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Rehabilitative training following unilateral pyramidotomy in adult rats improves forelimb function in a non-task-specific way

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ABSTRACT

Spontaneous functional recovery following injury to the adult central nervous system can be enhanced with increased and focused activity, either through altered behaviour (skill learning, exercise or training) or by artificial stimulation (magnetic or electrical). In terms of training, the choice of paradigm plays a key role in the recovered behaviour. Here we show that task-specific training leads to improved forelimb function that can be translated to a novel forelimb task. Adult Long–Evans rats received a unilateral pyramidotomy and we studied the effects of different post-lesion training paradigms for their ability to recover function in the impaired limb. We trained rats on either the single pellet grasping or the horizontal ladder task. Rats were tested on both tasks regardless of the training paradigm and also on a related, but novel forelimb task, the Staircase. Horizontal ladder training led to full recovery of this task, and also limited recovery on the familiar but untrained single pellet grasping task. In comparison, single pellet grasping training led to a smaller improvement on the horizontal ladder, but interestingly the same degree of recovery on the single pellet grasping task as horizontal ladder trained animals. Both training groups performed equally well on a novel, untrained forelimb grasping task. These results show that task-specific forelimb training can lead to functional recovery also in non-trained, complex, forelimb movements. Anatomically, only single pellet grasping training was associated with enhanced sprouting of the intact corticospinal tract across the cervical spinal cord midline to innervate the denervated side of the spinal cord.

Introduction

Functional recovery following injury to the adult central nervous system (CNS) is limited because severed axons are unable to regenerate spontaneously, and compensatory fibre growth is often spatially restricted (Schwab, 2004; Fawcett, 2006; Cafferty et al., 2008). Interestingly, following unilateral corticospinal tract (CST) lesions, enhanced activity has a significant impact on compensatory sprouting of the remaining CST, such that either electrical stimulation of the intact tract or forced-use of the impaired forelimb post-injury promotes the growth of collaterals across the spinal cord midline into the denervated side of the spinal cord (Brus-Ramer et al., 2007; Maier et al., 2008). At the same time improved recovery of CST-mediated behaviours on the denervated side was observed (Maier et al., 2008; Carmel et al., 2010). Both experimentally and clinically, activity and rehabilitative training have been shown to enhance recovery after CNS trauma (Edgerton et al., 2006; Frigon and Rossignol, 2006; Dietz, 2008). Locomotor training has been shown to have positive effects on locomotor ability after injury in animals (Barbeau and Rossignol, 1987; Barriere et al., 2008; Ichiyama et al., 2008; Courtine et al., 2009) and humans (Wernig et al., 1995; Harkema et al., 1997; Wirz et al., 2005). Also, forced-use of the impaired upper limb is correlated with functional recovery in rodents (Jones and Schallert, 1994; Maier et al., 2008) and is being used as an effective therapy in humans, e.g., after stroke (Taub et al., 1993; Kunkel et al., 1999; Liepert et al., 2000; Johansen-Berg et al., 2002). Specific motor-skill training also provides substantial functional benefits after injury to the CNS (Nudo et al., 1996; Kleim et al., 2004; Maldonado et al., 2008). Rehabilitative training may lead to reorganisation of neuronal circuits that could contribute to recovery of sensory–motor functions after injury.

The type of rehabilitative training applied is an important factor to consider and depends on the behaviour or function that needs to be recovered (Edgerton et al., 1997; De Leon et al., 1998; Girgis et al., 2007; Krajacic et al., 2009). A number of studies show that when a behaviour is not trained, ability in the task can be lost (De Leon et al., 1998; Girgis et al., 2007; Garcia-Alias et al., 2009). The question of whether general activity is sufficient to promote functional recovery or whether one should train specific tasks remains to be answered as some studies have suggested that certain types of training can

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interfere with the performance of other tasks (Edgerton et al., 1997; Grasso et al., 2004; Girgis et al., 2007; Garcia-Alias et al., 2009). In this study we investigated whether task-specific training of adult rats following a unilateral CST lesion results in recovery of only the trained task (task-specific recovery) or whether the improved forelimb skill can be translated to untrained tasks. We observed that training-induced improvements in the performance of two different tasks were successfully transferred to a similar but novel task. We also found that grasping training was associated with significant plastic sprouting of the intact CST across the spinal cord midline into the denervated hemi-cord in a segmentally specific manner.

Materials and methods

Animals

Adult female Long–Evans rats (200–250 g, $n=24$) were obtained from a specific pathogen-free breeding colony (Charles River, Germany). Animals were kept in groups of five in standardised cages (type 4 Macrolon) on a standard regimen of 12-h light/dark cycle and received food and water *ad libitum*. All experiments were performed in accordance with the guidelines of the Veterinary Office of the Canton Zurich, Switzerland.

Experimental groups—pre-training

Animals were divided into 4 experimental groups: intact controls ($n=3$), lesioned untrained ($n=6$), lesioned horizontal ladder trained (Ladder, $n=7$) and lesioned single pellet grasping trained (Grasping, $n=8$). Animals in the Untrained, Ladder and Grasping groups were all pre-trained on the single pellet grasping task and the horizontal ladder. Training consisted of daily (5 times per week for 4 weeks) training on the single pellet grasping task (25 pellets per day) followed by the horizontal ladder (crossing of irregular spaced horizontal ladder 3 times per day) as described previously (Whishaw et al., 1993; Metz and Whishaw, 2002; Whishaw et al., 2008). All animals included in the study were handled, using the same protocol, prior to whichever intervention they received for 1 week prior to the start of training.

