Research Report

Axonal plasticity is associated with motor recovery following amphetamine treatment combined with rehabilitation after brain injury in the adult rat

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ABSTRACT

Clinical and laboratory studies have suggested that amphetamine treatment when paired with rehabilitation results in improved recovery of function after stroke or traumatic brain injury. In the present study, we investigated whether new anatomical pathways developed in association with improved motor function after brain damage and amphetamine treatment linked with rehabilitation. Following a unilateral sensorimotor cortex lesion in the adult rat, amphetamine (2 mg/kg) was administered in conjunction with physiotherapy sessions on postoperative days two and five. Physiotherapy was continued twice daily for the first 3 weeks after injury, and then once daily until week six. Performance on skilled forelimb reaching and ladder rung walking was used to assess motor improvement. Our results show that animals with sensorimotor cortical lesions receiving amphetamine treatment linked with rehabilitation had significant improvement in both tasks. Neuroanatomical tracing of efferent pathways from the opposite, non-damaged cortex resulted in the novel finding that amphetamine treatment linked with rehabilitation significantly increased axonal growth in the deafferented basilar pontine nuclei. These results support the notion that pharmacological interventions paired with rehabilitation can enhance neuronal plasticity and thereby improve functional recovery after CNS injury.

1. Introduction

Damage to the adult sensorimotor cortex following stroke or trauma can result in permanent motor deficits. Past clinical studies suggest that a combination treatment of d-amphetamine and rehabilitation increases the rate and extent of motor recovery in patients following stroke and traumatic brain injury (Crisostomo et al., 1988; Walker-
Batson et al., 1995; Hornstein et al., 1996; Grade et al., 1998). Similarly, animal studies have shown improved motor function during beam walking tests when training was combined with amphetamine treatment after sensorimotor cortex lesion (Feeney and Sutton, 1988; Sutton et al., 1989; Goldstein and Davis, 1990). More recently, Adkins and Jones (2005) showed that amphetamine paired with daily rehabilitation sessions after cortical ischemic damage significantly enhanced performance in a skilled reaching task. Yet other investigators reported a lack of motor recovery after stroke with physical therapy and the combined use of d-amphetamine (Treig et al., 2003) or the racemic mixture d,l amphetamine (Sonde et al., 2001). The variations in testing procedures and amphetamine administration are likely to account for differences in behavioral outcome. Nonetheless, the mechanism by which amphetamine treatment combined with rehabilitation facilitates functional recovery following brain injury is not entirely clear.

Previous clinical studies have shown that spontaneous functional recovery after brain injury is often associated with neuronal plasticity and reorganization of the cortex around the lesion site and also in the undamaged, contralateral cortex (for review see Cauraugh and Summers, 2005). Thus, it has been suggested that treatments aimed at enhancing neuronal plasticity, such as amphetamine treatment, might improve functional outcome after brain damage. Transcranial magnetic stimulation studies have shown that amphetamine treatment in conjunction with motor training enhances the reorganization of the human primary motor cortex (Sawaki et al., 2002; Butefisch et al., 2002; Tegenthoff et al., 2004). Such short-term changes in cortical motor output might be related to the influence of amphetamine on synaptic efficacy (Gold et al., 1984), neurotransmission (Carr and Moore, 1969; Hoffman, 2001) or growth factor expression (Flores and Stewart, 2000). Following brain injury, amphetamine treatment in conjunction with motor training has been shown to induce long-term neuronal changes including an increased expression of GAP-43 and synaptophysin, proteins that are associated with axonal growth and synapse formation (Stroemer et al., 1998), a possible mechanism of remodeling in motor pathways as necessary for recovery.

Based on these previous studies, we hypothesized that amphetamine treatment linked to rehabilitation following sensorimotor cortex lesion in the adult rat would result in improved skilled motor function in parallel with remodeling of cortico-efferent motor pathways. Motor improvement after cortical lesion was evaluated using the skilled forelimb reaching test and the skilled ladder rung walking test. At the conclusion of behavioral studies, the anterograde neuroanatomical tracer, biotinylated dextran amine (BDA) was used to examine axonal growth in the cortico-pontine tract.

