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Effects of electroacupuncture at BL33 on detrusor smooth muscle activity in a rat model of urinary retention

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ABSTRACT

Background Detrusor smooth muscle (DSM) underactivity may lead to urinary retention (UR). Electroacupuncture (EA) at BL33 may be effective in improving DSM contractions.

Objectives This study aimed to investigate: (1) the effect of EA at BL33; and (2) the effect of different manipulation methods at BL33 on the modulation of DSM contractions in UR rats.

Methods 30 male Sprague-Dawley rats were anaesthetised with urethane and modelled by urethral outlet obstruction. First, 2 Hz EA at BL33, SP6 and LI4 wasrandomly applied to the UR rats for 5 min to observe the immediate effects (n=10); second, manual acupuncture (MA) (n=10) and 100 Hz EA (n=10) were applied with the same programme. DSM electromyography (EMG) and cystometrogram data were evaluated.

Results (1) 2 Hz EA at BL33 and SP6 significantly increased DSM discharging frequency (0.80±0.10 Hz, P<0.001, and 0.22±0.14 Hz, P=0.038), shortened micturation intervals (65.67±20.65 s, P=0.008, and 35.62±15.84 s, P=0.042), prolonged the duration of voiding (2.13±0.61 s, P=0.005, and 0.47±0.16 s, P=0.015), and reduced residual pressure (−0.91±0.31 mmHg, P=0.019, and −0.66±0.27 mmHg, P=0.046). EA at LI4 was not associated with any functional effects (P>0.05). Compared with SP6, EA at BL33 had greater positive effects on DSM discharging frequency, duration of discharging, and duration of voiding (all P<0.05). (2) No statistically significant differences were shown between MA, 2 Hz EA and 100 Hz EA interventions when stimulating at BL33, SP6 or LI4.

Conclusions EA at BL33 improved DSM contractions to a greater degree than EA at SP6 or LI4. There were no differences in effect when stimulating using 2 Hz EA, 100 Hz EA and MA.

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INTRODUCTION

Urinary retention (UR) is a common symptom in lower urinary tract dysfunction. Detrusor underactivity is one of the most common causes of UR and is defined as contraction of reduced strength and/or duration, resulting in prolonged bladder emptying and/or a failure to achieve complete bladder emptying within a normal time span.[1] As a result, UR can cause a prolonged increase in preload and damage the function of the bladder.[2]

Physiologically, detrusor smooth muscle (DSM) contraction can be activated by parasympathetic motor neurons via the pelvic nerve when receiving an efferent signal from the pontine micturation centre (PMC) or spinal micturation centre.[3–5] Current first-line pharmacotherapy includes muscarinic receptor agonists or acetylcholine esterase inhibitors, but curative effects are far from expected.[6 7] Recently, the effects of posterior tibial nerve stimulation (PTNS) and sacral neuromodulation on UR have been well validated in clinical trials.[8 9] However, PTNS requires regular office visits and costs both time and money. Neuromodulation requiring an invasive surgical operation may be accompanied by complications such as pain, stimulator malfunction or loss of battery function.[10] Also, the long-term effects still need to be investigated further.[11]

Electroacupuncture (EA) is a convenient and safe therapy that has long been used to treat UR.[12 13] BL33 (*Zhongliao*) and SP6 (*Sanyinjiao*) are the most commonly used traditional acupuncture points in clinical trials.[14] However, the effects of acupuncture at these particular locations on DSM contractions are unknown. Also, comparisons of the effects of acupuncture at these two different points are limited. From a neurophysiological perspective, the muscle tissues at BL33 and SP6

are innervated by the same segments (S2–S4) as the spinal micturation centre.[15] EA can activate afferent nerve fibre conduction.[16] Therefore we supposed EA at BL33 or SP6 may regulate DSM contractions. In addition, BL33 is located at the third posterior sacral foramina. EA at BL33 with deep insertion can directly stimulate the sacral nerve, and may therefore have a similar mechanism of action to conventional sacral neuromodulation.[17 18] Therefore, we hypothesised that acupuncture at BL33 may have a better effect than that at SP6. In addition, we selected LI4 (*Hegu*), given its location in different somatic segments (C5–C7), as a control and thereby aimed to assess whether EA at BL33 and/or SP6 has a better effect on the putative modulation of DSM contractions and voiding dysfunction than LI4. Furthermore, we tested out different acupuncture manipulations (manual acupuncture (MA) versus 2 Hz EA versus 100 Hz EA) to determine which manipulation method produces the best effect in UR rats.

METHODS

Ethical statement

The experiment was conducted in accordance with the National Research Council's "Guide for Care and Use of Laboratory Animals" (National Academies Press, Washington, DC, USA). It was also approved by the Institutional Animal Welfare and Use Committee of Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences (CACMS, reference no. 2015051501).

Animals

Specfic-pathogen-free adult male Sprague-Dawley rats weighing 180–220 g (Beijing Vital River Laboratory Animal Technology, China) were obtained from the laboratory animal feeding room at the China Academy of Chinese Medical Sciences (CACMS). They were reared among 120 cages of companion rats and housed six per cage. The animals had free access to food and water. The room temperature was maintained at 21±2°C, with a 12-hour light-dark cycle.

