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Mapping plasticity in the forepaw digit barrel subfield of rat brains using functional MRI

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Abstract

The topographic organization of the forepaw barrel subfield in layer IV of rat primary somatosensory cortex (S1) is a good model for studying neural function and plasticity. The goal of this study was to test the feasibility of functional MRI (fMRI) to map the forepaw digit representations in the S1 of the rat and its plasticity after digit amputation. Three dimensional echo-planar imaging with 300 micron isotropic resolution at 11.7 T was used to achieve high signal-to-noise ratios and laminar layer resolution. By alternating electrical stimulation of the 2nd (D2) and 4th (D4) digits, functional activation in layer IV of the barrel subfields could be distinguished using a differential analysis. Furthermore, two and a half months after the amputation of the 3rd digit in baby rats, the overlapping area between D2 and D4 representations was increased. This indicates that the forepaw barrel subfield previously associated with the ablated digit is now associated with the representation of nearby digits, which is consistent with studies using electrophysiology and cytochrome oxidase staining.

Keywords

functional MRI; high resolution; forepaw digit barrel subfield; electrical stimulation

Introduction

The forepaw barrel subfield is an organized region in layer IV of the rodent primary somatosensory area (S1). The most noticeable features of the forepaw barrel subfield are four centrally located bands of barrels orientated along a mediolateral plane. Each band consists of three to four barrels, which corresponds to digits from the 2nd digit (anterior) to

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the 5th digit (posterior). Each band is about 200 – 300 microns in width and 500 – 800 microns in length (Watres et al., 1995; Welker, 1976; Woolsey and Van der Loos, 1970). The well-defined relationship between the cortical barrels and the forepaw digits makes this system a good model for the study of neural function and plasticity. For example, it has been reported that removal of a peripheral afferent input to the forepaw barrel subfield prior to postnatal day 5 or 6 results in a disorganized forepaw barrel subfield, whereas deafferentation at later times produces little or no alteration of the forepaw barrel subfield (Dawson and Killackey, 1987; McCandlish et al., 1996). Although the representation of digit columns has been investigated by optical imaging of intrinsic signals (Gochin et al., 1992) and electrophysiological studies (Li et al., 1996; McCandlish et al., 1996), non-invasive mapping remains a challenge.

Functional magnetic resonance imaging (fMRI) allows noninvasive mapping of brain function based on the blood oxygenation and flow changes following neural activation (Kwong et al., 1992; Ogawa et al., 1992). This blood oxygenation level dependent (BOLD) fMRI method has been used in numerous studies in human and animals (Hennig et al., 2003; Ugurbil et al., 1999). However, several factors, such as the signal-to-noise ratio (SNR), the large vein effect, and the vascular point spread function (Kim and Ogawa, 2002; Menon and Goodyear, 1999), make it challenging to apply this method to resolve neural function in cortical columns or cortical layers. With high-resolution imaging at high field, the responses of the entire olfactory bulb in rats (Schafer et al., 2006), single whisker representations in rodent S1 (Yang et al., 1996), laminar layer dependent responses in rats (Silva and Koretsky, 2002), and cortical plasticity in rats (Pelled et al., 2009; Pelled et al., 2007; Pelled et al., 2006; Yu et al., 2010) have been differentiated using BOLD fMRI. Other research groups have been working on high-resolution fMRI studies mapping the forepaw barrel subfield (Li et al., 2010; Pawela et al., 2008) and the finger areas of monkeys (Chen et al., 2007; Zhang et al., 2010). Utilizing other measurements, such as cerebral blood flow (CBF) or cerebral blood volume, the orientation columns in the cat visual area have also been differentiated (Duong et al., 2001; Fukuda et al., 2006). Several such studies in humans have detected the ocular dominance column in the primary visual area (Cheng et al., 2001; Menon et al., 1997) and single digit representations (Francis et al., 2000; Nelson and Chen, 2008; Schweizer et al., 2008).

