



Recovery from ischemia in the middle-aged brain: a nonhuman primate model

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Abstract

Studies of recovery from stroke mainly utilize rodent models and focus primarily on young subjects despite the increased prevalence of stroke with age and the fact that recovery of function is more limited in the aged brain. In the present study, a nonhuman primate model of cortical ischemia was developed to allow the comparison of impairments in young and middle-aged monkeys. Animals were pretrained on a fine motor task of the hand and digits and then underwent a surgical procedure to map and lesion the hand-digit representation in the dominant motor cortex. Animals were retested until performance returned to preoperative levels. To assess the recovery of grasp patterns, performance was videotaped and rated using a scale adapted from human occupational therapy. Results demonstrated that the impaired hand recovers to baseline in young animals in 65–80 days and in middle-aged animals in 130–150 days. However, analysis of grasp patterns revealed that neither group recover preoperative finger thumb grasp patterns, rather they develop compensatory movements.

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1. Introduction

Strokes primarily occur in late middle age and beyond and compromise blood supply either through thrombotic occlusion or hemorrhagic damage from rupture of cerebral blood vessels. This results in varying degrees of brain damage depending on the size and location of the vascular compromise. Strokes that produce damage to the cortical gray matter of the motor cortices can affect motor function of the upper or lower extremities. Functional effects may include extremity weakness, spasticity, and impaired motor coordination, any of which may affect an individual's ability to perform activities of daily living and negatively affect

quality of life in stroke survivors (Nichols-Larsen et al., 2005; Ward and Frackowiak, 2003; Woodson, 2002). While partial recovery of motor activity often occurs spontaneously and may be enhanced by occupational therapies, most individuals who suffer stroke that affects the extremities are left with a long term chronic disability (Cirstea and Levin, 2000; Fridman et al., 2004; Noskin et al., 2008; Raghavan et al., 2010; Sunderland, 2000) and a complete return to pre-injury levels of function is a rare event (Gowland, 1987; Nakayama et al., 1994).

It is clear from clinical case studies and studies with animal models of cortical ischemia that cortical reorganization in the intact cortical areas may contribute to the recovery of function that is observed after stroke (Frost et al., 2003; Gonzalez et al., 2004; Green, 2003; Nudo, 1999; Nudo and Friel, 1999; Ward, 2004). In animal models of cortical injury it has been demonstrated that following damage in the motor cortex, representation of the upper limb

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expands into cortical areas surrounding the lesion, as well as in the nearby ipsilateral premotor cortex and possibly even into contralateral motor and premotor cortices (Butefisch et al., 2005; Frost et al., 2003; Green, 2003; Green et al., 1999a, 1999b; Miyai et al., 2002; Nudo, 1999; Nudo and Friel, 1999; Ward, 2004). However, the precise nature and extent of postinjury plasticity and cortical reorganization and their relationship to functional recovery still remains unclear.

Animal models of stroke and ischemia are critical to further our understanding of the processes of plasticity and cortical reorganization that underlie the recovery of function and to the development and testing of therapeutic interventions to enhance recovery. Various models of ischemia have been developed in rodents (Chen et al., 2002; Zai et al., 2009) and in the new world monkey (Barbay et al., 2006; Dancause, 2006; Eisner-Janowicz et al., 2008; Friel and Nudo, 1998; Friel et al., 2000; Frost et al., 2003; Plautz and Nudo, 2005). These models have provided considerable insight into the time course and parameters of recovery. However, despite the fact that aging is a significant risk factor for stroke (Bacigaluppi et al., 2010; Statler et al., 2001) and may alter the efficiency of physiological and metabolic processes involved in plasticity (Dinse, 2005), these animal models have only included young animals. Recently, several reviews have concluded that animal models of ischemia and stroke that include aged animals will provide the most clinically and experimentally relevant data about cortical reorganization and mechanisms underlying recovery of function (Buga et al., 2008; Lindner et al., 2003; Subramanyam et al., 1999). The goal of this study was to develop a nonhuman primate model of cortical ischemia that includes young and middle-aged rhesus monkeys with quantifiable and reproducible ischemic injury in the physiologically defined area of the primary motor cortex (M1) that controls the digits and hand. This model allows for the multidimensional assessment of the recovery of motor function and will allow future studies to assess the adaptive plasticity and cortical reorganization of the cortex and therapeutic interventions in both young and aged rhesus monkey brains.

2. Methods

Nine young and middle-aged male rhesus monkeys (*Macaca mulatta*; a gyrencephalic species) between the ages of 6 and 20 years of age were used. The young group consisted of 5 animals (6–9 years old) and the middle-aged group consisted of 4 animals (14–20 years old) (Table 1). Monkeys attain sexual maturity at about 5 years of age and the oldest rarely live beyond 30, suggesting an approximate relationship to humans of 1:3 (Tigges et al., 1988). Hence the monkeys between 6 and 9 years of age correspond roughly to humans between 18 and 27 years of age and

Table 1
Identification, age, and handedness for each animal in the study

Monkey	Age, years	Dominant hand	Presence of hand/digit injury or disease
Young			
SM002	6	Left	None
SM003	9	Left	None
SM004	6	Left	None
SM011	7	Right	Healed fracture to index finger (dominant hand)
SM012	9	Left	None
Aged			
SM006	16	Left	None
SM007	14	Left	Healed fracture to index finger (dominant hand)
SM008	19	Left	Mild arthritis (bilateral)
SM010	20	Left	Missing index finger and thumb (dominant hand)

those between 14 and 20 years of age to humans between 42 and 60 years of age.

All of the monkeys were obtained from a national primate research facility or breeding facility and had known birth dates and complete health records. Before entering the study, monkeys received medical examinations that included serum chemistry, hematology, and urine and fecal analysis. In addition, explicit criteria were used to exclude monkeys with a history of any of the following: splenectomy, thymectomy, exposure to radiation, cancer, organ transplantation, malnutrition, chronic illness including viral or parasitic infections, diabetes, neurological diseases, or chronic drug administration. Once entered into the study, all monkeys were individually housed in colony rooms in the Laboratory Animal Science Center at Boston University School of Medicine where they were in constant auditory and visual range of other monkeys. This facility is fully Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)- approved and animal maintenance and research is conducted both in accordance with the guidelines of the National Institutes of Health Committee on Laboratory Animal Resources and with the procedures approved by the Institutional Animal Use and Care Committee of the Boston University Medical Campus. Diet consisted of Purina Monkey Chow (Purina Mills Inc., St. Louis, MO, USA) supplemented with fruit. Feeding took place once per day, immediately following behavioral testing. Water was available continuously. The monkeys were housed under a 12-hour light/dark cycle with cycle changes occurring in a graded fashion over the course of an hour.

2.1. Behavioral testing procedures

Behavioral testing procedures are explained in detail in Moore et al. (2010). In brief, monkeys were trained preop-

eratively on a task of fine motor function of the hand and digits, the Hand Dexterity Task (HDT), and then retested on this task in the postoperative period. The HDT is a modified version of the Kluver board that requires the monkeys to retrieve small food rewards (M&M's [Mars Inc., McLean, VA, USA] and Reese's Pieces [Hershey Co., Hershey, PA, USA]) from wells of various diameters and depths with each hand (see Moore et al., 2010). It is administered in a Wisconsin General Testing Apparatus that has been modified to accommodate a specially designed apparatus consisting of a clear plexiglass box that is divided into right and left sides. The box is placed directly in front of the monkey with access limited to a small opening located on each side that forces the use of the right or left hand for the right and left openings respectively. Each side of the box was fitted with a square tray containing 4 wells. The openings on either side of the apparatus were fitted with a photocell that triggers a timer to begin recording when the animal's hand enters the box and stops when the hand is removed from the box (with or without the reward, recorded independently by the tester); thus establishing a reward retrieval latency that provides a quantitative measure of the efficiency of digit use for successful reward retrieval as well as the number of successful and unsuccessful responses. In addition, video cameras located immediately over each of the trays recorded the topography of each response.

