

Sacral nerve stimulation with optimized parameters improves visceral hypersensitivity in rats mediated via the autonomic pathway

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Liuqin Jiang^{1,2} , Nina Zhang¹, Shengai Zhang¹, and Jiande DZ Chen¹

Abstract

The purpose of this study was to determine effects and mechanisms of sacral nerve stimulation (SNS) on visceral hypersensitivity in rodent models of colonic hypersensitivity. SNS was performed with different sets of parameters for 30 min in six regular rats. Visceral sensitivity was assessed by the measurement of electromyogram and abdominal withdrawal reflex before and after SNS. Real/sham SNS with optimized parameters was performed in 8 restraint stress-induced visceral hypersensitivity rats and 10 neonatal acetic acid-treated colonic hypersensitivity rats; acute effect of SNS was assessed by comparing electromyogram and heart rate variability. Neonatal acetic acid-treated rats were treated by SNS ($n = 10$) or sham-SNS ($n = 10$) daily for seven days for the assessment of the chronic effect of SNS. (1) When the stimulation amplitude was reduced from 90% of motor threshold to 65% or 40% motor threshold, SNS with certain parameters showed an inhibitory effect on abdominal withdrawal reflex. The best stimulation parameters for SNS were “14 Hz, 330 μ s, and 40% motor threshold.” (2) SNS significantly reduced visceral hypersensitivity and improved autonomic function in restraint stress-induced rats. The inhibitory effect was blocked by naloxone. (3) Acute and chronic SNS significantly reduced visceral hypersensitivity and improved autonomic function in acetic acid-treated rats. SNS with reduced stimulation strength may be used to treat colonic hypersensitivity and the best stimulation parameters seem to be “14 Hz, 330 μ s and 40% motor threshold”. SNS with optimized parameters improved visceral hypersensitivity in rodent models of colonic hypersensitivity mediated via the autonomic and opioid mechanisms.

Keywords

Sacral nerve stimulation, visceral hypersensitivity, colorectal distention, autonomic function

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Irritable bowel syndrome (IBS) is one of the most common gastrointestinal diseases, with a prevalence rate of 3% to 22% in the general population.^{1,2} With reference to the Roman III diagnostic criteria,³ IBS is defined by recurrent symptoms of abdominal pain or discomfort associated with alteration in bowel traits or habits. At present, the treatment of IBS mainly focuses on improving symptoms and is often unsatisfactory, and symptoms are often recurrent.

Sacral nerve stimulation (SNS) is a minimally invasive treatment that was proposed in 1995 by Matzel⁴ to treat fecal incontinence. SNS is achieved by placing bipolar electrodes in the sacral hole or around the sacral nerve

and connecting the electrodes to an implantable impulse generator positioned underneath the skin. Currently, SNS has been used widely in the treatment of bladder dysfunctions and fecal incontinence and occasionally in

¹Division of Gastroenterology and Hepatology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

²Department of Gastroenterology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China

Corresponding Author:

Jiande DZ Chen, Division of Gastroenterology and Hepatology, Johns Hopkins University School of Medicine, Baltimore, MD 21224, USA.
Email: jchen184@jhu.edu



the treatment of patients with refractory constipation.^{5–10} SNS was reported to be effective in treating pelvic pain after abdominal surgery¹¹ and anal pain with controversial results.^{12–14}

There are few research works of SNS on IBS or visceral hypersensitivity. Fassov et al.¹⁵ applied SNS to patients with IBS and reported some improvement in symptoms and the quality of life. However, it was an open label study that could not exclude possible placebo effect, and most importantly, they made no efforts in optimizing stimulation parameters and simply adopted the stimulation parameters used for treating fecal incontinence. Langlois et al.¹⁶ performed SNS in regular rats under anesthesia and reported improvement in visceral hypersensitivity induced by colorectal dilation. Similarly, no efforts were made to optimize SNS methodologies for treating visceral pain, and the experiment was performed in a non-conscious condition. It is conceivable that before SNS can be reliably applied for treating visceral pain, its methodology, such as stimulation parameters, must be optimized for this particular application, physiological improvement must be achieved, and mechanisms involved in the ameliorating effect of SNS on visceral hypersensitivity must be understood, at least partially.

Accordingly, the purposes of this study were as follows: (1) to determine the best stimulation parameters for SNS to reduce visceromotor reflex induced by colorectal distention (CRD) in regular rats, (2) to prove that SNS with optimized stimulation parameters improve visceral hypersensitivity induced by acute stress and study mechanisms involving autonomic functions, and (3) to investigate effects and mechanisms of optimized SNS on colon hypersensitivity in a previously validated rodent model of IBS.

Materials and methods

Animal preparation

Twelve adult male Sprague–Dawley (SD) rats (250–300 g, purchased from Charles River, USA) and 40 six-day-old male SD rats (Harlan, USA) were housed under controlled illumination (light/dark cycle, lights on/off: 12 h/12 h), humidity (40%–50%), and temperature ($22 \pm 2^\circ\text{C}$). Each adult rat was housed in a cage provided by the laboratory animal center and was free to drink and feed. Ten pups and one mother rat were housed in one cage and would be housed separately in a cage when they were four weeks old. Water and food were provided by the staff at the Animal Resource Center at Johns Hopkins University Bayview Medical Center.

All adult rats were acclimated to the environment for one week without any procedures after arrival to the facility. Surgery began at around 7 weeks of age, and experiments were carried out at the age of 8 weeks to 12 weeks. The Animal Care and Use Committee of the

Johns Hopkins Hospital (Baltimore, MA) approved the protocol of this research.

Surgery for electrode placement

Implanting electrodes round sacral nerve. Animals were operated under anesthesia induced by 1.5% to 2% isoflurane (Terrell, Piramal Critical Care, Inc., USA). A dorsal midline incision was performed to expose the right sacral nerve. One pair of electrodes and stainless steel cardiac pacing wires (A&E Medical, Farmingdale, NJ, USA) were placed around right sacral nerve (S3), and the dental cement was used to isolate the exposed wires from circumjacent tissues. The electrical connecting wires were fixed in the muscle layer and brought out at the back of the neck via a tunnel underneath the skin.