This study tests the feasibility of BOLD fMRI in mapping the forepaw digit representations in the S1 of the rat and applies this technique to study the cortical plasticity after digit amputation at early age. The results show that individual digit representations can be reliably identified and the functional reorganization after amputation can be detected.

Materials and Methods

Animal Preparation

All experiments were approved by the Animal Care and Use Committee of the National Institute of Neurological Disorders and Stroke, National Institutes of Health (Bethesda, MD, USA). Ten adult male Sprague Dawley rats were imaged at two and a half months of age (about 250 g body weight). In five rats, the 3rd digits (D3) were cut at approximately postnatal day 3, whereas the other 5 rats served as controls. For amputation, the rats were anesthetized with pentobarbital sodium (35 mg/kg) and with a local injection of lidocaine (Xylocaine, 2%) in the skin proximal to D3. A tourniquet was placed around the proximal part of D3, and the digit was amputated at the metacarpophalangeal joint. The wounded skin was sutured and stabilized using cyanoacrylate and was cleaned with gentamicin to prevent bacterial infection. The animals were then returned to their cages, after which the wound site was examined daily. No infection was observed in any of the animals. Animals remained caged for an average of two and a half months prior to functional imaging.

Before imaging, the rats were anesthetized with 5% isoflurane mixed with air, N₂, and O₂, and maintained at 1.5% isoflurane during the following surgical procedures. Each rat was orally intubated and placed on a mechanical ventilator (CWE SAR-830/P, Ardmore, PA, USA) throughout the surgery and the experiment. Polyethylene catheters were inserted into the right femoral artery and vein to allow monitoring of arterial blood gases and administration of drugs. After surgery, the rat was given an i.v. bolus of α -chloralose (80 mg/kg; Sigma, St Louis, MO, USA), and isoflurane was discontinued. Anesthesia was maintained with a constant α -chloralose infusion (27 mg/kg/hr). Rectal temperature was maintained at $\sim 35.5^{\circ}\text{C}$. End-tidal CO₂, rectal temperature, tidal pressure of ventilation, heart rate, and arterial blood pressure were continuously monitored. Arterial blood gas levels were checked periodically, and corrections were made to maintain normal levels. For example, sodium bicarbonate (14 mg/kg) was injected intravenously when the blood pH was too low. Pancuronium bromide (4 mg/kg) was given once per hour by i.v. to prevent motion.

Digit Stimulation

During the functional imaging, electrical stimulation was applied to the rat's 2nd (D2) and 4th (D4) digits. Two ring pairs were attached to D2 and D4, respectively, and were connected to a stimulator (World Precision Instruments, Sarasota, FL, USA) that applied serial rectangular pulses of 2 mA current, 0.33 ms duration, and 3 Hz frequency (Keilholz et al., 2006). A block-design paradigm was used to stimulate the rat's D2 and D4 alternatively (Fig. 1a). Each digit was stimulated with five cycles of 15-s electrical stimulations with 75-s resting periods in between, and the digit that was stimulated first was randomized across animals. Stimulation of one of the digits was preceded by a 15 s resting period, whereas stimulation of the other digit was preceded by a 60 s resting period. Therefore only one digit was stimulated at a time and the digit that was stimulated first was randomized across animals. The experimental time of each run was 465 s. The animal was allowed to rest for 3 to 5 min, and then the stimulation paradigm was repeated. At least 6 runs and, on average, 8 runs of the experiment were conducted in each rat.

