NeuroImage 128 (2016) 209–217

Contents lists available at ScienceDirect

NeuroImage

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

Laser-evoked cortical responses in freely-moving rats reflect the activation of C-fibre afferent pathways

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ARTICLE INFO

Article history:
Received 2 October 2015
Accepted 23 December 2015
Available online 30 December 2015

Keywords:
Pain
Electrocorticography (ECoG)
Animal models
Aδ-fibres
C-fibres
Laser-evoked potentials (LEPs)

ABSTRACT

The limited success of translating basic animal findings into effective clinical treatments of pain can be partly ascribed to the use of sub-optimal models. Murine models of pain often consist in recording (1) threshold responses (like the tail-flick reflex) elicited by (2) non-nociceptive specific inputs in (3) anaesthetized animals. The direct cortical recording of laser-evoked potentials (LEPs) elicited by stimuli of graded energies in freely-moving rodents avoids these three important pitfalls, and has thus the potential of improving such translation. Murine LEPs are classically reported to consist of two distinct components, reflecting the activity of Aδ- and C-fibre afferent pathways. However, we have recently demonstrated that the so-called "Aδ-LEPs" in fact reflect the activation of the auditory system by laser-generated ultrasounds. Here we used ongoing white noise to avoid the confound represented by the early auditory response, and thereby comprehensively characterized the physiological properties of C-fibre LEPs recorded directly from the exposed surface of the rat brain. Stimulus–response functions indicated that response amplitude is positively related to the stimulus energy, as well as to nocifensive behavioral score. When displayed using average reference, murine LEPs consist of three distinct deflections, whose polarity, order, and topography are surprisingly similar to human LEPs. The scalp topography of the early N1 wave is somatotopically-organized, likely reflecting the activity of the primary somatosensory cortex, while topographies of the later N2 and P2 waves are more centrally distributed. These results indicate that recording LEPs in freely-moving rats is a valid model to improve the translation of animal results to human physiology and pathophysiology.

Introduction

Pain is an increasingly important healthcare issue, with dramatic costs for both patient wellbeing and the society (Breivik et al., 2008). Animal models are widely used to understand fundamental mechanisms of chronic pain and identify new analgesic targets. However, the limited success of translating basic findings in animals into effective, clinical analgesics can be largely ascribed to the use of sub-optimal animal models of pain (Mogil, 2009). In this respect, three important limiting factors are (1) the still surprisingly common use of somatosensory stimuli that are neither nociceptive-specific nor quantifiable (e.g., pinching or heating the skin with hot water) (Bastos and Tonussi, 2010; Hernandez et al., 1994; Toda et al., 2008; Uchida et al., 2012), (2) the recording of 'threshold' measures (e.g., the tail-flick reflex), instead of suprathreshold responses that allow deriving stimulus–response functions (Carstens and Wilson, 1993; Danneman et al., 1994), and (3) the use of anaesthetized animals when the neural activity of the central nervous system is sampled using electrophysiology or functional magnetic resonance imaging (Ando et al., 2004; Becerra et al., 2011; Toda et al., 2008; Yen and Shaw, 2003). These three important issues can be satisfactorily addressed by combining the selective laser stimulation of skin nociceptors with the recording of the cortical activity using electrodes placed directly on the exposed surface of the brain (electrocorticography, ECoG) in freely-moving rats. Considering that the electrocortical responses elicited by nociceptive stimuli (laser-evoked potentials, LEPs) are also widely used to study pain in healthy individuals and patients (Cruccu et al., 2008; Haanpaa et al., 2011; Treede et al., 2003), the use of similar setups in animal and human studies presents the additional advantage of facilitating successful translation.

Abbreviations: LEPs, laser-evoked potentials; ECoG, electrocorticography; LMM, linear mixed modeling; FDR, false discovery rate; S1, primary somatosensory cortex; S1FL, the forelimb areas in the S1; S1HL, the hindlimb areas in the S1; S2, secondary somatosensory cortex; ACC, anterior cingulate cortex.