Implanting electrodes at abdominal wall for recording electromyography. A skin oblique incision was made, and the tips of two cardiac pacing wires (A&E Medical, Farmingdale, NJ, USA) were implanted at the external oblique muscles in the lower left abdomen to record electromyogram (EMG).^{17,18}

Implanting electrodes at chest wall for recording electrocardiogram. Three skin incisions were made, and the tips of three cardiac pacing wires (A&E Medical, Farmingdale, NJ, USA) were implanted at muscles: two in the chest wall and one in abdomen wall near the heart to record electrocardiogram (ECG).^{18,19}

The connecting wires for the EMG and ECG electrodes were subcutaneously tunneled to the back of the neck, affixed with sutures, and externalized for recording. Sham-SNS animals and control animals underwent the same surgical procedures for the placement of electrodes. After the surgery, the rats were given a sufficient time for a complete recovery (7–10 days).

Assessment of visceromotor reflexes

Previously established methods were used to assess visceromotor reflex to CRD.^{20,21} Under mild sedation with 1% methohexital sodium (Brevital; EliLily & Co, Indianapolis, IN, USA) 25 mg kg⁻¹ intraperitoneally, a flexible balloon (5 cm) constructed from a surgical glove finger attached to a Tygon tubing was inserted into the descending colon and rectum 8 cm from the anal verge and held in place by taping the tubing to the tail. The rat was then placed in a small Lucite cubicle (20 × 8 × 8 cm) and allowed to adapt for 30 min before the test. CRD was performed by rapidly inflating the balloon to predefined constant pressures of 20, 40, 60, and 80 mm Hg for a 20-s period, each followed by a 2-min rest at a pressure of 0. The EMG was recorded continuously during the experiment using a Biopac system EMG 100 C (Biopac Systems Inc., Goleta, CA, USA). It was amplified in a range of 1 Hz to 500 Hz and digitized

using the Acknowledge (Biopac Systems, Inc.). The area under the curve (AUC) of the EMG signal during each 20 s of distention was calculated using an in-house written computer program. The net EMG value for each distention which represented strength of visceromotor reflexes was calculated by subtracting the baseline value derived from the AUC for the 20 s predistention period.¹⁸ The abdominal withdrawal reflex (AWR), which represented characteristic behaviors by involuntary visceromotor reflexes, was assessed according to the animal's behavioral response to graded CRD (20, 40, 60, and 80 mm Hg) by two colleagues who were blinded to the study protocol and graded as follows: 1, no behavioral response to CRD; 2, contraction of abdominal muscles; 3, lifting of abdomen; and 4, body arching and lifting of pelvic structures.²⁰

Measurement and analysis of autonomic functions

The autonomic functions were assessed from the spectral analysis of heart rate variability (HRV) derived from the ECG signal (Figure 1).^{22,23} The ECG signal was recorded for 30 min via a special one-channel amplifier with a cutoff frequency of 100 Hz (Fetrode Amplifier, model 2283 ft/I, UFI, Morro Bay, CA, USA). The HRV was analyzed using a previously validated software.²² The software identifies R waves in the ECG signal, calculates R-R interval, interpolates

the R-R interval data, and finally, saves the HRV data at a sampling frequency of 8 Hz for spectral analysis. The overall power spectrum of the HRV signal was divided into two-frequency sub-bands, including low frequency (LF) and high frequency (HF).²³ LF was defined as the frequency range of 0.3 to 0.8 Hz, representing mainly sympathetic activity. HF was defined as the frequency range of 0.8 to 4.0 Hz, representing purely parasympathetic or vagal activity. The ratio LF/HF reflects the balance between sympathetic activity and vagal activity.

Experimental protocols

Three experiments were included in this study. In the experiments, SNS was performed with weak electrical current produced by a universal pulse generator (Model DS8000, World Precision Instruments, Sarasota, FL, USA) through the electrodes placed around the sacral nerve. The experimental protocols are shown in Figure 2.

Experiment 1: Effects of SNS on visceromotor reflexes in regular rats (parameter optimization)

The aim of this series of experiments was to find the best stimulus parameters of SNS for improving visceral pain

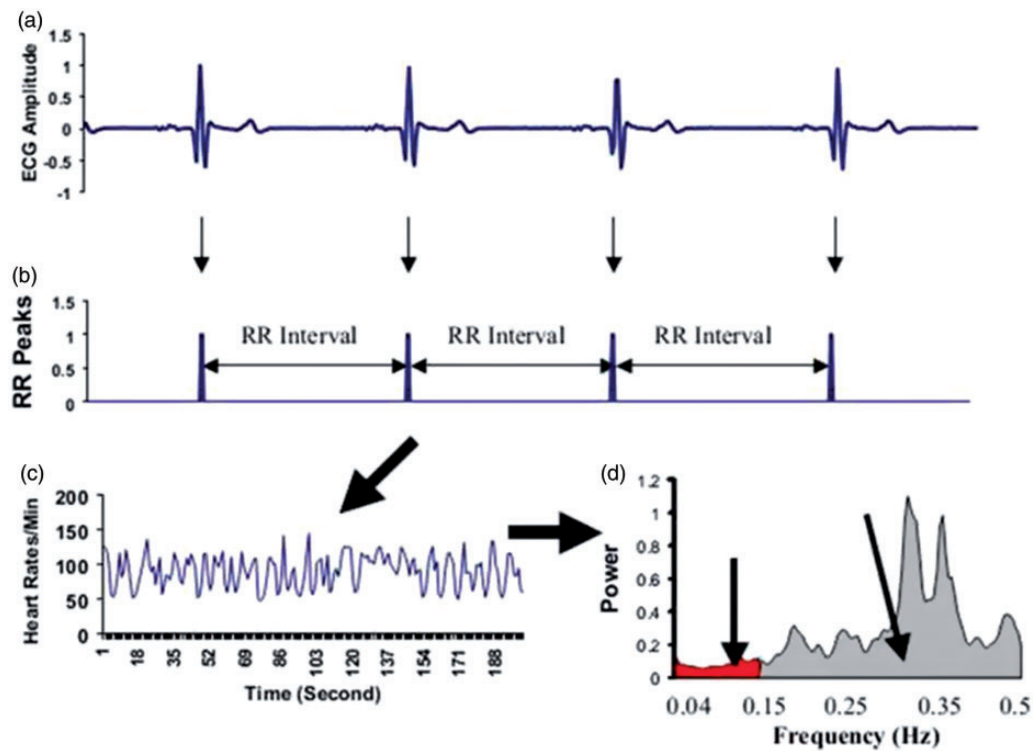


Figure 1. The spectral analysis of heart rate variability (HRV) derived from the ECG signal. (a) The ECG signal is recorded. (b) R waves are identified in the ECG signal and R-R interval is calculated. (c) Changes in heart rate at different times. (d) The overall power spectrum of the HRV signal.

