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Title page

Title:

The relationship between cortical activation in response to anorectal stimuli and continence behavior in freely behaving rats before and after application of sacral nerve stimulation.

Short Running Head: SNS in freely behaving rats

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Abstract

Background:

Changes in anorectal sensation have been reported in patients with fecal incontinence and there is limited evidence that sacral nerve stimulation can restore normal sensation.

Objective: The aims of the present study were to investigate changes in transmission of sensory anorectal stimuli in a rodent model of FI and to study the effects of SNS on defecation behaviour.

Design & Interventions:

An established model of fecal incontinence using pudendal nerve stretch and compression was used in 16 adult female Wistar rats and followed for 3 weeks: six rats received sacral nerve stimulation for 1 week using an implantable neurostimulator and ten rats had non-functioning 'dummy' devices inserted. Five additional rats were sham operated. Anorectal cortical evoked potentials were used as a surrogate marker for anorectal sensory function.

Main Outcome Measures: Faecal incontinence index, evoked potential amplitude and latency.

Results:

Fifty percent of rats showed behavioral signs of FI measured by the Fecal Incontinence Index(>0.20), calculated using the pellet distribution outside the cage's latrine area.

Anorectal evoked potential amplitude was reduced in rats with an fecal incontinence index >0.20($p=0.019$). The amplitude of forepaw evoked potentials recorded as a control was not different between groups. Sacral nerve stimulation using the fully implantable device and custom rodent lead was safe and stable during this prospective study. Incontinent rats($N=3$) that received sacral nerve stimulation showed an improvement of fecal incontinence

index and an increase of evoked potential amplitude to anorectal stimulation compared to the dummy implant controls (N=5).

Limitations: The main limitation is the small number of animals that received sacral nerve stimulation.

Conclusions:

Chronic sacral nerve stimulation is feasible in rats when miniature telemetric devices are used. Behavioral signs of fecal incontinence were positively correlated with latency of anorectal evoked potentials.

What does this paper add to the literature?

This is the first study to report SNS in rodents using a remotely programmable and rechargeable miniature device and the first to link loss of anorectal evoked potentials to continence behaviour in an animal model. This study may lead to a better understanding of the mechanism of SNS.

Introduction

Neuromodulation of the sacral nerve is an established surgical treatment for fecal incontinence(FI)^{1,2}, whereby the sacral nerves, usually at the level of S3, are continuously stimulated at 14 Hz. The cellular basis for the mechanism of action is not completely understood³. Fecal incontinence is a common distressing ailment that diminishes the social quality of life^{4,5}. It has recently been hypothesized that sacral nerve stimulation(SNS) may normalise anorectal hypo- or hypersensitivity which is common in FI patients⁶⁻¹⁰.

To study the mechanism of action of SNS in FI in rats, a model of pudendal neuropathy and simulated childbirth trauma has been used which employs retro-uterine balloon inflation that mimics intrapelvic pressures reported in human childbirth¹¹. This model causes anal sphincters^{11,12}, atrophy, denervation and reinnervation of the external anal sphincter(EAS) on electromyography¹¹, and interruption of both motor¹¹ and sensory function of the pudendal nerve^{13,14}. This model has been successfully applied in freely behaving rats and causes a proportion of injured rats to lose place preference for pellet deposition, as measured by the fecal incontinence index(FII)¹⁵.

During retro-uterine balloon inflation a progressive reduction in cortical activation has been observed^{16,17}, and acute SNS has been shown to restore diminished anal evoked potentials(EPs)¹⁷. Therefore, cortical EP recordings can also be used to study the relation between the level of sensory deficit and behavioral signs of incontinence and the possible restorative effect of SNS. It is an inherent feature of the balloon model that not all rats lose place preference for pellet disposal. The expected spread in continence might faithfully reflect the range of clinical outcomes in humans and will be used to correlate FII and cortical EPs.

Reasons for the inconsistencies might be slight differences in surgery between animals and/or variation of pudendal nerve anatomy.

A novel miniature, implantable, inductively rechargeable and externally programmable rodent neurostimulator (Medtronic Inc., Dublin, Ireland)¹⁸, and rat SNS leads based on the human SNS lead¹⁹, have been made available for this study.

The aims of this study were (1) to investigate the relationship between behavioral signs of FI and anorectal cortical activation, (2) to test the feasibility of a novel miniature rodent stimulator as a method for SNS in freely behaving rats and (3) to explore the effect SNS has on continence behavior and cortical activation.

