

Opposite parietal and midbrain BOLD patterns during inflammatory pain in the rat: A high-field fMRI study

A. Graff-Guerrero^{1,2}, J. Ortega-Legaspi³, C. De la Fuente-Sandoval⁴, F. Pellicer³, D. López⁵, A. Moreno⁵, R. Martín⁵, S. Solís⁵, S. Hidalgo⁵, and A. O. Rodríguez⁵

¹Instituto de Neurobiología, Universidad Nacional Autónoma de México, México, DF, México. ²PET Centre, Centre for Addiction and Mental Health, Toronto, Ontario, Canada, ³Laboratorio de Neurofisiología Integrativa, Instituto Nacional de Psiquiatría Ramón de la Fuente, México, ⁴Laboratorio de Psiquiatría Experimental, Instituto Nacional de Neurología y Neurocirugía, México, ⁵Electrical Engineering Department, Universidad, Autónoma Metropolitana-Iztapalapa, México, DF, México

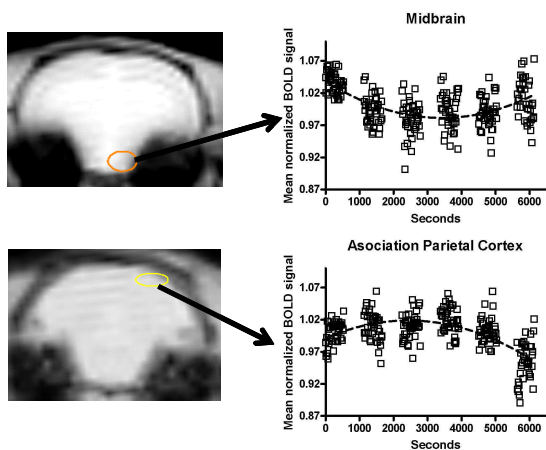
Introduction. Functional Magnetic Resonance Imaging (fMRI) is a tool that has allowed us to know the loci and the pathways involved in brain physiology under normal and pathological conditions. For example, this tool permits a deeper understanding of the brain mechanisms involved in pain perception. The first fMRI studies in pain were carried out in humans, which showed that pain perception is integrated in the brain within a series of distributed structures defined as the 'pain matrix'; including the primary somato-sensory cortex (S1), anterior cingulate cortex (ACC), midbrain and associative parietal cortex (PC) among others [1]. The availability of high-field MRI for small animals has redirected the study of pain (nociception) [2,3]. While human studies have advantages to study cognitive-psychological processes during pain perception, animal studies allow to correlate fMRI with neurotransmission systems, receptors or pharmacology in an experimentally controlled manner; impossible to perform in human studies [4]. In the present work, we describe the pattern of the blood oxygenation level-dependent (BOLD) changes related to inflammatory nociceptive stimulation in the rat. The BOLD changes are described for the S1, ACC, midbrain and PC.

Material and Methods. Four adult male Wistar rats (250-310g) were included in this study. The animals were anesthetized with isoflurane at 1% delivered through an oro-nasal mask. At the beginning of the experiment, the animals were injected in the right hind paw with 250 µl of carrageenan at 1% in order to induce a constant inflammatory nociceptive stimulus [5]. Immediately after injection, fMRI experiments were acquired during 100 minutes in groups of 40 sets of images with 10 minutes between each set with a final total of 280 images. All experiments were performed on a 7T/21 Varian system (Varian, Inc, Palo Alto, CA) equipped with DirectDrive technology and a transceiver birdcage coil. Rat's brain images were acquired using a standard gradient echo sequence and the following parameters: TR/TE=65.69/2.67 ms, Flip angle=10°, FOV=61, thickness=2mm, and NEX=1. Electrocardiogram and breathing were constantly monitored. Regions of interest were segmented according to the Paxinos and Watson atlas [6]. MR signals from the left S1, ACC, left midbrain and left PC were extracted with AMIDE software (Loening & Gambhir, UCLA School of Medicine, CA) from raw images and normalized to the mean. Data was then fitted to a non-linear regression over time.

Results. The pattern of BOLD change was different between the midbrain and PC. The BOLD signal from the midbrain decreased with a peak approximately 3500 s after the carrageenan injection. In contrast, PC increased with a peak at 2400 s after the injection. In both cases, the BOLD signals showed a trend to baseline at 6000 s after injection. Interestingly, the ACC showed a brief signal increase at 1500 s with a return to baseline at 2500 s. No changes were shown in S1. The plantar perimeter of the right hind paw increased in 42% (SD=20) after 100 minutes from injection. This result confirmed the success in the inflammatory induction. The midbrain ($R^2=0.30$) and PC ($R^2=0.38$) BOLD data were fitted to a second order

function as shown in eqs. (1) and (2): (1) $NS_{\text{midbrain}} = 1.03 - 3.34 \times 10^{-5} t + 4.89 \times 10^{-9} t^2$ and (2) $NS_{\text{PC}} = 0.99 + 2.26 \times 10^{-5} t - 4.64 \times 10^{-9} t^2$, where NS represents the normalized signal and t time in seconds and, their corresponding fittings were plotted in Fig. 1.

Conclusion. This fMRI study is the first performed in rats and illustrates a different pattern of BOLD changes between brain structures after inflammatory nociceptive stimulation. Moreover, suggests an early participation of the PC and a late participation of the midbrain during inflammatory nociceptive stimulation under inhaled anaesthesia. Previous studies in rats exclusively reported increases in the BOLD signal; however, studies in humans have reported both: increases and decreases. Our study suggests that with small animal fMRI, it is possible to image "deactivations" during nociceptive stimulation; opening an avenue to better understand the clinical findings using a pre-clinical model.



References

1. Peyron R. et al. Neurophysiol Clin. 2000;30:263.
2. Tuor UI. et al. Pain. 2000;87:315.
3. Hess et al. Eur J Pain 2007;11:109.
4. Zimmermann M. Pain. 1983;16:109.
5. López-Avila A. et al. Brain Res Brain Res Protoc. 2004;13:37.
6. Paxinos G, Watson Ch. The Rat Brain in Stereotaxic Coordinates, 4th Ed. Academic Press, 1998.

Figure 1. BOLD changes in the midbrain and left associative parietal cortex after a carrageenan injection in the right hind paw. The left figures illustrate the regions of interest in coronal projections of the brain's rat. The right figure illustrate the normalized mean BOLD signal over time.