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Chronic implantation of cuff electrodes on the pelvic nerve in rats is well tolerated and does not compromise afferent or efferent fibre functionality

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Abstract

Objective Neuromodulation of autonomic nerve activity to regulate physiological processes is an emerging field. Vagal stimulation has received most attention whereas the potential of modulate visceral function by targeting autonomic nerves within the abdominal cavity remains under-exploited. Surgery to locate intra-abdominal targets is inherently more stressful than for peripheral nerves. Electrode leads risk becoming entrapped by intestines and loss of functionality in the nerve-target organ connection could result from electrode migration or twisting. Since nociceptor afferents are intermingled with similar-sized visceral autonomic fibres, stimulation may induce pain. In anaesthetised rats high frequency stimulation of the pelvic nerve can suppress urinary voiding but it is not known how conscious animals would react to this procedure. Our objective therefore was to determine how rats tolerated chronic implantation of cuff electrodes on the pelvic nerve, whether nerve stimulation would be aversive and whether nerve-bladder functionality would be compromised.

Approach We carried out a preliminary de-risking study to investigate how conscious rats tolerated chronic implantation of electrodes on the pelvic nerve, their responsiveness to intermittent high frequency stimulation and whether functionality of the nerve-bladder connection became compromised.

Main results Implantation of cuff electrodes was well-tolerated. The normal diurnal pattern of urinary voiding was not disrupted. Pelvic nerve stimulation (up to 4mA, 3kHz) for 30min periods evoked mild alerting at stimulus onset but no signs of pain. Stimulation evoked a modest (<0.5^{o}C) increase in nerve temperature but the functional integrity of the nerve-bladder connection, reflected by contraction of the detrusor muscle in response to 10Hz nerve stimulation, was not compromised.

Significance Chronic implantation of cuff electrodes on the pelvic nerve was found to be a well-tolerated procedure in rats and high frequency stimulation did not lead to loss of nerve functionality. Pelvic nerve stimulation has development potential for normalizing voiding dysfunction in conscious rats.

Introduction

The concept of influencing physiological processes by modulating peripheral nerve activity is not new. Acupuncture and other counter-irritation measures have been utilised for millennia to lessen discomfort or irritation. However, in the 20th century the advent of transcutaneous electrical nerve stimulation (TENS) for pain relief (Melzack, 1975), based on physiological principles (Melzack and Wall, 1965), heralded the modern era of neuromodulation. Early neuromodulation techniques targeted somatic peripheral nerves but modulation of autonomic nerve activity has also been used for therapeutic gain. Stimulation of the vagus nerve is now a well-established procedure in the treatment of epilepsy but more recently is has been promoted as a treatment for a host of other disorders including depression, inflammatory disorders, heart failure and obesity (for recent reviews see Guiraud et al, 2016; Browning et al, 2017). Several recent studies have raised the possibility of developing novel approaches to treat bladder dysfunction via modulation of activity in other autonomic nerves. For example, suppression of imminent urinary voids and increased functional bladder capacity were reported following stimulation of the pelvic nerve in terminally anaesthetised rats (Jen et al, 2017; Langdale et al, 2017; Crook and Lovick, 2017).

Whilst such studies hold great promise for translation, there are potential pitfalls associated with chronic implantation of electrodes onto small autonomic nerves. Our recent experience that chronic implantation of cuff electrodes on the cervical vagus, a relatively large, straight nerve that is readily accessible, leads to loss of functionality within the efferent fibre population (Somann et al 2017) suggests that autonomic nerves may not be as robust as their somatic counterparts.

In view of these potential limitations associated with translating findings from acute studies to conscious animals, we carried out a preliminary de-risking study to investigate how conscious rats would tolerate chronic implantation of cuff electrodes on the pelvic nerve, and whether stimulation would evoke adverse behavioural responses and/or lead to a loss of nerve functionality.

