Acute lumbosacral nerve stimulation does not affect anorectal motor function in a rodent model

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Key Messages

• This study aimed to assess the effect of acute sacral nerve stimulation (SNS) on anorectal motor function in a rodent model.

• SNS was applied to 16 female rats and a novel technique was used to separately measure external and internal anal sphincter forces and rectoanal reflexes.

• Acute SNS has no motor effect on the anorectum in the rat which contrasts with a large sensory effect previously shown in the same animal.

• The mechanism of action of SNS is predominantly on sensory feedback mechanisms from the anorectum thereby increasing cortical awareness of the pelvic floor and improving symptoms of fecal continence.

Abstract

Background:

Sacral nerve stimulation has become a first line treatment for fecal incontinence, however, its effect on the motor function of the anorectum is uncertain. The aim of this study was to apply acute lumbosacral nerve stimulation in an animal model and to determine its effect on the external and internal anal sphincter forces, the rectoanal inhibitory and excitatory reflexes, and the slow wave frequency of the internal anal sphincter.

Methods:

Lumbosacral nerve stimulation was applied to 16 nulliparous female rats. A novel in vivo preparation was designed to allow simultaneous monitoring of external and internal anal sphincter forces. The effect of rectal distension on the two anal sphincters was also studied. Key Results:

Lumbosacral nerve stimulation delivered at either S1 or L6 in rodents did not affect sphincter forces,

rectoanal reflexes or slow wave frequency of anal canal smooth muscle.

Conclusions & Inferences:

The absence of effect on the motor pathways of continence suggests that the mechanism of action is predominantly on sensory feedback mechanisms from the anorectum, thereby increasing cortical awareness of the pelvic floor.

INTRODUCTION

In humans, normal anal sphincter function and rectoanal reflexes are integral to maintaining continence and abnormalities in patients with fecal incontinence are well described.[1] Although sacral nerve stimulation (SNS) has become a first line treatment for fecal incontinence, its effect on anorectal motor function is uncertain.[2]

The anal sphincter complex is composed of an inner layer of smooth muscle (internal anal sphincter, IAS) and an outer layer of striated muscle (external anal sphincter, EAS). In response to feces entering and distending the rectum, these muscles have opposite reflex actions on the anal canal, which are termed the rectoanal reflexes.

The rectoanal inhibitory reflex (RAIR) is the relaxation of the IAS in response to rectal distension. This is a local reflex mediated by enteric nerves and is thought to allow the rectal contents to descend into the sensate anal canal for discrimination between solid, liquid, and gaseous material; a 'sampling reflex'.[3] To counteract IAS relaxation and to prevent expulsion of rectal contents, the EAS contracts in response to the same stimulus. This is termed the rectoanal excitatory reflex (RAER) and is a spinal reflex which can be consciously modulated.[4] At rest the IAS contributes to 85% of the pressure in the anal canal5 and this has been shown to be dependent on the slow wave frequency in the muscle.6 During the recto-anal reflex, the IAS's contribution to anal canal pressure drops to 40%.[5]

Similar to humans, rats exhibit the RAIR of the IAS in response to rectal distension7 and also the RAER of the EAS.[8] In humans, SNS is delivered to sacral nerve root that gives the best motor response, usually S3. The corresponding root in the rat is L6, where most motor fibers to the inferior rectal nerve are located, while most sensory fibers pass through the S1 nerve root.[9] As such, both of these roots were stimulated in separate groups in this study.

The aim of this study was to determine the effect of acute SNS on the external and IAS forces, the recto-anal inhibitory and excitatory reflexes and the slow wave frequency of the IAS in an animal model.

MATERIALS AND METHODS

All procedures were approved by the Animal Research Ethics Committee in University College Dublin, and licensed by the Irish Department of Health and Children (license B100/2665). Anesthesia and animal preparation Sixteen nulliparous female rats were anesthetized with intraperitoneal urethane (1.5 g/kg; Sigma, Arklow, Ireland). Tail pinch, pedal withdrawal, and corneal reflexes were regularly examined to determine the correct depth of anesthesia. Supplemental intraperitoneal urethane was administered if needed (10% loading dose). The perineum and dorsum were shaved and rats were placed on a homeostatic warming blanket (Harvard Apparatus, Holliston, MA, USA) to maintain a body temperature of 37 °C during experimentation. The femoral vein was cannulated to administer supplemental anesthesia and fluids during the experiment. A tracheostomy was performed and the trachea intubated to maintain a patent airway during spontaneous respiration.

