Hz) were tested and showed that 1 and 10 Hz stimulation significantly increased the amplitude of EPs compared to other frequencies (Table 7). Using a graph of non-linear curve fitting for each time point, a frequency-potentiation relationship parabolic in form with a clear optimum at 2 Hz in these healthy rats was reported [25]. These findings were further substantiated in a rodent model of faecal incontinence where SNM restored the decreased EPs after pudendal nerve injury and 2 Hz SNM also enhanced the decreased EPs more than 14 Hz [26].

Table 7. Frequency and outcome in bowel dysfunction

Frequency (Hz)	Anal canal EPs	Rectal volume	Reference
0.1 - 5	+	+	[25-27]
10 - 15	+	+	[25-27]
25 - 30	?	+	[25, 27]
100	0		[25]

0 = no changes; + = excitation or increase; ? = ambiguous outcome

In the treatment of constipation, SNM significantly increased rectal volume. All frequencies tested increased rectal volume in rodents, with a reported optimal frequency of 5 Hz and an optimal pulse width of 100 µs [27].

Effect of Intensity

Using the reported optimal frequency of 2 Hz, various stimulation intensities (0.25x, 0.5x, 0.75x, 1x T_{mot}, and sham) were investigated in healthy rats [25]. SNM stimulation at 0.5x, 0.75x, and 1x T_{mot} significantly increased the amplitude of EPs compared to sham stimulation (Table 8).

Table 8. Intensity and outcome in bowel dysfunction

Intensity (x T_{mot})	Anal canal EPs	Reference
0.25	0	[25]
0.5	+	[25]
0.75	+	[25]
1	+	[25]

0 = no changes; + = excitation or increase

Discussion

This systematic review aimed to investigate the effects of various stimulation parameters of SNM for urinary tract and bowel dysfunction in animals. In general, all studies reported an acute effect of SNM on urinary tract or bowel dysfunction while various stimulation settings were used.

LF-SNM of 7.5-15 Hz appeared to be optimal in inhibiting bladder contractile activity and increasing bladder capacity, which is useful for patients with urinary incontinence, whereas HF-SNM inhibited EUS activity and caused voiding. It is important to note that some SNM frequencies within this range have only been studied to a limited extent, which might over- or underestimate the effect. Ambiguous results were reported using LF-SNM. Three studies reported that LF-SNM increased bladder activity [14-16] and three studies reported that SNM inhibited bladder activity [8-10]. No clear explanation for these contradictory results was given causing the underlying mechanisms to warrant further research. Interestingly, studies that reported an increase in bladder activity applied SNM at L6 in female Sprague Dawley rats [9, 10] and S1 in male cats [8], whereas studies that reported a decrease in bladder activity applied SNM at S2 in male cats [14] and S1-S3 in male dogs [15, 16]. These results may be caused by the various animal species used. But these results may also suggest that location of SNM is important as SNM at L6-S1 may increase the EUS activity and concomitantly inhibit bladder activity, whereas SNM at S1-S3 may shift the balance in the opposite direction.

HF-SNM was reported to block or fatigue EUS pressure and allow voiding [14-16]. At the same time HF-SNM was reported to suppress acute imminent voids [17]. All studies reported low bladder pressure when HF-SNM was applied. It is highly probable that HF-SNM first increases EUS pressure and therefore suppresses voiding and after a short period decreases EUS pressure due to fatigue and thereafter allows voiding.

SNM at low frequencies that seem to be optimal in inhibiting bladder activity and increasing bladder capacity are similar to the conventional frequencies used in the clinic. Similarly, high frequencies that seem to cause voiding are not used. These high frequencies may have clinical utility for patients with voiding dysfunction. Moreover, in clinically related areas like anaesthesiology and pain management, the use of spinal cord stimulation in treatment of chronic neuropathic (low back) pain has been shown to benefit from HF (10 KHz) [28]. As the spinal network underlying bladder control and defecation has similarities with the nociceptive spinal network it is not unreasonable to speculate that similar HF paradigms may also achieve significant effects for lower urinary tract and bowel dysfunction [29].

For bowel dysfunction, the preferred SNM frequencies to increase anal canal EPs and rectal volume are lower than the conventional frequencies used in clinical settings. In addition, the preferred pulse width to increase rectal volume is lower, whereas the stimulus intensity seems equal to clinical settings.

For urinary tract dysfunction, values above 1x T_{mot} were optimal in achieving a positive acute effect. Only two papers showed an effect of sub-T_{mot} SNM [10, 24], and it is noteworthy that inhibition of bladder activity was reported in animals that received bilateral SNM [10, 24] but not in animals that received unilateral SNM [8, 10]. In additional papers, it was concluded that bilateral stimulation increased bladder pressure and decreased the number of hyperactive detrusor contractions more than unilateral SNM with similar stimulation settings [16, 23]. This may suggest that bilateral SNM is more effective at lower intensities than unilateral SNM. However in clinical studies, bilateral SNM was not reported to be more effective than unilateral SNM [30] and chronic SNM in clinical setting is always applied at (sub)sensory threshold which is significantly lower than the motor threshold [31].

Preferred SNM frequencies for bowel dysfunction (2-5 Hz) are lower compared to urinary tract dysfunction (10 Hz) in animal models, whereas bowel dysfunction (31 Hz) in clinical settings is treated with higher SNM frequencies compared to urinary tract dysfunction (16 Hz). In contrast, experimental studies on bowel dysfunction apply lower intensities to achieve a positive effect compared to urinary tract dysfunction. In this respect, it should be noted that most experimental studies were performed under anaesthesia which has been shown to affect outcome measurements. Under conscious conditions, stimulation at T_{mot} may cause an unpleasant perception or paraesthesia.

The majority of the SNM pulse widths applied in the experimental studies on urinary and bowel dysfunction are similar to conventional settings used in clinical settings. The inverse exponential correlation and the optimal pulse width based on T_{mot} (40µs) was later reported to be the identical in anaesthetized and awake sheep [32]. This inverse exponential correlation suggests that the SNM total charge per second is relevant for a positive effect [20]. However, when the total number of pulses for each frequency-duration combination was fixed at 180, not all combinations presented the same outcome in anal canal EPs. This suggests that SNM total charge is not important for the outcome. Only 0.1 and 1 Hz showed a significant effect, whereas 10 and 100 Hz did not. It is worth noting that the settings with a significant effect had the longest SNM duration; 30 min and 3 min, respectively. Other settings had a SNM duration of 18 sec and 1.8 sec [25]. This may suggest that a minimal SNM duration is required to detect a significant outcome and that SNM total charge is still important for clinical outcomes.

Furthermore, SNM during the last 50% of bladder filling appeared to be the most optimal [24]. In addition, a sinusoidal and quasi-trapezoidal signal seemed to be more organ-specific and inhibited induced detrusor overactivity more, respectively [21, 22]. Both results were only reported in one study making it hard to draw definite conclusions from this data.

Previous research in the field of urology improved the understanding of the mechanisms of action behind SNM. It was once thought that an efferent EUS motor response causing detrusor relaxation was involved in the mechanisms of action underlying SNM. This theory

solely is unlikely as SNM has shown to work when no EUS contractions are observed [33]. Another theory supports activation of sensory nerves; research into the latency of the motor response (i.e. anal sphincter contraction) measured in women implied that the response is reflex mediated [34]. In this context it is also important to note that, although with use of intravaginal electrical stimulation in cat, the involvement of reflex pathways when recording efferent nerves to the bladder was noted [35]. Research into cortical changes showed increased cortical activity after acute SNM and reduced cortical activity after chronic SNM. These observations were seen in both urinary and faecal incontinence [36]. In addition, areas involved in micturition, awareness, and alertness showed changes in regional cerebral blood flow (rCBF) in the brain following SNM. This results display an increased awareness of bladder filling and pelvic floor contraction after SNM suggesting SNM restores normal urinary continence [37].

In line with this hypothesis, in cats dorsal root SNM (afferent pathways) at intensity threshold inhibited bladder activity more than ventral root SNM (efferent pathways) [18]. Moreover, low intensity SNM in rodents showed increased bladder capacity which was reported to be afferent mediated, whereas high intensity SNM additionally attenuated the bladder contraction amplitude and was reported to be efferent mediated [9]. These results suggest that low intensity SNM only activates large myelinated fibers while high intensity SNM additionally activates unmyelinated C-fibers [9, 10, 38].

Limitations within the reviewed studies for both urinary tract and bowel dysfunction were, mainly, the methodological quality assessment [5]. Due to poor reporting most RoB items were scored with ‘unclear’ risk for all studies. Only a few studies reported randomization or blinding while most studies did not report such details. Likewise, data required for replication of the experiment was missing, such as stimulation settings, device specification, and animal characteristics. More precise reporting is required to achieve higher quality animal studies during future investigations.

Furthermore, the diversity in outcome parameters and SNM settings made it difficult to compare studies. Primary outcome parameters (bladder activity, bladder capacity, EUS

activity, and anal canal EPs) were not one on one comparable making it challenging to determine an optimal effect. Moreover, stimulation settings often differed. For example, stimulations with similar frequencies noted various results, which could be due to the large variability in other stimulation settings, such as intensity, duration, and location. Furthermore, use of heterogeneous animals across studies may result in differential effects of stimulation using similar stimulation settings. Looking forward, a more standardized methodological approach is needed for further research.

In summary, in animal studies we found that LF-SNM of 7.5 - 15 Hz appeared to be optimal for storage dysfunction. HF-SNM is shown to facilitate bladder evacuation. Bilateral SNM and pulse widths above conventional settings allow a reduction in stimulus intensity to diminish aberrant perceptions. For bowel dysfunction it was difficult to make any firm conclusions, but studies showed that a frequency of 2-5 Hz and a stimulus intensity below 1x T_{mot} was preferred for both storage and evacuation dysfunction. The findings of this review should be interpreted with caution since the methodologies within the studies analyzed were assessed with 'unclear' risk of bias and too diverse to present comprehensive and precise conclusions toward optimal settings in clinical practice. A more standardized methodological approach is needed for further research.

Acknowledgements

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Reference

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Appendix A: Search Terms

All search strategies are based on work published in:

Riemsma R, Hagen S, Kirschner-Hermanns R, Norton C, Wijk H, Andersson KE, Chapple C, Spinks J, Wagg A, Hutt E, Misso K, Deshpande S, Kleijnen J, Milsom I. Can incontinence be cured? A systematic review of cure rates [Internet]. BMC Med. 2017 [accessed 12.3.18];15(1):63. Available from:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5364653/

Embase (ovid): 1974-2020/01/13

Searched 14.1.2020

1. incontinence/
2. continence/
3. (incontinen$ or continen$ or obstipat$).ti,ab,ot.
4. urine incontinence/ or mixed incontinence/ or stress incontinence/ or urge incontinence/
5. ((Urine$ or urinary or urinat$ or micturat$ or bladder$) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or escaping or uncontrolled or trickl$ or "lack of control" or "no control" or "out of control" or "not voluntary" or involuntary or wetting or leaked or seeped or retention$ or retain$ or dysfunct$ or malfunct$ or obstruct$ or block$ or overactiv$ or over-activ$)).ti,ab,ot.
6. (bladder$ adj3 control$).ti,ab,ot.
7. (SUI or OAB or BPS).ti,ab,ot.
8. "giggle enuresis".ti,ab,ot.
9. "enuresis risoria".ti,ab,ot.
10. (incontinentia urinae or enuresis ureterica or ureter enuresis or enuresis diurnal).ti,ab,ot.
11. ((Unable or inabilit$ or abilit$ or able) adj3 control$ adj3 (urine$ or urinat$ or urinary or micturat$)).ti,ab,ot.
12. neurogenic bladder/
13. ((neurogenic$ or neurologic$ or spinal or spastic$) adj4 bladder$).ti,ab,ot.
14. neurogenic vesical dysfunct$.ti,ab,ot.
15. (Bladder sphincter dys?ynergia or detrusor sphincter dys?ynergia or neurogenic detrusor overactiv$).ti,ab,ot.
16. feces incontinence/
17. (Encopresis or incontinentia alvi).ti,ab,ot.
18. ((bowel$ or rectum or rectal$) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or uncontrolled or trickl$ or "lack of control" or "no control" or "out of control" or "not voluntary" or involuntary or control$)).ti,ab,ot.
19. ((Unable or inabilit$ or abilit$ or able) adj3 control$ adj3 (faeces or faecal$ or feces or fecal$ or stool$ or rectum or rectal$ or bowel$ or bladder$ or anal$ or anus or urine or urinary or diarrh$ or soiling)).ti,ab,ot.

20. ((feces or faeces or fecal$ or faecal$ or stool or stools or defecat$ or soiling) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or escaping or uncontrolled or trickl$ or "not voluntary" or involuntary or control$)).ti,ab,ot.
21. ((diarrh$ or Pseudodiarrh$ or Pseudo-diarrh$) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or uncontrolled or trickl$ or "not voluntary" or involuntary or control$)).ti,ab,ot.
22. ((Unable or inabilit$ or abilit$ or able) adj3 control$ adj3 (diarrh$ or Pseudodiarrh$ or Pseudo-diarrh$)).ti,ab,ot.
23. ((bowel$ or rectum or rectal$ or defecat$) adj4 (disorder$ or malfunction$ or dysfunction$ or evacuat$ or obstruct$ or block$)).ti,ab,ot.
24. ((feces or faeces or fecal$ or faecal$ or stool or stools or defecat$ or soiling) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or escaping or uncontrolled or trickl$ or "not voluntary" or involuntary or control$)).ti,ab,ot.
25. (urinary tract adj3 (dysfunct$ or disorder$ of syndrome$)).ti,ab,ot.
26. (LUTD or LUTS).ti,ab,ot.
27. (pelvic floor adj3 (dysfunct$ or disorder$ of syndrome$)).ti,ab,ot.
28. ((feces or faeces or fecal$ or faecal$ or stool or stools or defecat$ or soiling) adj2 (store or stored or storag$) adj2 (disorder$ or dysfunct$ or malfunct$ or syndrome$)).ti,ab,ot.
29. ((disorder$ or difficult$ or syndrome$) adj4 (urine$ or urinat$ or urinary or micturat$ or bladder$)).ti,ab,ot.
30. overactive bladder/
31. (detrusor adj2 (overactiv$ or over-activ$)).ti,ab,ot.
32. cystitis/ or interstitial cystitis/
33. ((pain$ or discomfort$ or inflamm$ or infect$) adj4 (urine$ or urinat$ or urinary or micturat$ or bladder$ or pelvis or pelvic)).ti,ab,ot.
34. (megacystitis or cystitis or pericystitis).ti,ab,ot.
35. (detrusor adj2 (overactiv$ or over-activ$)).ti,ab,ot.
36. ((bladder$ or hunner or hunneri or submucos$ or sub-mucos$) adj2 (ulcus or ulcer$)).ti,ab,ot.
37. or/1-36
38. sacral nerve stimulation/
39. InterStim.ti,ab,ot.
40. (SNS or SNM).ti,ab,ot.
41. (sacral adj3 (neuromodulat$ or neuro-modulat$ or deafferent$ or de-afferent$ or neurostimulat$ or neuro-stimulat$)).ti,ab,ot.
42. medical electrical stimulation therap$.ti,ab,ot.
43. ((bladder$ or sacral$) adj2 (Autoaugment$ or Auto-augment$)).ti,ab,ot.
44. (sacral nerve$ adj3 (modulat$ or stimulat$)).ti,ab,ot.
45. or/38-44
46. 37 and 45

Medline (Ovid): 1946-2020/01/13

Searched 14.1.2020

1. Fecal Incontinence/
2. exp Urinary Incontinence/

3. Urinary Bladder, Neurogenic/
4. Urinary Bladder, Overactive/
5. cystitis/ or cystitis, interstitial/
6. urination disorders/ or urinary retention/
7. (incontinen$ or continen$ or obstipat$).ti,ab,ot.
8. ((Urine$ or urinary or urinat$ or micturat$ or bladder$) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or escaping or uncontrolled or trickl$ or "lack of control" or "no control" or "out of control" or "not voluntary" or involuntary or wetting or leaked or seeped or retention$ or retain$ or dysfunct$ or malfunct$ or obstruct$ or block$ or overactiv$ or over-activ$)).ti,ab,ot.
9. (bladder$ adj3 control$).ti,ab,ot.
10. (SUI or OAB or BPS).ti,ab,ot.
11. "giggle enuresis".ti,ab,ot.
12. "enuresis risoria".ti,ab,ot.
13. (incontinentia urinae or enuresis ureterica or ureter enuresis or enuresis diurnal).ti,ab,ot.
14. ((Unable or inabilit$ or abilit$ or able) adj3 control$ adj3 (urine$ or urinat$ or urinary or micturat$)).ti,ab,ot.
15. ((neurogenic$ or neurologic$ or spinal or spastic$) adj4 bladder$).ti,ab,ot.
16. neurogenic vesical dysfunct$.ti,ab,ot.
17. (Bladder sphincter dys?ynergia or detrusor sphincter dys?ynergia or neurogenic detrusor overactiv$).ti,ab,ot.
18. (Encopresis or incontinentia alvi).ti,ab,ot.
19. ((bowel$ or rectum or rectal$) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or uncontrolled or trickl$ or "lack of control" or "no control" or "out of control" or "not voluntary" or involuntary or control$)).ti,ab,ot.
20. ((Unable or inabilit$ or abilit$ or able) adj3 control$ adj3 (faeces or faecal$ or feces or fecal$ or stool$ or rectum or rectal$ or bowel$ or bladder$ or anal$ or anus or urine or urinary or diarrh$ or soiling)).ti,ab,ot.
21. ((feces or faeces or fecal$ or faecal$ or stool or stools or defecat$ or soiling) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or escaping or uncontrolled or trickl$ or "not voluntary" or involuntary or control$)).ti,ab,ot.
22. ((diarrh$ or Pseudodiarrh$ or Pseudo-diarrh$) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or uncontrolled or trickl$ or "not voluntary" or involuntary or control$)).ti,ab,ot.
23. ((Unable or inabilit$ or abilit$ or able) adj3 control$ adj3 (diarrh$ or Pseudodiarrh$ or Pseudo-diarrh$)).ti,ab,ot.
24. ((bowel$ or rectum or rectal$ or defecat$) adj4 (disorder$ or malfunction$ or dysfunction$ or evacuat$ or obstruct$ or block$)).ti,ab,ot.
25. ((feces or faeces or fecal$ or faecal$ or stool or stools or defecat$ or soiling) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or escaping or uncontrolled or trickl$ or "not voluntary" or involuntary or control$)).ti,ab,ot.
26. (urinary tract adj3 (dysfunct$ or disorder$ of syndrome$)).ti,ab,ot.
27. (LUTD or LUTS).ti,ab,ot.
28. (pelvic floor adj3 (dysfunct$ or disorder$ of syndrome$)).ti,ab,ot.

29. ((feces or faeces or fecal$ or faecal$ or stool or stools or defecat$ or soiling) adj2 (store or stored or storag$) adj2 (disorder$ or dysfunct$ or malfunct$ or syndrome$)).ti,ab,ot.
30. ((disorder$ or difficult$ or syndrome$) adj4 (urine$ or urinat$ or urinary or micturat$ or bladder$)).ti,ab,ot.
31. (detrusor adj2 (overactiv$ or over-activ$)).ti,ab,ot.
32. ((pain$ or discomfort$ or inflamm$ or infect$) adj4 (urine$ or urinat$ or urinary or micturat$ or bladder$ or pelvis or pelvic)).ti,ab,ot.
33. (megacystitis or cystitis or pericystitis).ti,ab,ot.
34. (detrusor adj2 (overactiv$ or over-activ$)).ti,ab,ot.
35. ((bladder$ or hunner or hunneri or submucos$ or sub-mucos$) adj2 (ulcus or ulcer$)).ti,ab,ot.
36. or/1-35
37. InterStim.ti,ab,ot.
38. (SNS or SNM).ti,ab,ot.
39. (sacral adj3 (neuromodulat$ or neuro-modulat$ or deafferent$ or de-afferent$ or neurostimulat$ or neuro-stimulat$)).ti,ab,ot.
40. medical electrical stimulation therap$.ti,ab,ot.
41. ((bladder$ or sacral$) adj2 (Autoaugment$ or Auto-augment$)).ti,ab,ot.
42. (sacral nerve$ adj3 (modulat$ or stimulat$)).ti,ab,ot.
43. or/37-42
44. 36 and 43

Pubmed (NLM): 1947-2020/01/13

Searched 14.1.2020

#52 Search (#41 AND #46 AND #51)
#51 Search (#50 OR #49)
#50 Search (((pubstatusaheadofprint OR publisher[sb])))
#49 Search (#47 OR (#47 AND #48))
#48 Search human*[tiab]
#47 Search (((rat[tiab] or rats[tiab] or mouse[tiab] or mice[tiab] or murine[tiab] or rodent[tiab] or rodents[tiab] or hamster[tiab] or hamsters[tiab] or pig[tiab] or pigs[tiab] or porcine[tiab] or rabbit[tiab] or rabbits[tiab] or animal[tiab] or animals[tiab] or dogs[tiab] or dog[tiab] or cats[tiab] or cow[tiab] or bovine[tiab] or sheep[tiab] or ovine[tiab] or monkey[tiab] or monkeys[tiab])))
#46 Search (#42 OR #43 OR #44 OR #45 OR)
#45 Search ("sacral nerve"[Title/Abstract]) AND (modulat*[Title/Abstract] OR stimulat*[Title/Abstract])
#44 Search ((sacral[Title/Abstract] OR Bladder*[Title/Abstract])) AND (neuromodulat*[Title/Abstract] OR neuro-modulat*[Title/Abstract] OR deafferent*[Title/Abstract] OR de-afferent*[Title/Abstract] OR neurostimulat*[Title/Abstract] OR neuro-stimulat*[Title/Abstract] OR Autoaugment*[Title/Abstract] OR Auto-augment*[Title/Abstract])
#43 Search (medical electrical stimulation[Title/Abstract]) AND therap*[Title/Abstract]
#42 Search (InterStim[Title/Abstract] OR SNS[Title/Abstract] OR SNM[Title/Abstract] OR PTNS[Title/Abstract])
#41 Search (#21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31 OR #32 OR #33 OR #34 OR #35 OR #36 OR #37 OR #38 OR #39 OR #40)