Study design

The experiment comprised three parts, each of which used 10 rats (30 rats in total). All the rats were modelled for UR before the experiment, and DSM electromyography (DSM-EMG) and cystometrograms were collected as baseline data. For experiment 1, we needled BL33, SP6 and LI4 in turn on each individual rat using 2 Hz EA stimulation to determine each point's immediate effects on DSM contractions and voiding dysfunction (n=10). The sequence of the three points was randomly produced by the drawing of lots. Each point was stimulated for 5 min. The first DSM discharge and voiding signal were recorded as data after EA stimulation at the first point. Then, after at least three voiding cycles were observed, in order to make sure that the animal returned to the baseline level, EA was conducted at the next point allocated point. In experiments 2 (n=10) and 3 (n=10), MA and 100 Hz EA, respectively, were used and the other procedures were the same as those in experiment 1. During the experiment, the statistician was kept blinded to treatment group allocation.

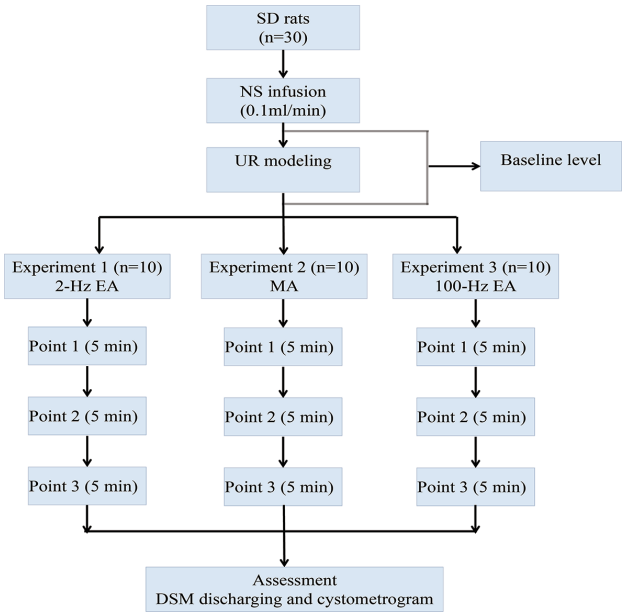

Figure 1 Flow chart of the study. Points 1, 2 and 3 refer to the three acupuncture points (BL33, SP6 and LI4). The sequence of the three points was randomly generated by the drawing of lots. DSM, detrusor smooth muscle; EA, electroacupuncture; NS, normal saline; SD, Sprague-Dawley; UR, urinary retention.

The flow chart for the study is presented in figure 1.

Experimental procedures

Anaesthesia

The rats were anaesthetised using an intraperitoneal injection of 10% urethane (1.0 g/kg, Sinopharm Chemical Reagent, China).[19] After the experiment, the dose of 10% urethane (1.0 g/kg) was continued to maintain the animal in a steady deep anaesthetic state, which was evaluated by the lack of a withdrawal response to paw pinch. The rats were then killed by cervical dislocation.

UR induced by urethral outlet obstruction

The distal urethra was clamped. Isotonic saline was infused into the bladder at the rate of 0.6 mL/min over the first 5 min. Then, the obstruction was sustained for another 55 min.[20 21] After that, the urethra was released and urine was allowed to drain.

Recordings of DSM discharge and cystometrogram

Each animal was placed on an electric heating pad (ALCBIO, China). The bladder was exposed by a midline abdominal incision. An intravenous infusion needle was inserted into the apex of the bladder dome, while the other end was connected to a syringe pump (Smith

