NeuroImage xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Metabolic brain activity suggestive of persistent pain in a rat model of neuropathic pain

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ARTICLE INFO

Article history:
Accepted 13 January 2014
Available online xxxx

Keywords:
MicroPET
FDG
Neuropathic pain
Spared nerve injury
Formalin model

ABSTRACT

Persistent pain is a central characteristic of neuropathic pain conditions in humans. Knowing whether rodent models of neuropathic pain produce persistent pain is therefore crucial to their translational applicability. We investigated the spared nerve injury (SNI) model of neuropathic pain and the formalin pain model in rats using positron emission tomography (PET) with the metabolic tracer [18F]fluorodeoxyglucose (FDG) to determine if there is ongoing brain activity suggestive of persistent pain. For the formalin model, under brief anesthesia we injected one hindpaw with 5% formalin and the FDG tracer into a tail vein. We then allowed the animals to awaken and observed pain behavior for 30 min during the FDG uptake period. The rat was then anesthetized and placed in the scanner for static image acquisition, which took place between minutes 45 and 75 post-tracer injection. A single reference rat brain magnetic resonance image (MRI) was used to align the PET images with the Paxinos and Watson rat brain atlas. Increased glucose metabolism was observed in the somatosensory region associated with the injection site (S1 hindlimb contralateral), S1 jaw/upper lip and cingulate cortex. Decreases were observed in the prelimbic cortex and hippocampus. Second, SNI rats were scanned 3 weeks post-surgery using the same scanning paradigm, and region-of-interest analyses revealed increased metabolic activity in the contralateral S1 hindlimb. Finally, a second cohort of SNI rats was scanned while anesthetized during the tracer uptake period, and the S1 hindlimb increase was not observed. Increased brain activity in the somatosensory cortex of SNI rats resembled the activity produced with the injection of formalin, suggesting that the SNI model may produce persistent pain. The lack of increased activity in S1 hindlimb with general anesthetic demonstrates that this effect can be blocked, as well as highlights the importance of investigating brain activity in awake and behaving rodents.

Introduction

Neuropathic pain related to peripheral nerve injury results from a variety of causes, including diabetes, shingles (herpes zoster), cancer treatments, and trauma. Neuropathic pain almost always involves sensory abnormalities, such as numbness and/or allodynia and hyperalgesia to touch or temperature (Maier et al., 2010). In addition, patients report pain in the absence of obvious externally applied stimuli. This pain may result from spontaneous activity in nerve fibers, or subtle stimulation resulting from normal daily activities. Thus, persistent pain experienced by patients is likely a mix of stimulus-independent pain and pain provoked by inadvertent stimulation. Neuropathic pain is studied using multiple nerve-injury rodent models (Bennett and Xie, 1988; Decosterd and Woolf, 2000; Kim and Chung, 1992; Seltzer et al., 1990). Unfortunately, assessing persistent pain using these models is difficult, since the animals frequently do not manifest the pain behaviors observed during acute injury. Attempts to measure persistent pain using ultrasonic vocalizations, facial expression, altered locomotion and altered sleep patterns have revealed few positive results (Jourdan et al., 2002; Langford et al., 2010; Mogil et al., 2010; Urban et al., 2011; Wallace et al., 2005). Thus, neuropathic pain models typically rely on measures of mechanical and/or thermal hypersensitivity (D'Amour and Smith, 1941; Le Bars et al., 2001; Woolfe and MacDonald, 1944), which may not reflect the persistent pain reported by chronic pain patients (Backonja and Stacey, 2004; Baron et al., 2009; Gottrup et al., 1998). Based upon behavioral results, it is unclear whether the assessment methods are inadequate or if the rodent models do not produce chronic persistent pain. In contrast, there are rodent pain models that result in overt short lived pain-related behaviors. As an example, the formalin tonic pain model results in a well characterized set of persistent pain-related behaviors that last for approximately 1 h (Dubuisson and Dennis, 1977).

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http://dx.doi.org/10.1016/j.neuroimage.2014.01.020

In humans, imaging has revealed brain regions commonly activated by pain, including the primary somatosensory cortex of the area affected by pain, secondary somatosensory cortex, prefrontal cortex, insular cortex, anterior cingulate cortex, and thalamus (for reviews see: (Apkarian et al., 2005; Schweinhardt and Bushnell, 2010)). These regions are also activated during ongoing, chronic pain in humans (Baliki et al., 2006; Howard et al., 2012). Rodent *in vivo* brain imaging has revealed activations of homologous brain regions in response to acute noxious stimuli (for reviews see: (Borsook and Becerra, 2011; Thompson and Bushnell, 2012)). Using *ex vivo* CBF imaging, Paulson et al. (2002) showed that 12 weeks after a chronic constriction nerve injury (CCI), somatosensory cortex showed increased CBF in the absence of stimulation. However, no *in vivo* brain imaging study has evaluated activations related to unstimulated, chronic persistent pain in awake rodents.

The current study tested the hypothesis that rats with a chronic nerve injury that produces cutaneous hypersensitivity also show a pattern of brain activity consistent with persistent pain. To test this hypothesis, positron emission tomography (PET) scans were performed on three cohorts of rats using the metabolic tracer [18F]fluorodeoxyglucose (FDG) (Ido et al., 1978; Kornblum et al., 2000). In the first group, formalin-evoked brain activity was assessed in awake and behaving rats (during the tracer uptake period) to identify the pattern of persistent pain-related activation. In a second group, the same scanning paradigm was used in rats three weeks post-nerve injury to measure ongoing nerve-injury-related brain activity. Finally, to examine whether activations related to nerve injury were influenced by the state of consciousness, a third group of nerve-injured rats was scanned after they had been anesthetized during tracer uptake.