Materials and methods:

Experiments were approved by the Animal Research Ethics Committee of UCD (AREC-15-42-Jones), Dublin, Ireland and licensed by the Health Products Regulatory Authority of Ireland (AE18982/IO67). Animals were housed in a 12-hour light and dark cycle with free access to water and rodent standard diet in a temperature ($22.3^{\circ}\text{C}\pm 0.06^{\circ}\text{C}$) and humidity ($44\%\pm 0.2\%$) controlled environment. From 6 weeks, rats were held in pairs and handled daily to familiarize the rats with the researcher. Thirty-one naïve female Wistar rats ($272\pm 4\text{g}$ at the beginning of the behavioral assay) were used.

Experimental design

Rats were followed for 3 weeks. In week 1, baseline continence behavior was established. Then, recovery surgery was performed (sham surgery in Group 1, balloon model and dummy implant in Group 2 or balloon model and neurostimulator implantation in Group 3). In week 2 and 3, continence behaviour was monitored. In Group 3 (Balloon-SNS) SNS was

delivered continuously in week 3. Lastly, EPs were recorded under terminal anaesthesia (Figure 1A). A block design was used to allow for the number of rats studied.

Behavioral assay

A metal tray (26x20cm, Drömmmer, Ikea Ireland Limited, Dublin, Ireland; 1/3 of the overall cage floor area) filled with wood shavings was placed in the corner, farthest away from the food and drink of a standard cage (floor area 30x50cm) as a latrine (Figure 1B). Each cage also contained two paper rolls for enrichment and paper strands as nesting material. Cages were placed close together to allow visual, auditory and olfactory cues between the rats. Fecal pellets were counted and cleaned out daily. During a 1 week training period, pairs were held in the modified cage and cleaned twice daily with some pellets left in the latrine area each time. All pairs deposited >80% of pellets within the latrine and were included in the study¹⁵. One rat needed to be excluded at a later stage due to a lack in place preference. For assessment, rats were held singly. Video-tracking as described by Devane et al.¹⁵, was used to determine the amount of time spent in each compartment (latrine&non-latrine). The fecal incontinence index(FII) was calculated from the number of pellets passed and the amount of time spent in each area. First, the defecation rate (pellets/hour) for each area was calculated. Finally, the FII was calculated by dividing the non-latrine defecation rate by the overall rate for the cage.

Recovery surgery

Recovery surgery was performed under Isoflurane anaesthesia(Iso-Vet, Chanelle, Loughrea, Ireland; 4.5% in 4 l/min oxygen for induction and 1.5 – 2% in 1 l/min for maintenance) with regular monitoring of the anaesthetic plane. The animal rested on a heating blanket to maintain a body temperature of 37°C

Rats received subcutaneous(s.c.) injection of prophylactic antibiotics (Gentamicin(6mg/kg) and Metronidazole(20mg/kg)) and pre-operative analgesia (Buprenorphine(0.05mg/kg; Buprenodale, Dechra Veterinary Products, Shrewsbury, UK) and Carprofen(5mg/kg; Caprievie Norbrook, Monaghan, Ireland)). Post-operative, rats received 10ml warmed saline solution s.c. For analgesia, rats received an additional dose of Buprenorphine in the evening and then for a minimum of 4 days a jelly or s.c injection (1ml jelly (containing 1.5mg Carprofen and 0.015mg Buprenorphine/300g rat).

First, the balloon model was performed as previously described ^{11,15}(Figure 1C.). A midline laparotomy was performed under sterile conditions and two silicone Foley catheters(Coloplast A/S, Humlebaek, Denmark) inserted deep into the retro-uterine space were inflated with 1.5mls each for 60min. In Group 1 (sham) balloons were inserted without inflation. The abdominal wall was closed in a cruciate pattern(Ethicon, 4-0) and the skin with a subcuticular suture(4-0 Vicryl suture, both Johnson+Johnson Intl, St Stevens Woluwe, Belgium).