#40 Search (#11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20)
#39 Search ((bladder*[Title/Abstract] OR hunner[Title/Abstract] OR hunneri[Title/Abstract] OR submucos*[Title/Abstract] OR sub-mucos*[Title/Abstract])) AND (ulcus[Title/Abstract] OR ulcer*[Title/Abstract])
#38 Search ((pain*[Title/Abstract] OR discomfort*[Title/Abstract] OR inflamm*[Title/Abstract] OR infect*[Title/Abstract])) AND (urine*[Title/Abstract] OR urinat*[Title/Abstract] OR urinary[Title/Abstract] OR micturat*[Title/Abstract] OR bladder*[Title/Abstract] OR pelvis[Title/Abstract] OR pelvic[Title/Abstract])
#37 Search ((disorder*[Title/Abstract] OR difficult*[Title/Abstract] OR syndrome*[Title/Abstract])) AND (urine* or urinat* or urinary or micturat* or bladder*)
#36 Search (((feces[Title/Abstract] OR faeces[Title/Abstract] OR fecal*[Title/Abstract] OR faecal*[Title/Abstract] OR stool[Title/Abstract] OR stools[Title/Abstract] OR defecat*[Title/Abstract] OR soiling[Title/Abstract])) AND (store[Title/Abstract] OR stored[Title/Abstract] OR storag*[Title/Abstract])) AND (disorder*[Title/Abstract] OR dysfunct*[Title/Abstract] OR malfunct*[Title/Abstract] OR syndrome*[Title/Abstract])
#35 Search (("urinary tract"[Title/Abstract] OR "pelvic floor"[Title/Abstract])) AND (dysfunct*[Title/Abstract] OR disorder*[Title/Abstract] OR syndrome*[Title/Abstract])
#34 Search (OAB[Title/Abstract] OR BPS[Title/Abstract] OR LUTD[Title/Abstract] OR LUTS[Title/Abstract])
#33 Search (((cystitis[Title/Abstract]) OR "overactive bladder"[Title/Abstract]) OR ("over-active detrusor"[Title/Abstract] OR "overactive detrusor"[Title/Abstract])) OR (megacystitis[Title/Abstract] OR pericystitis[Title/Abstract])
#32 Search ((((Unable[Title/Abstract] OR inabilit*[Title/Abstract] OR abilit*[Title/Abstract] OR able[Title/Abstract])) AND control*[Title/Abstract]) AND (diarrh*[Title/Abstract] OR Pseudodiarrh*[Title/Abstract] OR Pseudo-diarrh*[Title/Abstract]))
#31 Search (((diarrh*[Title/Abstract] OR Pseudodiarrh*[Title/Abstract] OR Pseudo-diarrh*[Title/Abstract])) AND (leak[Title/Abstract] OR leakage[Title/Abstract] OR leaks[Title/Abstract] OR leaking[Title/Abstract] OR seep[Title/Abstract] OR seepage[Title/Abstract] OR seeps[Title/Abstract] OR seeping[Title/Abstract] OR accident*[Title/Abstract] OR escap*[Title/Abstract] OR uncontrolled[Title/Abstract] OR trickl*[Title/Abstract] OR "not voluntary"[Title/Abstract] OR involuntary[Title/Abstract] OR control*[Title/Abstract]))
#30 Search (((feces[Title/Abstract] OR faeces[Title/Abstract] OR fecal*[Title/Abstract] OR faecal*[Title/Abstract] OR stool[Title/Abstract] OR stools[Title/Abstract] OR defecat*[Title/Abstract] OR soiling[Title/Abstract])) AND (leak[Title/Abstract] OR leakage[Title/Abstract] OR leaks[Title/Abstract] OR leaking[Title/Abstract] OR seep[Title/Abstract] OR seepage[Title/Abstract] OR seeps[Title/Abstract] OR seeping[Title/Abstract] OR accident*[Title/Abstract] OR escap*[Title/Abstract] OR escaping[Title/Abstract] OR uncontrolled[Title/Abstract] OR trickl*[Title/Abstract] OR "not voluntary"[Title/Abstract] OR involuntary[Title/Abstract] OR control*[Title/Abstract]))
#29 Search ((((Unable[Title/Abstract] OR inabilit*[Title/Abstract] OR abilit*[Title/Abstract] OR able[Title/Abstract])) AND control*[Title/Abstract]) AND (faeces[Title/Abstract] OR faecal*[Title/Abstract] OR feces[Title/Abstract] OR fecal*[Title/Abstract] OR stool*[Title/Abstract] OR rectum[Title/Abstract] OR rectal*[Title/Abstract] OR bowel*[Title/Abstract] OR bladder*[Title/Abstract] OR anal*[Title/Abstract] OR anus[Title/Abstract] OR urine[Title/Abstract] OR urinary[Title/Abstract] OR diarrh*[Title/Abstract] OR soiling[Title/Abstract]))

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Vaginal Distention Rodent Model for Faecal Incontinence: A Pilot Study on the Effect on Defecation Behaviour

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Chapter 3

Abstract

Objective: Vaginal balloon inflation simulates the compressive forces at the pelvic floor during the second phase of natural delivery. This animal model of vaginal distention (VD) is foremost used to study mechanisms underlying urinary incontinence. As damage to the pelvic floor during natural birth is a common cause of faecal incontinence, this paper aimed to investigate the effect of VD on defecation behaviour in adult rats.

Methods: VD was performed in eight rats for 2 hours, in three rats for 4 hours and sham inflation was performed in four rats. With use of a latrine box in the rat home-cage and 24/7 video tracking, defecation behaviour was examined. Time-spend in- and outside the latrine was monitored for two weeks pre-operatively and three weeks post-operatively and a defecation behaviour index (DBI; range 0 (continent) to 1 (incontinent)) was defined. Pelvic floor tissue was collected post mortem and stained with haematoxylin and eosin.

Results: Vaginal balloon inflation for 2 hours resulted in faecal incontinence in 29% of animals (responders) whereas non-responders (71%) and control animals did not change DBI in the post-operative phase compared to baseline. A 4h balloon inflation resulted in faecal incontinence in one animal and caused a humane endpoint in two animals with markedly more tissue damage in the 4h responder compared to the 2h responders.

Conclusions: Vaginal balloon inflation, with an optimum duration between 2 and 4 hours, can be used as a model to study pelvic floor damage induced changes in defecation behaviour in rats.

Keywords: animal, bowel dysfunction, balloon model, sacral nerve stimulation, vaginal balloon inflation

Introduction

Faecal and stress urinary incontinence are a possible consequence of natural birth due to excessive compressive forces at the pelvic floor that cause damage to the pelvic floor [1-3]. Present preclinical research into the treatment and mechanisms of action (MoA) of faecal incontinence is limited and only two preclinical models related to defecation behaviour have been published in literature to date. These studies utilized either retro-uterine balloon inflation [4] or transvaginal retro-uterine intrapelvic balloon inflation [5]. While both balloon inflation models showed signs of faecal incontinence the responder rate was limited as for instance with the retro-uterine balloon inflation model this did not exceed 32% of animals [4]. The retro-uterine intrapelvic balloon inflation model resulted in a small effect on behavioural outcome and this very small window did not allow to study future treatment effects [5].

Currently, the vaginal distention (VD) model is a commonly used model for stress urinary incontinence [6, 7]. This model is characterized by an intravaginal balloon inflation, which mimics the compressive forces at the pelvic floor during the second phase of natural delivery. The advantage of this model over the previous mentioned models is that it is induced using a physiological approach as it does not require an open procedure and at the same time it very closely resembles natural delivery in humans. Morphological studies into urinary incontinence have shown significant muscle disruption, inflammatory damage and acute edema as a result of VD [8, 9]. Remarkably, the VD model has been exclusively focused on urinary incontinence but not on faecal incontinence and defecation behaviour.

Therefore, the present pilot study aimed to investigate the effect of the relatively non-invasive vaginal balloon inflation on defecation behaviour in adult rats in order to establish a reproducible animal model for faecal incontinence.

Methods

Animals

All experiments were performed in accordance with the European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609/EU). The Central Authority for Scientific Procedures on Animals (CCD) gave ethical approval to all experiments (Project License 2018-005-018). Nineteen nulliparous female Sprague Dawley rats (160-200g, 8 weeks old at the start of the experiment) were used for this experiment. Animals were housed individually in custom-made cages designed for this experiment in a 12 h reversed day/night cycle with constant temperature (20°C) and humidity (55%). During the entire experiment, cages were randomly placed at the wall rack to avoid environmental influences. Food and water were available ad libitum. The present study consisted of two experiments. Initially before start of the experiments, we detected a loss of volume during balloon inflation in a pilot with three animals, and therefore a clamp was used right behind the balloon to prevent loss of volume due to high compliance in the tubing. In **Experiment A**, twelve Sprague Dawley rats (230-270g) were included, of which eight animals underwent balloon (Rüsch gold foley balloon catheter, CH8) inflation with 4 ml saline (at room temperature) for two hours (experimental group) and four animals underwent sham inflation (control group). In **Experiment B**, four Sprague Dawley rats (200-250g) were included, of which three rats underwent balloon inflation with 4 ml for four hours and one rat underwent sham inflation. All animals were randomly assigned to one of the groups using randomize software at the time of balloon inflation. Researchers were blinded for the condition of the animal during the entire experiment including analysis.

Vaginal distention and balloon inflation

Thirty minutes prior to surgery, buprenorfine (0.025 mg/kg s.c.) was administered. Rats were anaesthetized with isoflurance (4%) and anaesthesia was maintained with isoflurane (1.5-2.5%). The depth of anaesthesia was monitored during surgery. Body temperature was maintained at 37.5 ± 0.5 °C using an automated heating pad and heat lamp. Rats were placed on their back and the balloon was placed intravaginally. The balloon was then inflated using a constant inflation rate of 400 µL/min and counter pressure was given to prevent the balloon

of popping out easily. After 10 minutes the balloon was inflated with 4 ml and clamped to avoid loss of volume. A plunger of a 5ml syringe was placed against the balloon to keep the balloon in place. After two or four hours the balloons were deflated, carprofen (4-5 mg/kg) was administered and the rat was placed in the home cage to recover. When discomfort was observed after surgery, extra carprofen (4-5 mg/kg) was administered. Rats in the control group underwent the same procedure with a sham inflation (0 ml).

Figure 1. Home-cage of the rat.

Defecation behaviour task

The defecation behaviour task as used in this study was first published by Devane and colleagues [4]. In short: A latrine box was placed into the rat's home-cage (40x60 cm) in the edge furthest away from the food and water. The latrine box was filled with bedding material and the rest of the cage (non-latrine area) was filled with paper bedding, and contained nesting and playing material, shown in Figure 1. A one-week continence training period was performed, in which rats were trained in pairs to defecate in the latrine box by placing all pellets in the latrine twice daily (see timeline in Figure 2). Following one week of training, rats were housed individually and pellets in the latrine area and non-latrine area were counted daily. With use of infrared video-tracking the location of the rat was then tracked for 24 hours daily and time spend in- and outside the latrine was monitored. A defecatory behaviour index (DBI) was used to examine faecal incontinence. The DBI (range 0-1) was defined as the

amount of pellets per hour outside the latrine divided by the amount of pellets per hour in total [4]. A DBI of 0 implies a completely continent rat with all pellets in the latrine, whereas a DBI of 1 refers to a completely incontinent rat with all pellets deposed randomly throughout the cage. Baseline defecation behaviour was measured for two weeks and post-operative defecation behaviour was studied for three weeks. Animals were considered incontinent if the post-operative DBI is doubled as compared to the baseline DBI and if DBI is more than 0.3.

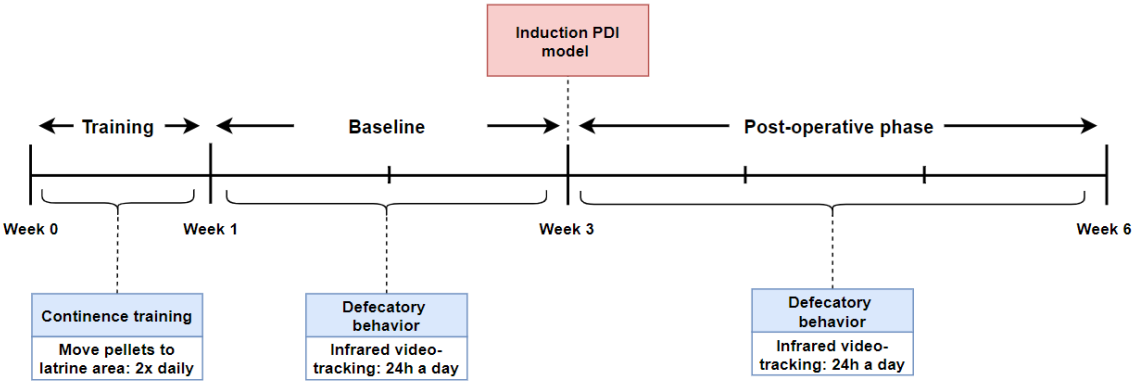

Figure 2. Timeline of experiments.

Post mortem analysis

Animals were euthanized using CO_2 and fresh pelvic floor tissue was collected. Tissue was further immersion fixed in paraformaldehyde (4%) for 14 days, decalcified in a solution containing formic acid (8%), and embedded in paraffin. Sections of 5 µm were cut using a microtome and mounted at polysine-coated glass slides. Sections were incubated overnight at a temperature of 37 ˚C and stained with haematoxylin and eosin (H&E).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com) and data are presented as mean ± SEM. Baseline data of all animals appeared to be normal distributed using visual inspection of the histogram. A repeated measures ANOVA was performed for significance of the DBI over time. Comparisons between groups were performed over the third post-operative week using an unpaired sample t-test.

Results

Experiment A: 2h balloon inflation

Out of 12 animals, two did not successfully complete continence training (DBI < 0.3) and were excluded from the study. From the remaining 10 animals, 7 were allocated to the experimental group and 3 to the control group (sham surgery). Results are shown in Figure 3a. The DBI of the control group did not change in the post-operative phase as compared to baseline (0.19 ± 0.03 vs 0.13 ± 0.04; p=0.4438). Likewise in the experimental group, 2h balloon inflation did not significantly change DBI (baseline: 0.16 ± 0.03 vs postoperative: 0.24 ± 0.11; p=0.4073). As already described by Devane and colleagues [4], within the experimental group subgroups of responders and non-responders could be discerned. The DBI in the subgroup of non-responders (n=5, 71%) did not significantly change in the post-operative phase as compared to baseline (0.11 ± 0.03 vs 0.07 ± 0.02; p=0.0671). The DBI in the subgroup of responders (n=2 29%) clearly increased in the post-operative phase compared to baseline (0.20 ± 0.02 vs 0.65 ± 0.12; p=0.0295) and was significantly higher than the DBI of the control group (0.65 ± 0.12 vs 0.13 ± 0.04; p=0.0150). Side effects seen in this responder subgroup were absence of defecation for two days post-surgery and small motor deficits of the hind paws.

Experiment B: 4h balloon inflation

Out of 4 animals, one animal did not successfully complete continence training (DBI < 0.3). The remaining 3 animals (n=3) were allocated to the experimental group. To enable blinding of the researcher during the experiment, the animal that was not continent underwent the whole procedure but was excluded from the analysis. Results of the 4h inflation are shown in Figure 3b. Two animals developed absence of defecation after surgery and reached a humane endpoint after two days. Absence of defecation, albeit to a smaller degree, was also seen in the responders of Experiment A (2h balloon inflation). The DBI of one animal increased after surgery compared to baseline (0.27 ± 0.05 vs 0.65 ± 0.04).

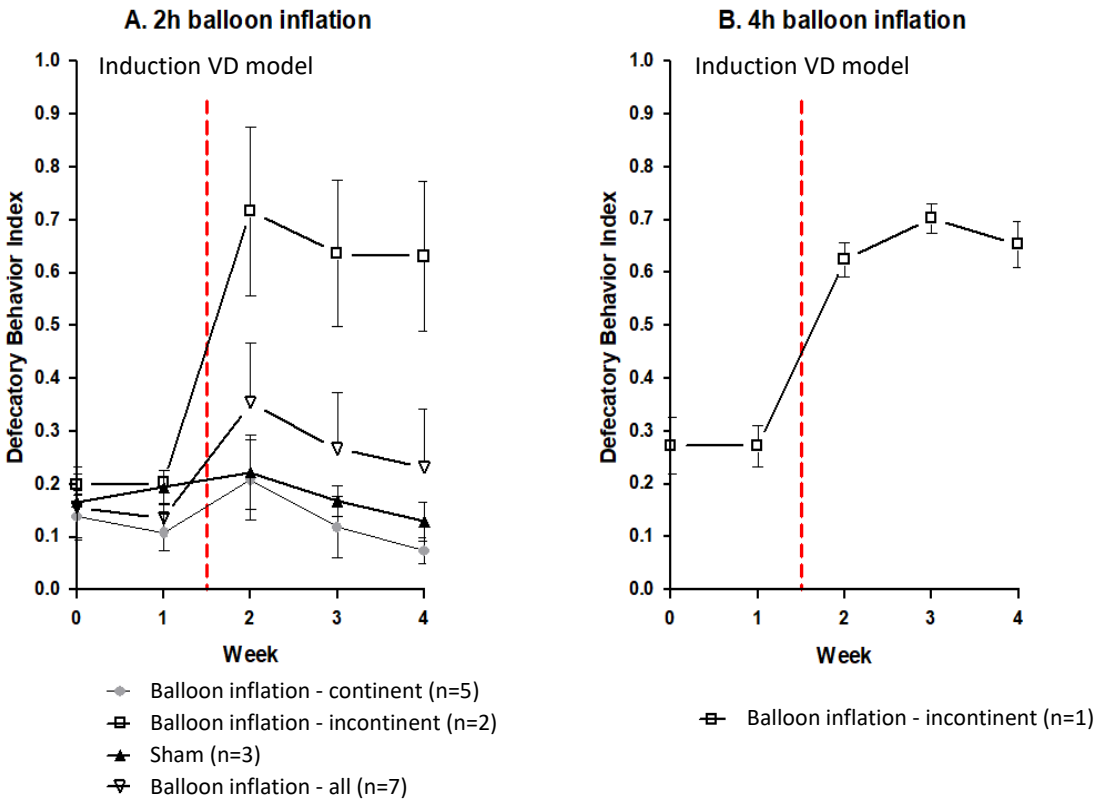

Figure 3. Effect of balloon inflation on defecation behaviour. (A) Represents the Defecatory Behaviour index of Experiment An overtime, and (B) represents the Defecatory Behaviour index of Experiment B overtime. Data is presented as the mean ± SEM.

Post mortem analysis

Sections of the pelvic floor stained with H&E are shown in Figure 4. Continent animals (VD non-responders; Fig. 4b) showed minor signs of inflammation, but no signs of fibrosis, oedema or dilatation as compared to control animals (Fig. 4a). In contrast, inflammation with fibrosis was seen in the musculus levator ani surrounding the colon, with markedly more damage after 4h balloon inflation (Fig. 4d) as compared to 2h inflation (Fig. 4c). Furthermore, signs of oedema in de submucosa and rectum dilatation were noted after 4h balloon inflation (Fig. 4d) and to a lesser extent after 2h inflation (Fig. 4c). In addition, a slightly thinner muscularis externa was seen after 4h balloon inflation (Fig. 4d). The mucosa did not seem to be affected in all animals, regardless of the animal condition.

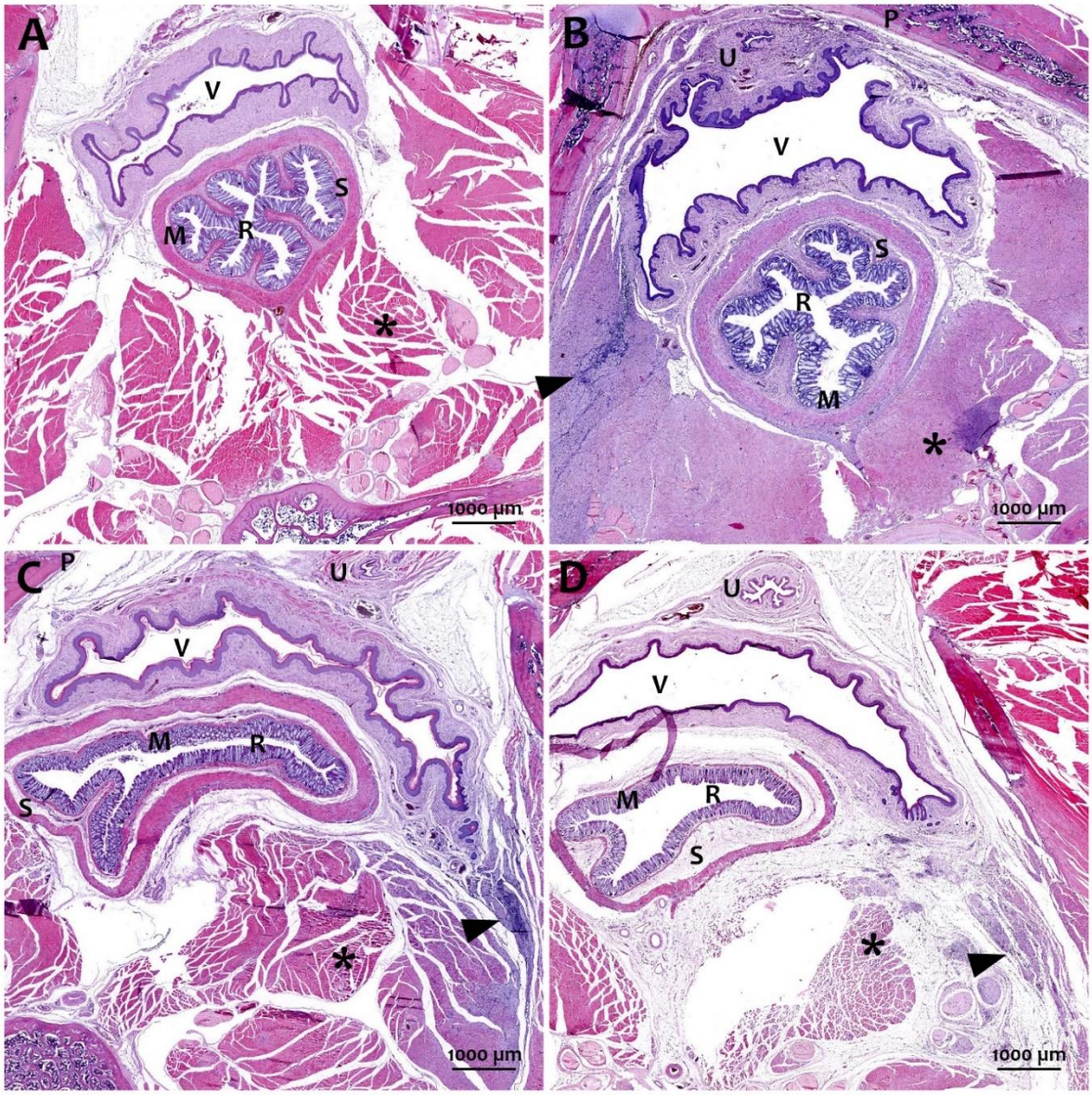

Figure 4. Sections of the pelvic floor with H&E Staining. A: Control group; B: 2h balloon inflation - continent group; C: 2h balloon inflation - incontinent group; D: 4h balloon inflation - incontinent group. ✱ = musculus levator ani; ▶ = inflammation; M = mucosa; P = pubic bone; R = rectum; S = sub mucosa; U = urethra; V = vagina.

Discussion

This methodological paper showed the effect of vaginal balloon inflation on defecation behaviour in adult SD rats. The experimental group did not significantly differ from the control group after a 2h balloon inflation. Within the experimental group, 29% of animals developed faecal incontinence and 71% did not develop faecal incontinence. After a 4h vaginal balloon inflation, all animals responded to the inflation of which one animal developed faecal incontinence but two animals reached a humane endpoint. In the post mortem analysis, dilatation of the colon and damage to the musculus levator ani were seen after 4h, and to a lesser extend after 2h of balloon inflation.

The VD model has the advantage of being more closely related to natural birth as compared to the retro-uterine balloon inflation model [4] or the transvaginal retro-uterine intrapelvic balloon inflation model [5]. The responder rate following 2h vaginal balloon inflation (29%) is comparable to the responder rate of the retro-uterine balloon inflation model (32%) as described by Devane and colleagues [4]. Unfortunately, the responder rate is relatively low and this makes the animal experiments, from an ethical point of view, rather complicated. Interestingly, the level of incontinence in responders following 2h vaginal balloon inflation (DBI=0.65 ± 0.12) seems higher as compared to the retro-uterine balloon inflation model. This profound increase in DBI with the VD model allows an adequate window of opportunity and this may be useful to investigate the effect of possible interventions. To further increase the responder rate and with that increase the usability of the VD model, Experiment B with an inflation duration of 4 hours was performed. Although the responder rate increased, but regrettably, severe complications occurred and some animals reached a humane endpoint. However, the sample size for the 4h vaginal balloon inflation group in this study is low, and future studies should therefore aim to replicate and extend our findings.

