Experimental Neurology 241 (2013) 84–94

Contents lists available at SciVerse ScienceDirect

Experimental Neurology

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

Serotonergic pharmacotherapy promotes cortical reorganization after spinal cord injury

Patrick D. Ganzer [a], Karen A. Moxon [a,b,*], Eric B. Knudsen [a], Jed S. Shumsky [b]

[a] School of Biomedical Engineering, Science and Health Systems, Drexel University, 3141 Chestnut St., Philadelphia, PA 19104, USA
[b] Department of Neurobiology and Anatomy, Drexel University College of Medicine, 2900 Queen Lane, Philadelphia, PA 19129, USA

ARTICLE INFO

Article history:
Received 28 November 2012
Accepted 6 December 2012
Available online 19 December 2012

Keywords:
Primary somatosensory cortex
Spinal cord injury
Pharmacotherapy
Transection
Cortical plasticity

ABSTRACT

Cortical reorganization plays a significant role in recovery of function after injury of the central nervous system. The neural mechanisms that underlie this reorganization may be the same as those normally responsible for skilled behaviors that accompany extended sensory experience and, if better understood, could provide a basis for further promoting recovery of function after injury. The work presented here extends studies of spontaneous cortical reorganization after spinal cord injury to the role of rehabilitative strategies on cortical reorganization. We use a complete spinal transection model to focus on cortical reorganization in response to serotonergic (5-HT) pharmacotherapy without any confounding effects from spared fibers left after partial lesions. 5-HT pharmacotherapy has previously been shown to improve behavioral outcome after SCI but the effect on cortical organization is unknown. After a complete spinal transection in the adult rat, 5-HT pharmacotherapy produced more reorganization in the sensorimotor cortex than would be expected by transection alone. This reorganization was dose dependent, extended into intact (forelimb) motor cortex, and, at least in the hindlimb sensorimotor cortex, followed a somatotopic arrangement. Animals with the greatest behavioral outcome showed the greatest extent of cortical reorganization suggesting that the reorganization is likely to be in response to both direct effects of 5-HT on cortical circuits and indirect effects in response to the behavioral improvement below the level of the lesion.

Introduction

Somatotopic reorganization of the primary somatosensory cortex after a major loss of sensory inputs is well established (Calford and Tweedale, 1988; Endo et al., 2007; Florence et al., 1998; Jain et al., 1997; Pons et al., 1991; Wall and Egger, 1971). This reorganization plays a role in recovery after spinal cord injury (SCI) (Darian-Smith and Ciferri, 2005; Ghosh et al., 2009; Giszter et al., 2008; Kao et al., 2011, 2009; Qi et al., 2011; Ghosh et al., 2010) and further promoting this cortical plasticity could facilitate improvements in behavioral outcome (Girgis et al., 2007; Engineer et al., 2011; Ghosh et al., 2009; Kaas et al., 2008; Kao et al., 2011; Nishimura et al., 2007; Ramanathan et al., 2006) Therefore, it is important to understand the effect, if any, of therapies known to improve behavioral outcome on cortical reorganization.

One promising therapy after a SCI is the administration of serotonergic (5-HT) receptor agonists. After a SCI and the concomitant loss of the descending 5-HT fibers, the administration of 5-HT receptor agonists promotes recovery of motor function (Antri et al., 2003; Antri et al., 2005; Courtine et al., 2009; Landry et al., 2006) by activating the circuitry of the lumbar spinal cord. At the same time, the 5-HT system plays a significant role in the regulation of cortical plasticity (for review see Whitaker-Azmitia, 2001; also Vitalis and Parnavelas, 2003). Specifically, activating the 5-HT system is associated with sprouting of neurites (Fricker et al., 2005), dendritic remodeling and synaptogenesis (Azmitia et al., 1995). Furthermore, modulation of the 5-HT system promotes plasticity associated with the recovery of visual function after an insult to the visual system (Jitsuki et al., 2011; Maya Vetencourt et al., 2008, 2011). However, the contribution of the 5-HT system to cortical reorganization after a SCI is unknown.

