Pain fMRI response in anesthetized rats correlates with behavioral response to pain in awake rats

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[INTRODUCTION] fMRI can non-invasively measure pain-related neural activities in humans and animals, providing a valuable tool to evaluate efficacy, and to study the mechanisms of action of analgesics. However, due to its extreme sensitivity to subject motion, pain fMRI studies in animals need to be performed under anesthesia. Since anesthesia would inevitably impact the pain processing in brain, it is unknown if fMRI responses to pain measured in anesthetized animals correlate with the behavioral response to pain measured in awake animals. With noxious electrical stimulation (NES) as a pain source, the effects of intravenous infused lidocaine on the NES-induced fMRI signals in different brain regions of anesthetized rats and on the NES threshold to induce vocalization of awake rats (a behavioral response to pain) were investigated. Our results suggest that 1) pain fMRI response in anesthetized animals correlates with the behavioral response in awake animals; and 2) the primary somatosensory cortex (S1) does not appear to be involved in pain processing.

[METHODS] fMRI study was carried out on a 7 T Bruker Biospec in rats (n=6) anesthetized with medetomidine (dormitor) and low isoflurane protocol previously described [1]. The NES (2 ms, 5 mA, 9 Hz) was delivered to bilateral forepaws. After administration of USPIO [1], blood volume (BV) fMRI data were acquired in sixteen consecutive coronal slices covering the brain from forelimb region to cerebellum using a single-shot GE EPI; matrix size = 64 × 64; TE=11 ms; FOV = 2.5 cm × 2.5 cm. Each fMRI run consisted of 10-**10**-20 image acquisitions (boldface represents stimulation on) with TR=4 sec. For the behavioral study, a separate set rats (n=6) were initially anaesthetized with isoflurane (3% in air) and then secured in a rat restrainer. The study started ~1 hour after cessation of isoflurane to allow the rats to recover. The vocalization thresholds of NES of the left hindpaw and right hindpaw were measured independently in each rat. A train of 10 electrical pulses (2 ms) lasting 0.5 sec was delivered to one hindpaw starting with a low current. If no vocalization was heard, the electrical current was increased at a step-size of 0.1 mA and delivered 1.5 sec later. The process was repeated until the vocalization was heard, and the pain threshold current recorded. Measurement of pain threshold in the other hindpaw immediately followed, and the pair of measurements repeated every 10 min. Lidocaine was diluted with saline to 0.5% and injected at the volume rate of 0.1 ml/kg/min (0.5 mg/kg/min) for 40 min during both fMRI and behavioral experiments. Student's t-test was used to compare data acquired before and at various times after lidocaine administration to evaluate lidocaine's effect on NES-induced neural activation.

[RESULTS] As shown in Fig. A, before lidocaine administration (left column), NES induces well-localized activations in S1, cuneate nuclei (CN), caudate putamen (CPu), and widespread activations in some cortical and subcortical regions (collectively referred to as 'Disperse' activations). During the lidocaine infusion (middle column), while the activations in S1 are not altered, the activations in all other regions are suppressed. After cessation of lidocaine (right column), the spatial pattern and strength of the activations return to pre-infusion levels, indicating that the effects of lidocaine disappear soon after stopping infusion. To quantify lidocaine's effects on the activations in different brain regions, the fMRI amplitudes were averaged in selected ROIs covering S1, cuneate nucleus, caudate putamen and an anatomically ill-defined 'dispersed' area. As shown in Fig. B, no statistically significant amplitude change was observed in S1 during the lidocaine infusion. In all other ROIs, however, the decrease in amplitude during lidocaine infusion can be clearly observed, reaching statistical significant difference ($p<0.05$, indicated by asterisks) from pre-infusion values. For the behavioral study shown in Fig. C, the vocalization threshold slowly increases during lidocaine infusion, and slowly recovers after stopping infusion, resembling the time course reported for lidocaine plasma concentration during a similar infusion protocol [2]. The time after infusion at which each readout reaches a statistically significant difference ($p<0.05$) from pre-infusion value is indicated in the figure by asterisks. Comparing the temporal profiles of fMRI amplitude in Fig B with that of vocalization threshold in Fig. C, the decreased fMRI amplitude in the caudate putamen, cuneate nuclei and the 'disperse' activation regions highly correlate with the reversed vocalization threshold changes (regression analysis p=~0), indicating that the pain-related neural activities measured by fMRI in anesthetized rats are related to those measured by behavioral vocalization in awake rats.

