Published in final edited form as:

Exp Brain Res. 2006 February ; 169(1): 106–116. doi:10.1007/s00221-005-0129-4.

Behavioral and neurophysiological effects of delayed training following a small ischemic infarct in primary motor cortex of squirrel monkeys

Scott Barbay^{1,2}, Erik J. Plautz¹, Kathleen M. Friel¹, Shawn B. Frost¹, Numa Dancause¹, Ann M. Stowe¹, and Randolph J. Nudo¹

¹ Landon Center on Aging and Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS, USA

² Mental Retardation Research Center, University of Kansas, Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160, USA, E-mail: sbarbay@kumc.edu, Tel.: +1-913-5887394

Abstract

A focal injury within the cerebral cortex results in functional reorganization within the spared cortex through time-dependent metabolic and physiological reactions. Physiological changes are also associated with specific post-injury behavioral experiences. Knowing how these factors interact can be beneficial in planning rehabilitative intervention after a stroke. The purpose of this study was to assess the functional impact of delaying the rehabilitative behavioral experience upon movement representations within the primary motor cortex (M1) in an established nonhuman primate, ischemic infarct model. Five adult squirrel monkeys were trained on a motor-skill task prior to and 1 month after an experimental ischemic infarct was induced in M1. Movement representations of the hand were derived within M1 using standard electrophysiological procedures prior to the infarct and again one and two months after the infarct. The results of this study show that even though recovery of motor skills was similar to that of a previous study in squirrel monkeys after early training, unlike early training, delayed training did not result in maintenance of the spared hand representation within the M1 peri-infarct hand area. Instead, delaying training resulted in a large decrease in spared hand representation during the spontaneous recovery period that persisted following the delayed training. In addition, delayed training resulted in an increase of simultaneously evoked movements that are typically independent. These results indicate that post-injury behavioral experience, such as motor skill training, may modulate peri-infarct cortical plasticity in different ways in the acute versus chronic stages following stroke.

Keywords

Squirrel monkeys; Stroke rehabilitation; Recovery of function; Motor learning; ICMS

Introduction

Experience-dependent, cortical plasticity has been well documented in both normal and injured brains (Kleim et al. 2003; Nudo et al. 2003). Efforts are being made to develop more efficient rehabilitative strategies that utilize current knowledge of cortical plasticity, bridging basic research with clinical application. The basic research in this area is broad, ranging from fundamental neurochemical or neurophysiological processes associated with experimental

brain injury to developing more specific models of brain injury and recovery that attempt to model clinical syndromes (Nudo et al. 2001). The present study addresses one aspect of cortical plasticity related to ischemic cortical damage. It is not intended as a model for stroke per se, but as an experimental model of focal ischemia. Basically, this study addresses reorganizational plasticity within the areas of M1 adjacent to an experimental, ischemic infarct and the possible temporal constraints on reorganizational capacity that occur in the absence of directed behavioral experiences such as post-infarct rehabilitative training.

An interest in temporal constraint on experience-dependent plasticity originates from clinical observations that stroke related motor impairments spontaneously recover to some extent without rehabilitative intervention, especially during the first several weeks after a stroke. In the absence of further therapeutic intervention, recovery usually plateaus within 3 to 6 months (Duncan et al. 2000). It has been difficult to distinguish the beneficial aspects of rehabilitative therapy initiated during this recovery period from the improvements gained spontaneously over time. However, using a nonhuman primate model of ischemic injury to the cerebral cortex, Nudo and associates (1996b) demonstrated that there are favorable changes in neocortical organization associated with rehabilitative training and recovery of skilled use of the hand. In that study, rehabilitative training was initiated at the end of the first week following an experimentally induced ischemic infarct within the primary motor cortex (M1) of the squirrel monkeys. Standard intracortical microstimulation techniques (ICMS) were used to determine (i.e., “map”) the relative location of movement representations in the cortex before creating a small infarct within the hand representation of M1 (approximately one-third of the hand representation). After approximately one month of rehabilitative motor skill training or spontaneous recovery, additional maps were derived. The results demonstrated that the spared hand representation within M1, adjacent to the damaged tissue, was diminished further following a spontaneous recovery. This decrease in the hand area within the peri-infarct region was not an enlargement of the ischemic lesion. Rather, adjoining proximal (elbow/shoulder) representations appeared to invade the original hand representation. In contrast, when motor skill training was initiated within 1 week after the infarct, the peri-infarct hand area was maintained, and in some cases extended further into proximal representations of M1.

This experience-dependent reorganization of movement representations may have been optimal due to the early initiation of intensive training (i.e., within 1 week after the infarct). It is as yet unknown whether there is an optimal time for implementing rehabilitative training after cortical injury. Although intense forced use of the forelimb contralateral to sensorimotor cortical damage has been shown to be deleterious to recovery in rodents (Kozlowski et al. 1996; DeBow et al. 2004), the vast majority of animal models of stroke and recovery have introduced therapeutic interventions within the first few days after an injury (Sutton et al. 1989; Goldstein and Davis 1990; Nudo et al. 1996b; Stroemer et al. 1998; Jones et al. 1999; Kleim et al. 2003). While acute post-injury physiotherapy has been shown to be effective in animal models in enhancing recovery and neuroplasticity, it has been difficult to distinguish the beneficial aspects of acute rehabilitative therapy from subacute and chronic therapy. A recent study using a rodent model of stroke found results consistent with a use-dependent hypothesis of neuronal sparing that demonstrates the benefits of early intervention (Biernaskie et al. 2004). In the Biernaskie study, the authors examined the differences in dendritic morphology in the cerebral cortex after early or late motor training following a middle cerebral artery occlusion. The authors found that when training began 5 or 14 days after the occlusion, improvement in reaching skills and locomotion were positively associated with an increase in dendritic growth within the intact, contralateral cortex. When training was delayed for 30 days neither improved motor function nor dendritic growth was observed. This study showed a clear relationship between the temporal constraints on cortical plasticity and recovery of function.

