Neural Substrate of Chronic Neuropathic Pain in Mice Revealed by Manganese-enhanced MRI

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Introduction Most of the previous neuroimaging studies investigating the neural substrate of pain used experimental stimuli inducing acute pain [1]. Only few studies have studied the neural substrate of chronic neuropathic pain, one form of pain that is the most clinically relevant and difficult to treat [1, 2]. Yet the findings from these studies are still far from conclusive. Manganese-enhanced magnetic resonance imaging (MEMRI) has been widely used to study functional neural activities in conscious free-moving animals [3]. In this study, MEMRI was employed to study the brain activation/deactivation patterns in a mice model of chronic neuropathic pain induced by chronic constriction injury (CCI).

Materials and Methods Female Kunming mice weighing 20-23 g were used. All procedures were conducted in strict adherence to the local guidelines on ethical use of experimental animals. Two groups of animals were studied: the CCI group (n=19) and the sham-operated group (n=13). The right sciatic nerves of the CCI mice were exposed and ligated according to a procedure developed by Bennett et al [4]. The sham mice received similar surgical procedures, but no ligation of the sciatic nerve. Spontaneous pain and thermal hyperalgesia behaviors (i.e., by a hotplate test [5]) were observed and measured before operation and between 3d-13d post-operation. On 13d post-operation, all mice received an intraperitoneal injection of 35 mM $MnCl_2$ solution (1.5 ml/100 g body weight). The mice were imaged 24 hrs later on a Bruker Biospec 4.7 T/30 cm spectrometer under a 10% urethane anesthesia. T_1-weighted images were acquired with a volume coil using a 3D gradient echo pulse sequence (TR/TE=35/5 ms). The spatial resolution of acquired images was 150 μm×150 μm×300 μm. Using landmark anatomical structures as the references, the 3D image datasets were coregistered to a standard stereotactic space built from the digital Paxinos mouse brain atlas, followed by spatial smoothing with a 0.4-mm FWHM kernel. For each individual mouse, the voxel signal intensity was normalized to the averaged signal intensity of the whole brain. The preprocessed images from the CCI and sham mice were then entered into a random-effect two-sample *t*-test in SPM2 to reveal inter-group differences.

Results The CCI mice displayed spontaneous pain behaviors throughout the observation period. The CCI procedure induced progressively-developing thermal hyperalgesia behaviors, which became statistically significant on 10d post-operation and thereafter (Fig.1, left). The MEMRI results showed that, compared to the sham-operated mice, the CCI mice had significantly increased neural activities (Fig.1, middle) in the contralateral primary/secondary somatosensory areas (S1/S2), amygdale (Amy), cingulate/retrosplenial cortical areas (cg/RS), insular cortex, primary and secondary motor areas (M1/M2), piriform cortex (PC) and ventral endopiriform nucleus (VEn), and decreased neural activities in bilateral laterodorsal thalamic nucleus, ventral tegmental area (VTA), dentate gyrus (DG), lateral periaqueductal gray (LPAG), anterior pretectal nucleus (APT), parabrachial pigmented nucleus (PBP), peripeduncular nucleus (PP) and pontine nuclei (Pn).

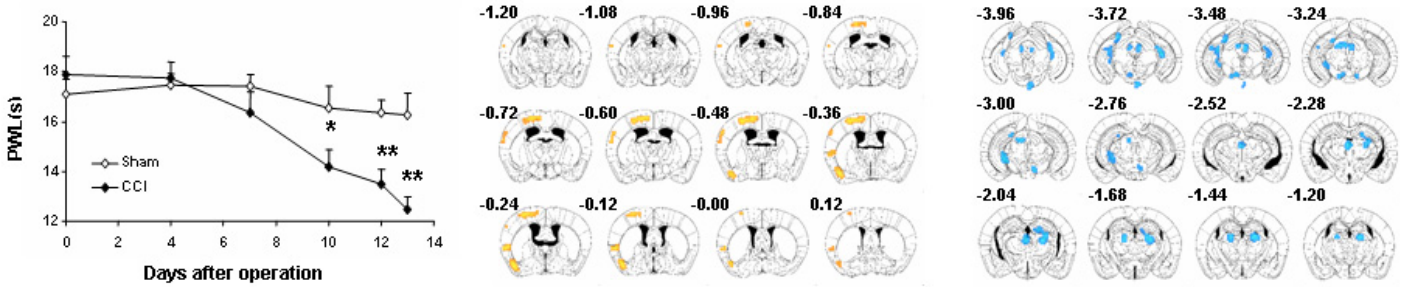

Fig. 1 Left: Hindpaw withdrawal latencies (PWL) (i.e., on a 46 °C hotplate) of the CCI and sham mice after operation. *p< 0.05 and **p<0.01 compared to the sham mice. Middle and right: Brain regions in the CCI mice showing significantly increased (middle panel, warm color overlay, CCI>sham) and significantly decreased (right panel, cold color overlay, CCI<sham) neural activities (p<0.005, uncorrected, cluster threshold=15 voxels), compared to the sham mice. The background figures of brain slices were taken from digital Paxinos mouse brain atlas, with the distance between the corresponding brain slice and bregma (i.e., in mm) labeled at the left upper corners. The left side of the image corresponds to the left side of the brain.

Discussion and Conclusion MEMRI results demonstrated that the CCI mice had significantly increased neural activities in the contralateral S1/S2, insula, cg/RS, Amy, M1/M2, and deactivation in the bilateral laterodorsal thalamic nucleus. These results are in good agreement with the results obtained in previous studies on neuropathic pain [2, 6-7]. In addition, it was found that the CCI mice exhibited significantly increased neuronal activities in the contralateral PC and VEn and significant deactivation in the endogenous antinociceptive system (LPAG, APT, PP, PBP and Pn) and in the brain regions involved in the reward and memory circuits (VTA and DG). The PC has been shown to participate in acute and deep pain [8, 9], and the VTA and DG have also been shown to be also involved in pain [10, 11]. Deactivation in PAG and habenular nuclei as structures in the endogenous antinociceptive system has been reported in neuropathic pain [12]. In summary, our data indicate that neuropathic pain induced by CCI involves not only neural activation of the supraspinal structures participating in pain perception and modulation but also deactivation of the descending antinociceptive system and the reward system.

Acknowledgements Supported by a 973 grant (2006CB705600) from the Ministry of Science and Technology of China.

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