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Short communication

Neurons in the nucleus tractus solitarius mediate the acupuncture analgesia in visceral pain rats

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ABSTRACT

The study investigated the role of nucleus tractus solitarius (NTS) neurons in electroacupuncture (EA) analgesia in colorectal distension (CRD) rats. NTS neurons responding to both CRD test and EA conditioning stimulations were considered somato-visceral convergent neurons. The neuronal activities evoked by graded CRD showed multiple firing patterns indicating multisynaptic connections. Some of the CRD excitatory neurons were inhibited by EA and vice versa. There was no discrepancy among different acupoints in inducing the changes of unit discharges. Conclusively, EA could regulate CRD related neurons in the NTS through polysynaptic cross-talk mechnism, which mediates EA analgesia on visceral pain in anesthetized rats.

Visceral hypersensitivity is the presence of sensitization of the neural pathways (including primary sensory afferents and spinal ascending neurons) involved in the transmission of visceral sensation to the supraspinal level and the dysregulation of descending pathways that modulate spinal signaling (Mayer and Liebeskind, 1974). Visceral nociceptive information ascends to the thalamus and medullary lateral reticular nucleus in the spinal cord by dorsal midline and lateral spinal pathways (Ness, 1999). Previous study has demonstrated electroacupuncture (EA) can alleviate CRD-induced visceral pain associated with the irritable bowel syndrome in rats (Cui et al., 2005). CRD may cause reduction in the arterial pressure and heart rate in intact rats (Li and Suzuki, 2006; Li et al., 2006). EA at ST36 and PC6 significantly counteracted CRD-induced changes in blood pressure, gastric blood flow, electrogastrogram and gastric tension (Chen et al., 2011). Colorectal discomfort caused by barostat-induced rectal distension was significantly reduced by EA at ST36, PC6 and LI4 compared to sham acupuncture (Leung et al., 2011). Nucleus tractus solitarius (NTS), as the first relay center of the visceral primary afferent, not only regulates respiratory, cardiovascular and gastrointestinal visceral functions, but also plays an important role in modulating visceral pain. The inputs both from viscera and somatic stimulations project to NTS via different pathways (Altschuler et al., 1989). Previous studies demonstrated that acupuncture could decrease the Fos expression and increase the substance P in NTS (Bai and Qiu, 1991; Kwon et al., 2001). It is still unknown whether EA can eliminate CRD induced visceral pain through somato-visceral convergent neurons in the caudal and medial NTS which has been proved to be the subnuclei related to gastrointestinal system and somatic inputs. Here we observe the different responses of NTS neurons to stepped CRD stimulations and compare the effects of acupuncture with different somatic segmental locations on the CRD responsive neurons to investigate the role of somato-visceral convergent NTS neurons in the acupuncture analgesia in visceral pain rat model.

Experiments were conducted in accordance with *the Guide for Care and Use of Laboratory Animals* issued by NIH of the USA and the Institutional Animal Care and Use Committee of the China Academy of Chinese Medical Sciences. Sixty-two male Sprague–Dawley (SD) rats (300–350 g) were kept in an animal house maintained at 21 ± 2 °C with a 12-hour light–dark cycle and given free access to food and water for 2–3 weeks. After the rat was initially anesthetized with an intraperitoneal injection of 10% urethane (1.0 g/kg, Sigma-Aldrich), the left common carotid artery was cannulated for arterial pressure (AP) monitoring. Additional anesthetic (urethane 0.3 g/kg) was given if the animal showed large fluctuations in baseline AP or a withdrawal response to a pinch of the paw. Single unit recording was conducted as described by Gao et al. (Gao et al., 2011). Animals were fixed on a stereotaxic frame (Narishige, Tokyo, Japan). The glass recording electrode was stereotaxically placed at 0.3–0.5 mm rostral to the CS, 0.4–0.8 mm lateral to the midline and 0.2–0.7 mm caudal to the CS, 0.1–0.6 mm lateral to the midline and advanced to a depth of 0.3–0.8 mm from the dorsal surface of the medulla (Gamboa-Esteves et al., 2001a). The signal was amplified with a pre-amplifier (AM-1800, USA) and collected through Micro1401 data acquisition unit and SPIKE2 software (CED, UK). A neuron was considered to be excited or inhibited if its firing rates were increased or decreased over 15%. The recording site in the brainstem was labeled and determined by microscopic examination. Only those data with histological confirmation were accepted for statistical analysis. Stepped CRD test stimulation was conducted with a 7 cm flexible latex balloon inflated to produce 20 mm Hg (non-noxious), 40,

