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Task Practice with or without Cellular Transplantation Promotes Recovery of Reach-to Grasp Function after Cervical Spinal Cord Injury

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Abstract

Background and Purpose: Cervical spinal cord injury results in specific deficits in forelimb function. In the rat, lesions to the rubrospinal tract impair forelimb function despite the presence of an intact corticospinal tract. Both functional and anatomical recovery have been promoted by transplantation of neuronal and glial restricted precursors (NRP/GRP) following injury, and task-specific practice is used clinically to maximize recovery of function. We tested the hypothesis that combination therapy of daily task practice and NRP/GRP cell transplants will improve reach-to-grasp function.

Methods: Forty-one adult female rats received a lesion to the right cervical dorsolateral funiculus. They were randomly divided into 4 groups for the study: Control (n=11), NRP/GRP Transplant (n=14), Task Practice (n=8), and Task Practice + NRP/GRP (n=8). All animals were assessed pre-injury and during weeks 1 and 8 postoperatively on two reach-to-grasp tests (Single Pellet and Staircase Reaching).

Results: Task Practice + NRP/GRP and Task Practice groups achieved significant recovery of function in the Staircase Reaching test at week 8 of recovery. Analysis of individual kinematic elements from the Single Pellet Reaching test allows detailed quantitation of specific movements. While major differences were not observed in the Single Pellet Reaching, the Digits Open and Pronation qualitative component scores were higher in the Task Practice + NRP/GRP group compared to controls at 8 weeks post injury.

Conclusions: While task practice improves recovery of forelimb function following incomplete spinal cord injury, combination therapy of daily task practice and cell transplantation practice did not result in superior recovery of reach-to-grasp function.

Keywords: Cell transplantation; Cervical; Forelimb; Reaching; Rehabilitation; Spinal cord injury

Introduction

Regaining arm and hand function is considered a high priority for improving the quality of life in people with cervical spinal cord injury (SCI) [1], because this can dramatically impact their level of independence. Loss of hand function translates into increased dependence on attendant care for activities of daily living and impacts on occupational/employment status [2]. Current clinical rehabilitation practices include teaching the injured individual compensatory strategies or using assistive devices for transfers, activities of daily living, or mobility. Restoration of a grossly functional palmar grasp is achievable by establishing tenodesis of the long finger flexors in people who have some residual active wrist extension (C6/C7 tetraplegia), but this does not allow fine digital manipulation or lifting heavy objects [3]. Because of the severe impact on societal roles and substantial economic burden, therapies for tetraplegia that have potential to restore function to the upper extremities are clearly needed.

Cellular/tissue transplantation into the site of an SCI has shown promise in enhancing the sprouting and regenerative capacity of injured adult rat spinal cord axons, providing neuroprotection, and supporting functional recovery [4]. Transplants of fetal tissue, Schwann cells, olfactory ensheathing cells, genetically modified fibroblasts that produce neurotrophins, and lineage-restricted neuronal (NRP) and glial (GRP) precursor cells have all been reported to induce axonal growth into [5,6] and beyond [4,7-12] the transplant site in adult animals. Pre-clinical studies using cell and tissue transplants have also shown promise in restoring reach-to-grasp function in adult rats after cervical dorsal corticospinal tract lesions [9,13,14] and combined with neurotrophins after cervical over-hemisection [15].

Other studies have used task practice, which targets activity-dependent mechanisms of neuroplasticity, to improve function after CNS injury [16-21]. Deficits in skilled/learned forelimb reaching function have been improved through forced- or encouraged-use of the impaired forelimb during reaching tasks after brain injury [16-19,22,23] and more recently after partial cervical spinal injury. Reaching practice has previously been shown to induce cortical plasticity [16-19,21].

Although previous studies were successful in restoring some ability to reach and grasp a food pellet, the movement quality was not

evaluated with quantitative kinematics nor did the animals recover to the baseline number of successful pellet retrievals [13,14].

Kinematic analysis allows the quantification of specific deficits in limb or digit position and trajectory, which may provide insight into aspects of motor control that are disrupted or recover when reaching toward a target after injury. Given that both transplants and task practice have improved forelimb reaching function, the main working hypothesis of this project, therefore, is that a combination therapy of task practice and cellular transplantation will enhance skilled forelimb function after cervical dorsolateral funiculotomy (disruption of the rubrospinal tract and lateral fibers of the corticospinal tract) over either therapy alone.