Methods

Electrode design for chronic implantation

Nerve cuffs were fabricated from 0.2" biomedical silicone tubing (A-M Systems, Carlsborg, USA), which was slit longitudinally so it could be slipped round the pelvic nerve. The electrodes were made of 7-stranded 75μm platinum/iridium wire with cobalt chrome core (custom made product, Fort Wayne Metals, Indiana, USA). The cuff was pierced to allow two wires, 1mm apart, to enter the lumen and run round the inner perimeter of the cuff. The wire was sealed to the lumen wall with silicone adhesive (Med-4213, Nusil, Carpinteria, USA) so that 1.25mm of bared wire could make contact the nerve (Fig 1a). The peripheral end of the wire was encased in silicone tubing and soldered to a gold-plated pin, which could be inserted into a Plastics One headcap (MS363, Plastics One, Roanoke, USA). The overall dimensions of the finished cuff were ID 0.5mm, OD 1.2mm, cuff length: 2mm, inter-electrode distance: 1mm, lead length: 200mm. For use in acute experiments the open cuff can be slipped under the pelvic nerve and does not move (Fig 1a), however for chronic implantation it was necessary to devise a method to hold the cuff in place. To achieve this we passed a silk ligature round the cuff, in between the leads (Fig 1b) and tightened it sufficiently to close the cuff without pinching the nerve (Fig 1c). The cuff and nerve were then 'potted' in a drop of dental silicone (Klasse 4, Augsburg, Germany).

Animals

Female Wistar rats (n=9), 238-287g on the day of surgery were chosen for this study as access to the pelvic nerve is easier in females than in males. Animals were housed in a temperature-controlled environment (20-25^{o}C) on a 12h light/dark schedule (lights on 4am-4pm) with free access to rat chow and water. Prior to surgery they were handled regularly. In the two weeks running up to surgery each rat was placed on two occasions in a metabolic cage for 20-22h periods (Raturn, Culex Automated In Vivo Sampling System; Bioanalytical Systems, Inc.) in order to obtain an indication of the normal voiding pattern. In order to estimate the timing and volume of voids the apparatus was modified to enable urine arriving in the terminal chute to be collected in a covered receptacle mounted on the pan of an electronic balance. For estimations of urine volume we assumed a specific gravity of 1.0. During the dark period the apparatus was illuminated with red light (which rats cannot see) allowing us to make video recordings of the rat's behavior. The first session was used to habituate the rat to the apparatus and the data on urine collection was not used.

The animals were re-tested 7-9 days following surgery to check that normal voiding had resumed. Additional sessions to test the effects of long periods of pelvic nerve stimulation were carried out between 11 and 19 days following surgery. All investigations conformed to the Guiding Principles for Research Involving Animals and Human Beings as adopted by The American Physiological Society.

Surgery

Full aseptic conditions were maintained throughout surgery. Animals were pretreated with butorphenol (400µg i.m.) and anaesthetised with isofluorane: 4% in oxygen for induction, reducing to 1.5-2.5% during surgery, according to individual animal's requirement to maintain a surgical level of anaesthesia. A small incision was made in the interscapular region; then the animal was turned to provide access for a midline laparotomy. A trochar was tunneled subcutaneously between the two incisions to allow passage of leads from the nerve cuff electrode. The abdominal wall and intra-abdominal fat were then retracted and the bladder deflected laterally to expose the left ureter. The junction of the ureter and bladder provided the landmark for locating the pelvic nerve, which runs medio-laterally round the wall of the uterus, initially posterior the ureter. A length of the preganglionic nerve bundle was carefully freed from the uterine wall and the cuff electrode hooked under it. The cuff was closed by tightening a 6.0 silk suture placed round the cuff, taking care not to pinch or kink the nerve (Fig 1c). The nerve-cuff assembly was then 'potted' in a drop of dental silicone. The abdominal muscle was closed using a continuous 4.0 Vicryl (Ethicon, Somerville, USA) suture, allowing space to exit the leads from the nerve cuff. Excess lead length was curved into a strain relief loop subcutaneously and loosely sutured to the abdominal wall to minimize movement of the cuff electrode. Staples (Vista skin staples, 3M, USA) were used to close the skin incision. Gold pins attached to the free end of the leads from the cuff electrode were inserted into a headcap (MS363, Plastics One, Roanoke, USA). The headcap was mounted on the skull using dental acrylic. Three stainless steel screws inserted into the skull served as anchor points. A dustcap fashioned from an E363 backmount (Plastics One) and dental Silicone (Klasse 4) prevented the contacts becoming blocked with bedding material. Wounds were treated prophylactically with topical antibiotic gel (400IU/g bacitran, 3mg/g neomycin, 5000IU/g polymyxin B). Once the surgery was completed, antibiotic

(Amoxicillin, 15mg/kg s.c.) and 5ml saline were administered subcutaneously and the animal placed in a warm environment to recover. The cage floor was lined with absorbent paper so that we could easily detect spontaneous urination (damp patches). Animals were monitored closely during recovery from surgery, and received supplementary analgesia (buprenorphine 18µg s.c. every 6-12 hours or as required) for 48 hours.