Few human studies have employed intensive skill training during the early stages after a stroke. Recently, Dromerick found that constraint-induced movement therapy (i.e., restricting the less affected, ipsilesional hand) could be applied beginning within 2 weeks after clinical stroke, and that improvements were seen in the Action Arm Reach Test (Dromerick 2003). Although constraint-induced movement therapy has also been successful in cases of chronic hemiparesis (Taub and Wolf 1997), it is possible that an early intervention may be more effective when employed before compensatory strategies are firmly established.

The purpose of this study was to determine the neurophysiological consequences of delaying rehabilitative training for 1 month in our nonhuman primate ischemic infarct model. Previous studies have shown that the small M1 infarcts used in the Nudo et al. (1996b) study produce transient behavioral deficits that may recover spontaneously over 2 months so that delayed training has no discernable benefit upon motor performance. Nevertheless, subtotal infarcts of the M1 hand area, leaving a significant portion intact allowed post-infarct physiological changes in the adjacent peri-infarct cortical tissue to be examined at various times after the lesion. Thus, the primary goal of the present study was to determine physiological changes within the spared M1 after delaying the initiation of post-infarct training for 1 month.

Methods

Subjects

Five male, adult squirrel monkeys (*Saimiri boliviensis*) ranging in weight from 655 to 1,035 g were used in this study. Monkeys were trained on a motor skill acquisition task requiring the skilled use of digits and wrist. Within a few days after learning the task, an experimental ischemic infarct was made in the hand representation of M1. The infarct was intended to destroy approximately one-third of the hand area, directed at caudal M1. Similar infarcts have been shown to disrupt performance on the type of motor skill task used in this study as well as altering M1 hand representations in adjacent cortex (Nudo and Milliken 1996). All monkeys in this study were individually housed in a climate-controlled vivarium. All procedures were in accordance with the NIH and the University of Kansas Medical Center institutional guidelines for the care and use of laboratory animals.

Experimental design

One month after an ischemic infarct in the M1 hand area, each of the five monkeys received rehabilitative training intended to facilitate reacquisition of skilled use of their affected hand contralateral to the infarct. Rehabilitative training was similar to the pre-infarct skill acquisition training experienced by the monkeys. Neurophysiological maps of the hand representation area in M1 were derived at three different time points in each monkey: (a) 2 to 3 days after skill acquisition training ended (pre-infarct map), (b) 1 month post-infarct (i.e., after 1 month of spontaneous recovery), and (c) 2 months post-infarct (i.e., after 1 month of rehabilitative training; see Fig. 1). Thus, monkeys in the present study experienced identical experimental procedures as those in a previous study (including a focal infarct of the same size and location; Nudo et al. 1996b) except that rehabilitative training began 1 month after the cortical infarct instead of 1 week.

Motor skill task

Training apparatus—Monkeys were trained to retrieve small, banana flavored food pellets (45 mg), delivered one at a time, from wells drilled into a Plexiglas board attached to each monkey's home cage (Klüver board). The board was attached to the cage so that the monkey had unrestricted access to each food well. Five wells were distributed across the Klüver board. All wells were 5 mm deep, but differed in diameter (well 1 = 25 mm, well 2 = 19.5 mm, well 3 = 13.5 mm, well 4 = 11.5 mm, and well 5 = 9.5 mm) and thus, varied in difficulty for the

monkey to retrieve a food pellet. To remove food pellets from the smaller wells (wells 3 to 5), the monkeys had to learn to use their digits differentially whereas on the larger wells (wells 1 and 2), synergistic movements of the digits and a prehensile grip were adequate to remove the pellets.

Hand preference—Prior to the pre-infarct training, each monkey was tested for hand preference on the Klüver board task over two consecutive days. In this procedure food pellets were randomly placed in each well for a total of 25 trials (5 trials per well) with the board in one position (e.g., well 1 to the right of the monkey) and 25 trials with the board reversed to counterbalance any possible position bias (well 1 repositioned to the left of the monkey). Two sessions were conducted per day, one in the morning and one in the afternoon (a total of 100 trials per day).

Constraint jacket—Once hand preference was determined, the monkey's non-preferred hand was restricted with a soft, cotton mesh sleeve sewn at the end to form a mitten and attached onto a lightweight nylon mesh jacket (Alice King Chatham Medical Arts mfr). The sleeved-jacket was designed to assist in rehabilitative training by restricting the hand ipsilateral to the lesion (i.e., the non-affected or less affected hand). The sleeved-jacket was worn continuously during the baseline and post-infarct training. The sleeve prevented the monkey from using the sheathed hand while performing the Klüver board task yet permitted the use of this hand during normal cage behaviors (e.g., climbing or grasping large food biscuits). The jacket was used during baseline (or pre-infarct) training to control for possible behavioral and physiological changes that may be caused by the introduction of the jacket.

Klüver board training—Motor skill training on the Klüver board was implemented prior to the infarct (baseline training) and 1 month after the infarct (rehabilitative training). During motor skill training periods, two 30-min sessions were conducted per day (morning and afternoon) in which the monkeys were trained to become increasingly efficient in retrieving pellets from a designated well. Efficiency was defined by the number of pellets a monkey was able to retrieve in 1 day. The criterion for monkeys to complete training on a particular well was 600 pellets/day. Monkeys were introduced to the task with the largest well and once the monkeys met the criterion number of pellets on one well they were trained on the next successively smaller well the following day. This procedure was carried out until the monkeys could meet the criterion on the smallest well (well 5) on two consecutive days. One monkey did not reach the criterion on well 5 (smallest well) during baseline training even though he could complete the task accurately (based on a low ratio of flexions/retrieval; see below). In this case, baseline training was considered complete after the criterion was met on well 4.