Lesion—unilateral pyramidotomy

Unilateral pyramidal tract lesions were performed as described previously (Thallmair et al., 1998; Maier et al., 2008). Briefly, animals were anesthetised with a subcutaneous injection of a mixture of Hypnorm/Dormicum (Hypnorm: 120 µl/200 g, Janssen Pharmaceutics, Beerse, Belgium; Dormicum: 0.75 mg/200 g, Roche Pharmaceuticals, Basel, Switzerland). Using a ventral approach, the skin was opened at the midline, the trachea and oesophagus were carefully displaced, the surface of the occipital bone was exposed and a small hole was drilled into the bone to reveal the medullary pyramids. The dura was opened and a fine tungsten needle was inserted into a depth of approximately 1 mm below the ventral brainstem surface just lateral to the basilar artery. The needle was laterally displaced and gently lifted, transecting the CST unilaterally just rostral to its decussation. Body temperature was regulated (37 °C) for the entire time the animals were anesthetised excluding the brief period for the surgery. After the lesion animals were sutured and warmed on a heating pad for 24 h before being returned to their home room.

Behavioural training and testing

Baseline scores were recorded 1 day prior to lesion for all animals on the horizontal ladder and single pellet reaching task. As the staircase task was used as a novel task at the end of the experiment there was no baseline recorded on this task. On the first day following the pyramidotomy, animals began rehabilitative training specified by their group for 6 weeks: the Untrained group received no training; the Ladder group was trained on the horizontal ladder 5 times per week with 10 crossings per day/training session; and the Grasping group animals were trained on the single pellet grasping task for 5 days per week for either 10 min or for 50 grasp attempts whichever was first. Our aim was that the Grasping group and the Ladder group had the same amount of training of their denervated forelimb. Animals from the Grasping group had to grasp for 50 pellets during each of their training sessions. Animals from the Ladder group each crossed the ladder 10 times per session. During a ladder crossing the animal grasps 14 rungs, thus 7 rungs per forepaw, thus 70 grasps per paw per session. However, animals mostly missed 1–2 rungs per crossing, bringing the number of grasps in line with the Grasping group. Animals in the Untrained, Ladder and Grasping groups were tested on the single pellet grasping task and the horizontal ladder twice, on day 1 and day 41 post-lesion. On days 42, 43 and 44 post-lesion the performance of the Untrained, Ladder and Grasp groups on the novel task, staircase grasping, was tested. As this was a novel task it allowed the assessment of whether the animals could adapt their training to a related task.

Single Pellet Grasping—training/testing

In the single pellet grasping test animals were placed in an open Plexiglas box (34×14 cm) with two openings on opposite sides (Whishaw and Pellis, 1990; Whishaw et al., 1993). During a training session rats had to grasp for 50 sugar pellets (45 mg dustless precision pellets, TSE Systems Intl. Group) presented at alternating sides of the grasping box. Animals were food deprived each evening for this task and their weight was checked and recorded daily. A maximum time of 10 min was given to grasp all pellets. Grasping performance was scored by the experimenter as follows: a trial, defined as a new pellet presented to the animal, was scored as 1 (successful grasp) if the animal retrieved the pellet with its impaired paw and brought it directly to its mouth. A score of 1 was given either if the animal succeeded on first attempt or if it used several attempts to grasp the pellet. An attempt was considered as the animal reaching with its paw through the slot. If the animal succeeded in grasping the pellet but dropped it inside the box before bringing it to its mouth, the trial was scored as 0.5. If the animal knocked the pellet off the shelf without retrieving it, the trial was scored as 0. The success rate in a testing session was calculated as the sum of the retrieved pellets divided by the number of trials.

In addition to this each testing session was filmed (Panasonic NV-GS500, 25 frames/sec) and single grasps were evaluated using frame-by-frame video analysis (Virtualdub; www.virtualdub.org). A detailed analysis of movement components was conducted at the end of the experiment by an experimenter blinded to the treatment group. Five successful and five unsuccessful grasps were analysed under the following criteria: body position, targeting, number of attempts, pellet position, supination, pellet sensing and pellet release (Table 1). Each sub-category contained "scores" although these were only used to categorise the movements components and were not added up to give a final grasping score at the end, for example, the "score" for a perfect grasp would be 1, 1, 1, 1, 1, 1, 1, 1, and not 8, whereas the "score" for a grasp where the targeting was too short and the pellet was not released from the paw but instead eaten from the floor would be 1, 1, 2, 1, 1, 1, 1, 2 and not 10. Grasps were "scored" from top to bottom of the table. Firstly, if the grasp ended in the pellet being eaten it was successful and thus a 1 and if not it was unsuccessful and thus 2. Next we scored the body position in relation to the pellet, either directly in front (1), next to (2) or opposite (3). Then we scored how the rat targeted the pellet with its paw, either directly on target (1), grasping too short (2), too long (3), too high (4), to either side (5), grasping the wall only (6), or too low so that the digit got caught on the shelf (7).

Table 1
Score sheet for frame-by-frame analysis of single pellet grasping. The scheme was adapted from Whishaw et al. (2008). Single grasps were separated into seven components which were scored using this scheme for frame-by-frame analysis.