It is complicated to compare the defecation behaviour of the VD model to the transvaginal retro-uterine intrapelvic balloon model, as the behaviour in these studies was examined with two different approaches [5]. Nevertheless, the treatment window for the transvaginal retro-

uterine intrapelvic balloon model appeared to be relatively small, whereas the treatment window for the VD model appears to be substantially wider.

In contrast to these other balloon models, in which balloons are inflated for a period of one hour, the present VD model has an inflation duration of two or four hours. The responder rate following 2h inflation was moderate (29%), whereas the responder rate following 4h inflation appeared to be substantially higher, but too severe in terms of discomfort. After 4h inflation, all animals showed signs of bowel dysfunction, of which one animal developed faecal incontinence, but two animals developed severe postoperative absence of defecation with signs of ileus and this did not allow to complete the analysis in 2 out of 3 animals.

Damage of the musculus levator ani was noted with the post mortem analysis of VD responders, and this confirms the importance of the muscle levator ani in bowel continence. Indeed, Fernandez-Fraga and colleagues showed that the severity of faecal incontinence is correlated to an impaired function of the musculus levator ani and less strongly related to EAS function [10]. This was supported by the clinical improvement after treatment which was seen without significant improvement of the EAS [10].

With the development of this VD model for faecal incontinence various technical aspects should be noted. As described, the balloon should be clamped to avoid loss of volume. In addition, it is important to apply counter pressure to the balloon from the perineal side in order to keep the balloon in place, as without this counter pressure the balloon will choose the path of the lowest resistance and pop out of the vaginal canal. A limitation of the present study might be the use of opioids, and the possible effect of these drugs on the gastrointestinal tract. In our study, buprenorphine (0,025 mg/kg s.c.) was applied to the animals preoperatively. Studies have shown that buprenorphine is associated with impaired gastrointestinal motility and postoperative ileus, especially after longer abdominal surgical procedures [11]. Several animals in both Experiment A and B developed absence of defecation, which in two of three animals from the 4h balloon inflation group even led to exclusion from the experiment. It is therefore of utmost importance to minimize the use of opioids in pre-operative pain. Hence, this might be related to the administration of opioids

such as buprenorphine and its effect on the gastrointestinal tract. However, absence of defecation can also be caused by pain induced reflex mediated pelvic floor hypertonicity. In the present study it is unlikely that pain induced hypertonicity as we administered peri-operative and post-operative analgesia.

From these experiments, it can be concluded that the vaginal distention model with a balloon inflation duration of 2 hours was not enough to cause faecal incontinence with a substantial responder rate. Besides a balloon inflation duration of 4 hours exceeded the discomfort needed to cause faecal incontinence. Probably, the optimum duration of inflation mimicking the physiological trauma caused by natural delivery will be between 2 and 4 hours. Importantly, responders showed a significant and adequate window of opportunity and responder rates are relatively low with an inflation duration of 2 hours. From this we presume that the VD model can be used in future studies on possible effect of interventions such as sacral neuromodulation. This will allow optimizing these therapies for clinical application and investigating the underlying mechanisms of action.

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Longitudinal Quantitative Evaluation of Bladder Storage and Evacuation Dysfunction for Preclinical Intervention Effect using Ultrasound Imaging in Awake Rats

P. Douven, S. Peter, G. Franken, J. Debets, W. Gerritsen, S.O. Breukink, E.A. Joosten, G.A. van Koeveringe

Under review.

Chapter 4

Abstract

Objective: Partial bladder outlet obstruction (pBOO) is a common condition related to an only partially specific complex of symptoms. Longitudinal evaluation of functional and structural alterations in pBOO can give insight into the disease progression overtime. This study aimed to monitor and evaluate longitudinal voiding behaviour and bladder capacity as related to terminal cystometric outcome in rats with pBOO.

Methods: Sprague Dawley rats underwent pBOO (n=8) or sham pBOO (n=4) surgery after which voiding behaviour (voiding spot assay) and bladder capacity (ultrasound imaging) was measured during a period of four weeks. Terminal cystometry and tissue collection for post mortem histology were performed at four weeks post-surgery.

Results: In pBOO rats, voiding behaviour did not change over time while bladder capacity increased. This increase was also seen with terminal cystometry, which also showed an increase in bladder compliance, residual volume and bladder weight, and a decrease in voiding pressure as compared to control rats.

Conclusions: Partial bladder outlet obstruction results in an increased bladder capacity. Bladder capacity measured with ultrasound imaging is related to terminal bladder capacity as measured with cystometry. This indicates that ultrasound imaging is a suitable technique for longitudinal evaluation and analysis of bladder storage and evacuation dysfunction.

Keywords: Voiding dysfunction, incontinence, ultrasound imaging, voiding spot assay, cystometry

Introduction

Anti-incontinence surgery, urethral stricture, benign prostatic hyperplasia or chronic functional non-relaxation of the urinary sphincter often cause an only partially specific set of lower urinary tract symptoms. These symptoms might be related to partial bladder outlet obstruction (pBOO). The most prevalent symptoms of pBOO are storage phase symptoms of urinary frequency and voiding symptoms (poor flow) [1, 2]. Current treatment options are predominantly based on treating these symptoms, and include pelvic physiotherapy, administration of α-blockers, (clean intermittent self) catheterisation, minimally invasive procedures like sacral neuromodulation or ultimately invasive surgery [3-5].

Several animal models have been used to investigate pBOO, its underlying pathophysiology and the effect of interventions [6-10]. Structural bladder alterations including increased bladder weight, reduced bladder compliance and micturition interval, high micturition pressure, and decreased micturition volume have been demonstrated in pigs, rabbits, guinea pigs, rats and mice (reviewed by Kitta and colleagues [11]). After longer periods of obstruction, a shift towards a more underactive detrusor dysfunction pattern can be observed in some models. Longitudinal evaluation of functional and structural alterations, such as voiding behaviour and bladder volume, in pBOO can give insights into the development of pBOO overtime. Eventually, this gives the opportunity to monitor the longitudinal effect of novel interventions and optimize these interventions to the need of the patient.

In the present study, a rat model of pBOO was investigated using a longitudinal approach in order to monitor lower urinary tract disease progression over four weeks. With use of voiding spot assay [12] and ultrasound imaging in awake rats, longitudinal voiding behaviour and bladder capacity were evaluated. The aim of the study was to investigate longitudinal voiding behaviour and bladder capacity as related to the terminal cystometric outcome in rats with pBOO.

Methods

Animals

Experiments were performed in accordance with the European Directive for Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (2010/63/EU). Ethical approval was provided by the Central Authority for Scientific Procedures on Animals, The Netherlands (Project License 2018-005-018). Twelve female Sprague Dawley rats (170-220g, 8 weeks old at the start of the experiment) were randomly divided in two groups using www.random.org: the experimental group (n=8) where animals underwent pBOO and the control group (n=4) where animals underwent a sham obstruction. Rats were socially housed in a 12:12h reversed day/night cycle with constant temperature (20°C) and humidity (55%). Home-cages contained bedding, nesting and cage enrichment. Food and water were available *ad libitum*.

Partial bladder outlet obstruction

Animals were anaesthetized using isoflurane (induction 4-5%; maintained 2-3%) and the depth of anaesthesia was frequently monitored during surgery. Body temperature was maintained at 37.5 ± 0.5°C using an automated heating pad connected to a thermal probe that was placed in the rectum. A midline abdominal incision was made and the bladder and urethra were visualized. A catheter (PE25 tubing, OD of 0.91mm) was inserted into the urethra and a 4-0 Silk suture was tied around the proximal end of the urethra so that the urethra was completely approximated to the catheter but not deformed i.e. the catheter could still be moved with light traction. Sham animals underwent the same procedure, but the suture was not tightened and immediately removed. The catheter was removed and the abdominal wall and skin was closed using 5-0 Polysorb sutures. Analgesia was applied peri-operative (buprenorphine 0.25mg/kg and carprofen 5mg/kg).

Voiding spot assay

Voiding behaviour was measured twice a week using a voiding spot assay (VSA) developed for mice [12], which was slightly adapted for use in rats. The test cage consisted of a standard cage (480x270x220mm - Floor area 1296cm2) with a walking grid floor with filter paper

underneath to collect the voided markings (spots). Sufficient drinking water and a wooden cube as cage enrichment were provided. Rats were habituated to the test cage a few days before baseline measurements. The test took place for 5 hours and each hour the filter paper was replaced via a slot underneath the walking grid floor of the cage. Subsequently, filter papers were dried for at least 24h. Behaviour of the individually placed rats was recorded using a custom-made infrared video tracking system, with an infrared light underneath, and a camera above each test cage. For analysis, voiding spots were visualized using ultraviolet light and the filter paper surface was divided into three areas; two corner zones and a central zone. The surface area of the central zone and the two corner zones combined were equal. The area in pixels and the number of voiding spots were quantified in each zone using Adobe Photoshop CC 2018. Spots were included when they had a diameter that exceeded 15 pixels. A spot that crossed one of the zone cut-off lines was assigned to the zone in which the middle of the spot was located. Using calibration with known volumes of urine, a linear relationship was found between urine volume and voiding spot size [12] and the volume of voiding spots was calculated. Filter papers were randomized before analysis and two blinded researchers (PD and SP) performed the analysis independently. Voiding pattern index (VPI) was defined as central zone divided by total to examine the number of spots, voided volume and voided volume per spot.

Ultrasound imaging

Ultrasound imaging of the bladder was performed weekly during the entire experiment. Conscious rats were fixed and the lower abdomen was covered with ultrasound transmission gel (Aquasonic 100, Parker Laboratories Inc., Fairfield, CT, USA). Ultrasound imaging (Vevo 2100 Imaging System, VisualSonics Inc., Toronto, Canada) of the bladder was performed in the sagittal and transversal plane. Diameters and surface area of both directions were measured using the Vevo LAB Analysis Software and bladder volume was calculated using the following formula:

$$V_{bladder} = \frac{4}{3}\pi \cdot \frac{1}{2}x \cdot \frac{1}{2}y \cdot \frac{1}{2}z$$

In this ellipse formula x, y and z are the diameters of the axis of the bladder.

Cystometry

Terminal cystometry was performed four weeks after induction of the pBOO. Rats were anaesthetized with urethane (20% weight per volume in saline, 1.5g/kg i.p.) and local application of lidocaine (20mg/kg, 2% s.c.). A midline lower abdominal incision was made and the bladder was visualized. An IV cannula (22g 0.8cm) was placed into the bladder and connected to an infusion pump and pressure transducer (DPT-6000, Codan pvb Medical GmbH, Germany). The transducer was calibrated before the start of the experiment using a watercolumn. The abdomen was closed using a 5-0 Polysorb suture to maintain abdominal pressure. Bladder filling was performed using saline at room temperature with an infusion rate of 190µl/min until voiding occurred. The bladder pressure was recorded (AcqKnowledge 4.2 version, BIOPAC system Inc., California, USA) and three successful micturition cycles were performed. For each rat, the average micturition parameters of the three micturition cycles were used for analysis. Cystometry data analyses were performed using an MP150 data acquisition system (AcqKnowledge 4.2 version, BIOPAC system Inc., California, USA). Recorded micturition parameters included bladder capacity (BC), bladder compliance, voiding pressure (the peak bladder pressure of each micturition cycle) and residual volume (RV).

Post mortem analysis

After cystometry, bladder and kidneys were collected and the bladder was weighed. Bladder and kidneys were fixated in PFA 4% for 48h followed by embedding in paraffin. Transverse sections of 5µm were cut using a microtome and subsequently stained using Haematoxylin and Eosin (H&E).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9.2.0 for Windows (GraphPad Software, San Diego California USA) and data are presented as mean ± SEM. Visual inspection of the histogram was performed to check for normal distribution and baseline data of all animals appeared to be normal distributed. A one-way repeated measures ANOVA was performed for significance of voiding behaviour and bladder volume over time and a two-way repeated measures ANOVA was performed over the post-operative data for significance

between groups. Additionally, an unpaired samples t-test was performed for comparison of bladder volume between groups at each time point. For cystometric measures, we choose to describe the results and not formulate null-hypothesis testing, due to the small number of animals.

Results

One animal of the experimental group was sacrificed two days post-surgery and excluded from the analysis due to an abdominal hernia and related risk of infection. From the remaining eleven animals, seven animals were randomly allocated to the experimental group and four animals were allocated to the control group. Leukocytes were found in the urine of one animal in the experimental group two weeks after surgery, which was treated with two doses of the antibiotic gentamicin (5mg/kg). Post-intervention no leukocytes were observed in the urine of this animal.

Voiding spot assay

Voiding behaviour based on number of spots (VPI; Fig. 1a) in both the experimental group ($p=0.1178$) and the control group ($p=0.2689$) did not significantly change over time and no significant difference between groups was found ($p=0.3177$). Likewise, no significant differences were found in the VPI of voided volume (Fig. 1b) neither in the experimental ($p=0.2648$) and control group ($p=0.5391$) over time, nor between groups ($p=0.3930$). Additionally, the VPI of the voided volume per spot (Fig. 1c) in the experimental group ($p=0.1538$) and the control group ($p=0.2707$) did not significantly differ over time and between groups ($p=0.7426$).

Ultrasound imaging

Ultrasound imaging of bladder volume of rats in the control group did not change during the post-operative phase as compared to baseline (Fig. 1d; baseline: 120.4 ± 37.94µl, week 1: 58.32 ± 15.54µl, week 2: 64.27 ± 26.25µl, week 3: 126.3 ± 45.86µl, week 4: 54.87 ± 8.23µl; $p=0.2060$). Conversely, an increase in bladder volume of rats in the experimental group was observed overtime, albeit this increase could not be detected by statistics due to the low power (baseline: 85.73 ± 20.31µl, week 1: 352.8 ± 124.4µl, week 2: 479.7 ± 218.2µl, week 3:

551.3 ± 176.6µl, week 4: 613.5 ± 215.9µl; p=0.0555). Between groups, no significant difference in bladder volume was found over time (p=0.1109), although a significance difference between groups was found in the fourth week (p=0.0413).

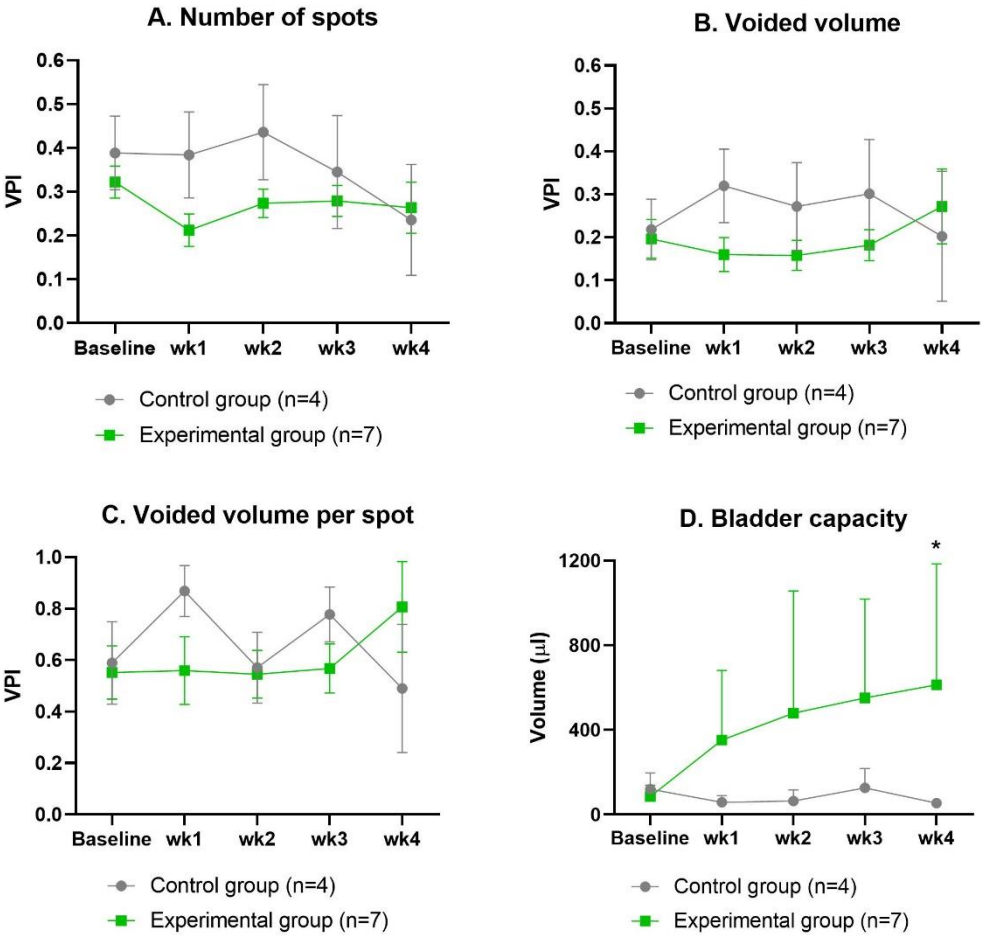

Figure 1. Effect of bladder outlet obstruction at voiding behaviour and bladder capacity. A) the number of spots, B) voided volume and C) voided volume per spot are examined with the voiding spot assay. D) Bladder capacity based on bladder volume as assessed with use of ultrasound imaging. Data is presented as the voiding pattern index as mean ± SEM. * p=0.0413

Cystometry

Cystometry was performed in seven rats, four rats of the experimental group and three rats of the control group. Two rats died under anaesthesia, and were excluded from the analysis. The experimental group showed an increased BC (Fig. 2a; experimental: 3276 ± 1512 µl vs control: 461.2 ± 256.8µl) and a slightly lower voiding pressure (Fig. 2b; 35.11 ± 0.79cmH2O vs 43.16 ± 0.07cmH2O) as compared to the control group. Bladder compliance (Fig. 2c; experimental: 0.219 ± 0.09, 0.072 ± 0.03 vs control: 0.025 ± 0.01, 0.033 ± 0.03Δml/cmH2O) an RV (Fig. 2d; 3213 ± 2319µl vs 5µl) was higher in the experimental group as compared to the control group especially in the first part of bladder filling. This RV was only collected in three rats; one rat in the control group and two rats in the experimental group.

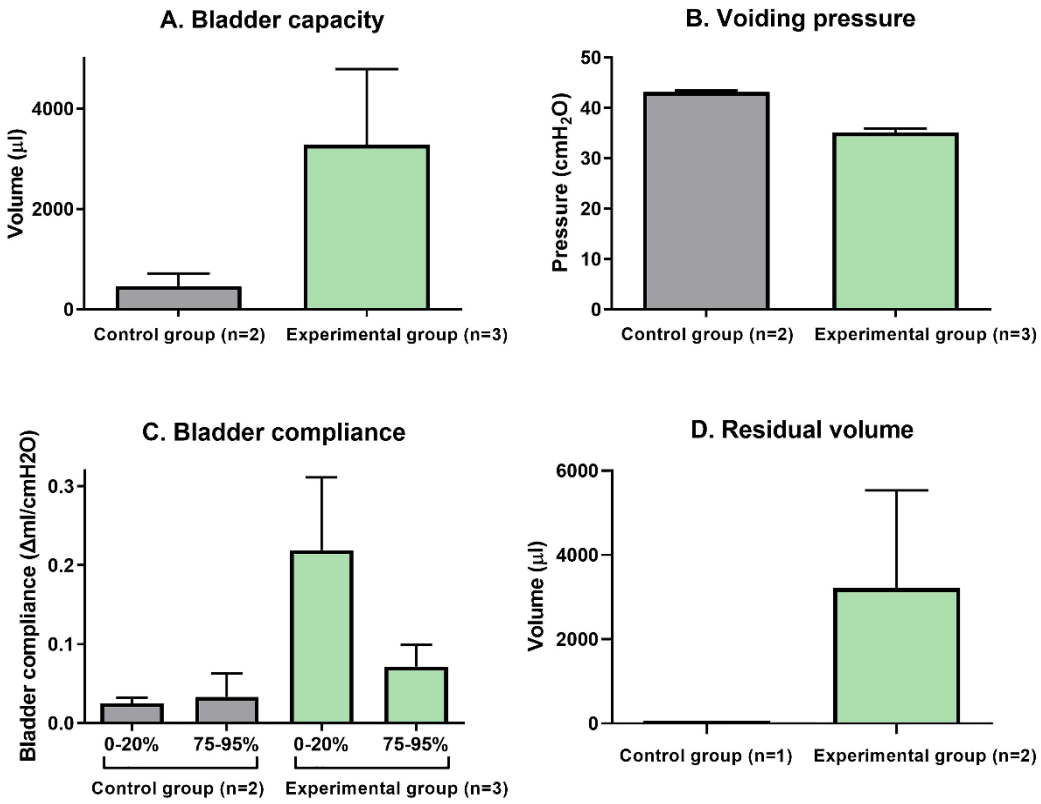

Figure 2. Effect of bladder outlet obstruction at cystometry. A) bladder capacity, B) voiding pressure, C) bladder compliance and D) residual volume. Data is presented as mean ± SEM.

A difference was noted in bladder pressure during the filling phase in the experimental group as compared to the control group (Figure 3). The filling phase in the control group can be divided in a passive phase and an active phase with bladder contractions. In the filling phase of the experimental group, the entire filling phase consists of passive bladder pressure.

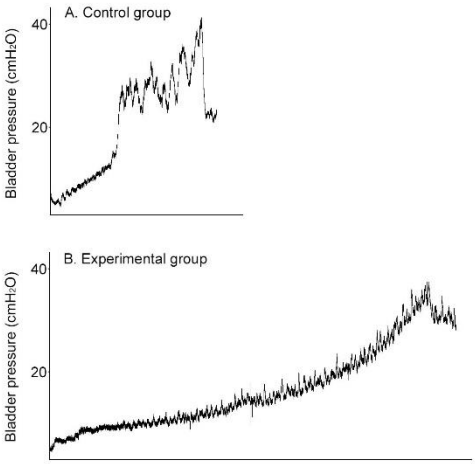

Figure 3. Micturition cycle of the control group (A) and the experimental group (B).

Post mortem analysis

Bladder weight increased in the experimental group as compared to the control group, albeit not significant (experimental (n=7): 0.42 ± 0.08 vs control (n=4) 0.25 ± 0.04; p=0.0846).

Bladder tissue of the experimental group (Fig. 4b) did not show damage as compared to the control group (Fig. 4a). In addition, no signs of damage were found in the kidneys. However, in the rat with leukocyturia, hyperplasia of bladder epithelium with some apoptotic cells (Fig. 4c) and a unilateral chronic nephritis in the left kidney were observed.

