CORRESPONDENCE OF FMRI AND 2-DEOXYGLUCOSE REPRESENTATIONS OF THE RODENT FOREPAW IN PRIMARY SOMATOSENSORY CORTEX UNDER DIFFERENT ANAESTHETICS

A. L. Dixon[1], D. Cash[1], S. C. Williams[1], G. T. Finnerty[1]
[1]Neurology, Institute of Psychiatry, London, United Kingdom

Introduction:
The sensory periphery is represented as a series of topographic representations in neocortex. These cortical maps alter following persistent changes in sensory inputs (Buonomano & Merzenich, 1998). However, the time course of cortical map reorganization is less well understood because the techniques e.g. single-unit recording, optical imaging, 2-deoxyglucose, that are used to generate high-resolution cortical maps are invasive typically and only allow study at one time point. Functional MRI (fMRI) offers a potential solution, but greatest insights will only occur when the relationship between the cortical maps generated by the blood oxygen-level dependent (BOLD) signal and other techniques is clear. Comparison of data is further complicated by the anaesthesia used to minimize distress and head movement during scans, particularly if the anaesthetic agent alters neurovascular coupling. To address these issues, we have studied the BOLD and ^{14}C-2-deoxyglucose autoradiography (2DG) representations of the rodent forepaw evoked by electrical stimulation using different anaesthetics.

Methods:
MRI: All MR experiments were conducted in a 4.7T Oxford 200/300 MkII (Oxford Instruments) super-conducting magnet interfaced with a UNITY Inova-200 imaging console (Varian, USA). Radio frequency excitation and signal detection were performed using a 40mm internal diameter quadrature coil. A total of 460 volumes covering 20 coronal 0.5mm slices were acquired in 197 min. The paradigm comprised 115 "on" and 115 "off" randomized epochs of electrical forepaw stimulation (2mA, 3ms, 3Hz). The MRI sequence was a multiecho gradient echo TR=0.38s, TE= 0.005, 0.01, 0.015s, Ernst flip angle, FOV 3.6cm x 3.6cm; 64 x 64 data matrix yielding 0.5 x 0.5 x 0.5mm voxels.
Anaesthetic protocols: chloralose (n=8): chloralose administered into the jugular vein (65mg/kg bolus then maintained at 30mg/kg/hr). Isoflurane (n=8): anaesthesia was induced with 2-3% isoflurane in 1L/min 90:10% air/oxygen then reduced to 1-1.5%. Sevoflurane (n=8): anaesthesia was induced 4-6% in 1L/min 90:10% air/oxygen then reduced to 2.5%. The animal was then positioned in head holder and placed in probe.
Data Processing: All raw data were converted into Analyze format. Only the mean TE images were analysed. SPM'99 software (Friston, et al 1999) was used for spatial preprocessing. All data were motion-corrected and a mean, realigned image created. Images were manually masked to exclude voxels outside the brain using Dispimage software (UCLH, London, UK). A non-biased template was used to normalise data sets into the same space. Data sets were then smoothed using a 2x pixel size Gaussian kernel.
Statistical Analysis: Data were analysed using SPM'99. Fluctuations in global intensity were removed by ANCOVA scaling. Each group was analysed separately using a statistical model of multi subject conditions & covariates.
^{14}C-2-deoxyglucose: Animals were anaesthetized and cannulae inserted into: the femoral artery for blood sampling; femoral vein for administration of ^{14}C-2-DG; and jugular vein for administration of chloralose. Animals were left undisturbed and anaesthetized with chloralose (n=6) or isoflurane (n=6) for 1 hr prior to commencement of stimulation (as above) was then commenced. We injected ^{14}C-2-DG after 20min of stimulation, then performed quantitative autoradiography (Sokoloff, 1977). Resulting autoradiographs were digitally captured from X-ray films, converted into glucose utilization maps (Glucalc software, D. Lythgoe, IOP) and regions of interest determined manually. Statistical comparisons of stimulated versus non-stimulated hemisphere in different groups were done by paired t-tests.

Results:

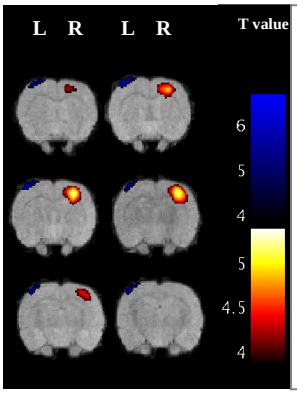
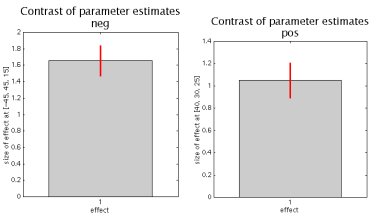


Fig. 1. BOLD responses evoked by left forepaw stimulation. The blue scale demonstrates the t value of the decreases and the red scale the t values of the increases in BOLD signal intensity.

Under chloralose anaesthesia, unilateral electrical stimulation (2mA, 3ms, 3Hz) of the left rat forepaw, yielded significant increases in BOLD fMRI (signal changes in the contralateral cortex, t=6.72, P<0.001 and decreases in ipsilateral cortices, t=8.92, P<0.001). This extended rostrocaudally through 5 slices (2.5mm). Bar charts show significant decreases (neg) and increases (pos) in BOLD signal in the rat somatosensory cortex.

Groups anaesthetised with either both isoflurane or sevoflurane failed to demonstrate statistically significant BOLD changes (P > 0.05).



Left Right

The maximal signal intensity change in both cortices was similar:
Left (ipsilateral) = -1.64 +/- 0.2%;
Right (contralateral) = 1.05 +/- 0.15%.

Discussion:
We found it easier to generate statistically significant maps of the rat forepaw using BOLD fMRI when using chloralose anesthesia compared with isoflurane or sevoflurane anaesthesia. The spatial extent of BOLD and 2-deoxyglucose representations of the rodent forepaw map imaged under chloralose anaesthesia were similar. Both results are consistent with forepaw maps generated by single-unit recordings in primary somatosensory cortex (Chapin & Lin, 1984). Whereas a robust increases in BOLD response is observed in somatosensory cortex contralateral to the stimulated forepaw, the ipsilateral supragranular cortex demonstrates negative BOLD as reported previously (Lowe et al, 2002). We believe that this is probably a result of the feedforward inhibition mediated by the transcallosal pathway linking primary somatosensory cortices (Shuler et al, 2001).

Volatile anaesthetics are non-invasive, give rapidly-controllable anaesthesia and allow recovery. Hence, they would appear to be ideal for longitudinal fMRI studies. However, our data indicate that isofluorane and sevofluorane attenuate BOLD responses. There are several possible explanations. Firstly, both isofluorane and sevofluorane reduce synaptic activity: glutamate release is reduced *in vitro* (Vinje et al, 2002); and isofluorane attenuates thalamic output when concentrations exceed 1% (Detsch et al, 1999). Secondly, reduced action potential firing (Richards, 1998) will lead to decreased synaptic activity. Thirdly, volatile anesthetics may alter neurovascular coupling (Lindauer et al, 1993).

References:
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