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Brainmapping of α-chloralose anesthetized rats with T_2*-weighted imaging: distinction between the representation of the forepaw and hindpaw in the somatosensory cortex†

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ABSTRACT: T_2*-weighted imaging at 4.7 T was used to identify the cortical areas activated by electrical stimulation of the forepaw and hindpaw of α-chloralose anesthetized rats. Variation of the coronal slice position relative to the bregma, showed that the forepaw representation in the somatosensory cortex is more frontal and lateral than that of the hindpaw. Overlap between both activation areas was observed only in a small region in the slice at the level of the bregma. Documented localizations of both representations are in good agreement with earlier observations using invasive techniques. The determination of the separate areas of both paws indicates the feasibility of more complex activation studies in anesthetized animals, such as combined stimulations for the investigation of potentiation or depression effects on individual stimuli. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: fMRI; forepaw; hindpaw; rat; somatosensory cortex

INTRODUCTION

The application of functional magnetic resonance imaging (fMRI) to animal models has recently been demonstrated in various publications.[1–4] In most of these studies somatosensory activation was performed with electrical forepaw stimulation of α-chloralose anesthetized rats. Recently, fMRI studies with this animal model were extended to investigations under pathophysiological conditions.[5–7] There, variations of the fMRI signal intensity were observed depending on the different physiological or pathophysiological conditions[5–7] or stimulation paradigm.[2,8] All these studies were limited to somatosensory activation of the forepaw with variations from left to right forepaw, or right and left somatosensory cortex, respectively.[2,3] The first report describing the activation of neighboring brain areas in the somatosensory cortex of the rat with fMRI was presented by Yang *et al.*[9] These authors were able to detect different activated regions in the somatosensory cortex during single barrel stimulation at 7 T.

In the present study, we demonstrate the feasibility to distinguish between different activated regions in the somatosensory cortex using fore- and hindpaw stimulations at 4.7 T. The cortical representations of both paws have been described to be direct neighbors to each other.[10] Together, they make up a large portion of the somatosensory cortex, S1. Separation of the activated regions in the somatosensory cortex will provide the basis for more complex stimulation paradigms in animals under different physiological conditions, such as interactions of stimuli or enlargement of representation area after conditioning.[11]

MATERIALS AND METHODS

Animal model

Five Sprague-Dawley rats were initially anesthetized with 1.5% halothane in a gas mixture of 70/30 v/v N_2O/O_2 for surgical preparation. The femoral artery and vein were catheterized with PE50 tubing for blood pressure monitoring, fluid and drug administration, and blood sampling. After a tracheotomy, the animals were paralyzed with pancuronium bromide (0.1 mg/kg/h) and

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†**Abbreviations used:** fMRI, functional magnetic resonance imaging; T_2*-WI, T_2*-weighted imaging.

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artificially ventilated with a small animal ventilator (RVS/1301, FMI-Medical Instruments, Seeheim, Germany). Then, anesthesia was switched to α-chloralose (80 mg/kg i.v.) for functional activation studies as already described in detail by Gyngell *et al.*[2] and Ueki *et al.*[12] Body temperature was measured via a rectal thermocouple and kept at 37°C with a feedback-controlled warm water blanket. Arterial blood pressure was monitored continuously, and arterial blood samples for blood gas analysis were taken in 30 min intervals.

For somatosensory stimulation, pairs of small needle electrodes were inserted into the skin of the left or right fore- and hindpaw. Electrical stimulation of the paws was carried out by applying square-wave pulses of 0.3 ms duration with a frequency of 3 Hz.[2] The current was 0.5 mA, unless stated otherwise.