In addition to the training trials described above, probe trials were conducted at the beginning of each session. During this phase of the session, a pellet was placed randomly into one of the five wells (25 trials per session; ten trials on each well per day). Probe trials were used to assess the skill level of each monkey prior to the daily training session. Probe trials were also employed once per week during the spontaneous recovery period in which the monkeys received no specific training. All trials were video taped with a Hi 8 video recorder (30 frames/s) for later performance analysis. The data from the probe trials were used for statistical analysis of daily performance. These data consisted of the number of finger flexions necessary for each monkey to retrieve food pellets from the smallest well on the Klüver board (i.e., flexions/retrieval).

Surgical techniques

Each monkey was initially anesthetized with ketamine (20 mg/kg i.m.), shaved (head and arm opposite the hemisphere to be mapped) and then intubated with a tracheal tube, outside diameter (O.D.) = 2.5 to 3.0 mm. The monkey was then placed on a gas mixture of 75% nitrous oxide

and oxygen with 1.5 to 2 % halothane. Intravenous fluids (3% dextrose in Ringers solution) were then administered and maintained throughout the procedure. Mannitol (8 ml) was administered through the i.v. line to control for possible edema. The monkey's head was stabilized in a stereotaxic frame, and under aseptic conditions, the scalp and the temporal muscle were reflected, exposing the skull. A craniotomy was then performed and a portion of the dura removed to expose M1 and the surrounding cortex. The segment of skull that was removed (skull cap) was maintained in sterile saline at 4°C during the ICMS procedure (see below). A sterile plastic chamber was secured over the opening and filled with sterile silicone oil to protect the cortex. Upon completion of the surgical opening, gas anesthesia was withdrawn and small doses of ketamine (~5 mg/15 min as needed) were delivered through i.v. to maintain a stable anesthetized state. Ketamine was occasionally supplemented with Valium 0.01 mg as needed to control excessive muscle tone. Vital signs (respiratory rate, expired CO₂ levels, oxygen saturation in blood, and heart rate) were monitored and recorded every 15 min. At the conclusion of the ICMS procedure, the monkey was again placed on the nitrous oxide and halothane gas mixture. Gelfilm was placed over the cortex as a dura replacement and to minimize the re-growth of tissue over the surface of the cortex and blood vessel anastomoses. The skull cap was then replaced and secured to the skull with dental acrylic. The scalp was then sutured and the incision anesthetized with lidocaine. A topical antibacterial spray (Furazolidone) was also applied to the scalp, followed by a subcutaneous injection of 0.1 ml penicillin (30,000 units). Dexamethasone (0.5 mg i.v.) was also given before and after surgery.

Neurophysiological mapping techniques

For each monkey, intracortical microstimulation (ICMS) techniques were used to define the hand representation in M1 contralateral to the preferred hand. Movements were evoked by delivering a small electrical current into the brain through a stimulating microelectrode. The microelectrode consisted of a platinum wire insulated within a glass micropipette (1.5 mm O.D.) filled with a 3.5 M NaCl solution. The pipette was tapered so that the electrode measured about 10–25 µm O.D. at the tip and about 100 µm O.D. 2 mm from the tip. The tip was then beveled to an angle of 25°. The ICMS stimulus was delivered as a train burst of 13, 0.2 ms cathodal monophasic pulses, delivered at 350 Hz. This pulse train was triggered through a stimulus pulse generator (Master-8; A.M.P.I., Israel) and delivered by a stimulus isolator (Model BSI-2, BAK Electronics Inc.) at a rate of one train per second. The microelectrode was held perpendicular to the cortex, suspended from a stereotaxic micropositioner (Narishige SM-11) and then lowered to ~1,750 µm below the surface with a hydraulic microdrive (Kopf Model 650) targeting layer V. Maximum current did not exceed 30 µA in order to limit the spread of activation to a radius of ~170 µm (Stoney et al. 1968; Nudo et al. 1990 see for further discussion). Stimulation was applied in increments of 1 µA. The M1 hand area was delineated by defining the borders between sites at which stimulation evoked digit or wrist–forearm movements and sites at which stimulation evoked more proximal (elbow, shoulder, and face) movements or by sites at which stimulation evoked no movement at the maximum current level. These latter sites are typically located caudal to M1, presumably in area 3a and 3b.

ICMS mapping procedures

A digital picture of the blood vessel pattern over the cortex was captured using a video frame-capture card (Scion Corp., Frederick, MD, USA) and an image analysis program (NIH Image). The file was saved and re-opened in an illustration program (Canvas, Deneba Inc.) where a computer-generated grid scaled to 250 µm × 250 µm was superimposed onto the digital image of the blood vessel pattern. The 250-µm resolution was used to achieve sufficiently detailed resolution of the motor map while minimizing the effective current spread between penetration sites relative to the maximum stimulus of 30 µA. This image was used to guide microelectrode penetrations with reference to the surrounding vasculature while accurately measuring the

distance between the microelectrode penetrations (interpenetration distance). Movements associated with stimulation at each of the penetration sites were designated on the digital image (see Fig. 2). One experimenter, who was blind to the location of the electrode, was primarily responsible for defining evoked movements, which were subsequently confirmed by a second experimenter.