2. Results

2.1. Sensorimotor cortex aspiration lesions

The area of cortical damage was primarily localized to the forelimb sensorimotor cortex in all four groups (Fig. 1). A one-way ANOVA revealed no statistical difference in lesion size among the treatment groups (Lesion-only=7 mm$^2$±2.11;
Lesion+Rehab = 9.15 mm$^2 \pm 3.3$; Lesion+Amph = 6.6 mm$^2 \pm 1.15$; Lesion+Amph+Rehab = 6.4 mm$^2 \pm 1.4$; $F_{(3, 20)} = 0.47, p = 0.70$.

2.2. Functional improvement after amphetamine treatment with rehabilitation

2.2.1. Skilled forelimb reaching test

Animals were scored on the number of pellets obtained in the skilled forelimb reaching test to assess recovery of motor function after sensorimotor cortical lesion (Fig. 2A). The level of skill in all animals was comparable before lesion surgery with all groups displaying a mean pre-operative reaching score greater than 16 out of 20 pellets. A one-way ANOVA showed no significant difference between groups ($F_{(3, 37)} = 1.82, p = 0.16$). Similar to our previous findings after sensorimotor cortex aspiration lesion (Emerick and Kartje, 2004), animals attempted to perform the reaching task but had difficulty using the affected forelimb to reach for and/or grasp the pellet in the days immediately after surgery. A one-way ANOVA on all groups showed a similar level of deficit at postoperative day one with no significant difference between groups ($F_{(3, 37)} = 3.37, p = 0.05$). The one-way ANOVA performed at each postoperative week revealed a treatment effect beginning at week two ($F_{(3, 37)} = 7.25, p < 0.05$) and continuing throughout the course of the study. A post hoc analysis indicated there was no significant difference between the Lesion-only, Lesion+Rehab, or Lesion+Amph groups at any of the post-operative weeks ($p > 0.05$). However, the Lesion+Rehab+Amph group was significantly better than Lesion-only and Lesion+Rehab beginning at week 2 and continuing throughout the course of the study ($p < 0.05$). Animals in the Lesion+Rehab+Amph group scored slightly better than the Lesion+Amph group, yet there was no significant difference between the two groups during any of the weeks tested ($p > 0.05$).

The forelimb reaching data were further assessed for the effect of treatment within each group by comparing each week to postoperative day one motor performance. A repeated measures ANOVA showed a significant effect of the lesion alone on skilled forelimb reaching ($F_{(7, 29)} = 28.68, p < 0.001$). Further post hoc comparison indicated no significant improvement in the Lesion-only group throughout the duration of the study ($p > 0.05$). Similar analysis revealed no effect of rehabilitation on motor recovery after sensorimotor cortex aspiration lesion ($p > 0.05$). In contrast, the Lesion+Amph group began improving at postoperative week two ($p < 0.001$) and remained improved through post-operative week six. Overall, the Lesion+Amph+Rehab group demonstrated the earliest recovery in the skilled forelimb reaching test beginning at week one after lesion surgery ($p < 0.01$). This motor improvement continued throughout the duration of the study.

2.2.2. Skilled ladder rung walking test

Animals were scored on the skilled ladder rung walking test for an additional assessment of motor function after sensorimotor cortical lesion (Fig. 2B). All animals were able to cross the walkway with less than 1 foot slip per 10 steps preoperatively, with no difference between groups ($F_{(3, 41)} = $).

![Graph A](image1)

Fig. 2 – Functional improvement after amphetamine treatment when paired with rehabilitation. (A) In the skilled forelimb reaching test, all animals showed a deficit after lesion surgery. The Lesion-only and Lesion+Rehab groups showed little improvement over the course of the study. The Lesion+Amph group significantly improved over post-op day one by week two, and continued this improvement until week six ($p < 0.001$). The Lesion+Amph+Rehab group demonstrated earlier improvement beginning at week one compared to its postoperative baseline ($p < 0.01$) and was significantly better than Lesion-only and Lesion+Rehab groups by week two ($p < 0.05$). (B) In the skilled ladder rung walking test, animals from the Lesion-only, Lesion+Rehab and Lesion+Amph groups showed only slight improvement and no significant difference among groups at week six ($p > 0.05$, one-way ANOVA). Treatment with Amph+Rehab, however, resulted in significant recovery ($p < 0.001$, repeated measures ANOVA) with no significant difference at week six from preoperative measures. Post hoc comparison of all groups at each time point indicated by #: *$p < 0.05$ and **$p < 0.001$. Post hoc comparison of each group as compared to day one postoperative performance indicated by *: *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. Data are represented as mean±SEM.
At postoperative day one, all experimental groups demonstrated a large motor deficit in the lesion-affected forelimb (average nearly 4 foot slips per 10 steps). A one-way ANOVA at each postoperative week revealed a treatment effect beginning at week four \((F_{(3, 41)}=28.25, p<0.001)\) and continuing through week six. A post hoc comparison confirmed that the Lesion+Amph+Rehab group was significantly better than Lesion-only, Lesion+Rehab and Lesion+Amph groups \((p<0.001)\).