Movement	Score	Description
Successful attempt	I	Yes
	2	No
Body position	1	Parallel
	2	Foot crosses line on side of pellet
	3	Foot crosses line on opposite to pellet
Targeting	1	On
	2	Short
	3	Long
	4	High
	5	Side
	6	Wall
	7	Low
1st attempt success	I	Yes
	2	No
Pellet position	I	Middle
	2	Digits
	3	No pellet
Supination	I	Paw turned 90 °
	2	Paw turned 45 °
	3	Paw not turned
	4	Wall/shelf used for support
	5	Floor used for support
	6	No pellet
Pellet sensing	1	Normal
	2	Abnormal I (no pellet, tries to eat)
	3	Abnormal II (got pellet, but does not eat it)
Pellet release	I	Normal (directly to mouth)
	2	Abnormal (dropped to the floor)
	3	No pellet

Next we scored whether the animal was successful at grasping the pellet on the first attempt (1) or whether more attempts were required or no pellet was grasped (2). We then scored where the pellet was positioned in the paw, either in the centre (1), between the digits (2) or in the case of a failed attempt (3). Next we scored how well the animal supinated. Perfect was when the paw was rotated 90 degrees after grasping (1), slightly less perfect but still successful was when the paw was rotated 45 degrees (2) whereas, if the paw was not turned the animal scored 3. Supination was assisted sometimes if the animal used the wall or shelf to help (4) or the floor (5). In the case where no pellet was grasped the animal scored 6. Some animals displayed sensory errors, if sensation was normal the animal scored 1, if the animal missed the pellet but tried to eat a non-existent pellet from its paw it scored 2 and if it grasped a pellet but then did not eat it, it scored 3. Finally we scored if the animal could release the pellet from its paw, normal scored 1, abnormal, i.e., the pellet was dropped, scored 2 and if no pellet had been grasped the animal scored 3.

Horizontal ladder—training/testing

Irregular horizontal ladder crossing was conducted as reported previously (Maier et al., 2008; Metz and Whishaw, 2009). During a training session rats had to cross the ladder 20 times in alternating directions and were motivated with treats (Choco Krispies, Kellog's Choco Krispies cereal, Bremen, Germany). Animals were food deprived each evening so that they were treated the same as the Grasping group and their weight was checked and recorded daily. Three runs per animal were recorded with a video camera (Panasonic NV-GS500, 25 frames/s) each day and analysed frame by frame with Virtual Dub. The performance was scored as reported (Maier et al., 2008). Briefly, a step was scored as *correct* if the animal placed the middle of the plantar surface of the paw on the rung and grasped it with all digits in front of the rung. Incorrect steps were categorised as *digit error* (if one or more digits were placed behind the rung), *wrist error* (if the animal placed its paw in front of the rung and weight support was provided by the wrist) and *slip error* (if the animal slipped off the rung on either side). The success rate on the test was calculated by dividing the amount of *correct* steps by the number of total steps taken with the respective paw × 100 in the 3 test runs. All post-lesion success rates were normalised to the pre-lesion level (baseline).

Staircase grasping—testing

The Montoya staircase test was performed as described previously (Montoya et al., 1991). For testing animals were placed in the staircase box for 10 min and were allowed to grasp for sugar pellets. The performance was scored such that pellets were counted as *remaining* if they were located on one of the steps either in the indentation or on the side of the indentation. Pellets were counted as *misplaced* if they were located on the ground of the staircase. The amount of *eaten pellets* was calculated as (*total pellets* − (*remaining pellets* + *misplaced pellets*) for each side/paw. The success rate in the test ((*eaten pellets*/*total pellets*) × 100) was normalised to the maximum possible score (30 pellets) in this test.

Anterograde tracing of the intact CST with biotinylated dextran amine (BDA)

At the completion of the behavioural training and testing the intact forelimb motor cortex of all animals was traced anterogradely with Biotinylated Dextran Amine (BDA). Stereotaxic injections of BDA (10,000 molecular weight, 10% solution in 0.01 M PBS, Invitrogen) were made through the dura using a 10 µl syringe (World Precision Instruments, Inc.) with a flow rate of 6 nl/s. A total volume of l.5 µl was injected at five injection sites (300 nl per site). The injection coordinates for the forelimb motor cortex were: 2 mm anterior to bregma (AP), 3 mm lateral to bregma (ML); 2.5 AP, 2.5 ML; 1.5 AP, 2.5 ML; 1.5 AP, 3.5 ML; 2.5 AP, 3.5 ML (Thallmair et al., 1998; Maier et al., 2008). All injections were at 1.5 mm depth and the syringe remained in place for 2 min after completion of each injection.

Tissue preparation

Three weeks after the BDA injections all animals were deeply anesthetised with pentobarbital (450 mg/kg body weight. i.p.; Abbott Laboratories, Cham, Switzerland), and perfused transcardially with 100 ml Ringer's solution (containing 100,000 IU/l heparin, Liquemin, Roche, Basel, Switzerland, and 0.25% $NaNO_2$) followed by 300 ml of 4% phosphate-buffered paraformaldehyde (pH 7.4). Spinal cords and brains were dissected and post-fixed in the same fixative over night at 4 °C before they were cryoprotected in phosphate buffered 30% sucrose for an additional 3–5 days.

Immunohistochemistry (PKCγ)

Completeness of the injury was confirmed in all animals using a histological approach. Transverse sections (40 µm) of spinal cord and brainstem were cut and immunostained with an antibody against PKCγ, a marker for CST axons (Mori et al., 1990; Bradbury et al., 2002; Starkey et al., 2005). Sections were incubated with rabbit anti-PKCγ (Santa Cruz Biotechnology, 1:200) overnight, followed by goat anti-rabbit Cy3 (Molecular Probes, Invitrogen, 1:300) for 2 h, then coverslipped in Mowiol mounting medium (Calbiochem, Darmstadt, Germany) and visualised under a Zeiss fluorescence microscope. The cross sectional area of the intact and lesioned CST tract in the cross sections of the brainstem were measured using ImageJ (Version 1.37c, National Institutes of Health, Bethesda, MD) to assess the lesion extent. Animals with incomplete lesions ($n = 3$) were excluded from the experiment.

DAB Staining (BDA)

The cervical spinal cord (C1–T1) and the brainstem were embedded in Tissue-Tec (O.C.T., Sakura Finetek, Zoeterwoude, NL) and frozen in isopentane (Sigma, Buchs, Switzerland) at −40 °C. Spinal cords and brainstems were cut in 40 μm thick cross-sections on a cryostat and mounted on objective slides (Superfrost, Menzel, Germany). Sections were processed using the nickel enhanced DAB protocol according to (Herzog and Brosamle, 1997).