Then, the rat was then turned around for implantation of the neurostimulator or dummy of the same dimensions and weight (Figures 1D, E). The miniature fully implantable rechargeable neurostimulator (Medtronic Inc, Dublin, Ireland) weighs 6g and has the following dimensions: 3.71cm x 1.65cm x 0.57cm. This corresponded to 2% of the rat's body weight at the time of implantation which was tolerated by the animals. A custom-made Medtronic rodent SNS lead with a platinum iridium monopolar plate electrode (0.75x2.5x0.025mm), which fitted the rat S1 foramina exactly. For implantation, a 3-4 cm skin incision over the sacrum was made. The latissimus dorsi fascia over the S1 foramen was cut and the muscle carefully separated from the spinal vertebrae. The small plate at the end

of the rodent lead inserted into the foramen and sutured to the fascia. Muscle and fascia were reattached using tissue glue (Liquiband, Advanced Medical Solutions, Winsford, UK). A subcutaneous pocket was created with blunt scissors and the neurostimulator fitted inside and fixated to the fascia. The skin was closed with an intracuticular suture(4-0 Vicryl, Ethicon). Rats' welfare was assessed post-surgery using the rat grimace scale.

Dummy implant fabrication

An approximation of the neurostimulator's shape was drawn in Google SketchUp and a mould created and printed on ZPrinter 250(ZCorporation, Rock Hill, South Carolina, USA). Dummies were cast in the mould using silicone(Ecoflex 00-50, Smooth-On, Macungie, PA, USA) and weighted down with stainless steel washers. The weight of the dummy was 5.6g. Implantation was as described above but no fixation was used.

SNS stimulation parameters

The motor threshold for SNS was noted and the correct placement of the lead and neurostimulator is confirmed by a tail twitch. The neurostimulator was then turned off. Sacral nerve stimulation was delivered in the 3rd week unilaterally and continuously at 14Hz, 210µs pulse duration, 75% of motor threshold.

Non-recovery EP recording

Rats were re-anaesthetised using urethane (Sigma, Arklow, Ireland) in a 20% solution at a dose of 1.5g/kg intra-peritoneal(i.p.). after induction with isoflurane(4%) in oxygen(4L/min). Urethane is a good anaesthetic for EP recording but unsuitable for recovery procedures. A top-up of 10% loading dose was administered either i.p. or i.v., if required. The animal rested on a heating blanket. Further preparation included femoral vein cannulation,

tracheotomy and intubation. On conclusion of each experiment the rat was euthanized using an overdose of urethane i.v.

Cortical EPs were recorded over the right primary somatosensory cortex while stimulation was applied either central (anorectum) or left-sided (extremities). During stereotaxic surgery, the anal canal representation was localized (-0.6mm anteroposterior $+2\text{mm}$ mediolateral from bregma)¹⁶ and EPs were recorded extradurally with a 32-channel multi-electrode array (MEA) (flexMEA). Recordings were processed through a 10-fold miniature pre-amplifier (MPA321) and a combined amplifier, filter and data acquisition system (USB-ME-FAI-System); MC_Rack 4.3.0 (all Multi channel systems, Reutlingen, Germany) was used for recording. Stimulation and recording were both triggered by a programmable stimulator unit (Master 8, Grass, Slough, UK). The sampling frequency was 10kHz. For each trial, EPs were recorded 1/s and averaged over 250s.

Cortical EPs were elicited by anorectal and, as control, fore paw stimulation^{16,17}. For anorectal stimulation, a cathode ($\varnothing 2\text{mm}$) in the anal canal and an anode ($\varnothing 2\text{mm}$) left of the EAS were used (stimulation parameters: 12V, 1Hz, pulse duration 1ms). A small EAS contraction was visible during stimulation. For fore paw stimulation, a concentric needle electrode was used (stimulation parameters: 15V, 1ms pulse duration, 1Hz).

Analysis

Behavioral data were used to calculate the FII. Groups were compared using a two-way repeated-measures ANOVA (Prism 5, GraphPad Software, San Diego, USA).

Evoked potentials were automatically averaged over all 32 channels. In channel with the greatest amplitude maximal amplitude from the baseline (maximum), onset latency (L1), and

area under the curve of the upward deflection(area1) were measured (Spike 2.6, C.E.D., Cambridge, UK). Subgroups were compared to Group 1(sham) using a one-way ANOVA (Prism 5). Linear curve fitting was used to correlate EP values to the FII(Prism 5).

Normally distributed data are presented as mean±S.E.M. (95% confidence interval). Power calculation performed in the study design phase (power (1-β=80%), criterion of statistical significance of α<0.05) was based on previous data and yielded N=5 for each subgroup (i.e balloon-dummy-continent etc.).