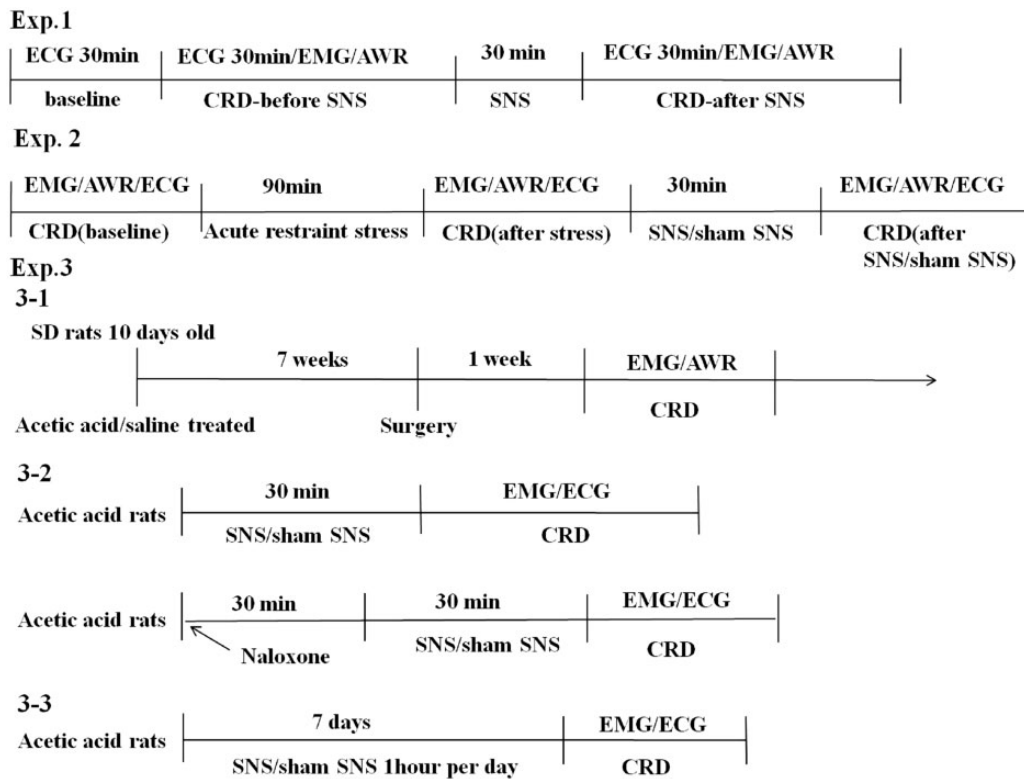


Figure 2. Experimental protocols.

in regular rats. Six adult male SD rats were used for this. Acute effects of SNS with different parameters were assessed as follows (Figure 2): behavioral testing before SNS by the EMG and AWR, SNS with one set of parameters for 30 min, and a repetition of the behavioral testing after SNS. Different sets of SNS parameters were tested on different days (one set at a time) with an interval of at least three days. To assess the effects of SNS on autonomic functions, the ECG was also recorded during the above behavioral testing.

Six sets of different parameters were included in this part: (1) “14 Hz 210 μ s,” which was the most commonly used parameters in SNS for treating various diseases, such as fecal incontinence and constipation^{8,24–26} and also used in SNS for treating IBS patients¹⁵; (2) “14 Hz 330 μ s,” which was used by Langlois et al. in treating visceral hypersensitivity induced by mechanical dilation¹⁶; (3) “10,000 Hz, 20 μ s,” which was in recent spinal cord stimulation for treating neuropathic pain^{27–29}; (4) “5 Hz, 500 μ s 10 s on 90 s off,” which was found to be effective in treating colon inflammation in rats in our lab³⁰; (5) “100 Hz, 250 μ s 0.25 s on 0.25 s off,” which was used for gastric electrical stimulation and was found to be effective in treating gastric hypersensitivity of rats in our lab (unpublished data); (6) “5 Hz, 100 μ s,” which was a set of parameters that was

found to be effective treating constipation (unpublished data). The stimulation amplitude was initially set at 90% of motor threshold (MT) that was the minimum SNS current to induce the movement of the rodent tail.¹⁶

Experiment 2: Effects of acute SNS on visceral hypersensitivity induced by restraint stress

The experiment was performed in six adult male SD rats. Behavior testing of the rat for visceral sensitivity was assessed in the resting state at first. The rat was then placed in a transparent plastic tube which was 60 mm in diameter and 150 mm in length in which the rat could only breathe freely and slightly move head and upper limbs but could not move their body or lower limbs.^{31,32} After 90 min, restraint stress behavior testing for visceral sensitivity was assessed, and then real/sham SNS with optimized parameters was performed to treat visceral sensitivity on model rats; after 30-min treatment, another behavior testing was made. ECG was recorded for 30 min at baseline after stress and after real/sham SNS. The rats were rested for three days at sacral nerve stimulus intervals; treatment was crossed over (sham/real SNS). Sham SNS was performed with same process but without current. Changes in visceral sensitivity and HRV of model rats were compared before and after SNS treatment.

Experiment 3: Effects and mechanisms of SNS on chronic visceral hypersensitivity induced by neonatal insult

Chronic visceral hypersensitivity induced by neonatal insult. Forty male SD rats were used in this series of experiments. Ten-day-old pups ($n=30$) received an infusion of a 0.2 mL of 0.5% acetic acid solution in saline into the colon 2 cm from the anal verge. Control animals ($n=10$) received 0.2 mL of saline.²¹ The surgical procedure for the placement of the electrodes was performed at seven weeks of age in each of these rats and after one week's rest, the behavior testing was performed. Then, the experiments were performed in the acetic acid-treated rats between 8 and 12 weeks of age described as follows.

Effects and mechanisms of acute SNS. This experiment was performed in 10 rats with chronic visceral hypersensitivity induced by neonatal acetic acid. Using the same protocol as shown in Figure 2, the effects of acute sham-SNS and SNS with the optimized parameters derived from the first series of experiments on visceromotor reflex were assessed in two randomized sessions on two separate days with an interval of at least three-days. The ECG was simultaneously measured in these sessions for the assessment of autonomic functions.