Methods

Subjects

All procedures followed NIH guidelines and were approved by Drexel University's Institutional Animal Care and Use Committee. Fifty adult female Sprague-Dawley rats (225-250 g; Taconic, Germantown, NY) that preferred to use their right forelimb to reach for single pellets received a lesion to the right cervical dorsolateral funiculus. Forty-one rats survived the surgery and were randomly divided into 4 groups for the study: Control (n=11), NRP/GRP transplant (n=14), Task Practice (n=8), and Task Practice + NRP/GRP (n=8). All animals were housed on a 12 hr light/dark schedule (lights on at 07:00) and maintained on a food-restricted diet of 12-15 g of standard rat chow per day per animal. This resulted in animals reaching approximately 90% of their free feeding body weight. Animals in the Control and NRP/GRP groups were group housed in standard cages for the duration of the experiment.

Task practice

Animals in the Task Practice and Task Practice + NRP/GRP groups were housed in multilevel cages that promoted climbing, wheel running, and foraging for food starting 7-days post-injury. Starting at day 9 post-injury, these groups also spent 20 minutes/day, 5 days/week (totaling 13 hours and 20 minutes) in a reaching trough apparatus that is similar to the staircase reaching apparatus described below. The reaching trough apparatus was set up so that only the impaired forelimb could be used to retrieve food pellets. Preliminary data collected on pilot animals suggested that food-restricted rats would make more than 100 reach attempts during the 20 minute access time in the trough, with a majority of attempts occurring in the first half of the session, this would equate to approximately 4,000 reach attempts over an 8-week training period. The arrangement of additionally living in an environment that promoted forelimb activity may afford the animals more opportunities to integrate impaired forelimb use in daily function that included climbing the wire mesh cage walls for food rewards, digging for food, and running wheel and multi-level cage locomotion. Animals in the Control and NRP/GRP group were given additional 45 mg pellets (Bio-Serv, Frenchtown, NJ) in their standard cages to match the extra food that the Task Practice groups received.

Behavior training and testing

Prior to surgery, all animals were trained on a single pellet reach-to-grasp task [24-26]. Following habituation to the Plexiglas® reaching chamber (45 x 40 x 12.5 cm), the animals were operantly trained to reach with the right forelimb through a slit in order to grasp and consume a food pellet that was placed in a food well on a shelf. Training was performed through successive approximation and described in detail elsewhere [25]. The animal was considered to be successfully trained when it reached with the right forelimb and grasped the food pellet with a success rate ≥50%.

Qualitative components of the single pellet reach-to-grasp task were scored by blinded raters using a movement rating scale [26,27], which assesses 10 different components of a reach. Each component was given a score of 2 if the movement appeared normal, 1 if the movement appeared somewhat abnormal but recognizable, or 0 if the movement was absent or was compensated for by moving other parts of the body. The ratings from each component of the reach were summed to get a deficit score from each trial (20=no deficit; 0=maximum deficit with no movement for any of the reaching phases). A total of 5 reaches from each animal were scored from video replay and averaged. The 10 components of the reach have been previously defined [25-27] and are: 1)limb lift; 2)digits close; 3)aim; 4)limb advance; 5)digits open; 6)pronation; 7)grasp; 8)supination I; 9)supination II; and 10)release.

Quantitative assessment of the single pellet reach-to-grasp task was assessed in the reaching chamber described above using a synchronized, high-speed (500 frames/sec) two-camera digital recording system (Redlake) by blinded raters. The two cameras were positioned to get a lateral view of the right side and a frontal view. The animals' right forelimbs were ink marked over the lateral wrist joint and the tips of the digits for kinematic analysis using WINanalyze tracking software (Mikromak). Quantitative measures that are assessed during the single pellet reach-to-grasp task are: 1) percent successful reaches; 2) amount of paw pronation excursion; and 3) the amount of spread between the 2nd and 5th digits during digits open and pronation phases. A successful reach was defined as a reach during which the rat made contact with the food pellet and grasped it to remove it from the pellet well on the platform. A reach during which the animal "raked" the pellet from the well and did not grasp the pellet with digit flexion was not counted as a successful reach. The percent of successful reaches was calculated as: (#successful reaches/ #unsuccessful reaches)X100. Both qualitative and quantitative assessments of the reach-to-grasp task were performed once during the pre-lesion phase after the animals were trained on the task. Post injury, qualitative and quantitative reaching assessment were performed at 1 and 8 weeks. During the post injury period, all study groups were given one practice session per week (20 attempts) to retrieve single pellets. This brief practice session was performed to ensure that the animals maintained familiarity with the single pellet reach-to-grasp test throughout the duration of the 8-week recovery period.

