Published in final edited form as:

Restor Neurol Neurosci. 2016 September 21; 34(5): 827–848. doi:10.3233/RNN-160661.

Inosine enhances recovery of grasp following cortical injury to the primary motor cortex of the rhesus monkey

Tara L. Moore^{a,b,*}, Monica A. Pessina^a, Seth P. Finklestein^d, Ronald J. Killiany^a, Bethany Bowley^a, Larry Benowitz^e, and Douglas L. Rosene^{a,c}

^aDepartment of Anatomy & Neurobiology, Boston University School of Medicine, Boston, MA, USA

^bDepartment of Neurology, Boston University School of Medicine, Boston, MA, USA

^cYerkes National Primate Research Center, Emory University, Atlanta, GA, USA

^dStemetix, Inc, Needham, MA, USA

^eDepartment of Neurosurgery and F.M. Kirby Neurobiology Center, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA

Abstract

Background: Inosine, a naturally occurring purine nucleoside, has been shown to stimulate axonal growth in cell culture and promote corticospinal tract axons to sprout collateral branches after stroke, spinal cord injury and TBI in rodent models.

Objective: To explore the effects of inosine on the recovery of motor function following cortical injury in the rhesus monkey.

Methods: After being trained on a test of fine motor function of the hand, monkeys received a lesion limited to the area of the hand representation in primary motor cortex. Beginning 24 hours after this injury and continuing daily thereafter, monkeys received orally administered inosine (500 mg) or placebo. Retesting of motor function began on the 14th day after injury and continued for 12 weeks.

Results: During the first 14 days after surgery, there was evidence of significant recovery within the inosine-treated group on measures of fine motor function of the hand, measures of hand strength and digit flexion. While there was no effect of treatment on the time to retrieve a reward, the treated monkeys returned to asymptotic levels of grasp performance significantly faster than the untreated monkeys. Additionally, the treated monkeys evidenced a greater degree of recovery in terms of maturity of grasp pattern.

Conclusion: These findings demonstrate that inosine can enhance recovery of function following cortical injury in monkeys.

Keywords

Inosine; cortical injury; rhesus monkey; recovery; motor function

*Corresponding author: Tara L. Moore, Tel.: +617 638 4054; Fax: +617 6384922; tmoore@bu.edu.

1. Introduction

Death of neural tissue occurs as the result of traumatic brain injury, stroke and neurodegenerative diseases. Depending on the location and extent of injury, impairments can include disruptions of cognitive, sensory or motor functions. Damage that includes the motor cortex can lead to disruption of motor function, especially fine motor control of the hand which can affect the ability of an individual to complete activities of daily living. While full recovery is rare, partial recovery of motor function often occurs over a number of weeks after cortical injury. Both clinical and animal studies suggest that recovery results from adaptive plasticity and reorganization in intact cortical areas (Carmichael et al., 2016; Caleo, 2015; Barbay et al., 2015; Gleichman & Carmichael, 2014; Di Pino et al., 2014; Starkey & Schwab, 2014; Grefkes & Ward, 2014; Nudo, 2013; Ko & Yoon, 2013; Milliken et al., 2013; Dancause & Nudo, 2011, Nudo, 2011). This has been documented in non-human primates (NHPs) following injury to primary motor cortex (M1).

Investigations have demonstrated reorganization in motor cortex immediately surrounding the site of injury, as well as in adjacent supplementary motor cortices, premotor cortices, and even somatosensory cortex in NHPs (Morecraft et al., 2016; Darling et al., 2016; Touvykine et al., 2016; Plautz et al., 2016; Wyss et al., 2013; Moore et al., 2012; Darling et al., 2011a,b, 2009; Eisner-Janowicz et al., 2008; Fukushima et al., 2007; Dancause et al., 2006, 2005; Plautz & Nudo, 2005; Frost et al., 2003; Roitberg et al., 2003; Nudo et al., 2003; Liu and Rouiller, 1999; Nudo & Milliken, 1996). In addition, NHP models have been utilized to test the efficacy of various therapies (e.g. cortical stimulation, rehabilitation training, constraint limb therapy, pharmaceutical agents) to enhance cortical reorganization and recovery of function following cortical injury (Plautz et al., 2016; Hoogewoud et al., 2013; Moore et al., 2013; Wyss et al., 2013; Milliken et al., 2013; Higo, 2010; Friel et al., 2007; Barbay et al., 2006a, b, Plautz and Nudo, 2005; Plautz et al., 2000, 2003; Friel et al., 2000; Friel & Nudo, 1998).

Recovery that has been found appears to correlate with a variety of mechanistic processes including dendritic remodeling, synaptogenesis, axonal sprouting and angiogenesis in peri-infarct regions. Nevertheless, our understanding of the mechanisms underlying reorganization and plasticity still remains unclear but points to plasticity within these areas as a target for therapeutic intervention (Carmichael et al., 2016; Iaci et al., 2016; Liu & Chopp, 2015; Meng et al., 2014; Barratt et al., 2014; Nouri & Cramer, 2011; Benowitz & Carmichael, 2010; Iaci et al., 2010; Benowitz & Carmichael, 2010; Beck & Plate, 2009; Zhang & Chopp, 2009; Cramer, 2008a,b, 2011; Buga et al., 2008; Carmichael, 2006; Li & Carmichael, 2006; Hayashi et al., 2003; Carmichael & Chesselet, 2002; Beck et al., 2000; Marti et al., 2000; Stroemer et al., 1998; Benowitz & Routtenberg, 1997). Indeed, a variety of agents that promote plasticity have enhanced functional recovery in rodent models of ischemic stroke (Duricki et al., 2016; George & Steinberg, 2015; Chollet et al., 2014; Dachir et al., 2014; Dhawan et al., 2011; Zai et al., 2009; Papadopoulos, 2009).

One such agent, inosine, enhanced the ability of neurons in undamaged contralesional hemisphere to extend axon collaterals into brainstem and spinal cord areas that have lost

normal innervation and this rewiring is accompanied by improved function of the impaired limb (Zai et al., 2011, 2009; Smith et al., 2007). Inosine is a naturally occurring purine nucleoside that is released by cells in response to metabolic stress, crosses the cell membrane and activates Mst3b, a protein kinase that plays a central role in the cell-signaling pathway through which trophic factors stimulate axonal growth (Lorber et al., 2009; Irwin et al., 2006; Benowitz et al., 1998). In cell culture, inosine stimulates neurons to upregulate expression of growth-associated genes and extend their axons (Conta & Stelzner, 2008; Petrusch et al., 2000; Benowitz et al., 1998; Zurn & Do, 1988). After spinal cord injury (SCI, dorsal hemisection) in rats, intravenously delivered inosine enhances collateral sprouting from injured corticospinal and raphespinal axons, leading to increased formation of “detour circuits” and improved use of the hindlimbs (Kim et al., 2013). To build upon the promising findings with inosine in rodent models of stroke, TBI and SCI, and following the recommendations of the Stroke Therapy Academic Industry Roundtable (STAIR, 1999) that promising therapies be tested in gyrencephalic species prior to human trials the current study explored, for the first time, the efficacy of daily oral doses of inosine on the recovery of motor function following cortical injury in the rhesus monkey.

2. Methods

2.1. Subjects

A total of 8 adult male rhesus monkeys (*M. mulatta*) were used ranging in age from 5–15 years (approximately equivalent to humans from 15–45 years of age, Tigges et al., 1988). Monkeys were obtained from the Caribbean National Primate Research Center and the National Institute of Mental Health. Before entering the study, they received medical examinations that included serum chemistry, hematology, and urine and fecal analysis. Health records were screened to exclude monkeys with a history of malnutrition, diabetes, neurological diseases or chronic illnesses. All monkeys were given initial MRI scans to ensure there were no occult brain abnormalities.

Once enrolled in the study, monkeys were housed in the Laboratory Animal Science Center of Boston University Medical Campus. This is an AAALAC accredited facility, managed by a licensed veterinarian. Experiments were conducted with the approval of the Institutional Animal Care and Use Committee (IACUC) of the Boston University Medical Campus.

2.2. Pre-operative training on fine motor function testing

As described in detail previously (Moore et al., 2010), monkeys were trained on tests of fine motor function of the hand using a testing apparatus that controls, quantifies and video records responses from each hand (Fig. 1). Using this apparatus, the monkeys were trained on the Hand Dexterity Task (HDT) for a total of 15 days (Monday, Wednesday and Friday each week for 5 weeks). The HDT is a modified version of a Klüver board (Klüver, 1935) and requires precise control of the digits, particularly apposition of the thumb and index finger, to retrieve a small, visible food reward (M&M's, Mars, Inc) from two different size round wells in a Plexiglas tray. Food rewards were round and approximately 1 cm in diameter. Both wells were 1 cm deep. The large well was 25 mm wide and the small well was 18 mm wide. Time to retrieve the food reward is recorded by a timer that is connected to

photocells that are located in the openings on each side of the apparatus (Fig. 1). The timer starts when the monkey puts a hand through one of the openings, triggering the photocells to start the timer. The timer stops when the monkey removes his hand. An experimenter records whether or not the reward was successfully retrieved and the response (time to retrieve) is recorded. The HDT has been used to assess fine motor function of the hand and digits in adult monkeys with and without injury to the hand representation in the motor cortex as well as to compare the performance of middle-aged rhesus monkeys to young adult monkeys (Moore et al., 2010, 2012). Each test day consists of 16 trials for each of the two well sizes and for each hand resulting in a total of 32 trials. The order of trials for each hand and well follows a pseudorandom balanced sequence to eliminate any order effects. Monkeys are given 30 seconds to complete a trial. If they do not or would not complete a trial in 30 sec, the trial is terminated and the monkey is given one additional opportunity to complete that trial. After a second failed attempt, a non-response is recorded, the monkey's difficulties are noted in the study record and the next trial is initiated.

2.3. Hand preference

At the completion of pre-training on the HDT, free choice trials with both sides of the apparatus baited and accessible are administered to determine which hand is "preferred". This assessment is also compared with the pre-operative acquisition rates for each hand. Based on this assessment, the cortical injury is targeted to the hand representation of the hemisphere controlling the preferred hand to ensure that monkeys are motivated to use the impaired hand during post-operative testing.

