Brain remodeling after chronic median nerve compression in a rat model
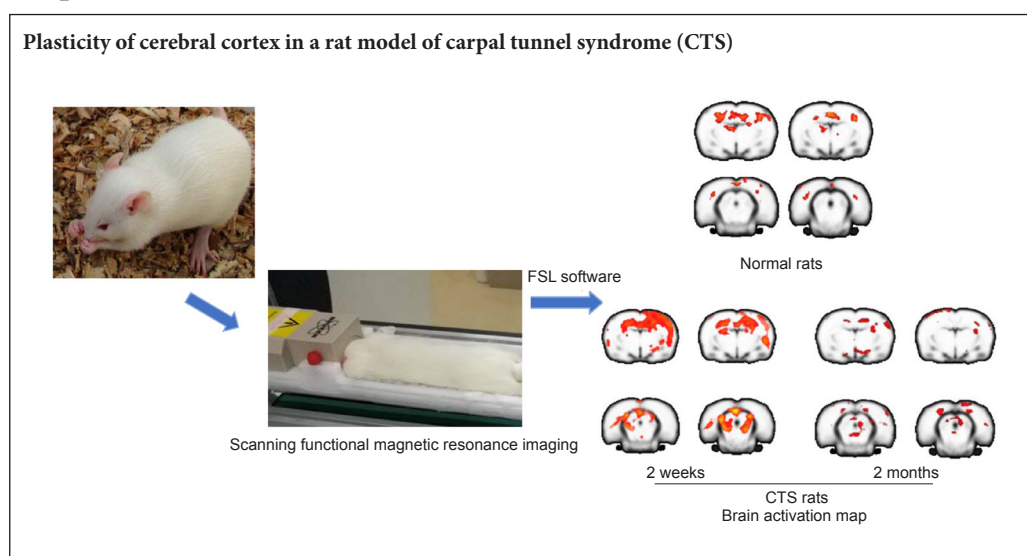
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Graphical Abstract

Plasticity of cerebral cortex in a rat model of carpal tunnel syndrome (CTS)

Scanning functional magnetic resonance imaging

FSL software

Normal rats

2 weeks — 2 months

CTS rats
Brain activation map

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Abstract

Carpal tunnel syndrome is the most common compressive neuropathy, presenting with sensorimotor dysfunction. In carpal tunnel syndrome patients, irregular afferent signals on functional magnetic resonance imaging are associated with changes in neural plasticity during peripheral nerve injury. However, it is difficult to obtain multi-point neuroimaging data of the brain in the clinic. In the present study, a rat model of median nerve compression was established by median nerve ligation, *i.e.*, carpal tunnel syndrome model. Sensory cortex remodeling was determined by functional magnetic resonance imaging between normal rats and carpal tunnel syndrome models at 2 weeks and 2 months after operation. Stimulation of bilateral paws by electricity for 30 seconds, alternating with 30 seconds of rest period (repeatedly 3 times), resulted in activation of the contralateral sensorimotor cortex in normal rats. When carpal tunnel syndrome rats received this stimulation, the contralateral cerebral hemisphere was markedly activated at 2 weeks after operation, including the primary motor cortex, cerebellum, and thalamus. Moreover, this activation was not visible at 2 months after operation. These findings suggest that significant remodeling of the cerebral cortex appears at 2 weeks and 2 months after median nerve compression.

Key Words: nerve regeneration; peripheral nerve injury; carpal tunnel syndrome; functional magnetic resonance imaging; remodeling; chronic nerve compression; cortical reorganization; sensorimotor function; brain; neural regeneration

Introduction

Carpal tunnel syndrome (CTS) is the most clinically common compressive neuropathy of the upper extremities, and affects many individuals (Stapleton, 2006). Specifically, it has a prevalence of 3–5% in the general population and 6% in females over the age of 40 years (Grace et al., 2010). It causes altered sensation, chronic pain, and partial thenar atrophy, which can lead to hand dysfunction (Kleopa, 2015; Padua et al., 2016; Dec and Zyluk, 2018). Previous studies have shown that CTS, accompanied by chronic nerve compression with compressive neuropathy, can induce changes in the structure and microvasculature of peripheral nerves (Bai et al., 2016; Chen et al., 2016). Further, CTS is also characterized by structural (Maeda et al., 2013, 2016) and functional (Druschky et al., 2000; Napadow et al., 2006; Dhond et al., 2012; Maeda et al., 2014) neuroplasticity in the primary somatosensory cortex (S1) of the brain.