Quantification of midline crossing CST fibres

CST fibre growth in response to injury was evaluated at spinal cord levels C1–T1 (every 3rd section), where motoneuron columns innervating shoulder and forelimb are located (McKenna et al., 2000). Fibres crossing the spinal cord midline were counted at a final magnification of 200x in the dorsal and ventral commissure, and branching of these fibres was evaluated at three defined distances from the midline in the grey matter (Maier et al., 2008). Intersections of CST fibres with vertical lines (M, D1, D2, D3, Fig. 3A–H) were counted (125 μm apart). To correct for variations in CST labelling, we normalised the data to the total number of BDA-labelled axons in the intact CST for each animal (CST axons: counted in three rectangular areas (200 μm^2) per slide on three sections at spinal cord levels C2, C5 and T1). Results are expressed as mean numbers of fibres crossing M, D1, D2 or D3 divided by the mean number of labelled fibres in the intact CST (per 200 μm^2) for each animal.

Statistical analysis

All data were analysed using parametric analysis of variance (ANOVA) of the appropriate design, followed by Bonferroni post-hoc pair-wise comparisons whenever a main effect or interaction attained statistical significance, or Mann–Whitney non-parametric test when appropriate. All statistical analyses were conducted using the software Graph Pad Prism. Data are presented as means ± SEM, single data points represent single animals, and asterisks indicate significances: $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

Results

Histological assessment of the lesion

With our lesion we aimed to fully transect the CST innervating the preferred paw at the level of the pyramids in the medulla oblongata, as described previously (Thallmair et al., 1998; Maier et al., 2008). The completeness of the pyramidotomy was determined using immunostaining for PKCγ in the brainstem and spinal cord (Fig. 1). In intact control rats positive PKCγ immunofluorescent staining of the CST was present bilaterally in the pyramidal tracts (Fig. 1D) and within the dorsal funiculi in the cervical spinal cord (Fig. 1E). However, in lesioned rats positive PKCγ immunofluorescent staining was only present unilaterally in the brainstem (Fig. 1F). Below the level of the CST decussation, in the cervical spinal cord, positive PKCγ immunofluorescent staining was present unilaterally in the dorsal funiculus (Fig. 1G). All brainstems were cut, stained and measured post-lesion to determine the extent of the damage. Three lesioned rats did not have a complete lesion meaning that the pyramidal tract was partially intact on the lesioned side (Fig. 1B). These rats were excluded from the study. All other rats were successfully lesioned with >90% of the lesioned pyramidal tract missing in the brainstem. Additionally, we found no evidence of damage to underlying medullary structures in the remaining animals which received complete lesions.

Effects of task-specific training on functional recovery after pyramidotomy

At day 1 post-lesion all groups showed significant deficits when crossing the horizontal ladder (Fig. 2A, $P<0.0001$, one-way ANOVA). Untrained animals showed no post-injury improvement on the horizontal ladder test (Fig. 2A). However, following 6 weeks of training, animals which had been trained on the horizontal ladder (Ladder group) showed recovery to baseline levels on this task (Fig. 2A, $P<0.0001$, one-way ANOVA). Whereas, animals which received single pellet reaching training (Grasping group) showed partial but significant recovery on the horizontal ladder (Fig. 2A, $P<0.05$, one-way ANOVA), despite being significantly worse than the Ladder training group (Fig. 2A, $P<0.05$, one-way ANOVA). Thus, single pellet reaching training was able to be translated to a significantly improved performance on the horizontal ladder, albeit to an inferior degree than that of the task-specific training group.

As has been reported in previous studies, unilateral pyramidotomy destroyed skilled grasping in the single pellet reaching task (Whishaw et al., 1993; Piecharka et al., 2005). Untrained animals showed no post-injury improvement (Fig. 2B). The type of training (either single pellet grasping or ladder training) did not affect the limited, non-significant (~25% success rate) improvement on the single pellet grasping task after 6 weeks (Fig. 2B). Again, this suggests that skills gained in one task (horizontal ladder) lead to the same degree of recovery in a related but untrained task (single pellet grasping) as the functional improvements seen when that task was trained. However, despite the similar overall success rates between the two training groups, when the targeting errors for the failed grasps were analysed in detail, the grasping trained group following training had become more similar to baseline, in terms of the lifting, aiming and advance of the paw, than the Ladder training group (Fig. 2D and F). Grasping trained rats had significantly fewer grasps that were too low than at day 2 post-lesion (black segment, $P<0.05$, one-way ANOVA), whereas, the Untrained group made significantly more too low grasps at 41 days post-lesion (black segment, $P<0.05$, one-way ANOVA). The targeting errors that resulted in a failed grasp for the Grasping group were most similar to baseline whereas the Ladder and Untrained groups were overall worse than at day 2 post-lesion (Fig. 2E, G and H). In this case we analysed failed grasps as both groups made very few successful grasps and those that they did make were, by virtue of being successful, very similar (upon detailed analysis) between the groups.

Post-training the Staircase was used as a novel, but related task that neither group had been trained on previously but all animals were familiar with. Both the Grasping and Ladder groups achieved significantly higher success rates on the Staircase test in comparison to the Untrained group (Fig. 2C, $P<0.05$, one-way ANOVA). Nevertheless, their performance was quite poor, confirming that this task is highly dependent on a functional CST (Stackhouse et al., 2008; Soleman et al., 2010).

