Brain Imaging and Behavior (2014) 8:359–369
DOI 10.1007/s11682-011-9144-1

Mean diffusivity as a potential diffusion tensor biomarker of motor rehabilitation after electrical stimulation incorporating task specific exercise in stroke: a pilot study

Erin L. Boespflug · Judd M. Storrs · Jane B. Allendorfer · Martine Lamy · James C. Eliassen · Stephen Page

Published online: 28 December 2011

Abstract Changes in diffusion tensor imaging (DTI) values co-occur with neurological and functional changes after stroke. However, quantitative DTI metrics have not been examined in response to participation in targeted rehabilitative interventions in chronic stroke. The primary purpose of this pilot study was to examine whether changes in DTI metrics co-occur with paretic arm movement changes among chronic stroke patients participating in a regimen of electrical stimulation targeting the paretic arm. Three subjects exhibiting stable arm hemiparesis were administered 30-minute (n=1) or 120-minute (n=2) therapy sessions emphasizing paretic arm use during valued, functional tasks and incorporating an electrical stimulation device. These sessions occurred every weekday for 8 weeks. A fourth subject served as a treatment control, participating in a 30-minute home exercise regimen without electrical stimulation every weekday for 8 weeks. DTI and behavioral outcome measures were acquired at baseline and after intervention. DTI data were analyzed using a region of interest (ROI) approach, with ROIs chosen based on tract involvement in sensorimotor function or as control regions. Behavioral outcome measures were the Fugl-Meyer Scale (FM) and the Action Research Arm Test (ARAT). The treatment control subject exhibited gains in pinch and grasp, as shown by a 5-point increase on the ARAT. The subject who participated in 30-minute therapy sessions exhibited no behavioral gains. Subjects participating in 120-minute therapy sessions displayed consistent impairment reductions and distal movement changes. DTI changes were largest in subjects two and three, with mean diffusivity (MD) decreases in the middle cerebellar peduncle and posterior limb of the internal capsule following treatment. No changes in fractional anisotropy (FA) were observed for sensorimotor tracts. Our preliminary results

E. L. Boespflug
Psychiatry and Behavioral Neurosciences & Center for Imaging Research & Neuroscience Graduate Program, University of Cincinnati College of Medicine,
231 Albert Sabin Way, Suite E685,
Cincinnati, OH 45267-0583, USA
e-mail: boespfel@mail.uc.edu

J. M. Storrs · J. C. Eliassen
University of Cincinnati, Center for Imaging Research,
231 Albert Sabin Way, Suite E685,
Cincinnati, OH 45267-0583, USA

J. M. Storrs
e-mail: judd.storrs@uc.edu

J. C. Eliassen
e-mail: james.eliassen@uc.edu

J. B. Allendorfer
Department of Neurology,
University of Cincinnati Academic Health Center,
260 Stetson St., Suite 2300, PO Box 670525, Cincinnati, OH 45267-0525, USA
e-mail: Jane.Allendofer@uc.edu

M. Lamy
Center for Imaging Research & Physician Scientist Training Program, University of Cincinnati College of Medicine,
231 Albert Sabin Way,
Cincinnati, OH 45267-0583, USA
e-mail: lamym@mail.uc.edu

S. Page (✉)
Occupational Therapy, The Ohio State University,
453 West 10th Street,
Columbus, OH 43210, USA
e-mail: page.61@osu.edu

suggest that active rehabilitative therapies augmented by electrical stimulation may induce positive behavioral changes which are underscored by DTI changes indicative of increased white matter tract integrity in regions specific to sensorymotor function.

Keywords Rehabilitation · Hemiplegia · Occupational therapy · Neuroimaging · Diffusion tensor imaging · Mean diffusivity

Introduction

Whereas conventional rehabilitation strategies have shown negligible efficacy, repetitive, task-specific practice (RTP) has been shown to produce neural and functional changes after stroke (Plautz et al. 2000; Lotze et al. 2003). These findings have led to the genesis of several promising rehabilitative regimens (Wolf et al. 2006; Page et al. 2005). Recently, a fitted neuroprosthesis providing electrical stimulation (NEURSTIM) during RTP was shown to reduce paretic arm impairment and disability and elicit functional improvement in patients initially exhibiting trace paretic wrist and finger movement (Alon et al. 2007; Weingarden et al. 1998; Dunning et al. 2008; Page et al. 2009). These findings are of potential significance, as individuals exhibiting trace levels of movement are frequently discharged from therapy regimens due to their moderate impairment levels.

Although the white matter that serves the sensorimotor systems may not be damaged in stroke, these tracts may be involved in the loss of function that is ultimately observed. A reason for this may include loss of functional integrity of axons secondary to the loss of origin or target grey matter regions (retrograde degeneration). Alternatively, loss of function could arise from a loss of sufficient connectivity among functionally related areas to maintain task abilities, otherwise known as disconnection syndrome (Catani and Mesulam 2008).

Diffusion tensor imaging (DTI) obtains information about the behavior of water molecules in biological tissue, making it possible to obtain information about the type, structure, and integrity of the tissue being imaged. Measuring water diffusion from multiple directions, and subsequent decomposition of each measurement permits various anisotropic indices to be calculated, including mean diffusivity (MD) and fractional anisotropy (FA) (Assaf and Pasternak 2008; Basser and Pierpaoli 1996; Melhem et al. 2002). High MD indicates less constrained diffusion of water molecules, such as in CSF as well as pathological conditions such as demyelination or edema. MD and FA correlate (MD, inversely) with axon count and myelin content in fixed postmortem brain tissue (Schmierer et al. 2008), providing strong evidence that this method is sensitive to the pathological status of brain tissue *in vivo*. DTI has been shown to be sensitive to changes in white matter (Yu et al. 2009; Werring et al. 2000), and studies performed longitudinally after stroke report retrograde and/or anterograde degeneration within the corticospinal tract, as indicated by the progressive decrease in FA (Moller et al. 2007; Thomalla et al. 2005). These measures correlate with changes in motor function and motor recovery post-injury as well as other neurological deficits (Puig et al. 2010; Radlinska et al. 2010; Watanabe et al. 2001; Jang et al. 2005). It is plausible that activity-based rehabilitation, including NEURSTIM, can influence white matter integrity as measured by DTI.

Precise measurement of the paretic arm's response to rehabilitative strategies is fundamental to cost effective, appropriate care. However, conventional measures of paretic arm impairment are behavioral, subjective, and susceptible to peripheral pathologies. For example, while change may be occurring, scores on a subjective behavior-based measure of arm impairment may be masked by a patient's spasticity, arthritis, and/or soft tissue changes. Anisotropic biomarkers would constitute a more valid representation of the efficacy of rehabilitative efforts. In the case of stroke rehabilitation, measures of brain change – and, in particular, DTI, may meet this need. The primary purpose of this pilot study was to examine whether changes in DTI measures co-occur with paretic arm movement changes among chronic stroke patients participating in NEURSTIM therapy. We hypothesized that anisotropic indices indicative of improved integrity (i.e., decreased MD or increased FA values) of tracts serving areas of the brain involved in sensorimotor performance would be observed in subjects receiving NEURSTIM therapy. To our knowledge, this is the first study prospectively examining DTI changes from participation in a rehabilitative intervention, and more specifically, following any form of peripheral electrical stimulation.