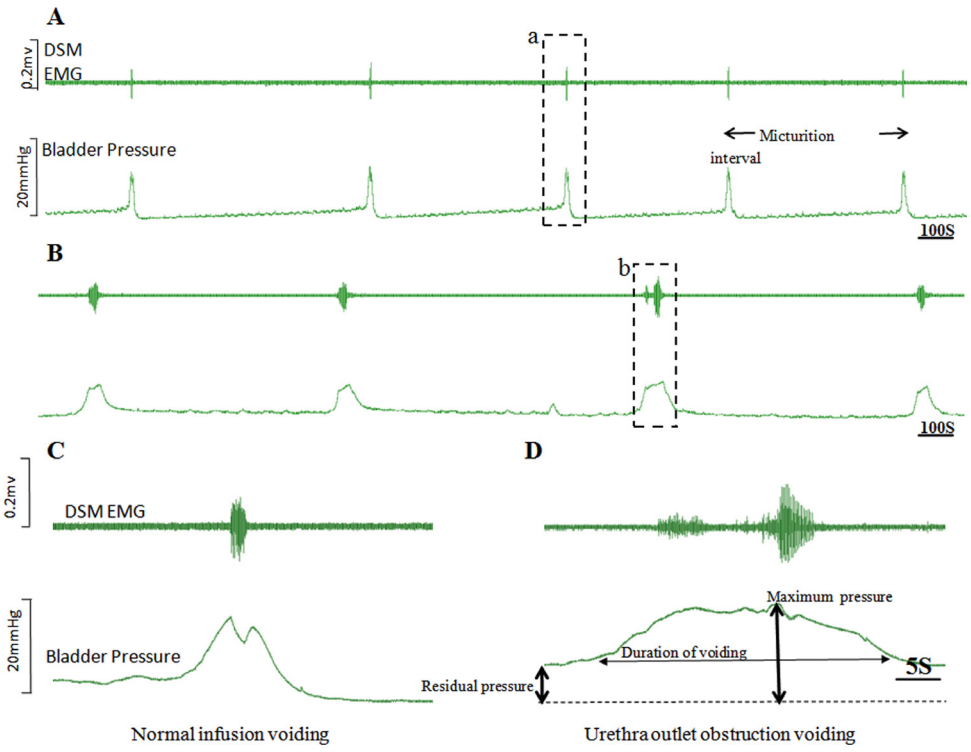

Figure 2 DSM-EMG and cystometrograms recorded before and after modelling. (A) Normal voiding cycles with continuous saline infusion (0.1 mL/min). (B) Voiding cycles after modelling. (C) Faster time tracing of normal voiding (a). (D) Faster time tracing of voiding after urethral outlet obstruction (b). After modelling, the DSM contractile ability was significantly decreased. DSM, detrusor smooth muscle; EMG, electromyography.

Medical, China) and a pressure transducer (BIOPAC, USA) via a three-way stopcock.[22] Two tungsten wire electrodes (0.05 mm diameter) were inserted into the layer of the DSM.[23] Paraffin oil was dripped adequately to keep the tissue moistened. Meanwhile, the wire electrodes were connected to Biopac Systems (BIOPAC, USA) and then connected to 1401 Expansion (CED, UK). A DSM-EMG and cystometrogram were simultaneously recorded. Isotonic saline (0.9% sodium chloride, Hengrui Medicine, China) was infused into the animal's bladder at the rate of 0.1 mL/min.

The experiment was carried out in the shielding laboratory at CACMS, where it could be guaranteed the electrophysiological signals were not disturbed.

Acupuncture intervention

Acupuncture points were located according to *Experimental Acupuncture Science*.[24] In humans, BL33 is located at the medial and inferior to the posterior superior iliac spine, over the third posterior sacral foramina. In rats, however, there are only three pairs of posterior sacral foramina, therefore the point was located at the

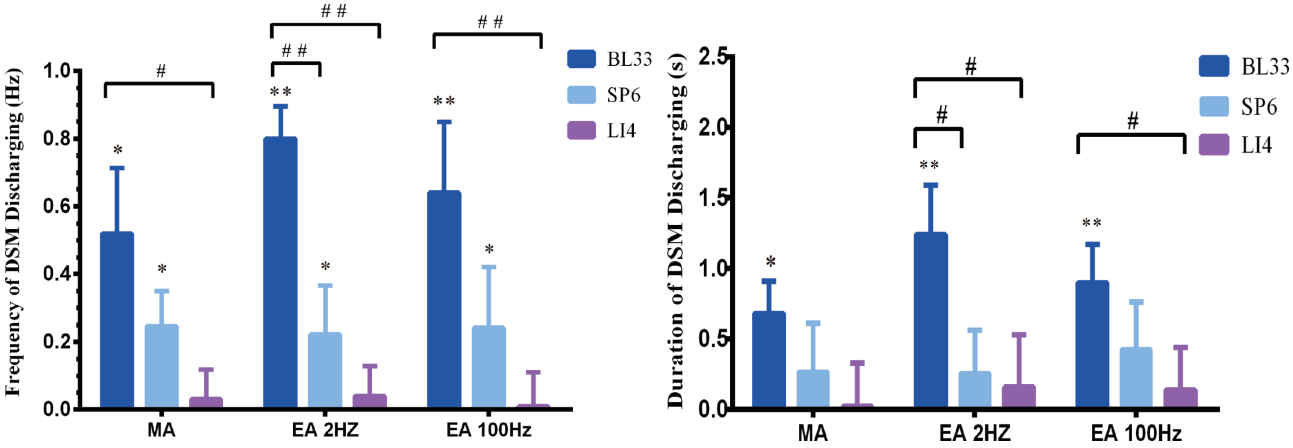

Figure 3 Effects of manual acupuncture (MA) and electroacupuncture (EA) at BL33, SP6 and LI4 on detrusor smooth muscle (DSM) discharging frequency (A) and duration of DSM discharging (B) in a rat model of urinary retention. *Refers to comparison between pre- and post-stimulation (*P<0.05; **P<0.01, n=10, paired t-test). #Refers to comparison of changes in different groups (#P<0.05; ##P<0.01, n=10, one-way ANOVA with LSD test).

spinous space between the second and third posterior sacral foramina.[22] Accordingly, we regarded the second posterior sacral foramina as BL33. SP6 was located at 10 mm above the tip of the medial malleolus and LI4 was located at the junction of the first and second metacarpal bones. Stainless steel acupuncture needles (0.25 mm×25 mm, Huatuo, China) were inserted into the second posterior sacral foramina to a depth of approximately 15 mm bilaterally at BL33 and vertically inserted into the skin to a depth of approximately 3 mm bilaterally at SP6 and LI4. The two needle handles were connected to the positive and negative electrodes of the EA stimulator (HANS-100, China). After stimulation with 2 Hz EA (continuous wave, 1 mA) for 5 min, the needles were removed.