2.2. Preoperative training

The tray for the HDT (see Moore et al., 2010) contained the following 4 wells that varied by depth and diameter: wide and deep (WD), diameter = 2.5 cm and depth = 1.6 cm; wide and shallow (WS), diameter = 2.5 cm and depth = 0.95 cm; narrow and deep (ND), diameter = 1.9 cm and depth = 1.6 cm; and narrow and shallow (NS), diameter = 1.9 cm and depth = 0.95 cm. These differences required the monkey to retrieve the food reward from the wide diameter wells using several fingers and the thumb together but required the use of the thumb and 1 finger to retrieve the reward from the narrow diameter wells. The type of food reward remained constant for all trials for all monkeys. An opaque screen occluded access to and sight of the box between trials when 1 of the 4 wells in either the right or the left side box was baited with a food reward. Once the appropriate well was baited, the opaque screen was then raised initiating a trial as it revealed the location of the baited well. The monkey was then allowed to retrieve the reward from the baited tray and the time required to retrieve the reward was recorded.

During preoperative training, a total of 32 trials were presented in a pseudorandom and counterbalanced fashion each day and were equally divided into 16 trials for each hand and 4 trials for each well. If an animal was unsuccessful at retrieving the food reward because the food reward was dropped inside the box, the latency data for that trial was discarded, the failure noted, and the trial was repeated.

All monkeys were trained on this task for 20 days. All trials during the last 5 days of preoperative testing were videotaped. These tapes were reviewed and used for comparison with video segments recorded during postoperative testing.

2.3. Determination of hand preference

At the completion of each day of testing, the experimenter placed several food rewards on the top and center of the plexiglass apparatus and then opened the occluding screen to allow the monkey access to the rewards. The hand that the monkey used to retrieve these rewards was recorded each day and the hand that was used for the majority of the reaches was deemed to be the preferred (dominant) hand. All monkeys in this study demonstrated a left-handed preference for retrieval of food reward except for SM011 who demonstrated a right-handed preference (Table 1).

2.4. Surgical procedure

All surgical procedures were carried out under aseptic conditions. Animals were food deprived 12 hours before surgery. For surgery, they were sedated with ketamine hydrochloride (10 mg/kg) and then anesthetized with intravenous sodium pentobarbital (15–25 mg/kg to effect). Heart rate, respiration, oxygenation, and muscle tone were monitored to ensure a safe surgical level of anesthesia. Once anesthetized, a midline incision of the skin and fascia was made followed by mobilization and reflection of the temporalis muscle from the skull, bilaterally. To map the hand-digit representation in the motor cortex controlling the dominant hand as identified during testing, a craniotomy was performed over the primary motor cortex (M1) contralateral to the dominant hand and the dura was opened to expose M1 between the central sulcus and arcuate sulcus. This area was visualized and a calibrated photograph taken and printed. M1 was then mapped onto this calibrated photograph using electrical stimulation delivered through a silver ball electrode placed gently on the surface of the pia. Monopolar stimulus pulses of 2 millisecond (ms) duration were delivered once every 1.5 seconds at each site. Nonresponsive sites were further tested with a 200 Hz train consisting of 4 or 8 pulses of 2 ms duration delivered over 20 or 40 ms respectively. Amplitude varied from 1 to 4 mA to identify the lowest threshold evoking the response. Stimulation sites were spaced approximately 2 mm apart in anterior to posterior rows and each row of stimulation points was separated from the next by approximately 2 mm (ventral to dorsal). Response intensity in the hand and digits was graded on a scale of 1 to 5 from barely visible to strong. Specific stimulation sites with the lowest threshold that control each digit and the hand were marked on the photograph (Fig. 1). This area represented the map of the hand-digit area for that animal. This map was then used to guide the creation of an ischemic lesion. This was accomplished by making a small incision in the pia at the dorsal limit of the motor representation area and by using a small micropi-

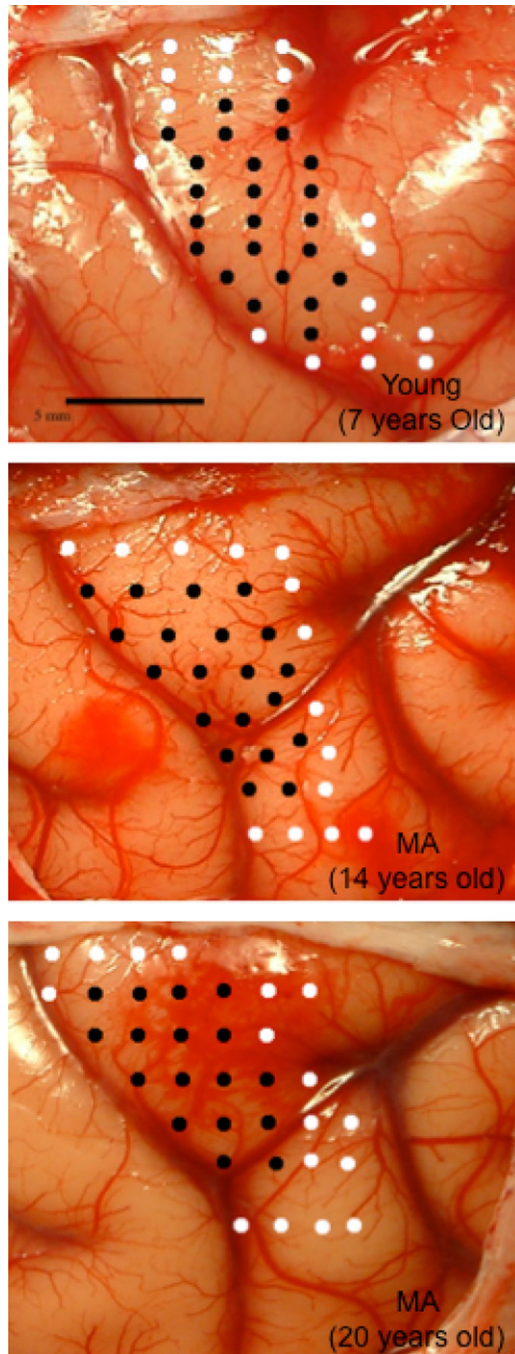


Fig. 1. Magnified photograph of lateral surface of motor and somatosensory cortices of 3 monkeys (1 young and 2 middle-aged) showing stimulation sites. Black circles represent sites that when stimulated produced a strong response of hand and/or digits and white circles represent sites that when stimulated produced weak or no response of hand and/or digits. Scale bar applies to all 3 maps.

pette suction tube to bluntly separate the pia from the underlying cortex, depriving the cortex of the blood supply from the arterioles that penetrate into it from the pia. In addition, the central sulcus was opened and the lesion was continued down to the fundus of the sulcus. This is com-

monly described as subpial aspiration, but it is important to note that the gray matter was not directly damaged and the suction was used only to control bleeding from the disrupted pial vessels. Hence this mimics the ischemic effects of an embolic stroke that occludes blood vessels to an area but does so in a controlled and completely reproducible fashion.

After the lesion was complete and bleeding stopped, the dura was closed, the bone flap was sutured in place using small burr holes placed in the flap and the skull, and muscle, fascia and skin were closed in layers. The monkeys were given prophylactic doses of antibiotics and analgesic. Buprenex, a narcotic analgesic, was used for 3 days or longer if medically indicated. Immediately following surgery, monkeys were kept in an incubator until they began to wake up, at which time, they were returned to their home cage and monitored on a regular basis by trained personnel for 2 weeks.

2.5. Postoperative testing

Postoperative testing began 14 days after surgery and consisted of retesting on the HDT. Animals were tested on the Monday, Wednesday, and Friday of each week, and each daily testing session consisted of 20 trials of the HDT (12 trials to the unimpaired hand and 8 trials to the impaired hand; equally divided across the 4 wells) for a total of 60 trials per week. This timetable of testing was based on the clinical experience of coauthor Dr. Monica Pessina, a licensed occupational therapist specialized in the upper extremity recovery of function after stroke, to replicate a typical therapy regimen (therapy 3–5 days/week) that may be used with human stroke patients. This schedule was also supported by a review article by [Steultjens et al. \(2003\)](#) that examined the efficacy of various occupational therapy regimens that found that the typical frequency of therapy sessions following stroke was 3–5 days per week. Postoperative testing continued until the animal's performance, in terms of latency to retrieve a food reward, returned to preoperative levels. This was determined as a latency to retrieve the food reward at or below the preoperative latencies for each well for 5 consecutive days. One young animal, SM004, died 6 months postoperatively from unrelated health issues and had not reached criterion on any well with his impaired hand at that time. However, performance with his intact hand had returned to preoperative levels and those data were included in this study.