Recovery from postoperative day one deficits in ladder rung walking was assessed within each group. A repeated measures ANOVA showed a significant effect of the lesion alone on the number of foot slips on day one after sensorimotor cortex lesion \((F_{(7, 49)}=14.65, p<0.001)\). The post hoc analysis indicated no significant improvement in the Lesion-only or Lesion+Rehab groups throughout the duration of the study \((p>0.05)\). The Lesion+Amph group eventually showed statistically significant improvement, but not until week six after sensorimotor cortex lesion \((p<0.05)\). In marked contrast, analysis in the Lesion+Amph+Rehab group revealed a significant improvement by week one after lesion \((p<0.01)\) and a full recovery at week six with no significant difference from preoperative baseline measurements of foot slips \((p=0.186)\).

### 2.3. Amphetamine treatment with rehabilitation results in axonal growth

To assess whether treatment with amphetamine linked with rehabilitation resulted in neuroanatomical plasticity in these animals, we examined BDA-positive labeled projections from the contralateral, non-injured primary motor cortex to the basilar pontine nuclei. The dense black BDA-positive labeled corticopontine projections to the non-affected basilar pontine nuclei appeared normal in all experimental groups (Figs. 3A–D). Upon qualitative analysis, the Lesion+Amph+Rehab group had a noticeable increase in corticopontine fibers crossing the anatomical midline at the level of the pons (Figs. 3D, E). A one-way ANOVA confirmed there was a significant effect of treatment \((F_{(3, 13)}=11.68, p<0.001)\) (Fig. 4A). The post hoc comparison revealed the Lesion+Amph+Rehab group had a significantly greater number of labeled fibers crossing the midline compared to all other groups \((p<0.001)\).

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**Fig. 3** – Cortico-efferent projections to the deafferented basilar pontine nuclei are increased in animals with amphetamine treatment linked to rehabilitation. (A) BDA-positive labeled cortico-efferent fibers project to the basilar pontine nuclei, with very few fibers crossing midline (dotted line) after lesion alone. Treatment with either rehabilitation (B) or amphetamine (C) resulted in a slight increase in labeled fibers crossing the midline. In contrast, many more fibers crossing the midline (arrows) were observed in animals with amphetamine treatment linked to rehabilitation after sensorimotor cortex lesion (D, E—higher magnification). Scale bar=50 μm.
We further analyzed the density of cortico-efferent labeled fibers within the basilar pontine nuclei ipsilateral to the lesioned motor cortex (Fig. 4B). A one-way ANOVA on all groups revealed a significant treatment effect ($F_{(3, 15)}=14.54$, $p<0.001$). The post hoc comparison indicated no significant difference between Lesion-only and Lesion+Rehab groups ($p>0.05$). Treatment with amphetamine after lesion showed a significant increase in fiber density in the lesion-affected pontine nuclei as compared to the lesion-only group ($p<0.05$, one-way ANOVA). Combined treatment with amphetamine and rehabilitation showed the most significant increase as compared to Lesion-only ($p<0.001$), Lesion+Rehab ($p<0.001$) and Lesion+Amph ($p<0.01$).

3. Discussion

The results of this study demonstrate that treatment with amphetamine linked with rehabilitation following brain injury in the adult rat results in a significant improvement in the skilled forelimb reaching test and in the skilled ladder rung walking test. Additionally, amphetamine treatment linked with rehabilitation enhances cortico-efferent plasticity as evidenced by the novel finding of increased axonal growth in the deafferented basilar pontine nuclei, an important relay center for motor control.

