Investigation of the BOLD contrast mechanisms initiated during prolonged trigeminal nerve stimulation

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Introduction: If sustained stimulation is straightforward in humans (1, 2, 3), it is not as evident in rodents due to anesthesia. Prolonged BOLD response in the somatosensory cortex of the rat was first shown in a forepaw rat model (4). Although other groups described a 10-minute BOLD response to forepaw stimulation (5), to our knowledge further investigations relative to the mechanisms initiated by a prolonged stimulation in rodents were not carried out. Different stimulation durations could potentially result in different activation maps themselves resulting from the recruitment of different neuronal, vascular or metabolic mechanisms (6). The aim of the present study was to characterize BOLD activation in the rat during prolonged trigeminal nerve stimulation.

Materials and Methods: Animal preparation: Male adult Sprague-Dawley rats (n=15; 350±40g) initially anaesthetized with isoflurane in a mixture of O_2 orally intubated were catheterized for □-chloralose administration and blood gas sampling. After fixing the rat head using ear and bite bars, the rat was positioned in a dedicated holder. The breathing rate and Body temperature were monitored simultaneously throughout the experiment. Body temperature was maintained at 37.5ºC ± 0.5ºC with temperature-controlled circulating water placed under the rat. Blood parameters were maintained at physiological levels (pH = 7.35-7.4, pCO2 = 39-45 mmHg and MABP = 90-130mmHg) throughout the experiment. After surgery, anesthesia was switched to α□-chloralose; an initial intravenous dose of 80mg/kg was administered followed by a continuous intravenous infusion of 27mg/kg/h at (2ml/hour). **Trigeminal nerve stimulation:** Two stainless steel electrodes were percutaneously inserted either in the left or right trigeminal nerve as described in (7) Electrical stimulation of one trigeminal nerve was performed by delivering square pulses using an external stimulator (8). During sustained TGN stimulation, the stimulus amplitude, frequency and duration were kept below the threshold of nociception where the stimulation induces systematic mean arterial blood pressure increases indicating painful stimulation.**fMRI protocol:** All the experiments were performed on an actively shielded 9.4T/31cm bore magnet (Magnex, Varian, Abingdon, UK) with 12 cm gradients (400mT/m in 120µs) with a quadrature Transmit/Receive 17mm surface coil. First and second order shims were adjusted using FASTMAP (9) resulting in water linewidths of 13-15Hz in a 216µl volume. The BOLD response was assessed using single shot gradient echo EPI (TR/TE=2500-2000/25ms; FOV=20x20mm; matrix=64x64; slice thickness = 1mm; 5-6 slices, Bandwidth=325 KHz, 300-960 volumes). **fMRI analysis:** Data were processed using STIMULATE (10) or with SPM8 (Matlab;; Statistical Parametric Mapping) using the General Linear model (GLM). T-maps and cross correlation maps were calculated on a pixel by pixel basis the motion-corrected and 3x3x3 Gaussian smoothed time series with a boxcar waveform representing the stimulation period. Only clusters comprising at least 5 pixels were considered significant. The activation threshold was set to 0.3 for cross-correlation maps and 3 for t-maps ($p<0.05$). Regions of interest (ROIs) encompassing the activated primary somatosensory barrel field cortex (S1BF) were drawn. ROIs varied among the animals. A representative average time-course was recorded for each animal. When needed baseline correction was performed. T-maps and Cross –correlation maps were overlaid on single shot gradient echo EPI images. Data were presented as means ± S.E.M.

Results and Discussion: Short TGN stimulation (30s ON-1min OFF-30sON-1min OFF) and sustained TGN stimulation (2minOFF-10minON-2minOFF) were compared to assess potential temporal and spatial changes in the BOLD activation of the rat barrel cortex. Upon onset of long TGN stimulation, an initial BOLD peak was found followed by a BOLD response that plateaus before returning to baseline. When moving across the activated area within the barrel cortex, the BOLD response to sustained TGN stimulation remained consistent across all slices with no significant spatial changes compared to short BOLD activation. However, for 20% of the rats in the present study, the BOLD response in the barrel cortex to long stimulation varied in shape across the slices with a BOLD plateau disappearing for the slices corresponding to the anterior part of the barrel cortex. The BOLD activation was localized within the contralateral primary somatosensory barrel field cortex (S1BF) as well as the secondary somatosensory cortex (S2). Strong BOLD activations (cross-correlation factor > 0.6; $p<0.01$) within the barrel cortex with short and sustained TGN stimulations were found.