To address this issue, we assessed the effect of different doses of 5-HT agonists on cortical reorganization in adult rats after a midthoracic (T8/9) spinal cord transection. We selected the complete spinal transection model to focus on reorganization in response to serotonergic (5-HT) pharmacotherapy without any confounding effects from spared fibers left after partial lesions. Our results demonstrate that chronic administration of 5-HT receptor agonists promotes the expansion of the intact forelimb somatosensory (FLS) cortex into the deafferented hindlimb sensorimotor (HLSM) cortex and into the intact forelimb motor (FLM) cortex. The magnitude of this expansion is dose dependent, has some topographic organization and, interestingly, is positively correlated with behavioral recovery.

* Corresponding author at: School of Biomedical Engineering, Science and Health Systems, Drexel University, 3141 Chestnut St, Philadelphia, PA 19104, USA. Fax: +1 215 895 0570.
E-mail address: km57@drexel.edu (K.A. Moxon).

0014-4886/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.expneurol.2012.12.004

Materials & methods

Experimental design

Transected animals were divided randomly into three groups (n = 8 animals per group): 1) animals receiving saline (Control), 2) those receiving a lower dose of 5-HT receptor agonists (LD) or 3) those receiving a higher dose of 5-HT receptor agonists (HD). Doses of drug (Sigma-Aldrich, St. Louis, MO) consisted of a combination of the 5-HT receptor agonists quipazine and 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin). Animals were handled daily and given passive hindlimb exercise to optimize hindlimb function below the level of the lesion. Drugs were injected once per day 5 days per week beginning at week 2 post SCI and continuing for 8 weeks. Open field testing was performed at 4 and 8 weeks post SCI. During the ninth week after SCI, animals were anesthetized and the sensorimotor cortex was mapped to identify responses to stimulation above the level of the lesion. Single neurons were identified, their responsiveness to light, tactile forelimb stimulation was assessed and their stereotaxic coordinates noted. Cortical reorganization and any resulting topography to that reorganization was measured by comparing changes in the proportion of cells responding to forelimb stimulation in the HLSM and the FLM cortices between groups.

Transection procedure and animal care

Adult female Sprague–Dawley rats weighing 250–300 g were used for this study (n = 24). Rats received a complete mid-thoracic transection at spinal level T8/T9. Briefly, animals were anesthetized with 5% isoflurane and 2-liters of oxygen and maintained at 2–3% isoflurane with 1-liter oxygen for the duration of the surgery. A laminectomy at the T8/9 level exposed one spinal cord segment. A #10 scalpel blade was used to open the dura and pia mater and #11 scalpel blade was used to make the complete transection of the spinal cord. A fine-tipped microaspiration device was then used to remove 2–3 mm of spinal cord. A collagen matrix, Vitrogen (Cohesion Technology, Encinitas, CA), was injected into the site of the transection. Following recovery from surgery, animals were given an IM injection of the antibiotic Pen-G and 5 ml of lactated ringer subcutaneously and returned to their home cages.

Animals were housed 2 per cage with highly absorbent Alpha-Dri bedding (Shepherd Specialty Papers Inc. Kalamazoo, MI) and cages were kept on warm water blankets. Animals were housed under a 12 h light/dark cycle (lights on at 07:00) with ad libitum access to food and water. To maintain hindlimb muscle mass, normalize hindlimb reflexes and indirectly favor CNS plasticity, starting one week after spinal transection all animals received the rehabilitative intervention of passive bicycling exercise three times a week (Monday, Wednesday & Friday) using a custom built motor-driven cycling apparatus (Dupont-Versteegden et al., 2000). Rats were suspended horizontally with their feet secured to the pedals. Cycling speed was maintained at 45 revolutions/min and each exercise bout consisted of two 30-min exercise periods with a 10-min rest period in between. In addition, bladder care was given 3 times daily for 2 weeks or until bladder control was regained. If there was any sign of infection, the rat was given subcutaneous injections of Baytril (0.06 mg/kg) twice a day for 7 days.