Data Acquisition

Images were acquired using an 11.7 T / 31 cm horizontal bore magnet (MagneX, Abingdon, UK) interfaced to an AVANCE console (Bruker BioSpin, Billerica, MA, USA). A homemade 10-mm-diameter receiving surface coil and a 90-mm-diameter birdcage transmitter coil were used to achieve high sensitivity. The reduce sensitivity area also helped to avoid fold-over artifact in the 3D imaging. Functional images were acquired by a custom-written 3D gradient-echo echo planar imaging (EPI) sequence with navigator echo guided reconstruction. The imaging parameters were: effective echo time = 30 ms, repetition time = 46.9 ms (effective repetition time of a whole volume = 1.5 s), flip-angle = 12° , bandwidth = 200 kHz, matrix size = $64 \times 64 \times 32$, field of view = $19.2 \times 19.2 \times 9.6 \text{ mm}^3$, yielding a 300 μm isotropic voxel resolution.

Data Analysis

Data processing and analyses were performed using in-house written software in MATLAB (MathWorks Inc., Natick, MA, USA). Movement was minimized by head fixation and muscle relaxant. The residual brain motion was further checked by inspecting the center of mass of each image. If certain translation or rotation displacement was found, that run of data was discarded. Temporal signal drift was corrected by linear fitting to the resting periods in each run before averaging. Then the data of different runs were averaged to improve the signal-to-noise ratio (SNR). Functional maps of D2 and D4 were generated by two kinds of correlation analyses: regular and differential. In the regular analysis, digit stimulation was compared with resting (i.e., D2 vs. rest or D4 vs. rest; Fig. 1a), and the data points corresponding to the other digit were removed. In the differential analysis,

stimulation of one digit was compared with the other (i.e., D2 vs. D4 or D4 vs. D2; Fig. 1b), and the data points corresponding to the rest period(s) were removed. The paradigm was shifted by 2 TRs (i.e., 3 s) to account for the delay of hemodynamic response in small animals (de Zwart et al., 2005). The correlation coefficient threshold was set at 0.2 with a minimum cluster size of 3 voxels in 3D. The percentage signal change maps were generated by subtracting the average signal intensity during the stimulation with the average intensity during the resting state (regular paradigm) and by subtracting between the averages of the two stimulation states (differential paradigm). A depth of 0.8 mm below the surface of cortex (layer IV) was chosen in coronal sections of the somatosensory area. Profiles of the activation in layer IV were drawn following the curvature of the brain on correlation coefficient maps and percentage signal change maps along the direction of largest activation extent. The mean profiles were calculated by aligning the central position between the peaks of D2 and D4. The full width at half maximum (FWHM) of the D2 and D4 profiles and the distance between the peaks of D2 and D4 on the correlation coefficient and percentage signal change profiles were calculated. Activation volumes of D2 and D4 were calculated from the 3D correlation coefficient maps, and the mean percentage signal change in these activation volumes was calculated. The Student's t-test was used for statistical analysis, and $p < 0.05$ was regarded as significant.

Results

By averaging at least 6 runs of fMRI data, an SNR of 54.6 ± 11.8 ($N = 10$) was achieved at 300 micron isotropic resolution. Significant and focal activation regions were observed using both regular and differential analyses where D2 activation (in red) was located more anterior and laterally than D4 activation (in green) (Fig. 2). This is consistent with the known relative location of these columns (Welker, 1976; Woolsey and Van der Loos, 1970). However, the activation areas of D2 and D4 were wider and overlapped when the digit activation was analyzed using the regular paradigm (Fig. 2, left column). Comparing the width of D2 and D4 activation and the distance between them (Table 1), the sum of half of the FWHM ($\text{FWHM}_{D2}/2 + \text{FWHM}_{D4}/2$) was 1.1 ± 0.3 mm, which was larger than the distance between the peaks in both representations (0.8 ± 0.3 mm).

Typically highest correlation was observed in the middle layer of the SI cortex, around the depth of the layer IV, similar to the observation reported by Silva et al. (Silva and Koretsky, 2002). Figure 3 shows examples of correlation line profiles from the upper, middle, and deeper parts in the S1. The peak correlation coefficients of these five depths were 0.38, 0.38, 0.42, 0.40 and 0.37, respectively. The smaller correlation coefficient of the deeper parts could be because of less draining vein effect, which makes the activation tends toward the surface and the small surface coil we used. To investigate the plasticity in the layer IV, line profiles were drawn at 0.8 mm below the surface of the brain.