In the second experiment, MA was applied for 1 min with continuous twisting and rotating at a rate of 120 rounds per minute. Then, the needles were retained without manipulation for 4 min. For 100 Hz EA stimulation, the methods and other parameters were the same as for the 2 Hz EA stimulation protocol.

Experimental outcomes

The DSM-EMG and cystometrograms were recorded using Spike 2 software (version 8.0, CED, UK). We collected the recordings both before and after each EA stimulus. According to these recordings, the DSM discharging frequency, the duration of DSM discharging, and the voiding assessments included evaluations of: (1) the micturation interval; (2) the duration of voiding; (3) the maximum pressure; and (4) the residual pressure.[21 25] In this study, we set the DSM discharging frequency as the primary outcome.

Sample size

The sample size was estimated with PASS 11.0 software (NCSS, USA). Based on our pilot study, we estimated that the difference in DSM discharging frequency between acupuncture at BL33 and SP6 would be approximately 0.69 Hz. Therefore, assuming a standard deviation of 0.27 Hz, it was estimated that 10 animals per group would be needed to detect a difference of at least 0.69 Hz at the 0.05 significance level with 80% power.[26]

Statistical analysis

Data were expressed as mean±standard error of the mean (SEM) and were analysed using the Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, IL, USA). Data were compared pre- and post- EA stimulation using the paired t-test. To compare different acupuncture points or manipulation methods, the data were analysed by one-way analysis of variance (ANOVA) with *post hoc* tests of least significance difference (LSD). A value of $P<0.05$ was considered to be statistically significant.