2.4. Group assignment and blinding

After the completion of pre-training, the monkeys were randomly assigned in a balanced fashion based on pre-operative performance (latencies) to receive either the experimental inosine treatment or vehicle/placebo. All study personnel (surgeons, technicians, behavioral testers, etc.) were kept blind to the assignment during surgery and during all post-operative *in-vivo* procedures, testing and terminal brain tissue harvest and processing.

2.5. Electrophysiological mapping of the hand representation in motor cortex

All surgical procedures were carried out under aseptic conditions. Each monkey was sedated with ketamine hydrochloride (10 mg/kg) and anesthetized with intravenous sodium pentobarbital (15–25 mg/kg) to effect and antibiotics and analgesics were given prior to and during surgery. Many anesthetics are known to be neuroprotective and/or differentially affect cortical excitability and this could potentially impact cortical mapping, the extent of damage that occurs and recovery of function. For our surgical procedure, we use sodium pentobarbital as the anesthesia as it has limited effect on cortical injury and allows for motoric responses to be elicited during the electrical stimulation to map the motor cortex. Heart rate, respiration, temperature, blood oxygenation and muscle tonus were monitored to ensure physiological homeostasis and a safe surgical level of anesthesia. The head was stabilized in a stereotactic apparatus, and a midline incision was made followed by reflection of the temporalis muscle. A bone flap approximately 40 mm in anterior to posterior extent and 35 mm in medial to lateral extent was made centered over the frontal and parietal lobes.

The bone was removed in one piece for later replacement. The dura was incised to expose the precentral sulcus and primary motor cortex.

To create reproducible cortical injury and motor deficits, a calibrated photograph of the precentral gyrus was taken and printed. The precentral gyrus was then systematically explored using electrical stimulation delivered through a small monopolar silver ball electrode placed gently on the surface of the pia to evoke movements. A surface electrode was used rather than a sharp electrode that could penetrate the cortex (e.g. Nudo & Milliken, 1996) in order to avoid extraneous damage to the motor cortex outside the hand representation. The stimulating electrode was moved across the precentral gyrus systematically in rows spaced approximately 2 mm apart (anterior to posterior) and separated from the next row by approximately 2 mm (medial to lateral) as shown in Fig. 2. Monopolar stimulus pulses of 250 msec duration at amplitudes from 2.0 to 3.0 mA were delivered at each site once every 2 seconds first singly and then in a short train of 4 pulses delivered over 40 msec at a rate of 100 Hz. Non-responsive sites were further tested with a 200 Hz train consisting of 4 or 8 pulses of 2 msec duration delivered over 20 or 40 msec respectively. During each stimulation, a trained observer noted muscle movements (eg. distinct movement or twitches of muscle) in specific areas of the digits, hand, forearm or arm, both visually and by palpation. The intensity of the motor response in the hand and digits was graded on a scale of 1 to 5 (barely visible to maximal). Specific stimulation sites with the lowest threshold and highest motor response were marked on the calibrated photograph creating a cortical surface map of the hand area that was used to guide placement of the lesion. (Fig. 2).

2.6. Placement of selective cortical injury

Using the map described above, cortical injury was induced by making a small incision in the pia at the dorsal limit of the mapped representation. A small glass suction pipette was then inserted under the pia and used to bluntly transect the small penetrating arterioles as they enter the underlying cortex. Suction and irrigation with sterile saline was sufficient to stanch any bleeding and maintain a clear field. Since the hand representation is known to extend down the rostral bank of the central sulcus, the sulcus was then opened down to the fundus along the length of the gyral hand representation by microdissection with a small glass pipette and a blunt periosteal elevator, taking care to leave the somatosensory areas on the caudal bank intact. The pia was then dissected with the glass pipette all the way down to the fundus of the sulcus. The hand area in the sulcus was not electro-physiologically mapped with the electrode to avoid inadvertent damage to the somatosensory areas on the caudal bank of the central sulcus as this mapping requires prolonged retraction. However, we have verified in terminal experiments the presence of the hand representation on the rostral bank (unpublished). This pial dissection of penetrating vessels removes the blood supply to the cortex of the hand representation, inducing damage that extends down to the underlying white matter. The cortical map and lesion for each monkey is shown in Fig. 2. An example of the area and extent of the lesion is shown in Fig. 3.

After the lesion was made and any bleeding stopped, the dura was closed, the bone flap was sutured back in place using small burr holes placed in the flap and the muscles, fascia and

skin were closed in layers. Immediately following surgery, antibiotics and analgesics were administered and the monkey was kept in an incubator and monitored continuously until anesthesia wore off. They were then returned to their home cage and monitored continuously until fully awake and able to eat and drink. For the next 3–7 days (or as needed) monkeys were given analgesics and monitored regularly for any signs of infection or complications.

2.7. Inosine administration

Beginning 24 hours after surgery, monkeys began receiving oral doses of inosine (Anabol Naturals, Santa Cruz, California) or placebo. Inosine was given in a single dose of 500 mg each day by mixing it with 4 ounces of flavored yogurt. The placebo consisted of 4 ounces of yogurt with 50 mg of powered sugar. The doses of inosine and placebo were prepared by a research technician not involved in post-operative testing and were coded so that other technicians feeding the mixture to the monkeys were also blinded to treatment group. The initial administration of either inosine or placebo was given 24 hours after the completion of surgery to induce cortical injury and was continued throughout the 14-week post-operative period with administration at the same time each day. On days of behavioral testing, a technician administered the yogurt compound to the monkeys immediately after the completion of testing. On all days, the technician verified that the monkeys consumed the entire portion of yogurt.

2.8. Initial cage-side post-operative assessment

Beginning immediately after recovery from surgery and continuing for 14 days, the upper extremity motor function of each monkey was rated daily in its home cage. Both upper limbs were rated for level of function in terms of tone, tremor, fine motor function of the hand, strength of the hand, digit flexion as well as movements of the forearm, wrist, arm and shoulder using our NHP Upper Extremity Motor Dysfunction Assessment Scale (Table 1). This scale was adapted from Zhang et al., 2000 and the National Institutes of Health Stroke Scale (<http://www.nihstrokescale.org/>).

2.9. Post-operative testing

Post-operative testing on the HDT began two weeks after surgery and continued for 12 weeks. Testing on the HDT was conducted on Monday, Wednesday and Friday of each week. Seventy percent of the trials required the use of the impaired hand, while 30% were given to the intact hand. The 30% of trials given to the unimpaired hand provide sufficient rewards to maintain motivation and sufficient data to demonstrate that effects are not due to generalized changes in motivation or motor function. Each animal was given 30 seconds to complete a trial as in pre-operative training. The forced use of the impaired hand on 70% of the trials is similar in nature to constraint-induced therapy used in human rehabilitation which forces use of the impaired limb (Kwakkel et al., 2016; Corbetta et al., 2015; Souza et al., 2015). Testing continued for 12 weeks, the time estimated for monkeys receiving placebo to achieve asymptotic stable performance.

2.10. Grasp pattern assessment

Performance on the HDT during pre-operative training and post-operative testing was recorded (up to 30fps) with fixed placement cameras (Logitech, Newark, CA). A licensed Occupational Therapist (M.P.) who has clinical experience in the treatment of patients with upper extremity impairment following stroke, and a trained research technician (B.B.) analyzed the video using our NHP Grasp Assessment Scale (Moore et al., 2012). This scale was adapted from the Eshkol-Wachman Movement Notation (Whishaw et al., 2002; Carr et al., 1985) and the Fugl-Meyer Motor Assessment scale (Fugl-Meyer et al., 1975). Our scale rates the position of the digits during grasp and the pattern of grasp and release to provide a semi-quantitative measure of maturity of the grasp pattern. The original scale consisted of 6 hierarchical stages that included distinct features for each stage (see Moore et al., 2012). In order to increase the sensitivity of this scale for detecting recovery of function, the scale was modified to include 8 divided hierarchical stages, for a total of 14 units with the maximum score of 8 reflecting normal grasp patterns (functional pinch between thumb and one individual digit: Table 2).

2.11. Perfusion and tissue processing

At the end of the 14 week post-operative period, monkeys were deeply anesthetized with IV sodium pentobarbital (25 mg/kg to effect) and were euthanized by exsanguination during transcardial perfusion of the brain, first for no more than 5 minutes with 4°C Krebs buffer at pH 7.4 and then with 8 liters of 4% paraformaldehyde, pH 7.4 over 10 minutes to completely fix the brain. The skull was opened and the brain was photographed *in situ* with the photograph aligned to the perspective of the cortical map used to create the lesion. The brain was blocked *in situ* in the coronal plane to ensure reproducible planes of section during later processing. The brain was removed from the skull, weighed and post-fixed overnight in 4% paraformaldehyde for no more than 18 hours. To eliminate freezing artifact the brain was then transferred to cryoprotectant solutions of glycerol and buffer and flash frozen at -75° C and stored at -80° C (Rosene, et al., 1986). Frozen blocks were later removed from storage and cut on a sliding microtome into interrupted series of coronal sections (eight series of 30 µm thick sections and one 60 mm thick series, with 300 µm spacing between sections). The 60 µm series was immediately mounted on microscope slides and stained with thionin for lesion reconstruction. Other series were collected in buffer with 15% glycerol, equilibrated overnight at 4°C and stored at -80°C for later histochemical processing.

2.12. Lesion volume

To reconstruct the lesion, all sections through the entire extent of the lesion (range of 23 to 31 sections spaced at 300 µm intervals) were digitized using an Epson 4490 Scanner to create high-resolution JPEGs of each section. These images were imported into Image J (Schneider et al., 2012). To estimate the volume of the lesion, the intact gray matter from the depth of the cingulate sulcus on the medial surface to the depth of the lateral sulcus on the lateral surface was outlined excluding the extent of the lesion as defined by the absence of tissue and the presence of intense gliosis or other scar tissue. The same procedure was done at the equivalent anatomical location in the opposite intact hemisphere. The total volume outlined in each hemisphere was determined using the Cavalieri estimator taking the sum of

the areas of each section and multiplying by the distance (0.30 mm) between sections (Rosen and Harry, 1990). This allowed us to estimate the volume of the lesion by subtracting the volume of the gray matter in the damaged hemisphere from the volume in the contralateral intact hemisphere (Table 3). Figure 4 shows representative thionin sections through the lesion from one monkey treated with inosine and one monkey given a placebo.