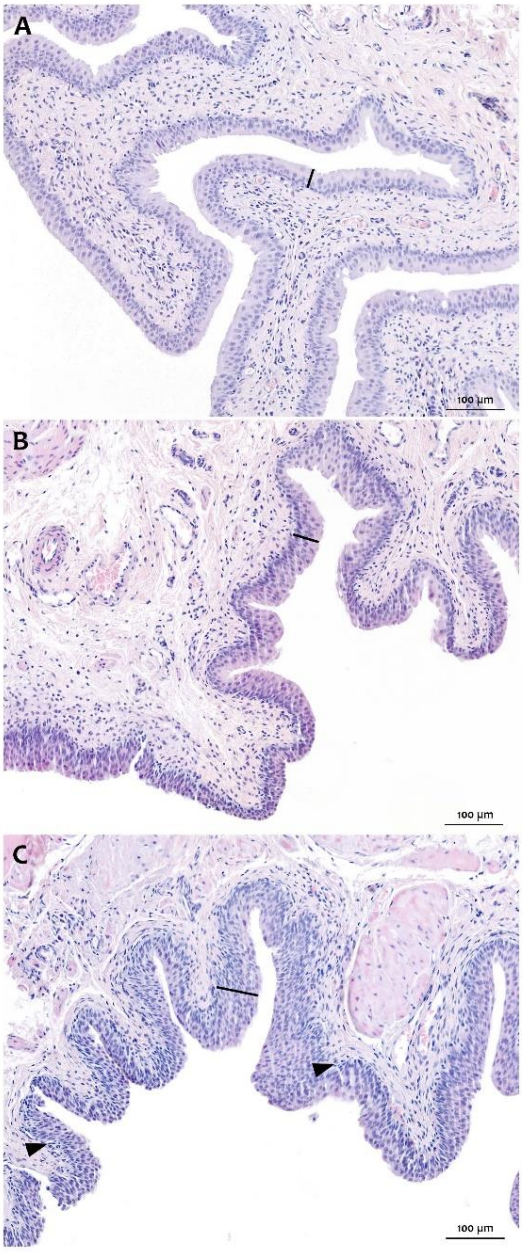

Figure 4. Sections of the bladder with H&E Staining. A: Control group, B: Experimental group, C: Experimental group, rat with leukocytes. — = bladder epithelium; ▶ = apoptotic cell.

Discussion

Disease progression of the pBOO model was examined using several approaches in a rat model of infravesical obstruction. Voiding behaviour parameters derived from the VSA did not show any differences between the experimental and control group over four weeks post-surgery. Interestingly, ultrasound imaging showed an increase in BC overtime in the experimental group, whereas BC in the control group did not change overtime. The larger BC in the experimental group was also observed during terminal cystometry four weeks post-surgery. Furthermore, rats in the experimental group showed a decreased voiding pressure, an increased bladder compliance, and a higher RV as compared to the control group. In post mortem analysis, rats in the experimental group showed an increased bladder weight over the control group, whereas no anatomical differences were observed in bladder and kidney tissue between groups.

The VSA is frequently used to investigate lower urinary tract dysfunction in mice, though it has hardly been examined in rats. In our study we did not find any differences in the voiding pattern measures with the VSA. In contrast an increase in number of spots, a lower voided volume per spot and an increase in voided volume in the center and decrease in the corner zones has been reported in a recent mouse study [13]. It should be taken into account that the latter mouse study used a different model to induce bladder obstruction. Furthermore, rats and mice are distinctive animal species, and therefore their voiding behaviour could potentially be different, although usually both mice and rats have a preference for corners with respect to micturition [12, 14, 15]. Additionally, voiding behaviour is highly sensitive to stress responses [16, 17]. As a consequence environmental differences, such as cage type and water bottle location, have been reported to affect VSA outcome [17]. Although all efforts were taken to minimize such environmental influences (e.g. same cage, constant water bottle location), the positioning of each cage relative to light source, door and background music might still influence voiding behaviour. It should be taken into account that stress sensitivity can differ between animal species or even per strain or sex which makes comparison among studies complicated [18]. Lastly, the absence of a significant change in VSA outcomes in this study could also be related to the absence of place preference for

voiding in the corners in the control group. Interestingly, it was reported that isolated housed rats did not show this place preference as compared to socially housed rats [19].

To the authors' knowledge, no literature described ultrasound imaging in awake rats with the pBOO model to evaluate BC longitudinally. Rats in the experimental group showed an increase overtime with a significant difference at four weeks post-surgery. Even though ultrasound imaging was not performed directly after voiding, a rise in BC was observed. This suggests that BC can be evaluated over time independent of the stage of the micturition cycle. One should take into account that imaging was always performed at the same time of the day, to prevent possible influences of circadian rhythm. In addition, it is recommended to perform ultrasound imaging regularly to reduce the possible variability as related to the stage of the micturition cycle.

Cystometric outcomes found in the present study are partly in accordance with the current literature. An increase in BC, voiding pressure, bladder compliance, RV and bladder weight was observed in pBOO rats in several studies [20-23]. In the present study, an increase in BC, bladder compliance, RV and bladder weight was observed in the experimental group, yet voiding pressure slightly decreased in this group. RV in pBOO rats was about 85-90% of BC, and this contributes to the strong increase in BC in these rats. The large RV and slightly lower voiding pressure suggest that pBOO rats show rather signs of urinary overflow incontinence instead of typical voiding. The passive pattern observed in pBOO rats corroborated this assumption as most experimental rats did not show a pressure increase as voiding occurred. This large RV indicates that the bladder is in a more or less decompensated stage [24], presumably a later stage in the sequence of detrusor changes that may occur after obstruction [10]. As the number of animals with cystometric evaluation was low, it is difficult to draw a definite conclusion from these findings.

Unfortunately, we were not able to observe changes in the VSA parameters that could be related to differences detected with cystometry. This suggests that the VSA is not the correct behavioural task to investigate longitudinal evaluation of the pBOO disease progression in rats. Nevertheless, ultrasound imaging did show similar results as cystometry regarding BC

and therefore ultrasound imaging appears to be a decent approach for longitudinal evaluation of BC.

In conclusion, our study shows that partial bladder outlet obstruction in rats results in an increased bladder capacity. Furthermore, we demonstrate that the use of ultrasound imaging to analyse bladder volume is a compelling technique for longitudinal evaluation of lower urinary tract dysfunction. Increased bladder capacity, the large residual volume, slightly lower voiding pressure and a passive pattern shown in terminal cystometry, suggest that the here presented bladder outlet obstruction model resembles a detrusor underactive pattern of dysfunction.

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Stimulation Parameters for Sacral Neuromodulation on Lower Urinary Tract and Bowel Dysfunction-Related Clinical Outcome: A Systematic Review

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Chapter 5

Abstract

Objective: Sacral neuromodulation (SNM) has been used to treat patients with lower urinary tract dysfunction and bowel dysfunction for many years. Success rates vary between 50% and 80%, indicating that there is much room for improvement. Altering stimulation parameters may result in improved outcome. This paper reports a systematic review of the clinical efficacy of nonconventional stimulation parameters on urinary tract and bowel dysfunction.

Methods: Three databases were used for the literature search: Ovid (Medline, Embase) and PubMed. Papers were screened by two independent reviewers, who also extracted data from these papers. Clinical papers studying SNM stimulation parameters, that is, intermittent stimulation, frequency, pulse width, and amplitude, in urinary tract and bowel dysfunction were included. Quality of included papers was assessed using standardized guidelines.

Results: Out of 5659 screened papers, 17 papers, studying various stimulation parameters, were included. Overall quality of these papers differed greatly, as some showed no risk of bias, whereas others showed high risk of bias. Stimulation parameters included intermittent stimulation, frequency, pulse width, amplitude, and unilateral vs. bilateral stimulation. Especially high frequency SNM and either a narrow or wide pulse width seem to improve efficacy in patients with bowel dysfunction. Additionally, implementation of short cycling intervals is promising to improve quality of life for patients with urinary tract or bowel dysfunction.

Conclusions: The results of our systematic review indicate that stimulation parameters may improve efficacy of SNM in treatment of both urinary tract dysfunction and bowel dysfunction.

Keywords: Fecal incontinence, sacral nerve stimulation, stimulation paradigm, voiding dysfunction

Introduction

Sacral neuromodulation (SNM) is used to treat both urinary and fecal storage and evacuation dysfunctions when conservative treatment options are not sufficient [1]. SNM efficacy, defined as >50% reduction in symptoms compared to baseline, varies between 50% and 80% in both urinary tract and bowel dysfunction and depends on interindividual characteristics and indication [2-8]. The fact that not all patients benefit from SNM underlines that there is much room for improvement to increase SNM efficacy. Furthermore, use of implantable pulse generators (IPGs) and SNM has shown to result in unwanted side effects including lead migration and pain surrounding the pocket. Recently, several technical improvements with respect to stimulation hardware have been introduced in SNM, for example, smaller IPGs, which resulted in a decrease of side effects. Additionally, staged implantation, as an alternative to percutaneous nerve evaluation (PNE), increased progression to IPG placement and decrease lead migration significantly [9, 10].

Up until now, SNM is most often applied as a rectangular signal, with a stimulation frequency of 14 Hz and a pulse width of 210 µs, also referred to as standard (conservative) stimulation parameters [11, 12]. Additionally, most patients have their IPG switched on 24/7, in contrast to the early years of SNM, when patients had their IPG switched off during the night. There is a continuing debate whether change of SNM stimulation parameters may result in improved outcome. Studies in other clinical fields of neuromodulation, such as spinal cord stimulation in treatment of neuropathic pain or deep brain stimulation in motor disorders, have shown that long-term efficacy can be improved with use of new stimulation parameters [13, 14]. In this context, it is important to review what is known about the efficacy of SNM in patients with urinary tract and bowel dysfunction as related to stimulation parameters, that is, intermittent stimulation, frequency, pulse width, amplitude and unilateral vs. bilateral stimulation. Insights into the underlying mechanism of action related to SNM stimulation parameters in preclinical studies have been reviewed in an accompanying paper [15]. However, to our knowledge, no systematic review has been conducted to determine whether these new stimulation parameters can improve the longterm efficacy of SNM in patients with

urinary tract and bowel dysfunction. As such, the aim of this review, and the afore mentioned accompanying paper [15], is to provide clinicians with new programming options regarding stimulation parameters and to provide pointers for future research focusing on SNM stimulation parameters in urinary tract and bowel dysfunction. In doing so, guidelines on trouble shooting, optimizing SNM efficacy and increasing battery life could be formed.

Methods

Search strategy

Two independent reviewers (PD, RA) performed extensive searches of the literature until January 14, 2020. This search was a shared search for both clinical and the preclinical literature on stimulation parameters for SNM on lower urinary tract and bowel dysfunction, meaning a systematic review on stimulation parameters in preclinical studies was performed in an accompanying paper. This review focuses only on human subjects and clinical outcomes. Due to the clinical heterogeneity across studies concerning study design, indication, outcomes, wash-out periods, and follow-up periods, a meta-analysis was not performed.

Three databases were used to conduct a systematic literature search: Medline (PubMed), Ovid (Embase), and PubMed. Appendix A includes all used search terms. Results of the search were uploaded to EndNote, in which articles were assessed for relevance. Abstracts and full text papers were screened by both reviewers (RA, PD). No language restrictions were used, but no foreign language papers were eligible for inclusion in the review. In case RA and PD were in disagreement on inclusion of a paper, a third author (EAJ) made the final decision.

Study selection and inclusion criteria

After final study inclusion, search results were allocated to either the clinical or the preclinical systematic review. Eligibility for inclusion of search results was evaluated based on the following criteria:

- Preclinical or clinical study
- Intervention of temporary or permanent SNM

- Comparison of various SNM stimulation parameters

The quality of included articles was assessed by two reviewers (PD, RA) using three Risk of Bias (RoB) tools:

- RoB 2.0 for randomized controlled trials (RCTs) [16]
- RoB 2.0 crossover for randomized controlled crossover trials
- The Joanna Briggs Institute Critical Appraisal tools for use in JBI Systematic Reviews-checklist for case series

If reviewers were in disagreement on RoB related to a certain category, a discussion was started until all issues were resolved. Data were extracted by two independent reviewers (RA and PD), and included first author, year of publication, indication of surgery, number of subjects, type of stimulation parameter, wash-out period, follow-up period, and primary outcome measure (Appendix B). A wash-out period of less than one week was considered very short, and may result in carry-over effects.

Due to the variety of outcome measures in the included studies, outcomes have been categorized as either objective or subjective. Objective outcomes are urinary voiding diaries, bowel habit diaries, pad changes, and anorectal measurements. Subjective outcomes consist of the following questionnaires: Cleveland Clinic Continence Score (CCCS), Patient Assessment of Constipation Symptoms Questionnaire (PAC-SYM), Patient Assessment of Constipation Quality of Life questionnaire (PAC-QOL), Fecal Incontinence Quality of Life Scale (FIQLS), St. Mark's Continence Score (SMCS), Gastrointestinal Symptom Rating Scale for Irritable Bowel Syndrome (GSRS-IBS), 11-point VAS scale for overall satisfaction, 101-point VAS scale for satisfaction, Wexner score.

Studies are discussed, based on the SNM stimulation parameter investigated, in the following order: 1) intermittent stimulation, 2) frequency and pulse width, and 3) unilateral vs. bilateral SNM, amplitude. For each SNM stimulation parameter, first those studies dealing with urinary tract dysfunction are discussed followed by those focused at bowel dysfunction.

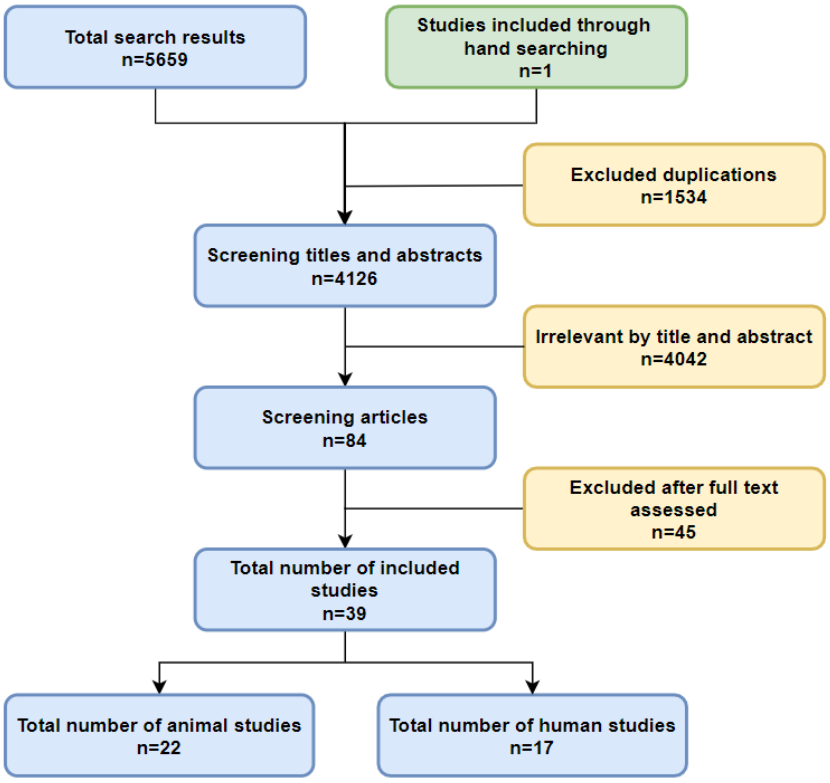

Figure 1. Flowchart of the included studies.

Results

A total of 5659 records were identified by the search strategy, of which 1534 were duplicates (Fig. 1). An additional 4042 papers were excluded after title and abstract screening. Screening of the 84 full papers resulted in exclusion of 45 additional papers, resulting in 39 papers that were included, of which 17 were clinical papers [17-33].

Risk of bias assessment

Four papers [24, 26, 27, 30] showed high RoB, mainly due to a very short washout period. Not all RCTs provided a method of randomization, although randomization itself was mentioned. Additionally, one case series showed a high RoB as well. Specifics of the RoB assessment are presented in Appendix C (Table C1-3).

Characteristics of included studies

The study characteristics are summarized in Appendix B (Table B1). In detail, 11 papers included were RCTs with a crossover design [17, 18, 21-23, 26, 27, 30-33]. One paper used a RCT design with parallel groups [29] and five papers were based on case series analysis [19, 20, 24, 25, 28], of which four prospectively [19, 20, 24, 25] and one retrospectively [28]. Eight papers studied the effect of cycling vs. continuous stimulation on urinary and bowel dysfunction [17, 18, 25, 27-29, 31, 32]. Two papers studied frequency [26, 30] and three papers studied both frequency and pulse width [19, 23, 33]. One paper studied unilateral vs. bilateral SNM [22]. Three papers assessed amplitude levels [20, 21, 24] (Appendix B).

Effect of Intermittent stimulation

Different types of interval stimulation were studied and compared to standard (conservative) stimulation (17-24). One stimulation interval with both an “on” and “off” component was defined as one cycling interval (Tables 1 and 2). Intermittent stimulation was investigated as a means to improve efficacy and in order to prolong battery life.

Table 1. Intermittent stimulation and outcome in SNM on Urinary tract dysfunction

Cycling interval	Time on, time off	Objective outcomes	Subjective outcomes	Reference
Short	16 sec on, 8 sec off; 20 sec on, 8 sec off	0 0 0 0	+ +	[17, 18, 31, 32]
Medium	10 min on, 10 min off; 1 hhour on, 2 hours off	0 0	0 0	[18, 32]
Long	8 hours on, 16 hours off; 30 min on, 23.5 hours off; 23 hours on, 1 hour off; On demand	0 0 0 0	+ − 0 0	[18, 25, 29, 32]

− = in favor of continuous stimulation; 0 = no difference between conditions; + = in favor of intermittent stimulation.

Table 2. Intermittent stimulation and outcome in SNM on Bowel dysfunction

Cycling interval	Time on, time off	Objective outcomes	Subjective outcomes	Reference
Short	20 sec on, 8 sec off	+	-	[28]
Long	Day on, night off	0	−	[27]

− = in favor of continuous stimulation; 0 = no difference between conditions; + = in favor of intermittent stimulation.

Intermittent stimulation and Urinary tract dysfunction

In patients with urinary tract dysfunction, objective outcome measures did not differ between standard (conservative) and intermittent stimulation [17, 18, 25, 29, 31, 32] (Table 1). Nevertheless, differences between standard and intermittent stimulation were noted as related to subjective measures, thereby indicating short cycling intervals to be favorable as compared to standard stimulation [17, 18]. Conflicting results were reported when comparing long cycling intervals with continuous stimulation: on the one hand a decreased quality of life was found, assessed by IIQ-7 score [18], on the other hand long cycling intervals seemed to result in less symptom severity for patients, assessed by PFDI-20 score [25].

Intermittent stimulation and Bowel dysfunction

Short cycling SNM stimulation intervals showed noninferiority on objective outcomes (bowel habit diary) when compared with continuous cycling in patients with bowel dysfunction (Table 2). It is concluded that based on objective measures intermittent stimulation is favored (over standard SNM) on every outcome measure [28]. It should be noted that this conclusion is based on one study which lacked a statistical analysis. Long cycling SNM stimulation intervals (day on, night off) showed similar effect when compared to continuous stimulation on objective outcomes (bowel habit diary). However, in a study with high risk of bias, they showed inferior efficacy on subjective outcomes (St. Mark's incontinence score, Wexner score) [27].

Effect of Frequency and Pulse width

Standard, conservative settings for SNM frequency and pulse width are 7–20 Hz and 100–300 µsec, respectively. The effect of low (<7 Hz) and high frequency (>20 Hz), and narrow (<100 µs) and wide (>300 µs) pulse width on SNM efficacy in both urinary tract dysfunction and bowel dysfunction were studied [19, 20, 23, 26, 30, 33].

Frequency and Urinary tract dysfunction

In urinary tract dysfunction, both low and high frequency were studied [26, 30] (Table 3). One study found no differences on either objective or subjective outcomes [26]. However, the other study found negative objective outcomes (increase in pad changes and number of

urinary incontinence episodes) when comparing low frequency with standard settings. It should be noted that both these studies showed high RoB due to a short wash-out period (one day in both studies).

Table 3. Frequency and outcome in SNM on Urinary tract dysfunction

Frequency	Objective outcomes	Subjective outcomes	Reference
Low: < 7 Hz	0 −	0 0	[26, 30]
High: > 20 Hz	0 0	0 0	[26, 30]

− = in favor of conventional frequency; 0 = no difference between conditions; + = in favor of intervention.

Frequency, Pulse width and Bowel dysfunction

Regarding bowel dysfunction, neither objective nor subjective outcomes differed when comparing low frequency with standard frequency settings [19, 23, 33] (Table 4). High frequency did show an improvement in both subjective and objective outcomes when compared to standard settings. Switching to high frequency resulted in a decrease of fecal incontinence (FI) episodes and bowel movements per day and an improved quality of life [19, 23].

Table 4. Frequency and outcome in SNM on Bowel dysfunction

Frequency	Objective outcomes	Subjective outcomes	Reference
Low: < 7 Hz	+ 0 0	+ − 0	[19, 23, 33]
High: > 20 Hz	+ 0 0	0 0 0	[19, 23, 33]

− = in favor of conventional frequency; 0 = no difference between conditions; + = in favor of intervention.

Narrowing the pulse widths, when compared to standard pulse width, results in improved objective outcomes (number of FI episodes) in one study [19], whereas others did not report this difference [23, 33] (Table 5). Subjective outcomes were contradictory: one study [19] showed improved quality of life, one [23] showed a decrease in quality of life, and one [33] found no differences. A wide pulse width was favorable over conventional pulse width in one study [19] on objective outcomes. No differences on objective and subjective outcomes as related to pulse width were reported in two other studies [23, 33].

Table 5. Pulse width and outcome in SNM on Bowel dysfunction

Pulse width	Objective outcomes	Subjective outcomes	Reference
Narrow: < 100 µs	+ 0 0	+ − 0	[19, 23, 33]
Wide: > 300 µs	+ 0 0	0 0 0	[19, 23, 33]

− = in favor of conventional pulse width; 0 = no difference between conditions; + = in favor of intervention.

Effect of SNM Amplitude

SNM amplitude is normally set at sensory threshold. In the outpatient clinic, amplitude is increased up to a point where the patient feels the tingling sensation of stimulation. However, there is no scientific evidence to back up setting SNM amplitude at this level. The effect of subsensory stimulation, as compared to SNM at sensory threshold, was analyzed in three studies [20, 21, 24].

SNM Amplitude and Bowel dysfunction

Subsensory stimulation at 50% of sensory threshold did not differ in SNM efficacy on objective and subjective measurements from stimulation at sensory threshold [20, 21]. No difference between stimulation at subsensory (75% of sensory threshold) and stimulation at sensory threshold is reported [21]. An earlier study [24] looked at amplitudes 0.6, 0.4, and 0.2 V below sensory threshold, but found only stimulation at sensory threshold decreased number of FI episodes significantly. This study only included eight subjects and scored a high RoB.

Effect of Unilateral versus bilateral SNM

With standard SNM, the electrode is implanted unilaterally to treat either urinary tract dysfunction or bowel dysfunction. From early studies [34, 35] on SNM in urinary tract dysfunction, it is deduced that bilateral SNM results in better treatment, since the bladder is bilaterally innervated [36, 37]. However, at this moment there is no data available to support this.

Unilateral versus bilateral SNM and Bowel dysfunction

No differences in effectiveness of unilateral SNM and bilateral SNM on either objective or subjective outcome measures are reported [22]. The study by Duelund-Jakobsen et al. was stopped after interim analysis of 20 patients showed there was no additional beneficial effect of bilateral stimulation. Moreover, the theoretical possibility of a doubling of infections and device-related pain or discomfort was ground for an early termination of the study.

Discussion

This systematic review provides an overview of the clinical efficacy of SNM related to its stimulation parameters on lower urinary tract and bowel dysfunction.