Effects of task-specific training on anatomical reorganisation of the CST

Injection of the anterograde tracer BDA into the sensorimotor cortex was used to assess axonal sprouting of the intact (labelled) CST into the denervated regions of the cord (Schwab and Brosamle, 1997; Thallmair et al., 1998). The number of BDA labelled midline crossing fibres of the intact CST, as well as branching of these collaterals, was evaluated as described previously (Maier et al., 2008) in sections of the cervical spinal cord (C3/C4, Fig. 3A–D, C7/C8, Fig. 3E–H). In intact, untrained rats (Control) very few labelled axons were found to cross the midline (M, Fig. 3D, H and I) and their arborisation was minimal (D1–D3, Fig. 3D, H and I). Additionally, we observed no sprouting of the ipsilateral (spared) ventral fibres in our animals.

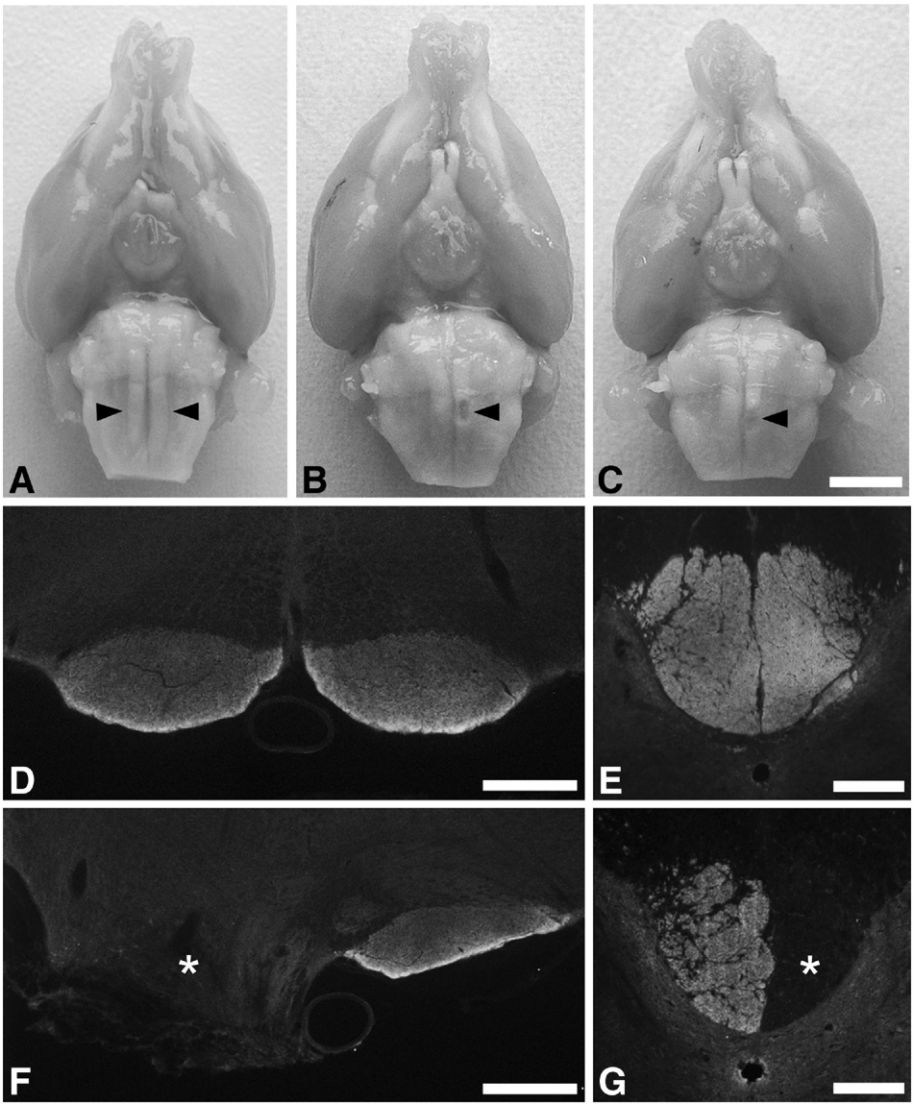

Fig. 1. Histological assessment of the unilateral pyramidotomy (CST) lesion. A, ventral view of the brainstem of an intact rat showing both portions of the CST running in the medullary pyramids (between arrows). B and C, ventral view of the brainstem of a rat following an incomplete (B, rat excluded from analysis) and complete (C) unilateral pyramidotomy lesion (arrows). D–G, PKCγ immunostaining of the intact CST in the pyramidal tract in the brainstem of an intact (D) and lesioned (F, asterisk) rat and in the main portion of the tract in the dorsal funiculus of the cervical spinal cord in an intact (E) and lesioned (G, asterisk) rat. Scale bars = A–C: 5 mm, D–G: 500 μm.

Following unilateral pyramidotomy and no training (Untrained) there was a slightly higher amount of axonal sprouting of the intact tract across the midline (M) into the denervated grey matter (Fig. 3C, G and I), but not significantly different to Control (intact, untrained) animals. Lesioned animals trained on the horizontal ladder (Ladder) also had increased numbers of midline crossing fibres (M) but they were not significantly different to either Control (intact, untrained) or Untrained (lesioned, untrained) animals (Fig. 3B, F and I). Rats in the Grasping group had significantly more midline crossing fibres in comparison to the Ladder, Untrained and Control groups (Fig. 3A, E and I, $P<0.001$, one-way ANOVA). Evaluation of the branching within the grey matter (D1, D2 and D3) revealed that the animals in the Grasping group had significantly more branching of midline crossing fibres than the Ladder and Untrained groups (Fig. 3A, E and I) at D1 ($P<0.001$, one-way ANOVA), D2 ($P<0.001$, one-way ANOVA) and D3 ($P<0.001$, one-way ANOVA). The presence of a lesion, but no training (Untrained group) produced the same amount of branching as a lesion followed by 6 weeks of training on the horizontal ladder (Ladder group, Fig. 3C, G and I).