3. Data analysis

3.1. Data analysis

All data analysis was conducted using Data Desk v 6.3 (Data Description, Inc, Ithaca, NY). The analysis showed no difference in the results between the small and large wells so only data from the large well is presented.

3.2. Pre-operative HDT

To ensure that pre-operative balancing of group assignment was effective, the mean time to retrieve a food reward from the large well of the HDT over the entire pre-operative testing was determined for each hand. An Independent Samples Student's *t*-test was used to compare the performance of placebo and treated monkeys on the large well.

3.3. Post-operative NHP upper extremity motor dysfunction scale

Beginning on post-operative day 1, the degree of motor impairment in the upper extremity was assessed for each monkey in its home cage using our adapted NHP Upper Extremity Motor Dysfunction Scale which assesses impairments in tone, tremor, fine motor function of the hand, strength of the hand, digit flexion as well as movements of the forearm, wrist, arm and shoulder. Each measure is rated on a scale of 0 (no impairment) up to impairments of 3, 4 or 5 as shown in Table 1. The mean rating for each animal on the first three (1–3) and last three (12–14) post-operative days prior to the commencement of HDT testing was determined for each measure. The difference in the mean rating between days 1–3 and days 12–14 was then calculated for each animal as a measure of recovery and compared between groups. Separate Independent Samples Student's *t*-tests were used to compare the recovery scores between groups on the measures of fine motor control, strength of the hands and digit flexion. The Bonferroni correction was used to correct for multiple comparisons.

3.4. Post-operative HDT

The mean time to retrieve the food reward from the large well on the HDT across the 12-week post-operative period was determined for the large well and the Independent Samples Student's *t*-test was used to compare the performance between placebo and treated animals on this measure. In addition, the mean time to retrieve a food reward on the HDT during the initial week of post-operative testing and the final week of testing on the HDT for the placebo and treated animals was compared using a two-way repeated measures ANOVA with group (placebo vs. treated) as a between-subject variable and time (initial testing week vs. final week) as a within-subjects variable.

3.5. Post-operative grasp assessment

The mean number of post-operative days required to return to asymptotic levels of grasp on the HDT with the impaired hand was determined. A return to asymptotic levels of performance was defined as a grasp assessment score at pre-operative levels (score of 8; functional pinch between thumb and one digit) for 5 consecutive days for each well. If a monkey did not return to pre-operative levels of grasp (score of 8), then the first 5 consecutive days at their highest rating was used as asymptotic performance. An Independent Samples Student's *t*-test was used to compare the number of post-operative days required to return to asymptotic levels of grasp on the HDT for the impaired hand of the placebo and treated monkeys. In addition, the highest grasp assessment rating achieved across three consecutive testing days during the post-operative period for the HDT was compared between the two groups using a Mann-Whitney U test.

3.6. Lesion volume

An Independent Samples Student's *t*-test was used to compare the volume of the lesion between the placebo and treated animals.

3.7. Correlations between lesion volume and motor function

Pearson's *r* was used to determine if there was a significant linear relationship between the number of post-operative days required to return to pre-operative levels of performance on the HDT, final grasp assessment on the HDT, and final lesion volume.

4. Results

4.1. Pre-operative HDT

The mean time to retrieve a food reward from the large well of the HDT with the preferred hand for the last 5 days of pre-operative testing was determined for each monkey (Table 3). An Independent Samples Student's *t*-test was used to compare the performance of monkeys assigned to the placebo and treatment groups on the large well. This analysis detected no significant difference between the placebo and treated monkeys ($p = 0.35$), confirming the effectiveness of pre-operative pseudo-random balancing of group assignment.

4.2. Post-operative NHP upper extremity motor dysfunction assessment scale

Separate Independent Samples Student's *t*-tests revealed a significant difference between the groups in terms of the change in the mean rating on the measures of fine motor function, strength of the hand and digit flexion between days 1–3 and days 12–14 ($p < 0.003$), with a greater degree of recovery in the inosine-treated animals (Fig. 5). A Bonferroni correction was used to correct for multiple comparisons.

4.3. Post-operative HDT

As shown in Fig. 6, there was no difference between groups on the mean time to retrieve the food reward from the large well of the HDT across the entire 12-week post-operative period ($p < 0.654$, Student's *t*-test). A two-way repeated measures ANOVA, comparing the time to retrieve the rewards during the initial week of testing and then during the final week of

testing with group (placebo vs. treated) as a between-subject variable and time (initial week vs. final week) as a within-subjects variable, revealed no overall effect of group [$F(1, 6) = 0.25$, $p = 0.623$] and no significant group by time interaction [$F(1, 15) = 0.03$, $p = 0.781$]. However, as expected, there was a significant effect of time [$F(1, 6) = 32.11$, $p = 0.001$] as both groups improved (Fig. 6). Figure 7 shows the mean time to retrieve a food reward each day of the pre- and post-operative testing period for each monkey.

4.4. Post-operative grasp assessment

An Independent Samples Student's *t*-test was used to compare the mean number of post-operative days required to reach asymptotic levels of grasp performance on the large well for the impaired hand of placebo compared to treated monkeys. As shown in Fig. 8, this analysis revealed a significant difference between groups ($p < 0.0001$), with treated monkeys showing a more rapid recovery of grasp pattern. In addition, the highest grasp assessment rating achieved across the post-operative period was analyzed with a Mann-Whitney U test. Figure 9 shows the mean grasp rating each day of the pre- and post-operative testing period for each monkey.

Finally, as shown in Fig. 10, this analysis revealed a significant difference between groups on the large well for the highest grasp rating achieved ($p < 0.02$), with three of the four treated monkeys returning to pre-operative grasp patterns compared to none of the placebo monkeys. Figure 11 shows video clips from one monkey that received placebo and one monkey that received inosine illustrating typical responses at the end of the 14-week evaluation. The monkeys that did not return to the precise finger-thumb pinch action, instead developed a compensatory "scooping" motion that involved using several fingers to push the food reward into the palm of the hand and then close the fingers around the food reward to retrieve it from the well. This represents a compensatory action rather than a more complete recovery as the "scooping" motion does not involve isolated digits as observed during pre-operative testing.

4.5. Lesion volume

Analysis of the volume of the lesions revealed no significant differences between groups (Table 3). Pearson's *r* Correlation revealed no significant linear association between lesion volume and the highest grasp rating ($r = -0.318$, $p = 0.54$) across the 12-week recovery period or between volume and the number of post-operative days required to return to pre-operative grasp levels of performance on the HDT ($r = 0.739$, $p = 0.09$) (Fig. 12)

5. Discussion

5.1. Summary of findings

During the first 14 days after surgery, there was a significant degree of recovery within the inosine-treated group on measures of fine motor function of the hand, measures of hand strength and digit flexion. There was no effect of treatment on the time to retrieve a reward from the large well on the HDT across the entire 12-week recovery period indicating that the between-group differences found in fine motor control do not reflect differences in motivation or gross movements. In terms of fine motor grasp pattern, the treated group

returned to asymptotic levels of grasp performance significantly faster than the untreated group. Additionally, the treated monkeys evidenced a greater degree of recovery in terms of maturity of grasp pattern with three of the four monkeys attaining pre-operative grasp positions. These findings suggest that while all monkeys returned to pre-operative latencies to respond, treatment with oral doses of inosine enhanced the recovery of function in terms of a return to normal finger-thumb grasp pattern rather than developing the compensatory “scooping” grasp pattern observed in monkeys that received placebo (Fig. 11). In the clinical context, the enhanced recovery of grasp pattern suggests that inosine facilitates greater recovery from this type of cortical injury and motor impairment. To our knowledge, this is the first study to demonstrate the positive effects of inosine for promoting recovery of function following cortical injury in a non-human primate.

5.2. Recovery of motor function: Full recovery versus developing compensatory movements

With advances in our understanding of neuroplasticity in the adult brain and growth in the field of neurorehabilitation, there has been a significant increase in the number of studies investigating new therapies for recovery from cortical injury. Recovery of motor function following damage to the motor cortex has been described as either compensatory motor movements, full recovery of normal (baseline) function, or a combination of both occurring during the recovery period (Moon et al., 2009; Metz et al., 2005; Whishaw, 2000). The distinction between full recovery and compensatory recovery is important as the development of compensatory movements in the upper extremity may not translate into functional use in human patients, as the development of compensatory strategies may inhibit full recovery (Lum et al., 2009).

In order to unify the use of these terms, Levin and colleagues (2009) offered the following definitions of each term; “recovery of motor performance is the reappearance of elemental motor patterns present prior to central nervous system injury” and “motor compensation is the appearance of new motor patterns resulting from the adaptation of remaining motor elements or substitution, meaning that functions are taken over, replaced, or substituted by different end effectors or body segments”. These definitions provide a more precise description of the variance of recovery of motor function that may occur after cortical injury or stroke and enable us to better understand outcomes in studies of treatments for cortical injury and stroke.

Numerous studies have demonstrated that a certain degree of spontaneous recovery occurs after cortical damage that usually consists of only compensatory motor movements. These movements can sometimes be enhanced by rehabilitative training (Nishibe et al., 2015; Barbay et al., 2006a; Nudo & Friel, 1999; Nudo et al., 1996). Full recovery to normal baseline function for upper extremity impairments in terms of both speed of performance and grasp patterns is still rare in humans and in animal models of cortical injury, even with training (Plautz et al., 2016). In the present study, all of the monkeys with a cortical injury to the hand area of primary motor cortex recovered to baseline levels of performance in terms of time to retrieve a food reward. However, only the monkeys treated with daily oral inosine demonstrated greater recovery in terms of a return to normal grasp patterns (finger-thumb

pinch; see Fig. 11H). Specifically, three of the four monkeys receiving inosine demonstrated a recovery of the precise finger-thumb grasp pattern that is typically observed during pre-operative training on our motor tasks. In contrast, the monkeys that received a placebo developed a compensatory “scooping” action that involved using multiple digits to draw the food reward out of the well and into the palm of the hand (Fig. 11C&D). While compensatory motor function may enable a patient to complete a task, a return to normal baseline movement patterns reduces the need for activity adaptations and may allow a more complete return to independence in activities of daily living.

