Pudendal but not Tibial Nerve Stimulation Inhibits Bladder Contractions Induced by Stimulation of Pontine Micturition Center in Cats

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Running head: Pudendal inhibition of efferent micturition pathway

Total number of words in abstract: 234
Total number of words in manuscript: 3361

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This study examined the possibility that pudendal nerve stimulation (PNS) or tibial nerve stimulation (TNS) inhibits the excitatory pathway from the pontine micturition center (PMC) to urinary bladder. In decerebrate cats under α-chloralose anesthesia, electrical stimulation of the PMC (40 Hz frequency, 0.2 ms pulse width, 10-25 second duration) using a microelectrode induced bladder contractions >20 cmH₂O amplitude when the bladder was filled to 60-70% capacity. PNS or TNS (5 Hz, 0.2 ms) at two and four times the threshold (2T and 4T) to induce anal or toe twitch was applied to inhibit the PMC stimulation-induced bladder contractions. Propranolol, a nonselective β-adrenergic receptor antagonist, was administered intravenously (1 mg/kg) to determine the role of sympathetic pathways in PNS/TNS inhibition. PNS at both 2T and 4T significantly (p < 0.05) reduced the amplitude and area under the curve of the bladder contractions induced by PMC stimulation, while TNS at 4T facilitated the bladder contractions. Propranolol completely eliminated PNS inhibition and TNS facilitation. This study indicates that PNS but not TNS inhibits PMC stimulation-induced bladder contractions via a β-adrenergic mechanism that may occur in the detrusor muscle as a result of reflex activity in lumbar sympathetic nerves. Neither PNS nor TNS activated a central inhibitory pathway with synaptic connections to the sacral parasympathetic neurons that innervate the bladder. Understanding the site of action involved in bladder neuromodulation is important for developing new therapies for bladder disorders.

KEYWORDS: bladder, pudendal, tibial, propranolol, cat
INTRODUCTION

Overactive bladder (OAB) is defined as urinary urgency, with or without urge incontinence, usually manifesting with frequency and nocturia. OAB is estimated to affect as many as 13% of women and 11% of men worldwide (1, 15). Current pharmacologic therapies are suboptimal as they have low efficacy with significant side effects (2, 4). Other effective OAB treatments include sacral neuromodulation and intra-detrusor botulinum-A toxin injection (24, 30). In addition to sacral neuromodulation, both pudendal (20, 21) and tibial (13, 19, 29) neuromodulation are also effective in the treatment of OAB patients. However, currently the mechanisms underlying bladder neuromodulation are not fully understood. Understanding the neural pathways involved in bladder neuromodulation is important for improving the treatment efficacy or developing new therapies for OAB.

Electrical stimulation of the pudendal or tibial nerves can inhibit bladder contractions by generating action potentials in sensory nerves, which propagate into the central nervous system leading to the release of neurotransmitters that inhibit the afferent and/or efferent limbs of the spinobulbospinal micturition pathway. Previous studies in cats have demonstrated the ability of tibial nerve stimulation (TNS) (27, 28) or pudendal nerve stimulation (PNS) (16, 23) to inhibit the spinobulbospinal micturition reflex passing through the pontine micturition center (PMC) and significantly increase bladder capacity during saline infusion of the bladder. It is assumed that this inhibition must have occur at least in part on the ascending afferent limb of the spinobulbospinal micturition pathway (9, 10), because clinical studies (19, 29) have demonstrated that PNS or TNS can reduce urgency sensations and increase bladder capacity without adversely affecting voiding, suggesting that the descending pathway from the PMC to the spinal cord which generates the bladder contractions is resistant to the stimulation. However,
the effect of PNS/TNS on the descending limb of the spinobulbospinal micturition reflex still needs to be examined.

Therefore, in this study using decerebrate, α-chloralose anesthetized cats the descending limb of the spinobulbospinal micturition reflex was directly activated by a microelectrode implanted in the PMC. Then, we examined the effect of PNS/TNS on the bladder contractions induced by PMC stimulation to determine if PNS/TNS can modulate the excitatory pathway from the PMC to detrusor. Since previous studies in cats (8, 11, 12, 17, 23) indicated that PNS could drive the sympathetic efferent output to inhibit the bladder contractions via β-adrenergic mechanism in the detrusor, propranolol (a nonselective β-adrenergic receptor antagonist) was administered intravenously in this study to determine if sympathetic inhibitory reflexes to the bladder were activated by PNS/TNS.

MATERIALS AND METHODS

All protocols involving the experimental use of animals in this study were approved by the Animal Care and Use Committee at the University of Pittsburgh.