2.6. Assessment of grasp patterns

To assess the recovery of grasp patterns, postoperative performance of the impaired hand in a subset of animals (3 young and 3 middle-aged) was scored by trained personnel using a scale we adapted from the Eshkol-Wachman Movement Notation ([Whishaw et al., 2002](#)) and the Fugl-Meyer Motor Assessment scales ([Fugl-Meyer et al., 1975](#)). This adapted scale, the Grasp Assessment Scale, assessed the initiation of movement and pattern of grasp and release. Six

Table 2
Description of stages 1–6 rating scale for Grasp Assessment Scale

Stage	Description
1	Complete absence of active finger movement.
2	Mild flexion and extension of digits as a group. Evidence of the development of compensatory “scooping” movement of reward toward palm of hand with multiple fingers and mass action of digits.
3	Moderate flexion and extension of digits and mass action of the digits. Evidence of the increased accuracy of compensatory “scooping” movement of reward toward palm of hand with multiple fingers.
4	Reaches toward reward with all digits in extension with evidence of occasional isolated digit movement but mass movement still predominates. The compensatory “scooping” movement toward palm increases in accuracy and occurs frequently with only 1 digit.
5	Reaches toward reward with all digits in extension. Isolated digit movement occurs on all trials. The compensatory “scooping” movement toward thumb increases in accuracy and efficiency with 1 digit directing food toward palm of the hand.
6	Normal movement of all digits and hand with no evidence of compensatory “scooping” movements. Functional pinch occurs between the thumb and 1 digit, usually the index finger.

hierarchical grasp stages were included each with distinctive features. The stages progressed from flaccidity to normal grasp and the key grading criteria was the relative amount of synergistic versus isolated movement observed in the digits and the extent of compensatory type movement that developed. See Table 2 for description of each stage of this scale. This new scale was applied to the video images from the monkeys in the current study to determine if typical grasp patterns are re-established when latencies are normal at the end of recovery; or if compensatory grasp patterns have replaced typical grasp patterns. Due to the variability in the animals' hands (e.g., healed digit fractures, missing digits and arthritis; Table 1) the rating scale used for each animal was slightly modified based on their preoperative performance. For example, SM010 was missing

the index finger and thumb on his left hand and therefore could not retrieve M&M's using his thumb and forefinger, which is the typical grasp used by monkeys on this task during preoperative training. Instead, he used his middle finger to push the M&M to the thenar eminence and hold the candy in that position. This was considered “normal” grasping for this monkey and this pattern was used to determine the rating scale used postoperatively for this specific animal.

Following completion of testing, 2 young and 2 middle-aged animals were deeply anesthetized with intravenous sodium pentobarbital (15 mg/kg to effect) and killed by exsanguination during transcardial perfusion of the brain with 4% paraformaldehyde. Following perfusion, both hemispheres were blocked, in situ in the coronal stereotactic plane for serial sectioning and then transferred to cryoprotectant solution to eliminate freezing artifact (Rosene et al., 1986). The cryoprotected blocks were then flash frozen and stored at -80°C until they were cut on a microtome into 9 interrupted series of 30- μm thick frozen sections and 1 60- μm thick series. The 60- μm series was immediately mounted on microscope slides, stained with thionin and used to verify the extent and location of the lesion (Fig. 2). Postmortem photographs of the intact brain were also used to determine the percent of the mapped hand representation area that was included in the lesion. From this small subset of animals it was determined that 94%–100% of the hand representation area was included in the lesion.

2.7. Data analysis

2.7.1. Preoperative testing

The mean latency to retrieve a food reward from each well with each hand for the last 5 days of preoperative testing was determined for each monkey. A 3-way repeated measures analysis of variance (ANOVA) with age as a between subject variable and hand and well as within sub-

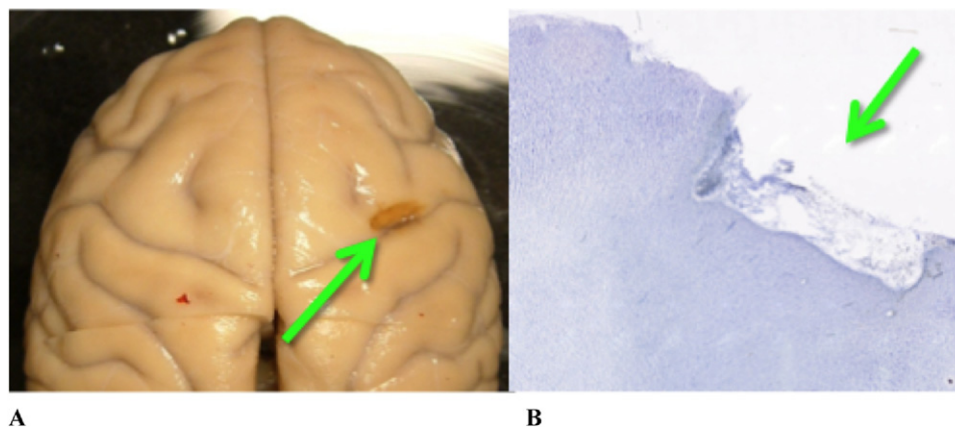


Fig. 2. (A) Postperfusion photograph of a brain from 1 young animal in this study demonstrating the location and extent of the lesion. (B) Representative thionin-stained coronal section from a young animal showing extent of lesion and pathological changes in region of ischemia.

ject variables was used to compare the performance of young and middle-aged monkeys on this measure.

2.7.2. Postoperative testing

The total number of postoperative days required to return to preoperative levels of performance for each well with each hand was determined. A return to preoperative levels of performance was defined as a latency to retrieve a food reward at or below the mean preoperative latencies for 5 consecutive days for each well. If a trial was not successfully completed (i.e., the monkey dropped the food reward before removing their hand from the apparatus) the trial was repeated. Unsuccessful trials occurred infrequently and did not account for a significant increase in the number of trials administered to each animal (i.e., fewer than 20 times over the 600–3000 trials administered over the postoperative testing period). Two separate multivariate analysis of variance (MANOVA) (1 for the young animals and 1 for the middle-aged animals) with hand (intact and impaired) as the independent variables and the number of postoperative days to return to preoperative latencies to retrieve a food reward for each well as the dependent variables were used to compare the postoperative days to criterion for the intact and impaired hands in young and middle-aged animals.

Then a MANOVA with age group (young and middle-aged) as the independent variable and latency to retrieve a food reward from each well as the dependent variables was then used to compare the young and middle-aged monkeys on the number of postoperative days required to return to preoperative levels of performance for the intact and impaired hand.

2.7.3. Hand dexterity task grasp patterns

Video clips of each animals' preoperative and postoperative testing were analyzed based on our Grasp Assessment Scale (Table 2). At the completion of preoperative testing, all animals consistently retrieved the food reward using the thumb and index finger (except for SM010; using a compensatory grasp pattern to accommodate for the loss of the thumb and index finger as described above) to reach into the well and grasp the reward and remove it from the well. During postoperative testing, no animal recovered "normal" function that they demonstrated during preoperative testing and therefore none received a rating score of 6 (normal grasp patterns with no evidence of compensatory movements) on any trial during their postoperative performance. However, all animals did eventually return to a grasp pattern that was scored as a 5 (effective retrieval of reward but with evidence of compensatory "scooping" movement towards thumb with increased accuracy and efficiency with 1 digit directing food toward the palm of the hand). Therefore, the number of days required to return to a pattern of grasping scored as 5 was determined. The criterion for this measure was 3 consecutive days of a rating of 5 for a given well. These data were analyzed using 1-way ANOVA with age as the between subject variable to determine the presence of an

age-related difference in the time required to reach criterion on each well. In addition, the postoperative time period that was required to return to 3 consecutive days of a rating of 5 per well was divided into 3 equal periods and the average rating for each period was determined in order to investigate the temporal aspects of recovery over the postoperative period. A Mann-Whitney *U* test was used to determine the presence of an age-related difference in the average rating for each time period for each well. Finally, the average rating across the entire postoperative period was determined and analyzed using separate 1-way ANOVAs with age as the between subject variable for each well.