5.3. Mechanisms of recovery: Cortical reorganization and cortical plasticity

There is increasing evidence that recovery of motor function following ischemic injury results from reorganization in intact cortical areas including surrounding primary motor, pre-motor and somatosensory areas and that the reorganization is related to dendritic remodeling, synaptogenesis, axonal sprouting and angiogenesis (Carmichael et al., 2016; Liu & Chopp, 2015; Caleo, 2015; Barbay et al., 2015; Meng et al., 2014; Barratt et al., 2014; Gleichman & Carmichael, 2014; Di Pino et al., 2014; Starkey & Schwab, 2014; Nudo, 2013; Kim et al., 2013; Ko & Yoon, 2013; Milliken et al., 2013; Hoogewoud et al., 2013; Ueno et al., 2012; Dancause & Nudo, 2011; Nudo, 2011; Benowitz & Carmichael, 2010; Beck & Plate, 2009; Zhang and Chopp, 2009; Buga et al., 2008; Carmichael, 2006; Li & Carmichael, 2006; Dancause et al., 2005; Carmichael et al., 2005; Hayashi et al., 2003; Benowitz et al., 2002; Carmichael & Chesselet, 2002; Beck et al., 2000; Marti et al., 2000; Liu & Rouiller, 1999; Stroemer et al., 1998; Benowitz & Routtenberg, 1997). It is hypothesized that these processes underlying cortical plasticity likely depend on endogenously secreted growth factors and changes in expression of particular growth associated proteins (Xiong et al., 2010; Chopp et al., 2008; Qu et al., 2007; Sofroniew, 2005; Mahmood et al., 2004, 2005; Chopp and Li, 2002). Inosine acts intracellularly on neurons by activating the protein kinase Mst3b and inducing the expression of GAP-43 and other gene products that promote axonal sprouting into denervated areas of the nervous system and this plasticity correlates with improved recovery of motor function following cortical or spinal cord injury (Kim et al., 2013; Zai et al., 2009; Lorber et al., 2009; Irwin et al., 2006; Chen et al., 2002). In a rodent model of ischemic stroke, rats that received inosine were able to retrieve food pellets at 80% of their pre-operative level while untreated rats only reached 40% of their pre-operative level of performance (Zai et al., 2009). In addition, in a model of spinal cord injury, rodents that received inosine showed a greater accuracy in hindlimb placement on the ladder-rung walking test (Kim et al., 2013). In both of these studies, there was a far greater degree of axonal sprouting in the animals treated with inosine. Interestingly, just as in the monkeys reported here, in both rodent models, there was no effect of inosine on lesion size. Together these findings suggest that the action of inosine in the present study likely facilitates restorative processes rather than having a neuroprotective function.

Support for plasticity as the mechanism of recovery in the present study also comes from studies investigating the efficacy of cell therapies as a treatment for stroke. Similar to inosine, it is thought that cell therapies do not repair damaged tissue, but instead, produce growth factors and may also induce intrinsic parenchymal cells to produce trophic factors

(Kaesler et al., 2010; Xiong et al., 2010; Chopp et al., 2008; Qu et al., 2007; Sofroniew, 2005; Mahmood et al., 2004, 2005; Chopp & Li, 2002). Specifically, Zhang et al. (2011) demonstrated that a cell therapy enhanced axonal sprouting, synaptogenesis and neural progenitor proliferation following stroke, similar to the reported effects of inosine. Further, using the same non-human primate model of cortical injury, we demonstrated that administration of a cell therapy after injury resulted in a pattern of recovery very similar to what we observed after administering inosine (Moore et al., 2013). Taken together, the findings with rodent models of cortical injury receiving inosine, and our findings in non-human primates receiving inosine or a cell therapy following cortical injury, support the hypothesis that recovery of motor function following cortical injury most likely results from cortical plasticity that facilitates a more complete recovery of motor function, including speed and grasp patterns.

5.4. Clinical translation of inosine

Complementing earlier studies showing that inosine enhances recovery of function following stroke, TBI and spinal cord injury in rodents, the present study shows for the first time that inosine is effective after cortical injury in the rhesus monkey. Inosine has also been administered in human clinical trials for multiple sclerosis and Parkinson's disease and has been proven to be safe in doses up 3000 mg/day (Schwarzchild et al., 2014; Markowitz et al., 2009). Athletes have used inosine as a nutritional supplement for decades, and inosine supplements are widely available commercially. Given the effectiveness of inosine in promoting cortical plasticity, axonal sprouting and dendritic branching (Cipriani et al., 2014; daRocha et al., 2013; Zai et al., 2009; Lorber et al., 2009; Irwin et al., 2006; Benowitz et al., 1998), the present evidence of efficacy after cortical injury in a non-human primate, combined with a long history of safe use, indicates a need for clinical trials with inosine after cortical injury and spinal cord injury.

5.5. Conclusions

Using the terms from Levin et al., 2009, three of the four monkeys in the current study that received daily doses of inosine following cortical injury demonstrated recovery of function in that they returned to pre-operative baseline motor movements of the hand and digits (finger-thumb pinch grasp). In contrast, the monkeys that received a placebo developed a compensatory grasp pattern that included using multiple digits to "scoop" the reward into the palm of their hand. While the monkeys receiving the placebo were able to perform this compensatory motor function as quickly as the monkeys that demonstrated more complete recovery of the finger-thumb pinch grasp, the return to a more mature grasp pattern would be more functional for human patients and should be the goal of therapeutics for cortical injury and stroke. Based on studies in rodent models of stroke, inosine appears to enhance neural plasticity, which may be the basis for the recovery of function observed in the present study. Further study of cortical tissue from these monkeys is currently being completed and may provide further insights into the mechanisms underlying recovery.

Acknowledgments

This study was supported by NIH-NINDS R21NS081261 and a grant from Advanced Technologies and Regenerative Medicine (ATRM), LLC. RR# 101115-PR. On December 30, 2012, ATRM merged into DePuy

Orthopaedics, Inc. We would like to thank Karen Slater, Reese Edwards, Penny Shultz for their technical assistance with this study.

References

- Barbay S, Plautz EJ, Zoubina E, Frost SB, Cramer SC, & Nudo RJ (2015). Effects of postinfarct myelin-associated glycoprotein antibody treatment on motor recovery and motor map plasticity in squirrel monkeys. *Stroke*, 46(6), 1620–1625. doi: 10.1161/STROKEAHA.114.008088 [PubMed: 25931462]
- Barbay S, Plautz EJ, Friel KM, Frost SB, Dancause N, Stowe AM, & Nudo RJ (2006a). Behavioral and neurophysiological effects of delayed training following a small ischemic infarct in primary motor cortex of squirrel monkeys. *Experimental Brain Research*, 169(1), 106–116. [PubMed: 16273404]
- Barbay S, Zoubina EV, Dancause N, Frost SB, Eisner-Janowicz I, Stowe AM, Plautz EJ, & Nudo RJ (2006b). A single injection of D-amphetamine facilitates improvements in motor training following a focal cortical infarct in squirrel monkeys. *Neurorehabil Neural Repair*, 20(4), 455–458. [PubMed: 17082500]
- Barratt HE, Lanman TA, & Carmichael ST (2014). Mouse intracerebral hemorrhage models produce different degrees of initial and delayed damage, axonal sprouting, and recovery. *Journal of Cerebral Blood Flow & Metabolism*, 34(9), 1463–1471. doi: 10.1038/jcbfm.2014.107 [PubMed: 24917041]
- Beck H, Acker T, Wiessner C, Allegrini PR, & Plate KH (2000). Expression of angiopoietin-1, angiopoietin-2, and tie receptors after middle cerebral artery occlusion in the rat. *American Journal of Pathology*, 157(5), 1473–1483. [PubMed: 11073808]
- Beck H, & Plate KH (2009). Angiogenesis after cerebral ischemia. *Acta Neuropathologica*, 117(5), 481–496. [PubMed: 19142647]
- Benowitz LI, & Routtenberg A (1997). GAP-43: An intrinsic determinant of neuronal development and plasticity. *Trends Neurosciences*, 20(2), 84–91. Review.
- Benowitz LI, Jing Y, Tabibiazar R, Jo SA, Petrusch B, Stuermer CA, Rosenberg PA, & Irwin N (1998). Axon outgrowth is regulated by an intracellular purine-sensitive mechanism in retinal ganglion cells. *Journal of Biological Chemistry*, 273(45), 29626–29634. [PubMed: 9792672]
- Benowitz LI, Goldberg DE, & Irwin N (2002). Inosine stimulates axon growth in vitro and in the adult CNS. *Progress in Brain Research*, 137, 389–399. [PubMed: 12440381]
- Benowitz LI, & Carmichael ST (2010). Promoting axonal rewiring to improve outcome after stroke. *Neurobiology of Disease*, 37(2), 259–266. doi: 10.1016/j.nbd.2009.11.009 [PubMed: 19931616]
- Buga AM, Dunoiu C, Bălan A, & Popa-Wagner A (2008). Cellular and molecular mechanisms underlying neurorehabilitation after stroke in aged subjects. *Romanian Journal of Morphology and Embryology*, 49(3), 279–302. Review. [PubMed: 18758632]
- Caleo M (2015). Rehabilitation and plasticity following stroke: Insights from rodent models. *Neuroscience*, 311, 180–194. doi: 10.1016/j.neuroscience.2015.10.029 [PubMed: 26493858]
- Carmichael ST, & Chesselet MF (2002). Synchronous neuronal activity is a signal for axonal sprouting after cortical lesions in the adult. *Journal of Neurosciences*, 22(14), 6062–6070.
- Carmichael ST, Archibeque I, Luke L, Nolan T, Momiy J, & Li S (2005). Growth-associated gene expression after stroke: Evidence for a growth-promoting region in peri-infarct cortex. *Experimental Neurology*, 193(2), 291–311. [PubMed: 15869933]
- Carmichael ST (2006). Cellular and molecular mechanisms of neural repair after stroke: Making waves. *Annals of Neurology*, 59(5), 735–742. Review. [PubMed: 16634041]
- Carmichael ST, Kathirvelu B, Schweppe CA, & Nie EH (2016). Molecular, cellular and functional events in axonal sprouting after stroke. *Experimental Neurology* 210 pii: S0014-4886(16)30029-2. doi: 10.1016/j.expneurol.2016.02.007. [Epub ahead of print]
- Carr JH, Shepherd RB, Nordholm L & Lynne D (1985). Investigation of a new motor assessment scale for strokepatients. *Physical Therapy*, 65, 175–180. [PubMed: 3969398]
- Chen R, Cohen LG, & Hallett M (2002). Nervous system reorganization following injury. *Neuroscience*, 111(4), 761–773. Review. [PubMed: 12031403]