Surgical Procedures

The experiments were conducted on a total of 12 cats (6 male, 6 female, 3.0-4.2 kg) under isoflurane (3-5% in O₂) anesthesia during surgery followed by α-chloralose anesthesia (65 mg/kg i.v. and supplemented as needed) during data collection. Systemic blood pressure was monitored throughout the experiment via a catheter inserted into the right carotid artery while the left carotid artery was ligated. Heart rate and oxygen saturation level were monitored by a pulse
oximeter (9847V, NONIN Medical, Plymouth, MN) attached to front limb. A tracheotomy was performed and an endotracheal tube was inserted to ensure airway patency. A catheter for intravenous infusion of fluid and drugs was inserted into the left cephalic vein. A laparotomy was then performed and the ureters were isolated, cut and drained externally. The urethra was exposed and a double lumen catheter was inserted through an urethrotomy into the bladder and secured by a ligature around the urethra. One lumen of the catheter was connected to a pressure transducer to monitor the pressure within the bladder and the other was connected to a pump to infuse (1-2 ml/min) the bladder with saline. The tibial nerve was isolated on the medial side of the left hindlimb above the ankle and the pudendal nerve was isolated in the region of right sciatic notch. Tripolar cuff electrodes (NC223pt, MicroProbe Inc., Gaithersburg, MD) were implanted around these nerves for stimulation.

The spinal cord and cauda equina were exposed between the L6 and Cx1 vertebrae via a dorsal laminectomy in a subset of cats (N = 4). The spinal dura was cut and the dorsal and ventral roots were separated. Each sacral (S1-S3) dorsal root was encircled individually with a suture so that the root could be identified for a complete transection at the end of the experiment. The spinal cord was then covered with saline-moistened cotton and the skin was closed by suture. The skin suture and cotton were removed when accessing the sacral dorsal roots for transection at the end of the experiment, and then re-applied after the transection.

Decerebration was then performed in all cats (N = 12) through a craniotomy by surgically removing brain tissue rostral to the superior colliculi. The animal’s head was fixed by a stereotaxic frame (1430, David Kopf Instruments, Tujunga, CA). Warm liquid agar gel was used to fill the space of the removed brain tissue and to close the craniotomy once the agar gel solidified. Subsequently a small portion of the agar gel was removed to expose the pons for
inserting a microelectrode to stimulate the PMC during the experiment. The exposed pons was covered with warm (36-38 °C) mineral oil. The temperature of the animal was maintained at 36-38 °C using a heating pad during the experiments. The data collection started after a waiting period of about 60 minutes in order to pass the acute neuronal shock stage caused by decerebration.

**Experimental Protocol**

At the beginning of the experiments, cystometrograms (CMGs) were performed in all cats by slowly infusing saline into the bladder to determine the bladder capacity defined as the minimal volume required to induce a micturition contraction of large amplitude (>30 cmH₂O) and long duration (>20 s). Multiple CMGs were performed to ensure reproducibility of the saline control capacity. Then, the saline volume in the bladder was maintained at 60-70% of the bladder capacity throughout the entire experiment in order to avoid reflex bladder contractions. PNS/TNS at 5 Hz frequency and 0.2 ms pulse width was used in the experiments to inhibit bladder contractions based on our previous studies (16, 23, 27, 28). Intensity threshold (T) for PNS or TNS to induce anal or toe twitch, respectively, was determined, and then 2T and 4T intensities were used throughout the experiments.

A tungsten concentric bipolar microelectrode (WE5CEA5, Micro Probe, Gaithersburg, MD) driven by a stereotaxic apparatus was advanced into the pons on the left side to locate the PMC. Based on the stereotaxic coordinates of the cat PMC (P=2.5, L=2.0, H=-2.0) (18) the microelectrode tip was initially advanced to a stereotaxic location 1 mm above the PMC. Then, stimulation of 40-80 V, 40 Hz, 0.2 ms was applied for 10-25 seconds to determine if this location was effective in inducing a bladder contraction of >20 cmH₂O pressure. If it was not effective,
the microelectrode was further advanced in steps of 200 μm and the same stimulation was
applied after every step until an effective stimulation location was found or the electrode tip
passed the estimated ventral border of the PMC (total length of the electrode track = 2-3 mm). If
the first microelectrode penetration failed to find an effective location, the microelectrode was
withdrawn and re-inserted into the pons at a different location 0.5 mm rostral/caudal or
medial/lateral to the previous location. Then, the same searching protocol was performed again.
Once an effective stimulation location was identified, the microelectrode remained at this
location for the rest of the experiment.

