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# Plasticity of subcortical pathways promote recovery of skilled hand function in rats after corticospinal and rubrospinal tract injuries

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ABSTRACT

The corticospinal and rubrospinal tracts are the predominant tracts for controlling skilled hand function. Injuries to these tracts impair grasping but not gross motor functions such as overground locomotion. The aim of the present study was to determine whether or not, after damage to both the corticospinal and rubrospinal tracts, other spared subcortical motor pathway can mediate the recovery of skilled hand function. Adult rats received a bilateral injury to the corticospinal tract at the level of the medullar pyramids and a bilateral ablation of the rubrospinal axons at C4. One group of rats received, acutely after injury, two injections of chondroitinase-ABC at C7, and starting at 7 days post-injury were enrolled in daily reaching and grasping rehabilitation (CHASE group, $n = 5$). A second group of rats received analogous injections of ubiquitous penicillinase, and did not undergo rehabilitation (PEN group, $n = 5$). Compared to rats in the PEN group, CHASE rats gradually recovered the ability to reach and grasp over 42 days after injury. Overground locomotion was mildly affected after injury and both groups followed similar recovery. Since the reticulospinal tract plays a predominant role in motor control, we further investigated whether or not plasticity of this pathway could contribute to the animal's recovery. Reticulospinal axons were anterogradely traced in both groups of rats. The density of reticulospinal processes in both the normal and ectopic areas of the grey ventral matter of the caudal segments of the cervical spinal cord was greater in the CHASE than PEN group. The results indicate that after damage to spinal tracts that normally mediate the control of reaching and grasping in rats other complementary spinal tracts can acquire the role of those damaged tracts and promote task-specific recovery.

## Introduction

Among the spinal cord motor pathways, the corticospinal tract (CST), and to a lesser extent the rubrospinal tract (RbST), play a major role in controlling manual dexterity (Lawrence and Kuypers, 1968). When these tracts are individually (Morris et al., 2011; Whishaw et al., 1993) or collectively (Girgis et al., 2007; Kanagal and Muir, 2009) damaged, forelimb skilled movements such as grasping, but not other gross motor functions such as reaching or ground locomotion, are impaired. Animal studies have consistently reported the limited capacities of CST axons to spontaneously regenerate, leading to chronic disruption of the direct motor cortex to spinal cord connections. An alternative strategy to axon regeneration is for undamaged axons to sprout and make new synaptic contacts with spinal neurons. This anatomical reorganization has been proposed to have contributed to motor recovery under different spinal cord injury conditions (Blesch and Tuszynski, 2009).

Anatomical and functional studies have demonstrated that the motor cortex is connected to the spinal cord not only via the CST but also indirectly via extrapyramidal routes (Bolzoni et al., 2013; Canedo, 1997). It is hypothesized that following CST damage the brainstem motor pathways “by pass” the direct corticospinal connections, by conducting the information indirectly from the cortex to the spinal cord. It has been shown that sprouting of sub-cortical, uninjured projecting axons in the spinal cord can occur in mice after a unilateral cortical stroke (Bachmann et al., 2014), and in rats after a spinal lateral hemisection (Filli et al., 2014). In both of these studies, the anatomical plasticity observed was thought to contribute to the spontaneous locomotor recovery achieved by the animals. It remains uncertain, however, whether or not plasticity of these sub-cortical pathways could be responsible for the recovery of skilled motor functions, such as reaching and grasping.

In the present study, we hypothesize that rats with complete CST and RbST injuries can recover the ability to reach and grasp by remodeling the cervical spinal circuitry. Rats received complete CST and RbST

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tract injuries and receive injections of either chondroitinase-ABC or penicillinase (as a control) into the caudal cervical spinal cord segments which host the spinal interneurons and motoneurons innervating the distal forelimb muscles important for grasping (McKenna et al., 2000). The removal of spinal chondroitin sulfate proteoglycans (CSPGs) via chondroitinase-ABC administration creates an environment permissive to axonal sprouting and synapse formation. It is important to note, however, that these same rats were enrolled in intensive reaching and grasping rehabilitation, that in itself, has been shown to actively strengthen newly formed functional connections and improve coordinated performance of a motor task (Girgis et al., 2007). These interventions were integrated into our CHASE intervention given our previously reported synergistic effects of chondroitinase-ABC treatment and rehabilitation on the recovery of reaching and grasping after a dorsal funiculi crush (Garcia-Alias et al., 2009). Since the reticulospinal tract (RET) plays a prominent role in motor control and is malleable to drive functional recovery (Ballermann and Fouad, 2006), the distribution of RET axons within the gray matter of the cervical spinal cord was analyzed and compared between the treated and untreated groups.

The results obtained show a significant recovery of reaching and grasping in the rats that received both interventions. The recovery was accompanied by increased density and expansion of RST projections into the areas of the gray matter that were vacated of CST and RbST input.

## Material & methods

All experimental procedures were performed in compliance with the University of California Los Angeles Chancellor's Animal Research Committee and complied with the guidelines of the National Institutes of Health.