Results

Of 31 rats used in this study, 5 did not proceed to surgery (no continent behaviour(N=1), reassigned to a different study(N=4)) and 5 rats had to be euthanized due to adverse effects (intestinal irritation/ileus and pain(N=3), intestinal damage during surgery(N=1), wound dehiscence(N=1). Minor events were wound licking(N=2) that led to secondary wound healing. Five rats in Group 1(sham), 10 in Group 2(balloon-dummy) of which 5 showed continent (balloon-dummy-continent) and 5 incontinent behaviour (balloon-dummy-incontinent) and 6 in Group 3(balloon-SNS) of which 3 showed continent (balloon-SNS-continent) and 3 incontinent behaviour (balloon-SNS-incontinent) completed the study (Figure 2).

Behavior pre-surgery

Rats showed a natural inclination to deposit the pellets in the latrine area provided. Rats were observed to enter the latrine area, defecate and leave immediately. In addition, single pellets were usually deposited in the cage corners. Rats slept in the cardboard rolls. Grooming

behaviour was most often observed in the corner next to the latrine and latrine. Baseline defecation rate was 1.58 ± 0.01 pellets/hour, equalling approximately 37 pellets passed per animal per day. In the latrine, the defecation rate was 21.73 ± 0.85 pellets/hour and in the non-latrine 0.19 ± 0.01 pellets/hour. The mean FII over 7 days was 0.12 ± 0.01 and showed no significant change over time ($p=0.06$, repeated measures ANOVA).

Behavior post balloon injury

In Group 1 (sham) the FII was stable and low over 3 weeks (0.10 ± 0.01 at baseline and 0.09 ± 0.02 after surgery) while the FII doubled after surgery from 0.11 ± 0.01 to 0.24 ± 0.04 in Group 2 (Balloon-dummy). Rats were divided into balloon-dummy-continent ($N=5$) and balloon-dummy-incontinent ($N=5$) rats (Figure 3A, Table 1). Incontinence was characterized by one weekly average that was double the baseline FII and >0.20 as defined previously¹⁵. Urine stains in their fur ($N=12$), fecal stains ($N=1$) and a visibly low EAS tone ($N=2$) were noted in some rats.

In Group 3 (Balloon-SNS), the group size was too low for comparisons, so data from each individual are presented (Figure 3B, C). The three incontinent rats (balloon-SNS-incontinent) treated with SNS all showed a small decrease in FII, but only one showed a return to continence.

SNS

Neurostimulators were implanted in 8 rats, two of which needed to be euthanized due to complications. Motor responses in the form of a typical tail twitch could be elicited in all rats during surgery, one week later when the device was turned on, 10 days later and in all but

one (lead breakage) 14 days later. The motor threshold was $0.38\text{mA} \pm 0.11\text{mA}$. The stimulation was set on $0.30\text{mA} \pm 0.1\text{mA}$ (75% of the motor threshold). This has been shown to be an effective stimulation amplitude previously¹⁹. No recharging was needed.

Cortical EP recordings

Evoked potentials were recorded from all but one animal (Group 2: balloon-dummy-continent). Fore paw EPs, recorded as a control, showed no significant differences between groups (amplitude: $P=0.93$, $F=0.15$; onset latency: $P=0.71$, $F=0.46$; area under the curve: $P=0.82$, $F=0.31$; one-way ANOVA) (Figure 4, Table 2).

Anorectal EPs were elicited by electrical stimulation of the anorectal area. Amplitude and area under the curve were significantly lower in incontinent rats of Group 2 (balloon-dummy-incontinent) but neither in continent (balloon-SNS-continent, $N=3$) nor incontinent (balloon-SNS-incontinent, $N=3$) rats that received SNS (amplitude: $P=0.03$, $F=3.91$; area under the curve: $P=0.02$, $F=4.73$; one-way ANOVA and Bonferroni post-test, comparing each group to Group 1 (sham))(Figure 5, Table 2). In addition, the onset latency was longer in these rats (balloon-dummy-incontinent) but not in rats that received SNS (onset latency: $P=0.002$, $F=7.82$; one-way ANOVA and Bonferroni post-test, comparing each group to Group 1 (sham))(Figure 5, Table 2).

Correlation between anorectal EPs and FI

As a last step, the severity of incontinence as described by the FI was correlated to the EP parameters. Evoked potential amplitude and area under the curve tended to be less when

the FII was high but only EP onset latency was significantly correlated with FII (R^2 value of 0.38; $p=0.019$; Figure 6).

Discussion and conclusion

The findings of this study show that half the rats that underwent balloon injury showed altered continence behaviour and these rats also had reduced EP amplitudes and longer EP onset latencies. This shows, for the first time in an animal model, the correlation between sensory cortical function and continence. Rats that received SNS, had EP amplitudes and onset latencies like those of sham control rats at the end of one week. This observation supports the hypothesis that SNS acts to restore anorectal sensory function.

