fMRI evidence of association between left and right forepaw stimulation in the α-chloralose anesthetized rat

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Introduction

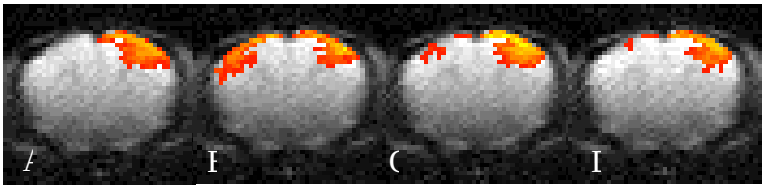
Electrical stimulation of the rodent forepaw is a well-studied model for fMRI (1-4). In these studies, activation maps have always been generated from signals that are correlated with the stimulation. However, detailed examination of the timecourse of the fMRI response to forepaw stimulation at 11.7T reveals in some rats an early pre-stimulation positive response in the primary somatosensory cortex, which usually occurs after many experimental runs. This result suggests that rats may learn to associate the electrical stimulation with some aspect of the MRI environment (such as the noise or vibration of the gradients during the EPI sequence) even while anesthetized with α-chloralose. Due to potential difficulties of determining what the cue may have been in the complex environment of the MRI scanner, we attempted to detect whether the anesthetized rat could develop an association between two well-defined stimuli. The results indicate that after one hour of stimulating two forepaws, stimulation of one forepaw leads to activation of both ipsilateral and contralateral cortex, indicating that an association had been formed under anesthesia and was detectable by fMRI.

Methods

BOLD fMRI studies of electrical forepaw stimulation were conducted in 12 rats on a 7T or an 11.7T scanner [4]. Rats were orally intubated and mechanically ventilated throughout the study. Under isoflurane, catheters were inserted into the femoral artery and vein. Anesthesia was switched to α-chloralose for imaging. Blood gases were measured and maintained at normal levels. Functional images were acquired with a single-shot EPI sequence, FOV 1.92 cm, matrix 64 x 64, TE 30 ms, TR 1.5 s, SW 200 kHz. For each epoch, 60 images were acquired during rest, 30 during stimulation, and 60 during rest. To verify normal response to stimulation of each forepaw, a set of functional images was acquired while the right paw was stimulated, and another was acquired while the left paw was stimulated. For the next hour, every stimulus epoch was applied simultaneously to both paws at intervals of 6-8 minutes (7-10 epochs presented). After the conditioning period, a set of functional images was acquired while only one paw was stimulated. In some rats, the dual-paw conditioning was repeated and followed by another image where only one paw was stimulated. A control group of three rats was established for the same paradigm described above, but with only one paw stimulated the entire time.

Results

Prior to conditioning, only the contralateral primary somatosensory cortex (SI) showed activation (Fig. 1A). During conditioning, in which both paws are stimulated simultaneously, SI activates on both sides (Fig. 1B). Immediately following the conditioning period, stimulation of only one of the paws caused activation in both contralateral and ipsilateral SIs in 8/12 rats (Fig. 1C). The activated ipsilateral SI was generally much smaller than the contralateral SI (7.4 ± 4 pixels vs. 61.7 ± 32 pixels) and showed a smaller percent signal change (1.6 ± 0.3% vs. 3.9 ± 1.7%). In 6 of the 8 conditioned animals, second stimulus epochs of only one paw showed activation only in the contralateral SI (Fig. 1D). In the three control rats that received only single-paw stimulation, activation in ipsilateral SI was observed in only 1 of 54 scans.



Discussion

Prior stimulation of both paws increases the likelihood of observing activation in the ispsilateral SI when only one paw is stimulated. These results may be interpreted as an indication that the rat associates the stimulation of one paw with the stimulation of the other. It is a short-term effect and easily extinguished by the repeated application of single paw stimulation. However, there is also a possibility that the repeated simultaneous stimulation of both paws, which gives more activation than the single-paw stimulation, causes the rat to become more responsive. Further experiments will be necessary to determine whether the results are due to association or increased sensitivity.

References: 1. Silva AC, Lee SP, Yang G, Iadecola C, Kim SG. *J Cereb Blood Flow Metab* 1999;19:871-879. 2. Silva AC, Koretsky AP. *PNAS* 2002;99:15182-7. 3. Brinker G, Bock C, Busch E, Krep H, Hossman KA, Hoehn-Berlage M. *MRM* 99;41:469-73. 4. Keilholz SD, Silva AC, Raman M, Merkle H, Koretsky AP. *MRM* 2004;52:89-99.

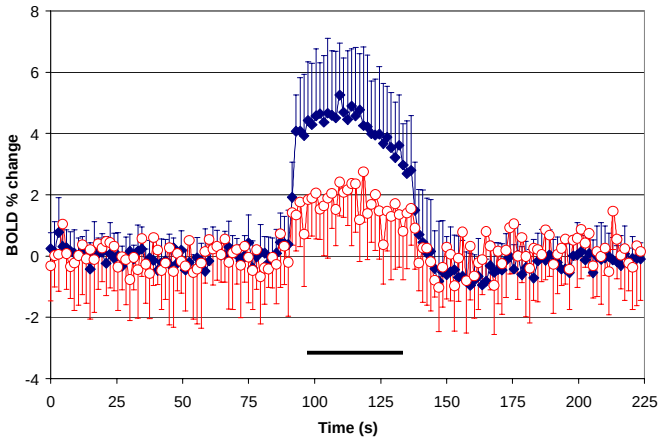


Figure 1 (top). Activation maps from one rat during single-paw stimulation before stimulation of both paws (A), during stimulation of both paws (B), during single-paw stimulation immediately after stimulation of both paws (C), and during a second single-paw scan after stimulation of both paws (D).

Figure 2 (bottom). Average time courses from contralateral SI (blue diamonds) and ipsilateral SI (red circles) during single-paw stimulation after conditioning. The black bar indicates the stimulation period.