In contrast, the activation areas of the two digits analyzed by differential analysis were narrower, extended deeper into the cortex and had a gap in between, which may be the representation of D3 (Fig. 2, right column). The widths of D2 and D4 activations were 0.8 ± 0.3 mm and 0.6 ± 0.1 mm, respectively (Table 1), which are about 2 times larger than the representations reported using cytochrome oxidase (CO) or other techniques (Watres et al., 1995). Nonetheless, the sum of half the FWHM for both digits (0.7 ± 0.1 mm) was significantly smaller than the distance between the peaks for the D2 and D4 representations (1.5 ± 0.3 mm; $p < 0.01$), and the difference (0.8 ± 0.4 mm) was about the size of another digit. This indicates that differential analysis enables more precise mapping of the digit representations. Therefore, this analysis was used to determine if plasticity could be detected in the amputated group.

Compared with the control group, activations of D2 and D4 in the amputated group had similar relative locations but with shorter distances in between (Fig. 4 and Table 2). Whereas the widths of both D2 and D4 activations did not change, the distance between their peak signals was significantly reduced to 0.9 ± 0.2 mm ($p < 0.01$, two-tailed unpaired t-test). This is consistent with the literature indicating that the nearby digit representations expand and take over the representation of the amputated digit (McCandlish et al., 1996). This can also be seen from the averaged profiles, crossing the centers of D2 and D4 activations in layer IV, from the correlation coefficient maps (Fig. 5) and percentage signal change maps (Fig. 6). Thus, in the amputated group, the D2 and D4 activations largely overlapped and the gap between them disappeared.

However, there wasn't much change in the representation areas in the amputated group. The mean D2 activation volume for the control group (26.7 ± 18.7 voxels; Table 3) was similar to that for the amputated group (26.0 ± 17.1 voxels; Table 4) ($p > 0.1$, two-tailed unpaired t-test). The mean D4 activation volume increased from 15.2 ± 11.6 voxels in the control group to 37.6 ± 28.4 voxels in the amputated group, but this change was also not significant ($p < 0.1$, two-tailed unpaired t-test). The percentage signal change of D4 increased to 1.1 ± 0.5 in the amputated group compared to 0.7 ± 0.3 in the control group ($p < 0.05$, two-tailed unpaired t-test), whereas that of D2 didn't change.

Discussions

This study demonstrates that BOLD fMRI activation of individual digits can be mapped in layer IV of the rat S1 region, which corresponds to digit representations in the forepaw barrel subfield, using high resolution 3D imaging and differential analysis. The cortical plasticity in the adult brain after digit amputation at a young age can also be detected. To the best of our knowledge, this is the first fMRI study that shows plasticity at the level of the cortical column.

Methods for digit fMRI

Different kinds of electrodes, such as clip, needle, and ring, were tested for reliable stimulation of individual digits. Consistent and robust responses were observed in the forepaw barrel subfield when a pair of ring electrodes was used; therefore, we used two pairs of ring electrodes to stimulate the two fingers.

Frequency-dependent BOLD responses of the rat S1 have been studied using electrical stimulation to the forepaw (Goloshevsky et al., 2008; Keilholz et al., 2006; Van Camp et al., 2006). To optimize the paradigm for digit stimulation, we repeated the experiment at different stimulation frequencies, waveforms, and durations. The greatest BOLD signal in the forepaw barrel subfield was observed at 3 Hz with serial rectangular pulses of 2 mA current and 0.33 ms duration. These optimal stimulation parameters are consistent with other reports on forepaw stimulation (Keilholz et al., 2006).