We next looked if there was any regional specificity as to where the CST fibres crossed the midline in the cervical spinal cord (Fig. 3J). Control, Untrained and Ladder trained rats had similar low numbers of midline crossing fibres in C2-T1 (Fig. 3J, $P>0.05$, Mann–Whitney test). In striking contrast, the Grasping trained animals had significantly higher amounts of midline crossing fibres in the C3–C6 region (Fig. 3J, $P<0.05$, one-way ANOVA), but not in the more caudal cervical spinal cord (C7-T1, Fig. 3J). C2–C6 (Fig. 3J) is the region containing motoneurons that control movements of the shoulder, and the upper and lower forelimb (McKenna et al., 2000). The low number of midline crossing fibres in the caudal cervical area (C7–T1), which is a region of the spinal cord that contains mostly the digit motoneuron-pools (McKenna et al., 2000) may correlate with the overall poor recovery of digit function.

Discussion

Training of either pellet grasping or walking across a horizontal ladder following unilateral pyramidotomy led to improved forelimb function on the horizontal ladder and in pellet grasping. Both trained groups also performed better than untrained animals on a novel, untrained, forelimb specific task, regardless of training paradigm. A high number of midline crossing sprouts of the intact CST into the

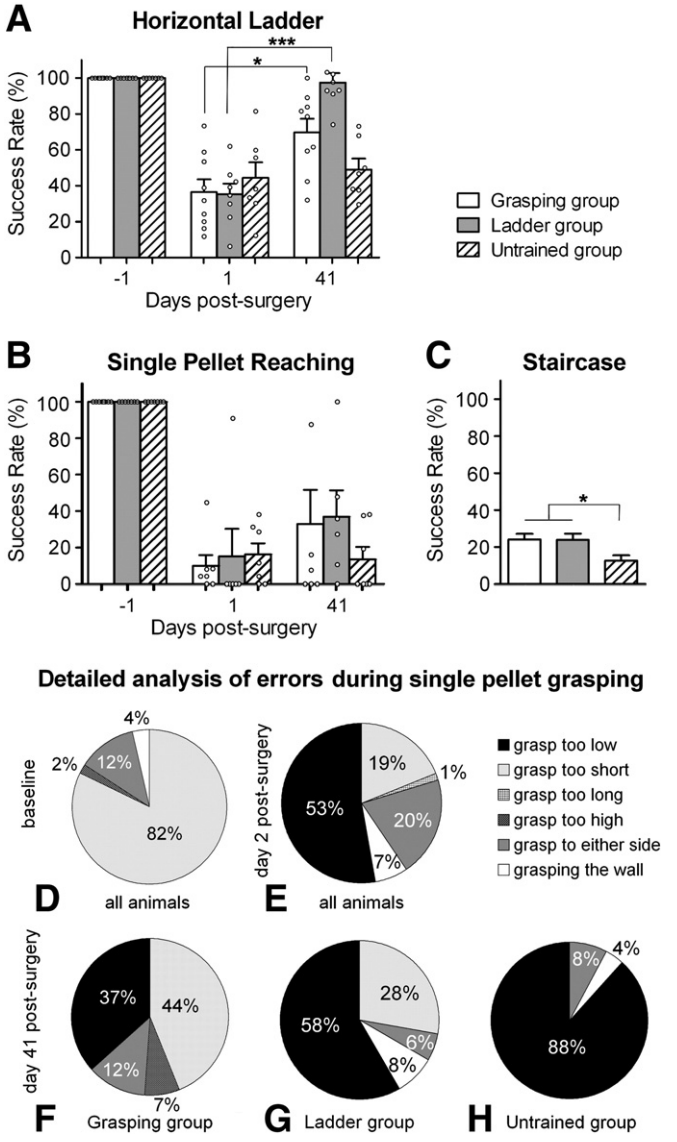

Fig. 2. Effects of task-specific training on functional recovery after pyramidotomy. A, Ladder training rats performed significantly better at crossing the horizontal ladder successfully than the other groups at day 41 post-lesion, with their success rate returning to baseline levels ($p<0.0001$, one-way ANOVA). Grasping trained animals also showed significant recovery on the horizontal ladder post-training ($p<0.05$, one-way ANOVA). B, both Ladder and Grasping trained rats performed equally well on the demanding single pellet grasping task, regardless of their training. Their success rate remained relatively low, however. C, both training groups were able to successfully complete a novel forelimb task with the same level of skill, but again, with a relatively low success rate. D–H, detailed frame-by-frame analysis of the targeting of failed grasping attempts. D, prior to lesion the most common targeting error was for the reach to be too short. E, early post-lesion the majority of failed grasps were due to the rats not lifting their paws high enough to grasp through the opening. F–H, training post-lesion strongly influenced the reasons for failing to grasp successfully: F, following 6 weeks of Grasping training the reasons for failure of a grasp were more similar to those at baseline than either the Ladder training (G) or the Untrained groups (**H**) where in both cases the most common reason for a failure (grasp too low) occurred more often than at day 2 post-lesion. Grasping trained rats (**F**) also had an increased number of grasps that were too short, the most common error at baseline. Data are presented as means ± SEM, asterisks indicate significances: $^{*}P\leq0.05$, $^{**}P\leq0.01$, $^{***}\leq0.001$.

denervated cervical spinal cord grey matter was associated with grasping training.