3. Results

3.1. Preoperative testing

3.1.1. Effect of age

A 3-way repeated measures ANOVA with age as a between subject variable and hand and well as within subject variables was used to compare the performance of young and middle-aged monkeys on the mean latency to retrieve a food reward from each well for the last 5 days of preoperative testing with each hand. This analysis revealed no significant difference between young and middle-aged animals with either hand on any well (Fig. 3A and B) though as a group the younger animals have overall lower latencies to retrieve a food reward across preoperative training than middle-aged animals.

3.2. Postoperative testing

3.2.1. Effects of lesion

As shown in Fig. 4 for the young monkeys, a MANOVA with hand (intact and impaired) as the independent variables and the number of postoperative days to return to preoperative latencies to retrieve a food reward for each well as the dependent variables revealed a significant effect of hand on the number of postoperative days to return to preoperative levels of performance on the WD well ($p < 0.02$), WS well ($p < 0.002$), ND well ($p < 0.04$), but not on NS well ($p < 0.26$) for the young animals. Similarly, as shown in Fig. 5 for the middle aged monkeys, a MANOVA revealed a significant effect of hand on the number of postoperative days to return to preoperative levels of performance on the WS well ($p < 0.01$), ND well ($p < 0.05$), and NS well ($p < 0.03$), but not for WD well ($p < 0.07$). Thus analyses for both young and middle-aged subjects revealed that both age groups required more postoperative days to return to criterion with the impaired hand than the intact hand. While there was a slight difference in preoperative performance between the young and middle-aged animals on the HDT, the analysis of postoperative rate of recovery reported here is a within subject measure, utilizing the preoperative baseline of each animal as a criterion score and therefore demonstrates the difference in recovery rates for the 2 age groups.

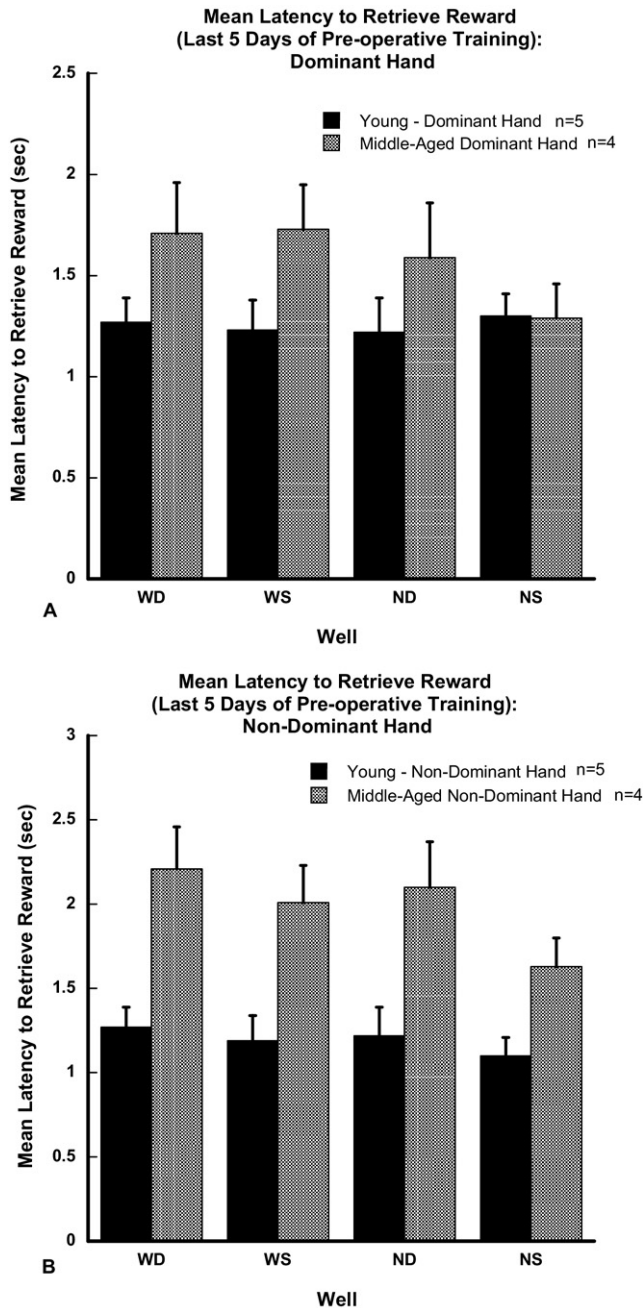


Fig. 3. A 3-way repeated measures analysis of variance (ANOVA) revealed no significant group differences with the dominant (A) or nondominant (B) hand for the mean latency to retrieve a food reward from each well for the last 5 days of preoperative training. Error bars represent standard error of the mean.

3.2.2. Effects of age on impaired hand

As shown in Fig. 6, a MANOVA with age group (young and middle age) as the independent variable and latency to retrieve a food reward from each well as the dependent variables revealed a significant effect of group on the number of postoperative days to return to preoperative levels of performance with the impaired hand on the WD well ($p <$

0.05), WS well ($p < 0.01$), ND well ($p < 0.03$), and NS well ($p < 0.02$).

3.2.3. Effects of age on intact hand

As shown in Fig. 7, a MANOVA with age group (young and middle age) as the independent variable and latency to retrieve a food reward from each well as the dependent variables revealed a significant effect of group on the number of postoperative days to return to preoperative levels of performance with the intact hand but only for the WS well ($p < 0.04$). There was no significant effect of group on the number of postoperative days to return to preoperative levels of performance with the intact hand for the WD well ($p < 0.08$), ND well ($p < 0.24$), and NS well ($p < 0.46$). Young animals required 30–40 days of testing postoperatively to return to criterion with their intact hand and the middle-aged animals required 60–80 days of testing postoperatively to return to criterion with their intact hand. While significantly less than what was required to reach criterion with the impaired hand it still represents a mild impairment in the intact hand. Review of human clinical data support these findings in that functional impairments, though mild, have also been well documented in the “intact hand” in humans following a stroke or ischemic event (Desrosiers et al., 1996; Noskin et al., 2008; Schaefer et al., 2007; Sunderland et al., 1999).

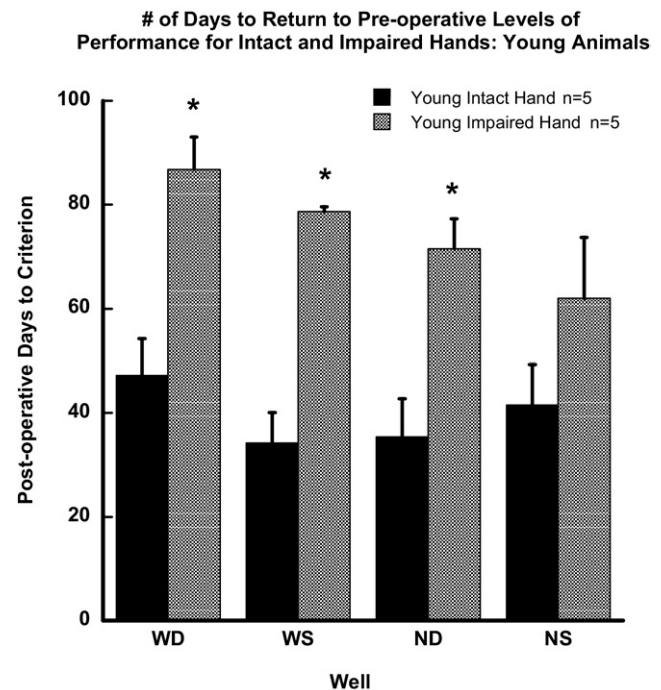


Fig. 4. A single multivariate analysis of variance (MANOVA) revealed a significant effect of hand on the number of postoperative days to return to preoperative levels of performance on the wide-deep (WD) ($p < 0.02$), wide-shallow (WS) ($p < 0.002$), and narrow-deep (ND) wells ($p < 0.04$), but not on the narrow-shallow (NS) well ($p < 0.26$) for the young animals. Error bars represent standard error of the mean. Asterisks indicate a significant difference.