Both high frequency and both a narrow and wide pulse width showed favorable objective outcomes in patients with bowel dysfunction when compared with standard SNM. In patients with either urinary tract dysfunction or bowel dysfunction, no differences between SNM intermittent stimulation and standard SNM stimulation on objective outcomes were reported. Bilateral SNM efficacy did not differ from unilateral SNM efficacy. The SNM efficacy of subsensory stimulation, at 50% and 75% of subsensory threshold, did not differ from standard stimulation at sensory threshold.

When compared to standard settings, high frequency, but not low frequency, resulted in improved SNM efficacy on bowel dysfunction. As opposed to results in patients with bowel dysfunction, high frequency did not show favorable results in urinary tract dysfunction. It is very easy to alter frequency for patients in the outpatient clinic. Therefore, increasing the frequency to a level that is still comfortable for the patient could be an easy to implement intervention to increase SNM efficacy. In patients with neuropathic pain, high frequency stimulation is a successful alternative to conventional stimulation [38]. It would be interesting to study whether such a high frequency would be feasible and effective in SNM patients.

Intermittent stimulation, and in particular short cycling intervals, seems to be a promising form of SNM in urinary tract dysfunction as related to subjective outcomes. On the one hand, short SNM cycling intervals improve quality of life for patients [17, 18]. On the other hand,

objective outcomes, that is, number of voids or leaks per day, did not improve using short cycling intervals [17, 18]. Studies investigating long SNM cycling intervals show conflicting evidence, with one study reporting a decrease in quality of life [18] and another reporting a decrease in symptom severity for patients [25]. Since the initial purpose of surgery is to improve quality of life for patients, this is a very interesting finding. In addition to the improved quality of life in patients with urinary tract dysfunction, short cycling intervals show a decrease in FI episodes as well [28], although this study lacked statistical analyses. All scores on intermittent stimulation were better than the scores on continuous stimulation on all domains. However, since no statistical analyses were performed, one can only draw the conclusion that intermittent stimulation is noninferior to continuous stimulation.

Even though the studies included and selected in this review regarding intermittent stimulation show similar results, the low number of studies, combined with different definitions of the duration of the intervals, make it difficult to provide conclusions. For example, short cycling intervals were defined as 16 sec [17, 18, 31, 32] or 20 sec [28] on and 8 sec off. A clear definition on certain cycling intervals, that is, in seconds on and seconds off, could improve homogeneity of studies and allow better comparison of results. Improved homogeneity of cycling intervals would consequently lead to a higher external validity and a stronger advice for clinical practice. Besides improving clinical efficacy, intermittent stimulation is often used as a way to improve battery longevity. Interestingly, Medtronic's manual [39] reports reduced longevity when using a 16 sec on, 8 sec off interval. Improved battery longevity is only 10–15% at a relatively high amplitude of 2.0 V with medium cycling intervals (i.e., 60 sec on, 60 sec off and 10 min on, 10 min off). Only when stimulating at 2.0 V using a long cycling interval (0.5 hour on, 23.5 hours off) a significant improvement in battery longevity of 40% was found. These numbers indicate that using intermittent stimulation is not a good means of prolonging battery life.

Bilateral SNM was studied, but showed no difference between unilateral and bilateral stimulation. One study, not included in this review, compared unilateral and bilateral stimulation using PNE instead of tined lead placement (TLP)[40]. No significant differences

were found between unilateral and bilateral stimulation and were thus in accordance with the paper included in this review. More studies comparing the unilateral and bilateral SNM in treatment of both urinary tract dysfunction and bowel dysfunction are needed to provide more conclusive results. However, due to the high costs of implanting SNM bilaterally, these studies are scarce.

With standard, conservative SNM, amplitude is set at sensory threshold during programming and is usually between 1 and 2 V. A downside of this way of programming is the fact that patients believe stimulation should always be at sensory threshold, instead of only during programming. As it is suggested in one pilot study (N = 17) and one follow-up study (N = 75) that stimulation below sensory threshold does not affect efficacy of SNM in bowel dysfunction [20, 21], subsensory stimulation could be used. However, one other study (N = 8) showed no effect of SNM using subsensory stimulation [24]. A clinically relevant advantage of SNM at subsensory threshold is the increase of battery life. To further substantiate the suggestion that SNM at subsensory threshold is as effective as SNM at sensory threshold, larger randomized trials are needed. Interestingly, McAlees et al. [41] are studying the effect of SNM in a sham controlled trial. To blind subjects, stimulation at subsensory threshold is used. It will be very interesting to see the results of this study, as this also might give more insight in the effect of stimulation at subsensory threshold.

Unfortunately, some studies were not included in this review due to high risk of bias. In particular, a short wash-out period led to exclusion of studies. In future studies, a wash-out period of at least one week is advisable in urinary dysfunction. In bowel dysfunction, a wash-out period of at least three weeks is advised. Another limitation of this review is the high heterogeneity in subjective measures due to a lot of different questionnaires in both the fields of urology and surgery, which leads to confusion. Consensus on one questionnaire for urinary tract dysfunction and one for bowel dysfunction would make comparison of data a lot easier.

In conclusion, the results of our systematic review indicate that stimulation parameters may improve efficacy of SNM in treatment of both urinary tract dysfunction and bowel

dysfunction. Especially implementation of short cycling intervals is promising for treatment of both urinary tract and bowel dysfunction. Additionally, high frequency SNM and either a narrow or wide pulse width seem to improve efficacy in patients with bowel dysfunction. Nevertheless, results should be treated cautiously, since the low number of small-scale studies and limited quality of studies makes it not possible to provide final conclusions. Hence, large-scale randomized studies are urgently needed.

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Appendix A: Search Terms

All search strategies are based on work published in:

Riemsma R, Hagen S, Kirschner-Hermanns R, Norton C, Wijk H, Andersson KE, Chapple C, Spinks J, Wagg A, Hutt E, Misso K, Deshpande S, Kleijnen J, Milsom I. Can incontinence be cured? A systematic review of cure rates [Internet]. BMC Med. 2017 [accessed 12.3.18];15(1):63. Available from:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5364653/

Embase (ovid): 1974-2020/01/13

Searched 14.1.2020

1. incontinence/
2. continence/
3. (incontinen$ or continen$ or obstipat$).ti,ab,ot.
4. urine incontinence/ or mixed incontinence/ or stress incontinence/ or urge incontinence/
5. ((Urine$ or urinary or urinat$ or micturat$ or bladder$) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or escaping or uncontrolled or trickl$ or "lack of control" or "no control" or "out of control" or "not voluntary" or involuntary or wetting or leaked or seeped or retention$ or retain$ or dysfunct$ or malfunct$ or obstruct$ or block$ or overactiv$ or over-activ$)).ti,ab,ot.
6. (bladder$ adj3 control$).ti,ab,ot.
7. (SUI or OAB or BPS).ti,ab,ot.
8. "giggle enuresis".ti,ab,ot.
9. "enuresis risoria".ti,ab,ot.
10. (incontinentia urinae or enuresis ureterica or ureter enuresis or enuresis diurnal).ti,ab,ot.
11. ((Unable or inabilit$ or abilit$ or able) adj3 control$ adj3 (urine$ or urinat$ or urinary or micturat$)).ti,ab,ot.
12. neurogenic bladder/
13. ((neurogenic$ or neurologic$ or spinal or spastic$) adj4 bladder$).ti,ab,ot.
14. neurogenic vesical dysfunct$.ti,ab,ot.
15. (Bladder sphincter dys?ynergia or detrusor sphincter dys?ynergia or neurogenic detrusor overactiv$).ti,ab,ot.
16. feces incontinence/
17. (Encopresis or incontinentia alvi).ti,ab,ot.
18. ((bowel$ or rectum or rectal$) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or uncontrolled or trickl$ or "lack of control" or "no control" or "out of control" or "not voluntary" or involuntary or control$)).ti,ab,ot.
19. ((Unable or inabilit$ or abilit$ or able) adj3 control$ adj3 (faeces or faecal$ or feces or fecal$ or stool$ or rectum or rectal$ or bowel$ or bladder$ or anal$ or anus or urine or urinary or diarrh$ or soiling)).ti,ab,ot.

20. ((feces or faeces or fecal$ or faecal$ or stool or stools or defecat$ or soiling) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or escaping or uncontrolled or trickl$ or "not voluntary" or involuntary or control$)).ti,ab,ot.
21. ((diarrh$ or Pseudodiarrh$ or Pseudo-diarrh$) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or uncontrolled or trickl$ or "not voluntary" or involuntary or control$)).ti,ab,ot.
22. ((Unable or inabilit$ or abilit$ or able) adj3 control$ adj3 (diarrh$ or Pseudodiarrh$ or Pseudo-diarrh$)).ti,ab,ot.
23. ((bowel$ or rectum or rectal$ or defecat$) adj4 (disorder$ or malfunction$ or dysfunction$ or evacuat$ or obstruct$ or block$)).ti,ab,ot.
24. ((feces or faeces or fecal$ or faecal$ or stool or stools or defecat$ or soiling) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or escaping or uncontrolled or trickl$ or "not voluntary" or involuntary or control$)).ti,ab,ot.
25. (urinary tract adj3 (dysfunct$ or disorder$ of syndrome$)).ti,ab,ot.
26. (LUTD or LUTS).ti,ab,ot.
27. (pelvic floor adj3 (dysfunct$ or disorder$ of syndrome$)).ti,ab,ot.
28. ((feces or faeces or fecal$ or faecal$ or stool or stools or defecat$ or soiling) adj2 (store or stored or storag$) adj2 (disorder$ or dysfunct$ or malfunct$ or syndrome$)).ti,ab,ot.
29. ((disorder$ or difficult$ or syndrome$) adj4 (urine$ or urinat$ or urinary or micturat$ or bladder$)).ti,ab,ot.
30. overactive bladder/
31. (detrusor adj2 (overactiv$ or over-activ$)).ti,ab,ot.
32. cystitis/ or interstitial cystitis/
33. ((pain$ or discomfort$ or inflamm$ or infect$) adj4 (urine$ or urinat$ or urinary or micturat$ or bladder$ or pelvis or pelvic)).ti,ab,ot.
34. (megacystitis or cystitis or pericystitis).ti,ab,ot.
35. (detrusor adj2 (overactiv$ or over-activ$)).ti,ab,ot.
36. ((bladder$ or hunner or hunneri or submucos$ or sub-mucos$) adj2 (ulcus or ulcer$)).ti,ab,ot.
37. or/1-36
38. sacral nerve stimulation/
39. InterStim.ti,ab,ot.
40. (SNS or SNM).ti,ab,ot.
41. (sacral adj3 (neuromodulat$ or neuro-modulat$ or deafferent$ or de-afferent$ or neurostimulat$ or neuro-stimulat$)).ti,ab,ot.
42. medical electrical stimulation therap$.ti,ab,ot.
43. ((bladder$ or sacral$) adj2 (Autoaugment$ or Auto-augment$)).ti,ab,ot.
44. (sacral nerve$ adj3 (modulat$ or stimulat$)).ti,ab,ot.
45. or/38-44
46. 37 and 45

Medline (Ovid): 1946-2020/01/13

Searched 14.1.2020

1. Fecal Incontinence/
2. exp Urinary Incontinence/

3. Urinary Bladder, Neurogenic/
4. Urinary Bladder, Overactive/
5. cystitis/ or cystitis, interstitial/
6. urination disorders/ or urinary retention/
7. (incontinen$ or continen$ or obstipat$).ti,ab,ot.
8. ((Urine$ or urinary or urinat$ or micturat$ or bladder$) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or escaping or uncontrolled or trickl$ or "lack of control" or "no control" or "out of control" or "not voluntary" or involuntary or wetting or leaked or seeped or retention$ or retain$ or dysfunct$ or malfunct$ or obstruct$ or block$ or overactiv$ or over-activ$)).ti,ab,ot.
9. (bladder$ adj3 control$).ti,ab,ot.
10. (SUI or OAB or BPS).ti,ab,ot.
11. "giggle enuresis".ti,ab,ot.
12. "enuresis risoria".ti,ab,ot.
13. (incontinentia urinae or enuresis ureterica or ureter enuresis or enuresis diurnal).ti,ab,ot.
14. ((Unable or inabilit$ or abilit$ or able) adj3 control$ adj3 (urine$ or urinat$ or urinary or micturat$)).ti,ab,ot.
15. ((neurogenic$ or neurologic$ or spinal or spastic$) adj4 bladder$).ti,ab,ot.
16. neurogenic vesical dysfunct$.ti,ab,ot.
17. (Bladder sphincter dys?ynergia or detrusor sphincter dys?ynergia or neurogenic detrusor overactiv$).ti,ab,ot.
18. (Encopresis or incontinentia alvi).ti,ab,ot.
19. ((bowel$ or rectum or rectal$) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or uncontrolled or trickl$ or "lack of control" or "no control" or "out of control" or "not voluntary" or involuntary or control$)).ti,ab,ot.
20. ((Unable or inabilit$ or abilit$ or able) adj3 control$ adj3 (faeces or faecal$ or feces or fecal$ or stool$ or rectum or rectal$ or bowel$ or bladder$ or anal$ or anus or urine or urinary or diarrh$ or soiling)).ti,ab,ot.
21. ((feces or faeces or fecal$ or faecal$ or stool or stools or defecat$ or soiling) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or escaping or uncontrolled or trickl$ or "not voluntary" or involuntary or control$)).ti,ab,ot.
22. ((diarrh$ or Pseudodiarrh$ or Pseudo-diarrh$) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or uncontrolled or trickl$ or "not voluntary" or involuntary or control$)).ti,ab,ot.
23. ((Unable or inabilit$ or abilit$ or able) adj3 control$ adj3 (diarrh$ or Pseudodiarrh$ or Pseudo-diarrh$)).ti,ab,ot.
24. ((bowel$ or rectum or rectal$ or defecat$) adj4 (disorder$ or malfunction$ or dysfunction$ or evacuat$ or obstruct$ or block$)).ti,ab,ot.
25. ((feces or faeces or fecal$ or faecal$ or stool or stools or defecat$ or soiling) adj4 (leak or leakage or leaks or leaking or seep or seepage or seeps or seeping or accident$ or escap$ or escaping or uncontrolled or trickl$ or "not voluntary" or involuntary or control$)).ti,ab,ot.
26. (urinary tract adj3 (dysfunct$ or disorder$ of syndrome$)).ti,ab,ot.
27. (LUTD or LUTS).ti,ab,ot.
28. (pelvic floor adj3 (dysfunct$ or disorder$ of syndrome$)).ti,ab,ot.

29. ((feces or faeces or fecal$ or faecal$ or stool or stools or defecat$ or soiling) adj2 (store or stored or storag$) adj2 (disorder$ or dysfunct$ or malfunct$ or syndrome$)).ti,ab,ot.
30. ((disorder$ or difficult$ or syndrome$) adj4 (urine$ or urinat$ or urinary or micturat$ or bladder$)).ti,ab,ot.
31. (detrusor adj2 (overactiv$ or over-activ$)).ti,ab,ot.
32. ((pain$ or discomfort$ or inflamm$ or infect$) adj4 (urine$ or urinat$ or urinary or micturat$ or bladder$ or pelvis or pelvic)).ti,ab,ot.
33. (megacystitis or cystitis or pericystitis).ti,ab,ot.
34. (detrusor adj2 (overactiv$ or over-activ$)).ti,ab,ot.
35. ((bladder$ or hunner or hunneri or submucos$ or sub-mucos$) adj2 (ulcus or ulcer$)).ti,ab,ot.
36. or/1-35
37. InterStim.ti,ab,ot.
38. (SNS or SNM).ti,ab,ot.
39. (sacral adj3 (neuromodulat$ or neuro-modulat$ or deafferent$ or de-afferent$ or neurostimulat$ or neuro-stimulat$)).ti,ab,ot.
40. medical electrical stimulation therap$.ti,ab,ot.
41. ((bladder$ or sacral$) adj2 (Autoaugment$ or Auto-augment$)).ti,ab,ot.
42. (sacral nerve$ adj3 (modulat$ or stimulat$)).ti,ab,ot.
43. or/37-42
44. 36 and 43

Pubmed (NLM): 1947-2020/01/13

Searched 14.1.2020

#52 Search (#41 AND #46 AND #51)
#51 Search (#50 OR #49)
#50 Search (((pubstatusaheadofprint OR publisher[sb])))
#49 Search (#47 OR (#47 AND #48))
#48 Search human*[tiab]
#47 Search (((rat[tiab] or rats[tiab] or mouse[tiab] or mice[tiab] or murine[tiab] or rodent[tiab] or rodents[tiab] or hamster[tiab] or hamsters[tiab] or pig[tiab] or pigs[tiab] or porcine[tiab] or rabbit[tiab] or rabbits[tiab] or animal[tiab] or animals[tiab] or dogs[tiab] or dog[tiab] or cats[tiab] or cow[tiab] or bovine[tiab] or sheep[tiab] or ovine[tiab] or monkey[tiab] or monkeys[tiab])))
#46 Search (#42 OR #43 OR #44 OR #45 OR)
#45 Search ("sacral nerve"[Title/Abstract]) AND (modulat*[Title/Abstract] OR stimulat*[Title/Abstract])
#44 Search ((sacral[Title/Abstract] OR Bladder*[Title/Abstract])) AND (neuromodulat*[Title/Abstract] OR neuro-modulat*[Title/Abstract] OR deafferent*[Title/Abstract] OR de-afferent*[Title/Abstract] OR neurostimulat*[Title/Abstract] OR neuro-stimulat*[Title/Abstract] OR Autoaugment*[Title/Abstract] OR Auto-augment*[Title/Abstract])
#43 Search (medical electrical stimulation[Title/Abstract]) AND therap*[Title/Abstract]
#42 Search (InterStim[Title/Abstract] OR SNS[Title/Abstract] OR SNM[Title/Abstract] OR PTNS[Title/Abstract])
#41 Search (#21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31 OR #32 OR #33 OR #34 OR #35 OR #36 OR #37 OR #38 OR #39 OR #40)

#40 Search (#11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20)
#39 Search ((bladder*[Title/Abstract] OR hunner[Title/Abstract] OR hunneri[Title/Abstract] OR submucos*[Title/Abstract] OR sub-mucos*[Title/Abstract])) AND (ulcus[Title/Abstract] OR ulcer*[Title/Abstract])
#38 Search ((pain*[Title/Abstract] OR discomfort*[Title/Abstract] OR inflamm*[Title/Abstract] OR infect*[Title/Abstract])) AND (urine*[Title/Abstract] OR urinat*[Title/Abstract] OR urinary[Title/Abstract] OR micturat*[Title/Abstract] OR bladder*[Title/Abstract] OR pelvis[Title/Abstract] OR pelvic[Title/Abstract])
#37 Search ((disorder*[Title/Abstract] OR difficult*[Title/Abstract] OR syndrome*[Title/Abstract])) AND (urine* or urinat* or urinary or micturat* or bladder*)
#36 Search (((feces[Title/Abstract] OR faeces[Title/Abstract] OR fecal*[Title/Abstract] OR faecal*[Title/Abstract] OR stool[Title/Abstract] OR stools[Title/Abstract] OR defecat*[Title/Abstract] OR soiling[Title/Abstract])) AND (store[Title/Abstract] OR stored[Title/Abstract] OR storag*[Title/Abstract])) AND (disorder*[Title/Abstract] OR dysfunct*[Title/Abstract] OR malfunct*[Title/Abstract] OR syndrome*[Title/Abstract])
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#34 Search (OAB[Title/Abstract] OR BPS[Title/Abstract] OR LUTD[Title/Abstract] OR LUTS[Title/Abstract])
#33 Search (((cystitis[Title/Abstract]) OR "overactive bladder"[Title/Abstract]) OR ("over-active detrusor"[Title/Abstract] OR "overactive detrusor"[Title/Abstract])) OR (megacystitis[Title/Abstract] OR pericystitis[Title/Abstract])
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#28 Search ((rectal[Title/Abstract]) AND (leak[Title/Abstract] OR leakage[Title/Abstract] OR leaks[Title/Abstract] OR leaking[Title/Abstract] OR seep[Title/Abstract] OR seepage[Title/Abstract] OR seeps[Title/Abstract] OR seeping[Title/Abstract] OR accident*[Title/Abstract] OR escap*[Title/Abstract] OR uncontrolled[Title/Abstract] OR trickl*[Title/Abstract] OR "lack of control"[Title/Abstract] OR "no control"[Title/Abstract] OR "out of control"[Title/Abstract] OR "not voluntary"[Title/Abstract] OR involuntary[Title/Abstract] OR control*[Title/Abstract]))
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#26 Search ((bowel*[Title/Abstract]) AND (leak[Title/Abstract] OR leakage[Title/Abstract] OR leaks[Title/Abstract] OR leaking[Title/Abstract] OR seep[Title/Abstract] OR seepage[Title/Abstract] OR seeps[Title/Abstract] OR seeping[Title/Abstract] OR accident*[Title/Abstract] OR escap*[Title/Abstract] OR uncontrolled[Title/Abstract] OR trickl*[Title/Abstract] OR "lack of control"[Title/Abstract] OR "no control"[Title/Abstract] OR "out of control"[Title/Abstract] OR "not voluntary"[Title/Abstract] OR involuntary[Title/Abstract] OR control*[Title/Abstract]))
#25 Search ((Encopresis[Title/Abstract] OR "incontinentia alvi"[Title/Abstract]))
#24 Search (("Bladder sphincter dyssynergia"[Title/Abstract] OR "detrusor sphincter dysynergia"[Title/Abstract] OR "Bladder sphincter dysynergia"[Title/Abstract] OR "detrusor sphincter dyssynergia"[Title/Abstract] OR "neurogenic detrusor overactivity"[Title/Abstract]))
#23 Search ((SUI[Title/Abstract] OR "giggle enuresis"[Title/Abstract] OR "enuresis risoria"[Title/Abstract] OR "incontinentia urinae"[Title/Abstract] OR "enuresis ureterica"[Title/Abstract] OR "ureter enuresis"[Title/Abstract] OR "enuresis diurnal"[Title/Abstract]))
#22 Search ((bladder*[Title/Abstract]) AND control*[Title/Abstract])
#21 Search "neurogenic vesical dysfunction"[Title/Abstract]
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#17 Search ((micturat*[Title/Abstract]) AND (leak[Title/Abstract] OR leakage[Title/Abstract] OR leaks[Title/Abstract] OR leaking[Title/Abstract] OR seep[Title/Abstract] OR seepage[Title/Abstract] OR seeps[Title/Abstract] OR seeping[Title/Abstract] OR accident*[Title/Abstract] OR escap*[Title/Abstract] OR escaping[Title/Abstract] OR uncontrolled[Title/Abstract] OR trickl*[Title/Abstract] OR "lack of control"[Title/Abstract] OR "no control"[Title/Abstract] OR "out of

control"[Title/Abstract] OR "not voluntary"[Title/Abstract] OR involuntary[Title/Abstract] OR wetting[Title/Abstract] OR leaked[Title/Abstract] OR seeped[Title/Abstract]))
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#12 Search (incontinen*[Title/Abstract]) OR continen*[Title/Abstract]
#11 Search (((((((("Urinary Incontinence"[Mesh]) OR "Fecal Incontinence"[Mesh:NoExp]) OR "Urinary Bladder, Neurogenic"[Mesh:NoExp]) OR "Urinary Bladder, Overactive"[Mesh]) OR "Cystitis"[Mesh:NoExp]) OR "Cystitis, Interstitial"[Mesh:NoExp]) OR "Urination Disorders"[Mesh:NoExp]) OR "Urinary Retention"[Mesh])