Task-specific training improves performance in a range of tasks

Some studies have shown that functional improvements after training of one task can come at the expense of ability in another task (De Leon et al., 1998; Girgis et al., 2007; Garcia-Alias et al., 2009; Krajacic et al., 2010), whereas others have been able to show a conversion of training in one paradigm to function in another (Lankhorst et al., 2001; Keiner et al., 2008; Krajacic et al., 2009; Singh et al., 2010). The present results show that training animals to cross a horizontal ladder post-lesion leads to excellent recovery on this task and a small but substantial recovery on a familiar but untrained grasping task. Grasping training post-lesion lead to good recovery on the horizontal ladder task but limited recovery on the grasping task, their success rate did not differ from animals trained to cross a horizontal ladder. Interestingly, the groups performed equally well on a novel, untrained grasping task and were both significantly better than control animals.

More work is certainly required to elucidate the exact mechanisms that lead to transfer of skills and thus the most appropriate training paradigm to be applied. The finding that following unilateral pyramidotomy animals trained on the single pellet reaching task showed similar but very limited recovery of both grasping and ladder tasks was unexpected. Whereas, the horizontal ladder trained animals showed full recovery on the horizontal ladder. This finding could be due to several factors: 1) the single pellet reaching task is a more difficult task than the horizontal ladder for the animals to perform. This is suggested by the extensive amount of pre-training required for animals to learn to grasp in comparison to the successful crossing of the horizontal ladder, and also the limited degree of functional recovery obtained; 2) motivation probably plays a crucial role, e.g., whereas it is easy to fail the single pellet reaching task (by knocking the pellet away), it is less easy to fail on the horizontal ladder because even if the paw is placed inaccurately or the animal slips then all other paws usually remain on the ladder so the animal can still cross and does not fail completely. For the grasping trained animals it might have been particularly frustrating when post-lesion they repeatedly miss the pellets and thus get no reward (rats were given either a maximum of 10 min or 50 pellets to grasp, whichever occurred first). Thus, the injured animals might have received more positive feedback from horizontal ladder training than they did from the pellet grasping training; 3) skilled hand and digit use may be more important for grasping than for ladder crossing (Metz and Whishaw, 2002). The very limited corticospinal connections remaining or reforming after the unilateral CST transection may only allow the observed low degree of recovery of grasping. The successful transfer of training induced functional improvements between ladder and pellet grasping may be due to the similarities of the movements and in the underlying motor programs. It is conceivable that training of very different movement patterns, e.g., treadmill running or swimming, would yield different results as has been observed previously (Edgerton et al., 1997; Grasso et al., 2004; Girgis et al., 2007; Garcia-Alias et al., 2009).

Grasping training induces sprouting of CST fibres across the spinal cord midline

The increased amount of CST fibres in the denervated spinal cord in the grasping trained animals following a unilateral pyramidotomy probably reflects growth of new midline crossing fibres, as well as sprouting of the few pre-existing ipsilateral and ventral fibres. Sprouting of ipsilateral ventral projections occurs spontaneously in response to lesions (Weidner et al., 2001; Bareyre et al., 2004; Fouad and Tse, 2008), and has been shown in previous studies to contribute to functional recovery (Weidner et al., 2001; Brus-Ramer et al., 2007). Reinnervation is enhanced by interventions that increase CNS fibre growth and plasticity such as suppression of myelin-associated growth inhibitors (Thallmair et al., 1998; Fournier et al., 2001), neurotrophins (Zhou and Shine, 2003), removal of chondroitin sulphate proteoglycans (Bradbury et al., 2002; Barritt et al., 2006) or enhanced activity (Brus-Ramer et al., 2007; Maier et al., 2008; Carmel