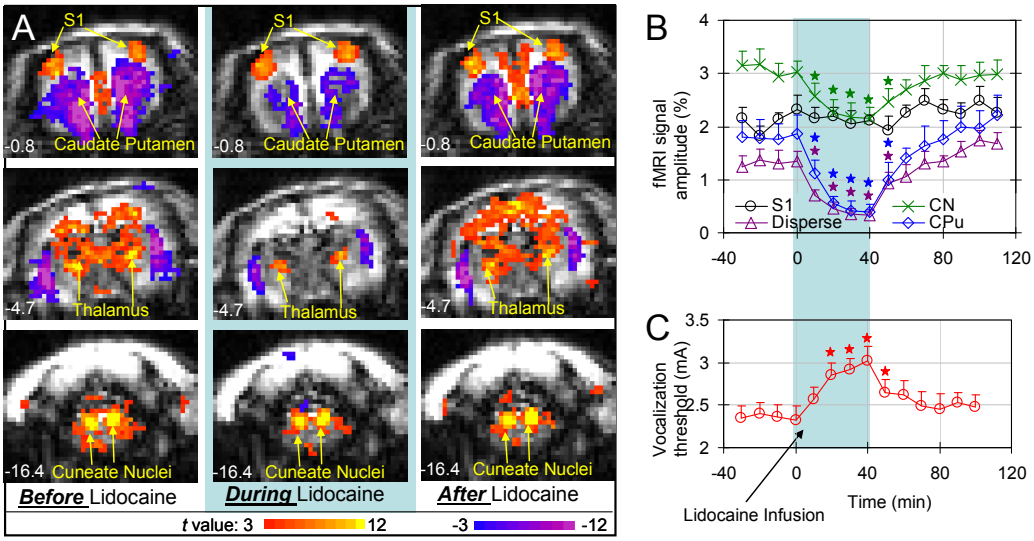

Fig. A Noxious electrical stimulation-induced BV fMRI activations in 3 slices covering primary somatosensory cortex (S1), thalamus, and cuneate nuclei (CN) from one animal before (left column), during (middle column) and after (right column) lidocaine infusion. Anterior-posterior coordinates with respect to bregma are indicated in the left-bottom corner of each image. **Fig. B** Temporal profiles of fMRI amplitudes in 4 selected ROIs (Mean ± SEM, n=6). CPu: caudate putamen. **Fig. C** Temporal profiles of the NES threshold to induce vocalization (Mean ± SEM, 6 rats, n=12 hindpaws). The shaded area indicates the time period of lidocaine infusion. * indicates times after treatment when statistical significant difference ($p<0.05$) from pre-treatment is reached.

[Discussion] Intravenous lidocaine is clinically used as an analgesic for pain treatment. Its suppression of NES-induced neural activities and behavioral response to pain were observed by fMRI and vocalization threshold, respectively. However, unlike fMRI, behavioral studies cannot provide the spatial information of the suppression effect. An important finding from this study is that pain fMRI signals in anesthetized rats highly correlate with the behavioral response (vocalization) in conscious rats, suggesting that anesthesia is not a confounding factor for monitoring analgesic action by fMRI. Furthermore, the results from this study imply that S1 may be not involved in the pain processing. Several anatomical structures including S1 referred to as 'pain matrix' [3] are presumed to be involved in pain perception. However, the role for S1 in pain processing is controversial [4]. Our result is consistent with previous electrophysiology studies in both human and conscious monkey [5, 6]. In those studies, the neural activities in S1 directly recorded by electrodes also fail to show its involvement in pain processing.

[**Reference**] 1. Zhao, F., Pain, 2009. 145: 110-9. 2. Puig, S., Pain, 1996. 64: 345-55. 3. deCharms, R.C., PNAS, 2005. 102: 18626-31. 4. Bushnell, M.C., PNAS, 1999. 96: 7705-9. 5. Klasen, J., Anesth Analg, 1995. 81: 332-7. 6. Chudler, E.H., Brain Res, 1986. 397: 47-60.