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# Contribution of $GABA_A$, Glycine, and Opioid Receptors to Sacral Neuromodulation of Bladder Overactivity in Cats

Xuewen Jiang, Thomas W. Fuller, Jathin Bandari, Utsav Bansal, Zhaocun Zhang, Bing Shen, Jicheng Wang, James R. Roppolo, William C. de Groat, and Changfeng Tai

*Department of Urology, Qilu Hospital, Shandong University, Jinan, P.R. China (X.J., Z.Z.); Department of Urology, University of Pittsburgh, Pittsburgh, Pennsylvania (X.J., T.W.F., J.B., U.B., Z.Z., B.S., J.W., C.T.); and Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, Pennsylvania (J.R.R., W.C.d.G., C.T.)*

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## ABSTRACT

In $\alpha$-chloralose–anesthetized cats, we examined the role of $GABA_A$, glycine, and opioid receptors in sacral neuromodulation-induced inhibition of bladder overactivity elicited by intravesical infusion of 0.5% acetic acid (AA). AA irritation significantly ($P < 0.01$) reduced bladder capacity to 59.5 ± 4.8% of saline control. S1 or S2 dorsal root stimulation at threshold intensity for inducing reflex twitching of the anal sphincter or toe significantly ($P < 0.01$) increased bladder capacity to 105.3 ± 9.0% and 134.8 ± 8.9% of saline control, respectively. Picrotoxin, a $GABA_A$ receptor antagonist administered i.v., blocked S1 inhibition at 0.3 mg/kg and blocked S2 inhibition at 1.0 mg/kg. Picrotoxin (0.4 mg, i.t.) did not alter the inhibition induced during S1 or S2 stimulation, but unmasked a significant ($P < 0.05$) poststimulation inhibition that persisted after termination of stimulation. Naloxone, an opioid receptor antagonist (0.3 mg, i.t.), significantly ($P < 0.05$) reduced prestimulation bladder capacity and removed the poststimulation inhibition. Strychnine, a glycine receptor antagonist (0.03–0.3 mg/kg, i.v.), significantly ($P < 0.05$) increased prestimulation bladder capacity but did not reduce sacral S1 or S2 inhibition. After strychnine (0.3 mg/kg, i.v.), picrotoxin (0.3 mg/kg, i.v.) further ($P < 0.05$) increased prestimulation bladder capacity and completely blocked both S1 and S2 inhibition. These results indicate that supraspinal $GABA_A$ receptors play an important role in sacral neuromodulation of bladder overactivity, whereas glycine receptors only play a minor role to facilitate the $GABA_A$ inhibitory mechanism. The poststimulation inhibition unmasked by blocking spinal $GABA_A$ receptors was mediated by an opioid mechanism.

## Introduction

Overactive bladder (OAB) symptoms are characterized by urinary urgency, frequency, and nocturia with or without incontinence (Abrams et al., 2002). OAB affects more than 30 million adults in United States (Coyne et al., 2011). Currently, antimuscarinic drugs are the first-line pharmacotherapy for OAB, but have a limited efficacy with significant adverse effect (Andersson and Pehrson, 2003; Andersson and Wein, 2004; Chapple et al., 2008). If pharmacotherapy fails, sacral neuromodulation is one of the alternative treatment options for OAB. Although this therapy has been approved by the Food and Drug Administration to treat OAB for more than a decade (Schmidt et al., 1999; van Kerrebroeck et al., 2007), its mechanism of action is still uncertain (Elkelini et al., 2010). The initial event in sacral neuromodulation is the activation of primary afferent nerves that project into the spinal cord and trigger the release of neurotransmitters that in turn modulate the neural pathways controlling bladder function. Unfortunately, little is known about the identity of the neurotransmitters, the receptors that they activate, or their site of action. The present experiments were undertaken to address these issues.

Our previous studies in cat revealed that spinal $GABA_A$ receptors play an important role in pudendal neuromodulation of bladder overactivity (Xiao et al., 2014), whereas opioid and glycine receptors have no or a minor role (Mally et al., 2013; Rogers et al., 2016). In contrast, we showed that opioid receptors have an essential role in tibial neuromodulation of bladder overactivity in the cat (Tai et al., 2012). Because afferent axons passing through the pudendal and tibial nerves enter the spinal cord through the sacral S1–S2 dorsal roots, it is possible that sacral neuromodulation activates these afferents in S1–S2 dorsal roots and might mimic some or all of the effects of pudendal/tibial neuromodulation. Therefore, in this study, we examined the effects of a $GABA_A$ receptor antagonist (picrotoxin), a glycine receptor antagonist (strychnine), and an opioid receptor antagonist (naloxone) on the modulation of bladder overactivity elicited by electrical stimulation of the S1 or S2 sacral dorsal roots.