- Chollet F, Tardy J, Albucher JF, Raposo N, Acket B, Sattler V, Pariente J, & Loubinoux I (2014). Monoaminergic drugs for motor recovery after ischemic stroke. *Annals of Physical and Rehabilitation Medicine*, 57(8), 509–519. doi: 10.1016/j.rehab.2014.08.002 [PubMed: 25438666]
- Chopp M, Li Y, & Zhang J (2008). Plasticity and remodeling of brain. *Journal of the Neurological Sciences*, 265(1–2), 97–101. Review. [PubMed: 17610903]
- Chopp M, & Li Y (2002). Treatment of neural injury with marrow stromal cells. *Lancet Neurology*, 1(2), 92–100. Review. [PubMed: 12849513]
- Cipriani S, Bakshi R, & Schwarzschild MA (2014). Protection by inosine in a cellular model of Parkinson's disease. *Neuroscience*, 22(274), 242–249. doi: 10.1016/j.neuroscience.2014.05.038
- Conta AC, & Stelzner DJ (2008). Immunomodulatory effect of the purine nucleoside inosine following spinal cord contusion injury in rat. *Spinal Cord*, 46(1), 39–44. [PubMed: 17406376]
- Corbetta D, Sirtori V, Castellini G, Moja L, & Gatti R (2015). Constraint-induced movement therapy for upper extremities in people with stroke. *Cochrane Database Systematic Reviews* 10 8; (10):CD004433. doi: 10.1002/14651858.CD004433.pub3. Review.
- Cramer SC (2011). An overview of therapies to promote repair of the brain after stroke. *Head & Neck*, 33(Suppl 1), S5–S7. doi: 10.1002/hed.21840 [PubMed: 21901776]
- Cramer SC (2008a). Repairing the human brain after stroke: I. Mechanisms of spontaneous recovery. *Annals of Neurology*, 63(3), 272–287. Review. [PubMed: 18383072]
- Cramer SC (2008b). Repairing the human brain after stroke. II. Restorative therapies. *Annals of Neurology*, 63(5), 549–560. Review. [PubMed: 18481291]
- da Rocha Lapa F, de Oliveira AP, Accetturi BG, de Oliveira Martins I, Domingos HV, de Almeida Cabrini D, de Lima WT, & Santos AR (2013). Anti-inflammatory effects of inosine in allergic lung inflammation in mice: Evidence for the participation of adenosine A2A and A3 receptors. *Purinergic Signal*, 9(3), 325–336. doi: 10.1007/s11302-013-9351-x [PubMed: 23355189]
- Dachir S, Shabashov D, Trembovier V, Alexandrovich AG, Benowitz LI, & Shohami E (2014). Inosine improves functional recovery after experimental traumatic brain injury. *Brain Research*, 1555, 78–88. doi: 10.1016/j.brainres.2014.01.044 [PubMed: 24502983]
- Dancause N, & Nudo RJ (2011). Shaping plasticity to enhance recovery after injury. *Progress in Brain Research*, 192, 273–295. doi: 10.1016/B978-0-444-53355-5.00015-4 Review. [PubMed: 21763529]
- Dancause N, Barbay S, Frost SB, Zoubina EV, Plautz EJ, Mahnken JD, & Nudo RJ (2006). Effects of small ischemic lesions in the primary motor cortex on neuro-physiological organization in ventral premotor cortex. *J Neurophysiology*, 96(6), 3506–3511.
- Dancause N, Barbay S, Frost SB, Plautz EJ, Chen D, Zoubina EV, Stowe AM, & Nudo RJ (2005). Extensive cortical rewiring after brain injury. *Journal of Neuroscience*, 25(44), 10167–10179. [PubMed: 16267224]
- Darling WG, Pizzimenti MA, Rotella DL, Hynes SM, Ge J, Stilwell-Morecraft K, & Morecraft RJ (2016). Sensorimotor cortex injury effects on recovery of contralesional dexterous movements in *Macaca mulatta*. *Experimental Neurology*, 281, 37–52. doi: 10.1016/j.expneurol.2016.04.004. [PubMed: 27091225]
- Darling WG, Pizzimenti MA, & Morecraft RJ (2011a). Functional recovery following motor cortex lesions in non-human primates: Experimental implications for human stroke patients. *J Integr Neuroscience*, 10(3), 353–384. Review.
- Darling WG, Pizzimenti MA, Hynes SM, Rotella DL, Headley G, Ge J, Stilwell-Morecraft KS, McNeal DW, Solon-Cline KM, & Morecraft RJ (2011b). Volumetric effects of motor cortex injury on recovery of ipsilesional dexterous movements. *Experimental Neurology*, 231(1), 56–71. doi: 10.1016/j.expneurol.2011.05.015. [PubMed: 21703261]
- Darling WG, Pizzimenti MA, Rotella DL, Peterson CR, Hynes SM, Ge J, Solon K, McNeal DW, Stilwell-Morecraft KS, & Morecraft RJ (2009). Volumetric effects of motor cortex injury on recovery of dexterous movements. *Experimental Neurology*, 220(1), 90–108. doi: 10.1016/j.expneurol.2009.07.034. [PubMed: 19679127]
- Dhawan J, Benveniste H, Luo Z, Nawrocky M, Smith SD, & Biegon A (2011). A new look at glutamate and ischemia: NMDA agonist improves long-term functional outcome in a rat model of stroke. *Future Neurology*, 6(6), 823–834. [PubMed: 22140354]

- Di Pino G, Pellegrino G, Assenza G, Capone F, Ferreri F, Formica D, Ranieri F, Tombini M, Ziemann U, Rothwell JC, & Di Lazzaro V (2014). Modulation of brain plasticity in stroke: A novel model for neurorehabilitation. *Nat Rev Neurol*, 10(10), 597–608. doi: 10.1038/nrneurol.2014.162. Epub 2014 Sep 9. Review. [PubMed: 25201238]
- Duricki DA, Hutson TH, Kathe C, Soleman S, Gonzalez-Carter D, Petruska JC, Shine HD, Chen Q, Wood TC, Bernanos M, Cash D, Williams SC, Gage FH, & Moon LD (2016). Delayed intramuscular human neurotrophin-3 improves recovery in adult and elderly rats after stroke. *Brain*, 139(Pt. 1), 259–275. doi: 10.1093/brain/awv341 [PubMed: 26614754]
- Eisner-Janowicz I, Barbay S, Hoover E, Stowe AM, Frost SB, Plautz EJ, Nudo RJ. (2008). Early and late changes in the distal forelimb representation of the supplementary motor area after injury to frontal motor areas in the squirrel monkey. *J Neurophysiology*, 100(3), 1498–1512. doi: 10.1152/jn.90447.2008.
- Friel KM, Barbay S, Frost SB, Plautz EJ, Stowe AM, Dancause N, Zoubina EV, & Nudo RJ (2007). Effects of a rostral motor cortex lesion on primary motor cortex hand representation topography in primates. *Neurorehabil Neural Repair*, 21(1), 51–61. [PubMed: 17172554]
- Friel KM, Heddings AA, & Nudo RJ (2000). Effects of postlesion experience on behavioral recovery and neurophysiologic reorganization after cortical injury in primates. *Neurorehabil Neural Repair*, 14(3), 187–198. [PubMed: 11272475]
- Friel KM, Nudo, & R.J (1998). Recovery of motor function after focal cortical injury in primates: Compensatory movement patterns used during rehabilitative training. *Somatosensory and Motor Research*, 15(3), 173–189. [PubMed: 9874517]
- Frost SB, Barbay S, Friel KM, Plautz EJ, & Nudo RJ (2003). Reorganization of remote cortical regions after ischemic brain injury: A potential substrate for stroke recovery. *Journal of Neurophysiology*, 89(6), 3205–3214. [PubMed: 12783955]
- Fugl-Meyer AR, Jaasko L, Leyman I, Olsson S, & Steglind S (1975). The post-stroke hemiplegic patient. *Scandinavian Journal of Rehabilitation Medicine*, 7, 13–31. [PubMed: 1135616]
- Fukushima J, Kasahara S, Asaka T, Saito H, & Yamanaka M (2007). Behavioral Findings during Recovery after Experimental Stroke in Monkeys: Assessment with Modified Hand Performance Test. *Journal of Physical Therapy Science*, 19(1), 33–40.
- George PM, & Steinberg GK (2015). Novel Stroke Therapeutics: Unraveling Stroke Pathophysiology and Its Impact on Clinical Treatments. *Neuron*, 87(2), 297–309. doi: 10.1016/j.neuron.2015.05.041 Review [PubMed: 26182415]
- Gleichman AJ, & Carmichael ST (2014). Astrocytic therapies for neuronal repair in stroke. *Neuroscience Letters*, 565, 47–52. doi: 10.1016/j.neulet.2013.10.055 [PubMed: 24184876]
- Grefkes C & Ward NS (2014). Cortical reorganization after stroke: How much and how functional? *Neuroscientist*, 20(1), 56–70. doi: 10.1177/1073858413491147 [PubMed: 23774218]
- Hayashi T, Noshita N, Sugawara T, & Chan PH (2003). Temporal profile of angiogenesis and expression of related genes in the brain after ischemia. *Journal of Cerebral Blood Flow & Metabolism*, 23(2), 166–180. [PubMed: 12571448]
- Higo N (2010). Training-induced recovery of manual dexterity after a lesion in the motor cortex. *Keio J Med*, 59(1), 4–9. Review. [PubMed: 20375652]
- Hoogewoud F, Hamadjida A, Wyss AF, Mir A, Schwab ME, Belhaj-Saif A, & Rouiller EM (2013). Comparison of functional recovery of manual dexterity after unilateral spinal cord lesion or motor cortex lesion in adult macaque monkeys. *Front Neurol*, 4, 101. doi: 10.3389/fneur.2013.00101. eCollection 2013. [PubMed: 23885254]
- Iaci JF, Ganguly A, Finklestein SP, Parry TJ, Ren J, Saha S, Sietsma DK, Srinivas M, Vecchione AM, & Caggiano AO (2010). Glial growth factor 2 promotes functional recovery with treatment initiated up to 7 days after permanent focal ischemic stroke. *Neuropharmacology*, 59(7–8), 640–649. doi: 10.1016/j.neuropharm.2010.07.017 [PubMed: 20691195]
- Iaci JF, Parry TJ, Huang Z, Pavlopoulos E, Finklestein SP, Ren J, & Caggiano A (2016). An optimized dosing regimen of cimaglermin (neuregulin 1b3, glial growth factor 2) enhances molecular markers of neuroplasticity and functional recovery after permanent ischemic stroke in rats. *Journal of Neuroscience Research*, 94(3), 253–265. doi: 10.1002/jnr.23699 [PubMed: 26660233]