In addition to the single pellet reach-to-grasp task, we also used the staircase-reaching test to examine performance [28]. The staircase-reaching test was used for several reasons: 1) The shelf of the single pellet reaching apparatus was 4.0 cm above the floor and requires control of shoulder muscles to aim the forelimb and permit contact with the pellet. The staircase-reaching test was able to test reaching function independent of shoulder control, which may be impaired after dorsolateral funiculotomy; and 2) The single pellet test allows the possibility for the rat to "rake" the pellet through the slot rather than grasping it. The staircase test, instead, requires the animal to make a coordinated grasp and lift the pellet to its mouth with no possibility for "raking". The animals were trained on the staircase-reaching test for two weeks prior to the lesion surgery (1, 15-min session/day). The

following week, the animals performed the test (2, 15-min sessions/ day) and the highest 3 sessions were averaged by raters blind to the animal groups to obtain the measure of forelimb reaching ability. Post-injury testing was performed at weeks 1 and 8

Cell preparation

Animals in the transplant groups were immunosuppressed throughout the study and NRP/GRPs were isolated from embryonic day 13.5 transgenic Fischer 344 rats [29-31] These cells were prepared for grafting, confirmed viable, and verified that they contained only precursor cells using methods described previously [6,31-33].

Lesion surgery

Cervical SCI was performed as previously described.25 Briefly, after deep anesthesia was achieved, animals received a partial laminectomy of the C3-4 level to expose one spinal cord segment and a lesion was created that disrupted the dorsolateral funiculus (Figure 1). The dura was closed with 9-φ sutures, and approximately μL of liquid collagen (Vitrogen, Cohesion, Palo Alto, CA) or NRP/GRP cells μ(~200,000 Lμg/ml+50 BDNcells/ F; 1NRP:2GRP) suspended in liquid collagen were injected using a gas-tightμL,syringeHamilton;(5 Reno, NV) to fill the lesion cavity. A fat pad was placed on top of the laminectomy site, the muscle and skin were closed in layers, and post-operative care was provided.

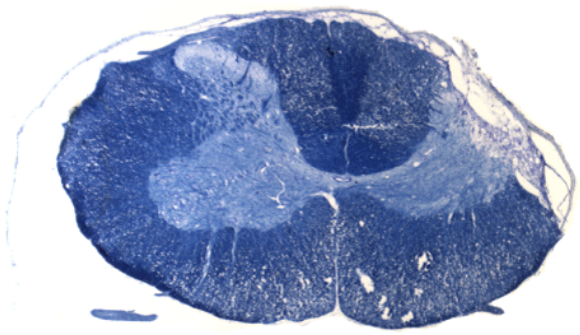

Figure 1: Nissl-myelin stain of the cervical dorsolateral funiculotomy lesion in cross section.

Statistics

Two-way analysis of variance (ANOVA) tests for treatment group and post injury time, with time taken as the repeated measure were conducted on each behavioral test with an alpha level set to 0.05. Main effects and interactions were identified. If a main effect was found to be significant, then Bonferroni-corrected post-hoc t-tests were performed to identify were the differences existed. Chi square analysis of final outcome data was applied to the staircase reaching data at week 8.