## Materials and Methods

The protocol and animal use in this study were approved by Animal Care and Use Committee at the University of Pittsburgh.

**Surgical Procedures.** A total of 20 cats (9 males and 11 females, 2.7–5.0 kg; Liberty Research, Waverly, NY) was used in this study. The

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**ABBREVIATIONS:** AA, acetic acid; CMG, cystometrogram; OAB, overactive bladder.

animals were anesthetized with isoflurane (2–5% in oxygen) during surgery and then switched to $\alpha$-chloralose anesthesia (initial 65 mg/kg followed by slow i.v. infusion at 2 mg/kg per hour) during data collection. Pancuronium (initial 0.1 mg/kg followed by slow i.v. infusion at 0.1 mg/kg per hour) was also given during data collection to prevent striated muscle contractions and movement of the animal. Right and left cephalic veins were catheterized for i.v. administration of drugs and fluid. A tracheotomy was performed, and a tube was inserted to keep the airway patent. A catheter was inserted into right carotid artery to monitor systemic arterial blood pressure. The mean systemic blood pressure was measured as the average of systolic and diastolic blood pressures. Heart rate and blood oxygen were monitored by a pulse oximeter (9847V; NONIN Medical, Plymouth, MN) attached to the tongue. Through an abdominal incision, the ureters were isolated, tied, and cut for external drainage. A double lumen catheter was inserted through the urethra into the bladder and secured by a ligature around the urethra. One lumen was connected to a pump to slowly (1–3 ml/min) infuse saline or 0.5% acetic acid (AA) in saline. The other lumen was attached to a pressure transducer to measure bladder pressure. After the surgery, the skin and muscle layers were closed by sutures.

The spinal cord and cauda equina were exposed between the L7 and Cx1 vertebrae via a dorsal laminectomy. The spinal dura was cut, and the S1 and S2 dorsal roots on the right side were separated for electrical stimulation. A bipolar stainless steel hook electrode was used during the experiment to stimulate individual S1/S2 dorsal roots by delivering electrical pulses that were generated by an electrical stimulator (S88; Grass Medical Instruments, Quincy, MA). The animal was mounted in a modified Narishige Eccles spinal cord frame in which the hip was supported by metal pins, and the spinous process at the rostral end of the laminectomy was secured with a clamp. The skin, cut midsagittally from L4 to S3, was tied along each margin to form a pool that was filled with warmed (35–37°C) mineral oil. The temperature of the animal was maintained at 36–38°C using a heating pad during the experiments. In five cats, a small catheter (PE10) was inserted rostrally underneath the dura to position the catheter tip between S1 and S2 spinal cord for i.t. administration of picrotoxin or naloxone. The location of the intrathecal catheter was confirmed by a postmortem laminectomy between the L5-L6 spinal processes.

**Stimulation Protocol and Drug Administration.** Our previous study in cats (Zhang et al., 2013) showed that reflex bladder activity could be inhibited by electrical stimulation (5 Hz frequency and 0.2 millisecond pulse width) of S1 or S2 dorsal roots at threshold intensity for inducing reflex twitching of the anal sphincter or toe, whereas stimulation of S3 dorsal root or S1–S3 ventral roots was not effective. Therefore, stimulation (5 Hz, 0.2 millisecond) of S1 or S2 dorsal roots at motor threshold intensity was used in this study to inhibit reflex bladder activity. The motor threshold was determined before administering pancuronium.

At the beginning of each experiment, multiple cystometrograms (CMGs) were performed by slowly infusing the bladder with saline to determine the bladder capacity that was defined as the bladder volume threshold to induce a bladder contraction of large amplitude (>30 cm$H_2O$) and long duration (>20 seconds). Then 0.5% AA was infused into the bladder to irritate the bladder and induce bladder overactivity. Once the control bladder capacity stabilized during repeated AA CMGs, the inhibitory effect of sacral dorsal root stimulation was determined by additional four AA CMGs: 1) control CMG without stimulation; 2) CMG during S1 dorsal root stimulation; 3) CMG during S2 dorsal root stimulation; and 4) control CMG again to examine any poststimulation effect. Then the animals were divided into three experimental groups.

