A reproducible experimental protocol for longitudinal rat fMRI studies: electrical mystacial pad stimulation under isoflurane anesthesia

Shin-Lei Peng[1,2], Ling-Yi Huang[1], Sheng-Min Huang[1], Yi-Chun Wu[3], Hanzhang Lu[2], Fu-Chan Wei[4], Chih-Jen Wen[4], Hui-Yu Cheng[4], Chih-Hung Lin[4], and Fu-Nien Wang[1]

[1]*Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, Hsinchu, Taiwan,* [2]*Advanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, Texas, United States,* [3]*Molecular Imaging Center, Chang Gung Memorial Hospital, Taoyuan, Taiwan,* [4]*Department of Plastic and Reconstructive Surgery, Chang Gung Memorial Hospital, Taoyuan, Taiwan*

PORPOSE: Functional MRI (fMRI) is playing an important role in studies of neuroscience research. In particular, understanding BOLD characteristics in the animal model is an essential step toward the clinical implications, such as the cortical reorganization after surgery (1,2). These researches emphasize the importance of longitudinal studies on individual animals. Most rodent fMRI studies have been performed under alpha-chloralose (AC) anesthesia, owing to the advantages of stability of baseline blood flow. However, the side effect of respiratory depression makes animals need to be euthanized after experiments, which is improper for longitudinal experiments (3). Isoflurane (ISO) has been suggested as an alternative anesthetic for electrical forepaw (FP) stimulation animal fMRI studies for years because animals can wake up within minutes regardless of the length of anesthesia (4,5). Nevertheless, induced BOLD signals from FP stimulation under ISO anesthesia are relative small when compared to those under AC anesthesia due to the suppression of the synaptic potentials (6). The insensitivity of BOLD signal detection may be not easy to recognize the subtle changes during functional recovery after surgery. According to the functional mapping of rodent brain, the cortical somatosensory representation of the rat mystacial pad (MP) is larger than that of FP. Thus, we hypothesize the induced BOLD activations may be greater and more sensitive by using electric MP stimulation even under ISO anesthesia. To verify a new protocol for electric MP stimulation under ISO anesthesia, the reproducibility of fMRI experiments of rat MP stimulation under ISO anesthesia was examined. For comparison, induced BOLD activations were also compared with those under standard AC anesthesia.

METHODS: Twelve Lewis rats (320-415 g) were scanned by using a 7T animal MRI scanner (Bruker clinScan 70/30). *Study 1: Reproducibility of electrical MP and FP stimulations under ISO anesthesia.* Rats (N=6) were anesthetized with 1% to 1.2% ISO during fMRI study. To map the somatosensory cortex, needle electrodes were inserted under the skin of the left MP and left FP. A stimulator supplied current of 3 mA and stimulation frequency of 3Hz to either the left MP or FP upon demand. Each rat was scanned thrice with an interval of 1 week by using following parameters: TR=1000 ms, TE=25 ms, flip angle=90°, FOV=30 mm, and matrix size=64×64. Reproducibility of active voxels among three sessions was examined within specific ROIs: primary somatosensory cortex barrel field (S1BF), second somatosensory cortex (S2) and primary somatosensory cortex upper lip region (S1ULp) for MP stimulation while primary somatosensory cortex forelimb region (S1FL) and S2 for FP stimulation. Four reproducibility categories were used: 1) no activation: the voxel was not activated in any session; 2) no reproducibility: the voxel was activated only in one session; 3) medium reproducibility: the voxel was active in two sessions; 4) high reproducibility: the voxel was activated in all three sessions. *Study 2: Comparisons of BOLD characteristics between AC and ISO anesthesia.* Another 6 rats were anesthetized with AC (60 mg/kg) during fMRI experiments. To compare BOLD characteristics from electrical MP under different anesthetics, the stimulation and fMRI scan parameters were identical to those used in Study 1. Averaged BOLD percentage changes were calculated in the ROI within the S1BF from rats in Study 1 and Study 2 under electrical MP stimulation.

RESULTS and DISCUSSION: *Study 1:* Figure 1(a) and (b) compare the reproducibility of activated voxels of one typical rat from MP and FP stimulations, respectively. The areas in purple, yellow and blue represent voxels activating with high reproducibility, medium reproducibility and no reproducibility, respectively. The highly reproducible activations (purple) in the S1BF were detected in the MP stimulation but not seen in S1FL during FP stimulation, suggesting that larger cortical somatosensory representation of MP stimulation would increase the sensitivity of BOLD signal detection and benefit the reproducibility test. The average of the mean percentages of no activation, no reproducibility, moderate reproducibility and high reproducibility for ROIs from six rats are showed in Fig. 2. In the MP stimulation (Fig.2 (a)), 31% of voxels in the S1BF could be activated across three sessions. Even for the S2, which is not easy to be detected in most animal fMRI studies (5, 7), 15% of voxels could be activated across three sessions. On the other hand, for FP stimulation (Fig. 2(b)), only 12% of voxels in the S1FL showed the repeatedly significant activation across three sessions. None of voxels in S2 could be repeatedly detected across three sessions and 83% of voxels in S2 showed no activation. In terms of activation in S2, our findings imply that MP stimulation is superior to FP stimulation, opening up the opportunity to further probing the somatosensory pathway.

