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Longitudinal Structural and Functional Brain Network Alterations in a Mouse Model of Neuropathic Pain

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Abstract—Neuropathic pain affects multiple brain functions, including motivational processing. However, little is known about the structural and functional brain changes involved in the transition from an acute to a chronic pain state. Here we combined behavioral phenotyping of pain thresholds with multimodal neuroimaging to longitudinally monitor changes in brain metabolism, structure and connectivity using the spared nerve injury (SNI) mouse model of chronic neuropathic pain. We investigated stimulus-evoked pain responses prior to SNI surgery, and one and twelve weeks following surgery. A progressive development and potentiation of stimulus-evoked pain responses (cold and mechanical allodynia) were detected during the course of pain chronification. Voxel-based morphometry demonstrated striking decreases in volume following pain induction in all brain sites assessed – an effect that reversed over time. Similarly, all global and local network changes that occurred following pain induction disappeared over time, with two notable exceptions: the nucleus accumbens, which played a more dominant role in the global network in a chronic pain state and the prefrontal cortex and hippocampus, which showed lower connectivity. These changes in connectivity were accompanied by enhanced glutamate levels in the hippocampus, but not in the prefrontal cortex. We suggest that hippocampal hyperexcitability may contribute to alterations in synaptic plasticity within the nucleus accumbens, and to pain chronification.

Key words: neuropathic pain, translational neuroimaging, magnetic resonance spectroscopy (MRS), voxel-based morphometry (VBM), resting-state functional magnetic resonance imaging (rs-fMRI), cold and mechanical allodynia.

INTRODUCTION

It has been suggested that central rather than peripheral factors are important in pain chronicity and that chronic pain is characterized by structural and functional changes within the central nervous system. The chronic pain state affects multiple brain functions, including motivational processing (Attal et al., 2011; Langley et al., 2013). Furthermore, increasing evidence suggests that the dopaminergic reinforcement system plays a critical role in mediating the negative affective perception of painful stimuli and altered motivational processing in a chronic pain state (Mitsi and Zachariou, 2016). This system evaluates the motivational value of a given stimulus, and depends primarily on the activity of dopaminergic neurons in the ventral tegmental area that project to the nucleus accumbens (NAc) and medial prefrontal cortex (PFC) (Spanagel and Weiss, 1999; Lammel et al., 2014). In addition to dopaminergic activity, glutamatergic input to the NAc is essential for motivational processing and mood regulation, especially from the PFC, hippocampus and other areas (Noori et al., 2012; Lammel et al., 2014; Thompson et al., 2015). Long-term increases in synaptic efficacy of glutamatergic input to the NAc may result in hyperexcitability and contribute to the plastic changes seen during pain chronification (Ji et al., 2003; Bleakman et al., 2006; Osikowicz et al., 2013; Zhuo, 2017). The impact of pain on the dopaminergic reinforcement system, including glutamate signaling from different brain sites, is supported by pharmacological, biochemical

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and electrophysiological data in various animal models (for review see Borsook et al., 2007; Navratilova and Porreca, 2014; Mitsi and Zachariou, 2016). There have been several longitudinal imaging studies performed in SNI animals (Seminowicz et al., 2009; Baliki et al., 2014; Hubbard et al., 2015) in which they performed fMRI and also MRS measurements at different time points before and after SNI.

These preclinical findings appear to translate to the human situation. For example, functional neuroimaging studies have shown negative changes in the BOLD signal in the NAc following the presentation of painful stimuli (Becerra et al., 2001), and the NAc is directly activated in anticipation of painful stimuli (Jensen et al., 2003; Baliki et al., 2010). Furthermore, high rates of comorbid chronic pain are common in disorders with deficits in the dopaminergic reinforcement system, including affective and substance use disorders (Jarcho et al., 2012). These psychiatric disorders are also accompanied by enhanced glutamate levels in the PFC and hippocampus (Hermann et al., 2012; McEwen et al., 2012; Frischknecht et al., 2017; Lefebvre et al., 2017). In summary, animal and human studies suggest that (i) the dopaminergic reinforcement system mediates the negative affective component of pain and altered motivational processing in the chronic pain condition and (ii) glutamatergic hyperexcitability in the PFC and hippocampus –regions that project to the NAc – may be one mechanism that leads to changes in synaptic plasticity within the NAc during pain chronification.

