NeuroImage 142 (2016) 431–453

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Spatiotemporal trajectories of reactivation of somatosensory cortex by direct and secondary pathways after dorsal column lesions in squirrel monkeys

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ARTICLE INFO

Article history:
Received 8 April 2016
Accepted 9 August 2016
Available online 12 August 2016

Keywords:
CBV
MION
Somatosensory cortex
Ascending spinal pathways
Spinal cord injury

ABSTRACT

After lesions of the somatosensory dorsal column (DC) pathway, the cortical hand representation can become unresponsive to tactile stimuli, but considerable responsiveness returns over weeks of post-lesion recovery. The reactivation suggests that preserved subthreshold sensory inputs become potentiated and axon sprouting occurs over time to mediate recovery. Here, we studied the recovery process in 3 squirrel monkeys, using high-resolution cerebral blood volume-based functional magnetic resonance imaging (CBV-fMRI) mapping of contralateral somatosensory cortex responsiveness to stimulation of distal finger pads with low and high level electrocutaneous stimulation (ES) before and 2, 4, and 6 weeks after a mid-cervical level contralateral DC lesion. Both low and high intensity ES of digits revealed the expected somatotopy of the area 3b hand representation in pre-lesion monkeys, while in areas 1 and 3a, high intensity stimulation was more effective in activating somatotopic patterns. Six weeks post-lesion, and irrespective of the severity of loss of direct DC inputs (98%, 79%, 40%), somatosensory cortical area 3b of all three animals showed near complete recovery in terms of somatotopy and responsiveness to low and high intensity ES. However there was significant variability in the patterns and amplitudes of reactivation of individual digit territories within and between animals, reflecting differences in the degree of permanent and/or transient silencing of primary DC and secondary inputs 2 weeks post-lesion, and their spatio-temporal trajectories of recovery between 2 and 6 weeks. Similar variations in the silencing and recovery of somatotopy and responsiveness to high intensity ES in areas 3a and 1 are consistent with individual differences in damage to and recovery of DC and spinocuneate pathways, and possibly the potentiation of spinothalamic pathways. Thus, cortical deactivation and subsequent reactivation depends not only on the degree of DC lesion, but also on the severity and duration of loss of secondary as well as primary inputs revealed by low and high intensity ES.

Introduction

The dorsal column of the spinal cord that terminates in the cuneate nucleus is the major activation pathway of the hand representations in primary somatosensory cortex (area 3b), the adjoining representation area 1, and the tactile component of areas 3a and 2. Thus, when the dorsal columns (DCs) are cut at high cervical levels (above the level where afferents from the hand enter the spinal cord), the hand region in area 3b and other somatosensory cortical areas become unresponsive to touch on the hand. However, over weeks to months of recovery in monkeys with spinal cord lesions, much of the hand representation in area 3b, and other cortical areas, becomes responsive to touch on the hand, and the pattern of reactivation in cortex preserves aspects of the normal somatotopic order (Bowes et al., 2013; Chen et al., 2012a; Jain et al., 1997; Qi et al., 2011). Some of this reactivation may originate from those few primary afferents that often survive such spinal cord lesions. However the spared afferents of the second order spinal cord neurons that normally provide sub-threshold or modulatory inputs to the cuneate nucleus may also become an effective source of cortical reactivation (Liao et al., 2015). The importance of these secondary inputs may increase with increasing severity of primary deafferentation. In addition, the relative contribution of these two components of cortical

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http://dx.doi.org/10.1016/j.neuroimage.2016.08.015
1053-8119/

reactivation, and their relation to functional recovery, remain less well understood, in large part because of the challenges and constraints imposed on longitudinal studies within individual animals by the invasive nature of electrophysiological mapping studies (Jain et al., 1997; Merzenich et al., 1983a; Merzenich et al., 1983b). Progress in high resolution functional MRI mapping now permits non-invasive longitudinal studies of cortical activation at high spatial resolution, with submillimeter coregistration of functional maps across sessions (Chen et al., 2012a; Corbetta et al., 2002; Frey et al., 2008; Lecoeur et al., 2011b; Lecoeur et al., 2011a; Makin et al., 2015a; Makin et al., 2015b; Moore et al., 2000; Yang et al., 2014; Zhang et al., 2010). Furthermore, by comparing cortical activation by low with high threshold pathways via low and high levels of tactile or electrocutaneous stimulation (ES), the relative contributions of dorsal and other pathways to local cortical innervation can be characterized (Dutta et al., 2014; Wang et al., 2012; Zhang et al., 2007). By combining these approaches, the spatiotemporal patterns of cortical reactivation as well as the relative contributions of reactivation of primary (low threshold dorsal column) and expansion of secondary (high threshold spinothalamic) inputs to cortical reactivation can be studied longitudinally in the same animal, thus controlling for inter-animal variations in somatotopy, and lesion severity and location (Chen et al., 2012a; Yang et al., 2014).