Pelvic nerve stimulation

Pelvic nerve stimulation (0.5-3kHz sinusoidal waveform) was delivered via a lead connected to the head cap using a stimulus isolation device (STMISOLA, Biopac Systems Inc., Santa Barbara, USA), driven by a digital waveform generator (M33622A, Agilent, Santa Clara, USA). As an initial test we investigated behavioral responses evoked by short periods of stimulation whilst the rat was confined in a high-sided hexagonal Perspex arena (diameter 40cm). Behavioural responses during stimulation were captured using a webcam (C120, Logitech, Lausanne, Switzerland). We were particularly alert to signs of pain and distress that could result from activation of pelvic nerve nociceptor afferent fibres. After this initial test session, subsequent stimulation was performed whilst rats were in the metabolic cage.

Although we used sinusoidal waveform in an effort to deliver zero net charge, we were mindful of reports that many stimulators do not achieve this (Franke et al, 2014). We found that our stimulator did in fact produce a small DC offset (0.18µA using 1-3kHz, 1-3mA). However, this current is orders of magnitude below that which might be expected to interfere with nerve conduction (Bhadra & Kilgore, 2004).

Acute experiments

Functional integrity of nerve-bladder connection

For terminal experiments the rats were anaesthetised with urethane (1.4g/kg i.p.). With the animal supine, the abdomen was opened and the tip of a 25G needle connected to a saline-filled catheter was inserted through the bladder dome. This was connected to a pressure transducer to record changes in bladder pressure evoked by stimulation of the pelvic nerve.

Measurement of nerve temperature during stimulation

High frequency nerve stimulation (up to 500Hz) has been shown to produce a local increase in temperature (Gerard et al, 1927). Since the presence of the nerve cuff would limit dissipation of heat and heating could be deleterious to the nerve, it was prudent to monitor nerve temperature. As the nerve and cuff assembly had been potted in silicone, it was not possible to gain access to the nerve after chronic implantation. We therefore carried out a separate series of experiments in which we prepared rats (n=10) for acute experimentation and positioned a miniature thermocouple parallel to the nerve inside the cuff electrode, in order to measure changes in nerve temperature during stimulation (2-4mA, 1 and 3kHz sine wave).

Statistical analysis

Statistical analysis was performed using Friedman's test followed by Dunn's test for multiple comparisons, normal Friedman's test followed by permutation paired t-test with Bonnferroni correction for multiple comparisons or Student's unpaired t-test as appropriate with Graphpad Prism v7 software, or Matlab R2016a (Mathworks, Natick, USA) to perform permutation tests. Mean values are presented ± S.E.M.

Results

Cuff electrodes were implanted on the left pelvic nerve of nine rats. One animal failed to thrive after surgery and was terminated. The remaining eight rats recovered promptly from anaesthesia; righting usually occurred within 15-30min of switching off the anaesthetic vapouriser. By the next morning rats displayed normal gait and were eating and drinking readily. No signs of distress were evident when the animals were handled. The fur round the urethra was also clean and dry, indicating that the animals were making discrete voids rather than 'dribbling' from the bladder. Well-formed faecal pellets were observed in the home cage litter.

Urine output and pattern of voiding was tested 9-11 days post operatively by placing the rat in a metabolic cage for a 20-22h period. Each rat displayed a normal diurnal pattern of voiding similar to that seen in the same animal prior to surgery with smaller and more frequent voids made during the dark period, when rats are more active, compared to the light (Fig 2).

