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Neuronal loss in anterior cingulate cortex in spared nerve injury model of neuropathic pain

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1. Introduction

Damage or disease that affects peripheral nerves frequently leads to development of neuropathic pain, which is characterized by hyperalgesia, allodynia, and spontaneous pain (Woolf and Mannion, 1999). Despite intensive research on neuropathic pain over the past few decades, the precise mechanisms involved are not fully understood, and current treatment for neuropathic pain has limited effectiveness (Wallace, 2007). Therefore, further understanding of the basic mechanisms underlying neuropathic pain development and maintenance in preclinical animal models is critical to providing promising and efficient therapies in patient populations.

It is well known that modulation of pain occurs peripherally at the nociceptor, in the spinal cord, or in the supraspinal structures (Campbell and Meyer, 2006). Several cortical areas are believed to be important for supraspinal nociceptive processing. The anterior cingulate cortex (ACC), an important structure of limbic system, is a key is a key cortical area in processing the affective-motivational pain perception. Despite being controversial (LaGraize et al., 2004; LaBuda and Fuchs, 2005), cumulative evidence from both human and animal studies has indicated that neurons in ACC are important for mediating the sensational aspects of pain (Johansen et al., 2001; Weston, 2012). Tactile allodynia in persistent neuropathic pain engages neural circuits that regulate the affective and motivational components of pain (Chang et al., 2017). Electrical stimulation of the ACC can largely attenuate mechanical allodynia in rats with neuropathic pain (Park et al., 2006).

Chen et al. reveal a direct ACC-spinal projecting pathway that provides rapid and profound modulation of spinal sensory transmission, including painful information (Chen et al., 2014). Stimulation of the ACC can facilitates the rat spinal cord neurons and nociceptive flexion reflex (Calejesan et al., 2000). Studies have shown that excitatory, especially glutamatergic, synaptic transmission in ACC neurons is significantly increased, and more importantly, pharmacologically blocking this synaptic strengthening can reduce behavioral hypersensitivity and prevent development of neuropathic pain (Li et al., 2010; Ning et al., 2013; Xu et al., 2008). In addition, the ACC has a substantial GABAergic inhibitory termination (Markram et al., 2004). However, few studies have investigated modulation of inhibitory transmission in the ACC. A recent study demonstrated that the nociceptive circuitry in the ACC is not exclusively mediated by glutamatergic neurotransmission (Zugaib et al., 2014). Glutamatergic/GABAergic balance is crucial. GABAergic disinhibition can facilitate glutamatergic excitatory transmission in the ACC, exacerbating nociceptive responses.

Peripheral nerve injury can lead to apoptosis of GABAergic neurons in the dorsal horn of the spinal cord (Moore et al., 2002; Scholz et al., 2005). Apoptosis in the cerebral cortex has also been reported in models of neuropathic pain (Fuccio et al., 2009; Leong et al., 2011). Moreover, patients with chronic neuropathic pain showed gray matter loss in some pain-processing cortical regions, including the ACC (Apkarian et al., 2004; DaSilva et al., 2008). In the present study, we showed that peripheral nerve injury causes apoptotic loss of ACC neurons (including GABAergic neurons). This apoptosis is likely mediated by glutamatergic transmission and involves caspase-3 activation. Our data indicate that the pathology of neuropathic pain may be, at least in part, attributed to the death of inhibitory ACC neurons, which results in decreased ACC inhibition.

2. Materials and methods

2.1. Animals

We used male Sprague-Dawley rats (200–250 g; Tongji Medical College Experimental Animal Center, Wuhan, China) for these studies. All animal procedures were approved by the Animal Care and Use Committee of Tongji Medical College, Huazhong University of Science and Technology, and were performed in accordance with the guidelines of the Committee for Research and Ethical Issues of International Association for the Study of Pain (IASP). All experiments were performed blindly so that the investigators were not aware of which experimental group they were evaluating. Schematic diagram of study design is shown in Fig. 1.

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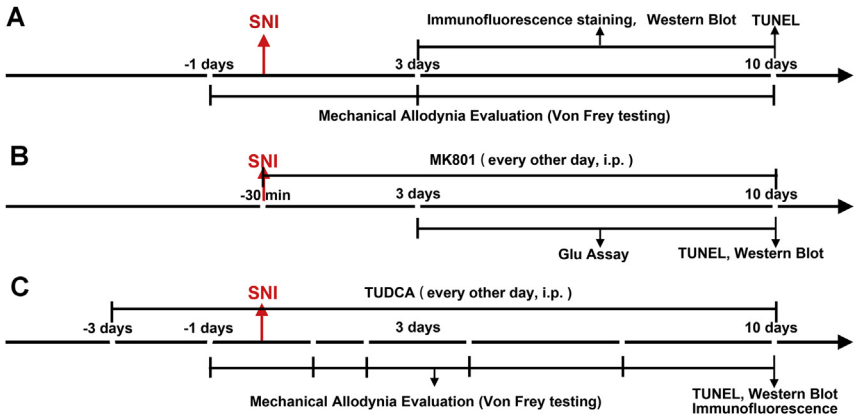

Fig. 1. Schematic diagram of study design. (A) Sprague-Dawley rats were randomly divided into Sham (n = 12) and SNI group (n = 12). Three and ten days after SNI, changes in ACC neurons were detected by immunohistochemistry and immunoblotting techniques using a monoclonal antibody against NeuN. And to detect apoptosis, double-staining with Casp-3a and NeuN antibodies, and TUNEL staining were performed. (B) Rats were randomly divided into 3 groups (n = 6): 1) Sham, 2) SNI + saline, and 3) SNI with MK801. Injections were administered 30 min before skin incision, and subsequent doses were given every other day. Glutamate concentration in bilateral ACC tissue was assayed spectrophotometrically. TUNEL staining was used to observe apoptosis, and expression of Casp-3a within ACC was measured by western blot 10 days after surgery. (C) Thirty-six Sprague-Dawley rats were randomly divided into 3 groups (n = 12): Sham group, SNI + saline group and SNI + TUDCA group. Rats in SNI + saline group and SNI + TUDCA group were intraperitoneally injected TUDCA (300 mg/kg) every other day, starting 3 day before SNI procedure. The mechanical withdrawal threshold was assessed with von Frey filaments in all groups. We immunolabeled makers of neurons and GABAergic inhibitory interneurons, respectively, and TUNEL staining was performed 10 days after SNI.