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60 and 80 mm Hg (noxious) pressures separately for 30 s and deflated to avoid wind-up firing of neurons (Juan et al., 2000; Ness, 1999).

EA (2 mA, 0.5 ms, 10 Hz) for 30 s was conducted on the following points as ST36 (Zusanli, located on the anterolateral side of the hindlimb near the anterior crest of the tibia below the knee under the tibialis anterior muscle, innervated by peroneal nerve), PC6 (Neiguan, proximal to the accessory carpel pad of the forelimb between ligaments of the flexor carpi radialis and palmaris longus, innervated by median nerve) and AA (auricular acupoint "Heart", at the inferior concha, with innervations of the auricular branch of the vagus nerve). Acupuncture needles (length, 13 mm; diameter, 0.25 mm; Hwato, China) were inserted perpendicularly 4–5 mm into the unilateral limb points or 1–2 mm into the AA. Data was shown as mean ± SEM. Data sets with normal distribution were analyzed by a paired *t* test for pre and post-stimulations or one-way ANOVA with Dunncan or LSD post hoc test for more than two groups. $P < 0.05$ was considered statistical significance.

Among the recorded one-hundred seventy-eight neurons with spontaneous discharges in NTS, fifty-four were somato-visceral convergent neurons and showed multiple responses to CRD. Twenty-one were CRD excitatory, twenty-four were CRD inhibitory and the other nine switched their responses during stepped CRD stimulations. The twenty-one CRD activated neurons could be further classified into a graded positive pattern ($n = 15$, Fig. 1A) if their firing rates increased in a graded pattern following stepped CRD stimulations as 20, 40, 60, 80 mm Hg, or a flat positive pattern ($n = 6$) if their firing rates increased not on a stepped pattern. Similarly, the twenty-four CRD inhibitory neurons could be further sorted as a graded negative pattern ($n = 17$, Fig. 1B) if their discharges decreased followed a stepped CRD increase, or a flat negative pattern ($n = 7$) if their firing rates decreased not on a stepped pattern. The characteristic description of the NTS CRD responsive neurons was in accordance with descriptions of the responsive patterns of raphe magnus neurons to CRD in the previous study (Brink and Mason, 2004).

Of the twenty-one excitatory neurons recorded mentioned above, EA at AA evoked different changes on them: the firing rates of eight neurons were decreased by EA, two neurons were further excited by EA and the firing rate of the other eleven CRD excited neurons were not changed. With regard to EA at PC6, the firing rates of five CRD excitatory neurons were decreased by EA; two were further excited by EA at PC6; and the other fourteen neurons did not show any changes to EA at PC6 statistically. For EA at ST36, the firing rates of ten CRD excited NTS neurons were decreased by EA; two were further excited; whereas the other nine CRD excited neurons did not exhibit significant changes to EA at ST36 statistically. The results indicate that EA produced reversible effect on some CRD excitatory neurons, but not all. The raw data example and summarized results were shown in Fig. 2A, C. There was no significant difference on the number of neurons on which CRD induced excitatory but EA produced reversible inhibitory changes among acupoints, and also no significant difference was found on their discharge rates ($P > 0.05$). Of the twenty four convergent neurons showing inhibitory responses to CRD, similar patterns of responses to EA at the three acupoints had been found. EA at different acupoints reversed some of the neuronal activities inhibited by CRD (AA: 15; PC6: 9; ST36: 12), but not all. The example and summarized results were shown in Fig. 2B, D. There was no significant difference on the number of neurons on which CRD produced inhibition but EA evoked excitation among acupoints, and also there was no significant difference on their firing rates ($P > 0.05$).