Results

Staircase reaching

Two-way ANOVA of group by post injury time, with time taken as a repeated measure, revealed a significant interaction [$F(6,72)=2.8$, $p<0.05$]. A main effect for post injury time revealed a significant deficit from baseline [$F(2,72)=207.6$, $p<0.001$] at week 1 post injury that partially recovered by week 8 in all groups. Post hoc analysis using one-way ANOVA revealed no difference among groups at baseline or at week 1 post injury, but a significant difference at week 8 [$F(3,36)=4.5$, $p<0.01$]. As shown in Figure 2, Bonferroni corrected t-tests confirmed that animals in both the Task Practice + NRP/GRP group and the Task Practice group retrieved significantly more pellets than SCI controls ($p<0.0083$). A threshold analysis was also performed to identify what percentage of animals in each group were able to reach 50% of pre-SCI performance by collecting 9 or more pellets. Chi square analysis of these data revealed that both the Task Practice + NRP/GRP group (63%) and the Task Practice group (63%) performed significantly better than both the NRP/GRP group (29%) and SCI controls (0%) at week 8 [$\chi2=0.387$, $p<0.01$]. Single Pellet Reaching Percentage of successful grasps was analyzed by two-way ANOVA of group by post injury time, with time taken as a repeated measure, and revealed no significant interaction [$F(6,72)=1.7$, n.s.]. A main effect for post injury time revealed a significant n.s. from baseline [$F(2,70)=47.8$, $p<0.001$] at week 1 post injury that did not recover by week 8 in all groups (data not shown).

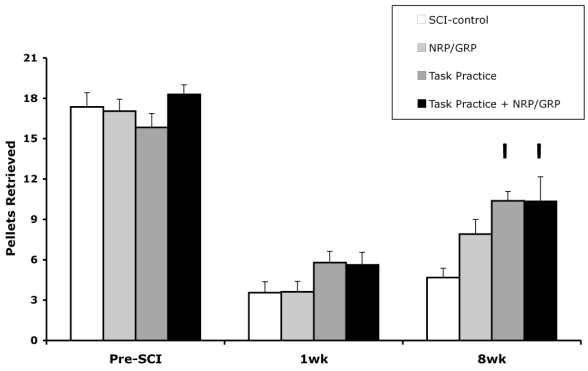

Figure 2: Staircase reaching: 1 wk after SCI, all rats displayed deficits in pellet retrieval. At 8 wks post injury, rats that had received Task Practice + NRP/GRP (n=8) or Task Practice (n=8), but not NRP/GRP alone (n=14) retrieved significantly more pellets (*$p < 0.0083$, Bonferroni correction) than SCI controls (n=11).

Qualitative Reaching

Total score: Qualitative reaching scores were analyzed by two-way ANOVA between group and time, with time taken as a repeated measure. A main effect for post injury time revealed a significant deficit from baseline [$F(2,70)=69.3$, $p<0.001$] at week 1 post injury that did not recover by week 8 in all groups (Figure 3). A significant main effect of treatment group was also found [$F(3,35)=4.1$, $p<0.05$] indicating that the SCI controls performed significantly worse than the three treatment groups. No significant interactions were found, indicating that the pattern of differences was maintained across all groups over time.

Component scores: Individual component reaching scores were analyzed by two-way ANOVA between treatment group and post injury time, with time taken as a repeated measure. Significant interactions were found for each component, thus individual component scores were analyzed across groups by one-way ANOVA

at each time point. Post hoc analysis using the Bonferroni correction revealed that the Digits Open and Pronation component scores exhibited significant differences ($p<0.0083$) between the Task Practice + NRP/GRP group and SCI controls at week 8 post injury (Figure 4). The Digits Open component, but not the Pronation component, was significantly different between both the NRP/GRP and Task Practice groups and SCI controls at week 8, as well.

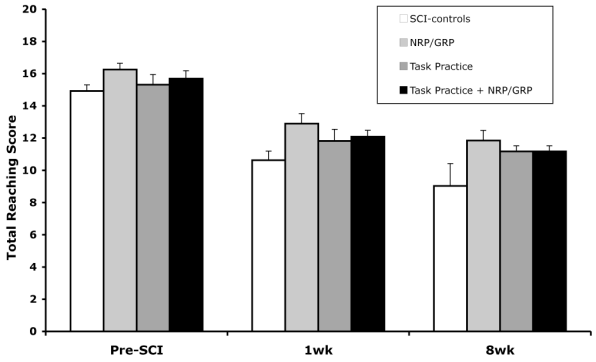

Figure 3: Single pellet reaching total component scores: 1 wk after SCI, all rats displayed deficits in pellet retrieval. By 8 wks post injury, there was no significant recovery of this measure in any group: SCI controls (n=11), NRP/GRP alone (n=14), Task Practice (n=8), or Task Practice + NRP/GRP (n=8), although a main effect of group was found indicating that the SCI controls were significantly worse than the three treatment groups.

