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INTRASTRIATAL DOPAMINE D1 ANTAGONISM DAMPENS NEURAL PLASTICITY IN RESPONSE TO MOTOR CORTEX LESION

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Abstract—Motor cortex lesions in rats partially denervate the striatum, producing behavioral deficits and inducing reactive neuroplasticity. Plastic responses include changes in growth-associated protein marker expression and anatomical restructuring. Corticostriatal plasticity is dependent on dopamine at the striatal target, where D1 receptor signaling reinforces behaviorally relevant neural activity. To determine whether striatal dopamine D1 receptor signaling is important for the growth-associated protein responses and behavioral recovery that follow unilateral motor cortex aspiration, the dopamine D1 receptor antagonist SCH23390 was intrastriatally infused in cortically lesioned animals. After a cortical aspiration lesion in Long Evans rats, the growth-associated proteins SCG10 and GAP-43 were upregulated in the cortex contralateral to the lesion at 30 days post-lesion. However, continuous unilateral intrastriatal infusion of SCH23390 prevented this aspiration-induced upregulation. Furthermore, lesioned rats demonstrated spontaneous sensorimotor improvement, in terms of limb-use symmetry, about 1 month post-lesion. This improvement was prevented with chronic intrastriatal SCH23390 infusion. The D1 receptor influence may be important to normalize corticostriatal activity (and observable behavior), either in a long-term manner or temporarily until other more permanent means of synaptic regulation, such as sprouting or synaptogenesis, may be implemented. © 2007 IBRO. Published by Elsevier Ltd. All rights reserved.

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The ability to reorganize neural connections according to changing environmental cues is essential to the formation of the wide network of limbic, striatal, and cortical circuits that regulate the complex array of human behaviors. Generally referred to as neural plasticity, these adaptive processes are most obvious in the developing brain, but are also utilized throughout adulthood as new skills and memories are acquired. Likewise, when the brain is damaged by trauma or disease, adaptive mechanisms of plasticity are brought into play and are critical for determining the limits of functional recovery (Ivanco and Greenough, 2000; Johansson, 2000; Nudo et al., 2001; Gonzalez and Kolb, 2003). These adaptive processes are governed by local extra- and intracellular signals and can be manipulated by various experimental interventions including: exercise (Cotman and Berchtold, 2002; Gomez-Pinilla et al., 2002), complex environments (van Praag et al., 2000), motor skills training (Plautz et al., 2000; Bury and Jones, 2002), and pharmacological treatment with monoamine affecting drugs (Feeney, 1997; Stroemer et al., 1998; Goldstein, 2003; Ivy et al., 2003). Understanding the cellular mechanisms and local factors that modulate neuroplasticity is the first step in developing novel treatment strategies that can facilitate the recovery of function after brain damage.

Evidence suggests that endogenous factors that regulate synaptic strength are likely to play key roles in the formation and maintenance of reactive innervation following injury (reviewed in Keyvani and Schallert, 2002; Butefisch, 2006). With strong influences on corticostriatal synaptic efficacy and striatal function, dopamine is one such putative factor. Nigrostriatal dopaminergic fibers densely innervate the striatum (Bjorklund and Hokfelt, 1984), and both dopaminergic and corticostriatal glutamatergic afferent axons synapse onto target medium spiny neurons (Bouyer et al., 1984; Groves et al., 1994; Smith et al., 1994; Centonze et al., 2003b). Within the striatum, dopamine is instrumental in determining the efficacy of glutamatergic signaling at corticostriatal synapses (Centonze et al., 1999, 2001; Cepeda et al., 2001; West et al., 2003), and striatal dopamine–glutamate interactions may play a role in the re-formation of behaviorally relevant functional connections after cortical injury. Indeed, as shown in previous studies from our laboratory, nigrostriatal denervation in combination with unilateral cortical lesion results in an aberrant nervous system response to the cortical lesion, evident in an elevated degree of reactive morphological change, altered growth-associated protein profile, and dampened behavioral improvement (Meshul et al., 2000; Hughes-Davis et al., 2005).

The effects of dopamine within the striatum are mediated by D1- and D2-type receptors located postsynaptically on striatal target neurons, as well as presynaptically by D2-type receptors. Although balance between D1 and D2 receptor signaling is vital for appropriate motor system function, the localization and normal role of D1 receptors support their differential involvement in reparative processes. In support of influential D1 receptor involvement, it has been observed that neighboring and contralateral cortical neurons that are recruited to participate in adaptive structural and functional plasticity after focal cortical injury include a corticostriatal population that preferentially contacts D1-expressing striatal neurons (Hersch et al., 1995;

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Lei et al., 2004). In addition, D1 receptor signaling is linked with several identified mechanisms of glutamate receptor regulation, as well as corticostriatal long-term potentiation (Dunah and Standaert, 2001; Flores-Hernandez et al., 2002; Centonze et al., 2003a; West et al., 2003; Pei et al., 2004; Cepeda and Levine, 2006; Hallett et al., 2006). Downstream consequences of striatal D1 receptor signaling include an influence on basal ganglia output, which ultimately feeds forward to the cortex (Steiner and Kitai, 2000; Blandini et al., 2002, 2003). Regulation of synaptic or motor loop activity by D1 receptors may act to sustain a degree of function within this system after lesioning, reflected in preserved or recovered motor abilities. Indeed, animals that have behaviorally recovered from unilateral frontal cortex aspiration rely on D1 receptor signaling to maintain this behavior (Vargo et al., 1995). Given the evidence supporting dopaminergic involvement in maintaining corticostriatal and basal ganglia activity, and the role of D1 receptors in regulating these effects, we wanted to determine the importance of this activity in supporting recovery following denervation.