In the first group ($N$ = 9 cats), cumulative doses (0.01, 0.03, 0.1, 0.3, and 1.0 mg/kg, i.v.) of picrotoxin (Sigma-Aldrich, St. Louis, MO) were given. In the second group ($N$ = 6 cats), strychnine (Sigma-Aldrich) was administered in cumulative doses (0.001, 0.003, 0.01, 0.03, 0.1, and 0.3 mg/kg, i.v.) followed by picrotoxin (0.3 mg, i.v.). In the third group ($N$ = 5 cats), a single dose (0.4 mg in 0.2 mL saline, i.t.) of picrotoxin was given, which was followed by a single dose (0.3 mg in 0.1 mL saline, i.t.) of naloxone. The dosage of each drug is chosen based on our previous studies (Hisamitsu and de Groat, 1984; Xiao et al., 2014; Rogers et al., 2016). After administering each dose of drug, the four CMGs (control, S1 stimulation, S2 stimulation, control) were repeated to determine the drug effects. A 10-minute waiting period for each i.v. dose of picrotoxin or strychnine and a 5-minute period for i.t. picrotoxin or naloxone were used to allow time for the drugs to take effect. A waiting period of 2–3 minutes was also used between CMGs to allow the bladder reflex to recover. Our previous studies (Xiao et al., 2014; Rogers et al., 2016) showed that the effects of picrotoxin or strychnine lasted long enough to perform the four repeated CMGs that required about 30–40 minutes.

**Data Analysis.** The bladder capacity was measured from each CMG and normalized to the capacity measured during the first control CMG in different test groups. Repeated measurements in the same animal under the same conditions were averaged. The normalized data from different animals were presented as mean ± S.E. Statistical significance ($P < 0.05$) was determined by a paired Student $t$ test or analysis of variance followed by Bonferroni multiple comparisons.

## Results

**Inhibition of Bladder Overactivity by S1 or S2 Dorsal Root Stimulation.** AA irritation induced bladder overactivity and significantly ($P < 0.01$) reduced bladder capacity to 59.5 ± 4.8% of saline control capacity ($N$ = 20 cats; Fig. 1). S1 or S2 dorsal root stimulation at threshold intensity for

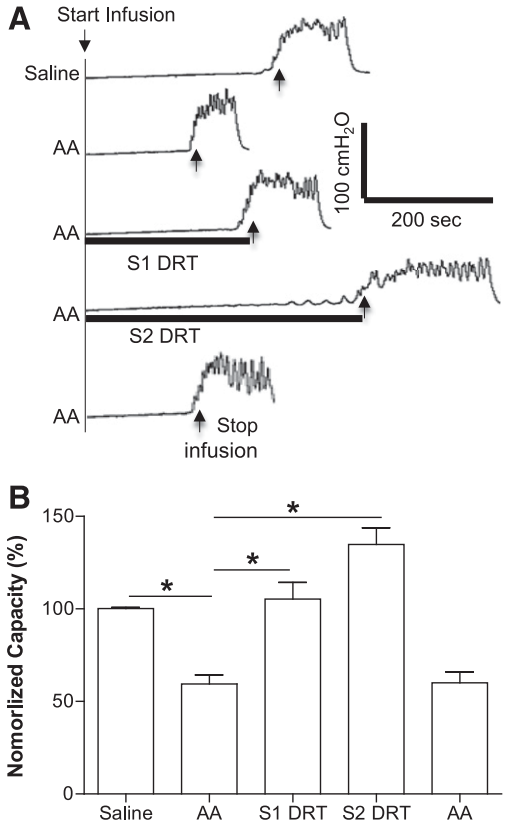


**Fig. 1.** Inhibition of bladder overactivity by S1 or S2 dorsal root (DRT) stimulation at motor threshold (T) intensity. (A) Repeated CMGs during saline or 0.5% AA infusion with or without DRT stimulation. Black bars under the bladder pressure traces indicate stimulation duration. Stimulation: 5 Hz, 0.2 millisecond, T = 0.3 V for S1, T = 0.18 V for S2. Infusion rate = 3 ml/min. (B) Normalized bladder capacity measured under different conditions ($N$ = 20 cats). *Indicates significantly ($P < 0.01$) different from the AA control (one-way analysis of variance).

inducing reflex twitching of anal sphincter or toe inhibited bladder overactivity and significantly ($P < 0.01$) increased bladder capacity to 105.3 ± 9.0% and 134.8 ± 8.9% of saline control, respectively. After the stimulation, AA control capacity returned to prestimulation level, indicating that there was no poststimulation effect (Fig. 1B).

