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Time-resolved functional ^{1}H MR spectroscopic detection of glutamate concentration changes in the brain during acute heat pain stimulation

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ABSTRACT

Non-invasive *in vivo* detection of cortical neurotransmitter concentrations and their changes in the presence of pain may help to better understand the biochemical principles of pain processing in the brain. In the present study acute heat pain related changes of the excitatory neurotransmitter glutamate were investigated in the anterior insular cortex of healthy volunteers by means of time-resolved functional proton magnetic resonance spectroscopy (^{1}H-MRS). Dynamic metabolite changes were estimated with a temporal resolution of five seconds by triggering data acquisition to the time course of the cyclic stimulus application. An overall increase of glutamate concentration up to 18% relative to the reference non-stimulus condition was observed during the application of short pain stimuli.

Introduction

Due to its protective function with respect to human survival, perception of pain represents one of the most important physiological senses. However, if acute pain turns to chronic pain its relevance for maintaining health is lost, and quality of life for the affected person may decline dramatically. Chronic pain is furthermore associated with high direct and indirect costs for health care systems due to potential invalidity and lifelong therapy. Therefore, improved understanding of the physiological processes underlying subjective pain perception may help to develop specific preventive or therapeutic methods for managing chronic pain disease (Borsook et al., 2007). The availability and application of modern functional imaging methods like functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) has already revealed new insights into human pain processing (Peyron et al., 2000, Apkarian et al., 2005, Melzack 2005). However, measuring changes of blood oxygenation level by fMRI or detecting glucose consumption alterations by PET only reflects changes of global neuronal energy uptake related to neuronal activity without a direct link to the underlying metabolic processes. Unlike these techniques, *in vivo* proton magnetic resonance spectroscopy (^{1}H-MRS) makes it possible to selectively detect specific excitatory or inhibitory neurotransmitters in the brain, such as glutamate (Glu), γ-aminobutyric acid (GABA) or intermediate neurotransmission products, like glutamine (Gln).

Few recently published *in vivo* ^{1}H-MRS studies reported on local changes of cortical Glu and Gln levels during acute painful stimulation (Mullins et al., 2005) as well as in the presence of chronic pain (Grachev et al., 2002, Siddall et al., 2006, Harris et al., 2008). The results suggest that spectroscopically estimated metabolic levels reflect interactions between excitatory neurotransmitter systems during cortical processing of pain stimuli and may thus allow a more objective evaluation of pain intensities compared to subjectively assessed intensities (Mullins et al., 2005).

Currently, the accuracy of *in vivo* ^{1}H-MR spectroscopic measurement of Glu, Gln and GABA is limited by their complex spectral multiplet structures and spectral overlapping with further signals of similar chemical shifts. Nevertheless, due to its relatively high concentration in the brain (ca. 8–10 mmol/l), Glu can be reliably quantified by using conventional ^{1}H-MRS localization techniques (PRESS, STEAM) on clinical whole body MR scanners with magnetic fields ≥3 T (Schubert et al., 2004, Mullins et al., 2008, Gussew

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et al., 2008). In contrast, the low cortical levels of Gln (2–4 mmol/l (Govindaraju et al., 2000)) and GABA (1–2 mmol/l (Govindaraju et al., 2000)) require improved detection sensitivity which can, for instance, be realized by using stronger magnetic fields (>3 T) (Tkáč et al., 2001). Other possibilities include specific MR spectroscopic techniques, like 2D J-resolved ^{1}H-MRS (Lymer et al., 2007), or methods based on spectral editing (Bogner et al., 2008).

Numerous fMRI and PET studies (Casey 1999, Craig et al., 2000, Baliki et al., 2006) have confirmed that the anterior insular cortex (*aIC*) is directly involved in the general cortical processing of pain. The goal of this study was to investigate changes of Glu concentrations in the *aIC* of healthy volunteers, induced by peripheral painful heat stimulation, by acquiring time-resolved, stimulus triggered ^{1}H-MR spectra. In contrast to the study of Mullins et al. (2005), who investigated the alteration of Glu and Gln in the anterior cingulate cortex (ACC) between a resting period and a continuously applied cold pain stimulus (10 min), we chose a cyclic application of short, repetitive heat stimuli. This way we intended to avoid adaptation processes due to nociceptor saturation which may affect metabolic changes during prolonged stimulation (Giove et al., 2003). Furthermore, temporal synchronization between repeated acquisitions and pain stimulation was implemented to acquire the spectra during exactly defined stimulation states.

Material and methods

Volunteers and heat stimulation

Six healthy, right-handed male subjects (mean age 31.1 ± 11.1 years) were recruited within the research group. Prior to the experiments volunteers were informed by a radiologist about all procedures and their possible risks and signed an informed consent. The study was approved by the local Ethics Committee.

Stimulation set-up

Painful heat stimuli were applied to the inner skin area of the left forearm (see Fig. 1a) by using a Peltier element thermode (Neuro Sensory Analyzer TSA-II, MEDOC Ltd., Ramat Yishay, Israel). Temperatures could be swept from 0 and 50 °C with a maximal rise time of 8 °C per second. The thermode (surface: 30×30 mm^2) was specially designed for applications within a MR scanner environment. Compatibility checks revealed neither distortions of the MR signals induced by the thermode nor any influences of the MR measurements on the thermal sensations.