To study a possible mechanism involving the opioid pathway, two additional experimental sessions were performed: naloxone + sham-SNS and naloxone + SNS. The experimental protocol of these sessions was the same except that naloxone was administrated 30 min before SNS or sham-SNS (1 mg/kg, i.p.).¹⁷

Effect of chronic SNS on chronic visceral hypersensitivity rats. This experiment was performed in 20 rats with chronic visceral hypersensitivity divided into two groups: SNS

group ($n=10$) and sham SNS group ($n=10$). The rats in each group received real SNS with the optimized parameters or sham SNS for 1 h once per day for seven-days. The visceromotor reflex and the ECG were recorded using the protocol shown in Figure 2 at the end of the seven-day SNS.

Statistical analysis

All data are expressed as means \pm SE. Analysis of variance (ANOVA) was used to study the difference among three sets of data or more. Student's paired/unpaired t test was performed to compare two paired/unpaired sets of SNS/sham SNS groups. Statistical significance was assigned at $P < 0.05$. Statistical analyses were performed using SigmaStat19.0 (SPSS, Chicago, IL, USA).

Results

Optimization of SNS parameters

Six sets of different parameters were tested in regular rats at a stimulation strength of 90% of MT and none showed any significant SNS effect on the AWR scores during CRD in regular rats ($n=6$) ($P > 0.05$; see Figure 3).

When the stimulation current amplitude was reduced to 40% MT, SNS with three sets of parameters reduced the AWR scores in response to CRD in the regular rats (see Figure 4(a) to (c)), including parameter sets of "5 Hz 500 μ s 10s on 90s off," "14 Hz 210 μ s," and "14 Hz 330 μ s." The parameter set "14 Hz and 330 μ s" was found to be the most effective among them according to the increase in AWR score compared with the reduction in the AWR score: 20 mm Hg: 38%, 40 mm Hg: 32%, 60 mm Hg: 27%, 80 mm Hg: 16% (Figure 4(g)).

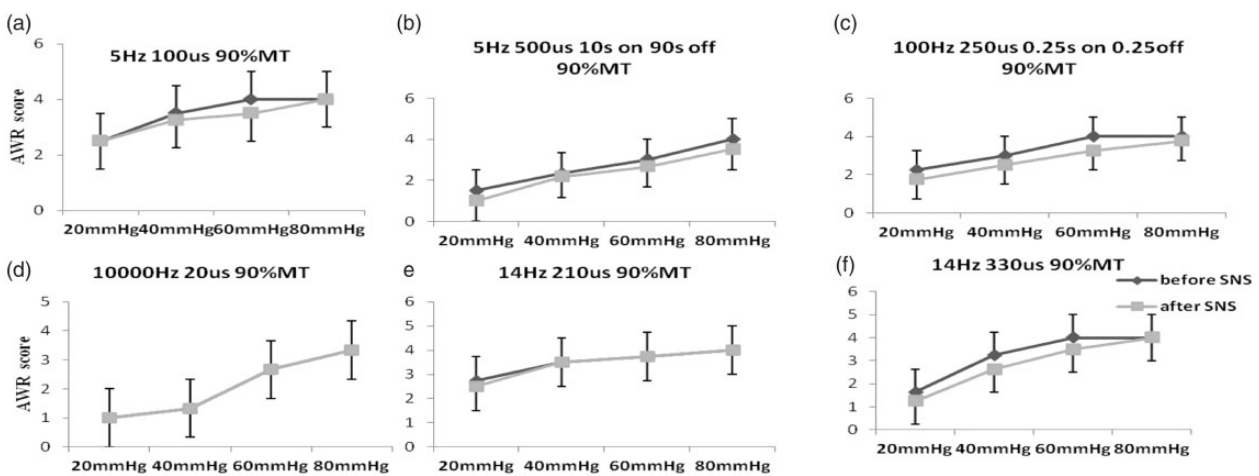


Figure 3. Sacral nerve stimulation (SNS) with different parameters with 90% motor threshold on the abdominal withdrawal reflex (AWR) in regular rats ($n=6$).