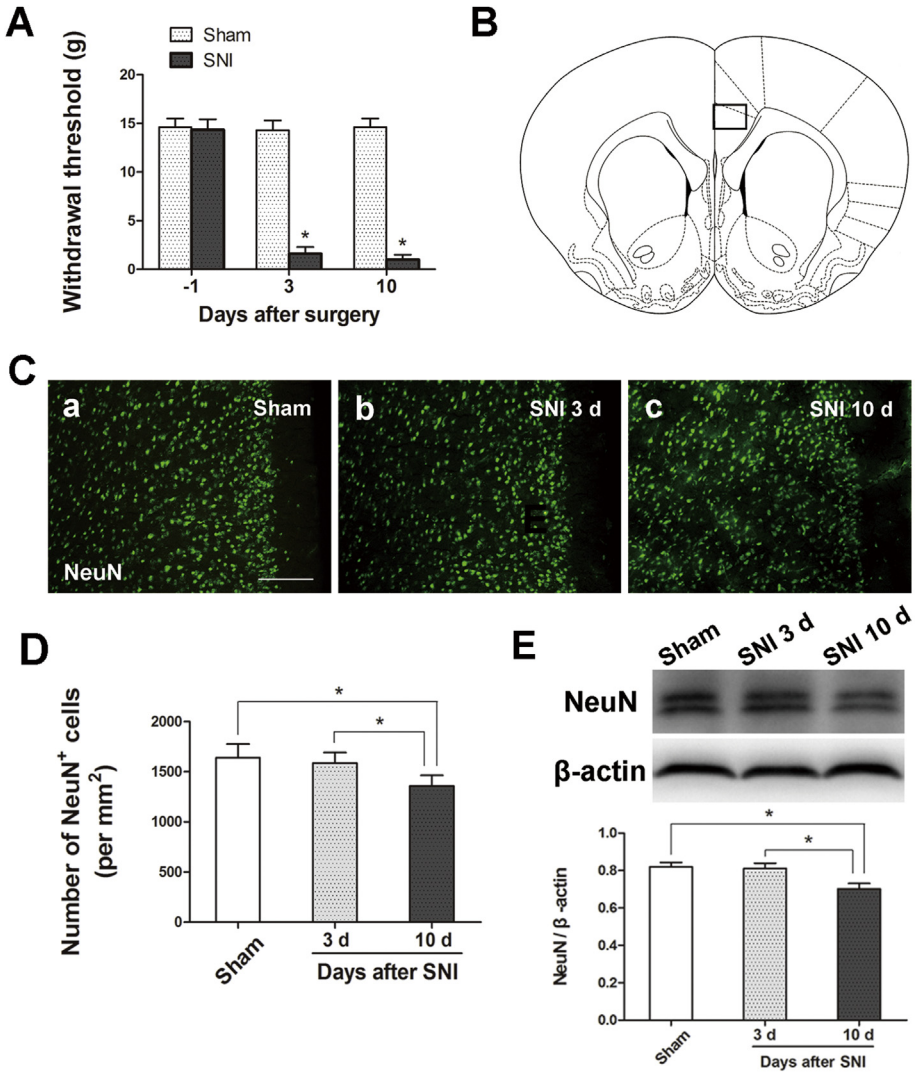

Fig. 2. SNI-induced ACC neuronal loss. (A) Mechanical withdrawal thresholds measured by von Frey filaments ipsilateral to the SNI were significantly decreased when compared with those observed in sham-operated rats. Results are expressed as mean ± SD (n = 6, for each group); *P < 0.01 *vs.* sham. (B) Representative cross section through the ACC, 1.7 mm rostral to bregma. Black box (1.026 mm × 0.769 mm) inset denotes the area from which images in (C, a-c) were obtained. (C) SNI resulted in a significant loss of ACC neurons. Images of NeuN immunoreactivity in the ACC of rats receiving sham surgery (a), 3 d after SNI (b), and 10 d after SNI (c). Scale bar, 200 μm. (D) NeuN-positive cells were counted on both sides of the ACC using the black box shown in B. *P < 0.01. (E) western blots of NeuN expression in the ACC of sham- and SNI-operated rats. Results are expressed as mean ± SD (n = 3, each group); *P < 0.01.

2.2. Spared nerve injury (SNI) model

A model of persistent peripheral neuropathic pain was induced by a SNI procedure according to the method described by Decosterd and Woolf (2000). Rats were anesthetized with sodium pentobarbital (40–50 mg/kg, i. p.). For the SNI, the common peroneal and tibial branches of the sciatic nerve were tightly ligated with a 5–0 silk suture and sectioned distal to the ligation, removing a 2–4 mm length of each distal nerve stump. Great care was taken to avoid damaging the intact sural nerve. The surgical procedure for sham-operated rats involved exposure of the sciatic nerve and its branches without manipulation.