At the effective location the stimulation intensity to induce a maximal bladder contraction
without any other motor response was first determined. At this intensity PMC stimulation of
different frequencies (10, 20, 30, 40, and 50 Hz) was tested to establish the frequency response
relationship (N = 11 cats). At the effective frequency (40 Hz) three different stimulation
intensities were used to induce bladder contractions about 30% (Min), 60% (Mid), and 100%
(Max) of the maximal contraction amplitude. Then, Min, Mid, and Max bladder contractions
were used as the control to examine the effect of PNS or TNS. For the purpose of comparison,
PMC stimulation (40 Hz, 0.2 ms) of a fixed duration (10-25 seconds) was used throughout the
same experiment to induce the 3 levels of bladder contractions. An interval of 2-3 minutes was
also used between stimulation applications for bladder to recover from the previous contraction.

To examine the effect of PNS (N = 12 cats), bladder contractions of three different
amplitudes (Min, Mid, and Max) were induced by PMC stimulation with a range of intensities
before PNS, during 2T PNS, during 4T PNS, and after PNS. The same test protocol was then
performed for TNS in the same animals (N = 10 cats) 2-3 minutes following PNS. TNS was
tested after PNS in order to avoid any potential post-stimulation effect of TNS (28). PNS/TNS
was applied continuously during the entire period of testing Min Mid, and Max bladder contractions. Following these tests, propranolol (a nonselective β-adrenergic receptor antagonist; Sigma Aldrich, St. Louis, MO) was administered intravenously (1 mg/kg) to a subset of cats (N = 5) to examine the influence of β-adrenergic receptors on the response to PMC stimulation and the contribution of these receptors to the modulatory effects of PNS and TNS. Ten minutes after propranolol treatment, the effects of PNS on PMC stimulation-induced bladder contractions were tested again, which was followed by testing the TNS effects. The effect of a large dose (1 mg/kg) propranolol (5, 8, 11,) can last much longer than the time (about 50-70 minutes) needed for testing PNS and TNS (25). In another subset of cats (N = 4), S1-S3 dorsal roots were transected bilaterally and the bladder responses induced by PMC stimulation were re-examined. The cats used for different subsets were selected randomly.

Data Analysis

The maximal amplitude and area under the curve were measured for each bladder contraction and were normalized to the average values of the maximal control contractions that were induced at least twice by PMC stimulation before each treatment (PNS, TNS, propranolol, or dorsal root transection). The data were averaged across animals under the same conditions and the results are presented as mean ± standard error. Statistical significance (p < 0.05) was determined by ANOVA analysis. The frequency response of PMC stimulation and the effects of PNS/TNS on different levels of bladder contractions were analyzed by one-way ANOVA followed by Dunnett’s post-tests. The effect of propranolol or dorsal root transection on bladder contractions was analyzed by two-way ANOVA followed by Bonferroni post-tests.
RESULTS

Maximal Bladder Contractions Induced by PMC Stimulation of Different Frequencies

At the maximal stimulus intensity (55.4±5.7 V, N=12 cats) that did not induce an observable motor response, PMC stimulation evoked bladder contractions ranging in amplitude from 20 to 100 cmH₂O presumably reflecting in part the different microelectrode locations relative to the PMC in different animals. The bladder response was dependent on stimulation frequency (Fig.1A). Stimulation at 40-50 Hz induced the maximal bladder contractions that was significantly (p < 0.05) larger in both amplitude and area under the curve than the contraction induced at 10 Hz (Fig.1B and Fig.1C, N=11 cats).

PNS and TNS Effect on Bladder Contractions Induced by PMC Stimulation

PNS or TNS of 5 Hz and 2-4T intensity did not induce any bladder contraction when they were applied continuously to modulate bladder contractions induced by PMC stimulation. The Min, Mid, and Max bladder contractions induced by PMC stimulation at three different intensities were suppressed during PNS (Fig.2, N=12 cats) but not during TNS (Fig.3, N=10 cats). No difference was observed when the data were separately analyzed between male and female cats. 2T and 4T PNS significantly (p < 0.05) reduced the maximal amplitude of the Min, Mid, and Max bladder contractions (Fig.2B) and the area under the curve of the Mid and Max bladder contractions (Fig.2C). The amplitude of the Max bladder contraction was significantly (p < 0.01) reduced by 26.8%±7.7% or 42.7%±7.5% during 2T or 4T PNS, respectively (Fig.2B); while the area under the curve of the Max bladder contraction was significantly reduced by 34.4%±7.0% (p < 0.05) or 47.9%±8.2% (p < 0.01) respectively (Fig.2C).
On the other hand, neither 2T nor 4T TNS suppressed the bladder contractions induced by PMC stimulation (Fig.3); but 4T TNS significantly (p < 0.01) increased the maximal amplitude of the Mid bladder contraction (Fig.3B) as well as the area under the curve of the Mid and Max bladder contractions (Fig.3C).