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http://dx.doi.org/10.1016/j.neuroimage.2015.12.042

Therefore, it is not surprising that laser-evoked cortical responses are being increasingly recorded in animals (Kalliomaki et al., 1993a; Kenshalo et al., 1988; Qiao et al., 2008; Shaw et al., 2001; Tsai et al., 2004). These responses are typically reported as consisting of two distinct components, whose latencies are compatible with the conduction velocity of Aδ-fibres ("Aδ-LEPs") and C-fibres ("C-LEPs") (Isseroff et al., 1982; Qiao et al., 2008; Shaw et al., 2001). However, we have recently demonstrated that the so-called "Aδ-LEPs", instead of reflecting the activation of the Aδ-nociceptive system (Hu et al., 2015), is actually consequent to the activation of the auditory system by laser-generated ultrasounds that can be detected by rats, but not by humans (Panksepp and Burgdorf, 2003; Scruby and Drain, 1990). This auditory response has been so far mistakenly interpreted as reflecting the Aδ-somatosensory input, thus undermining the conclusions of several previous investigations (Isseroff et al., 1982; Qiao et al., 2008; Shaw et al., 2001). Important from a practical perspective, this auditory response can be effectively eliminated by delivering laser pulses during ongoing auditory white noise (Hu et al., 2015).

Here, we delivered nociceptive-specific laser pulses to 12 awake, freely-moving rats. We recorded their behavioral and neurophysiological responses using direct recording of the electrical activity of the cerebral cortex, avoiding the confound represented by the laser-induced early auditory response. We aimed to test (1) whether reliable LEP responses can be obtained in single animals; (2) which population of peripheral nociceptors is reflected in the LEP responses; (3) the dependency of LEP responses on the stimulated territory (i.e., forepaws and hindpaws on the right and left sides); (4) the dependency of LEP responses on stimulus energy, and their relation with nocifensive behavior. Finally, we propose an optimal montage to isolate different LEP components arising from different neural generators.

Methods

Animal preparation and surgical procedures

We used 12 adult male Sprague–Dawley rats weighing between 300 and 400 g. Rats were housed in cages under temperature- and humidity-controlled conditions. All rats received food and water ad libitum, and were kept in a 12-h day–night cycle (lights on from 08:00 to 20:00). All surgical and experimental procedures were approved by the ethics committee of Southwest University.

Prior to the surgery, rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal injection: i.p.). Supplementary doses (12.5 mg/kg, i.p.) of sodium pentobarbital were given to maintain appropriate anesthetic depth during surgery, when necessary. During anesthesia the rat head was fixed using a stereotaxic apparatus. After the dorsal aspect of the scalp was shaved, the skull was exposed by a midline incision, as previously described (Qiao et al., 2008; Shaw et al., 1999, 2001). Fourteen holes were drilled on the skull, at defined locations on the stereotaxic reference system (Shaw et al., 1999). Stainless steel screws (diameter = 1 mm) were inserted into the holes, without penetrating the underlying dura mater. Twelve screws acted as active electrodes, and their coordinates in respect to the bregma were as follows (in mm; positive X and Y axis values indicate right and anterior locations, respectively). FL1: X = −1.5, Y = 4.5; FR1: X = 1.5, Y = 4.5; FL2: X = −1.5, Y = 1.5; FR2: X = 1.5, Y = 1.5; LFL: X = −4.5, Y = 0; RFR: X = 4.5, Y = 0; PL1: X = −1.5, Y = −1.5; PR1: X = 1.5, Y = −1.5; LPL: X = −4.5, Y = −3; RPR: X = 4.5, Y = −3; PL2: X = −1.5, Y = −4.5; PR2: X = 1.5, Y = −4.5. The reference and ground electrodes were placed on the midline, 2 mm and 4 mm caudally to the Lambda, respectively. The wires coming from each electrode were held together with a connector module fixed on the scalp with dental cement. To prevent post-surgical infections, rats were injected with penicillin (60,000 U, i.p.) immediately after the surgery. Following the surgery, rats were kept in individual cages for at least 7 days before the LEP experiments.