### *Surgical and drug injection procedures*

All survival surgical procedures were performed under aseptic conditions. Ten adult female Long Evans rats (280–300 g) were anesthetized with 1.5–2.5% isoflurane in 0.4% $O_2$. A ventral skin incision of the neck was made and the underlying muscles, trachea, and esophagus were displaced. A small piece of the ventrocaudal part of the occipital bone was removed and the medullary pyramids were exposed. The pyramids were incised bilaterally with a sharp scalpel blade. The esophagus, trachea, and muscles were repositioned, and the skin was sutured (Kanagal and Muir, 2009). A partial laminectomy at spinal segments C4–C5 was performed to expose the spinal cord. The dura was cut and an ~1.5 mm deep incision was made in the right and left lateral funiculi and the tissue displaced laterally using a sharp scalpel blade. This procedure damaged the most dorsal aspect of the dorsolateral funiculi. A laminectomy then was performed at C7 and two injections, each containing 1 μl of chondroitinase-ABC at 100 U/ml (Seikagaku) (CHASE group, $n = 5$) or Penicillinase (Sigma) (PEN group, $n = 5$), were made into the most rostral and caudal edges of the exposed spinal segment. A glass micropipette was lowered ~1 mm into the gray matter and the drug delivered over a period of 10 min (Garcia-Alias et al., 2011). The muscles and skin were sutured. The rats were allowed to fully recover in an incubator (37 °C) before being returned to their home cages. To evaluate the extension of CSPG digestion in the cervical spinal cord, three additional rats received a spinal cord injury followed by chondroitinase-ABC injection and were perfused the following day. Their tissue was processed as described below.

### *Training and testing of reaching and grasping*

The rats were placed individually inside a clear plastic box (18 cm × 15 cm × 31 cm) with a small opening in the front wall (3 cm × 1.5 cm) and trained to reach and grasp for chocolate pellets (45 mg, Bio-Serv) placed on a platform positioned 1 cm outside the window. The rats had to extend their preferred forelimb to reach and grasp the pellets. During each testing session, 20 pellets were presented to the rats and the ratio of the number of pellets eaten to the number of attempts was calculated (Whishaw et al., 2008). The rats were tested prior to the injury and weekly for 6 weeks post-injury. The rats were video recorded at 400 frames/s (Casio EX-FH25) and the movement elements during each attempt were analyzed following Whishaw's score (Piecharka et al., 2005). Five reaches with each limb by each rat were rated for qualitative features of the movement. A score of “0” was given if the movement was performed normally. A score of “2” was given if the movement was abnormal. A score of “1” was given in cases where there was some ambiguity concerning the normality of the movement. Seven component movements of a reach were rated: 1) advance: the limb is advanced directly through the slot toward the food target; 2) digits extend: during the advance, the digits extend so that the digit tips are pointing toward the target; 3) arpeggio: when the paw is over the target, the paw pronates from digit 5 (the outer digit) through digit 2 and, at the same time, the digits open; 4) grasp: the digits close and flex around the food, with the paw remaining in place, and the wrist is slightly extended to lift the food; 5) supination I: as the paw is withdrawn, the paw supinates by almost 90°; 6) supination II: once the paw is withdrawn from the slot to the mouth the paw further supinates by about 45° to place the food in the mouth; and 7) release: the paw contacts the mouth and the paw opens to release the food.

### *Grip strength*

At 6 weeks post-injury, the grip strength of each rat was measured with a custom designed grip strength meter. Rats were allowed to grip a horizontal bar connected to a force transducer with both forepaws and then pulled away until the grip was released. The force exerted on the bar at the time of release was measured in three consecutive trials (Garcia-Alias et al., 2009). The maximum force for the right and left forepaws was calculated and compared with a group of three intact, age and weight matched, control rats.

### *Assessment of overground locomotor ability*

Overground locomotor ability was tested prior to the injury and at 1 and 6 weeks post-injury. The forepaws and hindpaws were inked with different colors. The footprints were recorded on paper as the rats walked across a runway (8 cm wide and 100 cm long). The footprints then were used to determine mean stride length. The stride length was measured between the central pads of two consecutive prints on each side for the right and left forelimbs and hindlimbs. For each animal, the stride length was calculated as the average distance of 3–4 consecutive steps (Garcia-Alias et al., 2011).

### *Reaching and grasping rehabilitation*

Starting at 1 week post-surgery, rats in the CHASE group were subjected to reaching and grasping rehabilitation. Groups of 2–3 rats were placed in a cage with a plastic grid with square openings on the floor for 1 h/day, 5 days/wk. Seeds were placed in the square openings and the rats had to retrieve the seeds by extending their paws and grasping the seeds (Garcia-Alias et al., 2009). Rats in the PEN group did not undergo any rehabilitation regime; instead, the rats were placed in an empty cage without a grid on the floor, and with no seeds, for the same amount of time as the rats in the CHASE group were performing rehabilitation.

### *Histological analyses*

At the end of the functional evaluation, the rats received a unilateral stereotaxic injection of 1 μl of 10% dextran amine conjugated to 555-