One fundamental question in pain research is: What are the structural and functional changes involved in the transition from an acute to a chronic pain state? Answering this question requires longitudinal studies. Specifically, longitudinal multimodal neuroimaging studies are helpful for studying structural and functional variations in the pain and reinforcement network (Alomar and Bakhaidar, 2016). For example, neuroimaging studies have demonstrated a reorganization of brain structures associated with chronic pain (Baliki et al., 2006, 2008; Alomar and Bakhaidar, 2016), and two longitudinal multimodal neuroimaging studies in humans and rats revealed reduced gray matter density in the NAc and a greater functional connectivity of NAc and PFC, respectively, that were predictive of pain chronification (Baliki et al., 2012; Chang et al., 2014).

Despite these advances in understanding pain chronification, the degree to which alterations within the pain and motivational neuronal networks are related to the affective/motivational component of chronic pain remains unclear. Hence, clear relationships between structural and functional brain changes and affective/motivational alterations occurring during pain chronification has yet to be demonstrated. This aspect is particularly important, as ongoing pain, which involves negative affective components and changes in motivational processing, is the predominant complaint of clinical patients.

Here we studied in a longitudinal fashion pain-related behavioral changes with metabolic, structural and functional alterations within the pain and motivational/affective neuronal networks. We used the chronic neuropathic pain model of partial denervation in mice, known as spared nerve injury (SNI), and monitored stimulus-evoked pain responses at an early state and up to twelve weeks following injury. In parallel, we monitored changes in brain glutamate levels and other metabolites, structure and connectivity in pain and motivational/affective neuronal networks by longitudinal multimodal neuroimaging with magnetic resonance spectroscopy (MRS), voxel-based morphometry (VBM), and resting-state functional magnetic resonance imaging (rs-fMRI), respectively.

EXPERIMENTAL PROCEDURES

Animals: Forty C57BL/6N male mice from our breeding colony at the CIMH, Mannheim, Germany were used (8–10 weeks old at the beginning of the experiments). Mice were single-housed in standard hanging cages at 21 ± 1 °C and 50 ± 5% relative humidity on a reversed 12-h light/dark cycle, with lights on at 7:30 p.m. The animals were provided with standard rodent food and tap water *ad libitum*. All mice were handled on a daily basis before starting the experiments and were habituated to the behavioral testing environments. All experiments were performed during the dark cycle, between 10:00 a.m. and 15:00 a.m. Procedures for this study complied with the regulations covering animal experimentation within the European Union (European Communities Council Directive 86/609/EEC) and Germany (Deutsches Tierschutzgesetz) and the experiment was approved by the German animal welfare authorities (Regierungspräsidium Karlsruhe).

Neuropathic SNI model

The SNI model of chronic neuropathic pain was induced by the axotomy of the tibial and common peroneal nerves, leaving the sural nerve intact, as described in detail previously (Decosterd and Woolf, 2000). The SNI is a robust model for partial nerve injury leading to early (within few days) and prolonged (over months) increased cold allodynia and mechanical hypersensitivity mostly in the sural territory of the hindpaw.

Reflexive pain measurements

The testing of cold and mechanical allodynia took place one week prior to SNI surgery (Pre), and one (Post_1w) and twelve weeks (Post_12w) following surgery, during the dark/active phase of the day.

Cold-plate test. For assessing cold allodynia (described in detail in Pitzer et al., 2016), mice were placed on a cold plate (Bioseb, Vitrolles, France) at 2 °C and paw withdrawal latencies of the injured hindpaw were measured. Cut-off latencies were set at 30 s.

Von Frey's test. Mechanical sensitivity was assessed as previously described (Pitzer et al., 2016). Mice were placed briefly on an elevated wire mesh grid and the sural nerve territory of the hindpaw was stimulated with

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