CTS leads to altered afferent processing throughout the somatosensory system (involving both the peripheral and central nervous systems), as measured by somatosensory evoked potentials in the spinal cord, brainstem, and
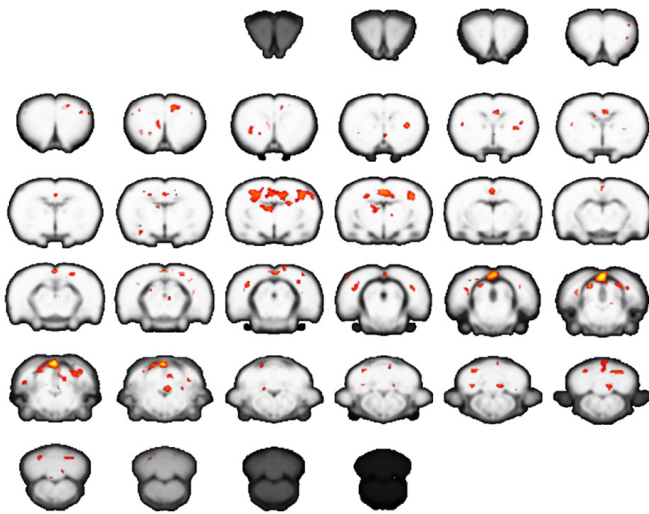
Figure 1 Brain activation map for normal control rats (functional magnetic resonance imaging).
Sensory stimulation to either forepaw generates significant activation of the contralateral sensorimotor cortex. Red-yellow color demonstrates significant positive activation within groups.
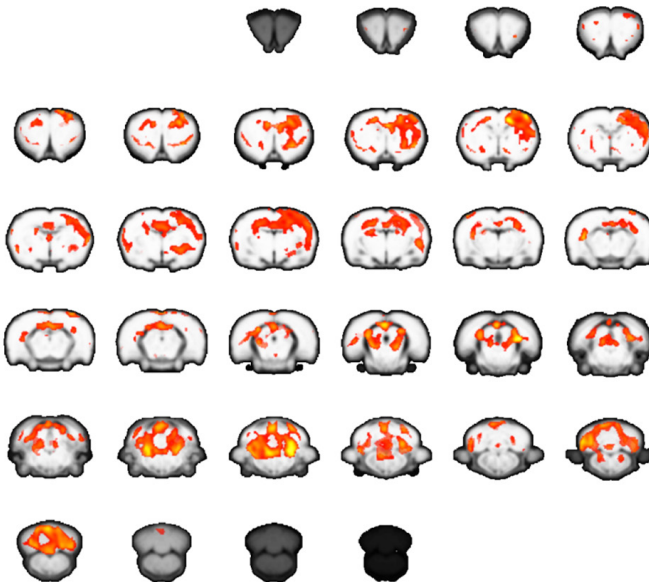

Figure 2 Brain activation map for carpal tunnel syndrome rats at 2 weeks after operation (functional magnetic resonance imaging).
Sensory stimulation to the affected right forepaw generates a strong signal change in the contralateral primary motor area (M1) and sensory cortex. Additional activation was found in the cerebellum and thalamus. Red-yellow color demonstrates significant positive activation within groups.
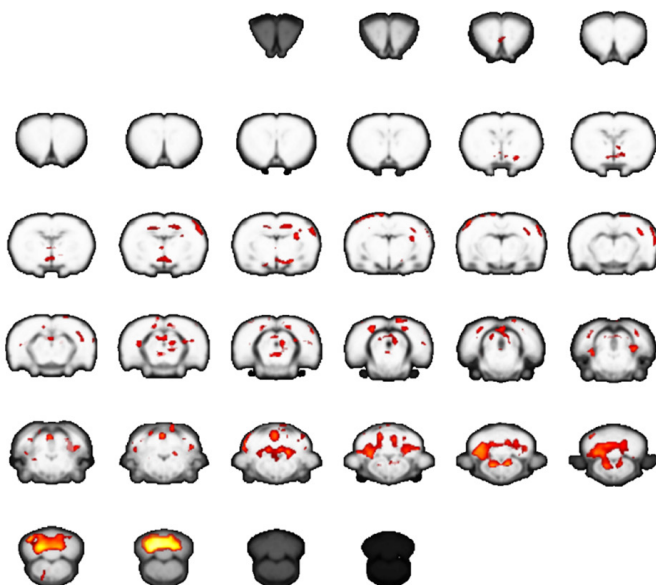

Figure 3 Brain activation map for carpal tunnel syndrome rats at 2 months after operation (functional magnetic resonance imaging).
Higher-level analysis between groups shows considerably weaker activation in the contralateral primary motor area (M1) and sensory cortex at 2 months in carpal tunnel syndrome rats than normal control rats. Red-yellow color demonstrates stronger activation in the control group compared with the carpal tunnel syndrome group.