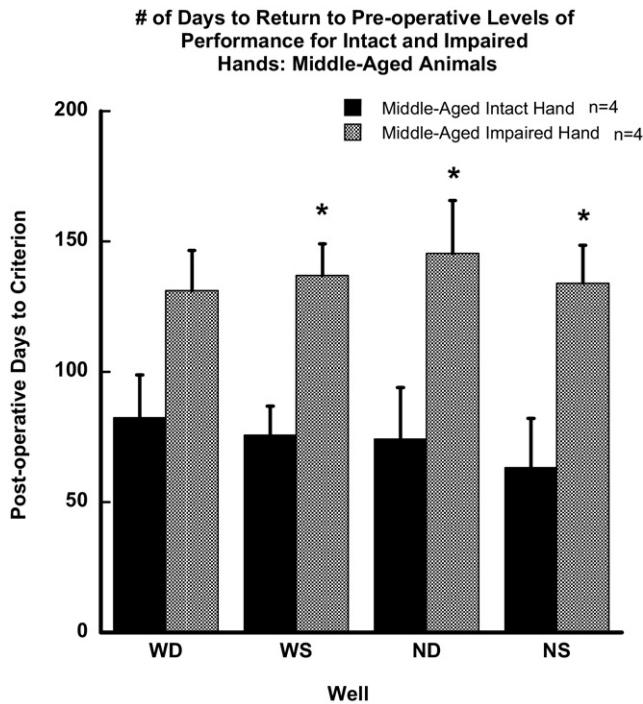


Fig. 5. A single multivariate analysis of variance (MANOVA) revealed a significant effect of hand on the number of postoperative days to return to preoperative levels of performance on wide-shallow (WS) ($p < 0.01$), narrow-deep (ND) ($p < 0.05$), and narrow-shallow (NS) wells ($p < 0.03$), but not for the wide-deep (WD) well ($p < 0.07$) for the middle-aged animals. Error bars represent standard error of the mean. Asterisks indicate a significant difference.

3.2.4. Assessment of grasp patterns

In addition to determining the latency to retrieve food wells, trials of the HDT were analyzed with our Grasp Assessment Scale to determine the nature of the grasp pattern demonstrated by each monkey during the recovery period. Separate 1-way ANOVAs to determine the presence of an age-related difference in the time required to reach criterion for recovery of grasp pattern (3 consecutive days of a rating of grade 5 for a given well) with the impaired hand revealed no effect of age for any of the 4 wells (WD well, ($F(1,4) = 5.11$, $p = 0.08$); WS well, ($F(1,4) = 1.25$, $p = 0.32$); ND well, ($F(1,4) = 4.03$, $p = 0.11$); NS well, ($F(1,4) = 3.51$, $p = 0.13$)).

To further explore the rate of recovery of grasp patterns in young and middle-aged monkeys, the number of days required to reach criterion with the impaired hand for each monkey was divided into 3 equal blocks (each consisting of 1 third of the total days to return to criterion) and the average rating for each period was determined. As shown in Fig. 8A, Mann-Whitney U test revealed significant age-related differences on the WD well for the 2nd ($p = 0.05$) and 3rd ($p = 0.05$) time periods. Analyses for the other wells showed significant differences on WS well for the 1st ($p = 0.05$) and 2nd ($p = 0.05$) time periods (Fig. 8B), ND well for the 1st ($p = 0.05$) time period (Fig. 8C), and for the

NS well for the 2nd ($p = 0.05$) time period (Fig. 8D). This suggests that during the initial stages of recovery both young and middle-aged animals perform in a similar manner on the test in terms of recovery of grasp function. However, the young monkeys appear to achieve a higher rating score in the second 2 time periods than the aged animals. It should be noted though, that the aged animals actually demonstrate the greatest level of recovery relative to their initial impairment, though this is due to the fact they were more impaired at the task in the initial stages of recovery than the young animals.

Finally, the mean rating across the postoperative period for the impaired hand was determined and a 1-way ANOVA revealed a significant effect of age for all wells: WD well, ($F(1,4) = 13.16$, $p = 0.02$); WS well, ($F(1,4) = 8.55$, $p = 0.04$); ND well, ($F(1,4) = 22.33$, $p = 0.009$); and NS well, ($F(1,4) = 28.65$, $p = 0.005$) (Fig. 9). This supports the findings from above that the young monkeys demonstrated less impairment in the recovery of grasp function with the impaired hand than older monkeys.

4. Discussion

4.1. Summary

We have demonstrated in this study that both young and middle-aged monkeys are severely and significantly im-

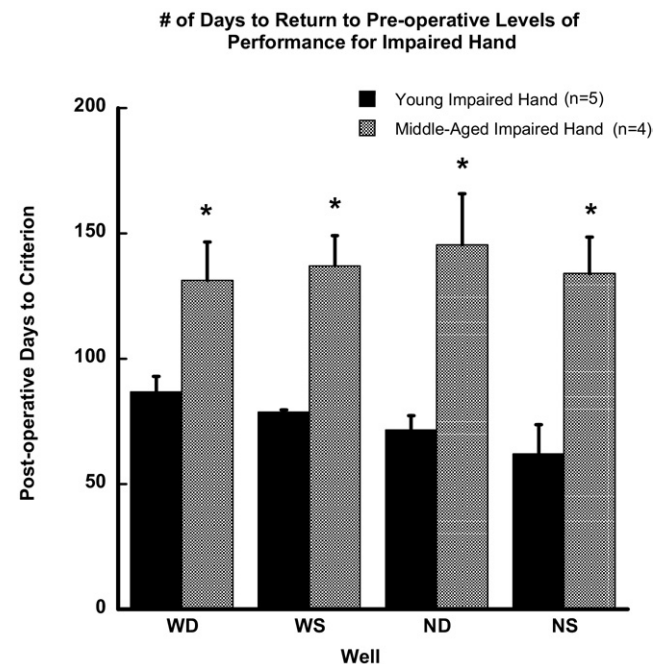


Fig. 6. A single multivariate analysis of variance (MANOVA) revealed a significant effect of group on the number of postoperative days to return to preoperative levels of performance with the impaired hand for the wide-deep (WD) ($p < 0.05$), wide-shallow (WS) ($p < 0.01$), narrow-deep (ND) ($p < 0.03$), and narrow-shallow (NS) wells ($p < 0.02$). Error bars represent standard error of the mean. Asterisks indicate a significant difference.

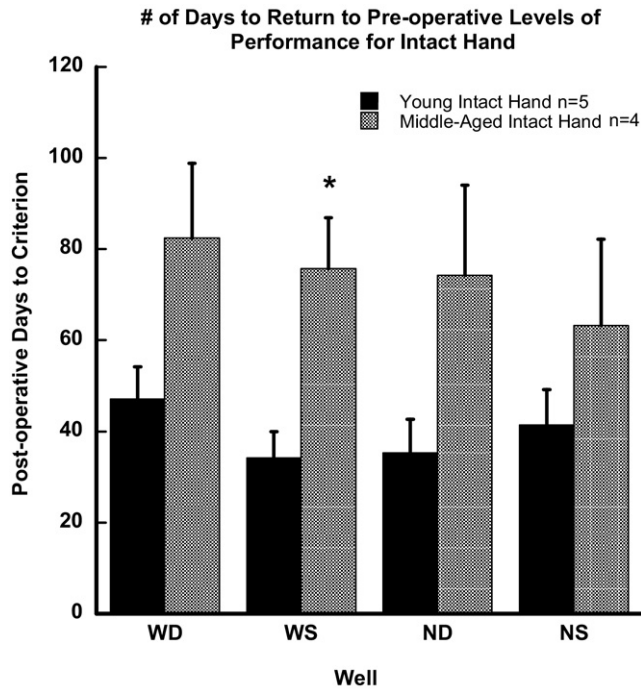


Fig. 7. A single multivariate analysis of variance (MANOVA) revealed a significant effect of group on the number of postoperative days to return to preoperative levels of performance with the intact hand for the wide-shallow (WS) well ($p < 0.04$). There was no significant effect of group on the number of postoperative days to return to preoperative levels of performance with the intact hand for the wide-deep (WD) ($p < 0.08$), narrow-deep (ND) ($p < 0.24$), and narrow-shallow (NS) wells ($p < 0.46$). Error bars represent standard error of the mean. Asterisks indicate a significant difference.