The balloon model used reproduces many features of FI in rats: motor and sensory damage to the anal sphincter^{11,13,14,20}, and FI like behaviour in a subset of animals¹⁵. The behavioral results of this study were similar to the study previously published by Devane et al¹⁵. While 50% of rats showed behavioral signs of FI in this study, 33% of rats became incontinent in the previous study.

Anorectal EPs are a useful measure to test the integrity of the whole sensory pathway, from the receptor in the anal canal, the inferior rectal nerves, the spinal cord and thalamus to the sensory cortex. Anorectal EP amplitude was reduced and onset latency increased in rats with an FII >0.20 indicating FI while the amplitude and latency of fore paw EPs recorded as a control was not different between groups. From previous studies of the balloon model, it is known that the inferior rectal nerve and its nerve endings deteriorate decreasing propagation of

anorectal stimuli to the primary somatosensory cortex as seen in diminished anorectal EPs, damage to the dorsal root ganglion and anal sphincter atrophy^{11,13,14,20}. Reduced EPs after the balloon injury have been previously reported after 4 weeks¹⁴, and were already present 2 weeks post injury in this study. The authors postulate that the chronic changes seen in the somatosensory cortex represent additional reorganisation at a cortical level due to inactivity of the pathways. It is not known if any reorganisation on the spinal cord level takes place.

Chronic SNS using a fully implantable device has proven its safety, stability and long-term utility. A minor complication that occurred was a mild seroma formation around the implant in two cases. For longer implantation durations this will need to be closely monitored. Sacral nerve stimulation at 14Hz for >2 weeks is easily achieved with a single battery without additional charging. It is advantageous that charging is required infrequently because even wireless charging requires sedation or anaesthesia. None of the rats that received SNS, neither continent (N=3) nor incontinent (N=3), showed a reduction in anorectal EP amplitude. This hints at an ameliorating action of SNS on anorectal sensory function but the number of experiments which were simultaneously successful in model creation and neuromodulation was small.

Sacral nerve stimulation was applied at the clinically used frequency of 14Hz although the authors' previous work showed an optimal frequency of 2Hz for acute SNS in rats¹⁹. The 14Hz frequency was chosen because it is clinically relevant and the superiority of 2Hz stimulation had not yet been shown in a behavioural setting. It was impossible to study several stimulation frequencies in the current animal model.

Limitations

The inherent limitation in the balloon model that not all rats lose place preference for pellet disposal was leveraged as a method to correlate FI and cortical EPs. In the rat a cut off for continence behavior of 80% pellets within the latrine was chosen in contrast to 100% continence expected for patients because fecal pellets have additional functions including marking of their territory and coprophagia which might displace pellets. The number of rats that received SNS was too low to allow for statistical analysis. This is partly due to technical difficulty and partly due to the limited number of neurostimulator devices available. Despite these of shortcomings the research has demonstrated that chronic SNS with implantable neurostimulators is feasible and offers direction for future work.

The timeline used in this study was kept deliberately as short as possible to facilitate testing of the neurostimulator and minimize laboratory time. Longer stimulation durations will be required in future studies. Evoked potentials were recorded only once at the end of the experiment and not tracked over time. Continuous recording of EPs would require more sophisticated equipment. In the present study all possible measures were taken to record EPs as uniformly as possible: the same stimulus, MEA location, the actual MEA, level of anaesthesia and equipment was used in each rat. Also, a control somatosensory region (the fore paw) was used to record EPs which remained stable following SNS.

In conclusion, this study has shown for the first time that neuropathic FI including sensory dysfunction can be re-created in a rodent model and deficits in the anorectal sensory function can be linked to FI behaviour in rats. It has also shown the feasibility of chronic SNS with a fully implantable neurostimulator in rats. Further work needs to include longer application of SNS and larger group sizes.

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Tables:

Group	Baseline FII (mean, SEM (95% confidence interval))	FII post-surgery (mean, SEM (95% confidence interval))
Group 1 (Sham) (N=5)	0.10 ± 0.01 (0.08 – 0.13)	0.09 ± 0.02 (0.06 – 0.11)
Group 2 (Balloon-dummy-continent) (N=5)	0.08 ± 0.02 (0.07 – 0.11)	0.12 ± 0.03 (0.09 – 0.14)
Group 2 (Balloon-dummy-incontinent) (N=5)	0.16 ± 0.01 (0.14 – 0.19)	0.44 ± 0.07 (0.36 – 0.49)

Table 1: FII at baseline and post-surgery.