Study 2: Group-level activation maps of the MP stimulation under ISO and AC anesthesia are displayed in Fig. 3. The significant activations in S1BF, S2, and S1ULp during MP stimulation were found for both ISO-fMRI and AC-fMRI. Under ISO anesthesia, the extensions of activations were in excellent agreement with those under AC anesthesia. Figure 4 shows the average BOLD signal changes in S1BF during MP stimulation under both ISO and AC anesthesia. The BOLD signal change in S1BF was 3.03± 0.54 % in the first ISO-fMRI session, 3.46 ± 1.48 % in the second ISO-fMRI session, 2.78 ± 1.42% in the third ISO-fMRI session, and 2.5 ± 0.92 % in the AC-fMRI session. There were no significant differences in BOLD signal changes between 4 sessions (ANOVA, P=0.54).

In summary, we demonstrate the feasibility of electrical MP stimulation in rat fMRI study under ISO anesthesia. ISO-fMRI for MP stimulation achieves a high level of competence in the inter-scan reproducibility, showing reproducible activations in S1BF, S2 as well as S1ULp in 3 successive sessions. The stable stimulus-related activation could benefit the comparisons of long-term brain plasticity research. Moreover, in terms of the extension of activations and the induced BOLD signal changes, results from MP stimulation under ISO anesthesia are comparable with those under AC anesthesia. Findings in this work suggest that MP stimulation under ISO anesthesia could serve as a new approach in longitudinal animal fMRI studies.

REFERENCES: 1. Pelled et al., Neuroimage, 2007; 37: 262. 2. Xin et al., Neuroimage, 2010; 49: 1667. 3. Silverman et al., Laboratory Animal Science, 1993; 43: 210. 4. Liu et al., MRM, 2004; 52: 277. 5. Sommers et al., NMR Biomd, 2009; 22: 414. 6. Richards et al., BJA, 2002; 89:79. 7. Kim et al., Neuroimage, 2010; 52: 224.

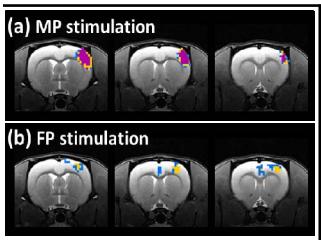

Fig. 1: Reproducibility of activation maps from one rat for (a) MP and (b) FP stimulations. Purple areas are voxels activated in all three sessions, yellow are voxels activated in two sessions, and blue are voxels activated in only one session.

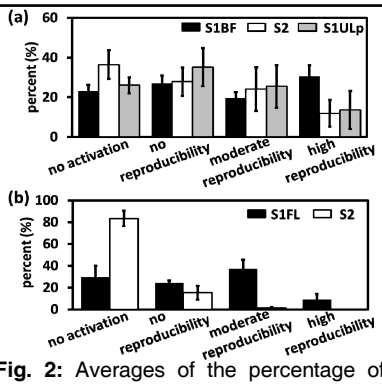

Fig. 2: Averages of the percentage of no activation, no reproducibility, moderate reproducibility and high reproducibility for (a) MP and (b) FP stimulations.

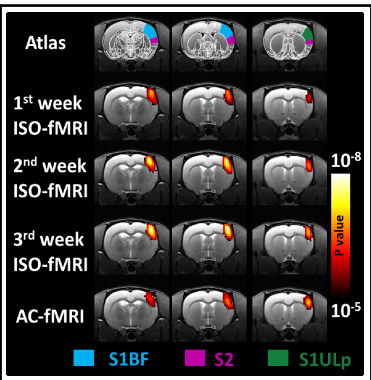

Fig. 3: Group activation maps from MP stimulation under both ISO and AC anesthesia.

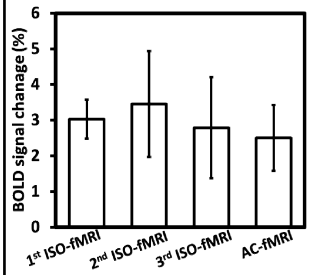

Fig. 4: BOLD signal changes from S1BF under ISO and AC anesthesia. No significant differences among sessions (ANOVA, P=0.54).