**Effect of i.v. Picrotoxin on Sacral Inhibition of Bladder Overactivity.** Picrotoxin (i.v.) slightly increased the prestimulation bladder capacity at 0.3–1.0 mg/kg doses (first column CMGs in Fig. 2A), but the increase was not statistically significant ($P > 0.05$, $N = 9$ cats; Fig. 2B). The 0.3 mg/kg dose of picrotoxin blocked ($P < 0.05$) the increase in bladder capacity elicited by S1 dorsal root stimulation, but not the increase elicited by S2 dorsal root stimulation (Fig. 2B). Picrotoxin at 1 mg/kg blocked ($P < 0.05$) the increase in bladder capacity induced by either S1 or S2 dorsal root stimulation (Fig. 2B). After the stimulation, the bladder capacity returned to prestimulation level at every dose of picrotoxin, that is, no poststimulation effect. Mean systemic blood pressure (control: 163.9 ± 4.6 mmHg) was not changed after any dose of picrotoxin (162.0 ± 4.5 mmHg).

**Combined Effect of i.v. Strychnine and Picrotoxin on Sacral Inhibition of Bladder Overactivity.** Strychnine at 0.03–0.3 mg/kg (i.v.) significantly ($P < 0.05$) increased the prestimulation bladder capacity without affecting the increase in bladder capacity caused by S1 or S2 dorsal root stimulation ($N = 6$ cats; Fig. 3). Mean systemic blood pressure (control: 154.2 ± 1.8 mmHg) was not changed after any dose of strychnine (150.6 ± 3.8 mmHg). Following strychnine treatment, picrotoxin (0.3 mg/kg, i.v.) further significantly ($P < 0.05$) increased the prestimulation bladder capacity and blocked the inhibition induced by S1 or S2 dorsal root stimulation ($N = 6$ cats; Fig. 4), whereas the same dose of picrotoxin without strychnine pretreatment only blocked the inhibition induced by S1 but not S2 dorsal root stimulation (see Fig. 2B). There was no poststimulation effect at any dose of the drugs.

**Effect of i.t. Picrotoxin and Naloxone on Sacral Inhibition of Bladder Overactivity.** Picrotoxin (0.4 mg, i.t.) did not significantly change the prestimulation bladder