Response to high frequency pelvic nerve stimulation

Brief periods of stimulation

On the 5th or 6th day post-operatively each rat (n=8) was placed in a high-walled Perspex arena to observe its response to short periods (10-20s) of high frequency sine wave stimulation of the pelvic nerve. The stimulation parameters chosen (1kHz or 3kHz, 0.5-5mA baseline-to-peak) were in the range that had been found to suppress imminent voids in anaesthetised rats (Crook and Lovick, 2017). As the stimulus intensity was increased the first response observed in all eight rats was a contraction of the musculature in the sacral region, which caused the base of the tail to rise and the tail to extend. In 6 of the animals thresholds for this effect ranged from 0.25-1.0mA at 3kHz (mean 0.67±0.10mA), 0.125-0.5mA at 1kHz, (mean 0.31±0.06mA). In three of these six animals stimulation at slightly higher intensities (0.5-1mA at 3kHz; mean 0.67±0.16mA); 0.25-0.5mA, mean 0.38±0.12mA at 1kHz) evoked transient (2-3s) licking of the pelvic region at the onset of the stimulus. Stimulation at similar intensities (0.5-1.5mA 3kHz, mean 0.67±0.11m; 0.125-1mA 1kHz, mean 0.38±0.06mA) often interrupted ongoing behavior. Arousal from sleep, onset of walking/running occurred in five out of six animals. On one occasion vocalization (in the audible range for humans) was evoked in one animal (1mA 3kHz). Otherwise, no signs of pain and distress e.g. vocalization, struggling on being handled, adopting a hunched posture were displayed by the rats at any time.

The two remaining rats much higher stimulus intensities (2-5mA) were required before the first signs of a behavioural response were observed. In terminal experiments under anaesthesia we were unable to evoke contraction of the bladder in response to low frequency (10Hz) pelvic nerve stimulation. We concluded that the integrity of the pelvic nerve was compromised in these two animals. Data from these rats was excluded from analysis. One further rat did not complete further stages of the study due to a breakage of leads to the headcap.

Prolonged stimulation

We tested the effect of longer periods (30min) of high frequency (1 and 3kHz) stimulation 11-19 days following surgery whilst rats were confined in the metabolic

cage. Stimulation was performed during the dark period when rats were most active (Fig 4a). Each rat received up to 3 x 30min periods of stimulation during a 6-9h observation period. The stimulus parameters (0.5-5.0 mA, 1-3kHz sine wave) were based on parameters we found previously to be optimal for suppressing imminent voids in anaesthetised rats (Crook and Lovick, 2017). Examination of the video record revealed no untoward behavioural response to nerve stimulation. Some rats showed a transient cessation of ongoing activities as the stimulation was switched on. Our apparatus did not allow quantification of movement. However, we were able to make a general assessment of activity from the video record by scoring the time that animals appeared asleep ie. motionless with head bowed. During pelvic nerve stimulation the proportion of the time rats adopted this posture was lower compared to the pre-stimulation period (Fig 4a). This was followed by a significant rebound increase in sleeping time during the hour after the stimulator had been switched off. There was no difference between the response to stimulation at 1 or 3kHz ($p>0.05$, Mann-Whitney U test for void frequency or voided volume, permutation Student's t-test for time spent sleeping), nor could any difference be detected between the response to stimulation at high (2.5-5mA) or low (0.5-2.0mA) intensities ($p>0.05$, Mann-Whitney U test for void frequency or voided volume, permutation Student's t-test for time spent sleeping). The results were therefore pooled.

The urine output log from the digital balance revealed the expected diurnal variation in voiding pattern (compare Figs 2 and 5, data from the same animal). However, during periods of pelvic nerve stimulation rats produced more frequent, smaller voids compared to the hour prior to stimulation, although the voided volume did not change significantly (Figs 4b and c). Once the stimulator was switched off voiding frequency decreased during the next hour in parallel with the increase in sleep-like behaviour (Figs 4a, 4c and Fig 5).

