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Role of cortical reorganization on the effect of 5-HT pharmacotherapy for spinal cord injury

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ABSTRACT

Cortical reorganization or expansion of the intact cortical regions into the deafferented cortex after complete spinal transection in neonatally spinalized rats was shown to be essential for increases in weight-supported stepping at adulthood. The novel somatotopic organization identified in these animals can be induced by exercise or spinal transplants that bridge the site of injury. However, the role of cortical reorganization in increased weight-supported (WS) stepping after pharmacotherapy is unknown. For the neonatally spinalized rat model, the 5-HT_{2C} receptor agonist 1-(*m*-chlorophenyl)-piperazine hydrochloride (mCPP) increases the number of WS steps taken when administered to adult rats spinalized as neonates (mCPP+) though not all animals showed this effect (mCPP−). Since no differences in the behavior of the animals off-drug has been demonstrated, it is unclear why acute administration of 5-HT affects only a subset of animals. One possibility is that differences in cortical organization between mCPP+ and mCPP− may contribute to the differences in the functional effect of mCPP. To test this, we recorded from single neurons in the deafferented hindlimb sensorimotor cortex during passive sensory stimulation of the cutaneous surface of the forepaws and during active sensorimotor stimulation of the forepaws while the animals locomoted on a motorized treadmill. Our results show that neurons recorded from mCPP+ animals increased their responsiveness to both passive and active stimulation off-drug in comparison to neurons from mCPP− animals. These data suggest that differences in the cortical organization of mCPP+ compared to mCPP− animals may be at least partially responsible for the effect of a 5-HT_{2C} receptor agonist on functional outcome.

Introduction

The neonatally spinalized rat model of spinal cord injury (SCI) is an effective model to assess the impact of therapies on functional outcome because these animals can achieve weight-supported (WS) stepping (Giszter et al., 1998a,b; Kao et al., 2009; Miya et al., 1997). Studies using this model support hypotheses from clinical observations that reorganization in the brain is important for completely understanding the mechanisms underlying functional recovery (Cramer et al., 2005; Curt et al., 2002a; Hoffman and Field-Fote, 2007; Lotze et al., 2006). For example, treadmill exercise induces cortical reorganization that is well correlated to the number of weight-supported steps that these animals take (Giszter et al., 1998a; Kao et al., 2009, 2011), and destruction of this reorganized cortex attenuates the effect (Giszter et al., 2008).

In addition to exercise therapy, pharmacotherapy, especially in the form of serotonergic (5-HT) receptor agonists has been shown to improve functional outcome in spinal injured animals (Antri et al., 2002, 2003; Barbeau and Rossignol, 1990; Jackson and White, 1990). Descending 5-HT projections into the spinal cord have been implicated in regulating the output of the central pattern generators in the spinal cord during locomotion (Cazalets et al., 1995; Kjaerulff and Keihn, 1996; Rossignol et al., 2001; Schmidt and Jordan, 2000) and it is hypothesized that, after SCI when these projections are lost, pharmacologic stimulation of the 5-HT system enhances recovery of function. In the neonatally spinalized rat model, improvement in weight-supported stepping can be accomplished by activation of the 5-HT2C receptor using the agonist 1-(m-chlorophenyl)-piperazine hydrochloride (mCPP) (Kao et al., 2006; Kim et al., 2001; Shumsky et al., 2005). However not all animals respond to treatment; approximately half of the animals challenged with a dose of mCPP respond by increasing their percentage of weight supported steps (mCPP+ animals) while the remaining animals do not increase their weight-supported steps (mCPP− animals). Since no behavioral differences in these animals were identified off-drug, we hypothesized that differences in the cortical organization of these animals may be related to the different effect of mCPP.

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To test this, differences in sensorimotor processing in the deafferented hindlimb sensorimotor cortex (HL-SMC) between mCPP+ and mCPP− animals were assessed. We chronically implanted arrays of microwires into the infragranular layer of the HL-SMC of neonatally spinalized rats and measured the response of neurons to passive sensory stimulation of the cutaneous surface above the level of the lesion and to active sensorimotor stimulation in response to forepaw footfalls on a motorized treadmill. We compared the responses of neurons recorded from mCPP+ animals to those of mCPP− animals after an injection of saline (off-drug) and after an injection of mCPP (on-drug). Results show distinct differences in the responsiveness of HL-SMC neurons both off- and on-drug that may be related to the improvement in functional outcome.

Materials and methods

The present study used 9 adult Sprague Dawley rats that received a complete mid-thoracic transection (TX) on postnatal days 2–3. The complete TX eliminates hindlimb input to the HL-SMC while leaving forelimb input intact. At adulthood, animals were tested on the treadmill after an injection of saline (1 ml/kg) and after an injection of mCPP (0.075 mg/kg, 1 ml/kg) on separate days. The percentage of weight-supported steps on each day was determined and if the animal increased its percentage after mCPP compared to after saline then it was classified as mCPP+. If the percentage of weigh-supported steps did not increase, then it was classified as mCPP−. Five animals were mCPP+ while four were mCPP−. Then animals were chronically implanted with arrays of microwires and allowed one week to recover. To compare differences in neuronal activity patterns between the two groups, two experiments were performed: passive sensory stimulation and active, sensorimotor stimulation. Each experiment was performed after an injection of saline (1 ml/kg) and after an injection of mCPP (0.075 mg/kg, 1 ml/kg) on separate days. For passive sensory stimulation, we recorded neuronal activity while specific locations on cutaneous forelimb above the level of the lesion were tapped when the animal was lightly anesthetized. For active sensorimotor stimulation, we recorded neuronal activity in response to paw placements while the animals performed treadmill induced locomotion. To identify differences in the cortical organization between the two groups, differences in neuronal response properties between groups (mCPP+ vs. mCPP−) were assesses off- and on- drug.