Appendix B: Characteristics of included articles

Table B1. Characteristics of the included studies

Study ID	Indication	No. of subjects	Stimulation parameter	Cycling interval	Freq (Hz)	Pulse width (µs)	Amplitude	Primary outcome	Wash-out	Follow-up period
Beer 2016 [17]	OAB	23	Intermittent stimulation	16s on, 8s off				OABq SF scores	3 months	3 days
Cadish 2016 [18]	OAB	23	Intermittent stimulation	16s on, 8s off; 23h on, 1h off; 1h on, 2h off				IIQ-7 score, Likert score; number of voids/day and number of leaks/day	13 days	1 day
Hoen 2007 [25]	OAB	19	Intermittent stimulation	8h on, 16h off	14	210	1.1-2.9 V	UDI-6, IIQ-7, PFDI-20, PFIQ-7, PISQ-12, FIQLS, FISI; Voiding diary	2 weeks	12 weeks
Price 2015 [31]	OAB	32	Intermittent stimulation	16s on, 8s off	14	210	ST	UIIQ, UDI; Voiding diary	4 weeks	3 days
Siegel 2018 [32]	OAB	28	Intermittent stimulation	16s on, 8s off; 10m on, 10m off; 30m on, 23.5h off;				GRA scores; Incontinence episodes/day	3 weeks	1 week
Oerlemans 2011 [29]	OAB	21	Intermittent stimulation	On demand vs continuous (min. 4h off)				UMQ; Voiding diary		Not mentioned
Michelsen 2008 [27]	FI	19	Intermittent stimulation	Day on, night off				Wexner score, St. Mark's continence score; Bowel habit diary	No wash-out	3 weeks
Norderval 2013 [28]	FI	29	Intermittent stimulation	20s on, 8s off				Bowel habit diary		3-34 months
Marcelissen 2011 [26]	OAB	50	Frequency		5.2, 10, 21, 40	210	ST	Questionnaire; Voiding diary	1 day	6 days
Peters 2013 [30]	OAB	12	Frequency		5.2, 14, 25			11-point VAS scale on pelvic pain; Voiding and bowel habit diary	1 day	6 days
Dudding 2009 [19]	FI	12	Frequency + pulse width		6.9, 14, 31	90, 210, 450	ST	St. Mark's continence score, FIQLS; Bowel habit diary		2 weeks
Dueland-Jakobsen 2012 [23]	FI	15	Frequency + pulse width		6.9, 14, 31	90, 210, 330	ST	FIQLS, CCCS, SMCS, GSRS-IBS, 11-point VAS scale for overall satisfaction; Bowel habit diary, anorectal measurements	1 week	3 weeks
Dueland-Jakobsen 2020 [20]	FI	17	Frequency		14, 31	90, 210		FIQoL, CCCS, VAS scale for satisfaction; Bowel habit diary	2 months	12 months

Thomas 2015 [33]	Constipation	11	Frequency + pulse width		6.9, 14, 31	90, 210, 450	ST	CCCS, PAC-SYM, PAC-QOL, 101-point VAS scale for satisfaction; Bowel habit diary	2 weeks	3 weeks
Dueland-Jakobsen 2015 [22]	FI	27	Bilateral vs unilateral SNM		14	210	ST	Wexner score, SMCS, GSRS-IBS, FIQLS; Bowel habit diary, anorectal measurements	1 week	3 weeks
Dueland-Jakobsen 2013 [21]	FI	17	Amplitude				50% ST, 75% ST, ST	Wexner score, SMCS, GSRS-IBS, FIQLS, 11-point VAS score on satisfaction; Bowel habit diary, anorectal measurements	1 week	3 weeks
Dueland-Jakobsen 2020 [20]	FI	75	Amplitude		14,31	90,210	50% ST, ST	FIQoL, CCCS, VAS scale for satisfaction; Bowel habit diary	2 months	12 months
Koch 2005 [24]	FI	8	Amplitude				0.6V<ST, 0.4V<ST, 0.2V<ST, ST	Bowel habit diary		4 weeks
OAB, overactive bladder; FI, faecal incontinence; ST, sensory threshold.										

Appendix C: Risk of Bias Assessment

Table C1. Risk of Bias assessment: Randomized Controlled Trials

Study ID		Bias from randomization	Bias due to deviations from intended interventions	Bias due to missing outcome data	Bias in measurement of outcome	Bias in selection of reported results	Overall bias
Oerlemans et al [29]	2011	!	!	✓	✓	✓	!

✓ = low risk; ! = some/moderate risk

Table C2. Risk of Bias assessment: RCT with cross-over design

		Bias from randomization process	Bias due to deviations from intended interventions	Bias due to missing outcome data	Bias in measurement of outcome	Bias in selection of reported result	Overall bias
Beer et al. [17]	2016	✓	✓	✓	✓	✓	✓
Cadish et al. [18]	2016	!	✓	✓	✓	!	!
Duelund-Jakobsen et al. [23]	2012	✓	✓	✓	✓	✓	✓
Duelund-Jakobsen et al. [21]	2013	✓	✓	✓	✓	✓	✓
Duelund-Jakobsen et al. [22]	2015	✓	✓	✓	✓	✓	✓
Marcelissen et al. [26]	2011	!	✗	✓	✓	!	✗
Michelsen et al. [27]	2008	✓	✗	✓	✓	✓	✗
Peters et al. [30]	2013	!	✗	✓	✓	✓	✗
Price et al. [31]	2015	✓	✓	!	✓	✓	!
Thomas et al. [33]	2015	✓	✓	✓	✓	✓	✓
Siegel et al. [32]	2018	!	✓	✓	✓	✓	!

✓ = low risk; ! = some/moderate risk; ✗ = high risk

Table C3. Risk of Bias assessment: Case series

Study ID		Clear criteria for inclusion	Condition measured in standard, reliable way	Valid methods used for identification of the condition	Consecutive inclusion of participants	Complete inclusion of participants	Clear reporting of demographics	Clear reporting of clinical information	Outcomes clearly reported	Clear reporting of presenting sites	Statistical analysis appropriate	Wash-out period sufficient	Overall bias
Dudding et al. [19]	2009	✓	✓	✓	−	−	✓	✓	✓	✓	✓	N/A	−
Dueland-Jakobsen et al. [20]	2020	✓	✓	✓	−	✓	X	✓	✓	✓	✓	✓	✓
Hoen et al. [25]	2017	✓	✓	✓	−	−	✓	✓	✓	−	✓	✓	−
Koch et al. [24]	2005	✓	✓	✓	−	−	✓	✓	✓	−	✓	X	X
Norderval et al. [28]	2013	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	N/A	✓

✓ = yes/low risk; − = unclear/moderate risk; X = no/high risk.

Burst Paradigms Evoked Bladder Responses in Sacral Neuromodulation Patients

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Submitted.

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Chapter 6

Replacement Adaptor 09106 for Patients With a Dynamic Graciloplasty or Patients With Sacral Neuromodulation and Abdominal IPGs: A Safety and Feasibility Study

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Under review.

* These authors contributed equally to this manuscript

Chapter 7

Abstract

Objective: Due to the introduction of a new implantable pulse generator (IPG), the Interstim II, patients with either a dynamic graciloplasty or an abdominally placed IPG for sacral neuromodulation could not undergo surgery to replace their IPG in case of end of battery life. For these patients, the Medtronic Replacement Adaptor 09106 was created. This retrospective case series aims to study safety and feasibility of the Medtronic Replacement Adaptor 09106 in patients with abdominally placed IPG's.

Methods: Seventeen patients (11 female, 6 male) received a replacement adaptor with a follow-up of six months. Outcome measures were objectified using a bowel habit diary. Adverse events were classified using the Clavien-Dindo classification.

Results: Outcome measures in the bowel habit diaries after replacement (feasibility) did not differ significantly from outcome measures before replacement. Adverse events occurred in 4 out of 17 patients (24%): two patients initially showed pocket site pain (Clavien-Dindo level I), which was resolved without intervention. One patient suffered from poor wound closure (Clavien-Dindo level II), and one patient had persisting pocket pain (Clavien-Dindo level IIIa), for which a pocket revision was performed.

Conclusions: The Medtronic Replacement Adaptor 09106 is a valuable option for patients with dynamic graciloplasty or sacral neuromodulation and abdominal IPG and has complication rates similar to replacement of the Interstim without Replacement Adaptor 09106.

Keywords: Faecal incontinence, neuromodulation, neurostimulation, adaptor

Introduction

Faecal incontinence is a great burden, for which several treatment options have been developed. A few decades ago, two treatment options involving electrical stimulation were introduced: dynamic graciloplasty (DGP) and sacral neuromodulation.

Graciloplasty was introduced in 1952 to create a neo rectal sphincter and restoration of anal continence by transplanting the gracilis muscle [1]. However, the results of this technique were mixed due to the presence of a majority of type II muscle fibres in the gracilis muscle [1]. These type II muscle fibres, also known as fast-twitch muscle fibres, use anaerobic metabolism to create adenosine triphosphate (ATP). Anaerobic metabolism is ideal to fuel short bursts of power, but is not ideal for endured exercise, like sustaining sphincter tone [2]. In 1988, Baeten and colleagues proposed to stimulate the transplanted gracilis muscle using neurostimulation to induce change from type II to type I (slow-twitch) muscle fibres [3]. At the same time, Williams et al. developed a similar neurostimulation technique with use of an implantable pulse generator (IPG) to stimulate the gracilis muscle [4]. While the group of Williams chose to indirectly stimulate the obturator nerve by fixing the electrodes directly onto the terminal nerve branches, the group of Baeten fixed the electrodes close to the nerve branches inside the muscle [5, 6]. Both techniques yielded similar results on muscle contraction force, but Williams' technique required more reoperation than Baeten's technique, partly due to leakage of IPGs [7, 8].

Sacral neuromodulation (SNM) as a treatment modality for faecal incontinence was introduced in 1995 and improved long-term continence in over 50% of patients [9, 10]. In SNM, an electrode is placed in sacral neural foramen S3 and powered by the aforementioned IPG, also used in dynamic graciloplasty [10].

Since its first introduction in the early 90s, several types of IPGs have been released. The first patients were implanted with the Itrel-I, which only allowed for parameter changes during surgery. In 1994, the Itrel-II (Fig. 1a) was introduced, which allowed the physician to alter parameters using an external programmer. Additionally, this Itrel-II IPG allowed patients to switch the IPG on and off and to choose between two programs using a magnet. In 1999, the

Interstim I (model 3023; Fig. 1b) IPG was introduced, which empowered patients to switch the IPG on and off using a remote control. This remote control also enabled patients to switch between pre-programmed parameters and alter amplitude within a certain range [11]. Although IPGs for DGP were always placed abdominally, the placement of the leads for SNM in the sacral foramina allowed for gluteal placement of the IPG, leading to a lower complication rate. In 2006, the Interstim I was replaced by the Interstim II (model 3058; Fig. 1c), which was smaller in size (22g vs 42g, 51x44x7.7mm vs 60x55x10mm) [12].

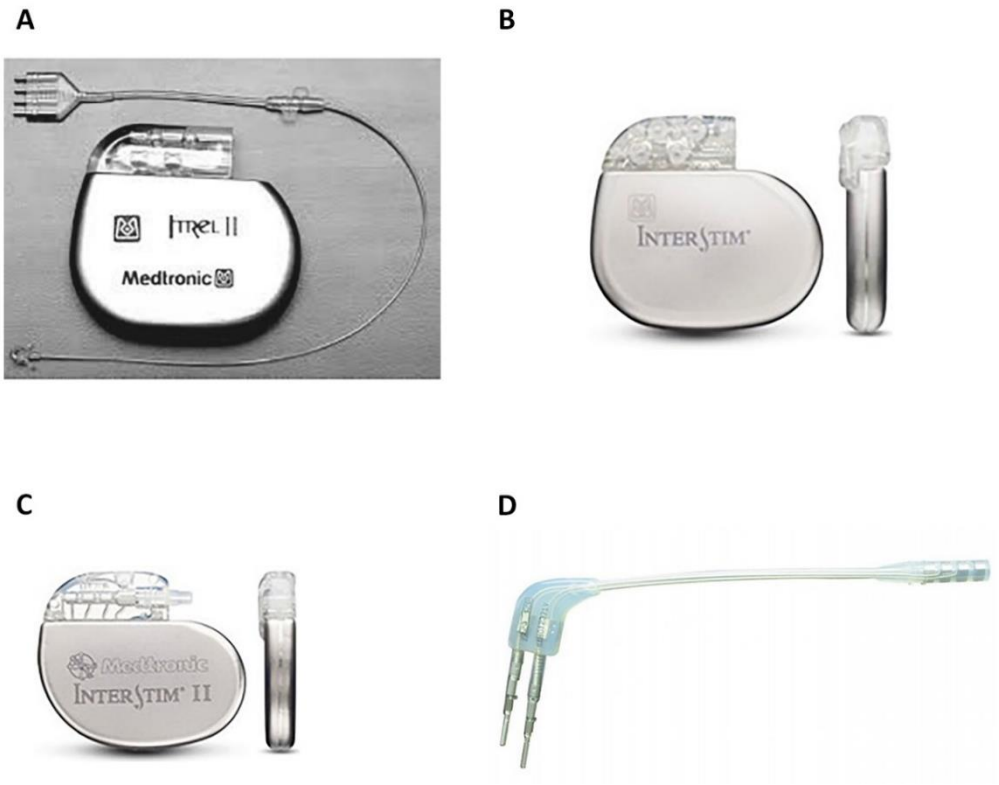

Figure 1. Implantable pulse generators A) Itrel II IPG. B) Interstim I IPG, 60x55x10mm, 42g. C) Interstim II IPG, 51x44x7.7mm, 22g. D) Model 3095 quadripolar extension.

Since 2017, patients with an abdominal IPG for either SNM or DGP to treat faecal incontinence encountered a problem in case of end of service (EOS), i.e. end of battery life, as the former IPGs (DGP: model 4300/4350 leads; SNM: model 3095 quadripolar extension,

Fig. 1d) were not produced anymore and the newer Interstim II was not compatible with the leads. One of the main differences between the Interstim I and Interstim II is the reduction in access points from two to one. On the one hand, the DGP consists of two leads placed in the gracilis muscle, which made it perfectly compatible with the Interstim I. On the other hand, SNM uses only one lead, making it compatible with the Interstim II.

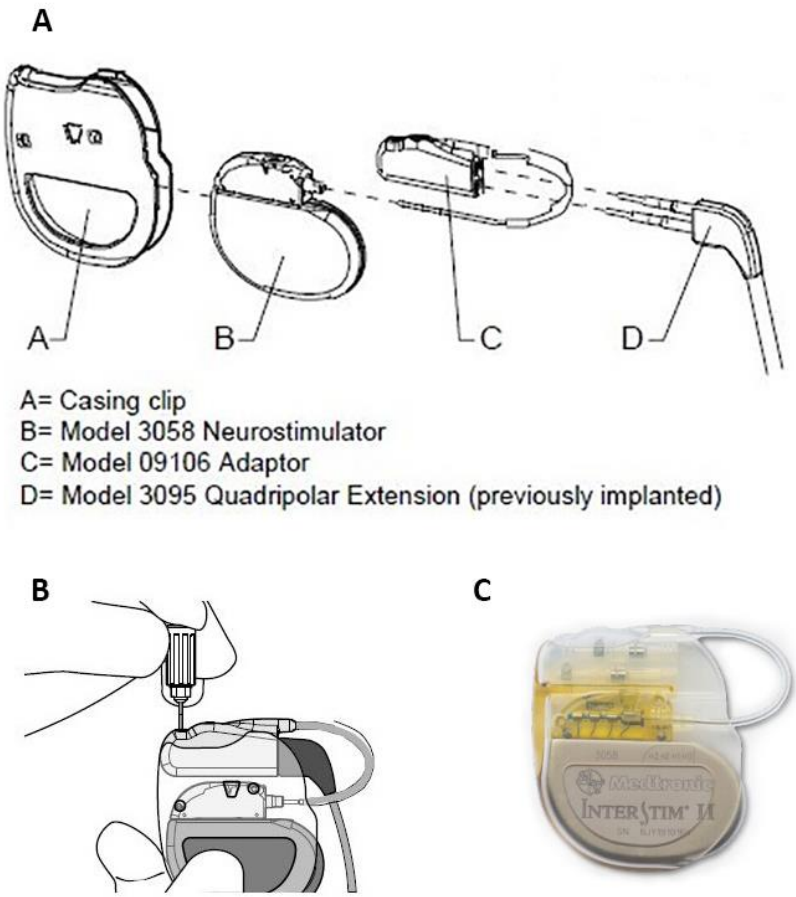

Figure 2. Replacement adaptor 09106. A) Components of the replacement adaptor. B) Schematic assemblage of components into replacement adaptor. C) Components assembled to the replacement adaptor.

To overcome differences between access points and number of leads, a connection piece was needed to connect the single SNM lead to the two Interstim I access points. In patients with the IPG placed in the buttocks, replacement of the Interstim I by the Interstim II does not pose a problem, since the connection piece can easily be removed in order to connect the single SNM lead to the single access point of the Interstim II. However, in patients with abdominally placed IPGs, this connecting piece also serves as an extension piece. Therefore, it cannot be removed since the lead would be too short.

As a solution to these problems for patients with IPGs that reached EOS, a collaboration between Maastricht University Medical Center+ (MUMC+) and the Medtronic Bakken Research Center created the Medtronic Replacement Adaptor 09106® (RA; Fig. 2). One of the goals when creating this adaptor was to create it with a volume similar to the Interstim I. The RA 09106 has received CE marking January 30th 2018.

This study aimed to determine safety and feasibility of this new RA 09106 in patients with either a dynamic graciloplasty or patients with sacral neuromodulation and abdominal IPGs.

Methods

Study group

All patients with faecal incontinence, who received an IPG Interstim II with RA 09106 in MUMC+ from February 2018 till April 2020 were included in the study, with a follow-up of at least six months. Ethical approval was granted (METC 2020-1320) and the research was performed in accordance with the Declaration of Helsinki. Clinical outcome before and after placement of the RA were reviewed retrospectively. Patient characteristics, clinical outcome and adverse events were reported.

Patient characteristics

For all patients, gender, age, treatment, type of anaesthesia and operating time were recorded.

Clinical outcomes and Feasibility

Patients reported defecation frequency, faecal incontinence episodes and ability to delay defecation both before and after replacement by using a bowel habit diary

Clinical outcome and Safety (adverse events)

Adverse events were device- and procedure-related events. All adverse events were scored using the Clavien-Dindo classification [13]. Serious adverse events were scored Clavien-Dindo Grade III or higher and required direct surgical, endoscopic or radiologic intervention. Adverse events were scored Clavien-Dindo grade II or lower.

Procedure for implantation RA

Since patients were already implanted with an IPG (Interstim I), the same pocket was used to place the Interstim II + RA. The abdominal scar was opened under local or general anaesthesia. Then, the pocket, situated behind the anterior wall of the rectus sheath, was opened and the old IPG Interstim I was released from the leads. Subsequently, the leads were connected to the replacement adaptor and the Interstim II. The combined Interstim II + RA were repositioned in the pocket. The pocket, anterior wall of the rectus sheath and wound were subsequently closed. Patients were contacted two weeks and three months after surgery, after which the regular, yearly check-ups were resumed.

Statistical analysis

All statistical analyses were performed using Prism 5 (GraphPad Software, San Diego, CA, USA). All reported data were continuously and presented as mean ± SD. Data were tested for normality using a Shapiro-Wilk normality test, and were found to be non-normally distributed. Hence, paired comparison was performed using a Wilcoxon signed rank test. A P-value < 0.05 was considered statistically significant.

Results

Patient characteristics

Twenty-two patients underwent successful replacement of the Interstim I by Interstim II + RA between February 2018 and April 2020. Five patients disclosed to their doctor to have no difference in bowel habit with the Interstim II + RA as compared to Interstim I, but did not

complete a bowel habit diary. Therefore, these five patients have been excluded from the analysis. Patient characteristics of 17 patients are shown in Table 1. The mean age was 64.8 (± 10.7; 38.0 – 81.7) years old and 64.7% were female (11 female, 6 male). All patients suffered from faecal incontinence, two patients were treated with SNM and fifteen patients with DGP. Sixteen patients were operated under general anaesthesia and one was operated under local anaesthesia. All patients were treated in day care and were able to leave the hospital the day of admission. Follow-up of patients was six months.

Table 1. Patient characteristics	
Gender	
Female, n (%)	11 (64.7%)
Male, n (%)	6 (35.3%)
Age, years (mean, range)	64.8 (± 10.7; 38.0 - 81.7)
Treatment	
DGP, n (%)	15 (88.2%)
SNM, n (%)	2 (11.8%)
Anaesthesia	
General, n (%)	16 (94.1%)
Local, n (%)	1 (5.9%)
Operating time, minutes (mean, range)	45.1 (± 11.4; 29-79)
n = amount; DPG = dynamic graciloplasty; SNM = sacral neuromodulation	

Clinical outcomes and Feasibility

All patients verbally mentioned to be satisfied with the replacement of the Interstim II and RA one month after surgery. Clinical outcome parameters are shown in Table 2. Defecation frequency per day did not significantly differ before and after replacement (1.91 ± 1.36 vs 1.72 ± 1.16; p=0.20). In addition, no significant differences were found in the number of faecal incontinence episodes per week before and after replacement (1.12 ± 1.81 vs 0.52 ± 1.09; p=0.22). Moreover, the replacement had no significant impact on the ability to delay defecation before and after replacement (median of 3.5 for both; p=0.50). Moreover, there was no difference between impedance before and after surgery (425.6 ± 200.2 vs 427.4 ± 106.4; p=0.97) and amplitude used to power the electrodes (2.20 ± 0.81 vs 1.63 ± 0.85; p=0.056).

Table 2. Efficacy of Interstim II + Replacement Adaptor 09106			
	Before replacement	**After replacement**	**P-value**
Defecation frequency per day (mean ± SD)	1.91 ± 1.36	1.72 ± 1.16	0.20
Faecal incontinence episodes per week (mean ± SD)	1.12 ± 1.81	0.52 ± 1.09	0.22
Impedance of IPG (mean ± SD)	425.6 ± 200.2	427.4 ± 106.4	0.97
Amplitude used for IPG (mean ± SD)	2.20 ± 0.81	1.63 ± 0.85	0.056
Ability to delay defecation in minutes (median, range)	3.5 (0-720)	3.5 (0-720)	0.50
Adverse events (n, %)	NA	4 (23.5%)	NA
SD = standard deviation; n = amount; NA = not applicable			

Clinical outcome and safety (adverse events)

Adverse events were recorded in four patients. Two patients suffered from pocket site pain initially, but this was resolved without intervention (Clavien-Dindo grade I). One patient had poor wound closure, a common complication for this diabetes patient after every procedure. This patient was treated with ciprofloxacin and clindamycin prophylactically and did not suffer from persisting complaints (Clavien-Dindo grade II). At 3 months follow-up, one patient suffered from pocket site pain, for which she underwent a pocket revision under local anaesthesia (Clavien-Dindo grade IIIa). After this procedure, the patient was relieved from the pain.

Discussion

The replacement of Interstim I by Interstim II with RA 09106 is a valuable solution for faecal incontinence patients with EOS of the IPG for DGP or SNM. No significant differences were found in bowel habit diary, impedance of IPG and amplitude applied before and after replacement. Complications occurred in four out of seventeen patients (23.5%). Two of these patients required intervention (antibiotics and reoperation), which classified the complications grade II and IIIa respectively. Two patients were relieved of pocket site pain without intervention (grade I complication). To date, no literature was published regarding

the RA and therefore we are not able to compare our results to previous studies. However, earlier studies have shown complications in SNM surgery without the RA 09106 in approximately 30% of patients, similar to the percentage in this study [14, 15].

