FREQUENCY DEPENDANT BRAIN ACTIVATION DURING SOMATOSENSORY STIMULATION IN ANAESTHETISED RATS: A COMPARISON USING EVOKED POTENTIALS, QUANTITATIVE 2-DEOXYGLUCOSE AUTORADIOGRAPHY AND FMRI

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Introduction

Electrical stimulation of the forepaw in rodents leads to a localized increase in neuronal activity, cerebral blood flow and glucose metabolism[1] in the forepaw somatosensory cortex; this is due to neurovascular coupling which is the basis of many brain imaging technique. The application of functional magnetic resonance imaging (fMRI) with blood oxygenation level-dependent (BOLD) contrast in this paradigm has been robustly demonstrated [2,3] and is used as a reliable model of somatosensory stimulation in rodents. Stimulation-induced fMRI is anaesthesia dependent and α-chloralose (α-chl) anaesthetic is commonly used in the forepaw fMRI model as it preserves neurovascular coupling and increases afferent input to the cortex[1]. Under α-chl, however, the BOLD response is only observed in a small range and over relatively low optimal frequencies of 1-3Hz[3] as compared to other anaesthetics[5,6]. The goal of the current study was to investigate the effect of stimulus frequency on the different components of the brain response to forepaw stimulation by measuring neuronal electrical activity (evoked potentials, EP), regional cerebral metabolism (2-deoxyglucose uptake, 2DG), and BOLD fMRI in α-chl anesthetized rats.

Methods

SD rats male rats 280-320g. Electrical stimulation used subcutaneous needle electrode in the forepaw and a TENS pad under the paw. Pulse duration 400μs, current 2mA, frequency was either 0.5 or 3Hz. Animals initially canulated under isoflurane (1.5%) anesthesia, then switched to iv α-chl 65mg/kg bolus, followed by continuous infusion at 30mg/kg/hr.

EP: N=8, alternating frequency (0.5 and 3Hz), 5 runs of each freq, 200 stimuli per run (0.5Hz = 6.7 min, 3Hz = 1.1min)**.** Reference electrode in the neck, two recording electrodes implanted over somatosensory cortex**.** Electrodes were made of stainless steel screws with Teflon coating.

2DG: N=4 rats per each frequency, stimulation started 10min before 2DG administration and continued for 15min thereafter. Stimulation sequence was matched to fMRI. After an iv bolus of 2-deoxy-D-[1-^{14}C]glucose (1000*u*Ci/kg),14 timed arterial blood samples were collected over 45min, brains harvested and frozen and cryosectioned, sections exposed to X-ray film and captured, converted to regional glucose utilization maps in μmol/100g tissue/min according to the operational equation[7].

BOLD fMRI: N=9 0.5Hz and N=6 0.5Hz. Multi echo gradient echo, TR= 360ms, TE's=5,10,15ms (mean TE=12.5 images analysed), NT=1, voxel 0.5x0.5x1, 20 slices, time per scan=23". Acquired 100 volumes with random off-on stimulation sequence. SPM8 (FIL, UCL, London) was used for pre-processing and statistical analysis: images were realigned and spatially normalised to a rat brain template; each individual rat was analysed by GLM, modelling the realignment parameters as nuisance covariate; for group analysis the individual results were analysed by one sample t test; resulting statistical parametric maps (SPM's) are group maps of significant (p<0.01) of BOLD contrast.

Results

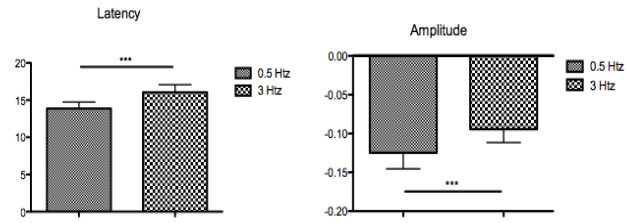

Fig 1 Evoked potentials at 0.5Hz have an increased amplitude and a decreased duration, compared to 3Hz, t test p<0.001.

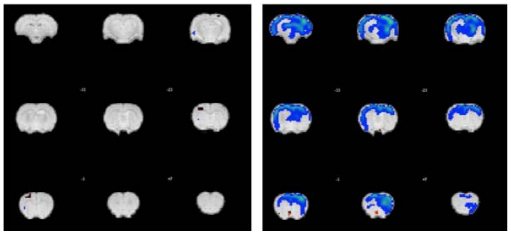

Fig 3. BOLD response to FP stimulation at 3Hz (left) and 0.5Hz (right), one sample t test. Group maps of significant (p<0.01) increases (red-yellow) and decreases (blue-green) in BOLD contrast. 3Hz stimulation causes a BOLD contrast increase in the FP sensory area. At 0.5Hz stimulation there is a widespread bilateral negative BOLD.

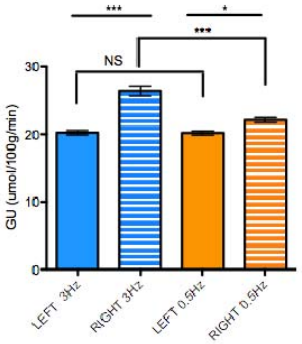

Fig 2. Regional glucose uptake (GU) is significantly increased in both frequencies in the contralateral (stimulated) cortex, greater at 3Hz (30.26% P<0.001) than at 0.5Hz: (9.69 % P=0.002).

Conclusion

A greater increase in *neuronal electrical activity* (EP) at lower frequency was as expected due to an increased synchrony amongst neurons which are allowed to rest between stimulations, so that none are in refractory period[5]. At higher frequency some neurons might remain in refractory period and the response is reduced due to less synchrony. The increased *metabolic* response at 3Hz, measured by 2DG, is indicative of the greater metabolism demand and probably recruitment of more cells. fMRI response to 3Hz stimulation was also as expected, with a focal activation in the contralateral forepaw somatosensory cortex. At lower frequency however, despite the higher neuronal electrical activity and a small but significant metabolic effect, observed BOLD response was negative and bilateral. These results indicate that neurovascular coupling may be affected, or abolished, by experimental conditions such as stimulation frequency and this has important implications for the interpretation of fMRI data. At suboptimal stimulation frequencies where neuronal activity and metabolism are responsive, neurovascular coupling may not operate[8], instead resting CBF may be able to cope with smaller demands (i.e luxury perfusion[9]).

References: 1. Ueki et al, 1992, Acta Anaesth Scand 36(4) 318, **2.** Hyder et al, 1994 JCBFM 14(4) 649, **3.** Gyngell et al, 1996, Mag Res Med 36(1) 13, **4.** Balis et al, Psychopharmacologia, **5.** Masamoto et al, 2007, Cereb Cortex 17(4) 942, **6.** Sheth S et al, 2003, Neuroimage 19(3) 884, **7.** Sokoloff et al, 1977, J Neurochem 28(5) 897, **8.** Ngai et al, 1999, Brain Res 837(1-2)221, **9.** Lassen, 1966, Lancet 2(7473) 1113.