PNS and TNS Effect after Propranolol Treatment

Propranolol (1 mg/kg, i.v.) slightly increased the amplitude and the area under the curve of the bladder contractions induced by PMC stimulation (Fig.4A). On average these increases were about 20% but not statistically significant (p>0.05) (Fig.4B and Fig.4C, N=5 cats). After the propranolol treatment, PNS did not suppress the bladder contractions induced by PMC stimulation (Fig.5), and 4T TNS did not increase the maximal amplitude and the area under the curve of the bladder contractions (Fig.6). During 2T/4T PNS, the Max or Mid bladder contractions before propranolol treatment (i.e. the amplitude and AUC in Fig.2 B-C) were significantly (P<0.05) smaller than the contractions after propranolol treatment (i.e. the amplitude and AUC in Fig.5 B-C), while the Min bladder contractions did not show significant differences probably due to the small contraction amplitudes.

Effect of Dorsal Root Transection on Bladder Contractions Induced by PMC Stimulation

Bilateral transection of the S1-S3 dorsal roots did not suppress the bladder contractions induced by PMC stimulation (Fig.7A). On the contrary, the maximal amplitude and the area
under the curve of the bladder contractions were enhanced slightly although they were not statistically significant (Fig.7B and Fig.7C, N=4 cats).

DISCUSSION

This study in decerebrate cats revealed that PNS but not TNS can inhibit bladder contractions induced by electrical stimulation of the PMC (Figs.2-3). The evoked bladder contractions are not sensitive to bilateral transection of S1-S3 dorsal roots (Fig.7) indicating that they are mediated by an excitatory pathway from the brain stem to the spinal cord and are not dependent on activation of the micturition reflex by bladder afferent activity entering the sacral spinal cord. Propranolol, a nonselective β-adrenergic receptor antagonist, eliminated the PNS inhibition (Fig.5) without significantly changing the bladder contractions induced by PMC stimulation (Fig.4). These data are consistent with the results of previous studies (18, 27, 32) indicating that PNS and TNS modulate reflex bladder activity via different mechanisms.

In this study the afferent pathway of the spinobulbospinal micturition reflex was intact in the majority of the experiments; and the sacral S1-S3 dorsal roots were only cut at the end of a subset experiments. Therefore, the bladder contractions induced by PMC stimulation could activate the bladder afferents that might enhance the PMC stimulation-induced bladder contractions. However, the contribution of bladder afferent activity to PMC stimulation-induced contractions was minimized by maintaining the bladder at only 60-70 % of the bladder volume required to trigger a micturition contraction. The efficacy of this strategy in eliminating bladder reflexes during PMC stimulation is evidenced by: (1) the PMC stimulation-induced bladder contractions were not reduced by transecting of the S1-S3 dorsal roots bilaterally to eliminate
bladder afferent input (Fig.7); (2) the bladder contractions stopped at the end of PMC stimulation (Figs.1-7); (3) TNS failed to inhibit the bladder contractions (Fig.3) although it is known that TNS can inhibit micturition reflex contractions triggered by bladder afferent activity (27, 28). Therefore, it is reasonable to conclude that the PMC evoked bladder contractions are mediated primarily by electrical stimulation of the descending pathways from the brain stem to the sacral parasympathetic neurons (SPN) and not dependent on reflexes that occur secondarily in response to an initial bladder contraction (see Fig.8). Unfortunately, it was not possible to test PNS inhibition after completely eliminating the afferent limb of the micturition reflex pathway because transecting the sacral dorsal roots would also eliminate the PNS afferent input to the spinal cord.

Neuroanatomical tracing indicates that descending excitatory pathways from the PMC to sacral spinal cord make direct connections with the SPN innervating the bladder (Fig.8) (3). Thus, neuromodulation of the PMC-to-bladder contractions might be mediated by activation of glycineergic or GABAergic inhibitory synapses (22, 31) on the bladder preganglionic neurons (see the dashed lines in Fig.8). However, under the conditions of our experiments activation of these inhibitory synapses cannot be a major contributor to the PNS inhibition because the inhibition was completely removed by propranolol treatment (Fig.2 and Fig.5). Although a propranolol-sensitive, PNS inhibitory mechanism in the central nervous system cannot be entirely excluded, these data suggest that the inhibition occurs mainly via PNS induced reflex activation of sympathetic nerves and inhibition in the periphery at the level of the detrusor muscle (see Fig.8). This mechanism would be consistent with previous experiments showing that: (1) stimulation of pudendal nerve afferents can elicit reflex firing in the hypogastric nerve (17, 23) and (2) electrical stimulation or reflex activation of sympathetic axons in the hypogastric
nerve inhibits bladder contractions via activation of β-adrenergic receptors in the detrusor muscle (8, 11, 12), an effect blocked by propranolol.