Previous study reported that electrical stimulation of the tip of D3 for a period of 2 h in an anesthetized rat produces an expanded representation of the stimulated digit in forepaw barrel subfield in S1 cortex (Li et al., 1996). This phantom expansion of digit representation from electrical stimulation itself may confound the observed activation area. However, the stimulation used here is much shorter than the long stimulation reported and the increase in the area of that phantom expansion was too small to be detectable by the resolution used. Besides, the activation pattern in the amputated group was not just larger than the control group. The D2 and D4 activations in the amputated group largely overlapped and the gap between them disappeared, which can not be merely due to phantom expansion by the stimulation.

It has been shown that differential analysis is more appropriate than regular analysis for mapping representations of cortical columns (Cheng et al., 2001), the advantage being that the overlapped areas due to the blurring of vascular responses in BOLD can be suppressed. In this study, the activation detected by differential analysis was more localized, with almost no overlap between the two digit activations, than that obtained by regular analysis. However, this method requires the cortical columns to be well organized rather than intervening together. Besides, the D2 and D4 representations observed using differential analysis may still have contributions from overlapping vasculature of adjacent digits, e.g., D3 and D5, which would lead to larger activated areas than those reported by histological staining.

In our study, two digit stimulations did not always elicit detectable BOLD activations, and the BOLD response of one digit was sometimes much higher than the other. The limited detectability of digit representations may be due to inter-subject variations in hemodynamic responsiveness and digit-specific differences in neuronal activity.

Spatial resolvability

The spatial resolution of fMRI is ultimately limited by the space between the vessels that primarily contribute to the signals. It can also be limited by motions of the head and brain and by signals from large veins. Moreover, geometric constraints are imposed by the morphology of the functional architectures being mapped (Cheng et al., 2001). Because the digit representations in the forepaw barrel subfield are only about 200 – 300 microns wide and orient in a lateral-anterior direction in layer IV of the S1, a 3D EPI of 300 micron isotropic resolution was used to minimize partial volume effects in the curved cortical layer, thereby permitting better localization of activations. However, the measured distance between the D2 and D4 activations in the control group, which is about 1.5 mm, is larger than those reported in electrophysiological studies (Li et al., 1996; McCandlish et al., 1996; Watres et al., 1995). The activation area size is also larger than that estimated from electrophysiological and histological studies. This could be due to the vascular point spread function extending beyond the column, as well as the partial volume blurring by the resolution used, which was about the width of a single digit representation. Although the resolution of the current study is sufficient for detecting changes after digit amputation, higher spatial resolution may be needed for more accurate quantification of the digit representations.

The spatial resolution of electrophysiology is limited by the dimension of individual electrode, the precision of electrode positioning, and the distribution of local field potential. It can be down to 75 μ m or less and to a distance that avoids surface vessels. In fMRI, the spatial resolution is limited by SNR, contrast mechanism, and, ultimately, the vascular organization (Kim and Ogawa, 2002). The SNR in MRI is proportional to the voxel size. Doubling spatial resolution in all 3 dimensions reduces SNR by a factor of 8. Therefore, better coil, higher magnetic field, etc are needed to achieve enough SNR. The size of vessels giving rise to the observed fMRI signal can vary from the capillary bed in the cortex (< 10 μ m) to draining veins (a few mm) (Lai et al., 1993; Menon et al., 1993). Signal changes from the capillary bed are more accurately co-localized with the site of neural activity, but the signal is much weaker compared to those from the draining veins which can be millimeters away (Kim et al., 1994). Spin-echo based imaging method at high field is less sensitive to the macrovasculature and hence could provide better specificity (Yacoub et al., 2003). As opposed to BOLD, CBF and CBV imaging are more sensitive to the changes in the capillary bed, and have been shown in mapping activity in cortical layers and columns (Duong et al., 2001; Zhao et al., 2006). Besides those methods relying on hemodynamic response as signal source, an alternative method is to utilize Mn^{2+} as a Ca^{2+} analog to map neuronal activation without confounding and limitation from the vasculature (Lin and

Koretsky, 1997). Recently it is demonstrated that activity at the level of individual olfactory glomerulus in the mouse olfactory bulb can be detected by this technique (Chuang et al., 2009).

