Nicotinic acetylcholine signaling is required for motor learning but not for rehabilitation from spinal cord injury

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Abstract

Therapeutic intervention for spinal cord injury is limited, with many approaches relying on strengthening the remaining substrate and driving recovery through rehabilitative training. As compared with learning novel compensatory strategies, rehabilitation focuses on reconstituting movements lost to injury. Whether rehabilitation of previously learned movements after spinal cord injury requires the molecular mechanisms of motor learning, or if it engages previously trained motor circuits without requiring novel learning remains an open question. In this study, mice were randomly assigned to receive intraperitoneal injection with the pan-nicotinic, non-competitive antagonist mecamylamine and the nicotinic α7 subunit selective antagonist methyllycaconitine citrate salt or vehicle (normal saline) prior to motor learning assays, then randomly reassigned after motor learning for rehabilitation study post-injury. Cervical spinal cord dorsal column lesion was used as a model of incomplete injury. Results of this study showed that nicotinic acetylcholine signaling was required for motor learning of the single pellet-reaching task but it was dispensable for the rehabilitation of the same task after injury. Our findings indicate that critical differences exist between the molecular mechanisms supporting compensatory motor learning strategies and the restoration of behavior lost to spinal cord injury.

Key Words: acetylcholine; basal forebrain; corticospinal tract; dorsal column lesion; mecamylamine; methyllycaconitine; motor control; rehabilitation; rotarod; single pellet-reaching task

Introduction

Spinal cord injury (SCI) results in the lasting impairment of the motor and sensory functions that underlie movement. The majority of clinical cases of SCI are incomplete, allowing for a limited capacity for spontaneous or rehabilitation-mediated functional recovery (Fawcett et al., 2007). This capacity for partial recovery may be achieved through reinforcement of spared sensory and motor axons, compensatory activities of indirect pathways, or reorganization of supraspinal command centers (Hollis et al., 2016; Li and Hollis, 2017). The corticospinal tract is critical to restoring supraspinal command of voluntary movement. Following stroke, the extent of spared corticospinal tract is proportional to the amount of spontaneous recovery that individuals experience (Stone et al., 2007). In chronic SCI, the use of rehabilitation and epidural electrical stimulation to restore voluntary locomotion likely leverages preserved corticospinal circuitry (Wagner et al., 2018). These spared circuits can be activated by cortical stimulation even in motor complete, chronically injured individuals (Edwards et al., 2013).

Both rodent and non-human primate models have been used to demonstrate the innate plasticity of corticospinal axons after injury (Rosenzweig et al., 2010; Modberger et al., 2017). Within the injured spinal cord, corticospinal axons sprout locally and form novel axon collaterals, many of which are pruned back over time (Bareyre et al., 2004). The removal of intrinsic branches on axons regeneration can enhance corticospinal axon plasticity and connectivity; however, the contribution of such connections to behavioral recovery is not always apparent (Liu et al., 2010; Hollis et al., 2016; Jayaprakash et al., 2016). Rehabilitative training drives recovery of previously trained, corticospinal tract-dependent, single pellet reach behavior in animal models of corticospinal tract injury (Wahl et al., 2014; Hollis II et al., 2016). Clinically, training after SCI can be used to either rehabilitate movements lost to injury, or to train compensatory strategies to improve mobility and independence (Behrman and Harkema, 2007). Animal models used to study recovery from SCI often rely on trained behavior; however, questions remain as to whether rehabilitation-mediated recovery represents novel learning, or a re-emergence of patterned movements using the remaining motor circuitry.