Terminal experiments under anaesthesia

3 weeks after implantation of electrodes we tested the functional integrity of the nerve-bladder connection in terminal experiments under anaesthesia. The stimulation parameters used (10s trains of 1ms rectangular pulses) were based on those we had found previously to be optimal for evoking contraction of the bladder in urethane-

anaesthetised preparations (Crook & Lovick, 2017). In five out of seven animals tested stimulation evoked a graded increase in bladder pressure (Fig 5). In two of the rats, stimulation failed to evoke a response. Interestingly, these were the same animals (rats 1 and 3) that had failed to show behavioural responses to high frequency pelvic nerve at the early stage of the study until high intensities were applied (see above). Gross examination of the abdominal cavity post mortem showed that the nerve cuff assembly had been encapsulated with connective tissue but there were no obvious signs of pinching or twisting of the nerve.

Change in pelvic nerve temperature during pelvic nerve stimulation

We measured the temperature change inside the nerve cuff during 5min periods of sine wave stimulation at two intensities (2mA and 4mA) and in the frequency range (1kHz and 3kHz), which have been shown in acute studies to be optimal for inhibiting voiding (Crook and Lovick, 2017). The effects evoked by stimulation at either 1 or 3kHz at a given intensity were similar so these data sets were pooled. Stimulation at 2mA, which represents the high end of the range of intensities tested in the chronically implanted conscious rats, did not evoke a significant increase in nerve temperature (+0.36±0.11^{o}C). Stimulation at 4mA did however evoke a modest but significant rise in temperature (+1.1±0.24^{o}C; p=0.01, Student's t-test).

Discussion

Overall, implantation of cuff electrodes on the pelvic nerve in rats was well tolerated. With the exception of two animals that were prepared at the beginning of the series, when our technique was still being refined, terminal experiments carried out 3 weeks post-surgery showed that the functionality of the nerve-bladder motor connection remained intact. This finding was important in view of our recent finding that functionality of efferent fibres of the vagus nerve becomes compromised following chronic implantation of nerve cuffs in rats (Somann et al, 2017).

Our specific interest was in responsiveness to high (1-3kHz) frequency stimulation, which in acute studies has been shown to inhibit imminent voids (Crook and Lovick, 2017). We noticed that kHz stimulation evoked a mild behavioural alerting at stimulus

onset. This is consistent with the 'on response', a brief intense burst of axonal firing that appears when kHz frequency alternating current is first applied to a nerve (Kilgore et al, 2009). Although the rats appeared to be otherwise unperturbed during prolonged stimulation, there was an absence of sleep-like behaviour and a concomitant increase in voiding frequency, which suggests that they were experiencing a sensation that kept them alert. These findings also suggest that sensory afferents in the pelvic nerve remained functional after implantation of the nerve cuff. Importantly, there was no indication that the stimulation was aversive, suggesting that nociceptive afferents were not recruited at the intensities of stimulation used in this study.

A concern to us was that the nerve might be susceptible to heat damage during stimulation. High frequency stimulation of nerves has been reported to generate a local increase in temperature (Gerard et al, 1927) and the nerve cuffs used in the present experiments would limit dissipation of heat. However, although we observed a rise in temperature, the increase was extremely modest ($0.36\pm0.11^{o}C$) and unlikely to be detrimental to the nerve.

The principal goal of this study was to determine whether rats would tolerate implantation of electrodes and high frequency stimulation of the pelvic nerve. The work represents the first step towards translating our finding in anaesthetized rats that a short (60s, 1-3kHz) period of high frequency pelvic nerve stimulation applied at the onset of an imminent void could suppress voiding (Crook and Lovick, 2017). We proposed that the stimulation induces a non-physiological pattern of firing in small diameter afferent axons found in the pelvic nerve (Hulsebosch and Coggleshall, 1982; Pelot et al, 2017), effectively imposing a 'nonsense' signal on the central circuitry, which make it impossible to set up the motor pattern needed to produce a co-ordinated void (Crook and Lovick 2017).

In contrast to the suppression of imminent voids that was produced by brief stimulation of the pelvic nerve in anaesthetised rats, voiding persisted during the long periods of stimulation used in the present study. Prolonged periods of nerve stimulation have been shown to lead to transmitter depletion in motor nerves (Ceccarelli et al, 1972). Thus bladder efferent fibres might become unable to evoke contraction of the bladder

muscle. Under physiological conditions pelvic nerve afferents do not fire at frequencies in excess of 15Hz (Shea et al 2000). Prolonged stimulation at kHz frequency might therefore be expected to lead to transmitter depletion and a reduction of transmission of signals to the spinal cord. The loss of functionality in the nerve on one side would not however, prevent voiding (Crook and Lovick, 2017). The pelvic nerve trunks on each side innervate the whole of the bladder surface (Carpenter and Rubin, 1967). In addition, gap junction coupling between cells (Fry et al., 2004) enables the detrusor to act as a functional syncytium. Thus voiding was most likely maintained by activity in the contralateral nerve.