The mechanism for pain modulation by acupuncture contains both spinal and supraspinal centers. The spinal mechanism for pain-relieving effects of acupuncture relays on gate-control theory. It lies that the wide dynamic range (WDR) neurons in the dorsal horn of spinal cord are inhibited by interneurons that are excited by non-noxious inputs in the same segment (Melzack and Wall, 1965). The supraspinal areas that contribute to acupuncture analgesia effects include the periaqueductal grey (PAG) (Mayer and Liebeskind, 1974), the nucleus raphe magnus (NRM), with axons projecting to the dorsal horn and using serotonin as the principal neurotransmitter (Fields et al., 1991). Others as rostral ventral medulla (RVM) neurons can potentiate or suppress central sensory transmission through descending pathways to the spinal cord (Wilder-Smith, 2011). In animal experiments, RVM neurons excited by somatic stimuli can be inhibited by visceral activity and vice versa. (Sikandar and Dickenson, 2011).

Diffuse noxious inhibitory controls (DNIC) system is another system contributed to pain modulation. And the brain structures involved in this modulation are confined to the most caudal part of the medulla (Bouhassira et al., 1995) and lesions of subnucleus reticularis dorsalis (SRD) located ventral to the cuneate nucleus, between trigeminal nucleus caudalis and NTS strongly reduce DNIC (Villanueva and Le Bars, 1995).

Visceral pain elicited from pelvic and most abdominal organs is mediated by spinal visceral afferent neurons but not by vagal afferents (Cervero, 1994). Neurons projecting to the NTS responsive to cutaneous

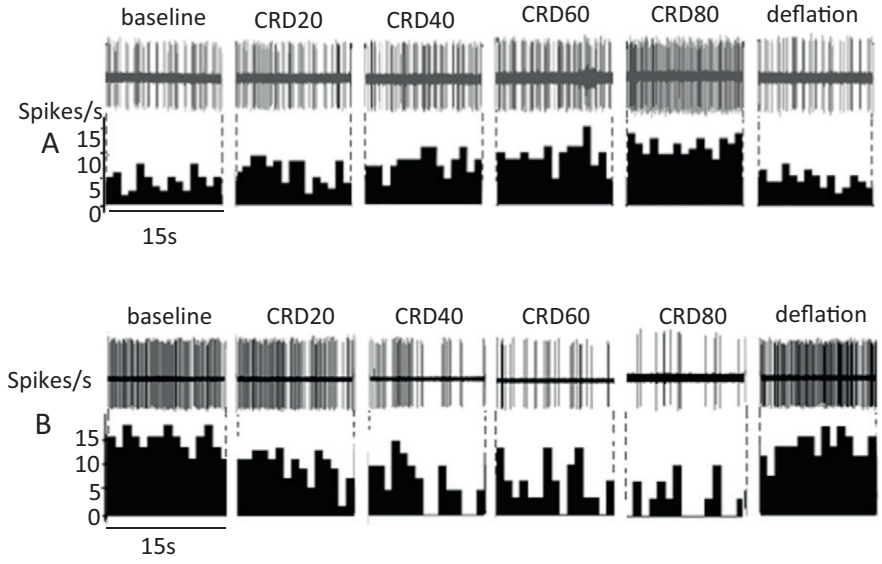

Fig. 1. Examples of the responses of graded positive and graded negative NTS neurons to stepped CRD stimulations. (A) An example of graded positive neuron of which the firing rate is in a stepped pattern; (B) An example of graded negative neuron. The firing rate is decreased by different intensities of CRD in a stepped pattern. For A and B, upper trace is raw data recorded by SPIKE2 software and the lower trace is peristimulus time histograms (PSTHs). Statistical data are not shown.