Experimental protocol

Radiant-heat stimuli were generated by an infrared neodymium yttrium aluminum perovskite (Nd:YAP) laser with a wavelength of 1.34 μm (Electronical Engineering, Italy). Nd:YAP laser pulses activate directly cutaneous nociceptive terminals in the most superficial skin layers (Baumgartner et al., 2005; Iannetti et al., 2006; Sikandar et al., 2013). The laser beam was transmitted via an optic fibre and its diameter was set at approximately 4 mm (~13 mm^2) by focusing lenses. A He–Ne laser pointed to the stimulated area. Laser pulses were delivered to four body territories (left forepaw, right forepaw, left hindpaw, and right hindpaw), using five stimulus energies (E1–E5: 1–4 J in steps of 0.75 J). The pulse duration was 4 ms, and the interval between two consecutive stimuli was never shorter than 30 s. To avoid nociceptor fatigue or sensitization, the target of the laser beam was displaced after each stimulus (Leandri et al., 2006).

During ECoG data collection, rats were placed into a plastic cage ($30 \times 30 \times 40$ cm^3), whose floor had a regular series of holes through which the laser beam could pass and reach the animal's skin (Hu et al., 2015). The diameter of each hole was 5 mm, and the distance between the borders of two nearby holes was 2 mm. The cage ceiling had a single, larger hole (diameter = 15 cm) through which ECoG cables were connected to the amplifier. Before the ECoG experiment, rats were placed for at least four slots of 1 h each in the same plastic cage, to familiarize them with the recording environment. In both pre-recording and recording sessions, rats could freely move in the cage. The skin area targeted by the laser was always within the paw. It was defined by the region of the paw that was visible through the holes in the bottom side of the cage, when the rat was spontaneously still. The distance between the laser end piece and the target site was kept constant at ~1 cm.

As demonstrated in our previous study (Hu et al., 2015), laser stimulation of the skin generates ultrasounds detected by the rat auditory system (Moller, 2013; Panksepp and Burgdorf, 2003; Scruby and Drain, 1990; Zhang, 1992). This has been further tested in the present study, by recording the thermoelastic response elicited by the laser stimulation of the plastic material of the cage surrounding the animal using a tunable ultrasound detector (Mini-2 Bat Detector, SUMMIT, Birmingham, UK). This recording showed a clear response in the ultrasound range (~40–60 kHz), graded with the energy of the laser pulse (Supplementary Fig. 1 and Supplementary audio files). Therefore, to avoid the activation of the auditory system by the laser-generated ultrasounds, the ECoG recording was performed during ongoing white noise, a procedure that allows selective recording of LEPs related to the activation of the nociceptive system. We delivered 10 laser pulses at each of the five stimulus energies (E1–E5) and each of the four stimulation sites (left forepaw, right forepaw, left hindpaw, and right hindpaw), for a total of 200 pulses. The order of stimulated sites was pseudorandomized. Animals were video-recorded throughout the experiment, and nocifensive behavioral scores were assigned after each laser stimulus, according to previously-defined criteria based on the animal movement (Fan et al., 2009; Fan et al., 1995), as follows: no movement (score = 0), head turning (including shaking or elevating the head; score = 1), flinching (i.e., a small abrupt body jerking movement; score = 2), withdrawal (i.e., paw retraction from the laser stimulus; score = 3), licking and whole body movement (score = 4). The effect of stimulus energy and stimulation site on behavioral scores was assessed using a two-way repeated-measures analysis of variance (ANOVA), with 'stimulus energy' (five levels: E1–E5) and 'stimulation site' (four levels: left forepaw, right forepaw, left hindpaw, and right hindpaw) as within-subject factors.

ECoG recording and data analysis

Data preprocessing

ECoG data were recorded with a sampling rate of 1000 Hz (Brain Products), and preprocessed using EEGLAB (Delorme and Makeig,