Movement analysis

Digit movements included all extensions, flexions, abductions (i.e., radial deviations), and adductions (i.e., ulnar deviations) of fingers and thumb. Wrist-forearm movements included all wrist extensions, wrist flexions, wrist radial deviations, wrist ulnar deviations, forearm supinations, and forearm pronations. Shoulder, elbow and oro-facial movements, evoked by stimulation of cortical regions that border the distal hand area, were not included in the data analysis. Movements of two independent joint categories within the distal hand area (e.g., digits and wrist-forearm) or between the distal hand and proximal arm (e.g., wrist-forearm and shoulder) evoked at the same stimulus threshold (or within 2 μ A) were separately identified and are herein referred to as dual responses. We use the absolute criterion of 2 μ A as a reasonable margin of error. As 2 μ A adds very little to the spread of current throughout the tissue, it is actually very difficult to discern differences in movements evoked within this range of current values. Each evoked response was stereotypical for digit, wrist-forearm and proximal movements and therefore clearly discernable from one another. Furthermore, when multiple responses were evoked within 2 μ A, individual responses could be isolated by stabilizing individual joints. For analysis purposes, the terms “total digits” and “total wrist-forearm” refer to all digit responses and all wrist-forearm responses, respectively, including those that are associated with dual responses. For example, areal measurements for digit/wrist-forearm and digit/proximal dual responses were added to the areal measurements of exclusive digit responses when referring to total digit area.

Stimulus thresholds were also defined and recorded at each stimulation site as the minimum intensity of stimulation between 1 and 30 μ A necessary to evoke a movement.

Ischemic infarct

Immediately after an ICMS baseline (pre-infarct) map was derived, an area of cortex confined primarily to the caudal aspect of the M1 hand representation was targeted for an ischemic infarct (see Fig. 2). The selection of this zone of cortex was based on the large number of digit representations compared to other possible targets in caudal M1, and the ability to occlude a contiguous vascular bed without destroying blood vessels supplying surrounding representations. The surface vasculature within this area was permanently occluded by microforceps connected to a bipolar electrocoagulator (Codman and Shurtle., Inc.). This technique produces a well-defined, focal infarct, confined to the targeted area of cortex, and sparing underlying white matter (Nudo and Milliken 1996). Subsequent studies in this laboratory applying laser Doppler analysis of blood flow have demonstrated that after this technique blood flow is eliminated within the defined lesion area but not in adjacent areas (Frost et al. 2003; Barbay et al. 2004). The area of the infarct was visually inspected for reperfusion 10 min after electrocoagulating the targeted vasculature. If reperfusion was observed, the electrocautery procedure was repeated over the reperfused vasculature to create a permanent occlusion of the targeted region.

Statistical analysis of behavioral data

The measure of each monkey's motor skill on each day was defined as the number of finger flexions necessary to retrieve the food pellets from the smallest food-well of the Klüver board during probe trials. An index of motor performance (finger flexions per food pellet retrieved during post-infarct assessment divided by baseline finger flexions per food pellet retrieved)

was derived to control for individual differences in baseline motor skill (Friel et al. 2000). A motor performance index of “1” means that pre- and post-infarct performance was equal, an index greater than “1” indicates that the monkey made more finger flexions to retrieve pellets after the infarct, and an index less than “1” indicates that post-infarct performance was improved from pre-infarct performance. Group data are shown as the mean $\pm$ SEM. One-group, two-tailed t tests were used to determine if motor skill on the Klüver board was different from baseline at various time points ($H_0=1$).

Statistical analysis of ICMS data

Physiological changes were measured in the peri-infarct hand area. M1 areal measurements and thresholds were subdivided into four categories: (1) Total hand area, including total digit and total wrist–forearm area; (2) Total digit area; (3) Total wrist–forearm area; (4) Dual response area. The dual response category was further subdivided into the digit/wrist–forearm, digit/proximal and wrist–forearm/proximal categories. One group, one-tailed t tests were performed to test the hypothesis that movement representations were reduced in size between the baseline and one-month spontaneous recovery maps (H_0 : %change < 0). This hypothesis was based on results obtained in a previous study in which hand representations were significantly reduced following spontaneous recovery (Nudo and Milliken 1996). In addition, one group, two-tailed t tests were performed to test the hypothesis that movement representations were the same size between the baseline and delayed training maps, again, based on a previous study (Nudo et al. 1996b; H_0 : %change = 0). Finally, one group, two-tailed t tests were performed to test the hypothesis that movement representations were the same size between spontaneous recovery and delayed training maps (H_0 : %change = 0). Percentage scores were normalized using the arcsine transformation (Zar 1984). All the monkeys reached training criterion within 1 month of initiating rehabilitative training (four monkeys on well 5 and one monkey on well 4); therefore, all post-rehabilitation maps were derived approximately 2 months post-infarct.

Results

Motor performance during spontaneous recovery and delayed training

Each animal showed deficits in retrieving food pellets from the smallest well on the Klüver board immediately after the infarct (range of week 1/baseline = 1.91 to 3.86; averages for raw flexions/retrieval by week are provided in Table 1). Flexions per retrieval were significantly higher (impaired motor performance) during each of the 4 weeks of spontaneous recovery (week 1: $t=5.07$, $P=0.007$; week 2: $t=3.53$, $P=0.021$; week 3: $t=3.68$, $P=0.021$; week 4: $t=3.73$, $P=0.020$). Following delayed training, there was no significant difference between the motor performance as compared to baseline ($t=-0.64$, $P=0.560$).

During the delayed training period, individual monkeys were able to attain a criterion level of 600 pellets per day for two consecutive days on the smallest well 26, 25, 24, 15 and 9 days, respectively, after training was initiated.