A large vein effect is known to be an issue when trying to identify finer neural structures using fMRI. Within the barrels of the S1, a highly organized distribution of radial blood vessels penetrating the cortex was observed (Patel, 1983). The longest set of radial vessels was found to overlap with the border zones of certain cortical areas rather than their centers (Eins et al., 1983). It was further observed that the distribution of pial vessels correlates with the position and expansion of functionally differentiated areas, i.e., one barrel accompanied by one draining vein (Ambach et al., 1986). Such close co-localization of functional area and blood vessels implies that the detection of activation based on vascular response may be biased toward the border of the functioning barrel and hence shown as wider than the actual size of a barrel. This may explain why the width of the BOLD activation is larger than the dimension reported in electrophysiology study. The large draining veins on the surface of the cortex have larger functional signal changes and cause the activation areas measured to extend away from the actual neural activation sites. This can introduce error in the estimation of activation area size and location. This may also be the reason that significant changes in the representation area size were not observed after digit amputation, though they were reported using electrophysiological recordings (McCandlish et al., 1996). To avoid this effect of large draining veins, line profiles were carefully drawn from layer IV of the S1. To further reduce the signal from large draining veins, imaging methods such as spin-echo acquisition (Lee et al., 1999), bipolar gradient (Michelich et al., 2006; Song et al., 2007) or perfusion (Duong et al., 2001; Sheth et al., 2004) can be used (rather than BOLD imaging).

Activation in deeper layers was not observed (Fig. 2 and 4). This could be due to smaller signal changes in the deeper cortical layers when gradient-echo based BOLD fMRI is used (Silva and Koretsky, 2002; Zhao et al., 2004). Comparison of line profiles drawn from the upper, middle, and deeper layers in the S1 (Fig. 3) showed the signal in the deeper layers to be noisier, making it difficult for signals of interest to pass the threshold. Spin-echo based imaging could be used to reduce the noise from pial vessels near the surface of the cortex. The SNR could be further improved by the use of more data averaging, more sensitive coils, and a higher magnetic field.

The plasticity in the rat forepaw barrel subfield reported in McCandlish et al corresponds to about 4.0 ± 0.8 pixel area (of a 300×300 micron² inplane resolution) in layer IV of the amputated group, vs., 2.2 ± 0.5 pixels in the normal group (McCandlish et al., 1996). In our study, the activated volumes of D4 of the amputated group increased but was not significant due to large individual variation. The change in the width of activation in layer IV was much smaller than the total volume – it could be merely a pixel change in our resolution. Therefore we didn't see any change in the width of activation. However, changes in the distance between the boundaries and the centroids of D2 and D4 are more likely to be detected because it shall be about the width of D3. In our study, significant and consistent decrease in the distance between D2 and D4 can be observed even with individual variation of about 1 pixel.

Mechanism for plasticity

The reorganization detected by the fMRI map differed from the maps reported by electrophysiological methods (McCandlish et al., 1996; Waters et al., 1990; Watres et al., 1995). Although the re-arrangement of activations in the barrel subfield was observed in our results, changes in the size and shape were not significant. The discrepancy between our findings and electrophysiological reports may be attributed to limitations of BOLD fMRI (as described above) and differences in peripheral receptor types (Iggo and Andres, 1982),

patterns of innervation (Munger and Rice, 1986; Waite and Cragg, 1979), the age of deafferentation (Jeanmonod et al., 1977; Killackey et al., 1976; Weller and Johnson, 1975; Woolsey and Wann, 1976), or the time period following deafferentation (McCandlish et al., 1996; Waters et al., 1990; Watres et al., 1995).