On the other hand a recent study (14) in rats showed that bladder inhibition induced by mechanical stimulation of the perineum which activates pudendal nerve afferents is not mediated by sympathetic reflex activity but rather occurs by inhibitory mechanisms in the central nervous system (see Fig.8). The different mechanisms might be due to species differences (rat vs. cat) or due to activation of different afferent fibers in the pudendal nerve. Perineal stimulation activates cutaneous afferents in the sensory branch of the pudendal nerve, while 2-4T PNS in our study could activate various populations of pudendal afferent fibers innervating skin, anal and urethral sphincter muscles, the urethra and anal canal (see Fig.8). Previous electrophysiological studies in cats also showed that activation of pudendal afferent nerves directly inhibits bladder SPN firing as well as activity in interneuronal pathways projecting to the bladder SPN (6, 7, 10). These results raise the possibility that different subpopulations of pudendal afferent axons can induce bladder neuromodulation by distinct central and peripheral mechanisms (Fig.8).

It is noteworthy that TNS which inhibits reflex bladder activity (27, 28) did not inhibit the PMC-induced bladder contractions (Fig.3). Thus it is likely that TNS produces inhibition by acting on the ascending afferent limb of the micturition reflex pathway or in the brain but not on the descending limb (Fig.8). However the present experiments also revealed a facilitatory effect of TNS that weakly enhanced the bladder contractions induced by PMC stimulation (Fig.3). This effect was eliminated after administration of propranolol which also produced a small but not statistically significant increase in the contractions evoked by PMC stimulation (Fig.4). These results raise the possibility that TNS reduces tonic sympathetic inhibition of the bladder and that this effect is occluded by propranolol treatment. Alternatively TNS might act in the central
nervous system to facilitate the descending limb of the micturition reflex pathway. However, these results about TNS should be interpreted carefully. TNS is a FDA-approved treatment for OAB. Our previous studies in cats also showed that TNS could inhibit the distention-induced bladder reflex and produce a long-lasting post-stimulation inhibition (27, 28). The results from current study imply that the TNS inhibitory effects on the ascending sensory limb of the spinobulbospinal micturition reflex pathway dominates the weaker facilitatory effects on the descending efferent limb of the pathway. Therefore, the net effect of TNS is inhibitory as shown clinically in the treatment of OAB.

This study only investigated PNS/TNS effects on bladder contractions while the effects on urethral functions were not investigated. It can be expected that 2T/4T PNS will certainly cause strong contractions of the external urethral sphincter and increase the urethral pressure, but the effects of TNS on the urethra are currently still unknown. Additional studies of PNS/TNS effects on urethral functions might provide useful information for understanding the mechanisms of lower urinary tract neuromodulation.

PERSPECTIVES AND SIGNIFICANCE

In conclusion, PNS but not TNS significantly inhibits bladder contractions induced by electrical stimulation of the PMC in decerebrate cats. The inhibition occurs via activation of a propranolol-sensitive β-adrenergic mechanism which is very likely produced in the detrusor muscle via reflex firing in lumbar sympathetic nerves (Fig.8). The experiments did not provide any evidence that PNS or TNS inhibits the descending limb of the micturition reflex pathway. These results provide new evidence in additional to previous reports (27, 18, 32) indicating that
different mechanisms are involved in the suppression of reflex bladder activity elicited by electrical stimulation of the pudendal nerve and the tibial nerve.

GRANTS

This study is supported by the National Institutes of Diabetes and Digestive and Kidney Diseases under Grants DK-094905, DK-090006, DK-102427, and DK-091253.

DISCLOSURES

The authors declare no actual or potential conflicts of interest.

REFERENCES


therapy for urinary voiding dysfunction: outcomes of a prospective, worldwide clinical study.


FIGURE CAPTIONS

Fig.1: Bladder pressure responses to different stimulation frequencies delivered by a microelectrode in the pontine micturition center (PMC). A: Bladder pressure tracings. The black bar under the pressure tracing indicates the duration of PMC stimulation. B: Normalized pressure amplitude. C: Normalized area under the curve (AUC). Bladder pressure response is normalized to the mean of the two responses induced at 50 Hz (Note: only one is shown in A). * indicates significantly (p<0.05) different from 10 Hz response (one-way ANOVA). PMC stimulation: intensity 57.7±5.7 V, pulse width 0.2 ms. N = 11 cats.

Fig.2: Pudendal nerve stimulation (PNS) suppressed the bladder pressure response induced by stimulation of pontine micturition center (PMC). A: Three levels (Min, Mid, and Max) of bladder pressure responses induced by PMC stimulation at three different intensities before, during, and after PNS. The black bar under the pressure tracing indicates the duration of PMC
stimulation. B: Normalized pressure amplitude. C: Normalized area under the curve (AUC).