- Irwin N, Li YM, O'Toole JE, & Benowitz LI (2006). Mst3b, a purine-sensitive Ste20-like protein kinase, regulates axon outgrowth. *Proceedings of the National Academy of Sciences of the United States of America*, 103(48), 18320–18325. [PubMed: 17114295]
- Kaerer M, Wyss AF, Bashir S, Hamadjida A, Liu Y, Bloch J, Brunet JF, Belhaj-Saif A, & Rouiller EM (2010). Effects of unilateral motor cortex lesion on ipsilesional hand's reach and grasp performance in monkeys: Relationship with recovery in the contralesional hand. *Journal of Neurophysiology*, 103(3), 1630–1645. doi: 10.1152/jn.00459.2009. [PubMed: 20071636]
- Kim D, Zai L, Liang P, Schaffling C, Ahlborn D, & Benowitz LI (2013). Inosine enhances axon sprouting and motor recovery after spinal cord injury. *PLoS One*, 8(12). doi: 10.1371/journal.pone.0081948
- Klüver H (1935) An auto-multi-stimulation reaction board for use with sub-human primates. *The Journal of Psychology: Interdisciplinary and Applied*, 1, 123–127.
- Ko SB, & Yoon BW (2013). Mechanisms of functional recovery after stroke. *Frontiers of Neurology and Neuroscience*, 32, 1–8. doi: 10.1159/000346405 Review. [PubMed: 23859958]
- Kwakkel G, Winters C, van Wegen EE, Nijland RH, van Kuijk AA, Visser-Meily A, de Groot J, de Vlugt E, Arendzen JH, Geurts AC, & Meskers CG (2016). EXPLICIT-Stroke Consortium. Effects of Unilateral Upper Limb Training in Two Distinct Prognostic Groups Early After Stroke: The EXPLICIT-Stroke Randomized Clinical Trial. *Neurorehabil Neural Repair* pii: 1545968315624784.
- Levin MF, Kleim JA, & Wolf SL (2009). What do motor “recovery” and “compensation” mean in patients following stroke? *Neurorehabilitation and Neural Repair*, 23(4), 313–319. doi: 10.1177/1545968308328727 [PubMed: 19118128]
- Li S, & Carmichael ST (2006). Growth-associated gene and protein expression in the region of axonal sprouting in the aged brain after stroke. *Neurobiology of Disease*, 23(2), 362–373. [PubMed: 16782355]
- Liu Z, & Chopp M (2015). Astrocytes, therapeutic targets for neuroprotection and neurorestoration in ischemic stroke. *Progress in Neurobiology*, pii: S0301-0082(15)30001-0. doi: 10.1016/j.pneurobio.2015.09.008. Review.
- Liu Y, & Rouiller EM (1999). Mechanisms of recovery of dexterity following unilateral lesion of the sensorimotor cortex in adult monkeys. *Exp Brain Res*, 128(1–2), 149–159. [PubMed: 10473753]
- Lorber B, Howe ML, Benowitz LI, & Irwin N (2009). Mst3b, an Ste20-like kinase, regulates axon regeneration in mature CNS and PNS pathways. *Nature Neuroscience*, 12(11), 1407–1414. [PubMed: 19855390]
- Lum PS, Mulroy S, Amdur RL, Requejo P, Prilutsky BI, & Dromerick AW (2009). Gains in upper extremity function after stroke via recovery or compensation: Potential differential effects on amount of real-world limb use. *Top Stroke Rehabil*, 16(4), 237–253. doi: 10.1310/tsr1604-237. [PubMed: 19740730]
- Mahmood A, Lu D, & Chopp M (2004). Intravenous administration of marrow stromal cells (MSCs) increases the expression of growth factors in rat brain after traumatic brain injury. *Journal of Neurotrauma*, 21, 33–39. [PubMed: 14987463]
- Mahmood A, Lu D, Qu C, Goussev A, & Chopp M (2005). Human marrow stromal cell treatment provides long-lasting benefit after traumatic brain injury in rats. *Neurosurgery*, 57, 1026–1031. [PubMed: 16284572]
- Markowitz CE, Spitsin S, Zimmerman V, Jacobs D, Udupa JK, Hooper DC, & Koprowski H (2009). The treatment of multiple sclerosis with inosine. *Journal of Alternative and Complementary Medicine*, 15(6), 619–625. doi: 10.1089/acm.2008.0513
- Marti HJ, Bernaudin M, Bellail A, Schoch H, Euler M, Petit E, & Risau W (2000). Hypoxia-induced vascular endothelial growth factor expression precedes neovascularization after cerebral ischemia. *The American Journal of Pathology*, 156(3) 965–976. [PubMed: 10702412]
- Meng Y, Chopp M, Zhang Y, Liu Z, An A, Mahmood A, & Xiong Y (2014). Subacute intranasal administration of tissue plasminogen activator promotes neuroplasticity and improves functional recovery following traumatic brain injury in rats. *PLoS One*, 9(9), e106238. doi: 10.1371/journal.pone.0106238 [PubMed: 25184365]

- Metz GA, Antonow-Schlorke I, & Witte OW (2005). Motor improvements after focal cortical ischemia in adult rats are mediated by compensatory mechanisms. *Behavioral Brain Research*, 162(1), 71–82.
- Milliken GW, Plautz EJ, & Nudo RJ (2013). Distal forelimb representations in primary motor cortex are redistributed after forelimb restriction: A longitudinal study in adult squirrel monkeys. *Journal of Neurophysiology*, 109(5), 1268–1282. doi: 10.1152/jn.00044.2012 [PubMed: 23236004]
- Moon SK, Alaverdashvili M, Cross AR, & Whishaw IQ (2009). Both compensation and recovery of skilled reaching following small photothrombotic stroke to motor cortex in the rat. *Experimental Neurology*, 218(1), 145–153. doi: 10.1016/j.expneurol.2009.04.021 [PubMed: 19409894]
- Moore TL, Pessina MA, Finklestein SP, Kramer BC, Killiany RJ, & Rosene DL (2013). Recovery of fine motor performance after ischemic damage to motor cortex is facilitated by cell therapy in the rhesus monkey. *Somatosens Mot Research*, 30(4), 185–196. doi: 10.3109/08990220.2013.790806.
- Moore TL, Killiany RJ, Pessina MA, Moss MB, Finklestein SP, & Rosene DL (2012). Recovery from ischemia in the middle-aged brain: A non-human primate model. *Neurobiology of Aging*, 33(3), 619.e9–619.e24.
- Moore TL, Killiany RJ, Pessina MA, Moss MB, & Rosene DL (2010). Assessment of motor function of the hand in aged rhesus monkeys. *Somatosensory & Motor Research*, 27(3), 121–130. [PubMed: 20653499]
- Morecraft RJ, Ge J, Stilwell-Morecraft KS, McNeal DW, Hynes SM, Pizzimenti MA, Rotella DL, & Darling WG (2016). Frontal and frontoparietal injury differentially affect the ipsilateral corticospinal projection from the nonlesioned hemisphere in monkey (*Macaca mulatta*). *Journal of Comparative Neurology*, 524(2), 380–407. doi: 10.1002/cne.23861. [PubMed: 26224429]
- Nishibe M, Urban ET, Barbay S, & Nudo RJ (2015). Rehabilitative training promotes rapid motor recovery but delayed motor map reorganization in a rat cortical ischemic infarct model. *Neurorehabilitation and Neural Repair*, 29(5), 472–482. doi: 10.1177/1545968314543499 [PubMed: 25055836]
- Nudo RJ (2013). Recovery after brain injury: Mechanisms and principles. *Frontiers in Human Neuroscience*, 7, 887. doi: 10.3389/fnhum.2013.00887. Review. [PubMed: 24399951]
- Nudo RJ (2011). Neural bases of recovery after brain injury. *Journal of Communication Disorders*, 44(5), 515–520. doi: 10.1016/j.jcomdis.2011.04.004 Review. [PubMed: 21600588]
- Nudo RJ, Larson D, Plautz EJ, Friel KM, Barbay S, & Frost SB (2003). A squirrel monkey model of poststroke motor recovery. *ILAR J*, 44(2), 161–174. [PubMed: 12652011]
- Nudo RJ, & Friel KM (1999). Cortical plasticity after stroke: implications for rehabilitation. *Revue Neurologique*, 155(9), 713–717. Review. [PubMed: 10528355]
- Nudo RJ (1999). Recovery after damage to motor cortical areas. *Current Opinion in Neurobiology*, 9(6), 740–747. Review. [PubMed: 10607636]
- Nudo RJ, & Milliken GW (1996). Reorganization of movement representations in primary motor cortex following focal ischemic infarcts in adult squirrel monkeys. *J Neurophysiology*, 75(5), 2144–2149.
- Nudo RJ, Milliken GW, Jenkins WM, & Merzenich MM (1996). Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. *Journal of Neuroscience*, 16, 785–807. [PubMed: 8551360]
- Nouri S, & Cramer SC (2011). Anatomy and physiology predict response to motor cortex stimulation after stroke. *Neurology*, 77(11), 1076–1083. doi: 10.1212/WNL.0b013e31822e1482 [PubMed: 21880996]
- Papadopoulos CM, Tsai SY, Guillen V, Ortega J, Kartje GL, & Wolf WA (2009). Motor recovery and axonal plasticity with short-term amphetamine after stroke. *Stroke*, 40(1), 294–302. [PubMed: 19038917]
- Petrausch B, Tabibiazar R, Roser T, Jing Y, Goldman D, Stuermer CA, Irwin N, & Benowitz LI (2000). A purine-sensitive pathway regulates multiple genes involved in axon regeneration in goldfish retinal ganglion cells. *Journal of Neuroscience*, 20(21), 8031–8041.
- Plautz EJ, Barbay S, Frost SB, Zoubina EV, Stowe AM, Dancause N, Eisner-Janowicz I, Bury SD, Taylor MD, & Nudo RJ (2016). Effects of subdural monopolar cortical stimulation paired with rehabilitative training on behavioral and neurophysiological recovery after cortical ischemic stroke