Combined anal sphincter recording

The distal 4–5 mm of the anal sphincter complex was mounted in vivo as a ring preparation (Fig. 1). A 3–0 silk suture (Fine Science Tools, Heidelberg, Germany) was placed through the anal sphincter complex, attached to a force transducer (Grass FT03, Astro-Med, Slough, UK), relayed to an analog to digital convertor (1401; Cambridge Electronic Design, Cambridge, UK) and displayed using Spike2 software (Cambridge Electronic Design). The position of the force measurement was orthogonal to the long axis of the anal canal. To maintain isometric conditions, a 4-mm-diameter silicone ring was placed in the anal canal and held in place with a metal rod.

The tension was adjusted until the baseline force was 10 mN. Inferior rectal nerve stimulation

The inferior rectal nerve (IRN) supplies the striated EAS. Stimulation of the IRN allowed separation of the external and IAS forces from a combined recording (Fig. 2). The IRN was identified bilaterally via incisions 0.5 cm lateral to the anal canal and vagina. Silver 125-lm electrodes were looped around each nerve. Correct placement was verified with EAS contraction prior to fixing in position with Kwiksil silicone adhesive (World Precision Instruments, Sarasota, FL, USA). The IRNs were stimulated in parallel at 1 Hz (Grass SD9 stimulator; Grass Instruments Co., Quincy, MA, USA). Stimulation voltage used ranged from 0.1 to 1.1 V and was adjusted to achieve the EF50 (the stimulus voltage producing 50% of peak force).

Recto-anal reflexes

The balloon of a pediatric Foley urinary catheter (6 French; Coloplast, Peterborough, UK) was placed in the rectum. Every 100 s this was inflated with 0.3 mL saline for 10 s to mimic the descent of a fecal pellet and evoke the recto-anal reflexes (Figs 1 and 2).

Lumbosacral nerve root stimulation

Animals were divided into two groups based on the nerve root stimulated. Eight animals were stimulated at L6 and eight animals at S1. The lumbosacrum was exposed via a 3-cm dorsal midline incision and a specially designed platinum iridium electrode (Medtronic, Minneapolis, MN, USA) was used to stimulate the selected nerve root on the left side. Contraction of the EAS was used to confirm placement at L6 and twitching of the tail was used to confirm placement at S1. The SNS parameters used were a frequency of 10 Hz (1 ms pulses) for 3 min at a voltage just above the motor threshold (0.5–1.1 V).

Analysis

Data were recorded for 3 min before SNS, 3 min during SNS and for 30 min post SNS. One-way ANOVA was performed on the data arranged in 3 min bins using Prism 5 (GraphPad Software, San Diego, CA, USA). The single factor was time and the criterion for statistical significance was $p < 0.05$.

RESULTS

Sphincter forces

Stimulation at S1 did not significantly affect the IAS or EAS force (Fig. 3). Stimulation at L6 produced a small decrease in EAS force due to striated muscle fatigue as EAS motor fibers originate at L6. Although this was a statistically significant finding, it was determined to be physiologically insignificant as fatigue of the EAS would not improve continence or explain the mechanism of action of SNS.

Recto-anal reflexes

Stimulation at L6 or S1 did not significantly affect the RAIR or RAER (Fig. 4).

Internal anal sphincter slow wave

Stimulation at L6 or S1 did not significantly affect the IAS slow-wave peak frequency (Fig. 5).

DISCUSSION

Using a rodent model enabled invasive recording of the combined internal and EAS force. This is a novel preparation that has allowed investigation of the separate effects of SNS on each sphincter during isometric contraction.

These results show no significant change in any of the motor or reflex parameters measured during or following SNS at either L6 or S1. While there was a statistically significant decrease in EAS tone following stimulation at L6, this was deemed to be physiologically insignificant as the magnitude was small and this would not explain the action of SNS. This is in contrast to the sensory system where SNS has been shown to result in a potentiation (up to 150%) of anal canal evoked somatosensory cortical potentials in a rat model.[10] The absence of a motor effect in contrast to the known effects on

the sensory cortex supports the theory that the mechanism of action of SNS is predominantly through modulation of sensory pathways.[11–13]

The animal model used in these experiments, while having similar recto-anal reflex patterns and anal sphincter musculature to humans, does have some limitations. Animals were investigated under anesthesia and while urethane has been shown to cause minimal physiologic disturbance compared to other anesthetic agents,[14,15] the preparation is not representative of a conscious animal. Sacral nerve stimulation may modulate feedback from the anorectum to higher neural pathways and alter the conscious control of the EAS and RAER. This may only be apparent on voluntary contraction, however, only resting sphincter forces and reflex patterns were recorded in this experiment. In addition, nerve stimulation was given acutely for 3 min, the period shown to have a lasting sensory effect,16 and may have a different action during chronic stimulation. Animals used were also healthy, whereas SNS may have a greater effect in the injured state.