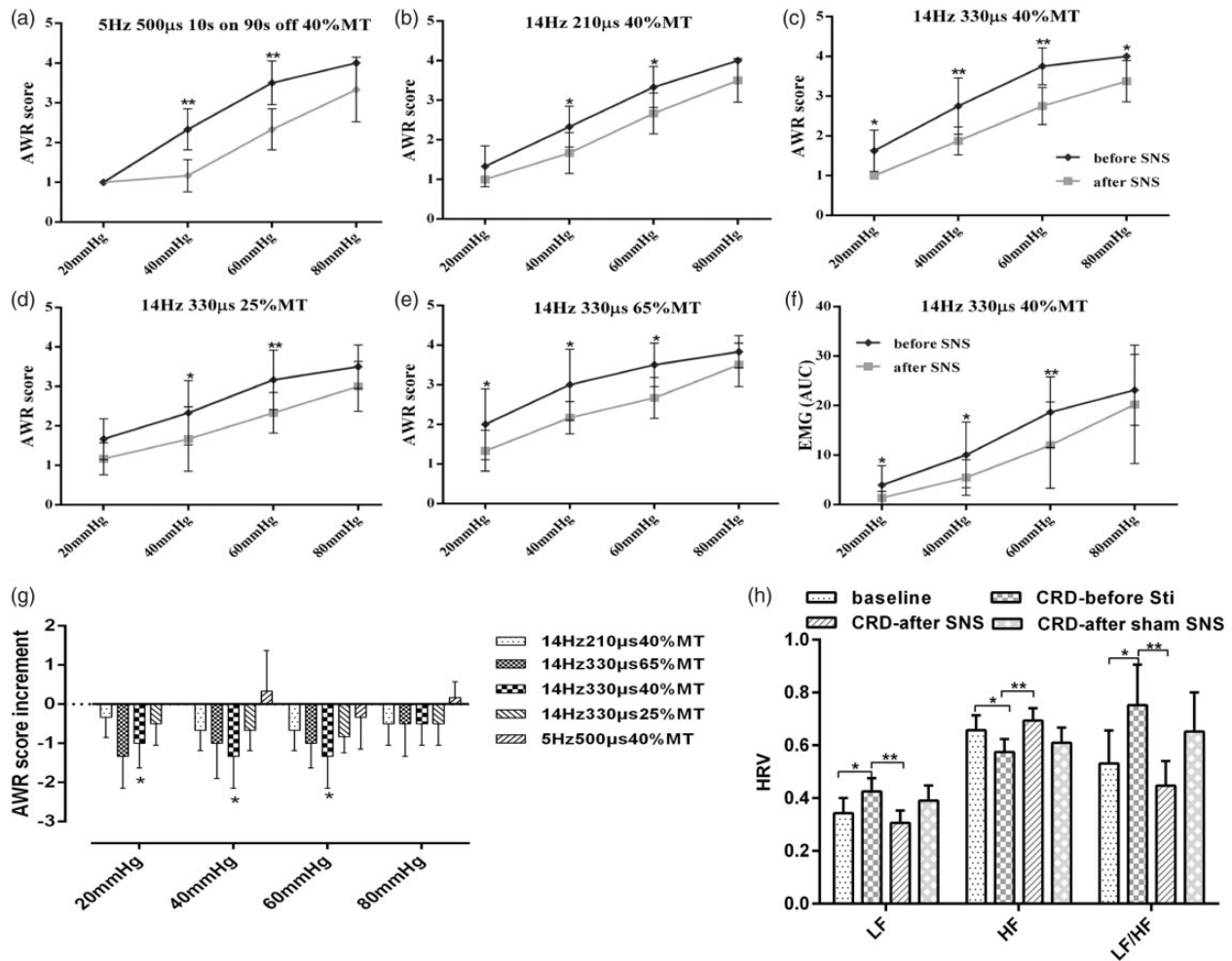


Figure 4. Optimization of sacral nerve stimulation (SNS) parameters in regular rats ($n = 6$). (a) to (e) SNS with different parameters was tested on the abdominal withdrawal reflex (AWR) during colorectal distention (CRD). (f) SNS with the parameters “14 Hz, 330 μ s and 40% MT” significantly reduced the electromyogram (EMG) response to CRD in regular rats. (g) AWR score increment was compared among different parameter sets. (h) Effects of CRD and SNS on autonomic function in regular rats. $*P < 0.05$, $**P < 0.01$, using two-way ANOVA.

Accordingly, further testing was performed using this set of stimulation parameters.

To find the best stimulation amplitude, SNS with 14 Hz and 330 μ s was further tested using stimulation amplitudes of 65% MT and 25% MT, respectively. The outcomes of SNS with all different stimulation outputs are presented in Figure 4(d) to (e) and (g). SNS at 40% MT was found to be the most effective, as it reduced the AWR score significantly at all distention pressures. Consequently, the parameter set, 14 Hz, 330 μ s and 40% MT, was considered as the optimized parameters and used in all subsequent experiments.

To show that SNS with the parameters optimized based on the AWR score was indeed effective in reducing visceromotor reflex, the experiment was repeated using the objective EMG assessment method and the results are presented in Figure 3(f). It was shown that

SNS with the optimized parameters significantly reduced the EMG responses to CRD at three distention pressures from 20 mm Hg to 60 mm Hg.

Mechanistically, the spectral analysis of the HRV derived from the ECG revealed an inhibitory effect on sympathetic activity and an enhance effect on vagal activity of SNS with the optimized parameters. As shown in Figure 4(h), the SNS decreased LF by 28%, increased HF by 20.8%, and decreased LF/HF by 40.6% ($P = 0.003$, 0.003 , 0.04 , respectively, vs. no-SNS).

SNS improved restrain-induced visceral hypersensitivity by normalizing autonomic functions

Restrain stress successfully induced visceral hypersensitivity assessed by the AWR and the EMG (Figure 5(a) to (c)).

