Functional Mapping of Rat Barrel Activation Following Whisker Stimulation Using Activity-Induced Manganese-Dependent Contrast

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Introduction

Cortical whisker barrels in the primary somatosensory cortex are a well-known example of brain function in rodents [1]. The well-defined relationship between barrels and whiskers makes this system a unique model to study neuronal function and plasticity. In this study, we sought to establish a feasible working protocol of applying activity-induced manganese-dependent contrast (AIM) [2] to map the cortical barrels following whisker stimulation.

Materials and Methods

Adult Wistar rats were initially anesthetized with 2% isoflurane mixed O_2 gas. $MnCl_2$ was given, 128 mM in 2 ml, via intraperitoneal (IP) injection. To break down the BBB, the rats were anesthetized with sodium pentobarbital, and mannitol solution, 30% in 2.1 ml, was injected into the right common carotid artery. The rats in the experimental group (N=6) received mechanical stimulation. The rats were kept under shallow anesthesia by injecting sodium pentobarbital several times during whisker stimulation. For each rat, whiskers on the left side were connected to a 2-inch speaker through a cotton thread. The whiskers were twitched with a series of rectangular pulses, 15 V and 8 Hz, generated by the speaker. The paper diaphragm of the speaker was removed to avoid auditory stimulation. The speaker was positioned so that the mechanical pulses pulled the whiskers anteriorly by approximately 2 mm. In this study the rats in the experimental group received whisker stimulation for three hours before MR scanning. The rats in the control group (N=6) underwent the same anesthesia as the experimental group without mechanical stimulation.

The experiment was performed on a 3T MRI system (Biospec, Bruker, Germany). A multislice multiecho spin echo sequence was performed to obtain T1WI, TR/TE = 500/10 ms; in-plane resolution = 187 μm; slice thickness = 1.5 mm. To improve detection sensitivity over the full extent of Mn^{2+} concentrations, image data for R1 mapping were acquired [3]. To obtain R1 mapping, the same sequence was performed to acquire 23 sets of images corresponding to 23 different TRs, ranging from 300 to 6000 ms, to sample along the recovery of longitudinal magnetization. Given the current field strength and scan time, cortical enhancement could not be visualized clearly in each individual rat; the enhancement could only be detected by averaging the rat images of the same group of rats. The post-processing of the image data entailed stereotactic coregistration, pixel intensity normalization, inter-subjects averaging and volume of interest (VOI) analysis. To highlight the activity-related functional enhancement following whisker stimulation, the averaged Mn^{2+}-enhanced T1WIs in the control group was subtracted from that in the experimental group after coregistration.

Results

In both experimental and control group, we noticed that right hemisphere, site of mannitol injection, was enhanced more than left hemisphere on T1WI (Fig. 1a, 2a) and R1 mapping (Fig. 1b, 2b). In the experimental group, cortical enhancement was localized in the barrel fields (Fig. 2a), whereas in the control group the enhancement was uniform throughout the cortex showing no specific localization (Fig. 1a). To localize the activity-related areas, R1 mapping was made. In the experimental group, the R1 mapping showed gradients of R1 with their peaks located at the right barrel fields (Fig 2b). In the control group, the R1 mapping showed no specific localization of the gradients (Fig. 1b). In the subtracted images the enhanced areas unrelated to whisker stimulation such as hippocampus, hypothalamus and amygdala were suppressed. Only the right barrel fields which were activated during whisker stimulation remained enhanced (Fig. 3a). The result was consisted with the brain atlas (Fig. 3b) [4].

Table 1 lists normalized intensities and R1 values calculated from T1WI and R1 mapping, respectively. Compared with the normalized intensities of T1WI, R1 values provided greater contrast between right and left hemispheres or between experimental and control groups. In both experimental and control groups, the normalized intensities and R1 values in the right barrel fields were higher than those in the left barrel fields. Comparing the enhancement of right barrel fields between the experimental and control groups, the normalized intensities and R1 values were both higher in the experimental group. In the left barrel fields, however, there was no significant difference between the experimental and control groups.

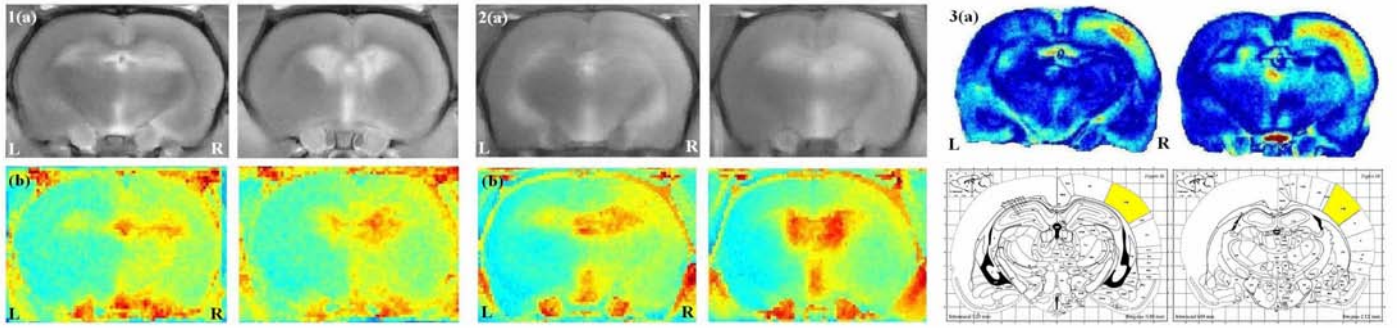

Fig. 1 Two consecutive slices of the averaged manganese-enhanced T1WIs (a) and R1 mappings (b) in the control group.
Fig. 2 Two consecutive slices of the averaged manganese-enhanced T1WIs (a) and R1 mappings (b) in the experimental group.
Fig. 3 Image subtraction (a) between experimental and control groups and the corresponding rat brain atlas (b).

Table 1

	T1WI (arbitrary unit)			R1 (sec^{-1})		
	Experimental	Control	Comparison	Experimental	Control	Comparison
Right	1.541 ± 0.167	1.177 ± 0.109	$p < 0.05$	1.120 ± 0.164	0.831 ± 0.215	$P < 0.05$
Left	1.152 ± 0.128	1.035 ± 0.062	$p > 0.05$	0.798 ± 0.179	0.647 ± 0.178	$P > 0.05$
Comparison	$p < 0.05$	$p < 0.05$		$p < 0.05$	$p < 0.05$	

Table 1. Volume of interest (VOI) analysis of bilateral cortical barrels in the experimental and control group.

Discussions

In this study a clear relationship between Mn^{2+}-enhanced cortical regions and whisker tactile-sense-evoked activity was demonstrated using the AIM method. Activity in barrel fields was recorded by IP injection of Mn^{2+} followed by whisker stimulation outside the MRI room for three hours and scanned. Mn^{2+} accumulation remained even after activity stopped demonstrating the memory capability of MEMRI. The fact that Mn^{2+} enters neurons during stimulation and does not leave rapidly, underlines its unique capability of mapping functioning neurons. Mn^{2+} can be injected into animals and taken up by the active neurons in response to stimulation closer to their normal behavior, which can be mapped and observed by MRI afterwards [5].

Conclusions

We have mapped the activated whisker barrels using the AIM method on a 3T MR system, showing a clear relationship between Mn^{2+} enhancement and whisker tactile-sense-evoked activity. Our results indicate that the AIM method is potentially useful to study plasticity in surgically- or genetically- manipulated rat brains.

References

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