in adult squirrel monkeys. *Neurorehabilitation and Neural Repair*, 30(2), 159–172. doi: 10.1177/1545968315619701 [PubMed: 26704255]

- Plautz E, & Nudo R (2005). Neural plasticity and functional recovery following cortical ischemic injury. *Conf Proc IEEE Eng Med Biol Soc*, 4, 4145–4148. [PubMed: 17281146]
- Plautz EJ, Barbay S, Frost SB, Friel KM, Dancause N, Zoubina EV, Stowe AM, Quaney BM, & Nudo RJ (2003). Post-infarct cortical plasticity and behavioral recovery using concurrent cortical stimulation and rehabilitative training: A feasibility study in primates. *Neurological Research*, 25(8), 801–810. [PubMed: 14669522]
- Plautz EJ, Milliken GW, & Nudo R.J. (2000). Effects of repetitive motor training on movement representations in adult squirrel monkeys: Role of use versus learning. *Neurobiol Learn Mem*, 74(1), 27–55. [PubMed: 10873519]
- Qu ZB, Liu LX, Wu LF, & Jiang HC (2007). Multiple littoral cell angioma of the spleen: A case report and review of the literature. *Onkologie*, 30, 256–258. [PubMed: 17460421]
- Rosen GD, & Harry JD (1990). Brain volume estimation from serial section measurements: A comparison of methodologies. *Journal of Neuroscience Methods*, 35(2), 115–124. [PubMed: 2283883]
- Rosene DL, Roy NJ, & Davis BJ (1986). A cryoprotection method that facilitates cutting frozen sections of whole monkey brains for histological and histochemical processing without freezing artifact. *J Histochem Cytochem*, 34(10), 1301–1315. [PubMed: 3745909]
- Roitberg B, Khan N, Tuccar E, Kompoliti K, Chu Y, Alperin N, Kordower JH, & Emborg ME (2003). Chronic ischemic stroke model in cynomolgus monkeys: Behavioral, neuroimaging and anatomical study. *Neurol Res*, 25(1), 68–78. [PubMed: 12564129]
- Schneider CA, Rasband WS, & Eliceiri KW (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671–675. [PubMed: 22930834]
- Schwarzschild MA, Ascherio A, Beal MF, Cudkowicz ME, Curhan GC, Hare JM, Hooper DC, Kieburtz KD, Macklin EA, Oakes D, Rudolph A, Shoulson I, Tennis MK, Espay AJ, Gartner M, Hung A, Bwala G, Lenehan R, Encarnacion E, Ainslie M, Castillo R, Togasaki D, Barles G, Friedman JH, Niles L, Carter JH, Murray M, Goetz CG, Jaglin J, Ahmed A, Russell DS, Cotto C, Goudreau JL, Russell D, Parashos SA, Ede P, Saint-Hilaire MH, Thomas CA, James R, Stacy MA, Johnson J, Gauger L, Antonelle de Marcaida J, Thurlow S, Isaacson SH, Carvajal L, Rao J, Cook M, Hope-Porche C, McClurg L, Grasso DL, Logan R, Orme C, Ross T Brocht AF, Constantinescu R, Sharma S, Venuto C, Weber J, & Eaton K (2014). Inosine to increase serum and cerebrospinal fluid urate in Parkinson disease: A randomized clinical trial. *JAMA Neurology*, 71(2), 141–150. doi:10.1001/jamaneurol.2013.5528
- Smith JM, Lunga P, Story D, Harris N, Le Belle J, James MF, Pickard JD, & Fawcett JW (2007). Inosine promotes recovery of skilled motor function in a model of focal brain injury. *Brain*, 130(Pt 4), 915–925. Epub 2007 Feb 9. [PubMed: 17293357]
- Sofroniew MV (2005). Reactive astrocytes in neural repair and protection. *Neuroscientist*, 11, 400–407. [PubMed: 16151042]
- Souza WC, Conforto AB, Orsini M, Stern A, & André C (2015). Similar effects of two modified constraint-induced therapy protocols on motor impairment, motor function and quality of life in patients with chronic stroke. *Neurology International*, 7(1), 5430. doi: 10.4081/ni.2015.5430 [PubMed: 26294941]
- Stroke Therapy Academic Industry R. Recommendations for standards regarding preclinical neuroprotective and restorative drug development (1999). *Stroke*, 30(12), 2752–2758. [PubMed: 10583007]
- Starkey ML, & Schwab ME (2014). How plastic is the brain after a stroke? *Neuroscientist*, 20(4), 359–371. Review. [PubMed: 24402612]
- Stroemer RP, Kent TA, & Hulsebosch CE (1998). Enhanced neocortical neural sprouting, synaptogenesis, and behavioral recovery with D-amphetamine therapy after neocortical infarction in rats. *Stroke*, 29(11), 2381–2393; discussion 2393–2395. [PubMed: 9804653]
- Tigges J, Gordon TP, McClure HM, Hall EC, & Peters A (1988). Survival rate and life span of rhesus monkeys at the Yerkes Regional Primate Research Center. *American Journal of Primatology*, 15, 263–273.

- Touvykine B, Mansoori BK, Jean-Charles L, Deffeyes J, Quessy S, & Dancause N (2016). The Effect of Lesion Size on the Organization of the Ipsilesional and Contralesional Motor Cortex. *Neurorehabil Neural Repair*, 30(3), 280–292. doi: 10.1177/1545968315585356. [PubMed: 25967757]
- Ueno Y, Chopp M, Zhang L, Buller B, Liu Z, Lehman NL, Liu XS, Zhang Y, Roberts C, & Zhang ZG (2012). Axonal outgrowth and dendritic plasticity in the cortical peri-infarct area after experimental stroke. *Stroke*, 43(8), 2221–2228. doi: 10.1161/STROKEAHA.111.646224 [PubMed: 22618383]
- Whishaw IQ, Suchowersky O, Davis L, Sarna J, Metz G, & Pellis S (2002). Impairment of pronation, supination, and body co-ordination in reach-to-grasp tasks in human Parkinson's disease (PD) reveals homology to deficits in animal models. *Behavioral Brain Research*, 133, 165–176.
- Whishaw IQ (2000). Loss of the innate cortical engram for action patterns used in skilled reaching and the development of behavioral compensation following motor cortex lesions in the rat. *Neuropharmacology*, 39(5), 788–805. [PubMed: 10699445]
- Wyss AF, Hamadjida A, Savidan J, Liu Y, Bashir S, Mir A, Schwab ME, Rouiller EM, & Belhaj-Saif A (2013). Long-term motor cortical map changes following unilateral lesion of the hand representation in the motor cortex in macaque monkeys showing functional recovery of hand functions. *Restor Neurol Neurosci*, 31(6), 733–760. doi: 10.3233/RNN-130344. [PubMed: 24064551]
- Xiong Y, Mahmood A, Meng Y, Zhang Y, Zhang ZG, Morris DC, & Chopp M (2010). Treatment of traumatic brain injury with thymosin beta(4) in rats. *Journal of Neurosurgery*, 114(1), 102–115. doi: 10.3171/2010.4.JNS10118 [PubMed: 20486893]
- Zai L, Ferrari C, Subbaiah S, Havton LA, Coppola G, Strittmatter S, Irwin N, Geschwind D, & Benowitz LI (2009). Inosine alters gene expression and axonal projections in neurons contralateral to a cortical infarct and improves skilled use of the impaired limb. *The Journal of Neuroscience*, 29(25), 8187–8197. [PubMed: 19553458]
- Zai L, Ferrari C, Dice C, Subbaiah S, Havton LA, Coppola G, Geschwind D, Irwin N, Huebner E, Strittmatter SM, & Benowitz LI (2011). Inosine augments the effects of a Nogo receptor blocker and of environmental enrichment to restore skilled forelimb use after stroke. *The Journal of Neuroscience*, 31(16), 5977–5988. [PubMed: 21508223]
- Zhang L, Li Y, Zhang C, Chopp M, Gosiewska A, & Hong K (2011). Delayed administration of human umbilical tissue-derived cells improved neurological functional recovery in a rodent model of focal ischemia. *Stroke*, 42(5), 1437–1444. [PubMed: 21493915]
- Zhang ZG, & Chopp M (2009). Neurorestorative therapies for stroke: Underlying mechanisms and translation to the clinic. *Lancet Neurology*, 8(5), 491–500. doi: 10.1016/S1474-4422(09)70061-4 [PubMed: 19375666]
- Zurn AD, & Do KQ (1988). Purine metabolite inosine is an adrenergic neurotrophic substance for cultured chicken sympathetic neurons. *Proceedings of the National Academy of Sciences of the United States of America*, 85(21), 8301–8305. [PubMed: 3186724]

Fig. 1.