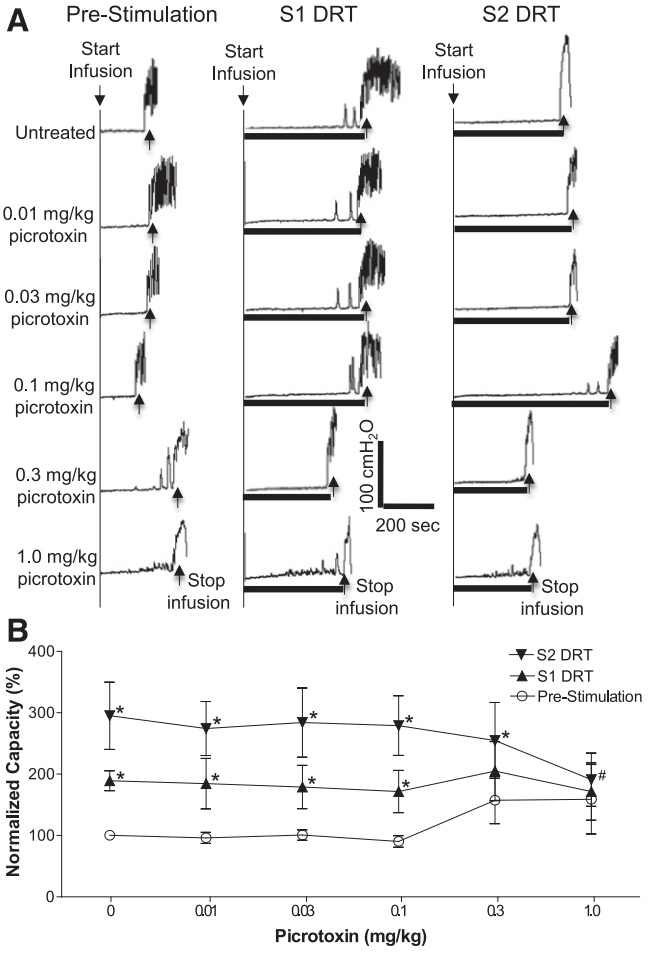


**Fig. 2.** Effect of picrotoxin (i.v.) on the inhibition of bladder overactivity by S1 or S2 dorsal root (DRT) stimulation at motor threshold (T) intensity. (A) Repeated CMGs at different cumulative doses of picrotoxin were performed during AA infusion with or without S1 or S2 DRT stimulation. Black bars under the pressure trace indicate stimulation duration. Stimulation: 5 Hz, 0.2 millisecond, T = 0.3 V for S1, T = 0.16 V for S2. Infusion rate = 1 ml/min. (B) Normalized bladder capacity measured under different conditions ($N = 9$ cats). *Indicates significantly ($P < 0.05$) different from the prestimulation group (two-way analysis of variance). #Indicates significantly ($P < 0.05$) different from the untreated condition in the S2 DRT group (one-way analysis of variance).

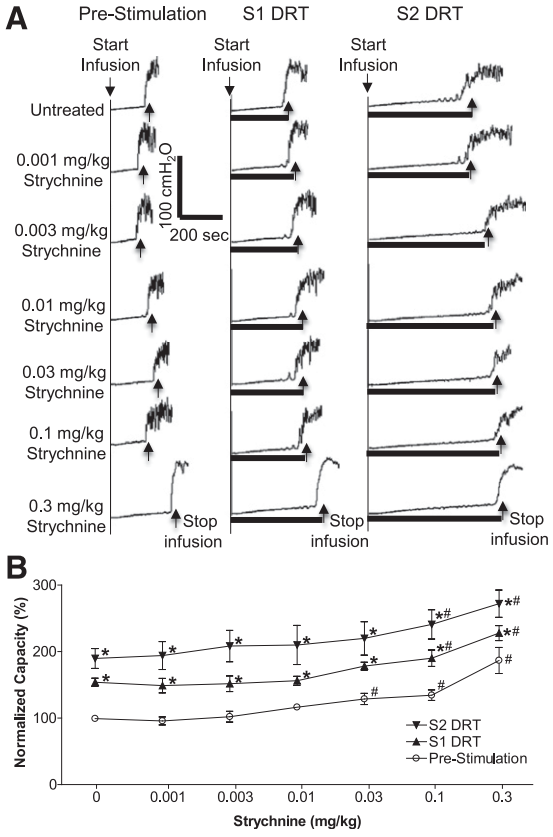


**Fig. 3.** Effect of strychnine (i.v.) on the inhibition of bladder overactivity by S1 or S2 dorsal root (DRT) stimulation at motor threshold (T) intensity. (A) Repeated CMGs at different cumulative doses of strychnine were performed during AA infusion with or without S1 or S2 DRT stimulation. Black bars under the pressure trace indicate stimulation duration. Stimulation: 5 Hz, 0.2 millisecond, T = 0.3 V for S1, T = 0.18 V for S2. Infusion rate = 3 ml/min. (B) Normalized bladder capacity measured under different conditions ($N = 6$ cats). *Indicates significantly ($P < 0.05$) different from the prestimulation group (two-way analysis of variance). #Indicates significantly ($P < 0.05$) different from the untreated condition in the same group (one-way analysis of variance).

