Longitudinal fMRI of Spontaneous Plasticity in Rats After Focal Stroke Under Alpha-Chloralose Anesthetic

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Introduction: Conducting longitudinal functional Magnetic Resonance Imaging (fMRI) studies in rats with stroke lesions requires careful choice of anesthetic and experimental paradigm in order to produce consistent, comparable results. Using a recently-developed[1] Alpha-Chloralose anesthetic regime compatible with repeated imaging we present such a study of recovery after stroke of activation in the area of the somatosensory cortex associated with the forepaw. The rats were lesioned in their dominant hemisphere and the level of activation compared when stimulating both the dominant and less-dominant forepaws.

Methods: 9 adult female Hooded Lister rats were imaged prior to (week 0), 7 days (week 1) and 56 days (week 8) after stroke was induced by an endothelin microinjection in the dominant hemisphere of their sensorimotor cortex[2]. This caused an ischemic lesion and persistent deficit in forelimb function. Rats were assessed for handedness via a postural support test; we recorded 4 left and 5 right-pawed rats, consistent with other literature indicating no species level preference in rats[3].

fMRI scans were acquired using a 7 Tesla scanner (Agilent) and a Multi-Gradient-Multi-Echo sequence (TR= 360 ms, TE's=5,10,15 ms, voxel size 0.5x0.5x1 mm, resolution 64x64x20, scan time 23 s) under Alpha-Chloralose anesthetic[1]. 100 volumes were acquired with a pseudo-random on-off stimulation of the forepaw at 3 Hz (400 µs, 2 mA pulse). The order of paw stimulation was also randomized. Scans with obvious imaging artifacts were discarded, leaving final group numbers of n=7,6,7 and n=6,7,7 for week 0, 1 and 8 for the left and right forepaws respectively. Individual masks for each rat brain at each time-point were generated from a FSE structural scan using a 3D Pulse-Coupled Neural Network[4].

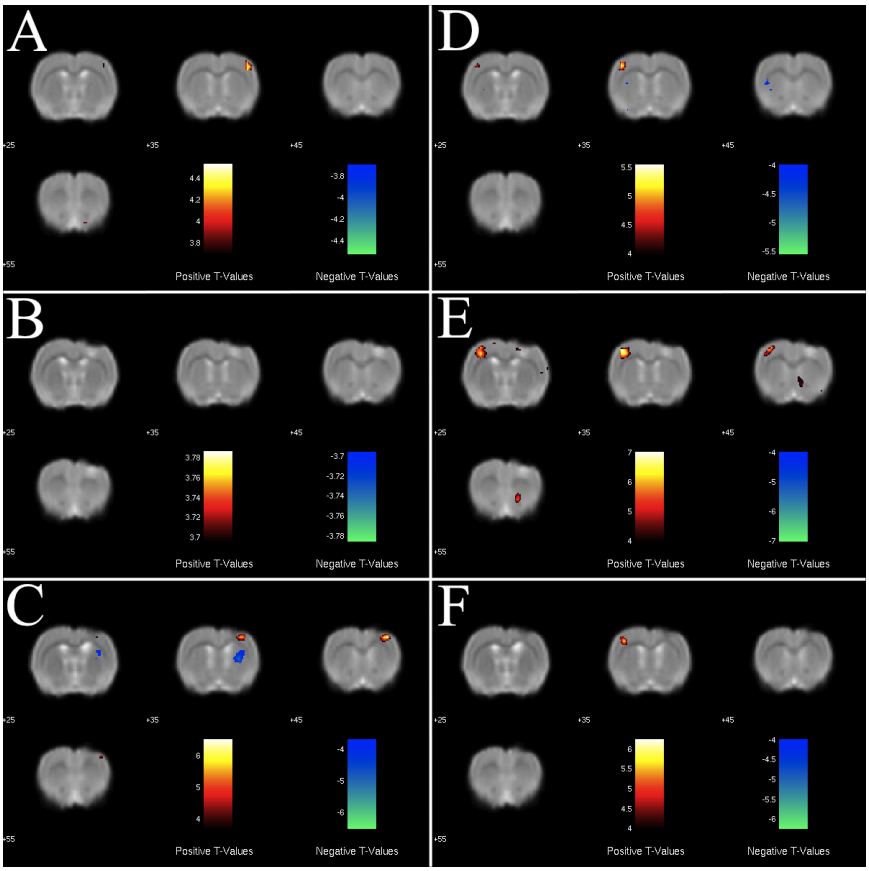

Figure 1 - Activation in the rat cortex for stimulation of the dominant (a-c) and less-dominant paw (d-f) at week 0, 1, and 8 respectively, overlaid on the mean structural image at each time-point.

The resulting images were analyzed with SPM-8 (Functional Imaging Laboratory, UCL). The scans from rats whose right paw was dominant were mirrored about the sagittal mid-plane so that the dominant hemisphere always appeared on the right. Each functional time-series was realigned to the mean image, masked and first-level analysis carried out using the Robust Weighted Least Squares toolbox for SPM-8[5]. Individual contrast images were linearly warped to a template brain to avoid deforming the lesion region. A group analysis was then carried out, the results being shown in figure 1.

Results: Figure 1 shows the group level activation for stimulation of each paw at each time point. Coronal slices shown are in caudal to rostral direction through the somatosensory region of the cortex. Significant activations were not seen outside of this region. The activations are thresholded at a T-value corresponding to a 10% False Discovery Rate. At week 0 a small amount of activation in the forepaw sensory cortex is seen when stimulating either paw (figures 1a&d). 1 week after stroke no activation is seen when stimulating the stroke-affected dominant paw (1b) while the less-dominant paw shows an increased area of activation (1e). 8 weeks after the stroke activation has returned when stimulating the dominant paw (1c), but appears to have moved due to the lesion. Activation when stimulating the less-dominant paw has also returned to similar levels seen at baseline (1f).

Conclusions: Alpha-Chloralose anesthetic at medium dose is compatible with both forepaw stimulation and serial imaging and is well tolerated by stroke-lesioned rats. This protocol hence reduces the number of animals required for longitudinal studies, facilitating improved sensitivity to test for spontaneous or drug-induced plasticity changes.

References: [1] de Celis Alonso et al (2011) On the use of Alpha-Chloralose for repeated BOLD fMRI measurements in rats. *Journal of Neuroscience Methods*
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