Alterations in hand representations in spared, adjacent M1 after spontaneous recovery and delayed training

Areal maps—When examined after 1 month of spontaneous recovery, significant decreases were found for total hand ($t=-2.43$, $P=0.036$) and digit ($t=-2.19$, $P=0.047$) representations. On average, the total hand area was reduced by 33% and the digit area by 40%. The decrease in the wrist–forearm representation approached significance ($t=-1.96$; $P=0.061$), averaging 28% less area than in baseline maps.

Subsequently, following 1 month of delayed training, total hand ($t=-3.07$, $P=0.037$) and digit ($t=-3.18$, $P=0.017$) representations remained significantly reduced compared with baseline maps. On average, total hand area was still 21% and digit area 39% below the baseline values. The wrist–forearm representation increased as a result of the delayed training, achieving a final area only 7% below its baseline size. After rehabilitation the wrist–forearm was not significantly different from baseline ($t=-0.80$, $P=0.24$).

Finally, changes in two movement categories were significant between the spontaneous recovery and the delayed training maps. The wrist–forearm/proximal ($t=9.07$, $P=0.0008$) and the total dual response ($t=4.17$, $P=0.014$) representations increased significantly following delayed training. In addition, there was a trend toward increased digit/proximal area after delayed training ($t=2.21$, $P=0.092$). See Fig. 3.

ICMS thresholds—A repeated-measures ANOVA revealed no significant effect of condition (baseline, spontaneous recovery, or delayed training map) in the minimum stimulation needed to evoke a response for any movement category. However, pooled across conditions, there was a significant effect of movement category ($F_{2,80}=15.5$, $P=0.0001$). Post-hoc comparisons using Fisher's LSD showed that thresholds for dual response sites (13 ± 0.8 μA) were significantly lower than digit response sites (19 ± 1.1 μA ; $P=0.0001$), wrist–forearm response sites (16 ± 0.07 μA ; $P=0.004$) and proximal-arm response sites (18 ± 0.7 μA ; $P=0.0001$). There was no significant difference between the other movement categories, though there was a trend for a significant difference between the digit and wrist–forearm response sites ($P=0.07$; Fig. 4).

Relationship between post-training motor maps and motor performance

To determine whether any of the map changes were directly related to the degree of motor recovery, we examined the relationship between the percentage change in movement representations and percentage improvement between the immediate post-infarct period and post-training. For this analysis, we used data from both the delayed (present study) and early training studies (Nudo et al. 1996b) to determine if the source of the variation was related to improvements in motor performance, regardless of the timing of rehabilitation. The relationship for one movement category, the wrist–forearm/proximal representation, was particularly strong (Fig. 5). The three monkeys from the early training study are represented at the low end of the abscissa (0, 2, and 4.7 %); one of the five monkeys in the present study is also on the low end of the abscissa (1.6%); four of the five monkeys in the present study, that received delayed training had a higher percentage of dual response sites within their maps (5, 9, 9.5, and 11.4%). A negative correlation was found between the percentage of wrist–forearm/proximal dual response area and motor performance ($r^2=-0.71$, $P=0.008$). Thus, motor performance gains were less when wrist-forearm/proximal representations comprised more of the post-rehabilitation M1 hand map.

Discussion

The present results demonstrate that delaying training for 1 month in a nonhuman primate focal ischemia model results in minimal restoration of total hand and digit areas that are lost during a one-month period of spontaneous recovery.

Behavioral recovery after delayed training

The results demonstrated that after a small infarct in the M1 hand area (so-called microinfarct) of squirrel monkeys, behavioral deficits in the skilled hand use persisted for at least 1 month in the absence of rehabilitative therapy. Subsequently, when delayed training was initiated at this one-month time point, skilled use of the hand in a pellet retrieval task was regained. At

the end of delayed training, motor performance scores were not statistically different from the baseline performance.

On the basis of the number of flexions per retrieval as an index of motor skill, the present model is limited due to a ceiling effect in the motor performance. Therefore we were not able to determine if there were behavioral advantages of early versus delayed training. Following post-infarct training, the monkeys in both the previous early training and the present delayed training studies performed slightly better, on average, than pre-infarct performance. It is difficult to determine any behavioral advantage or disadvantage of early training if all the groups recover equally well.

Further, after post-infarct rehabilitative training, the monkeys' performance improved beyond pre-infarct performance levels. The combined practice effects of rehabilitation and the transient effects of the lesion allowed the monkeys to perform slightly better than when they ended pre-infarct training. Extensive pre-infarct training, as experienced by the monkeys in this study, may have contributed to a pre-operative "overtraining" effect which has been shown to facilitate recovery of function compared to naive animals (Norrrell 1978; Schulkin 1989). Intensive training was used in the present study and in the Nudo et al. (1996b) study to maximize the initial effects of the lesion by reducing baseline variance, and to insure that the monkeys would be adequately adapted to their restraint jackets during post-infarct Klüver board training.

Several factors can contribute to the recovery rate in preclinical models of a stroke, including the pre-infarct exposure to the task, the frequency and intensity of periodic motor assessment during the post-infarct recovery period, and the size of the infarct. The effects of the first two factors have already been minimized to the degree that is possible in these experiments without sacrificing critical data regarding the early post-infarct performance. The third factor, infarct size, can easily be manipulated by creating infarcts that destroy a much larger portion of the M1 hand area (see Liu and Rouiller 1999). Infarcts that destroy the entire M1 hand area, as used in some of our more recent studies, may be better suited to examination of the timing of rehabilitation on behavior (Frost et al. 2003; Nudo et al. 2003; Plautz et al. 2003).