Skilled reaching is a complex movement requiring the coordination of multiple subcomponents (Whishaw, 2005), and has been shown to be compromised after injury to the motor cortex (Castro, 1972; Whishaw et al., 1991; Papadopoulos et al., 2002; Emerick and Kartje, 2004; Seymour et al., 2005; Markus et al., 2005). In agreement with previous findings, our animals with sensorimotor cortex aspiration lesion only and no further treatment showed enduring motor deficits in the skilled forelimb reaching test. Failure to score a success in the task most often resulted from difficulties in reaching the forelimb through the window or using the digits to grasp the pellet. We found that amphetamine treatment alone after lesion did result in gradual improvement in the skilled reaching test. Yet the pairing of amphetamine treatment with rehabilitation after lesion resulted in earlier improvement in success for retrieving pellets in the reaching test. In support of our work, a recent study using an endothelin-1 model of ischemic damage showed that a three-week regimen of amphetamine treatment initiated at 2 weeks after injury enhanced the effect of rehabilitation on skilled reaching (Adkins and Jones, 2005). Importantly we found early improvement with the administration of only two doses of amphetamine after brain injury.

We further examined the accuracy of limb placement and coordination after sensorimotor cortex lesion using the skilled ladder rung walking test. Our animals showed a general trend toward improvement in the skilled ladder rung walking test. Without further treatment were never fully recovered. The animals treated with amphetamine alone began to show significant improvement, but it was delayed until postoperative week six. This delay in improvement in our lesion with amphetamine treated animals is different from previous studies where animals received task-specific training while...
under the influence of amphetamine. Such task-specific training was found to be essential for motor recovery (Feeney et al., 1982; Hovda and Feeney, 1984; Schmanke et al., 1996). In fact, Feeney et al. demonstrated that physical restraint to prevent locomotion blocks amphetamine mediated recovery on the beam-walking test. In our study, amphetamine treated animals were tested prior to treatment with drug so that the amphetamine alone group would not have been influenced by the testing experience. Animals from that group were not restricted from locomotion upon being returned to their cages, however, the delay in recovery suggests that random locomotion after amphetamine injections was not a contributing factor. Alternatively, animals receiving amphetamine treatment linked with rehabilitation were under the influence of amphetamine during physiotherapy sessions. During these sessions animals were not necessarily repeating the same motions of the testing procedures; instead animals actively explored apparatuses that would strengthen the use of the impaired forelimb. It was this physiotherapy experience in combination with amphetamine that resulted in the most immediate and significant functional recovery in our animals.

Interestingly, animals receiving rehabilitation alone showed no significant motor improvement over the time course studied. This finding was surprising given the vast literature supporting the role of physical activity and enriched environment in modulating functional recovery after experimental brain injury (reviewed in Komitova et al., 2006). The severity of damage in our lesion model might contribute to the sustained motor deficit observed in the animals receiving rehabilitation alone. In our previous work we have found that other therapeutic interventions are far less effective in animals with motor cortex aspiration lesions (Emerick and Kartje, 2004) as compared to middle cerebral artery occlusion (Papadopoulos et al., 2002). In fact, more recent data has shown significant improvement in rats after ischemic stroke using the rehabilitation methods described in this study (Catherine M. Papadopoulos, personal communication). Perhaps with more focused rehabilitation utilizing the impaired forelimb more intensively, we might have seen a better outcome in the effect of rehabilitation alone on motor recovery.

The mechanism by which amphetamine treatment enhances the effect of rehabilitation on motor recovery after brain injury is still unclear. Experimental stroke studies have shown that rehabilitative training alone enhances synaptogenesis (Jones et al., 1999) and the growth of dendritic arbors (Biernaskie and Corbett, 2001), specifically within the motor cortex. In the normal adult rat, amphetamine administration similarly was found to alter synaptic connections as demonstrated by an increased number of dendritic spines on neurons located within the prefrontal cortex, nucleus accumbens (Robinson and Kolb, 1999), caudate putamen (Li et al., 2003) and ventral tegmental area (Mueller et al., 2006). In our studies the effect of combining amphetamine treatment with rehabilitation after lesion does not appear to be synergistic, as we did not observe a significant difference in axonal growth between lesion only animals and those treated with rehabilitation. But clearly amphetamine treatment when paired with rehabilitation resulted in significant neuroanatomical changes and ultimately the best behavioral recovery after cortical injury.