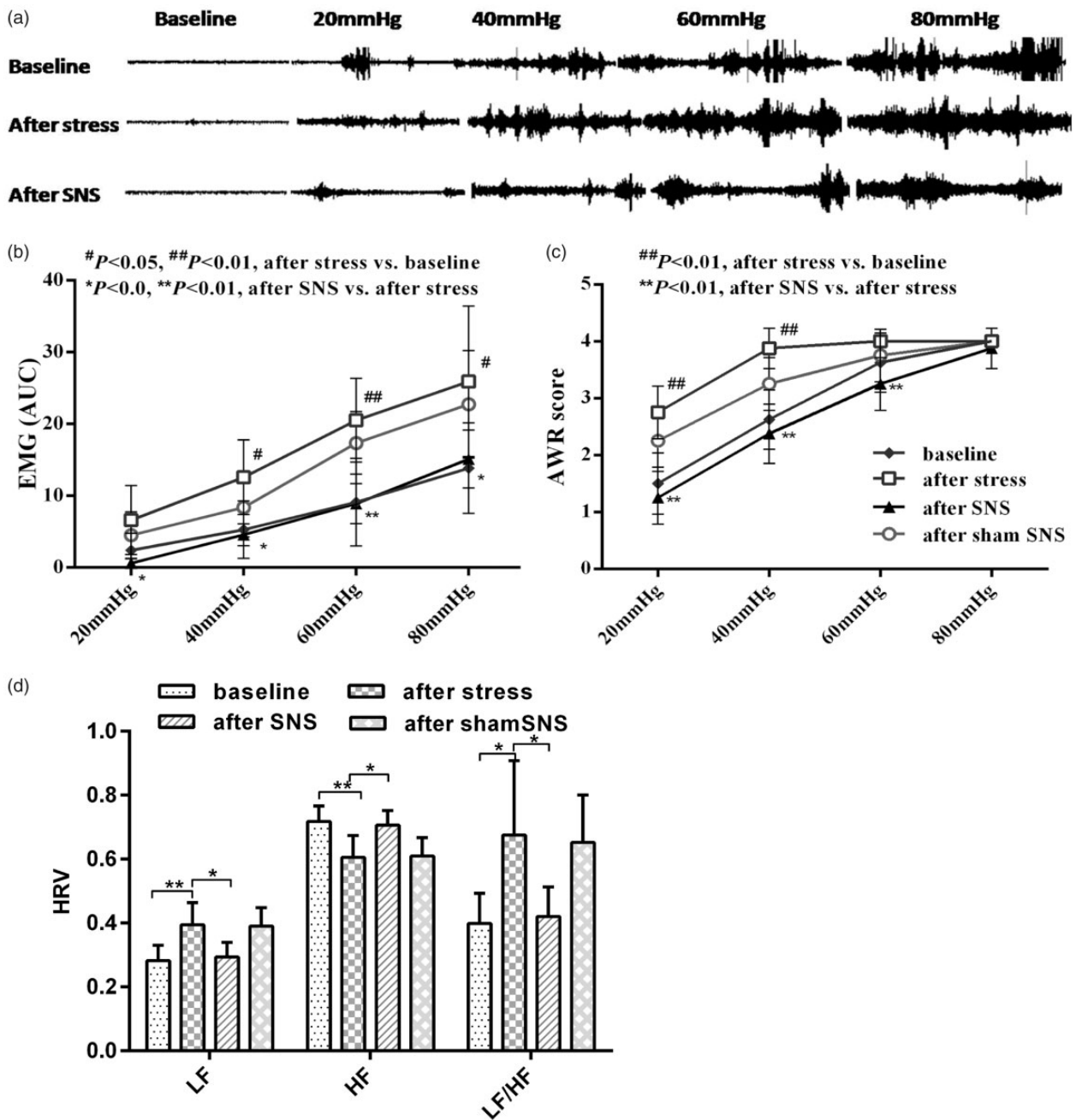


Figure 5. Effects of sacral nerve stimulation (SNS) in restrain-induced visceral hypersensitivity rats ($n = 6$). (a) Typical electromyogram (EMG) tracings at baseline or after stress or after SNS. (b) Restrain stress increased EMG response to colorectal distention (CRD) at 40 mm Hg ($P < 0.05$, vs. baseline), 60 mm Hg ($P < 0.01$, vs. baseline), and 80 mm Hg ($P < 0.05$, vs. baseline), while SNS reduced EMG response to CRD at 20 mm Hg to 80 mm Hg ($P < 0.05$, $P < 0.05$, $P < 0.01$, $P < 0.05$, respectively, vs. after stress). (c) Restrain stress increased abdominal withdrawal reflex (AWR) response to CRD at 20 mm Hg to 40 mm Hg ($P < 0.01$, vs. baseline), while SNS reduced AWR response to CRD at 20 mm Hg to 60 mm Hg ($P < 0.01$, vs. after stress). (d) Effects of restrain stress and SNS on autonomic function. $*P < 0.05$, $**P < 0.01$. EMG data and AWR score were compared using Student's paired *t* test. Autonomic function data were compared using two-way ANOVA.

All rats survived the restrain stress with no adverse events. After the 90-min restraint stress, the AWR score in response to CRD was increased significantly compared with that before the restraint (at 20 mm Hg: 2.75 ± 0.46

vs. 1.50 ± 0.53 , $P = 0.000$; 40 mm Hg: 3.88 ± 0.35 vs. 2.63 ± 0.5 , $P = 0.000$), Concurrently, the EMG in response to CRD was also increased (at 40 mm Hg: 12.59 ± 5.21 vs. 5.29 ± 3.99 , $P = 0.029$; 60 mm Hg: 20.52 ± 5.84 vs.

9.12 ± 6.09, $P=0.005$; 80 mm Hg: 25.93 ± 10.49 vs. 13.85 ± 6.31, $P=0.019$).

The 30-min acute SNS with the optimized parameters normalized the restraint stress-induced visceral hypersensitivity. As shown in Figure 5(b), the EMG responses to CRD at all pressures were reduced to the levels at baseline without stress. However, sham-SNS did not have any significant effects on the EMG. Similar results were also noted with the assessment of AWR (see Figure 5(c)).

Mechanistically, it was found that the restrain stress increased sympathetic activity and inhibited vagal activity, and these stress-induced alterations in the autonomic function were normalized with SNS with the optimized parameters. As shown in Figure 5(d), the LF assessed from the spectral analysis of HRV was significantly increased from the baseline after the restrain stress but returned to the baseline value after SNS. Similar effects can be appreciated from the HF and LF/HF. Sham-SNS, however, has no effect on the autonomic functions.

Effects and mechanisms of SNS on visceral hypersensitivity induced by neonatal acetic acid

Neonatal acetic acid treatment successfully induced chronic visceral hypersensitivity in the 30 rats. As shown in Figure 6(a) to (c), the AWR scores of the rats treated with acetic acid during the neonatal state in response to CRD were increased significantly compared to those of the control rats ($n=10$) (at 20 mm Hg: 3.14 ± 0.38 vs. 1.25 ± 0.46, $P=0.000$; 40 mm Hg: 4.00 ± 0.00 vs. 2.25 ± 0.46, $P=0.000$; 60 mm Hg: 4.00 ± 0.00 vs. 3.25 ± 0.46, $P=0.001$). Similarly, the EMG responses to CRD were also increased significantly in the acetic acid-treated rats in comparison with the control rats (at 20 mm Hg: 5.95 ± 2.54 vs. 0.75 ± 1.51, $P=0.001$; 40 mm Hg: 14.22 ± 4.58 vs. 3.68 ± 3.26, $P=0.000$; 60 mm Hg: 20.83 ± 4.52 vs. 7.18 ± 2.41, $P=0.000$; 80 mm Hg: 24.88 ± 3.61 vs. 10.95 ± 3.02, $P=0.000$).

Acute SNS (one time 30-min stimulation) improved visceral hypersensitivity in the rats with chronic visceral hypersensitivity. As shown in Figure 6(d), the EMG responses to CRD were decreased significantly after a 30-min SNS in comparison with those after a 30-min sham-SNS (at 60 mm Hg: 9.74 ± 1.68 vs. 27.41 ± 10.98, $P=0.018$; 80 mm Hg: 15.33 ± 3.38 vs. 36.07 ± 9.48, $P=0.008$). Similarly, the AWR scores in responses to CRD were also decreased significantly after the 30-min SNS in comparison with those after sham-SNS (at 20 mm Hg: 2.00 ± 0.00 vs. 3.38 ± 0.52, $P=0.000$; 40 mm Hg: 2.88 ± 0.3 vs. 4.00 ± 0.00, $P=0.000$; 60 mm Hg: 3.38 ± 0.52 vs. 4.00 ± 0.00, $P=0.011$).

The inhibitory effect of acute SNS on visceral hypersensitivity was blocked partially by the naloxone pretreatment (Figure 6(d)). In the session of naloxone + SNS, the

EMG response to CRD was significantly higher than those in the SNS session without naloxone (at 40 mm Hg: 16.34 ± 7.28 vs. 8.19 ± 4.71, $P=0.014$; 60 mm Hg: 24.07 ± 4.61 vs. 9.74 ± 1.68, $P=0.002$; 80 mm Hg: 28.73 ± 6.96 vs. 15.33 ± 3.38, $P=0.018$). Naloxone itself did not alter the EMG response to CRD at any pressure.

