

Effect of Isoflurane anesthesia on BOLD response to somatosensory stimulation: results from fMRI experiments in conscious rats

D. Welsh¹, A. Coimbra², A. Danziger², A. Rauch², C. Regan², J. J. Lynch², and D. S. Williams²

¹Merck & Co., Inc, West Point, Pa, United States, ²Merck & Co., Inc

INTRODUCTION: Translation of fMRI applications from preclinical to clinical experiments is confounded by the need for anesthesia in animal experiments. Anesthetic mechanisms remain poorly understood and may cause changes in cerebral metabolism, blood flow, and blood volume (CBV), which can obscure or alter the pharmacologic- or sensory- stimulated hemodynamic changes detected with fMRI. The complications of anesthesia in conducting these studies have prompted the development of techniques for fMRI in awake animals (1). Our previous work has demonstrated the feasibility of conducting pharmacologic fMRI in conscious animals and shown that elimination of anesthesia may allow for an increased dynamic range to detect small pharmacologic-induced changes in activity. As a next step, the use of a sensory stimulus in conducting conscious animal fMRI would be of great value in elucidating pain pathways and evaluating novel anti-nociceptive compounds. However, while conducting pharmacologic fMRI in conscious animals is challenging, applying a sensory stimulus to awake animal subjects adds another layer of complexity. In this work, comparison of the extent of cerebral activation following sensory stimulation is conducted in awake and anesthetized rats. This study describes the successful implementation of fMRI of sensory stimulation in conscious rats using a combined coil and restraint system (1), and a pneumatically controlled mechanical stimulation device similar to clinical von frey stimulation (~28 g of force). The activation patterns of conscious rats are compared with isoflurane anesthetized rats.

METHODS: Scanning was conducted on a Bruker Biospec 7T/30 MR imaging spectrometer and all animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee. For conscious animal imaging, female Sprague Dawley (SD) rats (280-310g) underwent one hour conditioning sessions to the restraint apparatus (Insight Neuroimaging LLC) and to recorded scanner noise for 3 consecutive days prior to the day of imaging (2). In previous studies, optimization of the acclimation protocol was achieved by measuring BP, heart rate, cortisol levels during acclimation and scanning sessions to determine the minimal number of acclimation periods needed to reduce stress levels. Conscious imaging was conducted using an Insight Neuroimaging resonator with a surface receive coil. BOLD measurements were made using a multi-slice GE EPI sequence with 6000/11 TR/TE (msec), 30 mm FOV, 64 x 64 matrix, and 1 mm slice thickness. Mechanical stimulation was conducted by placing the animals in a hindlimb holder customized to fit in the restraint device (Insight Neuroimaging LLC). The holder houses a filament, comparable to the 5.46 Von Frey filament, which is applied to the top of the right hind paw. An in-house device, consisting of a pneumatic regulator and solenoid gating valve controlled by pulse generator hardware, applied bursts of air pressure to a low friction, encapsulated glass syringe. The syringe plunger was coupled to a spring-return filament actuator which produced the mechanical stimulus to the paw. Quality control measurements confirmed that a consistent force of 26-29 g was delivered at a rate of 2 Hz. Awake rats (n=7) underwent 1 minute of baseline (no-stimulation) followed by 15 minutes of mechanical stimulation in a 30s on, 60s off paradigm. Isoflurane, 2% via a nose cone, was then administered to each animal and the mechanical testing was repeated. Anatomic images were acquired with a 2D RARE sequence, 5000/14 TR/TE (msec), 256 x 256 matrix, nectoies = 8, NA = 2, and 30 mm FOV, slice thickness = 1 mm. Following a General Linear Model analysis [FSL], group-wise activation maps (thresholded at $z>2.3$, $p<0.05$) were estimated and average percent-signal-change (PSC) were calculated for the left (contralateral) and right (ipsilateral) primary somatosensory cortices.

fMRI Activation Maps

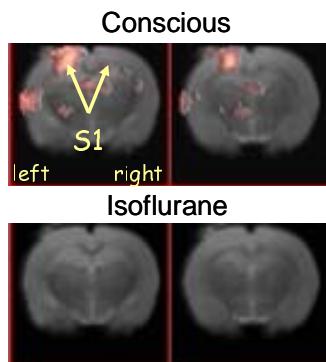


Figure 1. Average activation map for hindpaw mechanical stimulation in rats during the conscious vs isoflurane-anesthetized states.