Bladder pressure response is normalized to the mean of the two Max bladder pressure responses induced before PNS (Note: only one of the Max bladder pressure responses is shown in A). * indicates significantly (p < 0.05) different from the pressure responses induced before PNS (one-way ANOVA). PNS: frequency 5 Hz, pulse width 0.2 ms, T = 1.2 ± 0.31 V. T is the intensity threshold for PNS to induce anal twitch. PMC Stimulation: frequency 40 Hz, pulse width 0.2 ms, intensity (Min: 32.2 ± 3.8 V, Mid: 41.7 ± 4.9 V, Max: 55.4 ± 5.7 V). N = 12 cats.

Fig.3: Tibial nerve stimulation (TNS) facilitated the bladder pressure response induced by stimulation of pontine micturition center (PMC). A: Three levels (Min, Mid, and Max) of bladder pressure responses induced by PMC stimulation at three different intensities before, during, and after TNS. The black bar under the pressure tracing indicates the duration of PMC stimulation. B: Normalized pressure amplitude. C: Normalized area under the curve (AUC). Bladder pressure response is normalized to the mean of the two Max bladder pressure responses induced before TNS (Note: only one of the Max bladder pressure responses is shown in A). * indicates significantly (p < 0.05) different from the pressure responses induced before TNS (one-way ANOVA). TNS: frequency 5 Hz, pulse width 0.2 ms, T = 1.8 ± 0.17 V. T is the intensity threshold for TNS to induce toe twitch. PMC Stimulation: frequency 40 Hz, pulse width 0.2 ms, intensity (Min: 34.1 ± 4.4 V, Mid: 44.5 ± 5.4 V, Max: 60.0 ± 5.8 V). N = 10 cats.

Fig.4: Effect of propranolol (1 mg/kg i.v.) on bladder pressure responses induced by stimulation of pontine micturition center (PMC). A: Three levels (Min, Mid, and Max) of bladder pressure responses induced by PMC stimulation at three different intensities before and after propranolol treatment. The black bar under the pressure tracing indicates the duration of PMC stimulation. B: Normalized pressure amplitude. C: Normalized area under the curve (AUC). Bladder pressure
response is normalized to the mean of the two Max control bladder pressure responses (Note: only one of the Max controls is shown in A). PMC Stimulation: Intensity (Min: 25.8 ± 6.7 V, Mid: 33.0 ± 6.6 V, Max: 47.0 ± 6.6 V), frequency 40 Hz, pulse width 0.2 ms. N = 5 cats. No significant change was detected by two-way ANOVA followed by Bonferroni post-tests between control and propranolol data.

Fig.5: Propranolol treatment removed the inhibitory effect of pudendal nerve stimulation (PNS) on the bladder pressure response induced by stimulation of pontine micturition center (PMC). A: Three levels (Min, Mid, and Max) of bladder pressure responses induced by PMC stimulation at three different intensities before, during, and after PNS. The black bar under the pressure tracing indicates the duration of PMC stimulation. B: Normalized pressure amplitude. C: Normalized area under the curve (AUC). Bladder pressure response is normalized to the mean of the two Max bladder pressure responses induced before PNS (Note: only one of the Max bladder pressure responses is shown in A). PNS: frequency 5 Hz, pulse width 0.2 ms, T = 1.2 ± 0.25 V. T is the intensity threshold for PNS to induce anal twitch. PMC Stimulation: frequency 40 Hz, pulse width 0.2 ms, intensity (Min: 25.8 ± 6.7 V, Mid: 33.0 ± 6.6 V, Max: 47.0 ± 6.6 V). N = 5 cats.

Fig.6: Propranolol treatment removed the facilitatory effect of tibial nerve stimulation (TNS) on the bladder pressure response induced by stimulation of pontine micturition center (PMC). A: Three levels (Min, Mid, and Max) of bladder pressure responses induced by PMC stimulation at three different intensities before, during, and after TNS. The black bar under the pressure tracing indicates the duration of PMC stimulation. B: Normalized pressure amplitude. C: Normalized area under the curve (AUC). Bladder pressure response is normalized to the mean of the two Max bladder pressure responses induced before PNS (Note: only one of the Max bladder
pressure responses is shown in A). TNS: frequency 5 Hz, pulse width 0.2 ms, \( T = 1.3 \pm 0.21 \) V. T is the intensity threshold for TNS to induce toe twitch. PMC Stimulation: frequency 40 Hz, pulse width 0.2 ms, intensity (Min: 25.8 ± 6.7 V, Mid: 33.0 ± 6.6 V, Max: 47.0 ± 6.6 V). \( N = 5 \) cats.