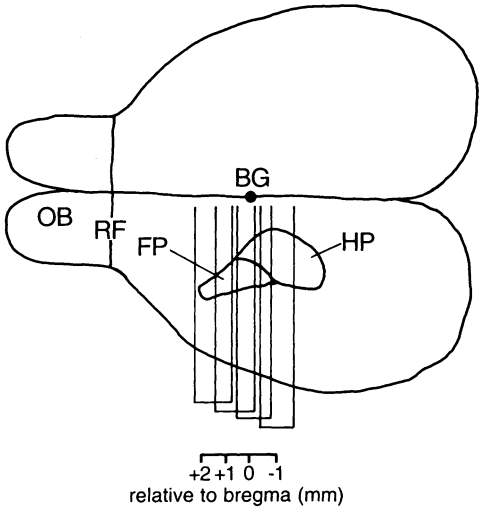

Figure 1. Schematic drawing of an axial orientation of the rat brain from the dorsal view. The olfactory bulb (OB) and the rhinal fissure (RF) are marked. The representations of the right forepaw (FP) and hindpaw (HP) in the somatosensory cortex of the left hemisphere are circumscribed. For orientation of the image positions of the fMRI experiments, the four chosen coronal slices are indicated. For localization of the image sections, their center positions are given relative to the bregma. The bregma (BG) is marked on the midline.

NMR methods

The NMR measurements were performed at 4.7 T using a 47/30 Biospec MSL-X11 system (Bruker Medizintechnik, Ettlingen, Germany) with actively shielded gradient coils (100 mT/m, 250 μs rise-time). Radio frequency pulses were transmitted using a 12 cm diameter Helmholtz coil, with the NMR signal reception via a 16 mm diameter inductively coupled surface coil placed over the skull and centered over the midline of the animal. Multi-slice FLASH pilot scans of brain anatomy were obtained in the sagittal planes, in order to place the functional imaging slice coronally through the fore- or hindpaw-area of the somatosensory cortex choosing the position relative to the rhinal fissure. For functional studies T_2*-weighted images (T_2*-WI) were acquired using a FLASH sequence (TR = 70 ms, TE = 60 ms, matrix size = 64 × 64, FOV = 2.4 cm, 2 mm slice thickness) with a flip angle α of 22.5°, resulting in a scan time of 4.5 s.[2] Anatomical coronal FLASH images of every fMRI slice (TR = 400 ms, TE = 8.4 ms, matrix size = 128 × 128, FOV = 2.4 cm, 2 mm slice thickness) were recorded for comparison with anatomical data from the literature by Paxinos[10] and Zilles,[13] and for data analysis.

Experimental protocol

The animals were fixed in a non-magnetic stereotactic headholder and positioned in the magnet. A set of eight T_2* -WIs were collected prior to stimulation (baseline) and were repeated during stimulation. All experiments started with the electrical stimulation of the forepaw in order to test the functional response of the somatosensory cortex in the T_2*-weighted images, and for comparison with established protocols of previous investigations.[2,3,7] After a resting period of 5 min electrical stimulation of the hindpaw was carried out. Then, the position of the imaging slice was shifted 1 mm posterior or anterior relative to the bregma and the set was repeated. In some cases, stimulation of the hindpaw was repeated as a function of the electrical current (I = 0.5, 0.75, 1.0, 1.25 and 2.0 mA).

T_2*-WI activation maps were obtained by subtracting averaged baseline images from averaged activation images, taking only those pixels into account with signal intensity 1.5 standard deviations (SD) above background noise.[2] Pixels were included only inside the brain and when forming spatial clusters of at least three pixels. Resulting activation maps were overlaid on anatomical FLASH images of the corresponding slice.

RESULTS

The physiological parameters remained within the normal range during the whole length of the NMR experiments. Blood pressure was kept between 90 and 100 mmHg, and arterial pCO_2 between 35 and 40 mmHg.

Figure 1 presents a schematic drawing of the localization of different somatosensory regions in the rat brain, based on the regional assignment by Paxinos.[10] The area of the forepaw is located more anterior and lateral in the somatosensory cortex than that of the hindpaw. For orientation purposes, the coronal image positions chosen for the activation experiments of fore- and hindpaw are also included in the drawing.

