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Manganese-enhanced magnetic resonance imaging depicts brain activity in models of acute and chronic pain: A new window to study experimental spontaneous pain?

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ABSTRACT

Application of functional imaging techniques to animal models is vital to understand pain mechanisms, but is often confounded by the need to limit movement artefacts with anaesthesia, and a focus on evoked responses rather than clinically relevant spontaneous pain and related hyperalgesia. The aim of the present study was to investigate the potential of manganese-enhanced magnetic resonance imaging (MEMRI) to measure neural responses during on-going pain that underpins hyperalgesia in pre-clinical models of nociception. As a proof of concept that MEMRI is sensitive to the neural activity of spontaneous, intermittent behaviour, we studied a separate positive control group undergoing a voluntary running wheel experiment.

In the pain models, pain behaviour (weight bearing asymmetry and hindpaw withdrawal thresholds (PWTs)) was measured at baseline and following either intra-articular injection of nerve growth factor (NGF, 10 μg/50 μl; acute pain model, n=4 rats per group), or the chondrocyte toxin monosodium iodoacetate (MIA, 1 mg/50 μl; chronic model, n=8 rats per group), or control injection. Separate groups of rats underwent a voluntary wheel running protocol (n=8 rats per group). Rats were administered with paramagnetic ion Mn^{2+} as soluble $MnCl_2$ over seven days (subcutaneous osmotic pump) to allow cumulative activity-dependent neural accumulation in the models of pain, or over a period of running. T1-weighted MR imaging at 7 T was performed under isoflurane anaesthesia using a receive-only rat head coil in combination with a 72 mm volume coil for excitation.

The pain models resulted in weight bearing asymmetry (NGF: 20.0 ± 5.2%, MIA: 15 ± 3%), and a reduction in PWT in the MIA model (8.3 ± 1.5 g) on the final day of assessment before undergoing MR imaging. Voxel-wise and region-based analysis of MEMRI data did not identify group differences in T1 signal. However, $MnCl_2$ accumulation in the VTA, right Ce amygdala, and left cingulate was negatively correlated with pain responses (greater differences in weight bearing), similarly $MnCl_2$ accumulation was reduced in the VTA in line with hyperalgesia (lower PWTs), which suggests reduced regional activation as a result of the intensity and duration of pain experienced during the 7 days of $MnCl_2$ exposure. Motor cortex T1-weighted signal increase was associated with the distance ran in the wheel running study, while no between group difference was seen. Our data suggest that on-going pain related signal changes identified using MEMRI offers a new window to study the neural underpinnings of spontaneous pain in rats.

Introduction

Chronic pain disorders are debilitating, costly to society and have a complex pathophysiology (Tracey and Bushnell, 2009). Imaging techniques are important tools in the study of chronic pain (Lee and Tracey, 2013) but the vast majority of imaging studies of pain focus on evoked responses rather than spontaneous pain, but the brain regions activated by painful stimuli only partially overlap with those attributed to

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spontaneous pain (Parks et al., 2011), with a shift to emotional brain regions such as the amygdala and putamen (Kulkarni et al., 2007; Howard et al., 2011; Cottam et al., 2016). Conversely experimental hyperalgesia induces upregulation of brain responses within known pain processing areas with significantly higher BOLD responses to cutaneous noxious stimuli in the dorsal anterior cingulate, bilateral anterior, left posterior insula, right inferior parietal lobule, right middle frontal gyrus and left striatum, based on a recent meta-analysis of 11 studies (Tanasescu et al., 2016).

Animal models offer a number of benefits to the study of chronic pain, including the evaluation of novel treatments and differentiation of underlying mechanisms across different types of pain (Borsook and Becerra, 2011). The clinical relevance of the models of pain employed, type of assessments of pain response (evoked versus on-going responses) and longitudinal testing of the models are fundamental to optimise translational validity of the findings (Tappe-Theodor and Kuner, 2014). Greater emphasis of pre-clinical studies on the neural mechanisms underlying spontaneous pain may provide targets for more effective treatment options in chronic pain disorders. Functional magnetic resonance imaging (fMRI) provides an important snap-shot of brain regional activity, however commonly images are acquired from anaesthetised animals which, whilst acceptable for the acquisition of structural data, can limit the information obtained from functional scans (Borsook and Becerra, 2011; Martin et al., 2006; Martin, 2014; Abaei et al., 2016) if not entirely obscure activity (Thompson et al., 2014). Although imaging in awake animals is feasible, it may introduce other confounds such as stress (Thompson and Bushnell, 2012). An alternative fMRI-based approach is to administer an activity-dependent magnetic resonance (MR) contrast agent with a long half-life whilst the animals are awake to capture waking-state activity when the animal is anaesthetised during read out. Mn^{2+} is a paramagnetic ion usually delivered as $MnCl_2$, which acts as a Ca^{2+} analog and has a half-life of approximately 12 days in cortex (Chuang et al., 2009). $MnCl_2$ enters excitable cells via voltage-, receptor- or non-specific calcium channels and can be used for functional contrast related to neural activity (Silva et al., 2004; Boretius and Frahm, 2011) that is not dependent on a haemodynamic response. Although early studies using acute $MnCl_2$ administration had issues with toxicity (Olanow, 2004; Wolf and Baum, 1983; Silva et al., 2004; Jackson et al., 2011), slower longer-term administration has overcome this problem (Eschenko et al., 2010; Hoch et al., 2013; Mok et al., 2012).