To test the hypothesis that striatal D1 receptor signaling regulates behavioral recovery and lesion-induced expression of genes thought to underlie neurite outgrowth and remodeling, we carried out a series of analyses on rats that underwent motor cortex lesioning in combination with intrastriatal infusion of the selective D1-type receptor antagonist SCH23390. Western blot analysis was used to examine changes in expression of two growth-associated protein genes previously linked with reactive structural adaptations in the deafferented striatum: SCG10 and GAP-43. Protein levels were assessed at three post-lesion time points. In addition, the time course of growth-associated protein changes was determined in parallel with the progression of sensorimotor recovery in an evaluation of spontaneous forelimb use for postural support.

EXPERIMENTAL PROCEDURES

Animals

Young adult male Long Evans rats (Harlan Sprague–Dawley, Indianapolis, IN, USA) were housed in pairs in a standard care facility with a 12-h light/dark cycle. Animals were fed standard rat chow *ad libitum* and weighed between 340 and 460 g at the time of surgery. Experimental groups consisted of rats that received unilateral intrastriatal infusions of either the D1 antagonist SCH23390 or saline, in combination with unilateral aspiration lesion of the forelimb representation area of motor cortex or no lesion. Subsets of each group were killed at 4, 10, and 30 days post-lesion (*N*=4–6 rats per group per time point). Animal care followed the guidelines set forth by the NIH Guide for the Care and Use of Laboratory Animals, and the Institutional Animal Care and Use Committee at the University of Southern California approved all experimental protocols. All efforts were made to minimize animal suffering and the number of animals used.

Surgery

At 12 weeks of age, animals (*N*=63) received ketamine/xylazine anesthesia (100 mg/kg ketamine and 20 mg/kg xylazine, i.p.) and underwent one of two surgical procedures: a cortical aspiration lesion in combination with intrastriatal cannula implantation and s.c. osmotic minipump insertion, or the cannulation procedure alone. For the cortical lesion, a craniotomy was performed to remove a rectangle of skull from 2 mm anterior to 1 mm posterior to bregma, spanning from 1 to 4 mm lateral to the midline on the right side. After cutting and gently removing the dura, a fine-tipped glass pipette connected to a vacuum pump was used to aspirate the forelimb representation area of the motor cortex from the exposed region (Swanson, 1998). All cortical layers were removed, with care taken not to penetrate the underlying corpus callosum (Fig. 1). A small piece of gelfoam (Pharmacia, Kalamazoo, MI, USA) was gently placed into the lesion cavity, which was then covered with a small piece of adhesive foil. For the infusion cannulation, an osmotic minipump (Alzet Model 2004, Durect, Cupertino, CA, USA) containing the D1 receptor antagonist SCH23390 (Sigma-Aldrich, St. Louis, MO, USA) dissolved in sterile 0.9% saline, or only vehicle was implanted in a s.c. pocket in the scapular region. SCH23390 is selective for the D1-like receptor family, but it also has affinity for serotonin receptors, and can inhibit some potassium channels (Millan et al., 2001; Kuzhikandathil and Oxford, 2002). The pump was connected via a catheter to a 5.0 mm long infusion cannula (Durect), which was stereotaxically implanted at 1.5 mm anterior and 4.0 mm lateral to bregma on the right side (ipsilateral to the lesion). These coordinates were chosen to allow for diffusion of SCH23390 into the dorsolateral striatum, a region receiving both ipsilateral and relatively dense crossed corticostriatal projections, without imparting local damage due to the cannula track (McGeorge and Faull, 1989; Ebrahimi et al., 1992). Minipumps infused SCH23390 into the striatum at a rate of 0.25 μl/h at a concentration (0.67 mg/ml) amounting to 4 μg/day delivered throughout the post-operative survival time (Caine et al., 1995; Steiner and Kitai, 2000; Baldo et al., 2002). The infusion assembly was cemented in place and anchored to a stainless steel surgical screw fastened to the skull. Following these procedures, the scalp was closed over the wound. If an animal underwent both the lesion and cannula implantation procedures, they occurred during the same surgical session. Animals recovered in their home cages without complications.

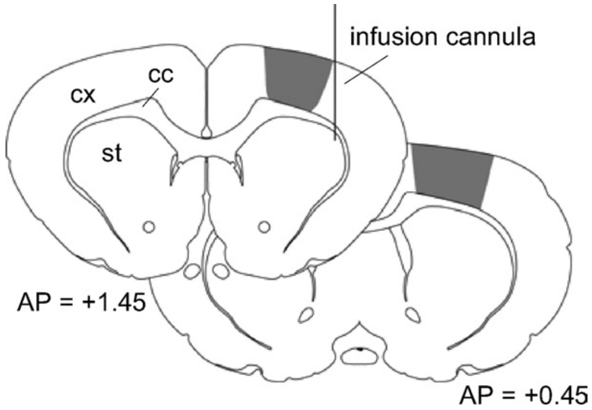

Fig. 1. Diagram depicting the cortical area targeted by aspiration (shading) and infusion cannula placement. Antero-postero levels relative to bregma. Coronal sections adapted from Swanson (1998). cc=corpus callosum, cx=cortex, st=striatum.

Forelimb-use asymmetry

Sensorimotor ability was monitored with the Schallert cylinder test (Schallert et al., 2000). Asymmetry in forelimb postural support was assessed once before surgery and at 4, 10, 20, and 30 days post-surgery. All animals participated in the cylinder test pre-surgery and at indicated time points up to the point at which they were killed. On each test day, rats were placed on the floor of a 30 cm tall clear acrylic cylinder (20 cm diameter) for a 2 min