The responses evoked by prolonged stimulation: behavioural alerting and continuation of voiding, although interesting, are to an extent peripheral findings. The current work represents the first step towards translating to humans the finding in anaesthetized rats that a short (60s, 1-3kHz) period of high frequency pelvic nerve stimulation applied at the onset of an imminent void could suppress voiding (Crook and Lovick, 2017). At this stage of animal testing we felt it important to 'overload' the system by stimulating beyond the limits that we anticipate would be necessary in a clinical situation. For this reason we investigated the effects of long periods of stimulation. The clinical application would be in urinary urge incontinence where sufferers experience 'a sudden intense desire to void, which is difficult to defer'. In order to suppress an imminent inappropriate void pelvic nerve stimulation would be starting at the onset of urge, and continued for only a few minutes to give the individual time to reach a suitable place, before turning off the stimulation to permit voiding.

Conclusion

Our study indicates that chronic implantation of cuff electrodes on the pelvic nerve is a well-tolerated procedure in rats. The effect of nerve stimulation is not aversive and the functionality of afferent and efferent fibre population does not appear to be compromised. The data suggest that modulation of intra-abdominal autonomic pelvic nerve activity offers potential for developing as a means to control visceral function in conscious rats.

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CONFLICT OF INTEREST

JC, CB, TL and PI declare no conflict of interest.

AUTHOR CONTRIBUTIONS

TL and PI conceived the study and obtained funding. JC and CB carried out the experimental work and analysed the data, with input from TL. TL prepared a draft of the manuscript with input from JC and CB. All authors approved the final version.

References

Bhadra N, Kilgore KL (2004). Direct current electrical conduction block of peripheral nerve. IEEE Trans Neural Syst Rehabil Eng. 313-324.

Browning KN, Verheijden S, Boeckxstaens GE (2017). The vagus nerve in appetite regulation, mood, and intestinal inflammation. Gastroenterology. 152, 730-744.

Carpenter, F. G., and Rubin, R. M. (1967). The motor innervation of the rat urinary bladder. J. Physiol. 192, 609–617.

Ceccarelli B, Hurlbut WP, Mauro A (1972) Depletion of vesicles from frog neuromuscular junctions by prolonged tetanic stimulation. J Cell Biol 54 30-38.

Crook JJ, Lovick TA (2016). Urodynamic function during sleep-like brain states in urethane-anaesthetised rats. Neuroscience 313 73-82.

Crook JJ, Lovick TA (2017). High frequency stimulation of the pelvic nerve inhibits urinary voiding in rats. Front Physiol – Integrative Physiology 8:623. doi: 10.3389/fphys.2017.00623 .

Franke M, Bhadra N, Bhadra N, Kilgore K (2014). Direct current contamination of kilohertz frequency alternating current waveforms. J Neurosci Meth. 232:74-83.

Fry, CH, Sui G-P, Severs NJ, Wu C (2004). Spontaneous activity and electrical coupling in human detrusor smooth muscle: implications for detrusor overactivity? Urology 63, 3–10.

Gerard RW, Hill AV, Zotterman Y (1927). The effect of frequency of stimulation on the heat production of nerve. J Physiol. 63 130-143.

Guiraud D, Andreu D, Bonnet S, Carrault G, Couderc P, Hagège A, Henry C, Hernandez A, Karam N, Le Rolle V, Mabo P, Maciejasz P, Malbert CH, Marijon E, Maubert S, Picq C, Rossel O, Bonnet JL (2016). Vagus nerve stimulation: state of the art of stimulation and

recording strategies to address autonomic function neuromodulation. J Neural Eng. 13:041002. doi: 10.1088/1741-2560/13/4/041002.

Hulsebosch CE, Coggeshall RE (1982). An analysis of the axon populations in the nerves to the pelvic viscera in the rat. J Comp Neurol 211 1-10.