Normal development of the forepaw barrel subfield requires an intact periphery prior to postnatal day 5. After this time, peripheral deafferentation does not appreciably alter the organization of the forepaw barrel subfield (Dawson and Killackey, 1987; Waters et al., 1990). Therefore, peripheral deafferentation in adult animals should have little effect on the anatomy of forepaw barrel subfields, as the period for map consolidation has already passed. However, physiological plasticity can still occur. Hence, digit removal in adult animals results in a mismatch between morphological maps and physiological maps (Watres et al., 1995). Because the rats in our study were amputated at postnatal day 3, reorganization of the morphological maps was expected and, thus, does not explain why the fMRI maps are different from the electrophysiology maps.

Several studies showed that while the functional map in amputated animals reorganized, many aspects of the brain morphology remained unchanged. For example, Wong-Riley and Welt reported that no obvious changes in the size, shape, or arrangement of barrels in the posteromedial barrel subfield was observed in mice 10 - 16 weeks after vibrissae removal (Wong-Riley and Welt, 1980). Dawson and Killackey reported that amputation of forepaw digits or transection of forelimb nerves after postnatal day 5 or 6 in neonatal rats leads to no apparent morphological alteration of the associated forepaw barrel subfield (Dawson and Killackey, 1987). Similar findings in the digit representation were reported by McCandlish et al. showing a normal-appearing forepaw barrel subfield morphology detected by CO and a reorganized digit representation within the D3 band in D3 amputated rats (McCandlish et al., 1996). Therefore it was assumed that the brain morphology was the same in both groups and the detected change in fMRI map reflected the functional reorganization after digit amputation. Nonetheless, it should be noted that in those studies a metabolic marker of neural activity, CO, was used to map the extent of barrel fields. There may be other morphological changes, such as cell density, vascular organization, not detectable by CO.

Reorganization of the somatosensory cortex following digit deafferentation has been documented by a number of investigators, with a consensus that reorganization results from the unmasking of previously existing input (Armstrong-James and Callahan, 1991; Armstrong-James et al., 1991; Istvan and Zarzecki, 1994). Some studies suggest a high degree of convergent input to individual cortical neurons in the S1 cortex. (Istvan and Zarzecki, 1994) reported that, using an in vivo intracellular recording approach, approximately 40% of the neurons within the D4 cortical representation also receive input from stimulation of neighboring D3 and D5. Other investigators attribute the post-deafferentation cortical changes to cortical-cortical connections (Armstrong-James and Callahan, 1991; Armstrong-James et al., 1991), describing a center-surround receptive field organization. Thus, when a neighboring whisker barrel is destroyed by lesion, the surrounding receptive field component from that associated whisker is also lost, suggesting a possible role of intra-cortical connectivity in shaping the surrounding component of the receptive field. Future studies, using electrophysiological manipulations, pharmacological blocking and intracellular recording, are needed to address the more subtle mechanisms that underlie forepaw barrel subfields.

Conclusion

We have demonstrated that the forepaw barrel subfields of single digits can be mapped using fMRI at 11.7 T. Alterations of the digit representations in the adult brain after digit

amputation at an early age was also detected, indicating that the reorganization of digit representations in layer IV of the S1 region can be resolved by fMRI. This method will be useful for studying neural plasticity in rat brains after surgical or pharmacological manipulations. Future studies will be performed to verify the identified digit representations using histological staining or manganese enhanced MRI.

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Research Highlights

- High resolution fMRI with 300 micron isotropic resolution was used to map the topographic organization of the forepaw barrel subfield in layer IV of rat primary somatosensory cortex.
- By alternating electrical stimulation of the 2nd (D2) and 4th (D4) digits, BOLD activation in layer IV of the barrel subfields could be distinguished with differential analysis.
- Two and a half months after the amputation of the 3rd digit in baby rats, the overlapping area between D2 and D4 representations was increased, indicating the representation previously associated with the ablated digit is now associated with that of nearby digits.

Fig. 1.

Regular (a) and differential (b) paradigms used in the correlation analysis. The activation of a digit was detected by comparing with the resting period (regular paradigm) or with the signal for the other digit (differential paradigm).