Methods

Subjects

Subjects were part of a larger NEURSTIM trial. To be eligible, volunteers had to meet the following inclusion criteria: (1) no active extension in the paretic wrist or fingers; (2) only one stroke experienced > 6 months prior to study enrollment; (3) a score≥70 on the Modified Mini Mental Status Examination (MMSE) (Teng and Chui 1987) (4) 18≤age≤85; (5) only one documented stroke; and (6) discharged from all physical rehabilitation. Subjects also had to exhibit at least partial ability to move outside of synergies at the paretic elbow, as indicated by Fugl-Meyer Scale items IV, movement mixing flexion and extension synergies or V, movements with little or no synergy

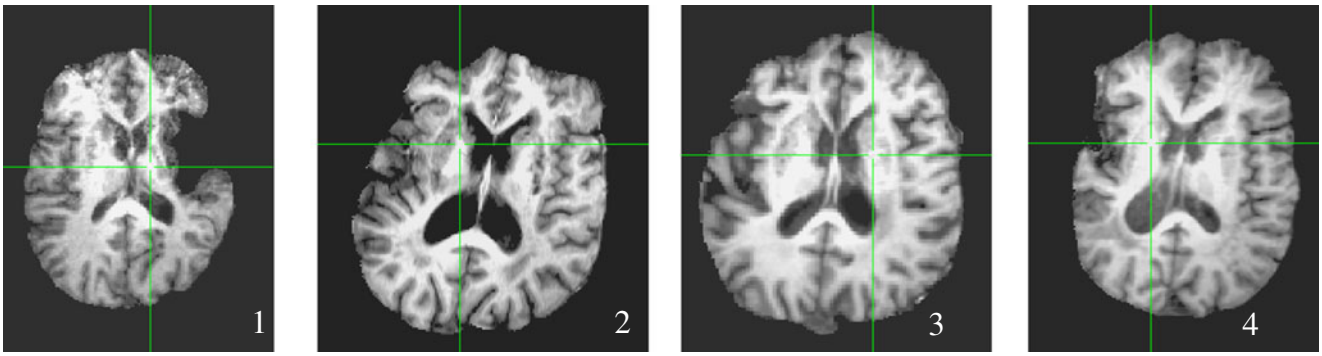

Fig. 1 High resolution anatomical scan for each subject, with corticospinal tract identified (crosshairs)

dependence (Fugl-Meyer et al. 1975). Although subjects met these inclusion criteria for functional impairment, the volume of their lesions were inhomogeneous for this pilot study, as shown in Fig. 1. Exclusion criteria were: (1) participating in any experimental rehabilitation or drug studies; (2) pregnant; (3) excessive spasticity at the paretic elbow, wrist, or fingers, defined as a score of≥2 on the Modified Ashworth Spasticity Scale (MAS) (Bohannon and Smith 1987); (4) excessive pain in the paretic upper extremity, as measured by a score≥4 on a 10-point visual analog scale; (5) mirror movements (i.e., involuntary movements by the nonparetic hand during attempts at unilateral movement by the paretic hand); (6) contraindication to MRI (e.g., metal implants, claustrophobia; pacemaker). The Institutional Review Board approved the study, and written consent was obtained from all patients.

DTI data were acquired on a subset of 5 stroke subjects who met the above study criteria. Inclusion of these subjects was arbitrary; they were the first subjects to join the study upon implementing the DTI protocol.

Intervention

The Bioness H-200 is a microprocessor based, FDA approved, NEURSTIM treatment device fitted to the forearm and provides electrical stimulation to the flexor and extensor muscles at set intervals. Features and use parameters in this study were identical to those used in previous NEURSTIM work and are described elsewhere (Page, Harnish et al. 2010).

Outcome measures

Behavioral outcome measures

Our primary behavioral outcome measure was the upper extremity section of the Fugl-Meyer Assessment of Impairment (FM), which discerned changes in arm impairment. Data arise from a 3-point ordinal scale (0 = cannot perform—2 = can perform fully) applied to each item. The FM has high test-retest reliability (total=0.98–0.99; subtests=0.87–1.00), inter-rater reliability, and construct validity (Di Fabio and Badke 1990; Duncan et al. 1983). To examine changes in functional limitation, we also administered the Action Research Arm Test (ARAT) as a secondary behavioral outcome measure (Lyle 1981). The ARAT is a 19-item test divided into four categories (grasp, grip, pinch, and gross movement), with 16 items measuring the distal arm. Items are graded on a 4-point ordinal scale (0 = can perform no part of the test—3 = performs test normally) with a total possible score of 57. The ARAT has high intra-rater (r=0.99) and retest (r=0.98) reliability and validity (Van der Lee et al. 2001; Hsieh et al. 1998). All outcome measures were administered by a physical therapist with over ten years of experience administering the measures.

MRI acquisition

Baseline scans were acquired 1 week after the second FM and ARAT assessments using a 4.0 Tesla Varian Unity INOVA Whole Body MRI/MRS system (Varian Inc., Palo Alto, CA). Although this scanner is a 100% research dedicated scanner and is about 2.5 times more powerful than typical 1.5 Tesla MRI used commonly in clinical centers, diffusion weighted imaging protocols are a common MRI sequence in clinical imaging. The FDA has approved MRI research at 4.0 Tesla in humans. Padding was inserted around each subject's head to minimize movement. A high-resolution, T1-weighted 3-D brain scan was obtained using a modified driven equilibrium Fourier transform (MDEFT) sequence (T_{MD}=1.1 s, TR=13 ms, TE=6 ms, FOV=25.6×19.2×19.2 cm, matrix 256×192×96 pixels, flip angle=20°) (Lee et al. 1995). A midsagittal localizer scan was obtained to place 30 contiguous 5 mm axial slices that extend from the inferior cerebellum to encompass the entire brain. A multi-echo reference scan was obtained and used for reduction of nyquist ghosting and distortion correction (Schmithorst et al. 2001). Finally, a single-shot echo-planar image sequence acquiring a 30-direction diffusion-weighted imaging series was collected (TR=10,000 ms, TE=89.2 ms, slice thickness=4 mm, FOV=25.6 cm×25.6 cm, flip angle=90°, slice orientation=axial, matrix size=256×256, maximum b-value=1000.65) with six

non-diffusion-weighted (B0) images. This scan sequence was modeled after the Jones, et al. paradigm (Jones et al. 1999).