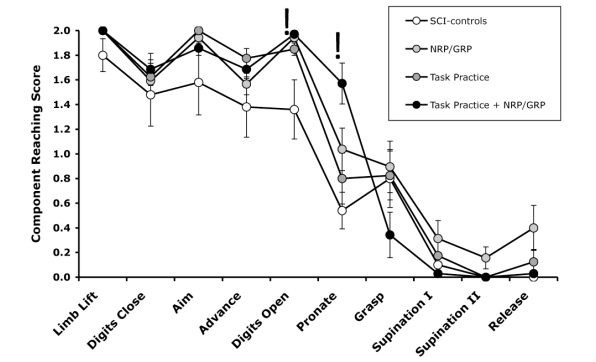

Figure 4: Single pellet reaching individual component scores: By 8 wks post injury, significant differences were identified in the Digits Open component (*$p<0.0083$, Bonferroni correction) in rats that had received Task Practice + NRP/GRP (n=8) or NRP/GRP alone (n=14), and nearly significant ($p = 0.0138$, Bonferroni correction) in rats that had Task Practice (n=8), compared to SCI controls (n=11). Significant differences were also found in the paw pronation component (*$p<0.0083$, Bonferroni correction) in rats that had received Task Practice + NRP/GRP compared to SCI controls.

Kinematic measure

Digit spread: Because of variability in the baseline values of this measure, data were transformed by dividing the individual animal's performance at both week 1 and week 8 by the baseline performance. Data were then analyzed by two-way ANOVA between treatment group and post injury time, with time taken as a repeated measure. ANOVA revealed a significant interaction [$F(3,35)=3.4$, $p<0.05$]. Post hoc analysis using the Bonferroni correction revealed that each group was significantly different from SCI controls at both 1 and 8 weeks post injury, but not different among the treatment groups (Figure 5).

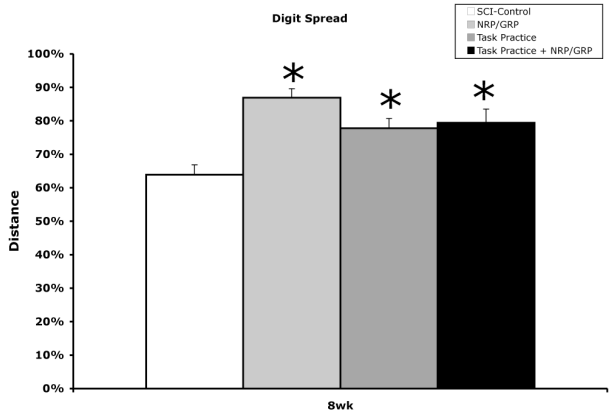

Figure 5: Single pellet reaching kinematics of Digit Spread: By 8 wks after SCI, rats that had received Task Practice + NRP/GRP (n=8), Task Practice (n=8), or NRP/GRP alone (n=14) displayed significantly greater Digit Spread (*$p < 0.0083$, Bonferroni correction) than SCI controls (n=11). Because of variance between the group baseline measures, data are presented as % Pre-SCI baseline.

Paw Pronation Excursion: Data were analyzed by two-way ANOVA between treatment group and post injury time, with time taken as a repeated measure. ANOVA revealed no significant interactions [$F(6,70)=0.65$, n.s.] or main effects.

Discussion

Our results confirm that task practice improves recovery of forelimb function following incomplete spinal cord injury and that cellular transplants may provide modest benefits. Both groups that received either Task Practice alone or Task Practice + NRP/GRP transplants achieved greater recovery of function than the NRP/GRP transplant and SCI control groups in the Staircase Reaching test, which emphasizes grasping and elbow flexion movements. These animals did not improve in single pellet reaching success, which emphasizes precision of aiming and grasping. While major kinematic differences were not observed in the single pellet reaching, the Digits Open and Pronation component scores showed partial recovery in the Task Practice + NRP/GRP group compared to controls at 8 weeks post injury.