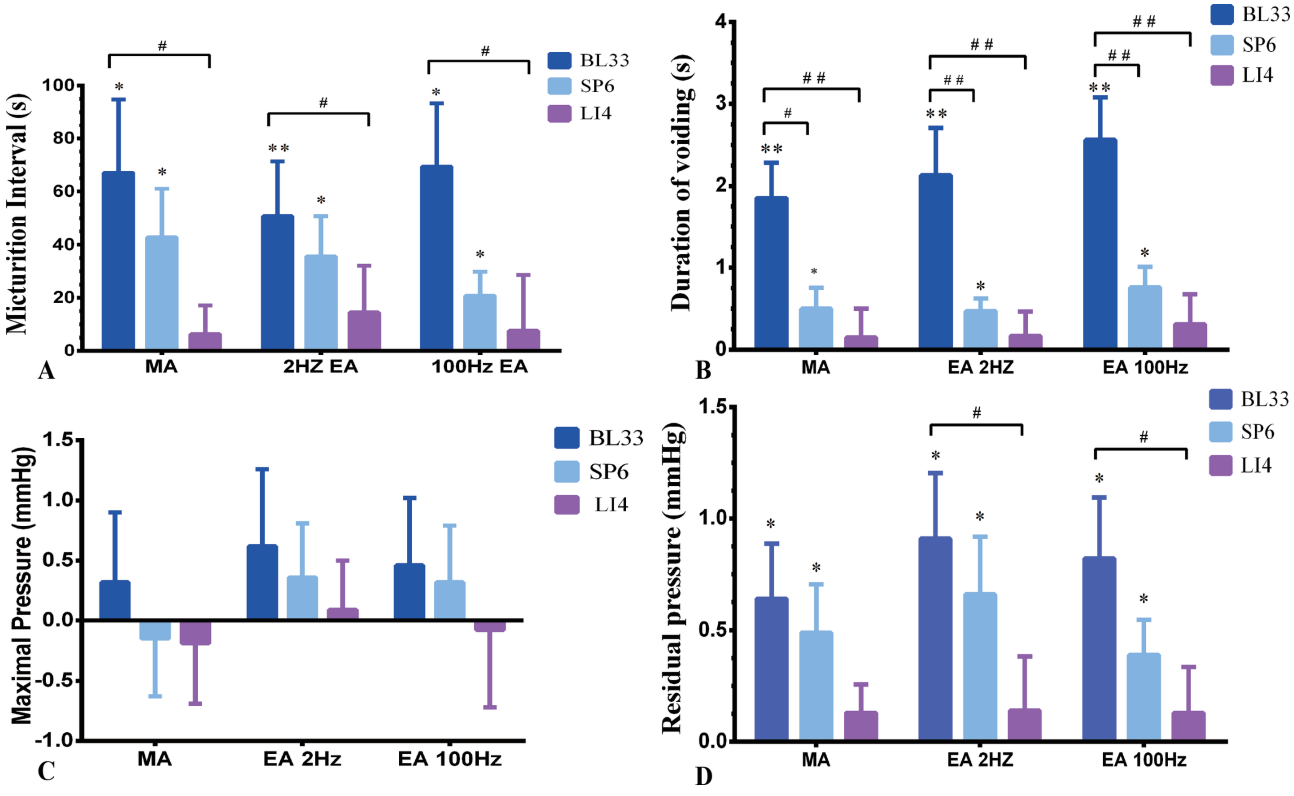

Figure 4 Effects of manual acupuncture (MA) and electroacupuncture (EA) at BL33, SP6 and LI4 on micturition interval (A), duration of voiding (B), maximal pressure (C) and residual pressure (D) in a rat model of urinary retention. *Refers to pre- and post-stimulation (*P<0.05; **P<0.01; n=10, paired t-test). #Refers to comparison of changes of assessments between groups (#P<0.05; ##P<0.01; n=10, one-way ANOVA with LSD test). ANOVA, analysis of variance; EA, electroacupuncture; LSD, least significant difference; MA, manual acupuncture.

RESULTS

Baseline data

Baseline measurements were obtained when the rats were kept in a steady state (figure 2A and C). After the urethral outlet obstruction, DSM-EMG recordings demonstrated a pathological pattern of discharge (figure 2B and D). The discharge frequency decreased from 6.80±0.15 Hz to 4.35±0.14 Hz (−2.45±0.18 Hz, P<0.001); the duration of discharging increased from 3.77±0.22 s to 5.59±0.42 s (+1.82±0.44 s, P=0.002). Regarding the cystometrograms, micturation interval increased from 517.38±24.71 s to 670.07±27.45 s (+152.69±26.08 s, P<0.001), duration of voiding increased from 16.56±1.28 s to 19.91±1.70 s (+3.34±1.36 s, P=0.036), and the residual pressure increased significantly from 1.56±0.85 mmHg to 6.93±0.67 mmHg (+5.37±0.34 mmHg, P<0.001). No significant difference was found in the maximum pressure (P>0.05).

OUTCOMES

Effects of 2 Hz EA at BL33, SP6 and LI4

With respect to DSM contractions, 2 Hz EA at BL33 increased the discharge frequency from 4.32±0.28 Hz to 5.11±0.33 Hz (+0.80±0.10 Hz, P<0.001), and 2 Hz EA at SP6 increased it from 4.21±0.17 Hz to 4.52±0.15 Hz (+0.22±0.14 Hz, P=0.038). 2 Hz EA at LI4 had no effect on the regulation of DSM discharging frequency (from 4.33±0.11 Hz to 4.37±0.14 Hz, 0.04±0.09 Hz, P=0.671). BL33 increased the duration of DSM discharging from 5.37±0.22 s to 6.61±0.39 s (1.24±0.34 s, P=0.005). 2Hz EA at neither SP6 nor LI4 prolonged the duration of DSM discharging (from 5.37±0.24 s to 5.63±0.23 s, 0.26±0.28 s, P=0.380; and from 5.50±0.25 s to 5.65±0.30 s, 0.16±0.37 s, P=0.471. respectively). Stimulation with 2 Hz EA at BL33 showed superiority when compared with stimulation at SP6 and LI4. Comparisons of the changes in DSM discharging between the different groups are presented in figure 3.

Cystometrograms were improved by 2 Hz EA stimulation at BL33 and SP6 (figure 4). As shown in figure 4A, BL33 shortened micturation intervals from 657.15±12.60 s to 591.48±23.02 s (+65.67±20.65 s, P=0.008) and SP6 shortened them from 642.17±16.06 s to 606.55±24.07 s (+35.62±15.84 s, P=0.042). As shwn in figure 4B, BL33 prolonged the duration of voiding (bladder contraction) from 19.72±0.31 s to 21.84±0.69 s (+2.13±0.61 s, P=0.005), while SP6 prolonged it from 19.60±0.24 s to 20.07±0.27 s (0.47±0.16 s, P=0.015). As shown in figure 4C, there was no difference in maximum pressure before and after 2 Hz EA stimulation at BL33 and SP6. Finally, as shownin figure 4D, BL33 reduced residual pressure from 7.88±0.38 mmHg to 7.06±0.60 mmHg (−0.91±0.31 mmHg, P=0.019) and SP6 reduced it from 7.66±0.29 mmHg to 7.07±0.19 mmHg (−0.66±0.27 mmHg, P=0.046). LI4 did not have any significant regulatory effect on voiding dysfunction (P>0.05 for all parameters). Comparisons of the changes of recordings in the different groups are shown in figure 5. Before and after 2 Hz EA stimulation at BL33 and SP6, DSM discharging frequency and duration of voiding were significantly increased, while EA at LI4 caused no significant change. In comparison, the changes between BL33, SP6, and LI4, BL33 showed a significant increase.