Amphetamine may play a role in the transcription of various proteins required for neuronal growth through a direct increase in the phosphorylation and activation of cAMP response element binding protein (Konradi et al., 1994). More recent in vitro evidence has shown that amphetamine acts through the norepinephrine transporter to increase protein expression related to neurite outgrowth (Park et al., 2002). Additionally, Stroemer et al., 1998 suggested the increased expression of neurite growth proteins resulting from amphetamine treatment paired with motor training might subserve recovery of behavioral function. Likewise, in the present study it is quite possible that the pairing of amphetamine treatment with rehabilitation has the effect of enhancing the expression of growth proteins allowing for the remodeling of cortico-efferent pathways important for motor recovery. While we did not study the timing of changes in axonal growth related to behavioral improvement, others have described axonal growth in the basilar pons occurring by 2 weeks after cortical lesion (Wenk et al., 1999) and pyramidotomy (Z'Graggen et al., 1998) in the adult rat treated with antibodies that promote neurite outgrowth. In the present study, early axonal growth might account for the continued improvement observed in the weeks after amphetamine administration.

The recovery process following ischemic stroke has been suggested to be modulated by neuroremodeling in the undamaged cortex contralateral to the stroke hemisphere (Nelles et al., 1999; Foltyts et al., 2003; Cramer, 2004, Song et al., 2005). We have previously reported that treatments aimed at enhancing neuronal plasticity after experimental stroke or cortical aspiration lesion, such as anti-Nogo-A antibodies, will increase cortico-efferent projections from the non-damaged contralateral to deafferented motor areas, including the striatum, red nucleus, basilar pontine nuclei and spinal cord (Kartje et al., 1999; Wenk et al., 1999; Papadopoulos et al., 2002; Emerick and Kartje, 2004; Seymour et al., 2005). In those studies, new growth was observed in parallel with motor recovery, and further electrophysiological testing confirmed that the pathways were indeed functional (Emerick et al., 2003). Likewise, the current study shows that amphetamine treatment when paired with rehabilitation after sensorimotor cortex lesion resulted in robust axonal growth in the cortico-pontine pathway originating from the non-injured motor cortex to the deafferented basilar pontine nuclei. Based on those previous findings, we suggest that the non-damaged contralateral cortex contributes to the observed behavioral improvement in animals with paired amphetamine treatment and rehabilitation through the long-distance remodeling of the cortico-pontine pathway and reinnervation of the deafferented pons. While we did not examine other sensorimotor pathways, previous studies suggest that amphetamine may also act on subcortical motor regions to enhance motor recovery (Sutton et al., 1989).

Several clinical studies have investigated the therapeutic benefit of amphetamine treatment combined with rehabilitation after stroke. However, to date there is no consensus
whether such therapy will benefit the stroke or brain injured patient. Here we demonstrate that following a focal cortical lesion in rats, amphetamine treatment linked with rehabilitation results in significant recovery from motor deficits. To our knowledge this is the first evidence that such combined treatment results in the long-distance remodeling of cortico-efferent pathways to subcortical areas important for motor function. These findings support the notion that pharmacological interventions such as a short course of amphetamine treatment can enhance neuronal plasticity when appropriately linked with rehabilitation, thus resulting in improved motor recovery after CNS injury.

4. Experimental procedures

4.1. Animals

All procedures were approved by the Joint Institutional Animal Care and Use Committee of Loyola University and Hines Veterans Affairs Hospital. Forty-five adult male Long Evans black-hooded rats were randomly assigned to the following groups: Lesion-only (L-only; n = 8), Lesion+Amphetamine (L+Amph; n = 10), Lesion+Rehabilitation (L+Rehab; n = 6), Lesion+Amphetamine+Rehabilitation (L+Amph+Rehab; n = 21). All animals in this study met the following inclusion criteria: post-operative motor deficit as measured by a score of <6 pellets obtained during the reaching test (described below); and histological evidence of damage to the forelimb motor cortex. The animals were housed singly, unless otherwise noted, and maintained on a 12:12 h light/dark cycle. During the behavioral training period, animals were reduced to 95% of their weight by food restriction. Water was available ad libitum.

4.2. Behavioral testing

4.2.1. Skilled forelimb reaching test

Forelimb coordination and fine digit motor control was measured in the skilled forelimb reaching test as previously described (Z’Graggen et al., 1998; Thallmair et al., 1998; Papadopoulos et al., 2002; Emerick and Kartje, 2004; Seymour et al., 2005). All animals were trained and tested in a clear Plexiglas box (30×36×30 cm) with a rectangular window (1.5×3 cm) open at the base of the floor and a platform attached outside of the window for the placement of small pellets. A success was scored when the animal retracted the forelimb and carried the pellet to its mouth. Water was available ad libitum.