Cervical injury produces forelimb motor deficits

Level and extent of injury are critical for predicting forelimb deficits [34], While we have demonstrated similar reaching deficits in both our unilateral C3/4 dorsolateral funiculotomy [25] and our unilateral C3/4 contusion model [24]. We chose the dorsolateral funiculotomy for this study, in order to focus on the rubrospinal tract. Although the corticospinal tract is often thought to be the primary descending

system involved in the control of skilled forelimb movements, [35] focal ablations of this tract do not prevent the directed aiming, transport of the arm and grasping in rats [36-39], monkeys [40,41], and even in humans [42]. Other descending systems that contribute to skilled reaching are the rubrospinal tract, [39] ventral corticospinal, [38] tectospinal tract and reticulospinal tract [43,44].

Schrimsher and Reier [37] showed that only lesions to the dorsolateral funiculus (rubrospinal tract) produced a persistent deficit in reaching success by impairing the ability to flex the digits during the reach-to-grasp task. The rubrospinal tract originates in the red nucleus and makes contralateral projections onto motoneurons of the forelimb musculature that controls wrist and digits [45]. Whishaw and colleagues [39,46] reported that ibotenic acid lesions to the red nucleus did not significantly impair reaching success in a reach-to grasp task, but deficits were noted in the Aim, Pronation, Grasp, and Supination phases of the reach. Similarly, our spinal lesion revealed deficits in reaching phases that involve distal motor control of the wrist and digits.

Task practice produced task-specific recovery

We designed our training approach based upon studies from the experimental stroke literature [16,17] showing that enriched environment and task specific forelimb training using a reaching trough similar to the staircase apparatus lead to improvement in both staircase and single pellet reaching performance. Thus our task practice paradigm involved two aspects of training to maximize their use of the affected forelimb. Rats that received Task Practice trained in an apparatus similar to the staircase reaching apparatus for 20 min/day, 5 days/week for a total of 13 hours and 20 minutes. In addition, rats in the Task Practice groups were provided with opportunities to increase general forelimb motor activity such as running, climbing, and foraging through living in an enriched environment (see Methods). We believed that this environment would allow rats to further integrate the use of the impaired forelimb in daily activity and thus promote greater recovery. In contrast to rats that had received a focal ischemic injury, [16,17] our spinal cord injured rats that experienced these interventions showed improvement in staircase reaching, but not in single pellet reaching, thus demonstrating task specificity of practice that did not generalize to other reach-to-grasp tasks. This reduction in generalization may be due to the loss of the fine motor control conferred by the rubrospinal tract [39,45,46] limiting the plasticity available following incomplete SCI compared to cortical injury. Additionally, dosing of rehabilitation interventions has not been commonly practiced in pre-clinical animal trials, specifically by counting the number of practice repetitions. Future study should include quantification of the number of reach-to-grasp attempts made during the rehabilitation training sessions to examine dose-response relationships. It is quite possible that driving reach-to-grasp attempts higher (5,000-10,000 attempts) may have resulted in better performance.

Effects of cellular transplantation after SCI on forelimb function

We chose NRP/GRP cells because of their potential to differentiate, survive long term, and integrate with host tissue [32,33,47] NRP/GRP cells transplanted into lesions of the lateral funiculus differentiated into neurons, oligodendrocytes, and astrocytes [33].

Furthermore, when NRP/GRP cells were suspended within a Vitrogen matrix containing BDNF (as we did in this study), they can produce process extension of approximately 2 mm in length in both the rostral and caudal white matter following a C4 dorsal column lesion [47]. Because NRPs stay localized in the lesion site and differentiate into mature neurons, these cells have the potential to become relays for reconstructing circuits, whereas GRPs may participate neuronal support and remyelination [33,47]. Our group has previously reported that when NRP/GRP transplants were made into a thoracic contusion injury, they promoted recovery of bladder, motor, and sensorimotor function.6 Our results of NRP/GRP transplantation into a cervical dorsolateral funiculotomy show significant improvements in distal muscle function as seen by increases in the Digits Open component score and Digit Spread kinematics at 8 weeks post transplant. Our behavioral results, which show improvements in elbow, wrist and digit movements, will help focus future anatomic studies on the control of the lower cervical motor pools that innervate the forelimb musculature [34].