Motor learning requires contributions from various brain areas, including motor cortex, cerebellum, striatum, and brainstem. Basal forebrain cholinergic neurons release acetylcholine in distinct targets and modulate a diverse array of functions, such as motor control, attention, cognition, and perception coding (Zaborszky et al., 2018; Boskovic et al., 2019). The cerebral cortex receives cholinergic input from nucleus basalis of Meynert (NBM). Primary motor cortex (M1) depends upon these basal forebrain cholinergic neurons for the maturation of cortical motor representations, or motor maps (Ramanathan et al., 2015). Ablation of NBM cholinergic neurons in rats attenuates skill acquisition in the single pellet-reaching task as well as the corresponding expansion of cortical forelimb motor representations and dendritic spine remodeling of corticospinal neurons that control the distal forelimb (Conner et al., 2003; Ang et al., 2016). Following cortical injury, rehabilitative training of skilled forelimb movements results in reconstitution...
of affected movement representations within adjacent, ectopic cortical areas (Castro-Alamancos et al., 1992; Castro-Alamancos and Borrel, 1995). As with motor learning, cholinergic input is critical for motor map reorganization and functional recovery after cortical injury (Friel et al., 2000; Conner et al., 2005). Previously, we observed similar rehabilitation-dependent cortical reorganization and functional recovery after SCI (Hollis et al., 2016). Unlike following stroke, cortical structures remain intact after SCI. Cortical and supraspinal motor centers are likely instrumental in driving rehabilitation-mediated recovery, but it remains unknown what role the molecular and cellular mechanisms of motor learning play in this recovery.

Nicotinic acetylcholine receptors expressed in the central nervous system are important for synaptic excitation, attention, and cognition (Dani, 2001). In primary visual cortex, cholinergic innervation is required for experience-dependent plasticity during the critical period for Fear and Singer, 1988) and closure of the critical period is associated with reduced nicotinic signaling (Morishita et al., 2010). Here we tested the role of nicotinic cholinergic signaling in skilled motor task acquisition and rehabilitation after SCI.

Methods

Animals

All animal experiments and procedures were approved by the Weill Cornell Medicine Institutional Animal Care and Use Committee (protocol # 2015-0042) on May 12, 2018. All mice were housed on a 12-hour light/dark cycle from 6 a.m. to 6 p.m., at 25°C with free access to food and water. Twenty-four male and female C57BL/6J animals (8–12 weeks old) were purchased from Jackson Laboratory. For forelimb reaching task, animals were food restricted to 80–90% of their free-feeding weight. Cervical spinal cord dorsal column lesion was used as a model of incomplete injury. Twelve mice used in this study performed the recessed single pellet-reaching task, the rotarod test, and the open field test. In each behavior test, six animals were injected with either nicotinic inhibitors or saline control. Experimental design is shown in Figure 1.

Drug administration

Mice were randomly assigned to drug or control groups prior to motor learning assays, then randomly reassigned after motor learning for rehabilitation study post-injection. Mice were injected intraperitoneally with the pan-nicotinic, non-competitive antagonist methyllycaconitine (MEC, 5 mg/kg; Torax, CA, USA) or the selective antagonist methyllycaconitine citrate (MLA, 5 mg/kg; MilliporeSigma, Burlington, MA, USA, Cat # M168) or vehicle (normal saline) 30 minutes before behavioral testing (Grottick and Higgins, 2000; Shi et al., 2011; Kita et al., 2013).

Recessed single pellet-reaching task

To test the effect of nicotinic inhibition on skilled motor learning, we employed a recessed single pellet-reaching task as described previously (Li and Hollis, 2021). Animals were calorie restricted to 80–90% of their free-feeding bodyweight by being given 1–3 g food before training. An acrylic behavior box (length × width × height: 29.5 cm × 21.9 cm × 21.6 cm) with three slots (7 mm wide) on the left, middle, and right sides of the front wall was used to train the mice. A recessed hole (3 mm wide, 2 mm deep) at 12 cm from the inside wall of the box was used to hold a 20-mg flavored food pellet (Bio-Serv, Flemington, NJ, USA), Cat# F50301). The dominant forelimb was identified during a single test session. Once the dominant forelimb was determined, it was trained over a total of 14 daily sessions consisting of 25 trials each. A trial was counted as a success if the mouse grasped, retrieved, and ate the food pellet. Only trials with pellet contact were counted. The open field test. In each behavior test, six animals were injected with either nicotinic inhibitors or saline control. Experimental design is shown in Figure 1.