Given the pilot nature of this study, as well as inclusion of only chronic stroke patients, we did not acquire a FLAIR sequence in this study, a sequence common to clinical and some research protocols used primarily to determine acute infarct (Kamran et al. 2000). Although future studies of larger scale would benefit from the use of an additional scanning sequence to evaluate chronic hemorrhagic changes, none was acquired in this pilot study.

Data processing

Raw MRI data were reconstructed with in-house software developed in IDL (www.ittvis.com). Subsequent processing, visualization and analyses were done using Analysis of Functional Neuroimages (AFNI) (Cox 1996). Alignment of pre- with post-treatment scans were performed with minimum spatial distortion by alignment tools in AFNI. The B0 images (the non-diffusion weighed images of the DTI dataset) were motion-corrected and averaged for alignment with the high-resolution anatomical MDEFT image using local Pearson correlation (Saad et al. 2009). Eddy current distortions and subject motion were corrected by 3D affine registration. The rigid-body component of the corrections were obtained by polar decomposition and used to motion correct the diffusion gradient vectors (Rohde et al. 2004). Finally, affine registrations were combined to generate DTI datasets for both sessions which were aligned to the first session anatomical dataset, corrected for gradient distortions, and only interpolated once. DTI parameter datasets (FA, MD) were computed using AFNI and used for region of interest analysis.

See Fig. 2 for a representative sample of ROI placement. ROIs were selected *a priori* based on known functional neuroanatomy (i.e., the relevance of the tract to the functional outcome), as well as ability to visualize the tract on DTI images. For these reasons, major white matter tracts known to be principally involved in the relay of sensorimotor information were selected as primary ROIs. The tracts included the posterior limbs of the internal capsule and the

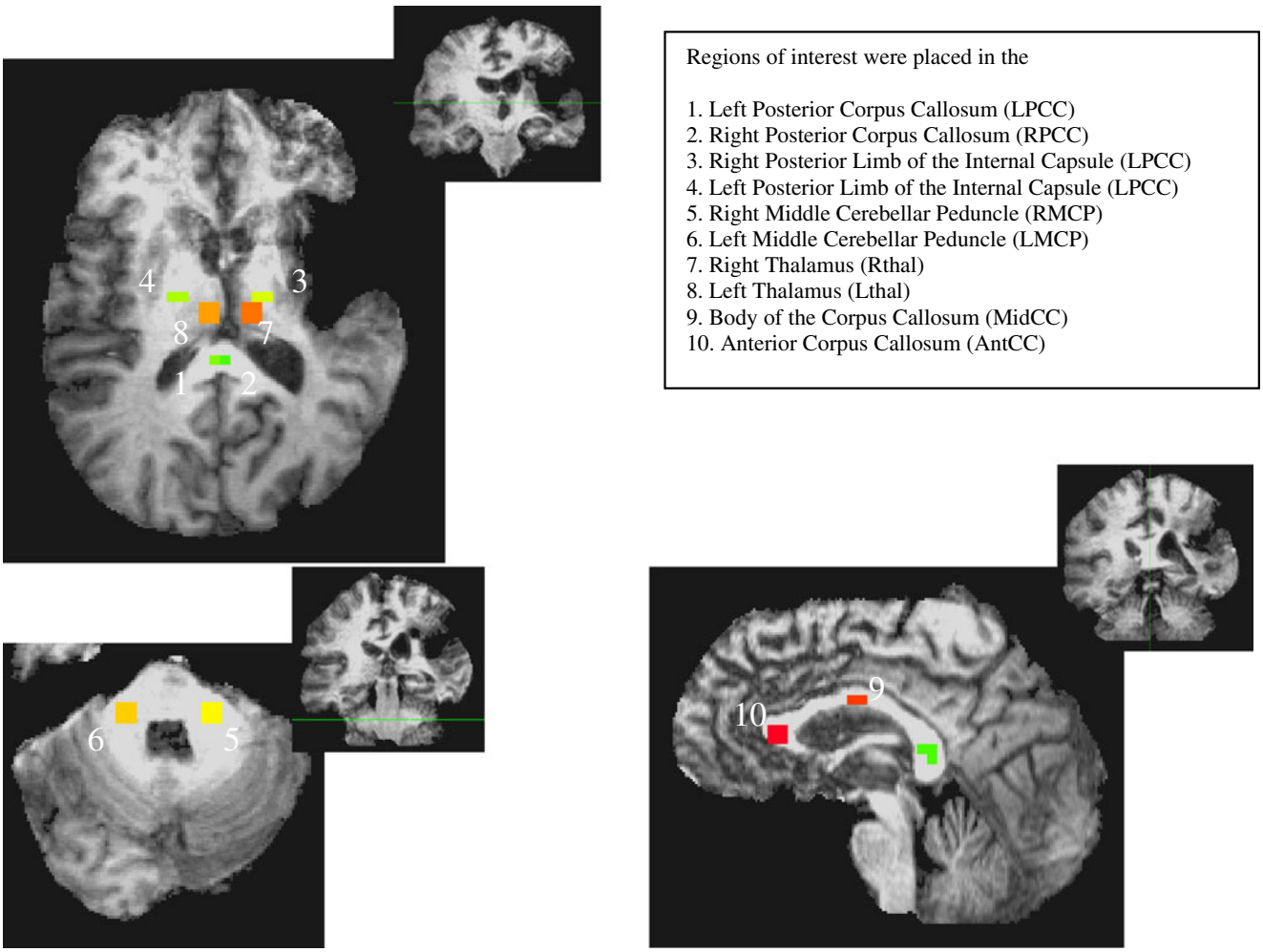

Fig. 2 ROI placement. After manual location of each of the ROIs, an 8 mm diameter sphere was placed at the center coordinates of each region, and ROIs were evaluated to assure that each ROI contained exactly 4 voxels, and that these were on the tract of interest

middle cerebellar peduncles. We also chose regions to serve as control regions, the corpus callosum and the thalami.

The posterior limbs of the internal capsule carry third order neurons from the medial lemniscus ascending pathway (contralateral discriminatory tactile sense and kinesthetic sense), as well as the lateral corticospinal (pyramidal) tracts governing volitional movement of the contralateral side. The ROIs of the posterior limbs of the internal capsule were placed lateral to the thalamus and medial to the lateral globus pallidus and putamen.

The middle cerebellar peduncles contain fiber tracts of many systems involved in sensory and motor integration, specifically the primary afferent fibers from the contralateral pontine nuclei, bilateral retrolenticular limb of the internal capsule, and ipsilateral raphe nucleus, serving principally as a route of motor and sensory information inflow into the cerebellum (Strong and Elwin 1943). Primarily, the pontocerebellar tracts transmit motor planning and initiation movement information arising in the cortical motor areas from the pontine nucleus to the contralateral cerebellar cortex (http://www.neuroanatomy.wisc.edu/virtual-brain/BrainStem/16Pontine.html). Additionally, afferent fibers from the pontine raphe nucleus, a nucleus implicated in the sensation of brush touch (Ghazni et al. 2009), travel in this peduncle. ROIs were placed in the middle cerebellar peduncles medial to branching point, when the superior cerebellar peduncles were still visible in the selected slice in the coronal view, and anterior to the anterior border of the fourth ventricle.