All procedures used in this study were performed under the guidelines of the National Institutes of Health and approved by the Institutional Animal Care and Use Committee of Drexel University College of Medicine.

Neonatal spinalization and post-operative care

The transection procedure for the pups was performed as in our previously published methods (Kao et al., 2006, 2009, 2011; Shumsky et al., 2005). Briefly, 2–3 day old Sprague Dawley female pups (Charles River) were anesthetized by hypothermia, the spinal cord was exposed by laminectomy at the T8/T9 level, and transected with iridectomy scissors followed by aspiration to ensure completeness. A collagen matrix, Vitrogen, was injected into the site of the transection to fill the cavity. The muscle and skin were sutured in layers with 5-0 silk. Pups were then warmed, and when they became active, were returned to the mothers and littermates. The pups were weaned at 4 weeks and housed in controlled conditions of temperature and humidity under a 12 hour light/dark cycle (lights on at 07:00) with free access to food and water.

Spinalized rats were handled and examined 5 days/week for skin lesions and other health concerns. After weaning, rats were placed on a motorized treadmill for 3 min/day at a speed of 6.5 m/min, 5 days per week. Previous work has shown that neonatally spinalized rats that received treadmill training can step at speeds of 6 m/min but not 12 m/min (Miya et al., 1997). Animals were continuously trained and their time on the treadmill was slowly increased to 10 m/day. This treadmill regimen was selected because this level of exercise is known to enhance the representation of the forelimb in HL-SMC of neonatally spinalized rats (Kao et al., 2009, 2011).

The spinal cords were assessed for completeness of the lesion (Fig. 1E). After the final recording session (see below), rats were perfused transcardially with buffered saline, followed by buffered 4% paraformaldehyde. Spinal cords were removed and placed in phosphate buffer containing 30% sucrose for 72 hours. Specimens were frozen in tissue freezing medium (Tissue-Tek) and sectioned on a freezing microtome at 20 μm. The lesion segments were sectioned parasagitally, and alternate sections were stained with Nissl-myelin stained or the polyclonal antibody to 5-HT to confirm completeness of transection (Kao et al., 2009). There were no differences between the lesions of mCPP+ animals and those of mCPP− animals. All transections were confirmed to be complete and no 5-HT was observed below the level of the lesion for any animals.

Implantation of electrode arrays

Six to eight months after spinalization, rats were chronically implanted bilaterally with arrays of microwires (stainless steel, 50 micron diameter insulated with Teflon, Neurolinc, Baskinridge, NJ) into the HL-SMC using the procedure from our previous hindlimb mapping study (Kao et al., 2009; Moxon et al., 2008). Briefly, rats were anesthetized by intraperitoneal injection of sodium pentobarbital (45 mg/kg), placed in a stereotaxic frame, and craniotomies were performed over both the right and left cortices to expose the hindlimb representation. A craniotomy was made between coordinates relative to bregma: (0 AP, 2.5 ML), (−0.5 AP, 3 ML), (−2 AP, 2 ML), (−2.5 AP, 2.5 ML), where ML is the medial–lateral coordinate and negative AP coordinates are posterior to bregma (units are in mm). These coordinates center the microwires over the sensory and sensorimotor overlap region of the hindpaw granular cortex (Chapin and Lin, 1984; Chapin and Woodward, 1986). Four screws were inserted into the skull to anchor the array and to act as an attachment point for ground wires. As the electrode array was slowly lowered, the signals were monitored, one channel at a time, on the oscilloscope and audio speakers. When the characteristic large amplitudes of layer V (infragranular layer) neurons were recorded on the majority of electrodes, the array was cemented in place with dental cement to the anchoring screws. Animals were allowed 7–10 days to recover from the implantation surgery before physiological evaluations of the neurons were performed. Recordings were completed within four weeks of implant.

Drug administration

mCPP (Sigma, St. Louis, MO) was dissolved in saline. Based on our previous study that identified the most appropriate dose of mCPP (Kao et al., 2006), saline (off-drug) or mCPP 0.15 mg/kg (on-drug) was injected intraperitoneally , 5 minutes before any electrophysiological recordings (either sensory maps or treadmill recording). All drugs were prepared fresh on the day of the experiment. On-drug and off-drug experiments were performed on different days with at least a 48 hour washout period.

Behavioral effect of mCPP

To determine if the animals responded to mCPP by increasing the probability of taking a weight supported step, treadmill testing was conducted under the same conditions as the training, allowing examination of step cycles and calculation of the percentage of weight-supported steps (%WSS). A step cycle is defined as a sequential flexion and extension of the hindlimb. Not all step cycles on a treadmill involve weight-supported stepping. Thus, we distinguished weight-supported step cycles, in which the hindlimb supported the hindquarters so that they were elevated above the surface of the treadmill, and non-weight-supported cycles in which the hindlimbs flexed and extended, but the