2 Hz EA versus 100 Hz EA versus MA

No statistically significant differences were shown between MA, 2 Hz EA and 100 Hz EA stimulations

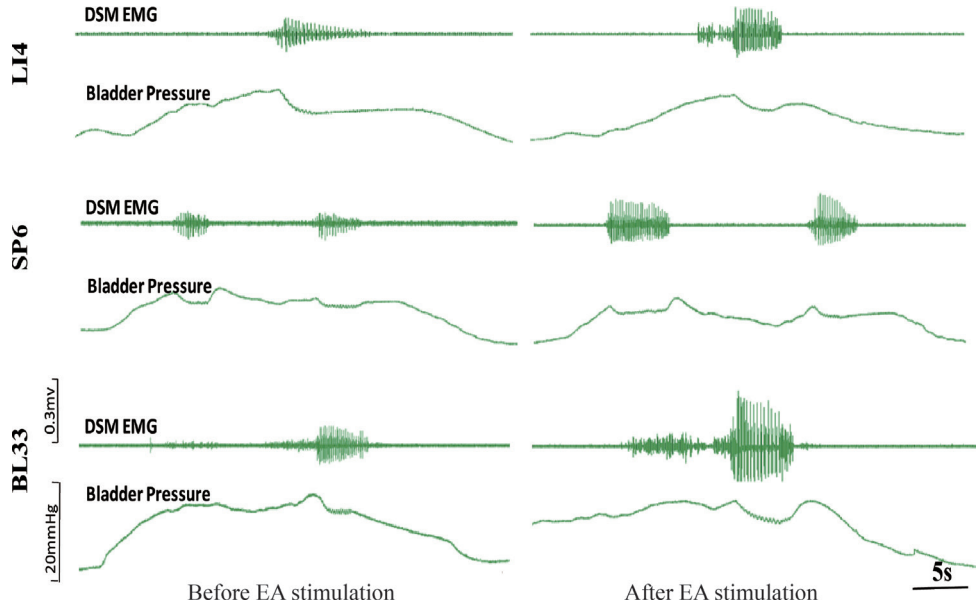

Figure 5 Detrusor smooth muscle electromyography (DSM-EMG) with corresponding cystometrogram before and after 2 Hz electroacupuncture (EA) stimulation at BL33, SP6 and LI4 in a rat model of urinary retention.

at BL33, SP6 or LI4 for either the DSM discharging frequency or the duration of DSM discharging (all P>0.05). With respect to the regulation of voiding dysfunction, no significant differences was shown in assessments between MA, 2 Hz EA and 100 Hz EA stimulations at BL33, SP6 or LI4 (all P>0.05). Comparisons of changes in DSM discharging and voiding assessments between the different manipulation methods are shown in figures 2 and 3. DSM-EMG with corresponding cystometrograms at BL33 with MA, 2 Hz EA and 100 Hz EA stimulations are shown in figure 6. Before and after acupuncture stimulation, the discharging frequency and duration obviously increased; however, no significant differences were in these changes were observed between the three groups.

DISCUSSION

The results of the present study showed that EA at BL33 and SP6 could enhance DSM contractions and improve voiding dysfunction in UR model rats, while LI4 had no such effect. Comparing the changes between groups, 2 Hz EA at BL33 was associated with a greater increase in DSM discharging frequency and a significantly prolonged duration of DSM discharging compared with 2 Hz EA at SP6. Similarly, 2 Hz EA, 100 Hz EA and MA at BL33 significantly prolonged the duration of voiding relative to SP6.

DSM contraction is mainly mediated through parasympathetic pathways. Efferent signals, which originate from the spinal micturation centre (S2–S4 in humans, L6–S1 in rats),[27 28] are sent to the post-ganglionic fibres or to the intramural ganglion of the bladder wall along with the pelvic nerve, and they act to contract the DSM. Previous studies have reported that acupuncture could activate afferent nerve fibres in the dorsal spinal root.[16] BL33 and SP6 share the same segment as the micturation centre. We propose that acupuncture at BL33 or SP6 may activate sacral afferent nerve fibres, therefore enhancing DSM contractions. BL33 is at the third posterior sacral foramina. Deep insertion at BL33 could stimulate the sacral nerve, and thereby work in a similar way to sacral neuromodulation. SP6 is located at 10 mm above the tip of the medial malleolus. Stimulation at SP6 is likely to be similar to PTNS. Currently, the exact mechanisms of action underlying the effects of sacral neuromodulation or PTNS on bladder dysfunction are still unknown. Van Kerrebroeck believed that sacral root electrical stimulation could enhance DSM contraction by strengthening somatic afferent inputs to the spinal micturation centre.[29 30] Kovacevic *et al* reported that PTNS could activate the voiding reflex and alter bladder function.[31] These studies are consistent with our results. In the present study, we chose acupuncture at LI4 (located in the area of segment C5–C7) as a control intervention and observed its lack of effect on DSM contraction. Overall, the results illustrate that the effect of acupuncture may correlate with the segmental innervation of the target tissues at the selected point.