Fig. 5 – Behavioral tests. (A) Deficits in fine digit motor use were measured in the skilled forelimb reaching test. Animals were trained to use the forelimb to reach through a window and grasp a small sucrose pellet (seen on the shelf outside the window). A success was scored when the animal retracted the forelimb and carried the pellet to its mouth. (B) Deficits in forelimb placement and accuracy were measured using the skilled ladder rung walking test. The number of lesion-impaired forelimb footslips (arrow) was measured per ten steps across the runway.
sucrose pellets (45 mg; Bilaney Consultants, NJ) (see Fig. 5A). All animals were trained daily (M–F) for 2 weeks prior to measuring the pre-operative baseline score. During the testing session, animals were presented with a total of 20 sugar pellets in succession. A score of 1 was given for each pellet the animal obtained by using the preferred forelimb to reach through the window, grasp the pellet with its digits and carry the pellet to its mouth. The maximum possible score per testing session was 20 pellets obtained. Animals were tested in the forelimb reaching task beginning on the first day after surgery. All animals receiving amphetamine treatment were tested prior to amphetamine injections given on postoperative days two and five. Testing continued daily (M–F) for 6 weeks. All testing sessions were video recorded for analysis.

4.2.2. Skilled ladder rung walking test

The skilled ladder rung walking test was used to evaluate accurate limb placement as described in our earlier work (Thallmair et al., 1998; Emerick and Kartje, 2004). The testing apparatus consisted of a horizontal ladder runway (1 m in length and 10 cm in width) with wooden rungs distanced 1–2 cm (see Fig. 5B). No training was necessary for animals to perform the test, yet animals were acclimated to the apparatus in two separate 10-min sessions. All animals were tested on the day before surgery, on post-operative day one and weekly thereafter for the duration of the study. Animals were video-recorded as they walked across the runway three times per session. The lesion-affected forelimb was analyzed for the number of errors in paw placement per 10 steps. A placement error was defined as either a total miss or a slip from the rung as described by Metz and Whishaw, 2002.

4.3. Sensorimotor cortical aspiration lesion

Following the establishment of the pre-operative baseline behavioral measurements (for timeline of manipulations, see Fig. 6A), animals underwent a unilateral sensorimotor cortical aspiration lesion. The cortical lesion was made in the hemisphere with motor control over the preferred forelimb (as determined in the earlier behavioral training sessions). Animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and ketamine HCl (20 mg/kg, i.m.) and secured in a stereotaxic apparatus. The scalp was incised along the midline and the skull overlying either the left or right sensorimotor cortex was opened. The caudal forelimb motor cortex, as described by Neafsey et al. (1986), was removed using gentle suction. The scalp was sutured and animals placed under a heating lamp and returned to their cages when awake.

Fig. 6 – (A) Timeline for experimental procedures. (B–D) The apparatus used for daily regimented physical rehabilitation sessions consisted of the vertical rope (B), inclined ladder (C) and vertical cylindrical grid (D).
4.4. **Rehabilitation**

Rehabilitation was composed of environmental enrichment and physiotherapy as described below:

1) Environmental enrichment. Animals were housed in groups of three in large cages (82 cm × 61 cm × 45 cm) with an enriched environment. This included a variety of objects such as ropes, running wheels, swings and ladders placed in the cages and changed daily.

2) Physiotherapy. Physiotherapy sessions consisted of daily regimented physical activity designed to encourage the use of the impaired forelimb. During physiotherapy sessions animals freely explored three apparatuses: a 30°-inclined ladder (200 cm in length and 5 cm in width), a vertical rope (100 cm in height), and a vertical cylindrical grid (100 cm in height and 10 cm in diameter) (see Figs. 6B–D). Physiotherapy sessions lasted 20 min. and were conducted twice daily for the first 3 weeks after sensorimotor cortex lesion surgery, and then once daily for the remainder of the study.

4.5. **Amphetamine treatment**

Although the optimal dosage schedule for amphetamine treatment to enhance recovery is not entirely clear, we chose a 2 mg/kg dose of D-amphetamine sulfate based on its reported efficacy for promoting recovery from paresis when paired with motor training after sensorimotor cortex ablation in rats (Feeney et al., 1982; Feeney and Sutton, 1987). Accordingly, animals received one injection of either D-amphetamine sulfate (2 mg/kg, i.p., dissolved in 0.9% buffered saline) or saline only on days 2 and 5 following aspiration lesions. Immediately following amphetamine injection, animals receiving rehabilitation were placed into physiotherapy sessions, so that animals were drug intoxicated while exploring the various apparatuses and thereby using the impaired forelimb during physiotherapy. On postoperative days 2 and 5 (when amphetamine injections were given) animals underwent behavioral testing prior to receiving amphetamine treatment, and therefore were not receiving behavioral tests while intoxicated on drug.