Stimulation of the right forepaw with a current of 0.5 mA resulted in a clear increase in T_2*-WI signal intensity

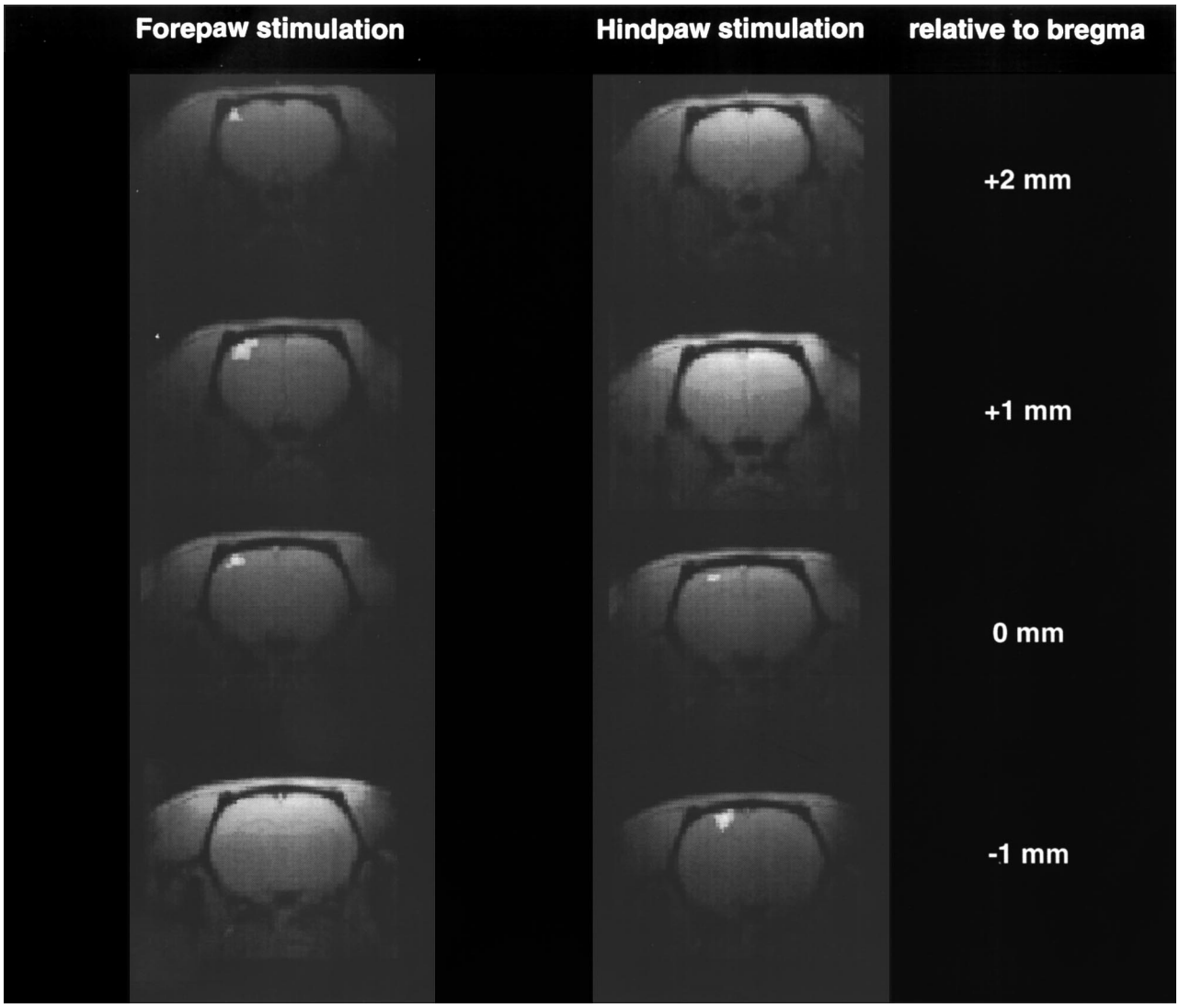

Figure 2. Coronal images of the rat brain at various positions anterior to posterior of the bregma. The activated areas, analyzed from the T_2^*-weighted image experiments, are overlaid on anatomical FLASH images. Activation of the forepaw is seen on the two frontal sections with the activation center being in the slice 1 mm anterior of the bregma. The activated area during hindpaw stimulation appears only when the imaged sections are shifted further posterior. Also, the hindpaw representation is more medial than that of the forepaw.

in the somatosensory cortex of the left hemisphere (Fig. 1). In contrast, the same stimulation of the hindpaw did not lead to any signal intensity changes of the T_2*-WI. Only when raising the current to 1.25 mA during hindpaw stimulation was an activated area detected. No significant difference in T_2*-WI signal intensity increase was observed between the activation of fore- and hindpaw.