Motor learning

Figure 2 shows the rotarod test (McCaffery et al., 2004) on May 12, 2018. All mice were housed on a 12-hour light/dark cycle from 6 a.m. to 6 p.m., at 25°C with free access to food and water. Twenty-four male and female C57BL/6J animals (8–12 weeks old) were purchased from Jackson Laboratory. For forelimb reaching task, animals were food restricted to 80–90% of their free-feeding weight. Cervical spinal cord dorsal column lesion was used as a model of incomplete injury. Twelve mice used in this study performed the recessed single pellet-reaching task, the rotarod test, and the open field test. In each behavior test, six animals were injected with either nicotinic inhibitors or saline control. Experimental design is shown in Figure 1.

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Statistical analysis

We used power analysis with a 40% effect size, an alpha of 0.05 and beta of 0.2, to generate our estimated group sizes based on our previous study (Li and Hollis, 2021). All procedures and analyses were performed blind to treatment assignment. Skilled pellet-reaching and rotarod tests were analyzed using two-way repeated measures analysis of variance with post hoc Sidak’s comparison test using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA, www.graphpad.com). The differences between two groups were compared by two-tailed unequal t-tests. The intra-group differences were analyzed using paired t-test. P < 0.05 was considered statistically significant.

Results

Systemic inhibition of nicotinic receptors impairs motor learning

We used a pharmacological approach to study the contribution of nicotinic receptors to motor learning in adult mice. MEC and methyllycaconitine were injected intraperitoneally 30 minutes prior to behavioral training. Control mice were injected with normal saline. Single injections of a skilled behavior used to measure dexterity of a single forelimb (Whishaw and Pellis, 1990). We employed a modified recessed single pellet-reaching task in which the food pellet is retrieved from a concave depression (Figure 2A); we previously found that the modification allows for a consistent learning curve in C57BL/6J mice (Li and Hollis, 2021). We found that systemic blockade of nicotinic receptors significantly attenuated skilled motor learning in the single pellet-reaching task (Figure 2B and C). MEC and MLA-treated mice showed smaller improvements over the course of training than controls. Washout of MEC and MLA over 5 days enabled the mice to learn the task with the same proficiency as controls (Figure 2B and C). The effects of systemic inhibition of nicotinic acetylcholine signaling were not specific for skilled forelimb motor learning. MEC and MLA administration also impaired coordinated motor learning on the accelerating rotarod task. Mice trained over nine trials (4 to 40 r/min, constant acceleration over 5 minutes) exhibited worse performance following intraperitoneal injection with MEC and MLA compared to control mice. MEC and MLA inhibition of nicotinic signaling resulted in significantly reduced latency to fall (Figure 2C). As a control, we included a washout of MEC and MLA over 5 days enabled the mice to learn the rotarod with the same proficiency as controls (Figure 2D). Additionally, MEC and MLA significantly reduced total walking distance in an open field test (Figure 2E).
Nicotinic signaling is not required for functional recovery following SCI
To test whether nicotinic signaling is also required for functional recovery, we randomly reassigned animals after motor learning, performed a cerebral SCI, and tested the effects of MEC and MLA on intensive rehabilitative training on the single pellet-reaching task. We performed a dorsal column lesion at cerebral spinal cord segment 5 (CS5) to transect the corticospinal and ascending dorsal column tracts, leaving most gray matter, lateral white matter, and ventral spinal cord intact (Figure 3A and B). One week after CS5 dorsal column lesion, intensive rehabilitative training was carried out in which animals were tested daily on the recessed pellet-reaching task. SCI significantly impaired task success (Figure 3C). Intensive rehabilitative training promoted recovery to levels similar to pre-injury, regardless of treatment group; MEC and MLA delivered no effect on the recovery of function (Figure 3C).