One of the main limitations of this study is the limited number of patients included. This can be explained as the total number of patients eligible for replacement of the Interstim I by the Interstim II plus replacement adaptor is low. Over the years, more patients will require replacement of the Interstim I with the RA 09106, which means a bigger cohort could be formed. Data of this bigger cohort could confirm the results in this study.

A second limitation of this study is the relatively short follow-up of six months. After these six months, patients had yearly check-ups and were instructed to contact the hospital in case of problems with their IPG, similar to the regular patient contacts. It needs to be emphasised that despite the limited follow-up period, none of the patients reported any problems. Follow-up until the next Interstim replacement would be optimal to study safety and feasibility.

In conclusion, this case series shows that the replacement adaptor 09106 for patients with a dynamic graciloplasty or patients with sacral neuromodulation and abdominal IPGs with end of service is a valuable option.

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General Discussion and Conclusion

Chapter 8

The primary aim of this academic thesis is to establish two experimental models for either faecal incontinence (FI) or lower urinary tract dysfunction (LUTD) in order to be able to study the effect and use of new SNM stimulation paradigms in the treatment of lower urinary tract or bowel dysfunction. The research questions formulated in *Chapter 1* and addressed in the subsequent chapters are evaluated in this general discussion.

Preclinical developments

Research Question 1 *'What are the recent developments concerning the use and effectivity of SNM stimulation parameters for the treatment of experimental lower urinary tract and bowel dysfunction?'* is reviewed and presented in ***Chapter 2*** *'Sacral neuromodulation for lower urinary tract and bowel dysfunction in animal models: a systematic review with focus on stimulation parameter selection'*.

In this systematic review, 22 animal studies that fit the inclusion criteria were included. Methodological quality assessment resulted in an 'unclear' risk of bias for most items, mainly due to poor reporting. The included studies showed a large variability in their methodology, use of specific stimulation parameters, outcome parameters and animal species. Overall, this systematic review indicates that SNM had a positive therapeutic effect in preclinical models of lower urinary tract and bowel dysfunction. For LUTD, low frequency (LF; 7.5-15Hz) stimulation in the same range as conventional stimulation (7-20 Hz) appeared to facilitate storage dysfunction, whereas high frequencies (100 Hz - 12.5 kHz) appeared to evoke bladder evacuation. Bilateral SNM and pulse widths above conventional settings (± 210 µs) may contribute to reducing stimulation intensity, and diminish unpleasant perceptions. For bowel dysfunction, it was shown that frequencies lower than those used with conventional (Con-) SNM (2-5Hz) and a stimulus intensity below motor threshold was preferred for both storage and evacuation dysfunction. The fact that most studies used a relatively tentative range of stimulation parameters, and only one study reported the use of high frequency (HF) SNM similar to that used in pain research (where frequencies are used up to 10kHz), makes meaningful comparisons of Con-SNM to both HF and alternative stimulation paradigms

almost impossible. This leaves a large unexplored area for more research into alternative SNM stimulation paradigms.

Methodological quality assessment for preclinical studies is not yet common practice, and thus often shows poor reporting on many items in the SYRCLE's risk of bias tool used for this systematic review [1], such as detailed information on randomization, concealment, blinding and missing data. This Risk of Bias tool was used to specify the quality of the articles included, and by enclosing the quality assessment we demonstrate the importance of detailed and uniform reporting of data which is needed to increase quality and reproducibility in future animal studies.

To answer ***Research Question 2*** *'Can we establish translational and reproducible animal models for faecal incontinence and lower urinary tract dysfunction?',* two animal models for either FI or LUTD were investigated. The data and research related to these animal models are presented in *Chapter 3 'Vaginal distention rodent model for faecal incontinence: effect on defecation behaviour'* and *Chapter 4 'Longitudinal quantitative evaluation of bladder storage and evacuation dysfunction for preclinical intervention effect in awake rats'*, respectively. In ***Chapter 3***, we designed an experimental animal study in which rats underwent vaginal balloon inflation to simulate childbirth as a model for FI (Vaginal Distention (VD) model) and assessed defecation behaviour, which has not been reported previously. Earlier described intrapelvic balloon models in which defecation behaviour was examined showed a relatively small window for treatment effect [2] or a relatively low responder rate [3]. The latter study used the same behavioural task set-up as in our present study. Our study showed that the VD model and a vaginal balloon inflation for 2 hours resulted in FI in 29% of rats (2 out of 7). In rats that underwent 4 hours of intravaginal balloon inflation, 33% (1 out of 3) developed FI and two rats (67%) showed signs of bowel dysfunction. In these two animals discomfort was too severe and these rats reached a humane endpoint. From these results, it became evident that an intravaginal balloon inflation duration between 2 and 4 hours seems to be optimal to induce FI, resulting in a substantial number of responders and avoiding excessive discomfort. Based on these

experiments, we conclude that additional research is needed to further titrate the most optimal duration of balloon inflation before the VD model can be used as a valid model for FI. Nevertheless, with these alterations and further fine-tuning we are convinced that the VD model can be used to adequately study FI, as the VD model not only closely mimics the pathology of the clinical phenotype, but also is a minimally invasive procedure (no incisions needed). Moreover, as reported in our study, the responders to the VD model showed a high defecatory behaviour index (DBI). These advantages are essential and make the VD model a very good candidate for mimicking and studying FI, especially when compared to other previously described models. The high FI rate as noted in our experiment in *Chapter* 3 also depends on the defecation behaviour task used. This behavioural task, in which defecation behaviour is assessed based on faecal pellet distribution throughout the cage with the use of a latrine box and continence training, was previously described by Devane and colleagues [3]. In contrast, Janssen and colleagues [2] investigated defecation behaviour using a different approach, in which rats did not undergo continence training before the start of the experiment. Their outcome was based on the place preference of defecation furthest from the food area. Furthermore, the control group did not show this assumed place preference, and this could possibly contribute to the small treatment window for defecation behaviour as reported by Janssen and colleagues [2].

The defecation behaviour task as developed by Devane and colleagues [3] and as used in our study, resulted in a wide and sufficient treatment window in responders. Moreover, our study even showed a slightly higher level of incontinence in terms of the DBI as compared to the study of Devane and colleagues. As the criteria to classify an animal as 'faecal incontinent' were considered sufficient, it now allows to study possible treatment effects. Furthermore, the defecation behaviour task is a partially automated, objective task with low between-researcher variability. All these advantages make the VD model in combination with the defecation behaviour task a promising method to investigate the efficacy and mechanisms of action of therapeutic interventions such as SNM in future studies.

In ***Chapter 4***, the partial bladder outlet obstruction (pBOO) model for LUTD, as previously described by Mattiasson and Uvelius [4], was further investigated for longitudinal monitoring and evaluation of voiding behaviour and bladder capacity. With this longitudinal approach, disease progression over time can be monitored and possible interventions can be adapted accordingly when needed. We designed an experimental animal study in which pBOO disease progression was investigated using longitudinal evaluation of voiding behaviour and bladder capacity. Bladder capacity was shown to increase over the four weeks post-surgery as measured with ultrasound imaging (US) in awake rats. This increase in bladder capacity was also observed with terminal cystometry. Additionally, terminal cystometry revealed an increase in bladder compliance and residual volume, as well as a decrease in voiding pressure. In accordance, bladder weight of pBOO rats was shown to increase during the four weeks post-surgery. No differences in voiding behaviour were observed during disease progression using the voiding spot assay (VSA), and no differences in post mortem assessment of bladder and kidney tissue were observed.

An increase in small voiding spots in the center zone was expected in pBOO rats as compared to control rats, resulting from an uncontrolled micturition pattern. Besides the low sample size as used in *Chapter 4*, multiple additional factors might be responsible for the lack of increase in small voiding spots with the pBOO model in our VSA set-up. Chen and colleagues reported that a sudden change of cage type could increase stress and influence voiding behaviour as measured by VSA in healthy mice [5]. In their study, the authors used different types of experimental cages in order to assess voiding behaviour by means of VSA [5]. The fact that our experimental test cage significantly differed from the standard housing cage of the rats (e.g. walking grid vs. soft bedding, less cage enrichment) might have induced stress in the animals, thereby affecting their voiding behaviour. On the other hand, the study by Chen was performed in mice, and previous VSA studies in mice have shown clear VSA differences related to pathology, despite these environmental influences [6]. Additionally, also other environmental factors, including the position of the cage in the testing chamber (e.g. close vs. far from the door, radio or light source), might have affected VSA outcomes, leading to increased variability in the data. Based on these data, certainly also other ways of

measuring voiding behaviour as well as optimized versions of the VSA should be considered. In this context, the metabolic cage might be an alternative option. A metabolic cage allows to measure micturition automatically over time with information on the number of spots, voided volume per spot and voided volume at specific time points. Using this method, intermittent voiding in several spots is seen as one void instead of separate voids for each spot as analysed by the VSA. Despite the fact that the metabolic cage also relies on the use of a walking grid, several studies showed an effect of the pBOO model on voiding behaviour using the metabolic cage [7-9]. Unfortunately, the use of a metabolic cage does not provide results on voiding location, an important aspect that is measured with the VSA set-up as used in our study.

It should be pointed out that the defecation behaviour task as mentioned in *Chapter 3* started with a training period to successfully acquire continence in rats, while the VSA, as used in this study is based on natural behaviour of the rat and as such no training period was included. Our voiding pattern data does not show place preference for voiding in the corners of the cage as might be expected for control animals. A 'marking' behaviour with approximately 90% of voids in the outer corners has been reported in control rats [10]. Interestingly, Richards and Stevens showed that singly housed rats did not show this 'marking' behaviour in a way that was observed with socially housed rats [11]. Hence, a training period to acquire urinary continence could possibly be an interesting addition to the current approach in measuring and analysing voiding behaviour. Although it is certainly more difficult to observe voiding spots in normal bedding as compared to faecal pellets, the use of hydrophobic sand [12] could serve as an alternative way to easily collect voids and subsequently place them in a latrine area for training purposes.

With our experimental design and study, we were the first to use longitudinal evaluation of bladder capacity with US in awake rats, allowing us to monitor increases in bladder volume over time. This US was performed at fixed time points during the experiment independent of the stage of the micturition cycle. The result based on US was related to the cystometric outcome, which revealed that US is a suitable technique for longitudinal evaluation of

bladder storage and evacuation dysfunction in small animals such as rats. Our results indicate a large window of opportunity using US and cystometry in pBOO animals, which indicates that the pBOO model can be used to monitor LUTD and future interventions. As we were not able to observe differences in voiding behaviour the question remains if and how voiding behaviour is related to bladder volume. As mentioned before, voiding behaviour is very sensitive to stress and environmental changes, and it is of utmost importance to either refine existing, or develop new tests in order to accurately measure voiding behaviour.

Clinical implementation

Research Question 3 *'What are the recent developments of SNM stimulation parameters for the treatment of lower urinary tract and bowel dysfunction in a clinical setting?'* is reviewed and presented in *Chapter 5 'Stimulation parameters for sacral neuromodulation on lower urinary tract and bowel dysfunction-related clinical outcome: a systematic review'*. This review is an accompanying paper to the systematic review on preclinical studies of SNM in *Chapter 2*. Together, these two chapters provide a solid state of the art overview of the effect of SNM stimulation parameters on lower urinary tract and bowel dysfunction.

In this review (***Chapter 5***), 17 articles meeting the inclusion criteria were included. Similar to what was observed in *Chapter 2*, altering SNM stimulation parameters in clinical lower urinary tract and bowel dysfunction indeed seemed to improve efficacy of SNM, with short cycling intervals being favourable over Con-SNM for LUTD. Unfortunately, the included clinical studies investigating LUTD only focussed on intermittent stimulation and stimulation frequency. In terms of the objective outcome parameters, no differences were noted between intermittent stimulation and conventional stimulation, whereas short cycling intervals did appear to be favourable over conventional stimulation in terms of subjective outcome parameters. Frequencies of SNM stimulation lower than the conventional frequency (defined as 7-20 Hz) showed a negative effect on the objective outcome parameters, and no effects of frequency alteration were observed for subjective outcome parameters. It should nevertheless be noted that these studies investigating SNM frequency showed a 'high' risk of bias due to the short washout period used.

For both reviews (*Chapter 2* (preclinical) and *Chapter 5* (clinical)), the best results for SNM in LUTD were observed in using conventional frequencies (7-20 Hz) in a range below 50 Hz. Unfortunately, none of the clinical studies investigated frequencies higher than 50 Hz, and might therefore be highly interesting as preclinical studies have shown bladder evacuation following HF stimulation. In closely related research fields, such as neuropathic pain, HF stimulation has shown compelling results as well. Spinal cord stimulation (SCS) using HF stimulation (up to 10 kHz) significantly induced pain relief, and patients often preferred HF-SCS over Con-SCS [13-15]. Whereas clinical studies have mainly focussed on symptomatic outcome parameters such as incontinence episodes, number of pads, and emotional outcome parameters as measured with questionnaires, preclinical studies have focussed on a broader set of functional outcome parameters such as bladder and urethral activity and bladder capacity. It is in this context that it is difficult to directly compare preclinical and clinical studies, given the different outcome measures that have been assessed. This stresses the importance and need for both clinical and preclinical research to move the field forward.

The clinical studies investigating bowel dysfunction showed that SNM with short cycling stimulation intervals did result in better objective outcomes as compared to Con-SNM. SNM stimulation frequencies (31Hz) higher than conventional (7-20Hz) or both narrow and wide pulse widths (90 µs, 300-450 µs) showed favourable objective outcomes over Con-SNM in some studies, whereas other studies did not show improvements over Con-SNM. In addition, effects of amplitude were investigated and subsensory SNM at 50% and 75% of the sensory threshold did not result in a better outcome. In both *Chapter 2* and *5*, a stimulation intensity below motor threshold, but likely higher than the sensory threshold, showed preferable results over higher intensities. Interestingly, preclinical studies showed that frequencies lower than Con-SNM were preferred, whereas the clinical studies showed that frequencies above Con-SNM were preferred. It should be noted that the preferred SNM frequencies in preclinical studies (2-5 Hz) are lower than the lowest frequency tested in clinical studies (6.9Hz). Frequencies in the same range as the preferred SNM frequencies in preclinical studies (2-5 Hz) were also found to be equally effective as conventional frequencies (20 Hz) in dorsal root ganglion (DRG) stimulation for pain research [16]. It is speculated that low-

frequency mechanoreceptors are involved in the underlying mechanism of LF-DRG stimulation (4 Hz) [17]. For bowel dysfunction, rectal low-threshold mechanoreceptors are involved in the first conscious awareness of influx of stool in the rectum and the urge to defecate [18]. It might well be possible that the low-threshold mechanoreceptors are involved in the underlying mechanism of LF-SNM and could therefore have a beneficial effect on patients with FI. These clinical findings make it very compelling to investigate LF-SNM (2-5 Hz) and possibly HF-SNM (> 1 kHz) as compared to conventional stimulation (14 Hz) in animal models such as the VD model as described in *Chapter 3*.

To answer ***Research Question 4*** *'What is the effect of Burst SNM on the urodynamic responses in patients suffering from overactive bladder dysfunction or non-obstructive urinary retention?',* we designed a clinical study in which the effect of SNM using Bursting patterns on urodynamic responses in patients suffering from overactive bladder dysfunction or non-obstructive urinary retention was evaluated. This study was presented in ***Chapter 6*** *'Burst paradigms evoked bladder responses in sacral neuromodulation patients'*. Burst SNM resulted in a substantial increase in both bladder and urethral pressure with increasing stimulation intensities. In contrast, Con-SNM resulted in a milder increase in urethral pressure with increasing stimulation intensities, and only one patient showed a modest increase in bladder pressure. Interestingly, SNM with Burst paradigms evoked a much higher increase in bladder and urethral pressure as compared to Con-SNM. Furthermore, SNM with Burst paradigms resulted in an increased pressure in the proximal urethra, and this pressure increase was higher than the pressure increase in the mid urethra. In contrast, the pressure increase using Con-SNM was similar for both the proximal and mid urethra. When comparing Burst paradigms with a uniform charge per second, Burst SNM with a 10 Hz interburst frequency and 4 mA amplitude resulted in the highest pressure responses in both the bladder and urethra, and this pressure slowly decreased when SNM intensity was lowered and SNM interburst frequency was increased.

Taken all stimulation parameters together, total charge per second can be calculated and depends on multiple variables, namely frequency, pulse width and amplitude. The same

charge per second can be achieved by adopting various dosing strategies or different stimulation waveforms. In the field of neuromodulation, one line of thought suggests that a stimulation's efficacy merely depends on the total charge delivered [19]. It is therefore interesting to recognize that in our study, different Burst SNM paradigms with a similar charge per second appeared to result in various pressure response in both the bladder as well as urethra. This might therefore implicate that a more complex interplay between individual parameters within the Burst waveform is responsible for at least some fundamental responses to SNM.

It is worth pointing out that preclinical research into the use and effect of Burst SNM paradigms is very compelling, especially to gain insights into the underlying mechanisms of action. In the field of chronic pain, both clinical and preclinical work indicated that Burst SCS resulted in a different effect on the nociceptive system as compared to Con-SCS, both in terms of patient outcome [20-22] as well as behavioural pain outcome in animal models [23]. Also from a mechanistic point of view, it was found that Con-SCS is only able to modulate areas of the brain that are important for the somatosensory aspect of pain (somatosensory cortex; location and intensity of pain) via activation of the lateral spinothalamic tract, whereas Burst SCS is also able to modulate areas of the brain that play an important role in affection/emotion of the pain experience (anterior-cingulate cortex, prefrontal cortex, insula, amygdala) via activation of the medial spinothalamic tract [21, 24-27]. Given the fact that pain and urinary tract dysfunction share many (supra)spinal pathways involved in both affective and sensory processing as part of their underlying mechanism of action (spinal cord, cingulate cortex, prefrontal cortex, insula and amygdala), one could speculate that Burst SNM (in line with Burst SCS) might also improve patient outcome by being capable of targeting both affective and sensory pathways [28, 29].

New technical advancements require adaptations for proper use in currently treated patients, and such adaptations should be tested for effectivity and safety before their introduction. An example of such a study is included in this thesis and addresses ***Research Question 5*** *'Is a novel adaptation to the existing SNM hardware safe and feasible for use in*

patients with bowel dysfunction?'. ***Chapter 7*** *'Replacement adaptor 09106 for patients with a dynamic graciloplasty or patients with sacral neuromodulation and abdominal IPGs; a safety and feasibility study'* describes the safety and feasibility of the Replacement Adaptor 09106 for the Interstim II in patients with dynamic graciloplasty or sacral neuromodulation with an abdominal-placed IPG as a replacement of the Interstim I. No significant differences were found in bowel habit diary and stimulation settings before and after replacement. Small adverse events occurred in a few patients, which were resolved after follow-up. This study showed that the Replacement Adaptor 09106 is a safe and feasible adaptation for patients with FI in case of end of service.

Future perspectives

Future preclinical studies should focus on optimization of the pBOO animal model for LUTD and the VD animal model for FI. This focus on optimization should specifically address the analysis of voiding behaviour in pBOO rats. As mentioned previously, the introduction of a continence training within the VSA protocol might improve the test as an outcome measure of LUTD, thereby providing an adequate window for pharmacological or SNM interventions. Additionally, also the use of alternative tasks should be explored and considered. Whereas the pBOO model did not result in changes in voiding behaviour as measured by the VSA test, the use of US in awake rats showed compelling results in terms of bladder volume over time, and therefore is likely to be a strong addition to future experiments into LUTD.

Preclinical studies into the VD model should focus on further refining the induction of the VD model to increase responder rates in VD rats. As described previously, an optimal balloon inflation duration between 2 and 4 hours might give an optimal balance between adequate responder rates and excessive discomfort. Furthermore, the fact that animals that did develop FI showed a sufficient treatment window makes the VD model a promising model to study the effect of novel interventions, including the use of novel SNM waveforms, in FI.

In addition, the parameter space of SNM in LUTD and FI, especially as related to clinical effectivity remains largely unexplored, which provides many opportunities for the implementation of novel SNM paradigms, such as Burst and HF-SNM. This research should

then be based on an orchestrated interplay between reproducible animal studies focussing on the underlying mechanisms of action, as well as clinical studies focussing on the (long-term) efficacy and feasibility of multiple SNM waveforms. Specifically, future work should definitely assess Burst SNM into more depth, especially with regard to individual parameter selection, as these were shown to result in at least some different fundamental responses using cystometry. Future studies should also investigate the effect of Burst SNM on (supra-)spinal areas related to affective and sensory processing as part of the underlying mechanism of action.

In order to personalize SNM therapy for specific indications or even individual patients, more sophisticated and mechanism-targeted diagnostic measurements will be necessary. New momentary and contextual patient complaint measurement and PROM tools can be very useful to increase objectivity of the assessments. In addition, functional imaging techniques using fMRI may better relate complaints to central nervous system connectivity changes during both diagnosis and treatment. Using these tools in combination with the newly developed treatment paradigms is expected to provide better patient specific treatments with sacral neuromodulation.

Conclusion

In summary, the results presented in this academic thesis show that both the pBOO and VD model are promising models to investigate LUTD and FI, respectively. This now allows future studies to investigate both the behavioural and mechanistic effects of SNM in the treatment of LUTD and FI. By optimizing relevant SNM stimulation parameters like stimulation frequency or use of new stimulation paradigms such as Burst SNM, the clinical efficacy as well as fundamental responses of SNM are likely to be improved. In this regard, the introduction of Burst SNM in the treatment of LUTD was shown to increase both bladder and urethral pressure in a frequency-dependent manner, which may provide a platform for future studies on Burst SNM in order to optimize clinical outcomes.

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General Summary

Chapter 9

English summary

Sacral neuromodulation (SNM) using electrical stimulation of a sacral nerve (S2-S4), is a treatment for lower urinary tract and bowel dysfunction (storage and evacuation complaints) and provides patients a better quality of life since the introduction of SNM in the early 80's. The research described in this thesis focused on sacral neuromodulation (SNM) in the treatment for lower urinary tract and bowel dysfunction. The project aimed to investigate in a translational and transdisciplinary way preclinical models for both lower urinary tract and bowel dysfunction and the application of various novel stimulation parameters for SNM.

A systematic review on the recent developments in the use and effectivity of SNM stimulation parameters for the treatment of experimental lower urinary tract and bowel dysfunction, was presented in ***Chapter 2*** and revealed a positive therapeutic effect of SNM in preclinical studies. For lower urinary tract dysfunction (LUTD), low frequency (7.5-15Hz) in the same range as conventional stimulation (7-20 Hz) appeared to facilitate storage dysfunction, whereas high frequencies appeared to evoke bladder evacuation. Bilateral SNM and pulse widths above conventional settings (± 210 µs) can contribute to reduction in intensity, to diminish unpleasant perceptions. For bowel dysfunction, it was shown that frequencies lower than conventional SNM (2-5Hz) and a stimulus intensity below motor threshold was preferred for both storage and evacuation dysfunction.