Group	Amplitude (μV) (mean, SEM(95% CI of the mean))	Onset latency (ms) (mean, SEM (95% CI of the mean))	Area under the curve (mVs) (mean, SEM (95% CI of the mean))	
Fore Paw EPs	Sham (N=5)	4.66 ± 0.84 (2.31 - 7.01)	10.11 ± 0.86 (7.72- 12.51)	2.8 ± 1.1 (0.38- 5.99)
	Balloon-dummy-continent (N=4)	4.98 ± 1.36 (0.645 - 9.31)	11.32± 1.64 (6.10- 16.54)	2.4 ± 1.3 (1.8- 6.6)
	Balloon-dummy-incontinent (N=5)	4.87± 0.89 (2.39- 7.35)	11.55 ± 1.42 (7.59- 15.51)	1.5 ± 0.6 (0.12- 3.21)
	Balloon-SNS (N=6)	5.52 ± 0.94 (3.09 - 7.94)	10.28 ± 0.31 (9.50- 11.07)	2.7 ± 1.0 (0.05- 5.31)
	One-way repeated-measures ANOVA	P = 0.93, F(3,16) = 0.15	P = 0.71, F(3,16) =0.46	P = 0.82, F(3,16) =0.31
	Anal canal EPs	Sham (N=5)	23.22 ± 3.67 (13.03 - 33.41)	8.55 ± 0.51 (7.142 - 9.962)
Balloon-dummy-continent (N=4)		16.76 ± 7.29 (3.496 - 37.01)	9.30 ± 0.28 (8.399 - 10.20)	10.7 ± 2.9 (1.351 - 20.05)
Balloon-dummy-incontinent (N=5)		5.70 ± 1.70 (0.9718 - 10.43)	12.72 ± 1.11 (11.56 - 15.37)	2.6 ± 0.6 (1.273 - 4.447)
Balloon-SNS (N=6)		22.80 ± 1.83 (18.09 - 27.51)	9.72 ± 0.33 (8.872 - 10.56)	15.0 ± 2.5 (8.583 - 21.38)
One-way repeated-measures ANOVA		P = 0.03, F(3,16) = 3.91	P = 0.002, F(3,16) =7.82	P = 0.02, F(3,16) =4.73

Table 2: Amplitude, onset latency and area under the curve of EPs for each group and results from one-way ANOVA.

Figures:

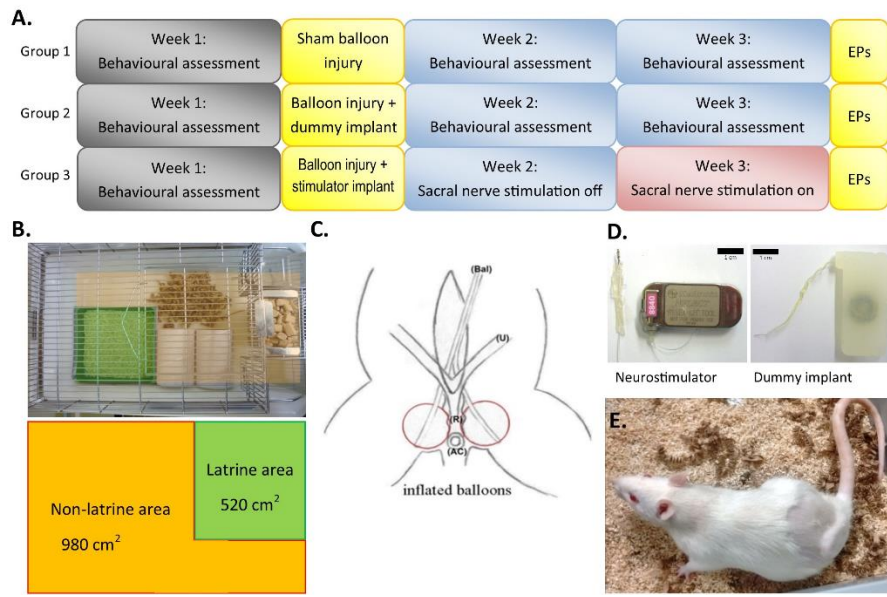


Figure 1: A. Study design. Timeline and interventions for each of the 3 groups studied. B. Cage design. C. Balloon model. Two balloons (bal) are inflated in the retro-uterine space for 60 min. (U) Uterus, (R) Rectum, (AC) Anal canal. D. Sacral nerve stimulation. Neurostimulator and dummy implant for comparison. E. Rat implanted with neurostimulator device subcutaneously in the back 14 days post-surgery.