Discussion
In this study, we used pharmacological tools to demonstrate that nicotinic acetylcholine signaling is required for the acquisition of motor skills but not the rehabilitation-mediated recovery of the previously trained skills after SCI. Recently, we have found that mice, unlike rats, do not require basal forebrain cholinergic input to cortex for the acquisition of skilled motor learning on the forelimb reach task (Li and Hollis, 2021). This leaves open the question of the locus of nicotinic activity during motor learning. In our previous study, we targeted both NBM cholinergic neurons directly, through targeted toxin, genetic, and optogenetic means, as well as the projections of NBM cholinergic neurons to motor centers in medial prefrontal cortex and primary motor cortex, leaving cholinergic innervation of striatum, brainstem, cerebellum, spinal cord, and periphery intact. Nicotinic signaling is likely active in one of these other motor loci during motor learning.

During rehabilitation from SCI, the extent to which rehabilitation either relies on the preservation of previously trained motor programs or else leverages the cellular and molecular mechanisms of motor learning is not known. Motor cortex plasticity occurs alongside the acquisition of skilled motor learning and we previously found that rehabilitation on the single pellet-reaching task after SCI shapes both behavioral recovery and cortical plasticity (Hollis, 2016). During motor learning, the role of motor cortex diminishes with the development of task proficiency. Inactivation of primary motor cortex early in training of a forelimb lever press task impaired performance; however, corticospinal silencing after an extensive training period had little effect on task success or movement kinetics (Hwang et al., 2019). In fact, the execution of a similar trained temporally precise lever press task is essentially unperturbed by inactivation of the motor cortex in mice (Cavaiola et al., 2015). The declining role of motor cortex in execution of learned behavior is reflected in the absence of a role for cholinergic signaling following training. We previously found that ablation of cholinergic innervation of motor cortex after coordinated motor learning of rotarod behavior had no effect on task execution (Li and Hollis, 2021), similar to the absence of effects on single pellet-reaching task success in rats when cholinergic neurons were ablated after training (Conner et al., 2003). Thus, when animals become proficient in a motor skill, motor cortex disengages from the behavior and subcortical structures (such as basal ganglia, red nucleus, brain stem, and cerebellum) are sufficient for maintenance of previously learned motor skills (Hikosaka et al., 2002).

The spinal cord receives multiple motor inputs and these supraspinal circuits are likely to control different aspects of movement execution. Our dorsal spinal cord SCI was limited to transection of the main body of the descending corticospinal tract and the ascending dorsal column-medial lemniscal sensory circuit, leaving other supraspinal pathways intact, including rubrospinal, reticulospinal, and the lateral and minor corticospinal tracts. It should be noted that the remaining supraspinal motor circuits retain the motor patterns encoded through training needed to compensate for the loss of dorsal column circuitry, or that nicotinic signaling plays no role in the shaping of these alternate motor pathways. Others have implicated a role for spared ventral corticospinal axons in rats and dorsolateral corticospinal axons in mice in the restoration of corticospinal-dependent behaviors, with limited effect on cortical motor representations (Weidner et al., 2001, Hilton et al., 2016). While we found no role for nicotinic signaling during intensive rehabilitative training to restore a previously trained, stereotyped movement, nicotinic signaling is likely to play a role in the learning of novel, compensatory movement strategies employed by individuals to regain independence after SCI.

Acknowledgments: The authors are grateful to the Burke Neurological Institute Structural and Functional Imaging Core for providing the resources used in image acquisition.

Author contributions: YL and ERH designed the study and wrote the manuscript. YL performed the experiments and analyzed data. Both authors approved the final version of the manuscript.

Conflicts of interest: The authors declare that there are no conflicts of interest associated with this manuscript.

Availability of data and materials: The complete dataset is available at doi:ng.g-node.org/10.1271/g-node.atjinn/.

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C-Editor: Zhao M; S-Editor: Li CH; L-Editor: Song LP; T-Editor: Jia Y