Here we have sought to address both the over-reliance on evoked data in pain studies and the limitations of acquiring imaging data from anaesthetised animals. We hypothesized that manganese-enhanced magnetic resonance imaging (MEMRI) could identify regional brain activity related to ongoing pain behaviour in the rat. To address this question a short-lasting, versus longer-lasting, model of pain behaviour was employed. Nerve growth factor (NGF) is a pro-inflammatory molecule that produces a relatively short lasting sensitization and activation of sensory nerves following subcutaneous or intra-articular injection into the knee joint (Lewin et al., 2014). The second model employed is associated with a longer-lasting period of nociceptive behaviour following the intra-articular injection of the chondrocyte toxin monosodium iodoacetate (MIA), which replicates aspects of the joint pathology and pain exhibited by human OA (Guingamp et al., 1997; Combe et al., 2004). Herein we report the first use of MEMRI to examine regional uptake of $MnCl_2$, and, by inference, activity, in the brain following a period of short term nociceptive behaviour or versus sustained nociceptive pain behaviour in separate groups of rats. As a control, the effects of running on regional uptake of $MnCl_2$ were also quantified in a separate group of rats.

Methods

The experiments described were approved by the local University ethical committee and all procedures were performed and specifically licensed following approval by the UK Home Office and in accordance with the Animals (Scientific Procedures) Act 1986 which incorporates Council Directive 2010/63EU of the European Parliament. Adult male Sprague Dawley rats (n=32) were used (Charles River, Margate, UK) and were housed on a reversed 12-h dark/artificial-light cycle in conventional cages at a temperature of 21 ± 2 °C and 55% humidity; food and water were available ad libitum.

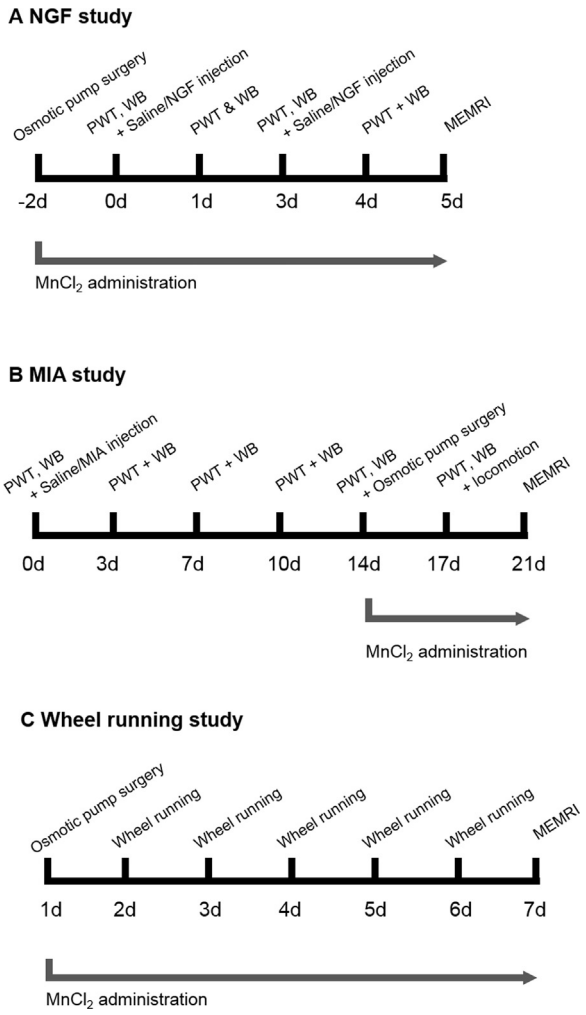

Fig. 1. Timelines of the acute intra-articular nerve growth factor (A) and chronic intra-articular monosodium iodoacetate (B) pain models and also the running wheel study (C). In the NGF model, two intra-articular injections of either saline or NGF were given on days (d) 0 and 3. In the MIA model, a single intra-articular injection of saline or MIA was given on d0. Behavioural pain assessments were performed as indicated and comprised of hindpaw withdrawal thresholds (PWT), weight bearing difference (WB) and locomotion. In the running wheel study rats had access to a running wheel for 3 h per day for 5 days before imaging. In all three studies the period of $MnCl_2$ (80 mg/kg) administration by implanted osmotic pump is indicated by gray arrows.

Intra-articular injections

Rats were randomly assigned to a treatment group briefly anesthetised with isoflurane (2.5–3% in O_2) and received either intra-articular injection through the infra-patellar ligament of the left knee of nerve growth factor (NGF 10 μg/50 μl in saline; Sigma, Gillingham, UK; dose based on (Ashraf et al., 2014), n=4), monosodium iodoacetate (MIA; 1 mg /50 μl in saline; Sigma; dose based on (Sagar et al., 2010), n=8), or saline (50 μl; n=12) using a 30 gauge hypodermic needle. For the NGF group, a second identical injection of NGF was given three days after the first injection (Fig. 1). Accuracy of injection was confirmed at the end of the study by the un-blinding of pain behaviour data. All