primary sensorimotor cortex (Maeda et al., 2013b, 2017). The finger and toe digits occupy a significant portion of the human somatotopic map in the primary somatosensory cortex, and are represented in consecutive order along the postcentral gyrus, with digit 1 (D1) being most ventrolateral and digit 5 (D5) most dorsomedial (Maeda et al., 2014, 2016, 2017). Although chronic nerve compression is a peripheral neuropathy, neuroimaging data suggests that irregular afferent signals of CTS produce maladaptive central neuroplasticity (Napadow et al., 2007). For example, spinal amplification of event-related potentials to ulnar nerve stimulation of the CTS-affected hand is thought to represent unmasking of secondary inputs that are normally silent in median nerve signaling. Similarly, studies have recently reported that during stimulation of median nerve-innervated digits, early cortical amplification can evoke responses and alter S1 digit somatotopy. Corroboratively, these findings have been verified by functional magnetic resonance imaging (fMRI) (Khosrawi et al., 2012; Beissner et al., 2013; Kim et al., 2015).

Nonetheless, there are limited longitudinal studies on plasticity in the somatosensory cortex, as it is difficult to acquire multi-point neuroimaging data clinically. Thus, to address this, we developed a rat model of CTS and investigated cerebral plasticity using small animal fMRI.

Materials and Methods

Animals

Forty female Sprague-Dawley rats weighing from 250 g to 300 g and aged 8 weeks were provided by the Animal Center of the Medical College of Shanghai Jiao Tong University, China (license No. SYXK (Hu) 2016-0020). All rats were housed at 20–25°C and 50 ± 5% humidity with free access to food and water in 12-hour light/dark cycles. All procedures and animal experiments were approved by the Animal Care and Use Committee of Shanghai Jiao Tong University, China (approval No. 2017-0124). Rats were randomly divided into a CTS group (*n* = 20; chronic median nerve compression) and normal group (*n* = 20).

Rat model establishment

Rats were intraperitoneally anesthetized with pentobarbital sodium (40 mg/kg; Shanghai Longsheng Chemical Co., Ltd., Shanghai, China). A dorsal gluteal splitting approach was used to expose the right median nerve of each rat. The right median nerve at the wrist level was mobilized and a 10-0 prolene suture used for median nerve ligation. This entire procedure was performed using a microscope (Shanghai Eder Medical Technology Inc. Shanghai, China) at 10× magnification. Finally, the incision was closed across all layers, with a tension-free skin closure performed in accordance with previously published methods (Atroshi et al., 1999b; Grace et al., 2010).

MRI acquisition

Rats received fMRI pre-operation, and 2 weeks and 2 months after operation. During the scans, their bilateral paws were stimulated by electricity for 30 seconds, alternating with 30 seconds of rest periods. Three blocks of each were used.

All fMRI scans were performed using a 7.0T horizontal-bore Bruker scanner (Bruker Corporation, Karlsruhe, Germany), which was equipped with a gradient system of 116 mm inner diameter and maximum gradient strength of 400 mT/m. fMRI scanning was performed to investigate cortical plasticity. Rats were anesthetized by sevoflurane inhalation (3% in oxygen) (Shanghai Longsheng Chemical Co., Ltd.), and then fixed on the scanner with the necessary ventilator support. A single transmitting and receiving surface coil consisting of a single copper-wire loop was used. For functional imaging, an interleaved single-shot echo planar imaging (EPI) sequence was applied with the following parameters: flip angle, 90°; slice thickness, 0.5 mm; repetition time, 3,000 ms; echo time, 20 ms; number of averages, 1; and field of view, 32 mm × 32 mm with 64 × 64 points. EPI fMRI volumes covered a relatively restricted area centered approximately on bregma point. The whole scan began with a dummy epoch of 8 seconds, which was automatically discarded by the system. Both "ON" and "OFF" epochs lasted for 30 seconds and these two epochs sequentially formed one cycle. A total of six cycles were performed in one stimulation session, during which only one side was stimulated with electric needles in the palm position.

Imaging preprocessing

There are several preprocessing steps that must be performed before data analysis. All images had their pixel dimensions scaled up by a factor of 10 in the Nifti header to avoid scale-dependent issues using standard FSL software (Oxford University, Oxford, UK). Apart from brain extraction and band-pass filtering, all steps were performed using the MELODIC graphical user interface. Preprocessing steps included:

(1) Brain extraction: brain extraction was manually performed. Specifically, masks were manually created by masking all slices from the first volume of each individual rat to generate a mask file. These mask files were then applied to all volumes in each functional image.

(2) Band-pass filtering: functional images were band-pass filtered between 0.01 and 0.1 Hz.

(3) Slice timing correction: because each slice was acquired in interleaved order (0, 2, 4, 6 …1, 3, 5, 7 …), interleaved slice timing correction was used.