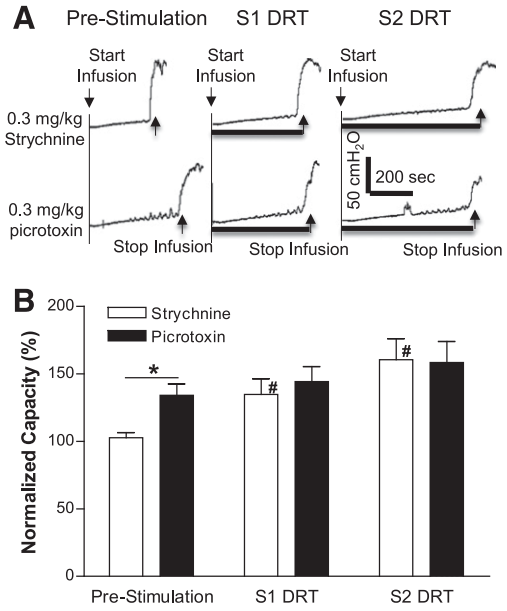


**Fig. 4.** Effect of picrotoxin (i.v.) on the inhibition of bladder overactivity by S1 or S2 dorsal root (DRT) stimulation at motor threshold intensity in strychnine (i.v.)-pretreated cats. (A) Repeated CMGs were performed during AA infusion with or without S1 or S2 DRT stimulation in a cat pretreated with strychnine (0.3 mg/kg, i.v.), followed by picrotoxin (0.3 mg/kg, i.v.). Note: the CMG traces for 0.3 mg/kg strychnine are from the same cat as shown in Fig. 3A. Black bars under the pressure trace indicate stimulation duration. Stimulation: 5 Hz, 0.2 millisecond, T = 0.3 V for S1, T = 0.18 V for S2. Infusion rate = 3 ml/min. (B) Normalized bladder capacity measured under different conditions ($N$ = 6 cats). *Indicates a significant ($P < 0.05$) difference between the prestimulation capacities before and after picrotoxin treatment ($t$ test). #Indicates significantly ($P < 0.05$) different from the prestimulation in strychnine-pretreated group (one-way analysis of variance).

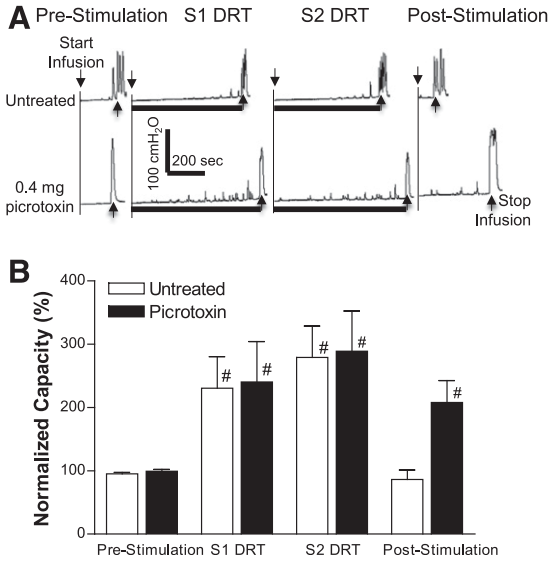


**Fig. 5.** Effect of picrotoxin (i.t.) on the inhibition of bladder overactivity by S1 or S2 dorsal root (DRT) stimulation at motor threshold (T) intensity. (A) Repeated CMGs were performed during AA infusion with or without S1 or S2 DRT stimulation. Black bars under the pressure trace indicate stimulation duration. Stimulation: 5 Hz, 0.2 millisecond, T = 0.4 V for S1, T = 0.18 V for S2. Infusion rate = 2 ml/min. (B) Normalized bladder capacity measured under different conditions. #Indicates significantly ($P < 0.05$) different from the prestimulation in the untreated or picrotoxin-treated group (one-way analysis of variance).

capacity and had no effect on the capacity increase induced by either S1 or S2 dorsal root stimulation ($N$ = 5 cats; Fig. 5). However, after sacral dorsal root stimulation, the poststimulation bladder capacity was significantly ($P < 0.05$) increased (about 100%), that is, the stimulation induced a significant poststimulation inhibitory effect (Fig. 5). Following picrotoxin treatment, naloxone (0.3 mg, i.t.) significantly ($P < 0.05$) reduced the prestimulation bladder capacity and removed the poststimulation inhibition induced by sacral dorsal root stimulation (Fig. 6). However, even after i.t. administration of both picrotoxin and naloxone, S1 or S2 dorsal root stimulation still significantly ($P < 0.05$) increased bladder capacity during stimulation (Fig. 6).

## Discussion

In this study, the effects of selective receptor antagonists administered alone or in combination revealed that GABA, glycine, and opioids contribute in varying ways to sacral neuromodulation of bladder overactivity in anesthetized cats. GABA acting on GABA$_A$ receptors at supraspinal sites plays a major role in sacral neuromodulation (Figs. 2 and 5), whereas glycine seems to have a minor role to facilitate the GABAergic inhibition (Figs. 3 and 4). In contrast, spinal opioid mechanisms have an unusual function. They do not contribute to the increase in bladder capacity elicited during stimulation of the S1 or S2 dorsal roots, but do contribute to the poststimulation increase in capacity that is unmasked by blocking GABA$_A$ receptors in the spinal cord with i.t. administration of picrotoxin (Figs. 5 and 6). The latter observation suggests that in anesthetized cats sacral neuromodulation activates a spinal opioid inhibitory pathway that, however, is suppressed by a tonic GABAergic mechanism. These results suggest that sacral neuromodulation of bladder overactivity is mediated by a complex interplay between multiple transmitter mechanisms at spinal and supraspinal sites.

