Quantification of neural activation in spinal cord by blood volume-weighted fMRI on a run-by-run basis – a viable pain assay for analgesics development

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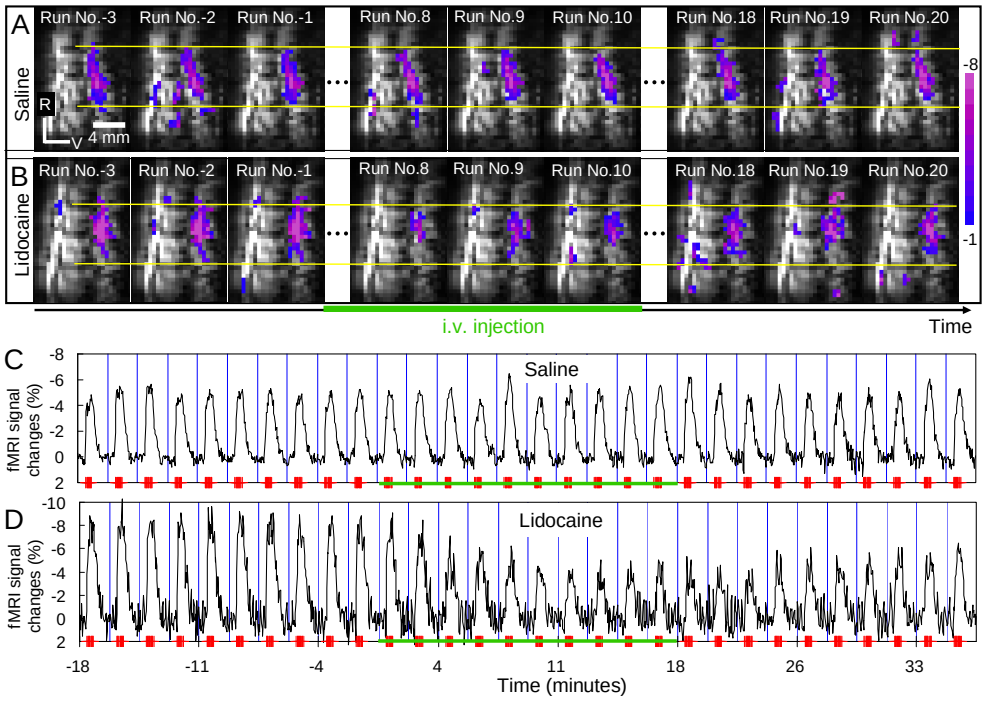
[INTRODUCTION] Spinal cord fMRI offers an excellent opportunity to quantify nociception using neuronal activation induced by painful stimuli. Measurement of the magnitude of stimulation-induced activation, and its suppression with analgesics can provide objective measures of pain and efficacy of analgesics (*1, 2*). To achieve this goal, it is preferred that the fMRI signals have high sensitivity such that they can be robustly quantified on a run-by-run basis. Such high sensitivity fMRI enables assessment of pharmacological modulation on stimulation-induced fMRI signals (pharmacodynamics (PD) of the analgesics) with temporal resolution in minute scale (the time needed for one fMRI run). In this study, a high sensitivity spinal cord fMRI technique by combining BV-weighted fMRI with optimum electrical stimulus was developed. Its utility is demonstrated by measuring analgesic effect of systemic lidocaine on noxious electrical stimulation-induced activation in spinal cord. The study also revealed that systemic lidocaine, which is clinically used for the treatment of neuropathic pain, and believed to only block the neural activity originating from the damaged peripheral nerves (*3, 4*), also blocks noxious electrical stimulation-induced activity.