A photograph of the plexiglass testing apparatus used to administer the Hand Dexterity Task (HDT). The red arrows indicate the right and left openings that the monkey must put the appropriate hand through in order to retrieve the food reward from the wells in each tray.

Fig. 2.

Photographs showing the stimulation sites on the hand representation maps and the intraoperative lesions for each monkey. On the hand representation maps, the black circles represent stimulation sites that generated a positive response in the hand or digits and the white circles represent stimulation sites that did not generate a positive response. Scale bar = 5 mm

Fig. 3.

Panel A: Lateral view of a rhesus monkey brain showing a representative example of the location of the lesion in the precentral gyrus for all subjects. The lesion as seen on the surface is shown outlined in black while its extension down into the anterior bank of the central sulcus is shown in gray as defined in dotted line cut-out below the brain. Panel B: Thionin stained coronal section (plane of section in A) showing typical cortical damage in the precentral gyrus. Abbreviations: CS – central sulcus; LS – Lateral Sulcus; iLAS – inferior limb of the arcuate sulcus; sLAS – superior limb of the arcuate sulcus; PS – principal sulcus.

Fig. 4. Representative thionin sections through the lesion from one monkey that received a placebo and one monkey that received inosine. Extent of lesion is visible to include surface representation of hand area and rostral bank of central sulcus. Scale bar = 10 mm.

Fig. 5.

Graph of difference scores on our Non-Human Primate Upper Extremity Motor Dysfunction Assessment Scale on measures of fine motor function, hand strength and digit flexion between the 1st 3 days (d1–3) and last 3 days (d12–14) of initial post-operative period. Asterisks indicate a significant group difference in recovery on all measures ($p < 0.003$). Error bars = Standard Error.

Fig. 6.

Graph of the difference in the mean time to retrieve the food reward between the placebo and treated groups during the initial week of testing and the final week of testing. There was no significant difference between the two groups at either time point in terms of the mean time required to retrieve the food reward. This graph also shows the mean time to retrieve rewards across the entire 12-week post-operative testing period on the HDT. There was no significant group difference in the time required to retrieve rewards between the placebo and treated group. Error bars = Standard Error.

Fig. 7.

Graphs of the daily mean time to retrieve a food reward across the pre- & post-operative testing periods for each monkey. The horizontal dashed line on each graph represents the mean time to retrieve across the entire pre-operative testing period.

Fig. 8.

Graph of the significant difference between groups for the number of post-operative days to attain asymptotic levels of grasp performance on the HDT for the impaired hand on the large well. The treated group required significant fewer days to return to asymptotic levels of grasp performance (* $p < 0.0001$). Error bars = Standard Error.

Fig. 9.

Graphs of the daily grasp assessment across the pre- & post-operative testing periods for each monkey.

Fig. 10.

Graph of the highest rating achieved on the Grasp Assessment Scale by each individual monkey. Three of the four monkeys treated with inosine returned to a score of 8 that represents a return to pre-operative grasp patterns. None of the placebo monkeys achieved a score of 8.

Fig. 11.

Sequences of grasp patterns from pre- & post-operative video from one monkey given a placebo and one monkey treated with inosine. Panels A&E show their pre-operative grasp patterns. Panels B-D show the compensatory “scooping” grasp involving all of the fingers of the impaired hand of a monkey in the placebo group. Arrow in Panel C shows fingers scooping candy into palm of the hand. Panels F-H shows the recovered finger-thumb grasp of the impaired hand of a monkey treated with inosine. This finger-thumb grasp (arrow in Panel H) is a return to pre-operative levels of performance in terms of quality of grasp (see Figs. 7 & 8).

Fig. 12.

A. Graph showing the relationship between final cortical lesion volume and days to return to criterion on the HDT. B. Graph showing the relationship between final cortical lesion volume and the highest grasp rating achieved by each monkey.

Table 1**Non-Human Primate Upper Extremity (UE) Motor Dysfunction Assessment Scale (From Moore et al., 2013)**

Observed Tone in Upper limbs	
0	Normal
1	Decreased muscle tone (flaccid, arm hangs limply at side)
2	Increased muscle tone (arm is held in flexed position)
Tremor in Upper Limbs (When taking food)	
0	Absent
1	Occasionally present
2	Present much of the time
3	Continuously Present
Fine-motor function of hands	
0	Normal use
1	Mild impairment in ability to retrieve food
2	Moderate impairment in ability to retrieve food
3	Severe impairment in ability to retrieve food
4	Unable or refuses to retrieve food
Functional Strength of Hand	
0	Normal – no signs of weakness
1	Mild weakness in UE when reaching or grasping
2	Moderate weakness in UE when reaching or grasping
3	Severe weakness in UE when reaching or grasping
4	Unable or refuses to reach or grasp
Flexion of Digits	
0	Normal flexion in digits
1	Mild impairment in flexion of digits
2	Moderate impairment in flexion of digits
3	Severe impairment in flexion of digits
4	No flexion observed
Movements of Forearm and Wrist	
0	Normal and full movement of wrist
1	Mild impairment/weakness/slowness of wrist

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

2	Moderate to severe impairment/weakness/slowness of wrist
3	Severe impairment/weakness/slowness of wrist
4	Unable to move UE at wrist joint
Movements of Arm and Shoulder	
0	Normal and full movement of arm and shoulder
1	Mild impairment/weakness/slowness of arm and shoulder
2	Moderate impairment/weakness/slowness of arm and shoulder
3	Severe impairment/weakness/slowness of arm and shoulder
4	Unable to move UE at arm and/or shoulder joints

Table 2**Modified Non-Human Primate Grasp Assessment Scale (From Moore et al., 2013)**

0.0	Complete absence of active digit movement and no attempt to use impaired UE to test.
1.0	Attempts to use impaired UE to retrieve food reward but no flexion and extension of digits either singly or as a group. Unsuccessful at retrieving any food rewards.
2.0	Flexion and extension of digits as a group using less than 15 degrees of digit MP flexion. No evidence of the purposeful “scooping” of reward. No movement of thumb towards palm. Unsuccessful at retrieving any food rewards.
3.0	Flexion and extension of digits as a group using less than 15 degrees of digit MP flexion. Evidence of the development of compensatory “scooping” movement of reward toward palm of hand with multiple digits and mass action of digits. Successful retrieval of food reward on at least 10% of trials. No movement of thumb towards palm.
3.5	Flexion and extension of digits as a group using less than 15 degrees of digit MP flexion. Evidence of the development of compensatory “scooping” movement of reward toward palm of hand with multiple digits and mass action of digits. Successful retrieval of food reward on at least 25% of trials. No movement of thumb towards palm.
4.0	Flexion and extension of digits as a group using greater than 15 degrees of digit MP flexion. Perform purposeful compensatory “scooping” movement of reward toward palm of hand with multiple digits and mass action of digits. Successful retrieval of food reward on at least 25% of trials. No movement of thumb towards palm.
4.5	Flexion and extension of digits as a group using greater than 15 degrees of digit MP flexion. Perform purposeful compensatory “scooping” movement of reward toward palm of hand with multiple digits and mass action of digits. Successful retrieval of food reward on at least 25% of trials. Begin to observe slight movement of thumb observed during testing session.
5.0	Flexion and extension of digits as a group using greater than 15 degrees of digit MP flexion. Perform purposeful compensatory “scooping” movement of reward toward palm of hand with multiple digits and mass action of digits. Slight movement of thumb towards palm with a web space no greater than one digit width. Thumb does not reach opposition. Successful retrieval of food reward on at least 75% of trials.
5.5	Flexion and extension of digits as a group using greater than 15 degrees of digit MP flexion. Perform purposeful compensatory “scooping” movement of reward toward palm of hand with multiple digits and mass action of digits. Thumb in opposition. Successful retrieval of food reward on at least 75% of trials.
6.0	Reaches toward reward with all digits in extension with evidence of isolated individual digit movement on at least 10% of trials per day. Mass movement of digits still predominates. The compensatory “scooping” movement toward palm is used.
6.5	Reaches toward reward with all digits in extension with evidence of isolated individual digit movement on 25% of trials per day. Mass movement of digits still predominates. The compensatory “scooping” movement toward palm is used.
7.0	Reaches toward reward with all digits in extension with evidence of isolated individual digit movement on 50% of trials per day. The compensatory “scooping” movement toward palm is used though becoming less predominant. Successful retrieval of food reward on 100% of trials.
7.5	Reaches toward reward with all digits in extension. Isolated individual digit movement occurs in greater than 50% of trials. The compensatory “scooping” movement toward palm is used though becoming less predominant. Successful retrieval of food reward on 100% of trials.
8.0	Pre-operative movement pattern of all digits and hand is observed with no evidence of compensatory “scooping” movements. Functional pinch occurs between the thumb and 1 individual digit, usually the index digit.

Table 3

This table outlines the weight of each monkey, their preferred hand, pre-operative mean time to retrieve on the HDT and the final lesion volume for each monkey

Animal	Weight at time of surgery (kg)	Preferred Hand	Pre-operative Mean Time to Retrieve (sec) on HDT	Final Lesion Volume (mm ³)
Placebo 1	10.94	Left	1.29	115.97
Placebo 2	8.2	Right	1.44	84.02
Placebo 3	8.3	Left	1.43	122.94
Placebo 4	10.5	Left	1.36	58.72
Mean			1.38	95.41
SE			0.03	14.88
Treated 1	12.2	Left	1.24	76.57
Treated 2	9.0	Left	1.17	117.48
Treated 3	13.8	Left	1.16	108.15
Treated 4	13.0	Right	1.38	99.32
Mean			1.24	100.38
SE			0.05	8.76