As shown schematically in Fig. 1b, a single cyclic period of the stimulation time course consisted of an temperature increase from baseline ($T_{baseline}$: 32 °C) up to the beforehand individually determined painful temperature level T_{Stim} (see below), which was then held constant for 1 s (t_{STIM}), followed by the return to the baseline temperature. Rise and decay times (t_{RISE}, t_{FALL}) were both limited to 2 s each. The cycle was repeated periodically with a resting period of five seconds between two subsequent cycles ($t_{INTERSTIM}$). To ensure comparable pain intensities among all volunteers the temperature level, T_{Stim}, was adjusted individually prior to the MR experiments. Subjects were instructed to rate the perceived pain intensity for incrementally increased temperatures (T_{Stim} values between 45 and 49 °C) using the visual-analogue pain scale (VAS) which ranges from one (corresponding to *no pain*) to ten (corresponding to *worst pain imaginable*) (Melzack 2005). The T_{Stim} value corresponding to the VAS interval between six and seven was chosen for the stimulation in the following MR-spectroscopic experiments. To monitor possible habituation effects during the experiments subjects were asked to rate the perceived pain intensity again immediately after the MR examinations.

MR-spectroscopy

All measurements were performed on a 3 T whole-body MR scanner (Magnetom Trio TIM, Siemens Medical Solutions, Erlangen, Germany) using a twelve channel phased array *receive-only* head matrix coil for signal detection. Spectra were acquired using a conventional PRESS sequence with single volume selection (TR/TE = 5000/30 ms; 4096 FID points, bandwidth: 4 kHz) which was modified for TTL-triggering by an external stimulation device. Prior to the MRS examination T_1-weighted MR images were reconstructed in three orthogonal orientations from a 3D dataset (MP-RAGE; TR/TE/TI = 2300/3.03/900 ms; $\alpha = 9°$; 192 sagittal slices, $FOV_{AP\times FH} = 256\times256$ mm^2, in-plane resolution: 256×256, slice thickness: 1 mm) and used for positioning the 25×10×10 mm^3 spectroscopic voxel in the left *aIC* (Figs. 2a and b). Zero- and first-order shim gradients were adjusted with an automatic

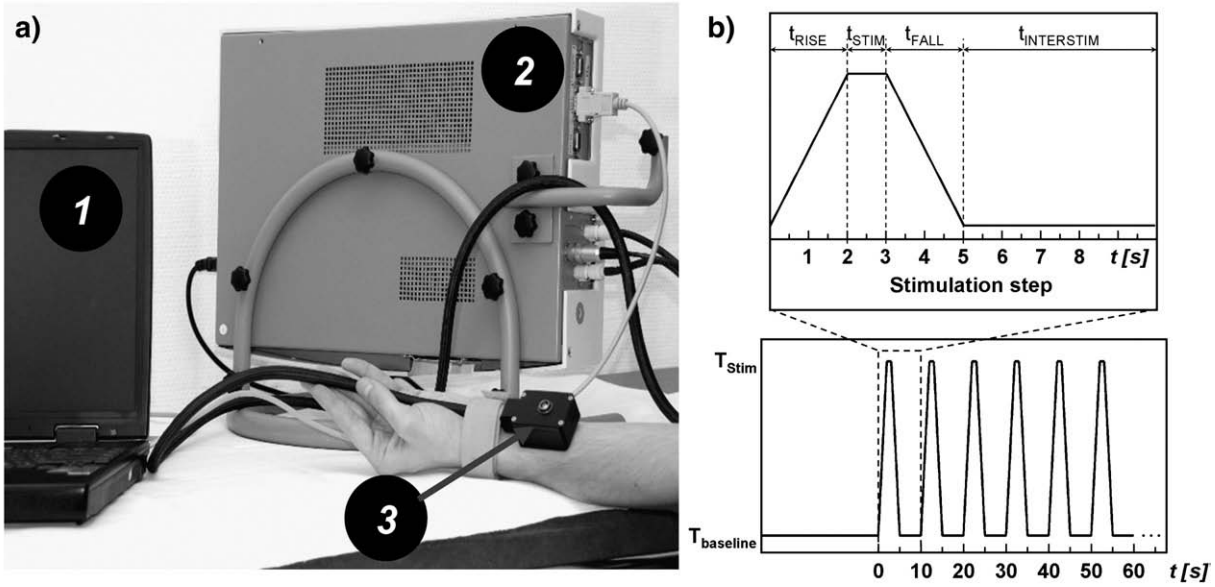

Fig. 1. Set-up used to apply heat stimuli (a): stimulation PC (1), temperature control device (2), and Peltier element thermode (3). Time course applied for heat pain stimulation (b) with cyclic temperature alterations between baseline ($T_{baseline}$) and stimulus levels (T_{Stim}). One complete stimulation cycle consists of an increase from $T_{baseline}$ to T_{Stim}, stimulus period (t_{STIM}: 1 s) followed by a return to $T_{baseline}$ and an inter-stimulus resting period ($t_{INTERSTIM}$: 5 s). The rise and fall times (t_{RISE}, t_{FALL}) are 2 s each.