Mechanistically, acute SNS improved autonomic functions in the rats with chronic visceral hypersensitivity, while the improvement of autonomic function could be blocked partially by the naloxone pretreatment (Figure 6(e)). Assessed from the spectral analysis of HRV, SNS for 30 min decreased LF by 25.6% ($P=0.000$), increased HF by 17.1% ($P=0.000$), and decreased LF/HF by 36.0% ($P=0.001$), compared with sham SNS. While naloxone + SNS increased LF by 31.4% ($P=0.001$), decreased HF by 10.6% ($P=0.003$), and increased LF/HF by 45.8% ($P=0.001$), compared with SNS.

Chronic SNS (1 h daily SNS for seven days) also significantly improved visceral hypersensitivity in rats treated with neonatal acetic acid. The EMG responses to CRD were decreased significantly after the chronic SNS ($n=10$) in comparison with those after chronic sham SNS ($n=10$) (at 60 mm Hg: 12.41 ± 1.92 vs. 17.18 ± 2.87, $P=0.016$; 80 mm Hg: 16.82 ± 2.29 vs. 22.35 ± 2.08, $P=0.001$) (Figure 6(f)).

Mechanistically, the chronic SNS improved the autonomic function in the rats with chronic visceral hypersensitivity (Figure 6(g)). Assessed from the spectral analysis of HRV, the chronic SNS decreased LF by 24.8% ($P=0.035$), increased HF by 22.5% ($P=0.024$), and decreased LF/HF by 44.0% ($P=0.035$) compared with the chronic sham SNS.

Discussion

In this study, we found that (1) the best stimulation parameters for SNS to reduce visceromotor reflex were as follows: frequency of 14 Hz, pulse width of 330 μ s, and stimulation amplitude of 40% MT; (2) an acute 30-min SNS with this optimized set of parameters normalized restrain-induced acute visceral hypersensitivity and improved chronic visceral hypersensitivity induced by acetic acid given at the neonatal state; similar ameliorating effects were noted with seven-day SNS in rats with chronic visceral hypersensitivity; and (3) mechanistically, the ameliorating effects of SNS on visceral hypersensitivity were mediated via the autonomic and opioid pathways.

This was the first study to report that SNS at a lower stimulation amplitude (40% of MT) was most effective in reducing visceromotor reflex in normal rats and suppressing visceral hypersensitivity. In almost all previous peripheral nerve regulation or spinal cord stimulation