paired on a task of hand-digit function following an ischemic event in the primary motor cortex (M1) as evidenced by the number of postoperative days required to return to criterion with the impaired hand. However, the middle-aged animals required significantly longer to recover fine motor function of the impaired hand than young monkeys. Additionally, our quantitative assessments also revealed that in both the young and middle-aged animals, there was a significant postoperative impairment of the intact hand, as evidenced by the number of postoperative days required to return to criterion with the intact hand, a finding similar to reports in humans with unilateral stroke of motor cortex (Desrosiers et al., 1996; Noskin et al., 2008; Schaefer et al., 2007; Sunderland et al., 1999). For the dominant hand, while both young and aged animals were severely impaired on this task relative to the nondominant hand controlled by the intact cortex, all subjects did return to preoperative levels of performance based on the latency to retrieve a food reward. However, they did this by developing compensatory “scooping” movements of the digits rather than through recovery of prestroke finger-thumb grasp patterns. As measured by our Grasp Assessment Scale, while the intact hand recovered normal grasp patterns in a few weeks, all subjects demonstrated less mature grasp patterns with the impaired

hand throughout recovery and even when preoperative latencies had been achieved. Compared with middle-aged monkeys, the younger subjects recovered a significantly greater degree of normal grasp pattern (i.e., a higher overall rating as assessed by our Grasp Assessment Scale), though the middle-aged monkeys demonstrated the greatest absolute amount of recovery due to the fact that as a group they started with a greater degree of impairment than the young and hence started from a lower baseline (see Figs. 8 and 9).

4.2. Comparison with human stroke

The nature of the impairment and subsequent recovery in our rhesus monkeys is very similar to what is observed in human stroke patients. In human patients recovering from strokes of M1, there is evidence of varying degrees of hemiparesis, with impairment of upper extremity function occurring most frequently (Cirstea and Levin, 2000; Duncan et al., 1983; Fridman et al., 2004; Noskin et al., 2008; Raghavan et al., 2010; Sunderland, 2000; Winstein et al., 1999). Impairments of the upper extremity in humans include difficulties with the execution of coordinated digit movement during grasping (such as finger-thumb grasping), reduced dexterity and grip strength, and decreased muscle tone (Heller et al., 1987; Jørgensen et al., 1995; Lum et al., 2009; Seo et al., 2010; Sunderland et al., 1989; Wade, 1989; Wade et al., 1983). The greatest degree of recovery of function of the upper extremity occurs in the first few months after the stroke. However, with continued rehabilitative therapy, improvements in function have been observed for months and years following the stroke (Carter et al., 2010; De Souza et al., 1980; Heller et al., 1987; Oujamaa et al., 2009; Schaechter, 2004; Wade et al., 1983; Wolf et al., 2006, 2008). In the present study, we observed a marked improvement in the latency to retrieve food rewards in the first 2–3 weeks of postoperative testing as control of the digits improved. This continued at a slower rate throughout the remainder of the postoperative testing period and ultimately performance latency returned to preoperative levels.

The testing sessions in this study required intensive use of the hand and digits in order to successfully retrieve the food rewards and therefore may be considered similar to rehabilitative therapy used with human stroke patients. Specifically, the HDT was developed to require the monkey to use the impaired hand on 40% of trials in order to approximate constraint-induced movement therapy (CIMT), a widely used therapy with human stroke patients. CIMT requires the individual to exclusively use their impaired extremity to complete tasks while the unimpaired arm is restrained in a sling. The use of CIMT for the enhancement of recovery of function of the upper extremity has been widely investigated, and to date, this type of therapy appears to result in significant and long term improvements in function (Langhorne et al., 2009; Liepert et al., 1998; O'Dell et al., 2009; Sirtori et al., 2009; Wittenberg and Schaechter,

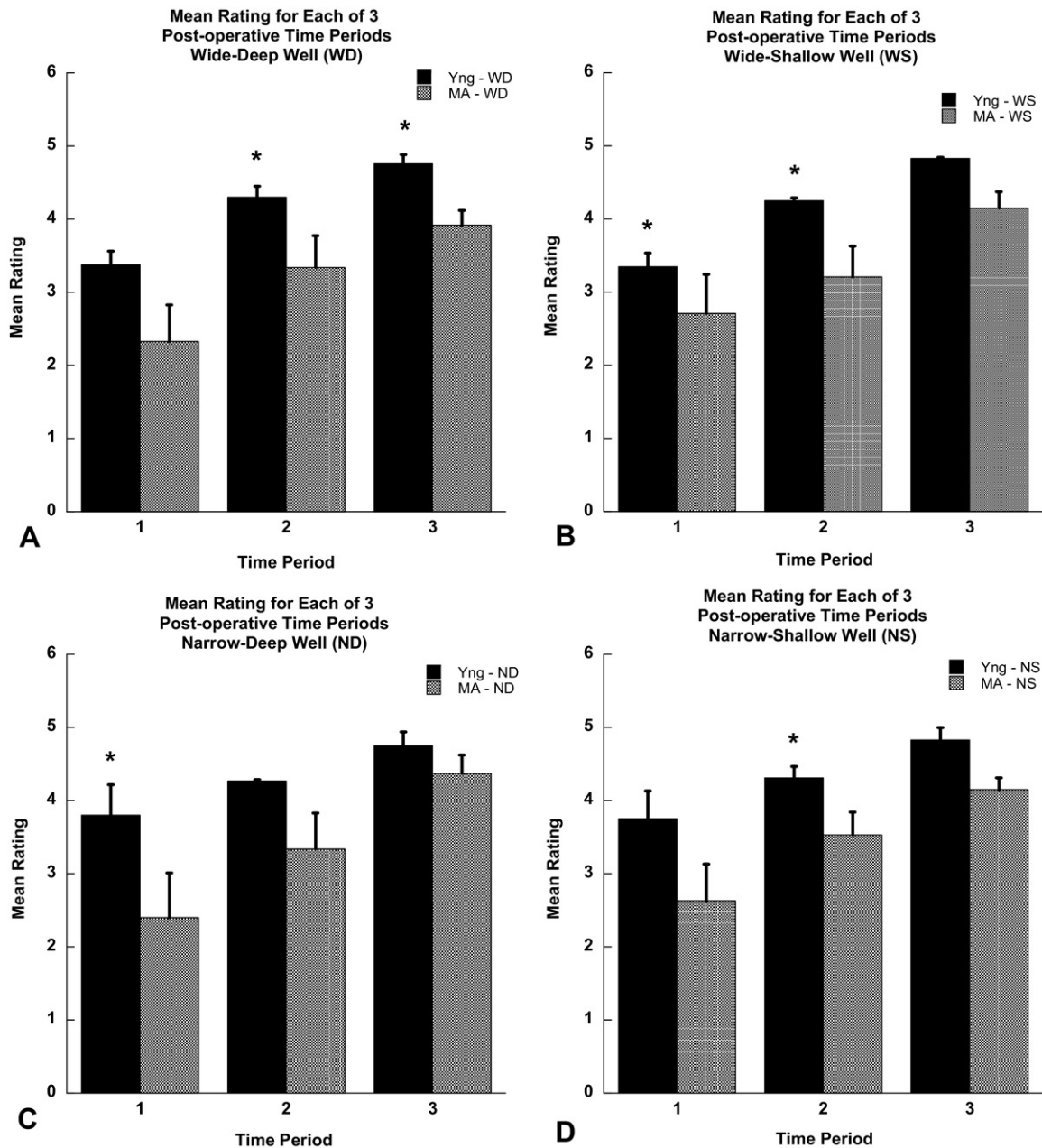


Fig. 8. The number of days required to reach criterion for the impaired hand for each monkey was divided into 3 equal blocks (each consisting of 1 third of the total days to return to criterion) and the average rating for each period was determined. A Mann-Whitney *U* test revealed significant age-related differences on the wide-deep (WD) well for the 2nd ($p = 0.05$) and 3rd ($p = 0.05$) time periods (A). Analyses for the other wells showed significant differences on the wide-shallow (WS) well for the 1st ($p = 0.05$) and 2nd ($p = 0.05$) time periods (B), the narrow-deep (ND) well for the 1st ($p = 0.05$) time period (C), and for the narrow-shallow (NS) well for the 2nd ($p = 0.05$) time period (D). Error bars represent standard error of the mean. Asterisks indicate a significant difference.