Fig. 2. Functional maps of D2 (red) and D4 (green) activations in two of the five rats (a, b) in the control group analyzed by regular (left 2 columns) and differential (right 2 columns) paradigms. The correlation threshold is 0.2. Two slice orientations were shown in each case: the left one is sagittal and the right one is horizontal. The slice locations were illustrated in the bottom row.

Fig. 3. Line profiles from the upper, middle, and deeper portions of the S1 were drawn on the correlation coefficient map of D2 activation in a rat. The peak correlation coefficient could be found in middle layers.

Fig. 4. Functional maps of D2 (red) and D4 (green) activations in two of the five rats (a, b) in the amputated group analyzed using the regular (left 2 columns) and differential (right 2 columns) paradigms. The correlation threshold is 0.2.

Fig. 5.

The averaged correlation coefficient profiles of the D2 (solid line) and D4 (dotted line) activations from the control (a) and amputated (b) groups. The distance between the peaks of D2 and D4 activations in the digit-amputated rats was smaller than that in the normal rats. The stars indicate significant differences in correlation coefficients between the two digits. (*: $p < 0.05$ and **: $p < 0.01$)

Fig. 6. The averaged signal change profiles of the D2 (solid line) and D4 (dotted line) activations from the control group (a) with the amputated group (b). The stars indicate significant differences in the signal change with respect to other digit. (*: $p < 0.05$ and **: $p < 0.01$)

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Table 1

The FWHM of D2 and D4 activations, and the distance between the peaks of D2 and D4 representations in the control group with regular and differential analyses.

Control group	Regular analysis			Differential analysis		
	FWHM _{D2} (mm)	FWHM _{D4} (mm)	Distance (mm)	FWHM _{D2} (mm)	FWHM _{D4} (mm)	Distance (mm)
Subject A	1.4	1.2	0.9	1.2	0.6	1.2
Subject B	1.2	0.9	0.6	0.6	0.9	1.2
Subject C	0.9	0.6	1.0	0.9	0.6	1.8
Subject D	0.6	1.2	1.0	0.6	0.6	1.5
Subject E	0.9	1.8	0.3	0.6	0.6	1.8
mean±std	1.0±0.3	1.1±0.4	0.8±0.3	0.8±0.3	0.6±0.1	1.5±0.3

Table 2

The FWHM of D2 and D4 activations, and the distance between the peaks of D2 and D4 representations in the amputated group with differential analysis.

Amputated group	Differential analysis		
	FWHM _{D2} (mm)	FWHM _{D4} (mm)	Distance (mm)
Subject A	0.6	0.6	0.6
Subject B	0.6	0.6	0.9
Subject C	0.6	0.9	0.9
Subject D	0.6	0.6	0.9
Subject E	0.9	0.6	1.2
mean±std	0.7±0.1	0.7±0.1	0.9±0.2

Table 3

The activation volumes and mean percentage signal changes of D2 and D4 in the control group.

Control group	Volume (voxels)		Mean signal change (%)	
	D2	D4	D2	D4
Subject A	56	14	2.1	1.2
Subject B	14	13	0.3	0.4
Subject C	32	8	1.4	0.7
Subject D	8	35	0.6	0.9
Subject E	28	6	0.3	0.5
mean±std	26.7±18.7	15.2±11.6	0.9±0.8	0.7±0.3

Table 4

The activation volumes and mean percentage signal changes of D2 and D4 in the amputated group.

Amputated group	Volume (voxels)		Mean signal change (%)	
	D2	D4	D2	D4
Subject A	42	60	1.9	1.8
Subject B	42	50	0.7	0.6
Subject C	8	64	0.6	1.4
Subject D	8	8	0.4	0.7
Subject E	30	6	1.5	0.9
mean±std	26.0±17.1	37.6±28.4	1.0±0.6	1.1±0.5
p-value	p > 0.1	p < 0.1	p > 0.1	*p < 0.05