4.6. **Biotinylated dextran amine (BDA) neuroanatomical tracing**

We used BDA to investigate long-term anatomical changes in the cortico-pontine pathway. Six weeks following sensorimotor cortex lesion, animals were re-anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and ketamine-HCl (100 mg/kg, i.m.) and secured in a stereotaxic frame. Using a Hamilton syringe, a 10% BDA solution in 0.01 M phosphate buffer was pressure injected (1 μL per injection at a rate of 0.2 μL per 1 min.) into four sites within the contralateral, non-lesioned motor cortex at a depth of 1.5 mm. Two weeks later, animals were sacrificed by sodium pentobarbital overdose (100 mg/kg, i.p.) and transcardially perfused with saline containing heparin and sodium nitrite, followed by 4% paraformaldehyde. Brains were removed, cryoprotected in 30% sucrose overnight, frozen in isopentane and stored at −80 °C, then processed in a “semifree-floating” technique as described by Herzog and Böösamle (1997). Tissue was cut on a cryostat and 50 μm coronal sections were serially collected on glass slides for processing with avidin-peroxidase complex (ABC kit elite, Vector Labs) to visualize BDA-positive fibers. In addition, every tenth section was serially collected on slides and stained with toluidene blue to examine the location and extent of the cortical lesion.

4.7. **Neuroanatomical analysis**

The brains used for neuroanatomical analysis were randomly selected from the animals in the behavioral study (L-only, n=4; L+Rehab, n=4; L+Amph, n=4; L+Amph+Rehab, n=5). The cortico-efferent projections to the basilar pontine nuclei were quantitatively analyzed ipsi- and contralaterally to the BDA injection site, as described in our earlier work (Wenk et al., 1999). Anatomical structures were identified using a rat brain atlas (Paxinos and Watson, 1998). All slides were coded so that investigators were blind to the experimental groups. Sections were captured with a digital camera (Hitachi model MOS VK-C150) attached to a Leitz DMR microscope and connected to a Macintosh computer (Centris 650) interfaced with a digitizing board. Sections were further analyzed by computer-aided image analysis using NIH Image version 1.51 (National Institutes of Health, Bethesda, MD).

4.7.1. **Standardization of cortico-efferent labeling**

In order to correct for BDA tracing variances among animals, the number of labeled cortico-efferent fibers in the cerebral peduncle ipsilateral to the injection site was first quantified. For each animal two consecutive sections at the midpontine level were analyzed. The number of BDA-positive fibers within a 3,015 μm² area of the cerebral peduncle was counted and extrapolated for each section. The average from the two sections was used to calculate the midline crossing fiber index described below.
4.7.2. Quantification of corticopontine plasticity

Anatomical plasticity in the corticopontine pathway was measured using two techniques. First, the midline crossing fiber index was calculated for each animal using the following equation:

\[
\frac{\text{number of BDA-positive fibers crossing the anatomical midline at the basilar pons}}{\text{number of corticofugal fibers in the cerebral peduncle}}
\]

Second, the density of BDA-labeled fibers within the lesion-affected pontine nuclei was analyzed. A 7500-μm² grid was placed over the nuclei ipsilateral and contralateral to the injection site. The optical density of the area was measured and normalized by subtracting the background density of surrounding tissue. To account for tracing variability among animals, the density measurements in the contralateral (lesion-affected) nuclei were divided by the ipsilateral nuclei on each section. An average of ten sections per animal was used to calculate the experimental group means.

4.8. Statistical analysis

All data were analyzed using SigmaStat Version 2.03 (SPSS Inc.). For the behavioral data a one-way analysis of variance (ANOVA) was used to compare the mean values across all groups in a single testing session. A repeated measures ANOVA was used for comparison of the mean values within the same group over time. Differences were further analyzed with the Bonferroni’s multiple comparison post-test. For the neuroanatomical data, a one-way ANOVA was used to compare all groups followed by Bonferroni’s multiple comparison post-test to analyze significance between groups. A p value less than or equal to 0.05 was considered significant. All data are presented as mean values±standard error of the mean (SEM).

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References


Hornstein, A., Lennihan, L., Seliger, G., Lichtman, S., Schroeder, K.,