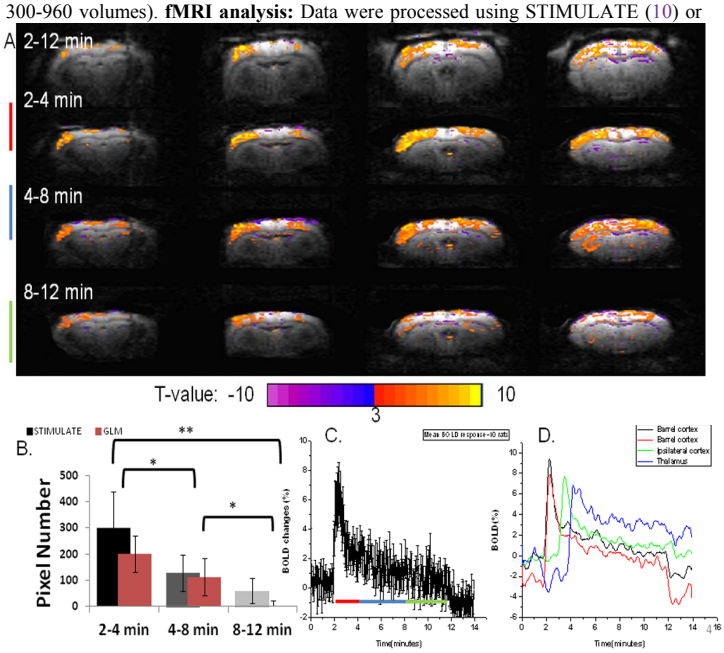

Figure 1: Stimulation time-dependent fMRI results. fMRI t-value maps generated from the images acquired during baseline vs all stimulation and 3 periods (2-4min, 4-8min, 8-12min) during the 10-minute TGN simulation. GLM analysis using the same stimulation periods was performed. BOLD t-value maps were overlaid over single shot gradient-echo EPI images (TE=25ms). **A**. t-value maps overlaid over single shot gradient-echo EPI images are shown for the baseline vs 2-12min, 2-4min stimulation period (red code), 4-8min stimulation period (blue code) and 8-12min stimulation period (green code). The t-maps demonstrate the decrease in activated pixels as a function of stimulation time within the rat barrel cortex. BOLD activation was detected in the thalamus as well as in the ipsilateral cortex. **B.** The average number of pixels activated and standard deviations (n=10 animals) is plotted. Statistical comparisons were performed between the 2-4min stimulus period and the 4min-8min stimulus period between the 4-8min and 8-12min stimulus period and between the 2-4min and 8-12min stimulus period. The GLM analysis was operated in the same way. **C**.Average BOLD timecourse ± SEM (10 rats) with color codes **D**. BOLD timecourses from ROIs drawn within the barrel cortex. the insilateral cortex and the thalamus.

Sustained BOLD activation was further characterized during the stimulation period (Fig1): the number of statistically significant active pixels was dependent on the duration of the stimulus period. In the barrel cortex, statistical values and the number of active pixels were the highest for t-maps obtained for the first 2 minutes of the long TGN stimulation. The number of active pixels within the barrel cortex decreased significantly during the next 4 minutes (pvalue~0.03) and the last 4 minutes of TGN stimulation (pvalue~0.03) (Fig1.A). The mean 10-minute BOLD (± SEM) response in the rat barrel cortex averaged across 10 rats is shown in Fig1.C. Color codes indicate the times given to obtain t-maps during sustained TGN stimulation (Fig 1.A, Fig 1.C). T-maps during the course of stimulation reveal that after 2 minutes of TGN stimulation the number of active pixels increased significantly in these areas. BOLD timecourses from ROIs drawn within the barrel cortex, the ipsilateral cortex and the thalamus reveal a delay period between the onset of positive stimulation in S1BF and the ipsilateral cortex as well as the thalamus (Fig1.A, Fig 1.D). The spread of activation invading both ipsilateral and contralateral cortical areas during whisker stimulation was observed in the past (11). In addition to local synaptic processing within the barrel cortex, excitatory neurons from the barrel cortex send and receive glutamatergic projections to and from a variety of specific brain regions. Little is known from these connections and their contribution to sensory processing. The rodent S1BF integrates somatosensory information from the ventral posterior medial nucleus (VPM) and the posterior complex (POm). The latencies between the hemodynamic responses of the thalamic nuclei and the barrel cortex could be explained by changes in oxygen consumption exceeding oxygen supply and resulting in a long initial dip (12). In addition, multiple-whisker stimulation induced by TGN stimulation may have introduced differences in the timing of activation modulated by GABAergic effects between the VPM and the POm (13). Moreover, functional connectivity changes during the long stimulation period may occur.

Prolonged stimulation appears to be an interesting and potentially promising way to investigate further the patterns of neuronal and vascular activity during brain activation. To understand the mechanisms of sensory processing during sustained barrel cortex and thalamic activations, activity-induced manganese dependent MRI and functional MRS are currently conducted during TGN stimulations.

References: 1.Mangia et al., 2007a, JCBFM; 2. Mangia et al., 2007b, JCBFM; 3.Bandettini et al., 1997, Hum Brain Mapp ; 4. Hyder et al., 1996, PNAS ;5.Xu et al., 2005, Neuroimage ;6.Hillmann E, 2011, ISMRM Workshop on Ultra-High Field Systems & Applications ;7.Nielsen et al., 2001, J. Physio.;8.Just et al., 2010 ; MRI ;9. Mlynarik et al., 2006, MRM ;10 Strupp, 1996, NeuroImage;11. Aronoff et al.; 2010, Neuron ;12. Schridde et al., 2008, Cereb. Cortex ;13. Diamond et al., 1992., J Comp Neurol.