Effects of delayed training on hand representations in M1

In an earlier study, when post-infarct rehabilitation was introduced during the first week after a small infarct in the M1 hand area, the spared hand representations adjacent to the injury were retained (Nudo et al. 1996b). This is in contrast to significant reductions in hand, especially digit representations following spontaneous recovery (Nudo and Milliken 1996 and present results). Spontaneous recovery resulted in an expansion of proximal representations (elbow/shoulder) into the former distal representations. When the squirrel monkeys experienced 1 month of spontaneous recovery and a subsequent period of delayed rehabilitative training in the present study, spared hand representations remained diminished relative to pre-injury maps (Fig. 4b).

It would seem that early versus delayed training have very different effects. First, total hand, especially digit representations, are retained after early training, but once lost during the spontaneous recovery period, digit representations do not appear to recover as a result of delayed training. Second, the total dual response area in which the multi-joint movements were evoked by ICMS, essentially remained the same size compared to baseline after early training, but increased significantly after delayed training. This was primarily due to an increase in wrist-forearm/proximal representation after delayed training that was not evident after the spontaneous recovery phase. Early training resulted in a smaller and statistically non-significant increase in wrist-forearm/proximal representation. Thus, it seems that one month of spontaneous recovery altered the potential for functional reorganization within the spared

hand representations. Subsequent training of the affected hand did not drive a re-emergence of lost digit representation, though it did drive functional improvements in motor performance.

Multi-joint movements elicited by ICMS (sometimes called dual responses) have been observed in squirrel monkeys with various experimental manipulations regarding Klüver board training and brain injury (pre-and post-training, with or without cortical infarcts). Although their occurrence is infrequent relative to single-joint responses (see Fig. 6) they have been shown to increase with training and parallel changes in the frequency of movement combinations used to perform a reach and retrieval task (Nudo et al. 1996a). In the present study, dual responses only accounted for ~15% of the distal hand area after post-infarct training (~8.4 sites per monkey). It is not clear how important these areas are to improvements in motor performance since most of the M1 hand area is composed of single joint movement representations using threshold currents as defined here. It is interesting that the dual response area can be increased by task specific activities on the Klüver board associated with simultaneous use of joints in the distal hand or combinations of distal and proximal arm movements. Kinematics of behavioral videotapes in the present study did not reveal appreciable differences between reach and retrieval movements from spontaneous recovery to post-training performance. It may be the case that the multi-joint movements used by the monkeys during spontaneous recovery to retrieve food pellets during the weekly probe trials were not sufficiently frequent or stereotypic to reveal a strict correlation between behavioral and physiological changes in specific dual response representations.

The emergence of dual response representations in the remaining hand area after delayed rehabilitative training may have been due to the temporally correlated activation of two or more movements during training. That is, the associated activation of overlapping single joint movement representation during skilled hand movements may become physiologically linked, resulting in a limited cortical area in which neurons contribute to specific conjoint movements. The presence of these dual response sites, however infrequent, may be important clinically in light of the extensive evidence that an abnormal joint torque coupling is observed in human stroke survivors (Dewald and Beer 2001).

Several studies using various techniques have demonstrated that the intrinsic neural connections within M1 support overlapping movement representations of distal and proximal arm movements (Sanes and Donoghue 2000). A recent study in our laboratory using ICMS has also shown the emergence of wrist–forearm representations within the proximal shoulder area of squirrel monkeys (Plautz et al. 2003). These novel wrist–forearm responses were related to therapeutic post-lesion cortical electrical stimulation using an implanted electrode (Northstar Neuroscience, Inc.) in the peri-infarct shoulder area. In addition to single neurons projecting to both distal and proximal arm motoneuronal pools (Park et al. 2001), there is also an abundance of horizontal fibers interconnecting digits to wrist, elbow, and shoulder M1 (Huntley and Jones 1991). These connections may support Hebbian-like associations between groups of neurons (Hess and Donoghue 1996; Rioult-Pedotti et al. 1998) that may contribute to the synchronized, simultaneous conjoint movements evoked by ICMS in the present study. However, the neurophysiological results in the peri-infarct hand area of M1 indicate that post-infarct training introduced at different time points alters the intracortical network differentially. Thus, the mechanisms underlying recovery of motor skills with respect to cortical network properties may be qualitatively different between early and delayed training.

Methodological considerations: comparisons to earlier data

Although data for the early and delayed training studies were collected at two different times (the first study was published in 1996 and the present study began in 1998) the same equipment and procedures were used for both studies and several of the same personnel were either directly involved or trained by personnel involved in data collection in both studies. Baseline, post-

infarct and pre-training data were similar between groups. Baseline flexions per retrieval for animals in the delayed and early training studies were 2.5 and 1.8 flexions per retrieval, respectively. Post-infarct flexions per retrieval in the two studies were 7.0 and 7.5 flexions per retrieval, respectively. The minimum current for evoking a response was also similar for each movement category, averaging below 20 μ A (see results). Finally, similar studies employing early training have been conducted in this laboratory throughout the interim period, and the quantitative results have not varied substantially. Thus, any differences between the two studies are unlikely to be due to the time difference between data collection. In the interest of limiting the number of primates used in these studies, we chose not to repeat the former experiments. However, to the extent that the time factor cannot be ruled out, the conclusions should be weakened accordingly.