Materials & methods

Experimental animals

Forty-six male Sprague–Dawley rats (150–200 g, Charles River, QC) were pair housed in temperature controlled (23 +/− 1 °C) ventilated racks with a 14-hour light, 10-hour dark cycle with lights on at 07:00. The rats had access to both food (Harlan Teklad 2920X) and water. Ethical treatment of animals was ensured; all procedures were approved by McGill University's Animal Care Committee.

PET imaging acquisition procedures

[18F]Fluorodeoxyglucose (FDG), an analog of glucose, was used as the PET tracer to yield a relative measure of glucose metabolism in the brain. As shown in Fig. 1, for the formalin and awake SNI scanning procedures, the FDG was injected in the tail vein while the rat was briefly anesthetized with sevoflurane (5% induction, 2.5% maintenance for ~3 min). The injection was made 45 min before PET scanning began, since the peak signal in rat brain occurs approximately 1 h after injection and represents an accumulation of the tracer that occurred from the time of injection (Ido et al., 1978). The anesthesia was quickly removed, the animal awoke, and was awake and behaving for the next 30 min before the animals was re-anesthetized and scanned. The use of this delayed scanning allowed us to capture metabolic activity that occurred while the animal was awake and behaving throughout 30 min of tracer uptake. Forty minutes after FDG injection, the animal was anesthetized (sevoflurane, 5% induction, 2.5% maintenance throughout the scan), placed in the PET scanner and a static 30-min scan was acquired. A single static scan was chosen over dynamic scanning, since maximizing signal-to-noise ratio was more important for this study than obtaining temporal information. For the SNI anesthetized scan, the rat was anesthetized (isoflurane, 5% induction, 2% maintenance) before the FDG injection and anesthesia was maintained with the rat resting on the scanner bed during the entire period of tracer uptake and scanning. Images were acquired using a microPET R4 (CTI Concorde, Knoxville, TN, USA). The scanner bed was equipped with a breathing rate monitor, rectal thermometer, and heating pad to maintain body temperature at 37 °C. Following standard procedures, rats were fasted for approximately 12 h prior to scanning as blood glucose levels can affect FDG uptake (Lindholm et al., 1993). The FDG tracer was obtained from on-site production at the Montreal Neurological Institute Cyclotron Facility using standard practices for the production of clinical FDG.

Formalin pain model

Sixteen rats in total (8 formalin, 8 controls) were randomly assigned to either a formalin (5%, 50 μL) or control (saline, 50 μL) injection. Injection of formalin results in a well-characterized behavioral response lasting approximately 1 h (Dubuisson and Dennis, 1977). On the day of the scan, each rat received a tail vein injection of a volume less than 0.2 mL and approximately 0.2 MBq of FDG, and a subcutaneous injection of formalin or saline into the plantar surface of the left hindpaw while briefly anesthetized with 5.0% sevoflurane (minute zero, see Fig. 1). The anesthetic was immediately removed after injections and the rats were placed in a ventilated clear Plexiglas observation chamber with a clear floor (30 cm × 30 cm × 30 cm). Beneath the floor, a mirror was mounted at a 45-degree angle allowing for an unobstructed view of the paws. Behavior was video recorded from minute 5 to minute 35. Behavior was not recorded minute 0 to 5 to allow for anesthesia to fully lift, nor at minute 35 to 40 because of scanning preparations requiring technician movement and noise, which could have modified behavior. At minute 40, the rat was removed from the observation apparatus, anesthetized with sevoflurane (5.0% for induction, 2.5% for maintenance) and placed on the scanner bed, with scanning starting at minute 45 and ending at minute 85 as shown in Fig. 1.

Neuropathic pain model

Eighteen rats were randomly assigned to either spared nerve injury (SNI) surgery (9 rats) or sham surgery (9 rats, control group). Surgery

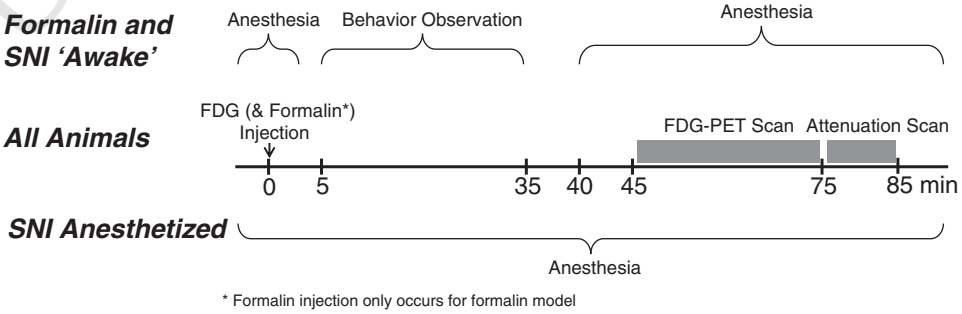

Fig. 1. Time course of small animal positron emission tomography (PET) scanning for the 3 experimental groups: formalin unanesthetized during uptake ('awake'), spared nerve injury (SNI) unanesthetized during uptake ('awake') and SNI anesthetized during uptake.

Please cite this article as: Thompson, S.J., et al., Metabolic brain activity suggestive of persistent pain in a rat model of neuropathic pain, NeuroImage (2014), http://dx.doi.org/10.1016/j.neuroimage.2014.01.020