In clinical research, despite efforts to find a measureable change in motor function following SNS treatment, there has been no consistent changes observed.[2] In one large study. however, squeeze pressures were significantly improved following SNS treatment.[17] This suggests that SNS may act on the conscious rather than the reflex control of continence and supports the theory of modulation through higher centers.

In summary, acute SNS at L6 or S1 does not have any effect on the RAIR, RAER, EAS or IAS tone, or IAS slow waves in a urethane-anesthetized rat. This lack of action on the motor pathways of continence is in contrast to the known effects of SNS on sensory evoked cortical potentials and suggests that SNS works predominantly by increasing cortical awareness of the pelvic floor.

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DISCLOSURE

No competing interest declared.

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FIGURES

Figure 1: Experimental setup. The anal sphincter is mounted as an isometric ring preparation in situ. The suture through the anal sphincter complex is connected to a force transducer to record the combined sphincter force. The inferior rectal nerve is stimulated at 1 Hz to enable separation of both sphincters from the recording. The balloon in the rectum is inflated every 100 s to elicit the recto-anal reflexes. IAS, Internal anal sphincter; EAS, External anal sphincter.

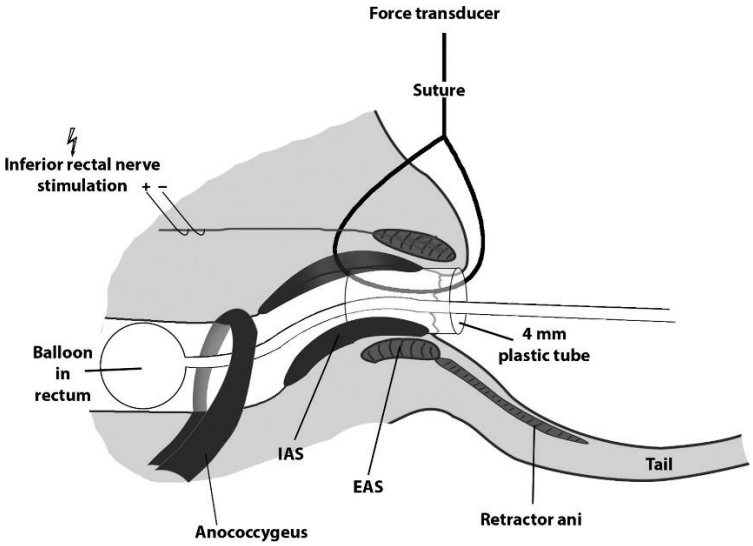

Figure 2: Separation of external and internal sphincter forces. The recording of the combined sphincters force is shown at the top. The amplitude of contraction of the external anal sphincter (EAS) spikes (red) is plotted separately. The internal anal sphincter (IAS) baseline (black) is also plotted. The recto-anal reflexes are evoked by balloon inflation in the rectum.

