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Rapid functional reorganization of the forelimb cortical representation after thoracic spinal cord injury in adult rats

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ABSTRACT

Thoracic spinal cord injured rats rely largely on forelimbs to walk, as their hindlimbs are dysfunctional. This increased limb use is accompanied by expansion of the cortical forelimb sensory representation. It is unclear how quickly the representational changes occur and whether they are at all related to the behavioral adaptation. Using blood oxygenation level dependent functional mangetic resonance imaging (BOLD-fMRI) we show that major plastic changes of the somato-sensory map can occur as early as one day after injury. The extent of map increase was variable between animals, and some animals showed a reduction in map size. However, at three or seven days after injury a significant enhancement of the forelimb representation was evident in all the animals. In a behavioral test for precise limb control, crossing of a horizontal ladder, the injured rats relied almost entirely on their forelimbs; they initially made more mistakes than at 7 days post injury. Remarkably, in the individual animals the behavioral performance seen at seven days was proportional to the physiological change present at one day after injury. The rapid increase in cortical representation of the injury-spared body part may provide the additional neural substrate necessary for high level behavioral adaptation.

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Introduction

Spinal cord injury (SCI) interrupts sensory and motor pathways resulting in dysfunctional body parts. The injured axons are unable to regenerate spontaneously and the cortex is permanently deprived of its normal sensory input (Kaas et al., 2008; Raineteau and Schwab, 2001). Spinal cord injured humans can partially compensate for the loss of function by increased utilization of injury spared body parts (Pentland and Twomey, 1994a,b). Interestingly, in rats and monkeys there are anatomical and physiological changes in the representation of the injury spared body part, in the sensory and motor cortices (Ghosh et al., 2010; Kaas et al., 2008; Raineteau and Schwab, 2001). For example, in the thoracic SCI rats the injury spared forelimb sensory representation expands into the area originally occupied by the hindlimb (Endo et al., 2007; Ghosh et al., 2010; Wall and Egger, 1971). Anatomical rewiring also occurs, the axotomised hindlimb corticospinal neurons sprout to the forelimb area of the spinal cord (Bareyre et al., 2004; Fouad et al., 2001; Ghosh et al., 2010). Whether and how the anatomical and physiological changes contribute to the behavioral compensation remains largely unexplored.

BOLD fMRI is a powerful method to non-invasively track the physiological changes after spinal cord injury (Endo et al., 2007; Ghosh et al., 2010; Ramu et al., 2006, 2007; Sydekum et al., 2009). In particular, expansion of the forelimb representation after thoracic spinal cord injury has been documented using BOLD fMRI (Endo et al., 2007; Ghosh et al., 2010). The expansion was evident at the first time points of evaluation in the earlier studies i.e. at three or seven days after injury. At seven days after the injury rats depend primarily on their injury spared forelimbs to perform skilled locomotion, which requires cortical control (Ghosh et al., 2010). Using electrophysiological methods, it has been shown that the cortex becomes more responsive to the injury-spared forepaw stimuli within 1 h after a thoracic SCI (Aguilar et al., 2010;

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Humanes-Valera et al., 2013). An objective of the current study was to analyze the sensitivity of BOLD fMRI for detecting early changes in the forepaw cortical sensory representation and to evaluate whether these changes are of predictive quality regarding the behavioral outcome in the rats following thoracic SCI. The latter aspects require analysis at the level of the individual, i.e. taking into account the intrinsic variability of sensory representations among animals both prior and post injury, and the variability in the dynamics and extent of behavioral or physiological changes. We therefore used non-invasive BOLD fMRI to monitor the forelimb sensory representations of individual adult rats prior to and at days 1, 3 and 7 following thoracic bilateral dorsal SCI. In addition, skilled and over ground locomotion was evaluated in these animals. Our results suggest that alterations in forelimb sensory representation occur already within 1 day after injury and thus could provide the basis for the skilled motor adaptation in the subsequent days.

Methods

Animals

Adult female Lewis rats (n = 8) of 230–250 g body weight were used in this study. Animals had free access to standard rat chow and tap water. All animal experiments were performed in strict adherence to the Swiss Law for Animal Protection and approved by the veterinary office of the canton of Zurich, Switzerland.

Spinal cord injury (SCI) model

Spinal cord dorsal bilateral hemisections at thoracic segment 8 (vertebra) were performed in rats as described elsewhere (Ghosh et al., 2010). Briefly, animals were deeply anesthetized with a subcutaneous injection of Hypnorm (120 μl/200 g body weight; VetaPharma LtD, Leeds, England) and Dormicum (0.75 mg in 150 μl/200 g body weight; Roche Pharmaceuticals, Basel, Switzerland). Upon dorsal bilateral hemisection of the spinal cord the retrograde tracer Fast Blue (0.2%) was injected into the lesion site and the excess dye was removed after 10–15 min. Post surgery, pain reducing and antibiotic medications were injected. Bladders were emptied twice a day until bladder function was completely recovered. At day 8 animals were sacrificed and the spinal cords and brains were cryoprotected in 30% sucrose solution and frozen. For visualization of retrogradely labeled cells in the cortex and evaluation of the injury site, animals were perfused with 4% paraformaldehyde (PFA) under deep anesthesia. At the time of perfusion, a small incision was made 1 mm lateral to bregma and 1 μl of the dye Fast Green was used to stain the position; this marked area was later used to determine the position of bregma in the reconstructions. 50-μm spinal cord and brain sections (100-μm gaps) were cut using a cryostat. Retrogradely labeled corticospinal cells were identified under a fluorescence microscope and cell positions were reconstructed using Neurolucida (ver 7.0; MicroBrightfield).