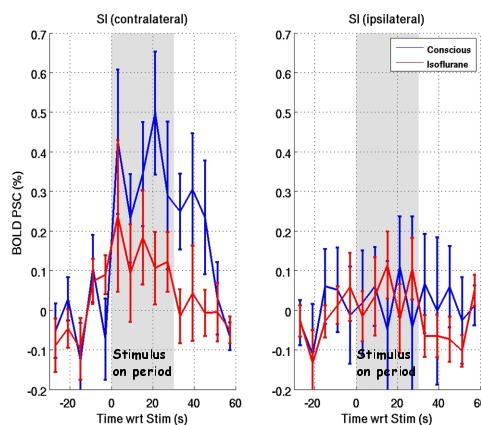


Figure 2. Average percent signal change from S1 regions contralateral and ipsilateral to stimulated hindpaw during mechanical stimulation. Averages are across all stimulus periods (n=10) and across subjects (n=7). Shaded areas depict period of stimulus on. Error bars are standard error of the mean.

Here hindpaw mechanical sensory stimulation also elicits reduced activation increases in anesthetized compared to conscious rats. Isoflurane anesthesia used by Lahti et al reduces CBF (4), while the isoflurane anesthesia used here is known to increase CBF. The different mechanisms and effects of various anesthetics make not just interpretation of fMRI results complex, but also the comparison of data across different studies difficult. While it has been possible to detect BOLD activation changes due to sensory stimuli in rats anesthetized with proper choice of anesthetic, the magnitude of fMRI signal in anesthetized animals is likely to be lower compared to conscious state. As Lahti et al showed with propofol, and here with isoflurane, anesthesia leads to a blunted hemodynamic response to neuronal activity and this results in a very small window in which to evaluate the efficacy of novel pain therapeutics. Conversely, conducting fMRI sensory stimulation experiments in conscious rodents imposes the complexity of additional activation due to behavioral and psychosomatic pathway stimulation. However, since these conditions more closely simulate the human condition under which these therapeutics will ultimately be applied, conscious animal fMRI offers a platform to better predict human fMRI behaviour. Our data demonstrates that sensory stimulation fMRI can be performed in conscious rats, and that a more robust somatosensory response is observed vs isoflurane anesthetized state. The conscious rat platform offers a potentially more physiologically and clinically relevant method for fMRI investigations. References: 1. Ferris CF, et al, J Neuroendocrin, 18: 307-318, 2006 2. King JA, et al, J Neurosci Methods, 148(2):154-60, 2005; 3. Luo F, et al, J Magn Reson Imaging, 26:557-63, 2007; 4. Lahti K, et al, Magn Reson Med, 41:412-16, 1999; 5. Sicard K, et al, J Cereb Blood Flow Metab, 23:472-81, 2003; 6. Borsook, D, et al, Drug Development Res, 68:23-41, 2007.

RESULTS/DISCUSSION: Visual inspection of images and motion correction parameters revealed minimal motion during scans. Figure 1 shows anatomic images from 2 successive slices overlaid with group average activation maps from mechanically stimulated conscious and isoflurane-anesthetized rats. Mechanical stimulation of the right hind paw evoked statistically significant signal intensity increases detectable, among other regions, in the contralateral primary somatosensory cortex (S1) of rats in the conscious state, but not in the anesthetized state. Figure 2 depicts the average percent increase in BOLD signal in the ipsilateral and contralateral primary somatosensory cortex during stimulation periods. Conscious rat fMRI has been performed following pharmacologic, hypercapnic, thermal, and electrical stimulation (3, 4, 5, 6), but to our knowledge this is the first comparison of a von frey type mechanical stimulation in conscious vs anesthetized rats. Lahti et al showed reduced BOLD activation after electrical hindpaw stimulation under propofol anesthesia compared to the conscious state in rats (4).

to conscious rats. Interestingly, the propofol