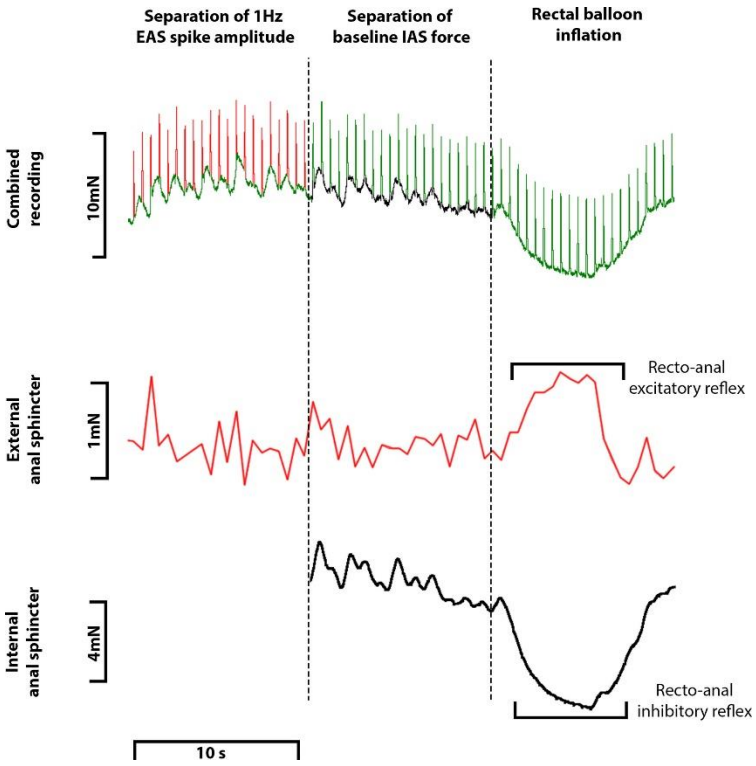

Figure 3: Effect of SNS on anal sphincter forces. SNS had no significant effect on the recorded force of either striated or smooth sphincter.

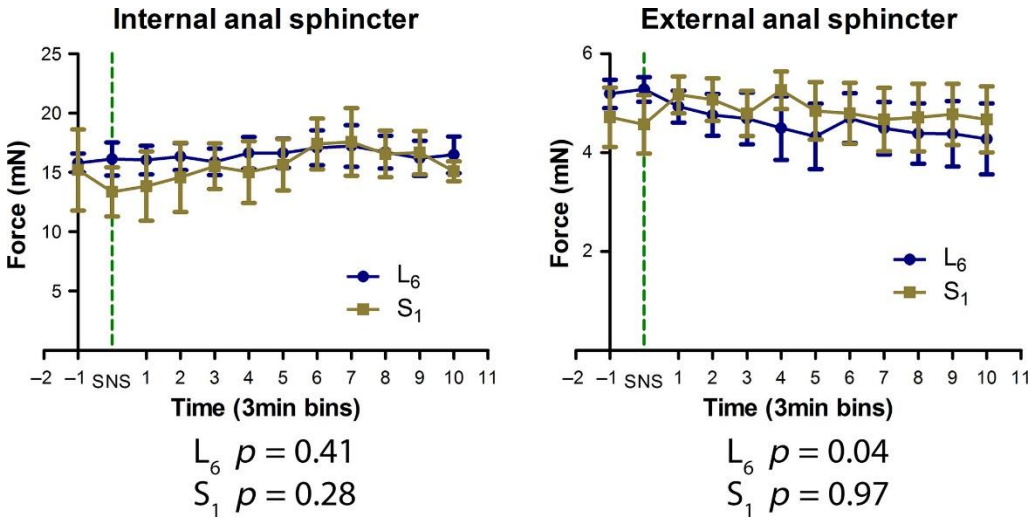

Figure 4: Effect of SNS on recto-anal reflexes. SNS had no effect on the degree of excitation or inhibition of the two rectoanal reflexes.

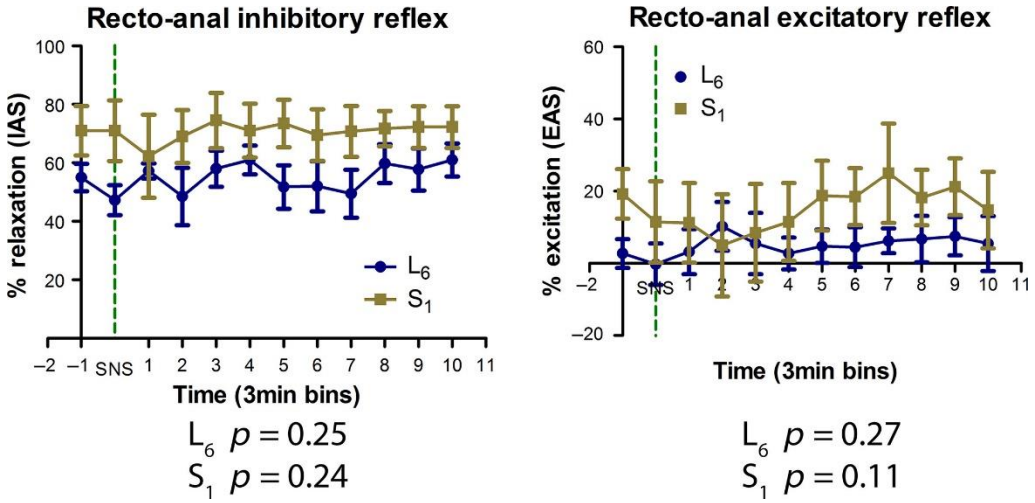

Figure 5: Effect of SNS on IAS slow-wave frequency. SNS had no effect on internal anal sphincter slow-wave frequency.