2009; Wolf et al., 2006, 2008). The improved hand function in the animals in this study after 4–8 months of testing on the HDT supports growing evidence in human literature of the significant benefit of rehabilitative therapy, such as CIMT, on recovery following stroke.

While the latency to retrieve a food reward did return to baseline levels of performance for all animals, the assessment of the nature of the grasp patterns used during the

postoperative period revealed that the monkeys progressed from severe deficits in the flexion and extension of the digits with evidence of mass action of the digits, to increasing flexion and extension of digits and isolated digit movement until effective compensatory “scooping” movements were established. These compensatory movements consisted of a scooping action with the middle and/or fourth finger moving the treat to the palm of the hand or to the thenar eminence

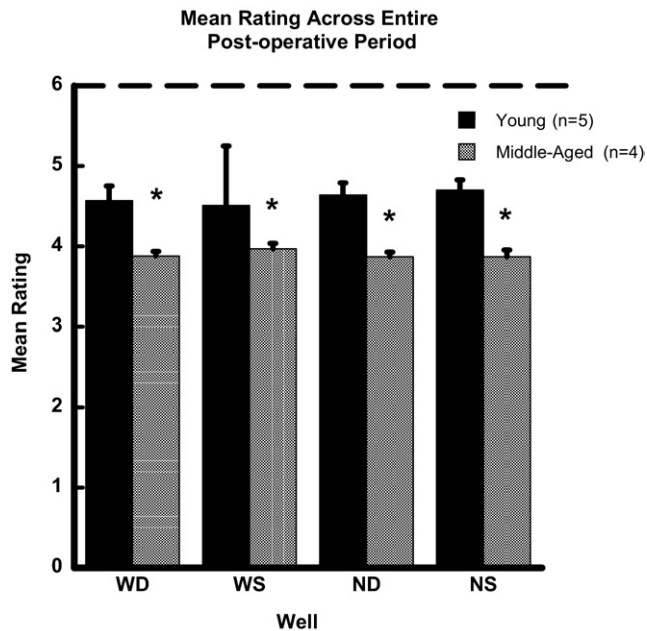


Fig. 9. Mean rating of grasp pattern of the impaired hand across the postoperative testing period (wide-deep, $p < 0.02$; wide-shallow, $p < 0.04$; narrow-deep, $p < 0.009$; narrow-shallow, $p < 0.005$). Error bars represent standard error of the mean. Asterisks indicate a significant difference.

rather than returning to the precise “finger-thumb” grasp that was used during preoperative training. The development of compensatory strategies for reaching and grasping has been defined as a qualitatively different approach to completing a motor function and has been repeatedly documented in human stroke patients (Metz et al., 2005; Michaelsen et al., 2004; Raghavan et al., 2010; Roby-Brami et al., 2003). A recent study investigating the development of compensatory hand movements in patients with hemiparesis reported prolonged reach-to-grasp time and reduced finger abduction, flexion of the proximal interphalangeal joints (PIP) and extension of the metacarpophalangeal joints (MCP) in patients completing reaching and grasping tasks. During recovery these patients developed compensatory strategies to complete the tasks that involved significantly increased flexion of the MCP joint and significantly reduced flexion of the PIP joint. This was in contrast to healthy, age-matched control subjects who demonstrated greater flexion of the PIP joint than the MCP joint, which is consistent with normal grasp patterns (Raghavan et al., 2010). The patients in this study developed compensatory use of alternative joints, whereas the monkeys in our study developed compensatory movements by utilizing various digits. However, it would be advantageous in future studies to attempt to assess the precise digit joint that is being used during postoperative testing in order to produce a more detailed analysis of their compensatory movements as was conducted by Raghavan and colleagues (2010).

Functional impairments, though mild and transient, are also observed in the “intact hand” in humans following a

stroke (Desrosiers et al., 1996; Noskin et al., 2008; Schaefer et al., 2007; Sunderland et al., 1999). Similarly, in this study, we observed that for both the young and middle-aged animals, there was an impairment in the intact hand as evidenced by the number of postoperative days required to return to criterion with the intact hand. Though still requiring less time to return to criterion than with the impaired hand, the young and middle-aged animals required an average of 35–80 days to return to criterion with the intact hand. Several explanations have been proposed to account for this effect. First, it has been suggested that each hemisphere may be specialized for different aspects of motor function and that optimal performance of some motor tasks requires bilateral cortical control. Therefore, a stroke in 1 hemisphere could disrupt performance on such tasks using either hand (Noskin et al., 2008; Schaefer et al., 2007). Second, disruption of ipsilateral descending projections of the corticospinal tracts that remain uncrossed may play a role in the impaired function of the unaffected hand (Desrosiers et al., 1996; Nathan and Tavi, 1990). Third, weakness and/or mild impairment in the ipsilateral hand may occur as a result of reorganization of function of the impaired hand into the contralesional intact cortex (Allred et al., 2010). Finally, the hand not used specifically in a motor movement is typically used to stabilize the body during that movement. If the stabilizing hand is paretic, this could impair stabilization and disrupt the ability of the unaffected hand to function normally (Allred et al., 2010; Desrosiers et al., 1996). We have observed in the animals in this study that they do in fact stabilize themselves during task performance with 1 hand. In the early stages of recovery, the time in which there is evidence of impaired use of the intact hand, we have observed that the animals have difficulties effectively stabilizing themselves with the impaired hand while performing the task with their intact hand. With increasing recovery of the impaired hand there is less difficulty using it for stabilization and that corresponds with the reduction in latencies to retrieve the food reward with the intact hand. Therefore it seems most likely that the difficulties noted with the intact hand may be related to the weakness and decreased coordination of the impaired hand in terms of stabilizing the body for reaching and grasping the food reward.

4.3. Comparison with other nonhuman primate models of ischemia

A small number of nonhuman primate models of ischemia and stroke currently exist and have provided a wealth of knowledge about ischemic injury, recovery of function, and cortical reorganization. Several groups, using cynomolgus monkeys, have investigated the efficacy of various therapeutic interventions after ischemic damage to several regions of cortex (Chin et al., 2010; Li et al., 2010; Sato et al., 2009). While providing valuable insight into the effectiveness of these treatments, the animals in these studies did

not complete extensive motor testing and there was considerable variability in lesion placement.