The involvement of GABA$_A$ receptors in sacral neuromodulation is very different from their involvement in pudendal neuromodulation. Our previous study in cats (Xiao et al., 2014) showed that spinal GABA$_A$ receptors play a critical role in pudendal inhibition of bladder overactivity because i.t. picrotoxin can completely remove the inhibition. In contrast, our current study indicates that spinal GABA$_A$ receptors are not involved in inhibition of bladder overactivity elicited by sacral neuromodulation (Fig. 5). However, this inhibition is reduced by high doses (0.3–1 mg/kg, i.v.) of picrotoxin (Fig. 2), whereas pudendal inhibition is reduced by low doses (0.01–0.1 mg/kg) (Xiao et al., 2014). These results indicate that GABA$_A$ receptors in the spinal cord are important for pudendal neuromodulation of bladder overactivity, whereas the receptors in the brain play a critical role in sacral neuromodulation of bladder overactivity. However, the exact sites of action in the brain still need to be determined in future studies.

The involvement of glycine receptor in sacral neuromodulation is also different from its involvement in pudendal neuromodulation. Our previous study in cats (Rogers et al., 2016) showed that strychnine at low doses (0.001–0.003 mg/kg, i.v.) reduces pudendal inhibition of bladder overactivity and at a high dose (0.3 mg/kg, i.v.) unmasks a poststimulation excitatory effect on the overactive bladder reflex. However, in this study, strychnine (i.v.) did not change the inhibition of bladder

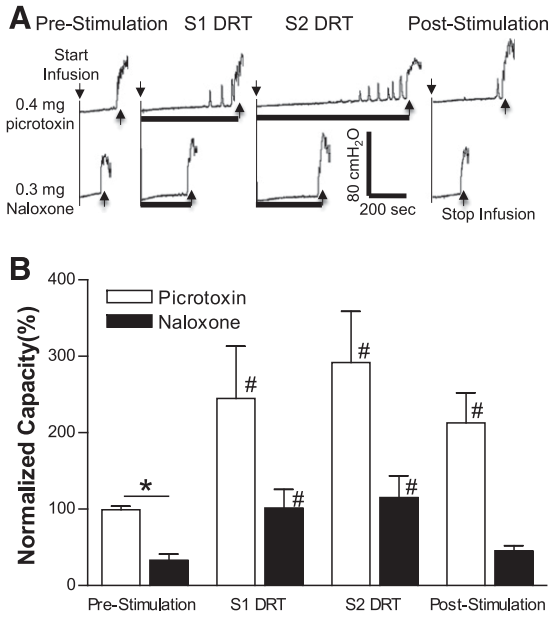


**Fig. 6.** Effect of naloxone (i.t.) on the inhibition of bladder overactivity by S1 or S2 dorsal root (DRT) stimulation at motor threshold (T) intensity in picrotoxin (i.t.)-pretreated cats. (A) Repeated CMGs were performed during AA infusion with or without S1 or S2 DRT stimulation in a cat pretreated with picrotoxin (i.t.), followed by naloxone (i.t.) treatment. Black bars under the pressure trace indicate stimulation duration. Stimulation: 5 Hz, 0.2 millisecond, T = 0.3 V for S1, T = 0.24 V for S2. Infusion rate = 2 ml/min. (B) Normalized bladder capacity measured under different conditions. *Indicates a significant ($P < 0.05$) difference between the prestimulation capacities before and after naloxone treatment ($t$ test). #Indicates significantly ($P < 0.05$) different from the prestimulation in picrotoxin- or naloxone-treated group (one-way analysis of variance).

overactivity elicited by sacral neuromodulation and did not cause poststimulation excitation (Fig. 3). Instead, strychnine (0.3 mg/kg, i.v.) reduced the i.v. dosage of picrotoxin from 1 mg/kg to 0.3 mg/kg necessary to completely eliminate sacral inhibition of bladder overactivity (Figs. 2 and 4), indicating a synergistic interaction between glycine and $GABA_A$ mechanisms. Please note that the 0.3 mg/kg picrotoxin is a cumulative dose in Fig. 2, but it is a single dose in Fig. 4, which includes the data for the interaction between strychnine and picrotoxin.

Due to the pharmacokinetics of the drug, a cumulative dose may not be exactly the same as the single dose. However, the different dosing methods should produce similar effects in this study because of the following: 1) a logarithmic increase of the drug dosage was used, which allowed an overlap of the effects of the two largest doses of the drug; 2) our previous study (Xiao et al., 2014) showed that the effect of picrotoxin can last more than 50 minutes for the period of performing the four repeated CMGs. A better designed dosing study is warranted to further determine the interaction between $GABA_A$ and glycine mechanisms.