An experimental animal study to investigate faecal incontinence (FI) was reported in ***Chapter 3***. The vaginal distention model simulated childbirth by intravaginal balloon inflation and induce FI. In this study the behavioural outcome of intravaginal balloon inflation was examined. Vaginal balloon inflation for 2 hours resulted in faecal incontinence in 29% of rats (2 out of 7). In rats that underwent 4 hours balloon inflation, 33% (1 out of 3) developed faecal incontinence and two rats (67%) showed signs of bowel dysfunction, yet discomfort was too severe and these rats reached a humane endpoint. From these results, it became evident that an intravaginal balloon inflation duration between 2 and 4 hours seems to be optimal to induce FI resulting in a substantial number of responders and to avoid severe discomfort.

In ***Chapter 4***, the partial bladder outlet obstruction (pBOO) model to study lower urinary tract disease progression was further investigated for longitudinal monitoring and evaluation of voiding behaviour and bladder capacity. With this longitudinal approach, disease progression over time can be monitored and possible interventions such as SNM can be adapted accordingly when needed. In this study, bladder capacity increased over the four weeks post-surgery and was measured with ultrasound imaging (US). This increase in bladder capacity was also observed with terminal cystometry, which indicates that US is a suitable technique to measure bladder capacity over time. In addition, terminal cystometry showed an increase in bladder compliance and residual volume and a decrease in voiding pressure was reported as well. In accordance, bladder weight of pBOO rats was increased four weeks post-surgery. No differences in voiding behaviour were observed during disease progression using the voiding spot assay (VSA) and no differences in post mortem tissue characteristics were observed. These consequences of the here presented bladder outlet obstruction model suggest that the pBOO rats resembles a more detrusor underactive pattern of dysfunction.

In addition to the preclinical review in *Chapter 2*, the recent developments in SNM stimulation parameters for the treatment of lower urinary tract and bowel dysfunction in a clinical setting, are reviewed and presented in ***Chapter 5***. This review is an accompanying paper to the systematic review on preclinical studies of SNM in *Chapter 2*, which together provide a solid state of the art overview of the effect of SNM stimulation parameters on lower urinary tract and bowel dysfunction. Overall, altering SNM stimulation parameters in clinical lower urinary tract and bowel dysfunction indeed seemed to improve efficacy of SNM, with short cycling intervals to be favourable over Con-SNM for LUTD. Frequencies of SNM stimulation lower than the conventional frequency (defined as 7-20 Hz) showed a negative effect on the objective outcome parameters, and no effects of frequency alteration were observed for subjective outcome parameters. The clinical studies investigating bowel dysfunction showed that SNM with short cycling stimulation intervals resulted in better objective outcomes as compared to Con-SNM. The use of either higher SNM stimulation frequencies (31Hz) or narrow and wide pulse widths (300-450 µs) was reported to result in favourable objective outcomes over Con-SNM in some studies, whereas other studies did not

observe such improvements. In addition, effects of subsensory SNM at 50% and 75% of the sensory threshold did not differ from conventional stimulation at sensory threshold.

In ***Chapter 6***, we studied the effect of new SNM stimulation paradigms based on a Burst stimulation pattern and its effect on bladder and urethral pressure in patients with LUTD. Burst SNM resulted in a substantial increase in both bladder and urethral pressure with increasing stimulation intensities. In contrast, Con-SNM resulted in a milder increase in urethral pressure with increasing stimulation intensities, and only one patient showed a modest increase in bladder pressure. Furthermore, SNM with Burst paradigms resulted in an increase in pressure in the proximal urethra, which was higher than the pressure increase in the mid urethra, whereas the pressure increase for Con-SNM was similar for both proximal and mid urethra. When comparing Burst paradigms with a uniform charge per second, Burst SNM with a 10 Hz interburst frequency and 4 mA amplitude resulted in the highest pressure responses in both bladder and urethra and the pressures slowly decreased when SNM intensity decreased and SNM interburst frequency increased.

Regarding hardware changes, ***Chapter 7*** describes the safety and feasibility of the Replacement Adaptor 09106 for the Interstim II in patients with dynamic graciloplasty or sacral neuromodulation with an abdominal-placed IPG as a replacement of the Interstim I. No significant differences were found in bowel habit diary and stimulation settings before and after replacement. Small adverse events occurred in a few patients, which were resolved after follow-up. This study showed that the Replacement Adaptor 09106 is a safe and feasible adaptation for faecal incontinence patients in case of end of service.

Overall, this thesis reports and summarizes the preclinical and clinical aspects of SNM as well as the effectiveness of new SNM stimulation paradigms in the treatment of lower urinary tract and bowel dysfunction. Preclinical results showed two promising animal models (pBOO and VD model) to investigate interventions, such as SNM, for LUTD or FI, respectively. For clinical implementation, adjusting relevant SNM stimulation parameters are likely to improve clinical efficacy as well as fundamental responses of this treatment. A new SNM paradigm, Burst SNM, is shown to modulate bladder and urethra responses in a distinctive way as

compared to conventional SNM, which gives a new perspective to optimizing clinical outcome.

Nederlandse samenvatting

Lage urinewegdisfunctie en darmfunctiestoornissen gaan gepaard met een verminderde controle over de mictie (urineren) en/of de stoelgang. Het zijn veel voorkomende problemen, die een negatief effect op de kwaliteit van leven van patiënten kunnen hebben en kunnen resulteren in een sociaal isolement. Als behandeling voor (opslag en evacuatie klachten bij) deze lage urinewegdisfunctie én darmfunctiestoornissen kan sacrale neuromodulatie (SNM) toegepast worden. Het is een vorm van elektrische stimulatie waarbij een elektrode via het intervertebraal foramen op de zenuwwortel van S3 wordt geplaatst. Het onderzoek beschreven in dit proefschrift richt zich op SNM bij de behandeling van lage urinewegdisfunctie en darmfunctiestoornissen. Het project is gericht op het op translationele en transdisciplinaire wijze onderzoeken van preklinische modellen voor zowel lage urineweg- als darmfunctiestoornissen en het toepassen van verschillende nieuwe stimulatie parameters voor SNM.

In ***Hoofdstuk 2*** wordt een systematisch overzicht gegeven van de recente ontwikkelingen met betrekking tot het gebruik en de effectiviteit van SNM stimulatie parameters voor de behandeling van lage urinewegdisfunctie en darmfunctiestoornissen bij dierstudies. Deze lieten een positief therapeutisch effect van SNM in preklinische studies zien. Voor disfunctie van de lage urinewegen, bleken lage frequenties (7.5-15Hz) in hetzelfde bereik als conventionele stimulatie (7-20 Hz) de urine-opslag te bevorderen, terwijl hoge frequenties de mictie bleken op te wekken. Bilaterale SNM en pulsbreedtes boven de conventionele instellingen (± 210 µs) zouden ervoor kunnen zorgen dat de intensiteit instelling verlaagd kan worden, om hiermee onaangename paresthesieën (tintelingen) te verminderen. Voor darmfunctiestoornissen werd aangetoond dat frequenties lager dan conventionele SNM (2-5Hz) en een stimulus intensiteit onder de motorische drempel het beste resultaat gaven voor zowel opslag als evacuatie klachten.

In ***Hoofdstuk 3*** wordt een experimentele dierstudie beschreven waarin fecale incontinentie (FI) onderzocht werd. In deze studie werd de gedragsmatige uitkomst van intravaginale balloninflatie bij ratten onderzocht, waarbij met behulp van een intravaginale balloninflatie

een bevalling werd gesimuleerd en FI geïnduceerd. Daaruit bleek dat een intravaginale balloninflatie van 2 uur, bij 29% van de ratten (2 van de 7) in FI resulteerde. Bij ratten die gedurende 4 uur een balloninflatie kregen, ontwikkelde 33% (1 van de 3) fecale incontinentie en twee ratten (67%) vertoonden tekenen van darmfunctiestoornissen. Echter werd het ongerief zo ernstig, dat deze ratten het experiment niet af konden maken. Uit deze resultaten werd duidelijk dat een intravaginale balloninflatieduur tussen de 2 en 4 uur optimaal lijkt te zijn om FI te induceren, waarbij een aanzienlijk aantal ratten fecaal incontinent wordt en ernstig ongerief wordt vermeden.

In ***Hoofdstuk 4*** wordt het model van een partiële blaasuitgang obstructie (pBOO) gepresenteerd, ten behoeve van het monitoren en evalueren van het plasgedrag en de blaascapaciteit van ratten over een langere periode. Met deze langdurige aanpak kan de ziekteprogressie over een langere tijd worden opgevolgd en kunnen mogelijke interventies zoals SNM indien nodig worden aangepast voor een beter resultaat. Deze studie liet zien dat de blaascapaciteit, gemeten met echografie, toenam gedurende de vier weken na de obstructie. Deze toename werd ook waargenomen met terminale urodynamica, wat aangeeft dat echografie een geschikte techniek is om blaascapaciteit gedurende een langere tijd te meten. Bovendien liet de terminale urodynamica een toename in de compliantie van de blaas en het restvolume zien en werd er een afname in de urinedruk gerapporteerd. In overeenstemming met de toegenomen blaascapaciteit, bleek het blaasgewicht van geobstrueerde ratten vier weken na de operatie toegenomen te zijn in vergelijking met de controle ratten. Er werden, met behulp van een test om het plasgedrag te observeren (VSA), geen verschillen in plasgedrag waargenomen tijdens de ziekteprogressie en ook het weefsel liet geen veranderingen zien ten aanzien van het geïnduceerde obstructie model. De resultaten van het hier gepresenteerde blaasuitgang obstructie model suggereren een meer onderactief patroon van detrusor (blaasspier) disfunctie bij de geobstrueerde ratten.

In aanvulling op het preklinische overzicht uit *hoofdstuk 2*, worden de recente klinische ontwikkelingen in SNM stimulatie parameters voor de behandeling van lage urineweg- en darmfunctiestoornissen in ***Hoofdstuk 5*** besproken en gepresenteerd. Deze recente klinische

ontwikkelingen in combinatie met het overzicht van preklinische studies uit *hoofdstuk 2*, geven een solide overzicht van het effect van SNM stimulatie parameters op lage urinewegdisfunctie en darmfunctiestoornissen. In het algemeen leek het wijzigen van SNM stimulatie parameters de werkzaamheid van SNM bij patiënten te verbeteren, waarbij korte cyclische stimulatie intervallen gunstiger bleken te zijn dan conventionele stimulatie voor patiënten met een disfunctie van de lage urinewegen. SNM frequenties lager dan de conventionele frequentie (gedefinieerd als 7-20 Hz) toonden een negatief effect op de objectieve uitkomstparameters. Daarnaast werd geen effect van frequentiewijziging op subjectieve uitkomstparameters waargenomen. De klinische studies waarin patiënten met darmfunctiestoornissen werden onderzocht, toonden aan dat SNM met korte cyclische stimulatie intervallen, betere objectieve uitkomsten gaf dan conventionele SNM. Het gebruik van hogere SNM stimulatie frequenties (31Hz) of smalle en brede puls breedtes (300-450 µs) resulteerde in sommige studies in betere objectieve uitkomsten dan conventionele SNM, terwijl andere studies dergelijke verbeteringen niet aantoonden. Bovendien verschilden de effecten van subsensorische SNM op 50% en 75% van de sensorische drempelwaarde niet van conventionele stimulatie gegeven op de sensorische drempel.

In ***Hoofdstuk 6*** bestudeerden we het effect van nieuwe SNM stimulatie paradigma's, gebaseerd op een Burst stimulatie patroon, en het effect daarvan op blaas en urethrale druk bij patiënten met lage urinewegdisfunctie. Bij het verhogen van de stimulatie intensiteit resulteerde Burst SNM in een substantiële toename van zowel blaas- als urethrale druk. Conventionele SNM daarentegen resulteerde in een mildere toename van de urethrale druk bij het verhogen van de stimulatie intensiteit, waarbij slechts één patiënt een bescheiden toename van de blaasdruk vertoonde. Bovendien resulteerde SNM in Burst patronen in een drukstijging in de proximale urethra, die hoger was dan de drukstijging in de mediale urethra, terwijl de drukstijging voor conventionele stimulatie vergelijkbaar was voor zowel de proximale als mediale urethra. Bij vergelijking van Burst patronen met een uniforme lading per seconde, resulteerde Burst SNM met een 10 Hz interburst frequentie en een 4 mA amplitude in de hoogste drukresponsen in zowel blaas als urethra. Wanneer de SNM intensiteit afnam en de SNM interburst frequentie toenam, namen de drukken langzaam af.

Wat hardwareveranderingen betreft, beschrijft ***Hoofdstuk 7*** de veiligheid en haalbaarheid van de vervangingsadapter 09106 van de Interstim II bij patiënten met dynamische graciloplastie of sacrale neuromodulatie met een abdominaal geplaatste stimulator als vervanging van de Interstim I. Er werden geen significante verschillen gevonden in de ontlastingsdagboeken tussen de stimulatie instellingen voor en na de vervanging. Bij enkele patiënten traden milde bijwerkingen op, die na follow-up verdwenen. Deze studie toonde aan dat de vervangingsadapter 09106 een veilige en haalbare toepassing is voor patiënten met fecale incontinentie waarbij normaliter het einde van het behandelproces bereikt is.

In het algemeen worden in dit proefschrift de preklinische en klinische aspecten van SNM gepresenteerd en samengevat, evenals de effectiviteit van nieuwe SNM stimulatie paradigma's voor de behandeling van lage urinewegdisfunctie en darmfunctiestoornissen. Preklinische resultaten toonden twee veelbelovende diermodellen (pBOO en VD model) aan om interventies, zoals SNM, te onderzoeken voor lage urineweg- en darmfunctiestoornissen. Voor klinische implementatie zal het aanpassen van relevante SNM stimulatie parameters waarschijnlijk zowel de klinische werkzaamheid als de fundamentele respons van deze behandeling verbeteren. Een nieuw SNM paradigma, Burst SNM, blijkt blaas en urethra responsen op een onderscheidende manier te moduleren in vergelijking met conventionele SNM, wat perspectief biedt om het klinisch resultaat verder te optimaliseren.

Impact Paragraph

Appendix

Lower urinary tract and bowel dysfunction (i.e. urinary incontinence, voiding dysfunction, faecal incontinence and constipation) is a significant burden for patients that become housebound with often desolation as a consequence. Sacral neuromodulation (SNM) can be a good therapy for these patients to reduce symptoms and return into society. Success rates for SNM in lower urinary tract and bowel dysfunction usually range from 50-80% for up to five years after implantation and are dependent on inter-individual variety and indication. Nevertheless, this leaves 20-50% of patients with no or a suboptimal treatment outcome. In addition, loss of efficacy is noted in 75-88% of patients overtime and only 40-75% of patients completely recover and achieve complete continence. Over the years, small hardware changes in SNM technology occurred, which resulted in a decrease in the occurrence of side effects. Interestingly, and important in view of the studies performed in this thesis, SNM stimulation parameters used for the treatment of lower urinary tract or bowel dysfunction have not changed significantly over time. In this thesis, we aimed to further facilitate SNM research by establishing two animal models of lower urinary tract dysfunction (LUTD) and faecal incontinence (FI) as well as gain more insights into alternative SNM stimulation paradigms.

In order to study the efficacy and underlying mechanisms of action of lower urinary tract and bowel dysfunction and SNM, good, reproducible preclinical animal models are required. To this end, we established two animal models for lower urinary tract dysfunction (LUTD) and faecal incontinence (FI), respectively. To our knowledge, we were the first to measure bladder volume in awake rats by means of ultrasound imaging for a period of 4 weeks in an animal model of LUTD. Additionally, using an animal model of FI (the vaginal distention or VD model), we showed for the first time that these animals develop FI as measured by defecation behaviour. These advancements in two commonly adopted models for LUTD and FI will now allow for studies to be conducted in order to better understand the mechanisms underlying LUTD and FI. By gaining more insight into the mechanisms that underlie LUTD and FI, new targets for intervention can be explored and developed in future research. Furthermore, by developing adequate, reproducible animal models for LUTD and FI, the effect and

mechanisms of action of pharmacological and neuromodulatory therapies such as SNM can not only be properly tested, but also implemented into the clinic.

Based on two literature reviews (one preclinical and one clinical review), we observed that the parameter space of SNM (''settings of the stimulation'') were hardly explored, especially in the clinical setting (e.g. only frequencies lower than 50 Hz were used). Nevertheless, first preclinical studies have shown that the implementation of high frequency protocols for SNM might benefit voiding dysfunction in preclinical animal models. In contrast, adjacent neuromodulation fields such as neuromodulation studies in the field of pain more often deploy such novel stimulation waveforms, which bear the potential to benefit patients that are refractory to conventional therapies. As the use of bursting paradigms has provided compelling results in refractory pain patients, we performed a pilot study using SNM with Burst patterns at different interburst frequencies in patients with LUTD. Under general anaesthesia, we found that Burst SNM, but not conventional SNM (Con-SNM), increased bladder pressure, especially at lower Burst SNM frequencies. Additionally, also urethral pressure was higher with Burst SNM as compared to Con-SNM. These results suggest that at least some differences exist in terms of fundamental responses to the Burst SNM waveforms as compared to Con-SNM, which may provide a platform for future studies on Burst SNM in order to optimize clinical outcomes. In the end, the results of this academic thesis will aid in improving SNM therapy for lower urinary tract and bowel dysfunction in patients and better understand its underlying mechanisms of action. New SNM stimulation paradigms developed using the here described models may allow for more disease specific and personalized therapies in patients.

Curriculum Vitae

Appendix

Appendix

Perla Douven was born on May 6th 1993 in Nederweert, The Netherlands. She attended secondary school at the Philips van Horne in Weert, where she obtained her Atheneum degree in 2011. That same year, she started her Bachelor study in Human Movement Sciences at the Vrije Universiteit in Amsterdam. During this Bachelor, Perla specialized in a minor Health, Sport and Sportpsychology and performed her research internship and literature thesis in size-weight illusion and 'inattentional blindness' respectively. During her time in Amsterdam, she joined the study association 'V.I.B.' where she joined and chaired several committees. She obtained her Bachelor degree in 2014, after which she enrolled in the Master program of Biomedical Sciences: Neurobiology with the specialization Basic and Applied Neuroscience at the University of Amsterdam. During her research internship, she joined the research team 'Genome Analysis' supervised by Prof. Dr. Frank Baas and Dr. Marianne Weterman at Academic Medical Center in Amsterdam. Here, Perla performed preclinical research into the genetic default of Charcot Marie Tooth disease type II (CMT2) and Pontine Tegmental Cap Dysplasia (PTCD). For her Graduation Project, Perla visited the Department of Health Science and Technology at the Eidgenössische Technische Hochschule (ETH) in Zürich, Switzerland supervised by Prof. Dr. Eling de Bruin and Federico Gennaro, to study the cause of falling in older adults by investigating the correlation between gait variability, brain activity (EEG) during a cognitive task and motor-evoked potentials using Transcranial magnetic stimulation (TMS). After successfully completing her Master studies in 2016, Perla started as a researcher at Health centre Honné in Horn and performed clinical research into a new rehabilitation device for stroke and traumatic brain disorder patients (hemi-inattention). Perla continued her career as a research assistant at the Department of Clinical Medicine and the Department of Forensic Medicine in Aarhus, Denmark. Here she performed preclinical research into nerve conduction of damaged peripheral nerves in forensic research under supervision of prof. dr. Michael Pedersen. Over the last four years, Perla worked as a PhD candidate at Maastricht University. This PhD project was a joint collaboration of the Department of Urology, the Department of Anaesthesiology and Pain Management and the Department of Surgery under supervision of Prof. Dr. Gommert van Koeveringe, Prof. Dr. Bert Joosten, and Dr. Stephanie Breukink. During her PhD, Perla studied

the experimental models for either faecal incontinence or lower urinary tract dysfunction and the effect of sacral neuromodulation stimulation paradigms in the treatment of lower urinary tract and bowel dysfunction. She collaborated with experts in the field of Neuromodulation and lower urinary tract and bowel dysfunction, including Prof. Dr. James Jones (University College Dublin, Ireland) and Prof. Dr. Stefan de Wachter (Universiteit van Antwerpen, Belgium), as well as world leading industry partners in the Neuromodulation field (Abbott inc.). The results of her studies are presented in this academic thesis.

List of Publications

Appendix

2020 **Douven P**, Assmann R, Breukink S O, Melenhorst J, Kleijnen J, Joosten E A, van Koeveringe G A. Sacral Neuromodulation for Lower Urinary Tract and Bowel Dysfunction in Animal Models: A Systematic Review with Focus on Stimulation Parameter Selection. Neuromodulation: Technology at the Neural Interface 2020; 23(8): 1094–1107. **Published.**

2020 Assmann R, **Douven P**, Kleijnen J, van Koeveringe G A, Joosten E A, Melenhorst J, Breukink S O. Stimulation Parameters for Sacral Neuromodulation on Lower Urinary Tract and Bowel Dysfunction-Related Clinical Outcome: A Systematic Review. Neuromodulation: Technology at the Neural Interface 2020; 23(8): 1082-1093. **Published.**

2022 **Douven P**, Franken G, Debets J, Joosten E A, van Koeveringe G A, Melenhorst J, Breukink S O. Vaginal Distention Rodent Model for Faecal Incontinence: A Pilot Study on the Effect on Defecation Behaviour. Journal of Coloproctology (Rio de Janeiro) 2022. **Accepted.**

2022 **Douven P**, Peter S, Franken G, Debets J, Gerritsen W, Breukink S O, Joosten E A, van Koeveringe G A. Longitudinal Quantitative Evaluation of Bladder Storage and Evacuation Dysfunction for Preclinical Intervention Effect using Ultrasound Imaging in Awake Rats. **Under review.**

2022 **Douven P***, Tilborghs S*, van de Borne S, van Koeveringe G A, de Wachter S. Burst Paradigms Evoked Bladder Responses in Sacral Neuromodulation Patients. **Submitted.**

2022 Assmann R*, **Douven P***, Joosten E A, van Koeveringe G A, Breukink S O, Melenhorst J. Replacement Adaptor 09106 for Patients with a Dynamic Graciloplasty or Patients with Sacral Neuromodulation and Abdominal IPGs: A Safety and Feasibility Study. **Under review.**

2021 Franken G, **Douven P**, Debets J, Joosten E A. Conventional Dorsal Root Ganglion Stimulation in an Experimental Model of Painful Diabetic Peripheral Neuropathy: A

Quantitative Immunocytochemical Analysis of Intracellular γ-Aminobutyric Acid in Dorsal Root Ganglion Neurons. Neuromodulation: Technology at the Neural Interface 2021; 24(4): 639-645. **Published.**

* These authors contributed equally to this manuscript

Dankwoord

Appendix

Als eerste wil ik graag mijn promotieteam bedanken, Prof. Dr. Gommert van Koeveringe, Prof. Dr. Bert Joosten en Dr. Stephanie Breukink, voor hun steun tijdens het gehele promotie traject.

Beste prof. van Koeveringe, beste **Gommert**, jouw enthousiasme voor onderzoek heeft er zeker toe geleid dat dit proefschrift een divers proefschrift is geworden. Altijd had jij wel een oplossing of een nieuw idee voor het een of het ander. Je had het vaak druk, maar je stond altijd klaar om de handen uit de mouwen te steken en mee te kijken in het lab of operaties voor te doen in de kelder. Dankjewel voor alle kansen die je mij geboden hebt en je vertrouwen in mij als onderzoeker!

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in het zwembad, talrijke creatieve dagen van schilderen tot macramé, en natuurlijk ook alle gezellige spelletjes/wijn/balkon-avonden. En naast al deze leuke momenten, natuurlijk ook hard werken om die PhD af te krijgen. Al waren we niet altijd even blij met de experimenten, we hebben het toch maar even allebei gehaald! Ik ben heel blij dat jij hier vandaag naast mij wil staan!

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