Fig.7: Effect of bilateral transection of S1-S3 dorsal roots on bladder pressure responses induced by stimulation of pontine micturition center (PMC). A: Three levels (Min, Mid, and Max) of bladder pressure responses induced by PMC stimulation at three different intensities before and after dorsal root transection. The black bar under the pressure tracing indicates the duration of PMC stimulation. B: Normalized pressure amplitude. C: Normalized area under the curve (AUC). Bladder pressure response is normalized to the mean of the two Max control bladder pressure responses (Note: only one of the Max controls is shown in A). PMC Stimulation: Intensity (Min: 41.3 ± 7.2 V, Mid: 57.5 ± 8.5 V, Max: 75.0 ± 9.6 V), frequency 40 Hz, pulse width 0.2 ms. \( N = 4 \) cats. No significant change was detected by two-way ANOVA followed by Bonferroni post-tests between control and root transection data.

Fig.8: Proposed mechanism for pudendal nerve stimulation (PNS) inhibition of the bladder contractions induced by stimulation (arrowhead) of pontine micturition center (PMC). Propranolol completely removed PNS inhibition indicating that PNS reflexly activated lumbar sympathetic pathways to inhibit bladder contractions via a \( \beta \)-adrenergic mechanism in the detrusor. Meanwhile, central inhibitory pathways with direct synaptic connections (represented by the dashed lines) to the sacral parasympathetic neurons (SPN) innervating the bladder do not play a major role in TNS or PNS inhibition under the conditions of our experiments because: (1) tibial nerve stimulation did not inhibit PMC stimulation-induced bladder contraction; (2) PNS inhibition was completely removed by propranolol treatment. The ascending and descending
limbs of the spinobulbospinal micturition reflex relayed through the PMC and activated by Aδ-fiber bladder afferents are shown on the right side of the figure.
Fig. 1: Bladder pressure responses to different stimulation frequencies delivered by a microelectrode in the pontine micturition center (PMC). A: Bladder pressure tracings. The black bar under the pressure tracing indicates the duration of PMC stimulation. B: Normalized pressure amplitude. C: Normalized area under the curve (AUC). Bladder pressure response is normalized to the mean of the two responses induced at 50 Hz (Note: only one is shown in A). * indicates significantly (p<0.05) different from 10 Hz response (one-way ANOVA). PMC stimulation: intensity 57.7±5.7 V, pulse width 0.2 ms. N = 11 cats.
Fig. 2: Pudendal nerve stimulation (PNS) suppressed the bladder pressure response induced by stimulation of pontine micturition center (PMC). A: Three levels (Min, Mid, and Max) of bladder pressure responses induced by PMC stimulation at three different intensities before, during, and after PNS. The black bar under the pressure tracing indicates the duration of PMC stimulation. B: Normalized pressure amplitude. C: Normalized area under the curve (AUC). Bladder pressure response is normalized to the mean of the two Max bladder pressure responses induced before PNS (Note: only one of the Max bladder pressure responses is shown in A). * indicates significantly (p < 0.05) different from the pressure responses induced before PNS (one-way ANOVA). PNS: frequency 5 Hz, pulse width 0.2 ms, T = 1.2 ± 0.31 V. T is the intensity threshold for PNS to induce anal twitch. PMC Stimulation: frequency 40 Hz, pulse width 0.2 ms, intensity (Min: 32.2 ± 3.8 V, Mid: 41.7 ± 4.9 V, Max: 55.4 ± 5.7 V). N = 12 cats.
Fig. 3: Tibial nerve stimulation (TNS) facilitated the bladder pressure response induced by stimulation of pontine micturition center (PMC). A: Three levels (Min, Mid, and Max) of bladder pressure responses induced by PMC stimulation at three different intensities before, during, and after TNS. The black bar under the pressure tracing indicates the duration of PMC stimulation. B: Normalized pressure amplitude. C: Normalized area under the curve (AUC). Bladder pressure response is normalized to the mean of the two Max bladder pressure responses induced before TNS (Note: only one of the Max bladder pressure responses is shown in A). * indicates significantly (p < 0.05) different from the pressure responses induced before TNS (one-way ANOVA). TNS: frequency 5 Hz, pulse width 0.2 ms, $T = 1.8 \pm 0.17$ V. $T$ is the intensity threshold for TNS to induce toe twitch. PMC Stimulation: frequency 40 Hz, pulse width 0.2 ms, intensity (Min: 34.1 ± 4.4 V, Mid: 44.5 ± 5.4 V, Max: 60.0 ± 5.8 V). N = 10 cats.