The ROIs of the corpus callosum were selected predominately due our ability to easily visualize the anterior, middle and posterior regions of this large fiber bundle. Although some sensorimotor information is carried in the corpus callosum, particularly in the middle (body) (Lee et al. 1995), the tract is not specialized for primary sensorimotor functions. In the event that DTI changes were evident in the corpus callosum, we investigated regions in the body, as well as anterior and posterior regions. ROIs for the anterior (genu) and body of the corpus callosum were placed in the most mid-sagittal slice. ROIs of the posterior (splenium) regions of the corpus callosum were placed just lateral to the midline.

Although there are numerous connections of sensory and motor systems throughout the thalamus, it was considered a comparison region; this collection of nuclei has predominately short, interconnected neuronal fibers, and therefore highly anisotropic (Mamata et al. 2004). Although not ideal, as discussed, we chose these control regions over homologue regions on the intact hemisphere given evidence of contralesional involvement in recovery after hemiparetic stroke. See (Judith 2004) for a review.

After manual location of each of the ROIs, an 8 mm diameter sphere was placed at the center coordinates of each region, and ROIs were evaluated to assure that each ROI contained exactly 4 voxels, and that these were on the tract of interest. We selected a diameter of 8 mm based on the spatial resolution of our data independent findings of tract diameter (Murata et al. 1998). Selection and placement was performed manually for each subject using the high-resolution anatomical scan as the visual reference and spatial coordinates of the ROIs were applied to the co-registered diffusion datasets. Placement of ROIs was made by a single rater blind to functional outcomes, and statistical analysis was performed by a separate author who was also blind to functional outcomes.

Interventions

Neurstim

Intervention for this study was identical to methods described previously (Alon et al. 2007; Weingarden et al. 1998; Dunning et al. 2008; Page et al. 2009; Page et al. 2010). Briefly, 1 week after the pre-treatment MRI, the H-200 electrical stimulation device was fitted on the three subjects who were administered NEURSTIM intervention. To maximize opportunity for reintegration into these patients' real world environments, the NEURSTIM intervention was primarily home-based and required use of the Bioness H-200 during practice of the activities that subjects had identified as important to them. One week after their education sessions, the NEURSTIM subjects began either 30-minute ($n=1$) or 120-minute ($n=2$) therapy sessions, occurring every weekday over an 8-week period. The reason for the varying NEURSTRIM durations was randomization for the parent study. These in-home sessions were complemented by 30-minute, clinically-based therapy sessions, occurring two weekdays every other week, (i.e., 1st, 3rd, 5th, and 7th week). The clinical sessions provided one-on-one training to augment the in-home sessions, check home compliance, adjust device parameters, and to make exercises more challenging. Challenge was modulated by increasing the temporal (i.e., repeat the task components or total task activity as many times as possible during a defined time interval) and/or spatial characteristics (i.e., increase the distance of the target from the patient and/or change the plane in which the activity was performed) characteristics of the task, as suggested in several RTP reviews (Mulder et al. 2002; Woldag and Hummelsheim 2002).

A fourth subject was also administered the above behavioral and neuroimaging procedures in the same fashion as previously described. Additionally, 1 week after the MRI session, the subject participated in a 1.5-hour education session which reviewed a home exercise regimen that was to be performed every weekday for 30 min. The treatment control subject was also given a sheet with a list of home exercises for the paretic arm, and a home use diary to record compliance with the home exercise program. This regimen is identical to exercises that discharged stroke patients would normally be practicing at home, and was an adequate treatment control condition.

Follow-up

All subjects returned to the research laboratory five weekdays post intervention to complete outcome tests,

administered by the same observer who performed pre-treatment testing. The research member was blinded to whether the subjects had participated in any intervention. The MRI protocol was also repeated during this same time period (i.e., within 5 days of completion of therapy).

Table 1 Subject demographics, diffusion values and functional scores before and after study treatment

Subject	1	2	3	4
Treatment	C	T×30	T×120	T×120
Age	58	68	71	52
Sex	M	M	M	F
Stroke type	I	I	H	I
Months since stroke	13	8	15	20
Side of lesion	R	L	R	L
LPLIC mean MD Pre	0.799	0.780	0.798	0.571
LPLIC mean MD Post	0.766	0.740	1.043	0.656
LPIC MD comparison (p)	*0.756*	*0.717*	*0.064*	*0.625*
RPLIC mean MD Pre	0.710	0.795	0.931	0.757
RPLIC mean MD Post	0.718	0.793	0.784	0.744
RPLIC MD comparison (p)	*0.903*	*0.927*	*0.048*	*0.875*
LPLIC mean FA Pre	0.330	0.453	0.519	0.539
LPLIC mean FA Post	0.330	0.503	0.440	0.630
LPLIC FA comparison (p)	*0.997*	*0.538*	*0.265*	*0.515*
RPLIC mean FA Pre	0.482	0.521	0.517	0.426
RPLIC mean FA Post	0.478	0.517	0.595	0.433
RPLIC FA comparison (p)	*0.903*	*0.908*	*0.278*	*0.685*
LMCP mean MD Pre	0.772	0.804	1.338	0.651
LMCP mean MD Post	0.840	0.725	0.985	0.751
LMCP MD comparison (p)	*0.146*	*0.272*	*0.041*	*0.375*
RMCP mean MD Pre	0.691	0.679	0.954	0.673
RMCP mean MD Post	1.007	0.547	0.508	0.829
RMCP MD comparison (p)	*0.180*	*0.025*	*0.126*	*0.625*
LMCP mean FA Pre	0.521	0.462	0.342	0.494
LMCP mean FA Post	0.388	0.420	0.428	0.479
LMCP FA comparison (p)	*0.146*	*0.703*	*0.146*	*0.748*
RMCP mean FA Pre	0.699	0.575	0.612	0.781
RMCP mean FA Post	0.523	0.707	0.767	0.740
RMCP FA comparison (p)	*0.178*	*0.225*	*0.324*	*0.646*
FM Pre-1	21	19	39	20
FM Pre-2	22	17	38	20
FM Post	22	18	44	25
ARAT Pre-1	3	6	25	6
ARAT Pre-2	3	8	24	6
ARAT Post	8	7	30	7

R right, *L* left, *PLIC* Posterior Limb of the Internal Capsule, *MCP* Middle Cerebellar Peduncle, *FA* Fractional Anisotropy, *MD* Mean Diffusivity, *C* treatment control, *I* Ischemic, *H* Hemorrhagic, *Tx* treatment group, minutes per day, *FM* Fugl-Meyer Scale, *ARAT* Action Research Arm Test

Statistical analyses

Behavioral analyses

Given the small sample size in this pilot study, inferential statistical analyses of behavioral outcome measures were not possible. Thus, after averaging the pre-intervention scores (i.e., PRE-1 and PRE-2), behavioral changes for each subject were determined by comparing total scores pre- and post-intervention.