An increase in blood pressure was not observed at this current during hindpaw stimulation, in contrast to the forepaw where the blood pressure increased when the stimulation current was raised above 1 mA.

In Fig. 2 the functional activation maps of different brain slices during the stimulation of the right fore- and hindpaw, respectively, are presented. On the left column fMRIs during forepaw stimulation are shown ($I = 0.5$ mA); activation maps during hindpaw stimulation ($I = 1.25$ mA) are given in the center column of Fig. 2. The corresponding positions of the image planes relative to the bregma are indicated on the right.

While a highlighted area in the left somatosensory cortex during forepaw stimulation occured in the most anterior slice (+2 mm relative to the bregma) and increased in size in the slice 1 mm anterior to the bregma, no activation was observed in these slices during hindpaw stimulation. In the following slice (at the bregma), posterior to the former two slices, the size of the activated area of the forepaw stimulation decreased again in the left somatosensory cortex. Stimulation of the hindpaw resulted in a small activated area in the somatosensory cortex of the same slice. This activated region was

located slightly more medial than for the forepaw. When the image plane was moved 1 mm posterior to the bregma, the activation during forepaw stimulation was no longer observed in the left somatosensory cortex, whereas the activated area during hindpaw stimulation increased in size.

Shifting the image plane even further posterior to the bregma, the T_2*-weighted images showed strong artifacts, because of susceptibility effects of the air-filled petrous bones of the animals. Comparison of the position of the activated regions as found from our fMRI data agreed very well with the assigment as described by Paxinos[10] (Fig. 1).

DISCUSSION

The aim of this paper was to demonstrate the capability of fMRI to achieve successful brainmapping in anesthetized animals at 4.7 T. The schematic functional architecture of the rat brain shows two separate, but closely neighboring, areas for the representation of the forepaw and hindpaw in the somatosensory cortex.[10] The locations of these areas differ in their position anterior and posterior relative to the bregma, but there is also a coronal section where both regions can be detected simultaneously. Furthermore, the shift of the activated areas from lateral to medial when going from fore- to hindpaw stimulation, was presented in the T_2*-WI activation maps of Fig. 2. Also, a small overlap of both regions could be observed in the T_2*-WI activation maps in the image plane at the bregma position (0 mm), indicating the good spatial resolution of fMRI at 4.7 T.

It should be pointed out that the stimulation currents of the activation paradigms of fore- and hindpaw were different. This is explained with the differing sensitivity of fore- and hindpaw to somatosensory stimulation. In man, the hands (and fingers, in particular) are known to have the highest somatosensory receptor density. Similar to that situation, the forepaw of the rat may be considered highly sensitive to somatosensory stimulations because these animals use their forepaws for all somatosensory activities, whereas the hindpaws are used predominantly for standing and running. Therefore, it is conceivable that the hindpaws require a higher electrical current for activation. Owing to the high sensitivity of the forepaw, a stimulation current above 0.5 mA induced a rise in blood pressure. This was in contrast to hindpaw stimulation, where no increase of blood pressure was observed. Only when the stimulation current was raised further to 2 mA, was an increase of blood pressure induced during hindpaw stimulation (data not shown), confirming the lesser sensitivity of the hindpaw to electrical stimulation. These differences indicate that the control of stimulation paradigms for functional activation studies in animals are important in order to avoid a misinterpretation of fMRI data, particularly for more complex stimulation protocols.

CONCLUSIONS

Stimulation of the fore- and hindpaw resulted in different activated areas in the somatosensory cortex of the rat brain observed with fMRI, demonstrating the capability of T_2*-weighted imaging and its sufficient spatial resolution for brainmapping studies in the rat brain at 4.7 T. Furthermore, the different stimulation currents for activation of fore- and hindpaw, indicated the necessity to control and/or adapt the stimulation paradigms for particular applications. The possibility for fMRI to distinguish different activated regions in the rat brain at 4.7 T prepares the ground for investigations of combined and more complex stimulation paradigms, including the result of somatosensory interactions between individual stimuli. Such experiments are at present under way in our laboratory.

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