Behavioral testing

Animals were evaluated on the Catwalk® (Noldus Information Technology) and on the irregular horizontal ladder, before and after injury (1 and 7 days immediately prior to fMRI acquisition). For the Catwalk® animals were trained for 3 weeks prior to baseline measurements (twice per week in the first 2 weeks, once in the following week) using a protocol described elsewhere (Hamers et al., 2006). Briefly, the animal is video recorded while traversing a glass runway and percentage of usage by each paw and the intensity of footprints during maximum paw contact at every step is automatically determined.

The horizontal ladder test is described in detail elsewhere (Maier et al., 2008). Briefly, animals were habituated to the testing apparatus until each animal crossed the ladder without any assistance, at slow, constant speed. The ladder was 1 m long and elevated 1 m over ground and encased in a glass to make it fall safe. Irregular bar spacing (1–6 cm) prevented habituation to a specific bar distance. Three trials over a defined 60 cm stretch were video recorded and evaluated offline (Virtualdub; www.virtualdub.org). Forepaw placements were judged as successful if a rat was able to target the rung and place its body weight without slipping. The percentage of successful placements for each animal was determined by averaging over three trials. Success rate was expressed as percentage of correct steps from all the attempted steps by the forelimb.

Animal preparation for MR experiments

Animal preparation for the fMRI study has been described previously (Sydekum et al., 2009). Briefly, anesthesia was induced with an initial dose of 4% isoflurane in an air/oxygen (4:1) mixture. After endotracheal intubation rats were stereotactically fixated and ventilated at a rate of 50 breaths per minute (BpM) using a small animal ventilator (Maraltec, Biel-Benken, Switzerland). Respiration frequency and tidal volume have been adjusted to maintain blood gases in physiological range. A single dose of 15 mg/kg of the neuromuscular blocking agent gallamine (Sigma-Aldrich, Germany) was administered intravenously to facilitate ventilation and to avoid motion artifacts. Anesthesia level was maintained at 1.5% isoflurane throughout the experiment. Body temperature and pCO2 (TCM4, Radiometer Copenhagen) were monitored and maintained at physiological levels.

MRI/fMRI experiments

Eight rats were scanned before, 1 and 3 days after SCI and 6, randomly chosen out of them, also on day 7. Measurements were performed on a Bruker Biospec 94/30 horizontal bore small animal MR system (Bruker BioSpin GmbH, Karlsruhe, Germany) operating at 400 MHz (BGA-12 gradient insert; maximum gradient strength of 400 mT m-1, minimum rise time of 80 μsec). A radiofrequency (RF) cross-coil setup was used with a linearly polarized birdcage resonator (inner diameter 67 mm, length of resonating structure 70 mm) for RF transmission and a quadrature surface coil (length 30 mm, width 26 mm) for signal reception. After acquisition of scout images in sagittal and coronal direction, horizontal anatomical reference images were acquired using a multi-slice rapid acquisition with relaxation enhancement (RARE) spin echo sequence (parameters: field of view (FOV) = 32.56 mm × 25.00 mm, matrix dimension (MD) = 163 × 124, spatial resolution 0.2 mm × 0.2 mm, slice thickness (SLTH) = 1.00 mm, inter-slice distance (ISD) = 1.00 mm, echo time (TE) = 25 ms effective echo time (TEeff) = 50 ms, repetition time (TR) = 1.50 s, RARE factor = 4, number of averages (NA) = 2, acquisition time Tacq = 93 s. BOLD-fMRI was performed using single shot gradient echo–echo planar imaging (GE-EPI) sequence (FOV = 32.56 mm × 25 mm, MD = 128 × 64, spatial resolution = 0.25 mm × 0.39 mm, SLTH = 1.00 mm, ISD =1.00 mm, TR = 1.25 s, NA = 8, NR = 56, Tacq = 560 s. Field homogeneity was improved by using the FASTMAP algorithm (Gruetter, 1993). Two horizontal images were acquired covering all cortical layers. Forepaws and hindpaws were stimulated using bipolar needle electrodes (Genuine Grass instruments, West Warwick, USA) placed subcutaneously. For hindpaw stimulation electrodes were placed in the pad between digits and thumb, in contrast to more distal placement in previous studies. A PowerLab (AD Instruments Inc., Spechbach, Germany) stimulator supplied rectangular pulses. Stimulation parameters were: current amplitude 1.6 mA and 6 mA for forepaw and 2.5 mA for hindpaw, pulse duration = 0.5 ms and frequency = 3 Hz. The forepaw stimulation intensities were the same as used in on our previous studies and the lower stimulation corresponded to the digit flexion threshold in 90% of the animals (Ghosh et al., 2009; Ghosh et al., 2010; Sydekum et al., 2009). For the hindpaw, pilot measurements revealed considerable measurement-to-measurement variation and digit twitch using the 1.6 mA current.

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