In this study, we observed the excitatory effect of EA on the UR rats. The results are consistent with previous studies.[31–33] Schultz-Lampel *et al* observed both excitatory and inhibitory reflex effects on the bladder following sacral nerve stimulation in anaesthetised cats. They also believed that this effect could be induced by different frequencies and intensities of electrical stimulation.[32] Qin *et al* reported that MA could inhibit bladder motility in the active state and enhance

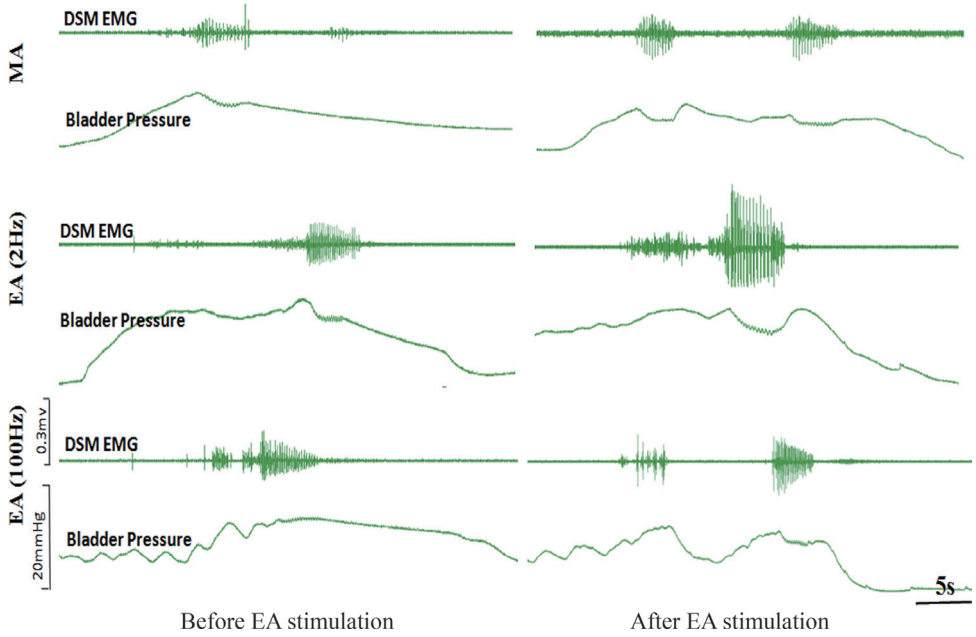

Figure 6 Detrusor smooth muscle electromyography (DSM-EMG) with corresponding cystometrograms of manual acupuncture (MA), 2 Hz electroacupuncture (EA) and 100 Hz EA stimulation at BL33 in a rat model of urinary retention.

bladder motility in the static state,[33] while in some studies BL33 has been used to suppress an overactive bladder (OAB).[34] In our previous study, we observed the inhibitory effects of EA in a rat model of OAB syndrome.[35] However, when we used acupuncture as an intervention in UR rats, we found that acupuncture could also enhance bladder activity. These findings seem contradictory at first glance and the underlying mechanism ultimately remains unknown. However, we think the effects of acupuncture are likely to be related to the baseline state of the bladder.

This study compared the influence of different acupuncture points and manipulation methods on UR rats. In this study, three points were used one by one on each rat. Although EA stimulation at the next point in the randomised sequence was postponed until the cystometrogram recording returned to its baseline level, the potential carry-over influence of needling at the former point cannot be completely excluded. However, we did not change the design because of ethical considerations.

Conclusion

Stimulation with 2 Hz EA at BL33 enhanced DSM contractile ability and improved voiding dysfunction to a greater degree than that at SP6 or LI4. No significant differences in DSM contractions were observed between MA, 2 Hz and 100 Hz EA stimulations at BL33.

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Contributors XL and KL performed the operations and recordings. MZ performed the acupuncture stimulation. QM and XYG performed the data analysis. ZL and Xinyan Gao designed the study. Xinyan Gao is another corresponding author of this study.