[METHODS] The animal protocol was approved by the IACUC of Merck Research Laboratories. The rats (n=10) were initially anesthetized with isoflurane in a mixture of O_2 and N_2 gases (3:7). A bolus of 0.05 mg/kg medetomidine (dormitor) was then injected subcutaneously, followed by continuous subcutaneous infusion at the rate of 0.15 mg/kg/hour. Isoflurane was then reduced to 0.4% and continuously delivered throughout the experiment. All MRI measurements were performed on a 7T Bruker Biospec system. A 2 cm diameter surface coil positioned beneath the lumbar spinal cord of the rat was used as the RF receiver, while an actively-decoupled 72-mm diameter volume coil was used as the RF transmitter. T2*-weighted images were acquired using a single-shot GE EPI with phase-encoding in the dorsal-ventral direction; matrix size = 64 × 64; TE=11 ms; FOV = 4 cm (rostral-caudal direction) × 3 cm (dorsal-ventral direction). Each run consisted of 40-**40**-80 image acquisitions (boldface represents stimulation on) with TR=0.5 sec (total time per run= ~2 min). To achieve the highest activation in spinal cord, an optimum electrical pulse strain (2ms, 5mA, 40Hz) (*5*) was applied to the bilateral hindpaws simultaneously. One single sagittal slice of 2 mm thickness covering the bilateral dorsal horns was chosen. Since the magnitude of the BV-weighted fMRI signal is a function of the concentration of USPIO in blood, in order to maintain sufficient levels of USPIO in blood, after the initial 15 mg Fe/kg bolus injection, USPIO (prepared in-house) was continuously infused into a tail vein at the rate of 7.5 mg Fe/kg per 2 hours to keep the blood USPIO concentration relatively constant. Lidocaine (Sigma) was diluted with saline to 1%, and injected at the volume rate of 0.1 ml/kg/min for 20 minutes (1 mg/kg/min, total 20 mg/kg). As a control, saline was infused at the same rate of 0.1 ml/kg/min in separate rats.



[RESULTS & DISCUSSION] Figure 1 shows illustrative data from two animals: one animal shows the effect of intravenous infused saline on the noxious stimulation-induced fMRI signals in spinal cord (**A, C**), and the other animal shows the effect of intravenous infused lidocaine on the noxious stimulation-induced fMRI signals in spinal cord (**B, D**). fMRI data were analyzed on a run-by-run basis. Bilateral hindpaw stimulation-induced fMRI signal changes were displayed as relative signal changes and were overlaid on the EPI images. Negative ΔS/S changes (blue/violet) detected in the BV-weighted fMRI indicate an increase in spinal blood volume. ***(A & B)*** show activation maps of different fMRI runs in saline and lidocaine infusions, respectively. The fMRI runs before intravenous infusion of saline and lidocaine are labeled by negative number, while fMRI runs after intravenous infusion of saline and lidocaine are labeled by positive number. Total of ten runs were acquired during the infusion. For saline infusion (A), robust activations can be detected for every single run and are highly reproducible in different runs with regard to both the activation pattern and activation strength. For lidocaine infusion (B), robust activations can also be detected for every single run, but the activation becomes weaker during the infusion of lidocaine, and slowly recovers after stopping infusion. The green bar on the time line indicates the 10-run period of saline or lidocaine injection. Two horizontal yellow lines indicate the positions of disk between T13 and T12 (top) and disk between L1 and L2 (bottom). R: Rostral; V: Ventral. ***(C & D)*** show averaged time courses over the pixels of common ROI for every single run of total 30 runs (10 runs before infusion, 10 runs during infusion, and 10 runs after infusion). A trace in one vertical grid (outlined by blue line in C & D) is a time course from one single run. The 20-seconds stimulation periods are marked by **red** bars. The **green** bars indicate the 10-run period of saline and lidocaine injection. For saline infusion (***C***), there is no significant difference in the amplitudes of stimulation-induced fMRI signals, indicating no effect of saline infusion on the noxious stimulation-induced activation. For lidocaine infusion (***D***), the amplitudes of stimulation-induced fMRI signals decrease during the infusion, and recovers after stopping infusion, indicating that the lidocaine infusion decreases the noxious stimulation-induced activation in spinal cord. In conclusion, noxious electrical stimulation-induced neural activity in spinal cord detected by BV-weighted fMRI can act as a robust biomarker for analgesic development. Combining with brain fMRI, spinal cord fMRI would be crucial in distinguishing pharmaceutical targets, hence would provide valuable information towards understanding mechanisms of action of the existing analgesics and in early proof-of-concept studies for new analgesic development.

[Reference] 1. R. G. Wise *et al.*, *Neuroimag* **16**, 999 (2002). 2. R. G. Wise, I. Tracey, *J Magn Reson Imag* **23**, 862 (2006). 3. S. Puig, L. S. Sorkin, *Pain* **64**, 345 (1995). 4. M. Devor, P. D. Wall, N. Catalan, *Pain* **48**, 261 (1992). 5. F. Zhao *et al.*, *Neuroimag* **40**, 133 (2008).