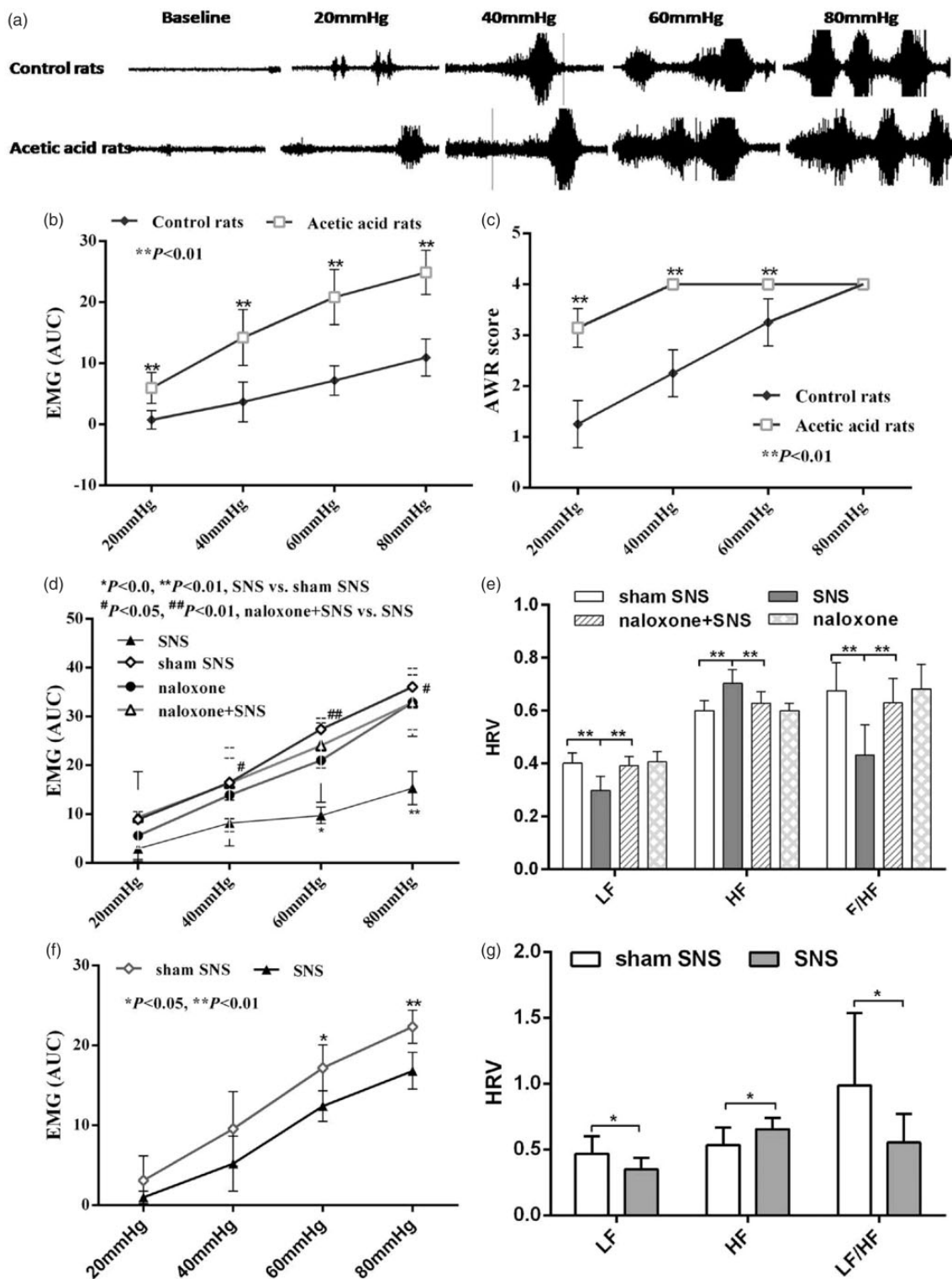


Figure 6. Neonatal acetic acid treatment induced visceral hypersensitivity, and sacral nerve stimulation (SNS) improved visceral hypersensitivity and autonomic function. (a) Typical electromyogram (EMG) tracings of control rats ($n = 10$) and acetic acid-treated rats ($n = 30$). (b) and (c) Neonatal acetic acid treatment increased EMG and abdominal withdrawal reflex (AWR) response to colorectal distention (CRD) of different pressure. (d) Acute SNS increased EMG response to CRD at 60 mm Hg ($P < 0.05$, vs. sham SNS) and 80 mm Hg ($P < 0.01$, vs. sham SNS), while naloxone+SNS could block the effect of SNS on EMG response to CRD at 40 mm Hg to 80 mm Hg ($n = 10$). (e) Acute SNS improved autonomic function in acetic acid-treated rats, while naloxone + SNS could block the improvement effect partially ($n = 10$, $*P < 0.05$, $**P < 0.01$). (f) Chronic SNS ($n = 10$) improved EMG response to CRD at 60 to 80 mm Hg compared with sham SNS ($n = 10$) ($*P < 0.05$, $**P < 0.01$). (g) Chronic SNS ($n = 10$) improved autonomic function compared with sham SNS ($n = 10$) ($*P < 0.05$, using Student's paired t test).

therapies, the intensity of stimulation output was set at 80% to 90% MT. However, in our study, we found that SNS at that high output was ineffective in reducing visceromotor reflex. This was a surprise to us and further mechanistic studies are needed to find out why a lower stimulation output was preferred for reducing visceral sensitivity to CRD.

Armed with the optimized set of parameters, SNS could effectively reduce visceromotor reflex in normal rats, normalize acute restraint stress-induced visceral hypersensitivity, and improve chronic visceral hypersensitivity induced by neonatal acetic acid. Although SNS was introduced for the treatment of visceral pain in both rats and humans,^{11,15,16} little efforts were made previously in determining most appropriate parameters. Previous studies simply adopted stimulation parameters used for treating bladder dysfunction and fecal incontinence. In this study, we investigated six sets of parameters used in previous SNS studies and in our lab. Logical and feasible systematic approaches were taken in this study. First, regular rats were used in the optimization process as the regular rats were stable and could give us more consistent outcomes. Then, the optimized SNS method was applied to acute and chronic rodent models of visceral (colonic) hypersensitivity. To make data more convincing, the assessment of visceromotor reflex or visceral hypersensitivity was accomplished by both the EMG and the AWR. These experimental results provided a strong evidence that SNS with the optimized parameters would have a therapeutic potential for treating visceral pain, such as pain in IBS and future clinical studies are warranted.

The pathogenesis of IBS are not fully understood, and various factors are involved in the development of IBS, including the abnormal function of the brain-gut axis. A number of brain nuclei were thought to be important components of the pain circuit, which played an important role in the processing of pain through a spinal cord sensation.³³ Spinal afferent nervous system is thought to be a classical method of visceral pain transmission, and the spinal cord stimulation treatment is widely reported in basic research and clinical practice.³⁴⁻³⁶ Sacral nerve is adjacent to the spinal cord; we therefore speculated that the ameliorating effect of SNS on colonic hypersensitivity might be mediated via both the spinal afferent pathway and the sacral efferent pathway. That is, SNS might improve impaired or altered brain-gut interactions in the animals with visceral hypersensitivity.

Indeed, in this study, we found that the autonomic function played an important role in visceromotor reflex and visceral hypersensitivity. We found that CRD increased the sympathetic function and reduced vagal function in regular rats, and that both acute restraint stress and neonatal treatment of acetic acid led to

sympathetic overactivity and vagal suppression. Most importantly, SNS was found to be capable of improving these altered autonomic functions in rodent models of colonic hypersensitivity and this was the first study to report such an important finding. Based on findings in this study, we believe that the ameliorating effect on visceral hypersensitivity was mediated through the regulation of the autonomic nervous function. At present, the role of vagus nerve function in the injury sensitivity adjustment is being more and more recognized,^{37,38} and the vagal neuromodulation was also closely followed in the treatment of visceral pain.³⁹ Putting together, we would speculate that on the vagal or parasympathetic arm, SNS might send a signal through the spinal cord to the brain center and the brain sends out an enhanced vagal efferent signal to the colon, and on the other hand, SNS might send a signal directly to the colon via the pelvic splanchnic nerve. The enhanced vagal activity to the colon might suppress neurotransmitters associated with visceral hypersensitivity, such as nerve growth factor⁴⁰ and/or special cells responsible for visceral hypersensitivity such as mast cells. On the other hand, the suppressive effect of SNS on sympathetic activity might block the spinal afferent signal carrying pain to the brain and also desensitize the spinal sensory pathway, such as dorsal root ganglion.⁴¹⁻⁴³ Further mechanistic studies are warranted to explore these speculations.

The opioid pathway is typically involved in the analgesic effects of various medications⁴⁴⁻⁴⁶ and in fact opioids are the most common medical therapies for pain. It had been reported that the sympathetic nervous system could be inhibited by opioid peptides produced in the heart through the respective receptors.⁴⁷ Chronic stimulation in μ -opioid receptor by methadone was found to reduce activity of resting sympathetic nerve to the muscles.⁴⁸ It is generally believed that the activation of the opioid pathway inhibits sympathetic outflow from the brain to the periphery. Previous studies had shown that the improvement effect of transcutaneous electrical nerve stimulation on analgesia was mediated via both the autonomic function and opioid pathways.⁴⁹⁻⁵¹ Our results found that naloxone could reverse the treatment effect of SNS on visceral hypersensitivity and autonomic function. Few studies discussed the opioid pathway of SNS on visceral hypersensitivity, only Langlois et al.¹⁶ found that the administration of naloxone could reverse the effect of SNS on visceral mechanosensitive rats. The findings of our study indicate that the improvement in autonomic function with SNS involved the opioid-mediated mechanism.

In conclusion, we have developed an effective method of SNS for suppressing CRD-induced visceromotor reflex. This optimized method of SNS is capable of ameliorating colon hypersensitivity induced by acute stress or neonatal treatment of acetic acid, mediated via the

autonomic-opioid mechanisms. In future studies, we would try to determine if the optimized stimulation parameters could be effective among different sensitized states of rats.

Declaration of Conflicting Interests

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ORCID iD

Liuqin Jiang  <https://orcid.org/0000-0001-5799-9220>

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