Differences in MD and FA values for each subject were calculated voxel-wise across scan sessions in AFNI using 3dDWItoDT (Basser and Pierpaoli 1996). Statistical comparisons were made by paired t-tests in GNU R version 2.10.1 (R Development Core Team 2009).

Results

One subject was excluded due to incomplete study data. For the four subjects included in the current analysis, the median age was 63 years, median time since stroke was 14 months, and three of the four subjects were male (Table 1).

During the pre-treatment phase, all subjects exhibited stable motor deficits, indicated by FM and ARAT scores that did not differ from each other between the two testing sessions prior to intervention (Table 1). Comparison of each subject's motor deficits at pre-treatment phase with those reported at therapy discharge in medical records further confirmed motor deficit stability. Subjects also each reported that their paretic arm movements had not changed substantively since outpatient therapy discharge. Representative MD images can be seen in Figs. 3 and 4.

Subject 1 S1, who received the home-based control treatment, showed nominal FM changes (21/22-22), although he did exhibit some noticeable changes in distal movement on the ARAT grasp and pinch scales (3–8). No significant changes in FA or MD were observed in any of the sensorimotor tracts over the treatment period for S1. There was a significant decrease in MD (p=0.03) and increase in FA (p=0.002) in the mid CC in S1.

Subject 2 S2, who received 30 minutes of NEURSTIM treatment also showed nominal change in the FM (19/17-18) and ARAT (6/8-7) scores, and reported no differences in his motor function from day to day. Pre/Post treatment diffusion for this participant exhibited a significant decrease in MD in the right MCP, the side contralateral to lesion, but did not show significant changes for any other ROI. This participant also showed a significant increase in FA in the mid CC.

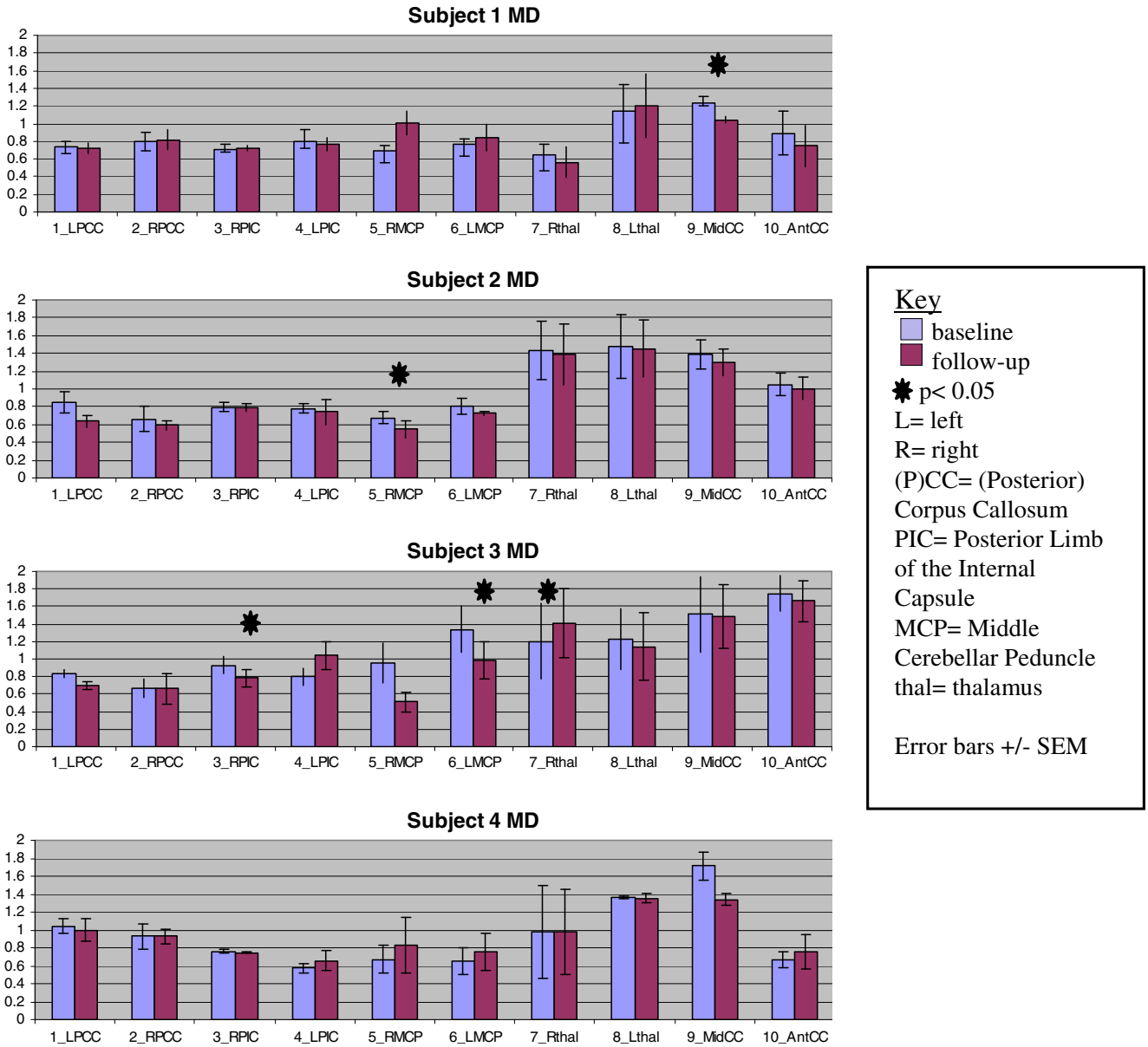

Fig. 3 Mean Diffusivity in Regions of Interest by Subject. A dose-dependent response to treatment was observed in subjects receiving control treatment to 30 min and 120 min of electrical stimulation treatment (subjects 1, 2 and 3, respectively). A statistically significant decrease in MD was observed post- versus pre-treatment in the cerebellar peduncle and the posterior limb of the internal capsule, regions of white matter known to be specifically involved in motor function

Subject 3 S3, who received 120 min of treatment, exhibited marked motor improvement in both the FM (39/38-44) and ARAT (25/24-30), outcomes which were underscored by significant decreases in MD in both the left MCP (p=0.041) and right PLIC (p=0.048), ROIs which were contra- and ipsi-lesional, respectively. Interestingly, this participant's diffusion data also indicated a significant increase of MD in the right thalamus ROI (0.035). No significant changes in FA were observed for any ROIs in S3.