Here we exploit differences in the fMRI response properties of cortical areas to dorsal column vs. spinothalamic inputs to track and characterize the afferents that drive cortical reactivation following varying degrees of loss of DC afferents. Thus, while tactile stimulation of the hand effectively activates low-threshold cutaneous afferents from the hand, contributions of higher threshold afferents from the hand and those of subsequent second and perhaps higher level afferents in the spinal cord and brainstem may not be revealed. Previous studies of blood-oxygen-level dependent (BOLD) contrast imaging have shown that nociceptive stimulation in humans and non-human primates can activate area 3b, as well as higher order areas of somatosensory cortex (Zhang et al., 2007), most likely via the spinothalamic pathway (Bingel et al., 2004; Chen et al., 2011; Chen et al., 2012b; DaSilva et al., 2002). Thus, pathways dependent on more intense and even nociceptive stimuli could contribute to cortical reactivations after DC lesions. ES levels can be adjusted to largely activate low threshold tactile afferents or higher threshold afferents as well (Wang et al., 2012). Co-activation of both sets of afferents may also more effectively activate higher order spinal cord and other sensory pathways.

To monitor the spatiotemporal evolution of cortical responses resulting from sensory loss and the origins of subsequent cortical reactivation, we collected high-resolution cerebral blood volume (CBV)-based functional maps of somatosensory cortex from three squirrel monkeys before and three times after a lesion of the contralateral dorsal columns at a mid-cervical level. To examine the potential contributions of low and high threshold sensory pathways in cortical activations and reactivation, we applied low and high intensity ES to the distal finger pads of digits 1–3 during imaging sessions in anesthetized monkeys. We reasoned that the low intensity innocuous ES would selectively activate pre-lesion and spared post-lesion low threshold primary Aβ afferents, and their secondary spinal cord neuronal targets, while high intensity nociceptive ES would recruit higher threshold Aδ and C-fibers and would more strongly activate lower threshold afferents and subsequent second order spinal cord relays. To gain insights into the roles of low and high threshold inputs to cortical reactivation, our study had three goals: first, to determine the spatial trajectories of recovery of cortical responsiveness to low and high intensity ES of digits 1–3 before, and 2, 4, and 6 weeks after a DC lesion; second, to compare temporal trajectories of activation response magnitudes in cortical areas 3b, 3a, and 1 at low and high levels of stimulation; third, to compare the somatotopic organization revealed by ES fMRI after 6 weeks of recovery to that obtained from microelectrode mapping using tactile stimulation.

As expected, high level ES to the digits effectively activated contralateral somatosensory cortex before and after a large unilateral lesion of the dorsal column at a mid-cervical level. Consistent with previous results, the larger, nearly complete lesion most effectively deactivated the contralateral hand region in area 3b and adjoining areas shortly after the lesion, while less complete lesions were less effective. Subsequent microelectrode mapping clearly matched those of the final imaging session. The results suggest that preserved DC afferents from the hand are important in maintenance and recovery of activation of cortex, and that second order spinal cord pathways likely play a role in reactivation after nearly complete lesions. In addition, the results reveal the usefulness of fMRI and electrocutaneous stimulation of the skin in evaluating the recovery process in primates with spinal cord injury.

Materials and methods

Animal preparation and DC lesion surgery

Three adult (4 years old) male New World squirrel monkeys (*Saimiri boliviensis*) were used in this study. The somatosensory cortex in these three subjects was scanned via high-resolution functional magnetic resonance imaging (fMRI) before, and 2, 4, and 6 weeks after a DC lesion. During the MRI scans, each monkey was anesthetized and mechanically ventilated, with isoflurane (0.5–1.1%) delivered by a mixture of N_2O/O_2 in a 70:30 ratio. The head and body were stabilized in an MRI compatible frame. Vital signs were monitored and maintained throughout the imaging sessions.

After pre-lesion imaging data were collected, all animals underwent a unilateral dorsal column lesion at mid-cervical level C4–C5 in the spinal cord, that possibly spares some of the primary afferent inputs from digit 1 and even digit 2 (Florence et al., 1991; Qi et al., 2011). A DC lesion was made under aseptic conditions and general anesthesia. The animal was intubated for ventilation, skin incision was made in the back of the neck, and muscles were separated to expose the dorsal vertebrae near cervical level C4–C5. A small opening was made on the dorsal arch of the vertebrae by first slicing the superficial connective tissue between the two vertebrae outside of the spinal cord, then carefully removing small pieces of bone on the dorsal arch of 1 segment using rongeurs. The dura and pia covering the cervical spinal cord were resected. Unilateral dorsal columns were sectioned on the right side of the spinal cord for all three monkeys with a pair of surgical iris scissors. To close, the dura was replaced by Gelfilm, and muscles were re-closed and sutured using absorbable suture. The skin was closed using surgical staples. The monkeys were carefully monitored until they fully recovered from anesthesia before they were returned to their home cage. The monkeys received antibiotics and analgesics for 3 days after surgery. All experimental procedures were approved by the Vanderbilt University Animal Care and Use Committee and followed the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

FMRI acquisition

MRI scans were acquired at 9.4 T (Agilent) using a 3 cm surface transmit-receive coil positioned over primary somatosensory cortex (Fig. 1).

Structural imaging

3D whole brain isotropic images were collected (TR/TE = 5/2.39 ms, 128 × 128 × 128 matrix; 0.5 × 0.5 × 0.5 mm^3) and used for planning of coregistered high resolution structural and functional images. These images were also used for coregistration of imaging studies between sessions (Lecoeur et al., 2011a, 2011c). High resolution T2*-weighted gradient echo structural images (TR/TE 400/16 ms, 16 slices, 512 × 512 matrix; 68 × 68 × 500 μm^3 resolution) were acquired as a multi-slice oblique stack positioned for maximal in-plane coverage of somato-sensory areas 3a, 3b and 1 (Fig. 1A). These images were used