Jen E, Hsieh T-H, Lu T-C, Chen M-C, Lee F-J, Lin C-T, Chen S-C, Chu P-Y, Peng C-W, Lin C-W (2017). Effects of pulsed-radiofrequency neuromodulation on the rat with overactive bladder. Neurourol Urodyn 36 1734-1741

Kilgore KL, Foldes EA, Ackermann FD, Bhadra N (2009) Combined direct current and high frequency nerve block for elimination of the onset response. Conf Proc IEEE Eng Med Biol Soc 197-9. doi: 10.1109/IEMBS.2009.5333642.

Langdale CL, Hokanson JA, Sridhar A, Grill WM (2017). Stimulation of the pelvic nerve increases bladder capacity in the prostaglandin E2 rat model of overactive bladder. Am J Physiol Renal Physiol doi:10.1152/ajprenal.00116.201

Melzack R (1975). Prolonged relief of pain by brief, intense transcutaneous somatic stimulation. Pain. 1 357-373.

Melzack R, Wall PD (1965). Pain mechanisms: a new theory. Science 150(3699) 971-979.

Negoro H, Kanematsu A, Yoshimura K, Ogawa O (2013). Chronobiology of micturition: putative role of the circadian clock. J Urol. 190 843-849.

Patel YA, Butera RJ (2015). Differential fiber-specific block of nerve conduction in mammalian peripheral nerves using kilohertz electrical stimulation J Neurophysiol 113: 3923–3929.

N A Pelot, C E Behrend and W M Grill (2017) Modeling the response of small myelinated axons in a compound nerve to kilohertz frequency signals. J. Neural Eng. 14 doi.org/10.1088/1741-2552/aa6a5f

Shea VK, Cai R, Crepps B, Mason JL, Perl ER (2000) Sensory fibers of the pelvic nerve innervating the rat's urinary bladder. J Neurophysiol. 84:1924-1933.

Somann JP, Albors GO, Neihouser K, Lu K-H, Liu Z, Durkes A, Robinson JP, Powley TL, Irazoqui PP (2017). Chronic cuffing of cervical vagus nerve inhibits efferent fiber integrity in rat model. J Neural Eng In Press.

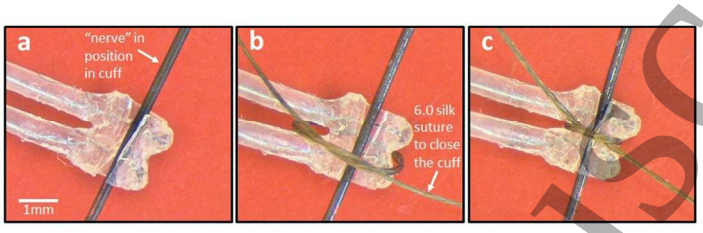
Fig 1. a) Cuff with nerve in place. Grey filament used as surrogate nerve for illustrative purposes; b) braided 6.0 silk suture thread looped round the cuff; c. tightening the suture thread closes the cuff, without pinching the nerve. The nerve and cuff are then potted in a drop of silicone glue (not shown)

Fig 2. Diurnal pattern of voiding in a rat before and 1 week after implantation of a cuff electrode on the left pelvic nerve.

Fig 3. Effect on a) time spent in sleep-like posture, b) void frequency and c) voided volume during 30min periods of pelvic nerve stimulation (1-3kHz sine wave; 0.5-5mA). a: Friedman's test followed by permutation paired t-test with Bonferroni correction for multiple comparisons, b, c: Friedman's test followed by Dunn's test for multiple comparisons. Note that in a) the error bar during stimulation is less than the thickness of the baseline. Data from 5 rats. * $p<0.05$; ** $p<0.01$; ***$p<0.001$.

Fig 4. Effect of 30 min periods of pelvic nerve stimulation on pattern of urine output. During periods of pelvic nerve stimulation indicated by pairs of vertical lines, rats produced more frequent, smaller voids compared to the preceding period. Zoom of one stimulation period shows individual voids as incremental steps in the record. Same rat as Fig 2.