Subject 4 Similar to S3, S4, who also received 120 min of treatment displayed marked improvement in FM (20/20-25) but marginal improvements in ARAT (6/6-7). In spite of these functional outcomes, there were no significant changes in MD or FA for any ROIs for S4.

In summary, a dose-dependent response to treatment was observed in subjects receiving control treatment to 30 min and 120 min of electrical stimulation treatment (subjects 1, 2 and 3, respectively). A statistically significant decrease in MD was observed post- versus pre-treatment in the cerebellar peduncle and the posterior limb of the internal capsule, regions of white matter known to be specifically involved in motor function (Fig. 3). Further, of all regions evaluated, only those regions known to be involved in relaying specifically

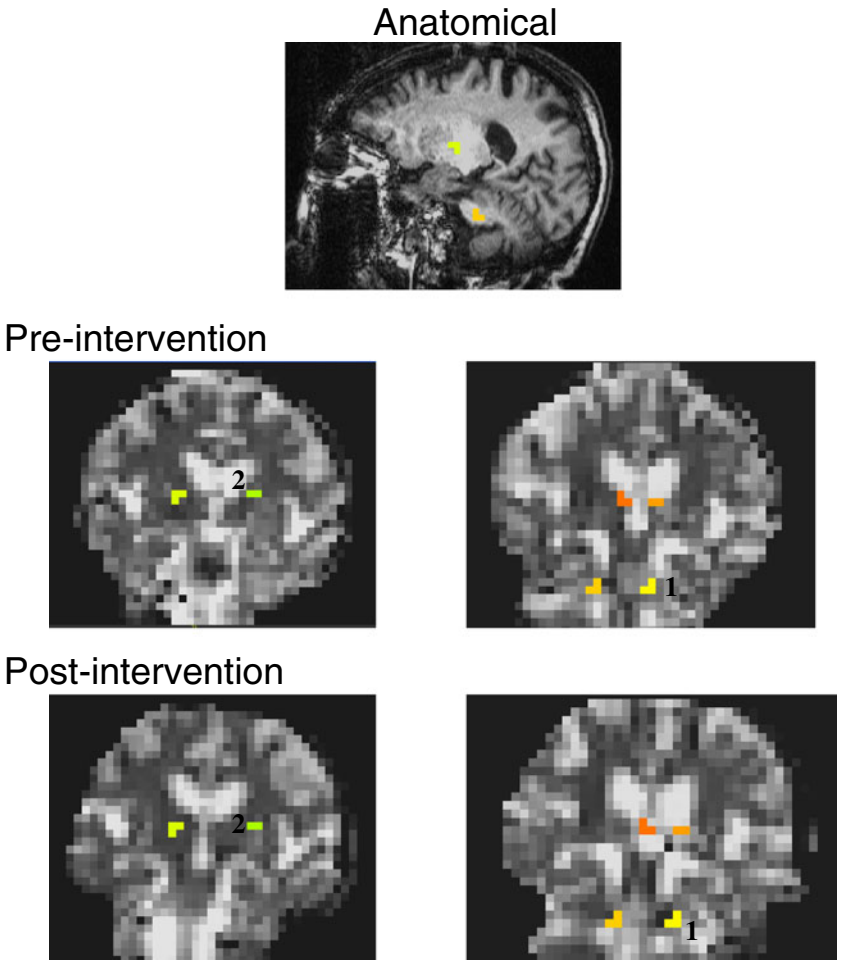

Fig. 4 Images of Pre- and Post- Mean Diffusivity. Anatomical image to localize ROIs and Pre- and Post-intervention MD images for Subject 3. Of particular interest, a decrease in MD was observed in ROIs placed in the left middle cerebellar peduncle (1) and the right posterior limb of the internal capsule (2). Also visible are ROIs placed in the right and left thalamus

sensorimotor information indicated a significant change in MD ($p<0.05$). While the linearity of this relationship was unexpected, this finding is consistent with our hypothesis.

Discussion

Our overall goal was to ascertain whether DTI, a clinically available scanning sequence, has utility as an imaging biomarker of response to a novel stroke rehabilitative intervention. The current study examined behavioral and cortical changes using DTI after NEURSTIM intervention in moderately motor-impaired stroke subjects. As was discussed, previous work has shown both behavioral and cortical changes following NEURSTIM participation in moderately impaired stroke patients (Alon et al. 2007; Weingarden et al. 1998; Dunning et al. 2008; Page et al. 2009). These findings are of potential significance, because (a) previous interventions with RTP alone have not been successful in this group, and (b) an improved understanding of the neural bases of intervention response in this group of patients may lead to more successful rehabilitative techniques.

Two subjects exhibited clinically significant motor changes in response to NEURSTIM participation. Interestingly, both were subjects receiving 120 min of NEURSTIM per day, a trend that was previously observed in the larger sample (Page et al. 2010). The subject who received 30 min of NEURSTIM showed nominal changes, and the control subject showed no FM changes and some marked ARAT changes. In speaking with the control subject, we learned that he had begun a home-based, supervised occupational therapy program focusing on RTP using the distal regions of the paretic extremity. Although he was asked by the research team not to participate in such programs during the intervention period, he mistakenly thought that the research team was only disallowing programs based in clinics, and that a home-based program was permissible. Regardless of his perceptions, this intervention is likely responsible for the positive ARAT changes that he exhibited.

Previous studies have suggested that diffusion measures stabilize prior to one year post-stroke (Choi et al. 2007) when no treatment is provided. This study provides evidence of the potential of targeted NEURSTIM intervention to induce changes in behavioral and DTI measures even in the chronic phase of stroke. Based on the success of the 120-minute treatment subjects who showed greatest behavioral improvement and concomitant MD changes in subject three, we surmise that heavy treatment with NEURSTIM may yield positive changes in white matter connectivity between regions supporting the practiced tasks. This speculation is supported by the observation that the tracts specifically carrying information to the cerebellum indicate increased integrity with increased treatment intensity. The reduced MD (indicative of increased integrity) observed in the subject with the highest dosage suggests that once the cerebellar pathway is involved, the cortical integration of motor and sensory systems may follow. This hypothesis is supported by the following observations: first, the effect was not seen in the subject receiving no treatment; second, the effect was seen exclusively in the white matter tracts specifically involved in motor function; and third, that the effect appeared to be dose-dependent.

Findings of significant differences in MD without concomitant changes observed in FA may be explained by the disparate calculation of each. MD is calculated from the mean of trace movement, providing an index of magnitude of diffusion within any voxel (Alexander et al. 2007; Pierpaoli et al. 1996). Conversely, calculation of FA provides an index of the directionality of diffusion within a voxel (Alexander et al. 2007; Pierpaoli et al. 1996). Our observation of MD changes over treatment may therefore indicate a reduction in gross (macrostructural) pathological conditions versus a microstructural change, such as axonal re-growth. We postulate that continued therapy causing the integration of novel connections within these tracts may result in observations of both FA and MD changes. Future analyses, including the use of additional anisotropic parameters, such as axial and radial diffusivity, may provide insight into a mechanism, as recent evidence suggests that these measures may be reflective of neuronal and myelin pathology, respectively (Alexander et al. 2007).