(4) Spatial smoothing: functional data were spatially smoothed to minimize minor registration imperfections. Because we were interested in large-scale networks across the whole brain of a young rat, Gaussian kernel full width

at half maximum (FWHM) of 0.7 mm was used to pre-process data and identify relatively large areas of coherent activity.

(5) Normalization to standard space: animals slightly differ in brain size, which must be taken into account. Therefore, before brain network analysis, individual brains were registered to a standardized anatomical image (see below). Registration of fMRI data to a standard space (in-house adult anatomical rat brain template) was performed using FSL's flirt, with a freedom affine transformation of 12° and resampling resolution of 0.4 mm. Consequently, for registration, affine transformation was used to ensure proper alignment of each individual rat to the adult rat brain atlas. This step is a pre-requisite for group analysis to identify common networks across all animals. Common expected minimal artifacts were detected across all animals in the brain.

(6) Post analysis: higher-level analysis was performed using a general linear model. One-sample t-test was first performed in each group for determining the significant area within the group (false discovery rate, FDR correction, $P < 0.05$). The significant area in each group was extracted and combined into one binary mask. Subsequently, a two-sample t-test was performed within the boundary of the previously-generated mask (FDR correction, $P < 0.05$). MRIcroGL software (Bonilha et al., 2016) was used to visualize the results.

Results

Intragroup differences in the sensory stimulus task at 2 weeks after operation
In control rats, stimulation to either forepaw generated significant activation of the contralateral sensorimotor cortex. However, in rats with CTS, stimulation to the affected right forepaw at 2 weeks after operation generated a strong signal change in the contralateral primary motor area (M1) and sensory cortex. Additional activation was observed in the cerebellum and thalamus.

Intergroup differences in the sensory stimulus task at 2 weeks and 2 months after operation
The extent of activation in the brain was greater in CTS rats than normal control rats at 2 weeks. However, activation in the contralateral primary motor area (M1) and sensory cortex at 2 months was much weaker compared with normal control rats. These results suggest there is dynamic plasticity in the sensorimotor cortex of CTS rats (**Figures 1–3**).

Discussion
Peripheral entrapment neuropathies are common sources of pain and paraesthesia (Neal and Fields, 2010) in clinical practice (Atroshi et al., 1999a; Wilson d'Almeida et al., 2008; Foley and Silverstein, 2015). Entrapment of

the median nerve at the wrist, called CTS, accounts for 90% of such neuropathies (Papanicolaou et al., 2001; Kleopa, 2015). Here, we demonstrate a dynamic plastic process of cortical reorganization in CTS rats using a long-term study. Our results show that the sensory map of the affected forepaw expands at the early stage, and then shrinks at the later stage. This suggests a compensatory process in the brain of CTS rats. Similarly, previous neuroimaging studies have shown that while CTS results from compression of the median nerve at the wrist, it is also characterized by structural and functional neuroplasticity in the brain (Tecchio et al., 2002; Maeda et al., 2017). Specifically, CTS patients show decreased primary somatosensory cortex (S1) gray matter volume and cortical thickness contralateral to the affected hand, which is pronounced in paraesthesia dominant symptom subgroups and associated with aberrant median nerve conduction. Further, fMRI shows reduced separation between S1 cortical representations of adjacent median nerve-innervated fingers, digits 2 and 3 (D2/D3), which is a reproducible finding in different CTS cohorts using both fMRI and magnetoencephalography. Reduced D2/D3 separation in S1 is associated with median sensory nerve conduction latency, symptom severity, reduced fine motor performance, and diminished sensory discrimination accuracy, demonstrating that functional brain neuroplasticity is indeed maladaptive (Baraban et al., 2016; Maeda et al., 2016, 2017).

In our present study, block-design stimulation of the affected forepaw generated significant activation in the contralateral sensorimotor cortex in normal control rats. However, the same stimulation in CTS rats at the early stage generated extended activation in the contralateral hemisphere, including the primary sensorimotor cortex, cerebellum, and thalamus. This suggests that the brain attempts to compensate for sensory loss after median nerve entrapment by enlarging central representation in the sensorimotor cortex and related brain regions of sensorimotor networks. Interestingly, brain activation decreased remarkably in CTS rats at the later stage. This suggests a maladaptive process in the brain after median nerve entrapment. Possibly with continuously decreased sensory input, the brain is unable to maintain control of the affected forepaw.

A limitation of our study is that the sensory nerve action potential test was difficult to perform in the rat model. Consequently, we were unable to obtain enough clinical neurophysiology data. Indeed, there were only two time-points in the follow-up investigation. In further studies, we would overcome this limitation by performing more investigations.

In conclusion, our results strongly support a dynamic plastic process after median nerve entrapment. Cortical reorganization is the foundation of sensorimotor func-

tion recovery and may be a treatment biomarker. Our future study will quantify the functional differences so as to objectively compare the temporal changes.

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