The re-establishment of forelimb function after SCI has also been investigated using other cellular transplants. Transplantation of neurospheres derived from rat embryonic spinal cord showed improvement in skilled target reaching following C4/5 cervical contusion [48]. Recovery of successful reach-to-grasp function has been promoted by olfactory ensheathing cell (OEC) transplants after a cervical CST lesion [9] and a dorsal funiculus lesion [14]. A subsequent study using delayed transplantation of OECs to further approximate a clinical model resulted in similar behavioral recovery associated with CST sprouting [13]. Most studies report partial recovery of forelimb function and no particular cell type appears to be capable of promoting recovery of reaching function to baseline pre-injury levels.

Combination treatments must be selected thoughtfully as they may interfere. Recently, groups have begun testing the hypothesis that combination therapies that address different mechanisms of recovery could result in more complete recovery; however, thus far results have been modest for forelimb reaching studies [15,48,49]. Our current study revealed similar effects of task practice alone and in combination with NRP/GRP transplants on the staircase pellet retrieval test, which were better than NRP/GRP transplants alone.

It may be that the timing of when individual elements of a combination therapy are applied is critical for the outcome. We used acute cellular transplantation combined with task practice starting one week after surgery. It is certainly possible that if we had

used delayed cellular transplantation, or if we had applied the task practice after the transplants had time to differentiate and integrate into host tissue, our results may have shown an enhanced effect of our combination treatments. Deciding when to apply specific elements in combination with other interventions has not been systematically studied. Early task specific training promoted recovery of single pellet reaching, but impaired ladder walking [50]. By delaying the start of task-specific training, the injured animals showed better single pellet reaching without impairing horizontal ladder walking [51]. These results suggest that the timing of interventions matters. Similar results on reaching outcome measures have been recently reported in an animal model of stroke, where sequentially applying pharmacotherapy followed by training improved forelimb outcomes, whereas applying them at the same time had no effect [52]. In addition to timing of training, the length of task training has not been extensively evaluated. Our group has shown that three months of task specific training

resulted in recovery that was retained for a month following the cessation of training [24,53].

The intent of this study was to highlight the specific aspects of behavioral recovery through both quantitative and qualitative assessments of reach-to-grasp function. Thus, assessment of multiple levels of the CNS (cortical, brainstem, spinal) and multiple systems (corticospinal, rubrospinal, corticobulbar, reticulospinal, propriospinal, etc) is required to fully understand the nature of motor control recovery. Task specific training may promote cortical plasticity and shift cortical control to other intact descending systems [50,51]. NRP/GRP transplants could promote relays or local sprouting of intact descending or priopriospinal pathways to increase synaptic contacts on motor neurons [6,13,32,33]. We may need to investigate plasticity at both the level of the spinal cord and cortex, especially given the nature of incomplete injury and the particular combination intervention strategy. However, it was beyond the scope of this work to perform this extensive type of anatomical analysis.

Limitations

Although common in research using animal models, this study was limited by a small sample size, which was only adequately powered to detect large differences. Additionally, our lack of quantification of reaching attempts during the trough training to establish a "dose of rehabilitation", investigation of all potential permutations of timing of the interventions, and a detailed neuroanatomical analysis of the spinal cord, brain stem, and cortex for plasticity all limit our ability to interpret our data fully. Nevertheless, we feel that the results contribute to the growing body of knowledge regarding combined interventions following spinal cord injury.

Conclusions and Future Study

Our model of cervical dorsolateral funiculotomy demonstrates persistent deficits in reaching function and acute task practice improves recovery of forelimb function. NRP/GRP transplantation had a modest effect on distal forepaw muscle function, which did not carry over to a significant change in pellet retrieval. Transplantation combined with task practice did not result in superior recovery of reaching function. Future studies should include evaluation of the number of reach-to-grasp attempts made during training to assess dose-response relationships, delivery timing of combination therapies needs exploration, and analysis of neuroanatomical plasticity at spinal, brain stem, and cortical levels. Thus, combination therapies designed to promote recovery of forelimb function must be carefully selected and systematically evaluated in order to identify those that are most likely to succeed in clinical translation.

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