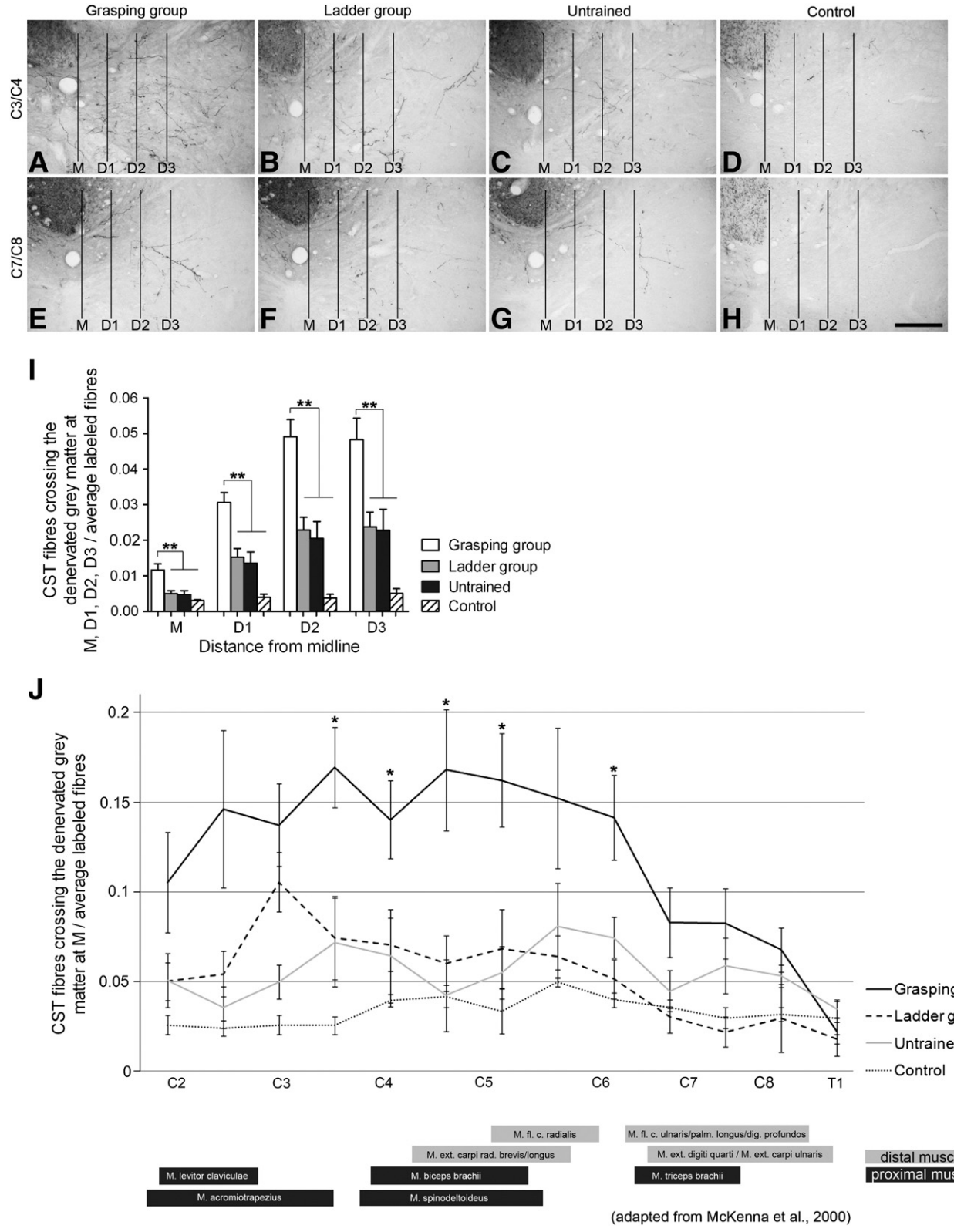

Fig. 3. Effects of task-specific training on anatomical reorganisation of the CST. Training for 6 weeks post-lesion differently influenced the sprouting of CST fibres from the intact side of the spinal cord across the spinal cord midline. A–H, photomicrographs of BDA-labelled intact CST fibres in the rostral (C3/C4, A–D) and caudal (C7/C8, E–H) cervical spinal cord. Fibres that crossed the midline (M) and then branched in the grey matter (D1, D2 and D3) were counted. I, Grasping trained animals had more fibres crossing the midline (M) than either of the two other lesioned groups (Ladder trained and Untrained) or unlesioned, untrained Control animals. Grasping trained animals also had a greater degree of branching of these fibres (D1, D2 and D3) than either of the lesioned (Ladder trained or Untrained) or unlesioned (Control) groups. Unlesioned, untrained Control animals had minimal numbers of fibres crossing the midline (M) and subsequently branching (D1, D2 and D3). J, segment-specific analysis of midline crossing fibres showed that the Grasping trained group had significantly more midline crossing fibres in the more rostral cervical spinal cord where motoneuron pools controlling proximal forelimb muscles are located, whereas they had fewer in the more caudal cervical spinal cord where motoneuron pools for the distal muscles are located. Ladder trained animals had a similar distribution but significantly less fibres. Data are presented as means ± SEM, asterisks indicate significances: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$. Scale bars = 200 μm, groups: Control ($n = 3$), Untrained ($n = 6$), Ladder group ($n = 7$), Grasping group ($n = 8$).

et al., 2010). Here, only the very demanding and complex single pellet reaching task, which is highly CST dependent (Whishaw et al., 1993; Whishaw et al., 1998; Stackhouse et al., 2008), appears to have generated a strong enough stimulus to induce significant sprouting of spared CST axons. We carried out correlation analyses between the number of midline crossing fibres per animal and their behavioural success score on all of the behaviour tasks at day 41 post-lesion. There were no significant correlations seen however it is common that the exact number of fibres does not correlate directly with the degree of recovery.

The distribution of midline crossing fibres correlated with the post-lesion upper limb movements that were observed (following detailed frame-by-frame analysis of either failed grasps and crossing of the horizontal ladder) in our study. Although not significant overall the Ladder trained animals had more midline crossing fibres in C2–C5 than in the more caudal regions of the cervical spinal cord. This is an area of proximal forelimb control (McKenna et al., 2000) and interestingly these animals showed good recovery on the horizontal ladder test. Grasping trained animals had 2–3 times higher number of fibres in C3–C6, which correlated with their recovery of the ability to lift the forelimb. All groups showed a reduced amount of fibres in C7–T1 where motoneurons required for fine digit control are located (McKenna et al., 2000; Kuchler et al., 2002), and none of the groups recovered fine digit control. Previous studies have shown that activity in selected muscles of the forelimb (Martin et al., 2004) or the remaining intact CST (Brus-Ramer et al., 2007; Maier et al., 2008; Carmel et al., 2010) is important for the topographic distribution of axon terminals in the spinal cord. These studies suggest the importance of activity, such as rehabilitative training, for recovery.

Our results suggest that post-lesion recovery of forelimb function is dependent on active, high-impact training of the forelimb. However, we also show that depending on the exact behaviour to be recovered the type of training does not play a huge role such that training on one task can lead to recovery of another, related task. In terms of anatomical changes, we show that Grasping training causes significantly more sprouting than walking across a horizontal ladder. In terms of the design of future rehabilitation studies these data have important implications. They suggest that firstly, the task that is trained requires active involvement. Secondly, they suggest that the training applied needs to be thought out carefully as some behaviours are more difficult to recover even with a high volume of training than others. Thirdly, they suggest that it is possible to transfer the skills learnt in one task to another, novel task. Finally, they suggest that some training paradigms lead to anatomical reorganisations of remaining projections to the spinal cord which could be enhanced to improve recovery profiles.

In conclusion, we show that following unilateral pyramidotomy different forelimb training paradigms influence functional recovery and show transfer between tasks. However, whether a similar transfer or interference exists between more distinct training paradigms remains to be studied. Training of the most demanding task, single pellet grasping, was associated with sprouting of the intact CST fibres across the midline to innervate the denervated side of the spinal cord. The detailed functional role of these fibres remains to be analysed. Our results have important implications for the design of rehabilitative therapies following CNS injury.

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