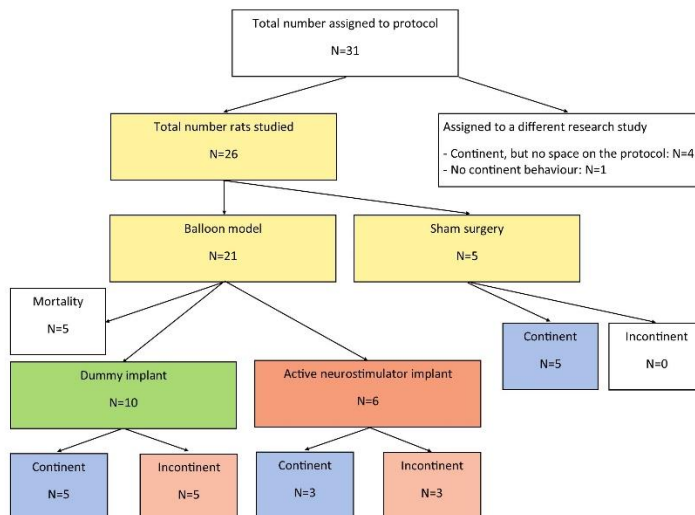
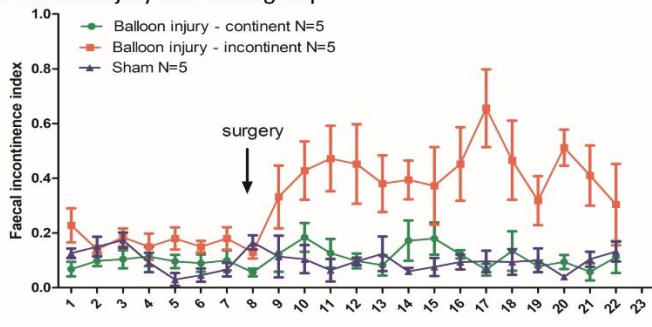
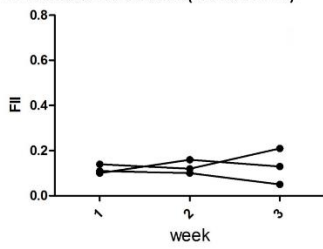


Figure 2: Allocation of animals and behavioral outcome.

A. Balloon injury and Sham group



B. Balloon and SNS(continent)



C. Balloon and SNS(incontinent)

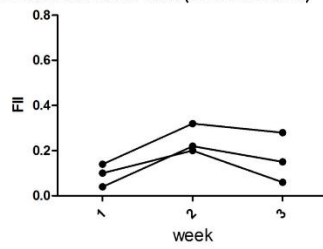


Figure 3: Faecal incontinence index (FI) A. Effect of balloon injury on FI in the Group 1 (Sham) and Group 2 (balloon-dummy-incontinent and balloon-dummy-incontinent). Mean \pm S.E.M. are shown. B. Effect of balloon injury and SNS in Group 3 (balloon-SNS) for each rat individually.