The small size of this study was the primary limitation of this study. These compelling findings, however, support the case for additional studies implementing a multicenter approach and a larger sample size to both confirm the results of this study and to further refine dosing and usage optimization.

It is important to note that there are many confounding variables and observations in this study. First, the stroke type for these subjects was not homogenous. Specifically, one subject receiving 120 min of treatment suffered a hemorrhagic stroke (ICH) (subject 3), whereas the other three discussed had experienced ischemic strokes. Although there is not consensus as to why functional outcome tends to be better in ICH than ischemic stroke (an observation we also found), there is speculation that the mechanism of functional deficits observed in ICH may be caused by compression of the brain, which is to some extent resolved with dissolution of the hematoma (Paolucci et al. 2003). It is also prudent to note the evaluation of diffusivity in one subject (subject 4) who received 120 min of therapy showed no significant changes in MD in any of the ROIs evaluated. There are a number of possible explanations for this. Although this subject showed marked improvement on the FM scale, she showed very little improvement on the ARAT measure. Additionally, this subject was the only female in the diffusion study sub-set. Sex differences in stroke recovery are well documented, such that functional outcomes after stroke are consistently poorer in female subjects (Reeves et al. 2008; Lai et al. 2005). It is reasonable, then, to postulate that these sex differences may account for the disparate imaging and functional outcomes observed.

Conclusion

Results of this preliminary study suggest that a targeted rehabilitative therapy can impact the integrity of white matter tracts, as measured by DTI in this pilot study. Given the limitations associated with traditionally-used, subjective measures in rehabilitative trials, the ability to detect anatomical changes responding to treatment is a promising diagnostic and prognostic tool in rehabilitation medicine that warrants further exploration in subsequent studies.

Acknowledgements This study was supported by a grant from the American Heart Association, and a University of Cincinnati Research Council Grant.

References

Alexander, A. L., Lee, J. E., Lazar, M., & Field, A. S. (2007). Diffusion tensor imaging of the brain. *Neurotherapeutics, 4*, 316–329.

Alon, G., Levitt, A. F., & McCarthy, P. A. (2007). Functional electrical stimulation enhancement of upper extremity functional recovery during stroke rehabilitation: a pilot study. *Neurorehabilitation and Neural Repair, 21*, 207–215.

Assaf, Y., & Pasternak, O. (2008). Diffusion tensor imaging (DTI)-based white matter mapping in brain research: a review. *Journal of Molecular Neuroscience, 34*, 51–61.

Basser, P. J., & Pierpaoli, C. (1996). Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. *Journal of Magnetic Resonance. Series B, 111*, 209–219.

Bohannon, R. W., & Smith, M. B. (1987). Interrater reliability of a modified ashworth scale of muscle spasticity. *Physical Therapy, 67*, 206–207.

Catani, M., & Mesulam, M. (2008). What is a disconnection syndrome? *Cortex, 44*, 911–913.

Choi, J. Y., Lee, K. H., Na, D. L., et al. (2007). Subcortical aphasia after striatocapsular infarction: quantitative analysis of brain perfusion SPECT using statistical parametric mapping and a statistical probabilistic anatomic map. *Journal of Nuclear Medicine, 48*, 194–200.

Cox, R. W. (1996). AFNI: Software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical Research, 29*, 162–173.

Di Fabio, R. P., & Badke, M. B. (1990). Relationship of sensory organization to balance function in patients with hemiplegia. *Physical Therapy, 70*, 542–548.

Duncan, P. W., Propst, M., & Nelson, S. G. (1983). Reliability of the fugl-meyer assessment of sensorimotor recovery following cerebrovascular accident. *Physical Therapy, 63*, 1606–1610.

Dunning, K., Berberich, A., Albers, B., et al. (2008). A four-week, task-specific neuroprosthesis program for a person with no active wrist or finger movement because of chronic stroke. *Physical Therapy, 88*, 397–405.

Fugl-Meyer, A. R., Jaasko, L., Leyman, I., Olsson, S., & Steglind, S. (1975). The post-stroke hemiplegic patient. 1. a method for evaluation of physical performance. *Scandinavian Journal of Rehabilitation Medicine, 7*, 13–31.

Ghazni, N. F., Cahill, C. M., & Stroman, P. W. (2009). Tactile sensory and pain networks in the human spinal cord and brain stem mapped by means of functional MR imaging. *AJNR Am J Neuroradio*l.

Hsieh, C. L., Hsueh, I. P., Chiang, F. M., & Lin, P. H. (1998). Inter-rater reliability and validity of the action research arm test in stroke patients. *Age and Ageing, 27*, 107–113.

Jang, S. H., Cho, S. H., Kim, Y. H., et al. (2005). Diffusion anisotrophy in the early stages of stroke can predict motor outcome. *Restorative Neurology and Neuroscience, 23*, 11–17.

Jones, D. K., Horsfield, M. A., & Simmons, A. (1999). Optimal strategies for measuring diffusion in anisotropic systems by magnetic resonance imaging. *Magnetic Resonance in Medicine, 42*, 515–525.

Judith, D. S. (2004). Motor rehabilitation and brain plasticity after hemiparetic stroke. *Progress in Neurobiology, 73*, 61–72.

Kamran, S., Bates, V., Bakshi, R., Wright, P., Kinkel, W., & Miletich, R. (2000). Significance of hyperintense vessels on FLAIR MRI in acute stroke. *Neurology, 55*, 265–269.

Lai, S. M., Duncan, P. W., Dew, P., & Keighley, J. (2005). Sex differences in stroke recovery. *Preventing Chronic Disease, 2*, A13.

Lee, J. H., Garwood, M., Menon, R., et al. (1995). High contrast and fast three-dimensional magnetic resonance imaging at high fields. *Magnetic Resonance in Medicine, 34*, 308–312.

Lotze, M., Braun, C., Birbaumer, N., Anders, S., & Cohen, L. G. (2003). Motor learning elicited by voluntary drive. *Brain, 126*, 866–872.

Lyle, R. C. (1981). A performance test for assessment of upper limb function in physical rehabilitation treatment and research. *International Journal of Rehabilitation Research, 4*, 483–492.

Mamata, H., Jolesz, F. A., & Maier, S. E. (2004). Characterization of central nervous system structures by magnetic resonance diffusion anisotropy. *Neurochemistry International, 45*, 553–560.

Medical Neurosciences Unit 2: Brainstem. Available at: http://www.neuroanatomy.wisc.edu/virtualbrain/BrainStem/16Pontine.html. Accessed August 25, 2011, 2011.