It is known that inhibitory interneurons in the spinal cord can synthesize and release GABA and glycine as inhibitory cotransmitters (Todd et al., 1996). A previous study in cat (Thomson and Franz, 1981) also revealed that neither picrotoxin nor strychnine administered alone increased firing in the parasympathetic efferent excitatory pathway to the bladder induced by stimulation of bulbospinal excitatory axons, whereas coadministration of the two antagonists markedly increased the firing. The authors concluded that GABA and glycine act synergistically to generate tonic inhibition of the micturition reflex. In addition, a patch clamp study in neonatal rat spinal cord slices showed that focal electrical stimulation in the region of the sacral preganglionic neurons elicited inhibitory postsynaptic currents mediated by glycine and GABA (Araki, 1994). However, the interaction between glycine and GABA mechanisms revealed in this study probably occurs in the brain rather than in the spinal cord because spinal $GABA_A$ receptors do not contribute to sacral inhibition of bladder overactivity (Fig. 5). Further studies to determine the supraspinal interaction between glycine and $GABA_A$ mechanisms in sacral inhibition of bladder overactivity are certainly warranted.

There are some similarities between sacral neuromodulation and tibial neuromodulation. Similar to tibial neuromodulation (Ferroni et al., 2015), sacral neuromodulation only produced poststimulation inhibition of reflex bladder activity during saline cystometry (Zhang et al., 2013), but not during AA cystometry that induces bladder overactivity (Fig. 1). However, blocking spinal $GABA_A$ receptors by picrotoxin (i.t.) unmasked a significant poststimulation inhibition during AA infusion (Figs. 5 and 6). Naloxone (i.t.) eliminated this poststimulation inhibition, indicating that the inhibition was mediated by spinal opioid receptors. Similarly, tibial neuromodulation in cats produced a significant poststimulation inhibition during AA infusion after activating opioid receptors by administration (i.v.) of tramadol (an opioid receptor agonist) (Zhang et al., 2012). These results indicate that opioid mechanisms play a critical role in the poststimulation inhibition induced by both tibial and sacral neuromodulation during AA irritation of the bladder. It is worth noting that the vehicle (saline) control for i.t. picrotoxin (Fig. 5) was not performed because it is assumed that the small amount (0.2 mL) of i.t. saline would have no effect. In addition, the interaction between $GABA_A$ and opioid mechanisms on poststimulation effect should be further investigated with systemic dosing of these drugs.

This study only tested a single drug (picrotoxin or strychnine) for each neurotransmitter mechanism. Further testing of different drugs is needed to confirm the results of this study and eliminate possible nonspecific effects related to testing a single drug. In addition, the small number of cats in each drug test (six or nine cats/group) cannot determine the variability related to sex, size, and age of the animals. More studies are certainly needed to further examine these issues.

In summary, comparing this study of sacral neuromodulation with previous studies of pudendal or tibial neuromodulation showed that the $GABA_A$ and glycine mechanisms are involved differently in sacral and pudendal neuromodulation, whereas the poststimulation inhibition induced by either sacral or tibial neuromdolation under different conditions is mediated by the same neurotransmitter (opioid) mechanism. These results suggest that the mechanisms underlying sacral neuromodulation could be different from pudendal/tibial neuromoduation, but at the same time they may also share some properties. Understanding neurotransmitter mechanisms involved in sacral neuromodulation could further improve this effective OAB treatment and identify molecular targets for developing new treatments for OAB.

### Authorship Contributions

*Participated in research design:* Jiang, Fuller, Bandari, Bansal, Zhang, Shen, Wang, Roppolo, de Groat, Tai.

*Conducted experiments:* Jiang, Fuller, Bandari, Bansal, Zhang, Shen, Wang, Roppolo, de Groat, Tai.

*Contributed new reagents or analytic tools:* Jiang, Fuller, Bandari, Bansal, Zhang, Shen, Wang, Roppolo, de Groat, Tai.

*Performed data analysis:* Jiang, Fuller, Bandari, Bansal, Zhang, Shen, Wang, Roppolo, de Groat, Tai.

*Wrote or contributed to the writing of the manuscript:* Jiang, Fuller, Bandari, Bansal, Zhang, Shen, Wang, Roppolo, de Groat, Tai.

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**Address correspondence to:** Dr. Changfeng Tai, Department of Urology, University of Pittsburgh, 700 Kaufmann Building, Pittsburgh, PA 15213. E-mail: cftai@pitt.edu