Summary

Behavioral experience such as rehabilitative training can have a differential effect upon reorganization of movement representations in M1 after a focal infarct, depending upon how soon after the infarct training is initiated. The present study demonstrates that delaying training by 1 month results in an expansion of proximal movement representations at the expense of distal hand movement representations in peri-infarct M1, and more distal/proximal dual responses evoked from peri-infarct M1 than expected from previous studies. It is believed that this is a combination of reorganizational constraints on cortical plasticity imposed during the period of spontaneous recovery. The functional significance of increases in dual response representations needs to be further examined with larger cortical infarcts since the average behavioral differences between the early and delayed training groups were not different. However, the negative correlation between dual responses and improvement in motor performance observed repeatedly in this laboratory suggests that early rehabilitative training is more beneficial than delayed training. If changes in neurophysiologic maps of movement representations are an indication of the functional capacity of the motor cortex, it would appear that delayed training is not as effective as early training in restoring the normal physiology in motor cortex controlling distal musculature.

Acknowledgments

This study was supported by NS30853 (RJN), HD02528 (NICHD center grant), and AG14635 (NIA Pepper Center grant). We would also like to thank Diane Larson for technical assistance.

References

- Barbay, S.; Zoubina, EV.; Needham, KD.; Frost, SB.; Eisner-Janowicz, I.; Stowe, AM.; Dancause, N.; Plautz, EJ.; Nudo, RJ. Society for neuroscience annual meeting. Society for Neuroscience, Online; San Diego, CA: 2004. Injury-induced early reduction and subsequent recovery of hand area representation in ventral premotor cortex following ischemic infarct in primary motor cortex.
- Biernaskie J, Chernenko G, Corbett D. Efficacy of rehabilitative experience declines with time after focal ischemic brain injury. *J Neurosci* 2004;24:1245–1254. [PubMed: 14762143]
- DeBow SB, McKenna JE, Kolb B, Colbourne F. Immediate constraint-induced movement therapy causes local hyperthermia that exacerbates cerebral cortical injury in rats. *Can J Physiol Pharmacol* 2004;82:231–237. [PubMed: 15181461]
- Dewald JP, Beer RF. Abnormal joint torque patterns in the paretic upper limb of subjects with hemiparesis. *Muscle Nerve* 2001;24:273–283.
- Dromerick AW. Evidence-based rehabilitation: the case for and against constraint-induced movement therapy. *J Rehabil Res Dev* 2003;40:7–9.
- Duncan PW, Lai SM, Keighley J. Defining post-stroke recovery: implications for design and interpretation of drug trials. *Neuropharmacology* 2000;39:835–841. [PubMed: 10699448]

- Friel KM, Heddings AA, Nudo RJ. Effects of postlesion experience on behavioral recovery and neurophysiologic reorganization after cortical injury in primates. *Neurorehabil Neural Repair* 2000;14:187–198. [PubMed: 11272475]
- Frost SB, Barbay S, Friel KM, Plautz EJ, Nudo RJ. Reorganization of remote cortical regions after ischemic brain injury: a potential substrate for stroke recovery. *J Neurophysiol* 2003;89:3205–3214. [PubMed: 12783955]
- Goldstein LB, Davis JN. Beam-walking in rats: studies towards developing an animal model of functional recovery after brain injury. *J Neurosci Meth* 1990;31:101–107.
- Hess G, Donoghue JP. Long-term potentiation and long-term depression of horizontal connections in rat motor cortex. *Acta Neurobiol Exp (Warsz)* 1996;56:397–405. [PubMed: 8787200]
- Huntley GW, Jones EG. Relationship of intrinsic connections to forelimb movement representations in monkey motor cortex: a correlative anatomic and physiological study. *J Neurophysiol* 1991;66:390–413. [PubMed: 1723093]
- Jones TA, Chu CJ, Grande LA, Gregory AD. Motor skills training enhances lesion-induced structural plasticity in the motor cortex of adult rats. *J Neurosci* 1999;19:10153–10163. [PubMed: 10559423]
- Klein JA, Jones TA, Schallert T. Motor enrichment and the induction of plasticity before or after brain injury. *Neurochem Res* 2003;28:1757–1769. [PubMed: 14584829]
- Kozlowski DA, James DC, Schallert T. Use-dependent exaggeration of neuronal injury after unilateral sensorimotor cortex lesions. *J Neurosci* 1996;16:4776–4786. [PubMed: 8764664]
- Liu Y, Rouiller EM. Mechanisms of recovery of dexterity following unilateral lesion of the sensorimotor cortex in adult monkeys. *Exp Brain Res* 1999;128:149–159. [PubMed: 10473753]
- Norrrell, U. Testing procedures and the interpretation of behavioral data. In: Finger, S., editor. *Recovery from brain damage: research and theory*. Plenum; New York: 1978. p. 199–215.
- Nudo RJ, Jenkins WM, Merzenich MM. Repetitive microstimulation alters the cortical representation of movements in adult rats. *Somatosens Mot Res* 1990;7:463–483. [PubMed: 2291378]
- Nudo RJ, Larson D, Plautz EJ, Friel KM, Barbay S, Frost SB. A squirrel monkey model of poststroke motor recovery. *Ilar J* 2003;44:161–174. [PubMed: 12652011]
- Nudo RJ, Milliken GW. Reorganization of movement representations in primary motor cortex following focal ischemic infarcts in adult squirrel monkeys. *J Neurophysiol* 1996;75:2144–2149. [PubMed: 8734610]
- Nudo RJ, Milliken GW, Jenkins WM, Merzenich MM. Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. *J Neurosci* 1996a;16:785–807. [PubMed: 8551360]
- Nudo RJ, Plautz EJ, Frost SB. Role of adaptive plasticity in recovery of function after damage to motor cortex. *Muscle Nerve* 2001;24:1000–1019. [PubMed: 11439375]
- Nudo RJ, Wise BM, SiFuentes F, Milliken GW. Neural substrates for the effects of rehabilitative training on motor recovery after ischemic infarct. *Science* 1996b;272:1791–1794. [PubMed: 8650578]
- Park MC, Belhaj-Saif A, Gordon M, Cheney PD. Consistent features in the forelimb representation of primary motor cortex in *Rhesus macaques*. *J Neurosci* 2001;21:2784–2792. [PubMed: 11306630]
- Plautz EJ, Barbay S, Frost SB, Friel KM, Dancause N, Zoubina EV, Stowe AM, Quaney BM, Nudo RJ. Post-infarct cortical plasticity and behavioral recovery using concurrent cortical stimulation and rehabilitative training: a feasibility study in primates. *Neurol Res* 2003;25:801–810. [PubMed: 14669522]
- Riout-Pedotti MS, Friedman D, Hess G, Donoghue JP. Strengthening of horizontal cortical connections following skill learning. *Nat Neurosci* 1998;1:230–234. [PubMed: 10195148]
- Sanes JN, Donoghue JP. Plasticity and primary motor cortex. *Annu Rev Neurosci* 2000;23:393–415. [PubMed: 10845069]
- Schulkin, J. *Preoperative events: their effects on behavior following brain damage*. Lawrence Erlbaum Associates; Hillsdale: 1989.
- Stoney SD Jr, Thompson WD, Asanuma H. Excitation of pyramidal tract cells by intracortical microstimulation: effective extent of stimulating current. *J Neurophysiol* 1968;31:659–669. [PubMed: 5711137]

