Two hour-sustained brain activation in the anesthetized rat

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Introduction :

The electrically stimulated rat forepaw is a widely known model used to investigate the anatomical organization of the somatosensory and motor cortices and the modulation of the parameters involved in the BOLD response. Network functionality, such as connectivity and plasticity mechanisms, can also be studied with BOLD fMRI, but long stimulation paradigms might be required to observe long-term modifications within small or large brain areas. The aim of the study was to develop a robust method to locally and sustainably activate the rat brain.

Material and Methods :

Animal preparation : Male adult SD rats (n=10, 322 ± 13 g) under α-chloralose anesthesia were stereotaxically fixed and positioned in a homebuilt holder. Physiological conditions were maintained during the entire experiment. **Electrical stimulation :** Left forepaw was electrically stimulated with two stainless steel electrodes inserted between the digits. Square pulses (0.5ms width) were delivered at constant current (2-3 mA) and constant (2 Hz) or variable frequencies (2-3 Hz switches at regular intervals) using an external stimulator. Several paradigms with variable interstimulus interval (ISI) (60sec, 30sec and 10sec) were applied and repeated for 2 hours in the left forepaw to find the one providing a sustained BOLD response with little habituation. **BOLD fMRI** : Experiments were performed on an actively shielded 9.4T/31cm horizontal bore magnet with a quadrature transmit/receive surface coil. First and second order shims were adjusted using FAST(EST)MAP resulting in water half linewidths of 16–21 Hz in a 270µl volume. The BOLD response was measured with single shot GE-EPI (TR/TE=2500/25ms ; FOV=30×30 mm; matrix=64×64; slice thickness=1 mm; 6 slices, bandwidth=300-350K Hz). Echo realignment was performed using a built-in reference scan**. Data analysis** : Motion correction was performed using SPM8 (MATLAB; The MathWorks; Natick, USA; Statistical Parametric Mapping). Then data was analyzed with STIMULATE (Strupp, 1996). Stimulation induced activation t-value maps were computed on a pixel by pixel basis from the comparison between the experimental fMRI data and the applied paradigm schemes. Only clusters including at least 3 pixels were considered significant. An average time-course from the activated region was then extracted and the relative BOLD response ($(S_{stim}-S_{baseline})/S_{baseline}*100$) was calculated. No other correction or filtering methods were applied.

Results :

While continuous stimulation (2.5 mA, 3 Hz) resulted in a rapid loss of the BOLD signal after 10 min (not shown), use of ISI resulted in sustained and localized cortical BOLD responses for 2 hours during electrical stimulation of the rat forepaw (Fig 1A-B). Activated regions encompassed contralateral S1, M1, M2 (Fig 1A) and sometimes S2. The activated area at the end of the 2 hour-stimulation compared to the beginning changed by 0 ± 28% and 14 ± 76% during variable and constant stimulation frequency, respectively (Fig 1C). The BOLD responses reached a maximum after the onset of the stimulation, then decreased and remained constant during the 2 hours of stimulation (1.3 ± 0.2% ; n = 5 at variable frequency).

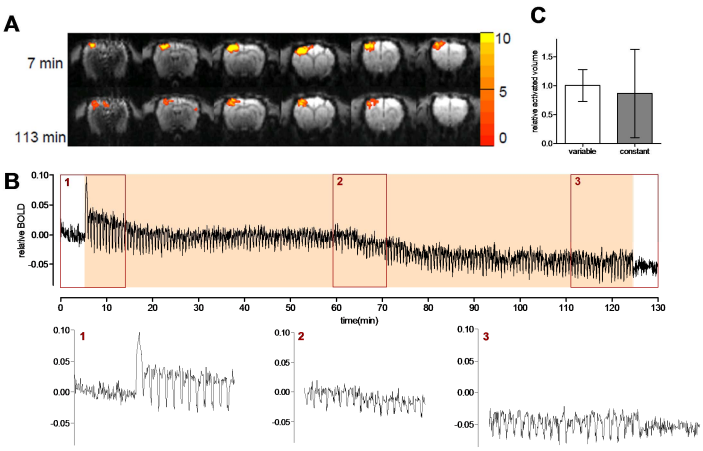

Figure 1 : (A) Representative t-test activation map showing activation of contralateral cortex overlaid on GE-EPI images after 7 min and 1.53 hour of left forepaw stimulation. The color scale represents t-values (from dark red = 0 to yellow = 10). (B) Relative BOLD signal during 2 hour-stimulation (30 sec ON – 10 sec OFF – 30 sec ON).The shaded area represents the stimulation period. Regions in red squares are expanded below. (C) Fraction of remaining activated volume at the end of the stimulation ($[1-(V_{beginning}-V_{end})/V_{beginning}$ at variable (n = 5) and constant (n = 5) frequency).

Discussion and Conclusion :

Contralateral cortical activation was maintained over 2 hours upon electrical stimulation of the left forepaw. Less variation in activated volume was observed in changing the frequency compared to constant frequency, and the steady-state BOLD amplitude was maintained during the entire stimulation period. We conclude that little adapation effects can be observed by using small ISI and changing the frequency of stimulation at regular intervals. Moreover the fact that the observed BOLD signal follows closely the applied paradigm increases the statistical power for functional network analysis.

References

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