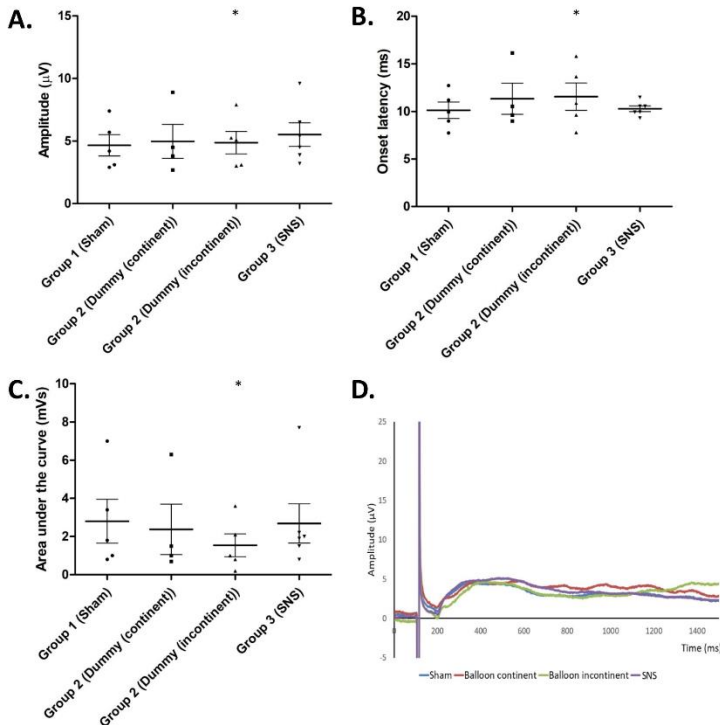


Figure 4: Graph of amplitude (A.), onset latency (B.) and area under the curve (C.) of the fore paw EP for each group. D. Average EP shape of sham-continent (N=5), balloon-dummy-continent (N=4), balloon-dummt-incontinent (N=5) and balloon-SNS (N=6) group.

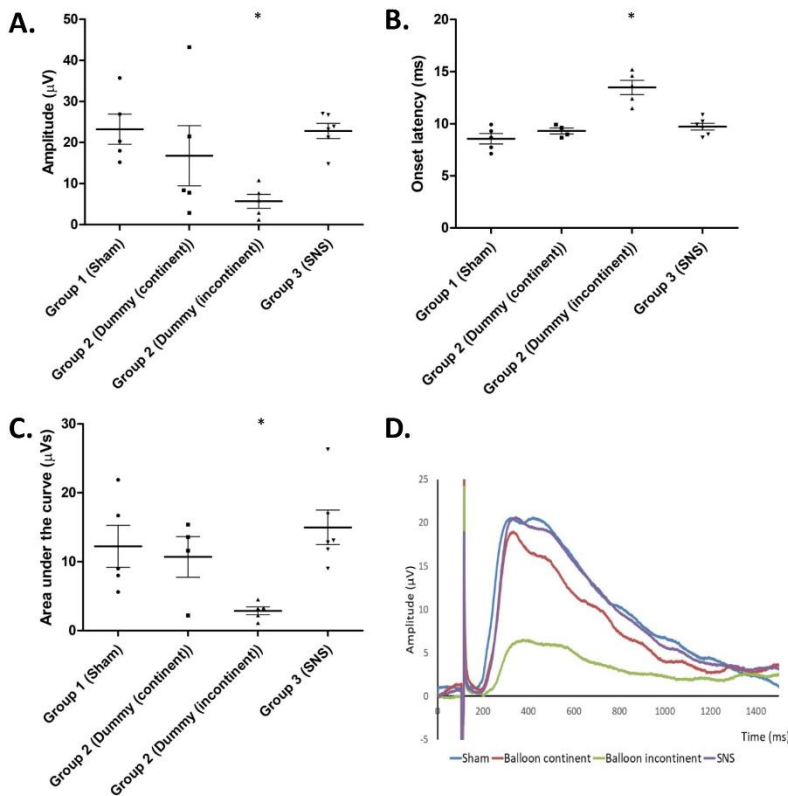


Figure 5: Graph of amplitude (A.), onset latency (B.) and area under the curve (C.) of the electrical anal canal EP for each group. D. Average EP shape of sham (N=5), balloon-dummy-continent (N=4), balloon-dummy-incontinent (N=5) and balloon-SNS (N=6) group. * denotes groups that show statistical difference to Group 1 (Sham).

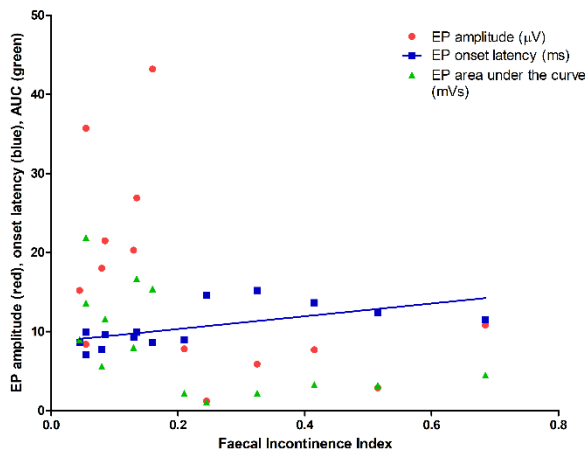


Figure 6: Correlation between EP amplitude, onset latency and area under the curve to FII in Groups 1 (sham) and 2 (balloon-dummy). Only the onset latency was significantly correlated with FII (blue line).