Melhem, E. R., Mori, S., Mukundan, G., Kraut, M. A., Pomper, M. G., & van Zijl, P. C. (2002). Diffusion tensor MR imaging of the brain and white matter tractography. *AJR. American Journal of Roentgenology, 178*, 3–16.

Moller, M., Frandsen, J., Andersen, G., Gjedde, A., Vestergaard-Poulsen, P., & Ostergaard, L. (2007). Dynamic changes in corticospinal tracts after stroke detected by fibretracking. *Journal of Neurology, Neurosurgery, and Psychiatry, 78*, 587–592.

Mulder, T., Zijlstra, W., & Geurts, A. (2002). Assessment of motor recovery and decline. *Gait & Posture, 16*, 198–210.

Murata, Y., Yamaguchi, S., Kawakami, H., et al. (1998). Characteristic magnetic resonance imaging findings in machado-joseph disease. *Archives of Neurology, 55*, 33–37.

Page, S. J., Levine, P., & Leonard, A. C. (2005). Effects of mental practice on affected limb use and function in chronic stroke. *Archives of Physical Medicine and Rehabilitation, 86*, 399–402.

Page, S. J., Maslyn, S., Hermann, V. H., Wu, A., Dunning, K., & Levine, P. G. (2009). Activity-based electrical stimulation training in a stroke patient with minimal movement in the paretic upper extremity. *Neurorehabilitation and Neural Repair, 23*, 595–599.

Page, S. J., Harnish, S. M., Lamy, M., Eliassen, J. C., & Szaflarski, J. P. (2010). Affected arm use and cortical change in stroke patients exhibiting minimal hand movement. *Neurorehabilitation and Neural Repair, 24*, 195–203.

Page, S., Dunning, K., Hermann, V., Boyne, P., & Levine, P. (2010). Poster 80: optimal daily duration of repetitive task specific training incorporating electrical stimulation in moderately impaired stroke. *Archives of Physical Medicine and Rehabilitation, 91*, e29.

Paolucci, S., Antonucci, G., Grasso, M. G., et al. (2003). Functional outcome of ischemic and hemorrhagic stroke patients after inpatient rehabilitation: a matched comparison. *Stroke, 34*, 2861–2865.

Pierpaoli, C., Jezzard, P., Basser, P. J., Barnett, A., & Di Chiro, G. (1996). Diffusion tensor MR imaging of the human brain. *Radiology, 201*, 637–648.

Plautz, E. J., Milliken, G. W., & Nudo, R. J. (2000). Effects of repetitive motor training on movement representations in adult squirrel monkeys: role of use versus learning. *Neurobiology of Learning and Memory, 74*, 27–55.

Puig, J., Pedraza, S., Blasco, G., et al. (2010). Wallerian degeneration in the corticospinal tract evaluated by diffusion tensor imaging correlates with motor deficit 30 days after middle cerebral artery ischemic stroke. *American Journal of Neuroradiology, 31*, 1324–1330.

R Development Core Team. (2009). R: A language and environment for statistical computing. Vienna, Austria;2.10.1.

Radlinska, B., Ghinani, S., Leppert, I. R., Minuk, J., Pike, G. B., & Thiel, A. (2010). Diffusion tensor imaging, permanent pyramidal tract damage, and outcome in subcortical stroke. *Neurology, 75*, 1048–1054.

Reeves, M. J., Bushnell, C. D., Howard, G., et al. (2008). Sex differences in stroke: epidemiology, clinical presentation, medical care, and outcomes. *Lancet Neurology, 7*, 915–926.

Rohde, G. K., Barnett, A. S., Basser, P. J., Marenco, S., & Pierpaoli, C. (2004). Comprehensive approach for correction of motion and distortion in diffusion-weighted MRI. *Magnetic Resonance in Medicine, 51*, 103–114.

Saad, Z. S., Glen, D. R., Chen, G., Beauchamp, M. S., Desai, R., & Cox, R. W. (2009). A new method for improving functional-to-structural MRI alignment using local pearson correlation. *NeuroImage, 44*, 839–848.

Schmierer, K., Wheeler-Kingshott, C. A., Tozer, D. J., et al. (2008). Quantitative magnetic resonance of postmortem multiple sclerosis brain before and after fixation. *Magnetic Resonance in Medicine, 59*, 268–277.

Schmithorst, V. J., Dardzinski, B. J., & Holland, S. K. (2001). Simultaneous correction of ghost and geometric distortion artifacts in EPI using a multiecho reference scan. *IEEE Transactions on Medical Imaging, 20*, 535–539.

Strong, O., & Elwin, A. (1943). *Human neuroanatomy.* Baltimore: Williams and Wilkins.

Teng, E., & Chui, H. (1987). The modified mini-mental state (3MS) examination. *The Journal of Clinical Psychiatry, 48*, 314–318.

Thomalla, G., Glauche, V., Weiller, C., & Rother, J. (2005). Time course of wallerian degeneration after ischaemic stroke revealed by diffusion tensor imaging. *Journal of Neurology, Neurosurgery, and Psychiatry, 76*, 266–268.

Van der Lee, J. H., De Groot, V., Beckerman, H., Wagenaar, R. C., Lankhorst, G. J., & Bouter, L. M. (2001). The intra- and interrater reliability of the action research arm test: A practical test of upper extremity function in patients with stroke. *Archives of Physical Medicine and Rehabilitation, 82*, 14–19.

Watanabe, T., Honda, Y., Fujii, Y., Koyama, M., Matsuzawa, H., & Tanaka, R. (2001). Three-dimensional anisotropy contrast magnetic resonance axonography to predict the prognosis for motor function in patients suffering from stroke. *Journal of Neurosurgery, 94*, 955–960.

Weingarden, H. P., Zeilig, G., Heruti, R., et al. (1998). Hybrid functional electrical stimulation orthosis system for the upper limb: effects on spasticity in chronic stable hemiplegia. *American Journal of Physical Medicine & Rehabilitation, 77*, 276–281.

Werring, D. J., Toosy, A. T., Clark, C. A., et al. (2000). Diffusion tensor imaging can detect and quantify corticospinal tract degeneration after stroke. *Journal of Neurology, Neurosurgery, and Psychiatry, 69*, 269–272.

Woldag, H., & Hummelsheim, H. (2002). Evidence-based physiotherapeutic concepts for improving arm and hand function in stroke patients: a review. *Journal of Neurology, 249*, 518–528.

Wolf, S. L., Winstein, C. J., Miller, J. P., et al. (2006). Effect of constraint-induced movement therapy on upper extremity function 3 to 9 months after stroke: the EXCITE randomized clinical trial. *Journal of the American Medical Association, 296*, 2095–2104.

Yu, C., Zhu, C., Zhang, Y., et al. (2009). A longitudinal diffusion tensor imaging study on wallerian degeneration of corticospinal tract after motor pathway stroke. *NeuroImage, 47*, 451–458.