More directly relevant to the current study, Nudo and colleagues have extensively investigated normal motor function of the hand and digits and the recovery of function, cortical plasticity, and cortical reorganization following ischemia in a squirrel monkey (lissencephalic) model of ischemia (Barbay et al., 2006; Dancause et al., 2005, 2006; Eisner-Janowicz et al., 2008; Friel and Nudo, 1998; Friel et al., 2000; Frost et al., 2003; Nudo and Milliken, 1996; Plautz and Nudo, 2005). Friel and Nudo (1998) assessed recovery of function following an ischemic injury to the hand area of M1 based on the number of finger flexions and the specific movement patterns used to retrieve pellets in comparison with preoperative movement patterns. They demonstrated that squirrel monkeys develop stereotypical retrieval patterns during pretraining and that after ischemic injury some of the monkeys develop alternate strategies for retrieval during rehabilitative therapy that include changes in the number of finger flexions and the occurrence of movements at the wrist and forearm. While the precise joint in the fingers that were flexed was not recorded, these findings are similar in nature to the results from the current study and the studies with human stroke patients discussed above. However, the use of our Grasp Assessment Scale adapted from human occupational therapy measures provides a more quantitative assessment of the precise hand and digit movements and in future studies can be expanded to provide even greater specificity of the precise digits and joints that are utilized during testing. Overall, there is evidence from studies with humans and nonhuman primates of the development of compensatory strategies that are coordinated movements that utilize alternative muscles and/or joints in order to complete reaching and grasp movements (Cirstea and Levin, 2000; Langhorne et al., 2009; Raghavan et al., 2010). While functionally useful, these compensatory movements are limiting and it has been suggested that compensatory movements may be maladaptive and limit the recovery of "normal" function (Cirstea and Levin, 2000) and therefore should be discouraged during therapy.

The goal of therapeutic intervention for stroke patients is to develop therapies that promote full recovery of "normal" function. The identification of the occurrence and the nature of compensatory strategies is therefore critical for rehabilitation in order to enhance the development of optimal motor strategies. The use of a measure of precise movement of the digits during recovery, such as the Grasp Assessment Scale adapted for this study, not only to provide an in-depth analysis of the nature of the compensatory movement but also because quantitative measures of performance alone (e.g., latency to retrieve or number of pellets retrieved) could fail to detect the development of compensatory movements and may lead to the incorrect conclusion about the nature of recovery. This issue has also been raised in several clinical studies assessing the efficacy of tests of arm func-

tion (Hammer and Lindmark, 2010; Heller et al., 1987; Lum et al., 2009; Sunderland et al., 1999; Wade, 1989). It has been suggested that many clinical assessments of arm function provide a pass/fail or a latency score but not a detailed assessment of the nature of upper extremity movements and that future studies should include more detailed analysis of these movements (Raghavan et al., 2010; Saver, 2007). Animal models of ischemia and stroke that employ the type of lesion utilized in this study and those of Nudo and colleagues (small, focal, and reproducible), along with the quantitative assessment of recovery in terms of both latency to respond and the nature of grasp patterns will be invaluable for both investigations of the recovery of function and cortical reorganization and the neural mechanisms underlying recovery.

4.4. Plasticity and the aging brain

In animal models of cortical injury it has been demonstrated that following damage to primary motor cortex involving the representation of the upper limb, that representation expands into cortical areas surrounding the lesion. There is also expansion of the representation in the ipsilateral premotor cortex as well as in the contralateral primary motor and premotor cortices (Butefisch et al., 2005; Frost et al., 2003; Green, 2003; Green et al., 1999a, 1999b; Jang et al., 2003; Miyai et al., 2002; Nudo, 1999; Nudo and Friel, 1999; Ward, 2004, 2005). In addition, there is an increase in neuronal activity in supplementary motor and premotor areas and an increase in the size of the hand-digit representation in the premotor area following recovery from stroke (Frost et al., 2003).

Insight into the basis for such changes in the cortical representation are suggested by studies investigating postinjury plasticity and the underlying cellular and molecular mechanisms. Like the physiological studies cited above, studies of plasticity have demonstrated functional alterations in regions of primary motor cortex surrounding the injury as well as in more remote areas such the premotor area ipsilateral to the lesion as well as in motor areas of the contralateral hemisphere (Biernaskie et al., 2005; Carmichael and Chesselet, 2002; Cramer et al., 2006; Dancause et al., 2005; Hsu and Jones, 2006; Nudo, 2003, 2006; Nudo and Milliken, 1996; Stroemer et al., 1995; Ward, 2004). Most frequently it has been demonstrated that following cortical ischemia there is evidence in peri-infarct regions of dendritic remodeling (Buga et al., 2008; Jones and Schallert, 1992), increased growth-associated protein 43 (GAP-43 immunoreactivity), and increased synaptophysin staining (Buga et al., 2008; Carmichael, 2006; Carmichael and Chesselet, 2002; Stroemer et al., 1998, 1995), axonal sprouting (Buga et al., 2008; Carmichael et al., 2001, 2005; Li and Carmichael, 2006), and angiogenesis (Beck and Plate, 2009; Beck et al., 2000; Hayashi et al., 2003; Krupinski et al., 1994; Marti et al., 2000). However, these

studies have been conducted in models of stroke that only include young animals.

The highest incidence of stroke occurs in aged individuals and considerable evidence exists demonstrating that aging may alter many physiological and metabolic processes in the nervous system that may play a role in plasticity and cortical reorganization (Badan et al., 2003, 2004; Dinse, 2005; Li and Carmichael, 2006; Li et al., 2005; Popa-Wagner et al., 2006, 2007a, 2007b; Wang et al., 2003). While there are conflicting data in regards to the effects of age on stroke size and the mechanisms of recovery, numerous studies have demonstrated age-related differences in functional recovery and the cytological response to stroke (Badan et al., 2003; Buga et al., 2008; Davis et al., 1995; Nagayama et al., 1999; Popa-Wagner et al., 2006, 2007a, 2007b; Wang et al., 2003). Rodent models of stroke and aging have demonstrated decreased expression of plasticity-associated genes, cellular proliferation in the early stages of recovery, increased vulnerability to ischemia, increased conversion of ischemic tissue to infarction, accelerated infarct development, and most commonly premature formation of a glial scar (Buga et al., 2008; Popa-Wagner et al., 2006; Roberts et al., 1997). It has been demonstrated that the accelerated formation of a glial scar is associated with cell proliferation that occurs very early in recovery originating from the vasculature in the infarct zone (Badan et al., 2003, 2004; Popa-Wagner et al., 2006, 2007a, 2007b). It has also been demonstrated that there is increased oxidative protein and DNA damage and reduced neuroprotective responses in peri-infarct tissue in aged animals (Li and Carmichael, 2006; Li et al., 2005). This group has suggested that as a result there may be reduced neuronal plasticity in this area that could limit functional recovery in the aged brain.

Despite recent evidence that increasing age effects outcome and recovery of function following cortical injury, the mechanisms underlying the response of the aged brain to injury and ischemia are still unclear (Hoane et al., 2004; Statler et al., 2001). It does appear though that the aging brain does produce a response to the ischemia however, this response appears to occur very early in recovery and is accelerated compared with in younger brains and therefore may affect the degree and rate of recovery. The present model affords the opportunity to further investigate the effect of age on the mechanisms underlying plasticity and the relationship of altered plasticity to the slower recovery observed in middle-aged monkeys. Future studies with this model will include remapping of motor cortices and surrounding tissue to determine areas of reorganization and immunohistochemical studies to investigate the mechanisms of plasticity in the aged brain.

4.5. Conclusions

This study has demonstrated significant age-related changes in the nature and rate of recovery of function of the

hand and digits following an ischemic lesion limited to the hand representation of M1 of the rhesus monkey. This is, to our knowledge, the first study to characterize the effect of age on recovery of motor function in a gyrencephalic non-human primate (rhesus monkey). Given that age is 1 of the most significant risk factors for stroke, these data should be of value in the development and testing of therapeutic interventions, both behavioral and pharmaceutical, aimed at facilitating the rate and degree of recovery. To this end, identifying the regions supporting this recovery and the underlying cellular mechanisms are critical next steps. As increasing evidence demonstrates age-related differences in the response of the brain to cortical injury, the development of animal models with older animals is critical to the investigation of the mechanisms underlying recovery of function and for the assessment of therapeutic interventions. This model will be used in future studies to investigate the mechanisms of recovery in middle-aged animals and the efficacy of various therapeutic interventions on recovery and the cellular response to ischemia.

Disclosure statement

There are no conflicts of interest for any author of this manuscript, including financial, personal or other relationships with other people or organizations within 3 years of beginning the work that could inappropriately influence the work.

Animal maintenance and research was conducted both in accordance with the guidelines of the National Institutes of Health Committee on Laboratory Animal Resources and with the procedures approved by the Institutional Animal Use and Care Committee of the Boston University Medical Campus.

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