Fig 5. Effect of 30 min periods of pelvic nerve stimulation on pattern of urine output. During periods of pelvic nerve stimulation indicated by pairs of vertical lines, rats produced more frequent, smaller voids compared to the preceding period. Zoom of one stimulation period shows individual voids as incremental steps in the record. Same rat as Fig 2.



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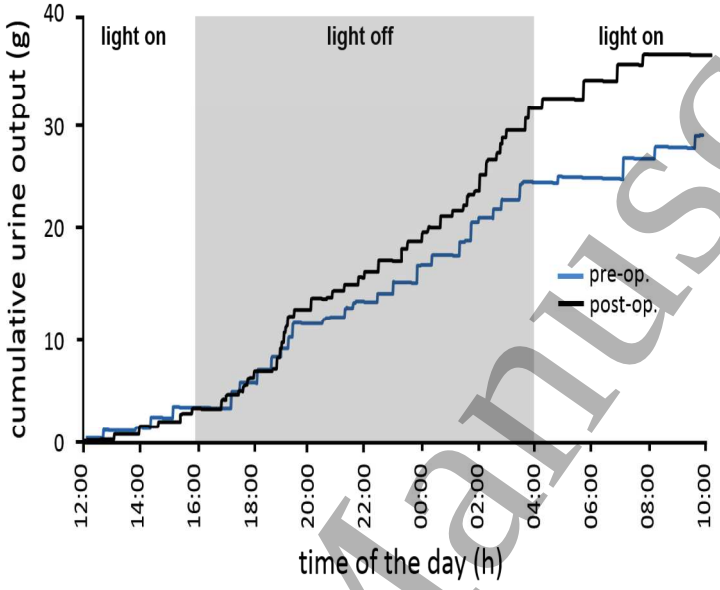

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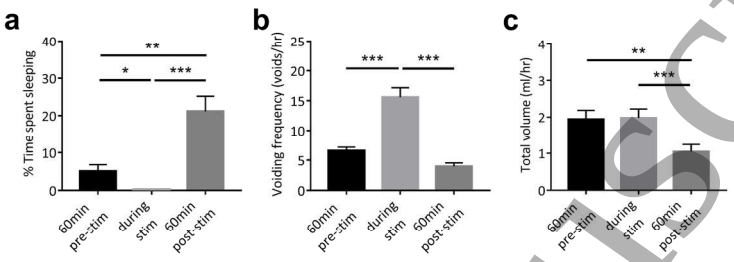

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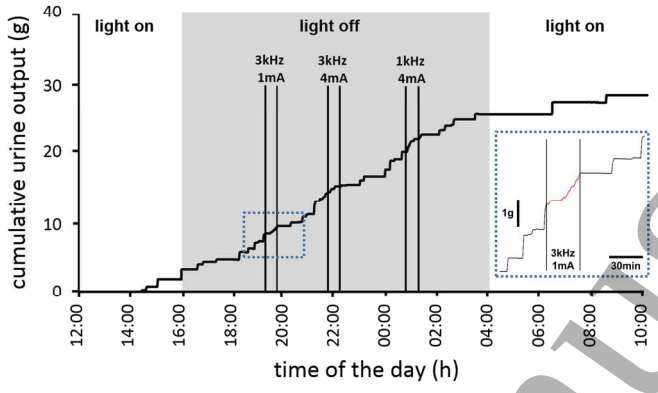

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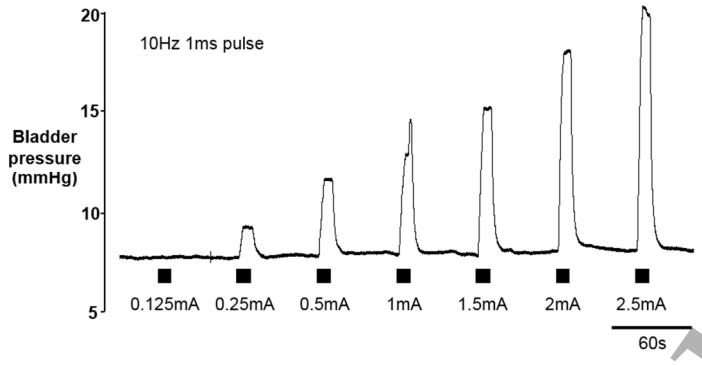

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