Fig. 4: Effect of propranolol (1 mg/kg i.v.) on bladder pressure responses induced by stimulation of pontine micturition center (PMC). A: Three levels (Min, Mid, and Max) of bladder pressure responses induced by PMC stimulation at three different intensities before and after propranolol treatment. The black bar under the pressure tracing indicates the duration of PMC stimulation. B: Normalized pressure amplitude. C: Normalized area under the curve (AUC). Bladder pressure response is normalized to the mean of the two Max control bladder pressure responses (Note: only one of the Max controls is shown in A). PMC Stimulation: Intensity (Min: 25.8 ± 6.7 V, Mid: 33.0 ± 6.6 V, Max: 47.0 ± 6.6 V), frequency 40 Hz, pulse width 0.2 ms. N = 5 cats. No significant change was detected by two-way ANOVA followed by Bonferroni post-tests between control and propranolol data.
Fig. 5: Propranolol treatment removed the inhibitory effect of pudendal nerve stimulation (PNS) on the bladder pressure response induced by stimulation of pontine micturition center (PMC). A: Three levels (Min, Mid, and Max) of bladder pressure responses induced by PMC stimulation at three different intensities before, during, and after PNS. The black bar under the pressure tracing indicates the duration of PMC stimulation. B: Normalized pressure amplitude. C: Normalized area under the curve (AUC). Bladder pressure response is normalized to the mean of the two Max bladder pressure responses induced before PNS (Note: only one of the Max bladder pressure responses is shown in A). PNS: frequency 5 Hz, pulse width 0.2 ms, T = 1.2 ± 0.25 V. T is the intensity threshold for PNS to induce an anal twitch. PMC Stimulation: frequency 40 Hz, pulse width 0.2 ms, intensity (Min: 25.8 ± 6.7 V, Mid: 33.0 ± 6.6 V, Max: 47.0 ± 6.6 V). N = 5 cats.
Fig. 6: Propranolol treatment removed the facilitatory effect of tibial nerve stimulation (TNS) on the bladder pressure response induced by stimulation of pontine micturition center (PMC). A: Three levels (Min, Mid, and Max) of bladder pressure responses induced by PMC stimulation at three different intensities before, during, and after TNS. The black bar under the pressure tracing indicates the duration of PMC stimulation. B: Normalized pressure amplitude. C: Normalized area under the curve (AUC). Bladder pressure response is normalized to the mean of the two Max bladder pressure responses induced before PNS (Note: only one of the Max bladder pressure responses is shown in A). TNS: frequency 5 Hz, pulse width 0.2 ms, T = 1.3 ± 0.21 V. T is the intensity threshold for TNS to induce toe twitch. PMC Stimulation: frequency 40 Hz, pulse width 0.2 ms, intensity (Min: 25.8 ± 6.7 V, Mid: 33.0 ± 6.6 V, Max: 47.0 ± 6.6 V). N = 5 cats.
Fig. 7: Effect of bilateral transection of S1-S3 dorsal roots on bladder pressure responses induced by stimulation of pontine micturition center (PMC). A: Three levels (Min, Mid, and Max) of bladder pressure responses induced by PMC stimulation at three different intensities before and after dorsal root transection. The black bar under the pressure tracing indicates the duration of PMC stimulation. B: Normalized pressure amplitude. C: Normalized area under the curve (AUC). Bladder pressure response is normalized to the mean of the two Max control bladder pressure responses (Note: only one of the Max controls is shown in A). PMC Stimulation: Intensity (Min: 41.3 ± 7.2 V, Mid: 57.5 ± 8.5 V, Max: 75.0 ± 9.6 V), frequency 40 Hz, pulse width 0.2 ms. N = 4 cats. No significant change was detected by two-way ANOVA followed by Bonferroni post-tests between control and root transection data.
Fig. 8: Proposed mechanism for pudendal nerve stimulation (PNS) inhibition of the bladder contractions induced by stimulation (arrowhead) of pontine micturition center (PMC). Propranolol completely removed PNS inhibition indicating that PNS reflexly activated lumbar sympathetic pathways to inhibit bladder contractions via a $\beta$-adrenergic mechanism in the detrusor. Meanwhile, central inhibitory pathways with direct synaptic connections (represented by the dashed lines) to the sacral parasympathetic neurons (SPN) innervating the bladder do not play a major role in TNS or PNS inhibition under the conditions of our experiments because: (1) tibial nerve stimulation did not inhibit PMC stimulation-induced bladder contraction; (2) PNS inhibition was completely removed by propranolol treatment. The ascending and descending limbs of the spinobulbospinal micturition reflex relayed through the PMC and activated by $A\delta$-fiber bladder afferents are shown on the right side of the figure.