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Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Abrams P, Cardozo L, Fall M, *et al*. The standardisation of terminology in lower urinary tract function: report from the standardisation sub-committee of the International Continence Society. *Neurourol Urodyn* 2002;2:167–78.
2 de Groat WC, Yoshimura N. Anatomy and physiology of the lower urinary tract. *Handb Clin Neurol* 2015;130:61–108.
3 Andersson K-E, Michel MC. *Urinary Tract*. Berlin: Springer, 2011.
4 Michel MC, Barendrecht MM. Physiological and pathological regulation of the autonomic control of urinary bladder contractility. *Pharmacol Ther* 2008;117:297–312.
5 Andersson KE, Arner A. Urinary bladder contraction and relaxation: physiology and pathophysiology. *Physiol Rev* 2004;84:935–86.
6 Andersson KE. Detrusor underactivity/underactive bladder: new research initiatives needed. *J Urol* 2010;184:1829–30.
7 Aggarwal H, Zimmern PE. Underactive bladder. *Curr Urol Rep* 2016;17:17:17.
8 Schultz-Lampel D, Jiang C, Lindström S, *et al*. Experimental results on mechanisms of action of electrical neuromodulation in chronic urinary retention. *World J Urol* 1998;16:301–4.
9 Yamanishi T, Kaga K, Fuse M, *et al*. Neuromodulation for the treatment of lower urinary tract symptoms. *Low Urin Tract Symptoms* 2015;7:121–32.
10 Joussain C, Denys P. Electrical management of neurogenic lower urinary tract disorders. *Ann Phys Rehabil Med* 2015;58:245–50.
11 Faris AER, Gill BC, Pizarro-Berdichevsky J, *et al*. Impact of age and comorbidities on use of sacral neuromodulation. *J Urol* 2017;198:161–6.
12 Yu KW, Lin CL, Hung CC, *et al*. Effects of electroacupuncture on recent stroke inpatients with incomplete bladder emptying: a preliminary study. *Clin Interv Aging* 2012;7:469–74.
13 Liu Z, Zhou K, Wang Y, *et al*. Electroacupuncture improves voiding function in patients with neurogenic urinary retention secondary to cauda equina injury: results from a prospective observational study. *Acupunct Med* 2011;29:188–92.
14 Peng X, Liang Q, Zhang Y, *et al*. Research on application of frequently-used acupoints for urinary retention. *Journal of Traditional Chinese Medicine* 2013;54:2046–8.
15 de Groat WC. Integrative control of the lower urinary tract: preclinical perspective. *Br J Pharmacol* 2006;147:S25–S40.
16 Kagitani F, Uchida S, Hotta H, *et al*. Manual acupuncture needle stimulation of the rat hindlimb activates groups I, II, III and IV single afferent nerve fibers in the dorsal spinal roots. *Jpn J Physiol* 2005;55:149–55.
17 Kessler TM, Fowler CJ. Sacral neuromodulation for urinary retention. *Nat Clin Pract Urol* 2008;5:657–66.
18 Krasmik D, Krebs J, van Ophoven A, *et al*. Urodynamic results, clinical efficacy, and complication rates of sacral intradural deafferentation and sacral anterior root stimulation in patients with neurogenic lower urinary tract dysfunction resulting from complete spinal cord injury. *Neurourol Urodyn* 2014;33:1202–6.
19 Chien CT, Yu HJ, Lin TB, *et al*. Neural mechanisms of impaired micturition reflex in rats with acute partial bladder outlet obstruction. *Neuroscience* 2000;96:221–30.
20 Saito M, Wada K, Kamisaki Y, *et al*. Effect of ischemia-reperfusion on contractile function of rat urinary bladder: possible role of nitric oxide. *Life Sci* 1998;62:PL149–PL156.
21 Saito M, Miyagawa I. Bladder dysfunction after acute urinary retention in rats. *J Urol* 2001;165:1745–7.
22 Herrera GM, Meredith AL. Diurnal variation in urodynamics of rat. *PLoS One* 2010;5:e12298–7.
23 Chang HY, Cheng CL, Chen JJ, *et al*. Serotonergic drugs and spinal cord transections indicate that different spinal circuits

are involved in external urethral sphincter activity in rats. *Am J Physiol Renal Physiol* 2007;292:F1044–F1053.
24 Li Z. *Experimental Acupuncture Science*. Beijing: China Press of Traditional Chinese Medicine, 2007.
25 Gao SH, Lu MX. Survey of point prescriptions of acupuncture treatment for urinary retention. *Zhongguo Zhen Jiu* 2006;26:681–4.
26 Hsu J. *Multiple Comparisons: Theory and Methods*. London: Chapman & Hall, 1996.
27 Mohan R, Tosolini AP, Morris R. Segmental distribution of the motor neuron columns that supply the rat hindlimb: a muscle/motor neuron tract-tracing analysis targeting the motor end plates. *Neuroscience* 2015;307:98–108.
28 Vera PL, Nadelhaft I. Anatomical evidence for two spinal 'afferent-interneuron-efferent' reflex pathways involved in micturition in the rat: a 'pelvic nerve' reflex pathway and a 'sacrolumbar intersegmental' reflex pathway. *Brain Res* 2000;883:107–18.
29 Van Kerrebroeck PE. Advances in the role of sacral nerve neuromodulation in lower urinary tract symptoms. *Int Urogynecol J* 2010;21(Suppl 2):467–74.
30 Jezernik S, Craggs M, Grill WM, *et al*. Electrical stimulation for the treatment of bladder dysfunction: current status and future possibilities. *Neurol Res* 2002;24:413–30.
31 Kovacevic M, Yoo PB. Reflex neuromodulation of bladder function elicited by posterior tibial nerve stimulation in anesthetized rats. *Am J Physiol Renal Physiol* 2015;308:F320–F329.
32 Schultz-Lampel D, Jiang C, Lindström S, *et al*. Experimental results on mechanisms of action of electrical neuromodulation in chronic urinary retention. *World J Urol* 1998;16:301–4.
33 Qin Q, Mo Q, Liu K, *et al*. Acupuncture at homotopic acupoints exerts dual effects on bladder motility in anesthetized rats. *BMC Complement Altern Med* 2015;15:267.
34 Zhang J, Cheng W, Cai M. Effects of electroacupuncture on overactive bladder refractory to anticholinergics: a single-blind randomised controlled trial. *Acupunct Med* 2015;33:368–74.
35 Yang L, Wang Y, Mo Q, *et al*. A comparative study of electroacupuncture at Zhongliao (BL33) and other acupoints for overactive bladder symptoms. *Front Med* 2017;11:129–36.