- Stroemer RP, Kent TA, Hulsebosch CE. Enhanced neocortical neural sprouting, synaptogenesis, and behavioral recovery with D-amphetamine therapy after neocortical infarction in rats. *Stroke* 1998;29:2381–2393. [PubMed: 9804653]discussion 2393–2385
- Sutton RL, Hovda DA, Feeney DM. Amphetamine accelerates recovery of locomotor function following bilateral frontal cortex ablation in cats. *Behav Neurosci* 1989;103:837–841. [PubMed: 2765187]
- Taub E, Wolf SL. Constraint induced movement techniques to facilitate upper extremity use in stroke patient. *Topics Stroke Rehab* 1997;3:38–61.
- Zar, JH. Biostatistical analysis. Prentice-Hall; New Jersey: 1984. p. 116

Fig. 1.

Motor skill performance associated with ICMS maps of hand representations in M1 were acquired for five squirrel monkeys that were trained on a motor skill task prior to an ischemic infarct, after 1 month of spontaneous recovery from the infarct and again after approximately 1 month of training (~2 mos. post-infarct)

Fig. 2.

a A magnified digital photograph of the surface of the cerebral cortex of a squirrel monkey brain after ICMS mapping. Each penetration site is marked with a filled, colored circle. Red, digit movements, Green, wrist-forearm movements, Blue, arm movements proximal to the forearm, and Black, non-responsive areas. Intended lesion is outlined within the mapped hand area. **b** Cortical regions with similar movement representations were delineated on a two dimensional representation of the M1 hand area. Borders were established between adjacent movement representations midway between microelectrode penetration sites using customized software. Color codes are the same as in **a** plus additional codes: yellow, digit/(wrist-forearm), light blue, digit/proximal and magenta, (wrist-forearm)/proximal

Fig. 3.

a Alterations in spared M1 hand area were analyzed 1 month after spontaneous recovery and 1 month after delayed training in the same monkeys. For each movement representation, and in each map, changes in M1 were described as the percentage change in area compared to the baseline condition. After 1 month of spontaneous recovery, total hand and digit representations were significantly reduced compared to baseline (indicated by *asterisks*). The reduction in hand area persisted after delayed training except for dual response representations that increased compared to spontaneous recovery maps (indicated by *S*). **b** Data from Nudo et al. 1996b showing alterations in spared M1 hand area analyzed after 1 month of early training that was implemented within 1 week after a cortical infarct in the M1 hand area. Early training was more beneficial than delayed training for maintaining distal hand area in M1 adjacent to the ischemic infarct, and did not result in a significant increase in dual responses above baseline values

Fig. 4.

The minimum stimulus necessary to evoke a movement via ICMS in motor cortex was defined for each movement (stimulus threshold). Stimulus thresholds did not change from baseline after spontaneous recovery or delayed training. Pooled across conditions, the average threshold was below 20 μA , the average dual responses thresholds were significantly lower than average distal hand response thresholds (digit or wrist) or proximal arm thresholds

Fig. 5.

Improvements in motor performance associated with the proportion of wrist–forearm dual response representations within the peri–infarct hand area of M1 in post–training maps. Monkeys from both the delayed (present study) and early training (Nudo et al. 1996) studies were combined for a linear regression analysis. A negative correlation is shown between improved performance and increases in wrist–forearm/proximal dual response area ($r^2=-0.72$, $P=0.008$)

Fig. 6.

Average percentage of digit, wrist-forearm and dual responses comprising post-infarct map after delayed training. The average total M1 hand area after delayed training = 4.69 mm^2 . The average number of penetration sites = 271.8. The average distal hand area is composed of 18.8 digit sites, 35.0 wrist-forearm sites, 3.4 wrist-forearm/proximal sites, 2.8 digit/wrist-forearm sites, and 2.2 digit/proximal sites. The remaining penetration sites included 89.2 proximal sites and 119.8 non-responsive sites

Table 1

Flexions/retrieval [86×22 mm (300×300 dpi)]

	Baseline	Spontaneous recovery			Training	
		Week 1	Week 2	Week 3	Week 4	
Mean	2.44	7.14	4.58	6.42	4.30	1.64
SEM	0.96	2.82	1.77	2.90	1.53	0.23