Drug administration

Drugs were prepared by dissolving in sterile physiological saline. Quipazine was injected intraperitoneally and 8-OH-DPAT subcutaneously (Antri et al., 2005). Drugs were injected once per day 5 days per week beginning at week 2 post SCI and continuing for 8 weeks. The 2 week lag time post injury allowed time for 5-HT receptor upregulation (Kim et al., 1999) in the spinal cord caudal to the lesion. Based on previous studies (Antri et al., 2005), the high dose was a combined injection of 0.125 mg/kg of quipazine (1 ml/kg) and 0.125 mg/kg of 8-OH-DPAT (1 ml/kg). To gain a more complete understanding of the effects of 5-HT receptor activation, the low dose was half this dose: 0.075 mg/kg of quipazine (1 ml/kg) and 0.075 mg/kg of 8-OH-DPAT (1 ml/kg).

Behavioral testing

BBB scoring in the open field was used to test hindlimb behavioral recovery (Basso et al., 1995, 1996) at three time points: 1) 4 weeks post-transection after a 3 day wash-out period and before the daily administration of 5-HT (off-drug), 2) 8 weeks post-transection immediately after drug administration (on-drug), and 3) 8 weeks post-transection (off-drug). Control (saline) animals received a challenge of a high dose of drug during the on-drug testing period. Spontaneous hindlimb motor activity was evaluated for 4 min in a 2.5×3 ft diameter enclosure and scored by two trained observers with an inter-rater reliability $\geq 95\%$. BBB scores of 8 or below describe various degrees of behavioral recovery of locomotor-like movements that do not include weight support. BBB scores of 9 or above (to a maximum of 21) indicate some degree of hindquarter weight support starting in stance and progressing to weight-supported stepping (Basso et al., 1995).

Single-neuron mapping procedure

Following the end of chronic therapy administration (week 9), a single-neuron mapping procedure of the hindlimb sensorimotor (HLSM) and forelimb motor (FLM) cortex was performed on the three groups: Control, LD and HD. Animals were anesthetized with urethane (1.5 mg/kg, i.p.) and placed in a stereotaxic frame. For all animals, the anesthesia level was maintained at Stage III-3 (Friedberg et al., 1999). This had the effect of reducing the responsiveness of cells and we therefore stimulated the cutaneous surface throughout the mapping procedure in order to identify single neurons (see below). Craniotomies were performed over the right or left cortex to expose: 1) the hindlimb (HLSM) and forelimb representations within the primary somatosensory cortex and/or 2) the forelimb representation in the motor cortex (FLM cortex) (Fig. 1A). Electrode penetrations were defined using stereotaxic coordinates (Leergaard et al., 2004). Penetrations in the forelimb somatosensory cortex (FLS) were used to verify electrode positioning. The stereotaxic coordinates for the HLSM cortex craniotomy were from 0 to 3 mm posterior to bregma and from 2 to 3 mm lateral. The coordinates for FLS cortex craniotomy were from 1.5 mm anterior to 3 mm posterior and 3 to 5 mm lateral. The coordinates for FLM cortex craniotomy were from 2 mm anterior to 0.5 mm posterior and 2 to 4 mm lateral.

A high impedance (4–10 MΩ) tungsten microelectrode (FHC, Inc. Bowdoin, ME) was mounted on a stereotaxic electrode manipulator. A ground wire was connected to a grounding screw adjacent to the craniotomies. The microelectrode was positioned to a random, preselected location above either the HLSM or FLM cortices (Fig. 1C). The dura was removed and the microelectrode was lowered, perpendicular to the surface of the brain, to penetrate the pia. The microelectrode was then slowly inserted into the brain.

The signals from the microelectrode were amplified (20 k), bandpassed filtered (0.7 Hz–6 kHz) and digitized at 40 kHz (Plexon Inc., Dallas TX). Neuronal activity was continuously monitored on an oscilloscope and through audio speakers as the electrode was lowered and the cutaneous surface of the animal was continuously stimulated. When a neuron was encountered, the depth of the cell with respect to the cortical surface was noted. Two experimenters then determined whether the identified cell responded to cutaneous stimulation. The first experimenter controlled the position of the electrode and used a wooden probe to touch the hair/skin of the animal. The second experimenter, blind to the position of the electrode and treatment group of the animal, determined if the cell responded to the