Changes in Cortical Activity in Stroke Survivors Undergoing Botulinum Neurotoxin Therapy for Treatment of Focal Spasticity

Kaleb Vinehout 1, Kelsey Tynes 1, Miguel R. Sotelo 1, Allison S. Hyngstrom 2, John R. McGuire 3 and Brian D. Schmit 1,3*

1 Department of Biomedical Engineering, Marquette University and the Medical College of Wisconsin, Milwaukee, WI, United States, 2 Department of Physical Therapy, Marquette University, Milwaukee, WI, United States, 3 Department of Physical Medicine and Rehabilitation, Medical College of Wisconsin, Milwaukee, WI, United States

Background: Botulinum NeuroToxin-A (BoNT-A) relieves muscle spasticity and increases range of motion necessary for stroke rehabilitation. Determining the effects of BoNT-A therapy on brain neuroplasticity could help physicians customize its use and predict its outcome.

Objective: The purpose of this study was to investigate the effects of Botulinum Toxin-A therapy for treatment of focal spasticity on brain activation and functional connectivity.

Design: We used functional Magnetic Resonance Imaging (fMRI) to track changes in blood oxygen-level dependent (BOLD) activation and functional connectivity associated with BoNT-A therapy in nine chronic stroke participants, and eight age-matched controls. Scans were acquired before BoNT-A injections (W0) and 6 weeks after the injections (W6). The task fMRI scan consisted of a block design of alternating mass finger flexion and extension. The voxel-level changes in BOLD activation, and pairwise changes in functional connectivity were analyzed for BoNT-A treatment (stroke W0 vs. W6).

Results: BoNT-A injection therapy resulted in significant increases in brain activation in the contralesional premotor cortex, cingulate gyrus, thalamus, superior cerebellum, and in the ipsilesional sensory integration area. Lastly, cerebellar connectivity correlated with the Fugl-Meyer assessment of motor impairment before injection, while premotor connectivity correlated with the Fugl-Meyer score after injection.

Conclusion: BoNT-A therapy for treatment of focal spasticity resulted in increased brain activation in areas associated with motor control, and cerebellar connectivity correlated with motor impairment before injection. These results suggest that neuroplastic effects might take place in response to improvements in focal spasticity.

Keywords: stroke, rehabilitation, BoNT-A, MRI, activation, connectivity
INTRODUCTION

Spasticity occurs in up to 40% of stroke survivors and is associated with functional loss, based on correlations with Barthel scores [see (1, 2) for review]. Botulinum Neurotoxin type A (BoNT-A), which acts by blocking the release of acetylcholine at the neuromuscular junction, temporarily relieves lower limb (3), and upper limb spasticity in patients with stroke (4–6); however, improvements in arm and hand function are not consistently observed across individuals (4, 7). Care and comfort of the hand are consistently improved with BoNT-A injections (8, 9), despite the observation that broader function of the hand is not reliably achieved (9, 10). When outcomes of BoNT-A treatments are considered in terms of passive and active function, passive functional goals are more often met compared to placebo controls, while no differences in active functional goals are observed (11). There is, however, some promise of BoNT-A for targeted improvements in function. A recent titration study demonstrated that increasing the dose of BoNT-A improves the attainment of individual goals (12). With improved goal attainment after BoNT-A treatment, it is possible that there are underlying neuroplastic effects that contribute to functional improvement. Thus, the purpose of the current study was to determine whether there are changes in brain activation or connectivity associated with hand function after BoNT-A that could serve as the basis for restoration of functional movement.

Investigations of brain activation following BoNT-A therapy have produced varying results. In contrast to dominant unilateral activation of motor areas during hand movements in controls, stroke survivors have extensive bilateral activation of primary sensorimotor, premotor and supplementary motor areas during movement of the affected hand (13–15). Changes in brain activity patterns following BoNT-A injections have shown variable results. Some reports indicate a reduction in the bilateral volume of activation in primary motor areas after BoNT-A injections during active (16–18) and imagined (19) movements, demonstrating a localizing and lateralization effect. Other studies have shown increases in activity in similar motor areas after passive wrist movement (20) and arm cycling (21) following BoNT-A therapy. In addition to changes in brain activity patterns after stroke, there are significant alterations in functional connectivity following stroke, including decreases in interhemispheric connections to somatomotor areas and increases in intrahemispheric connections (22–26); however, changes in connectivity with BoNT-A treatments have not been considered, to date.

In this study, we used functional magnetic resonance imaging (fMRI) to investigate the underlying changes in brain activation and connectivity following BoNT-A therapy to relieve upper limb spasticity after stroke. We examined changes in both brain activity and functional connectivity in participants undergoing BoNT-A therapy. To include stroke participants with severe limb spasticity, we developed a wrist-hand device that would allow active finger flexion while passively assisting the fingers to full extension. Note that Bergfeldt et al. (16) and Manganotti et al. (17) also used devices during fMRI measurements to minimize flexor synergies and large synkinetic movements; our device was unique in that it allowed full range-of-motion of finger flexion for stroke participants with mild-to-severe spasticity during fMRI scanning. Our analyses consisted of voxel-based activity measurements and region of interest (ROI) functional connectivity analyses of fMRI data. We hypothesized that BoNT-A's peripheral effects on the affected limb would increase brain activity in higher order motor control centers such as the premotor area and improve global connectivity between motor control centers.

METHODS

In this functional MRI study, we obtained blood oxygen level dependent (BOLD) images from a convenience sample of stroke participants undergoing botulinum toxin therapy for arm spasticity. The BoNT-A treatment was part of prescribed clinical care and was not modified for this study. We measured BOLD activation at the time of injection (W0) and 6 weeks (W6) later, at the peak effect of the botulinum toxin on alleviating arm spasticity. We performed voxel-level whole-brain activation and independent component analyses to identify changes in functional activation and connectivity associated with motor recovery due to botulinum toxin therapy. Nine people with chronic stroke were enrolled in the study (5 female; aged 58.2 ± 3.8, range 42–77). Stroke inclusion criteria included: undergoing BoNT-A therapy as part of clinical care; stroke onset more than 6 months prior to the study; wrist/finger impairment as determined by physical examination; no contraindication to MRI. All participants suffered from upper extremity spasticity following stroke and had previously undergone at least one session of BoNT-A treatment. Eight age-matched controls (3 female; aged 56.4 ± 2.2, range 47–70) were enrolled. Control inclusion criteria included: no known neurological or muscular disease and no MRI contraindication. All procedures were approved by the Institutional Review Board (IRB) of the Medical College of Wisconsin (MCW). All participants gave written informed consent to take part in this study and all procedures were conducted in accordance with the Declaration of the World Medical Association.

Study Set-Up

This study consisted of two test sessions scheduled 6 weeks apart for both control and stroke participants. For participants receiving BoNT-A therapy, each session included an MRI scan and a clinical assessment. At least 3 months had passed since the patients’ last BoNT-A injection before being enrolled in this study. The first session was conducted 1–4 days before participants received their BoNT-A injection (W0), and the protocol was repeated 6 weeks post-injection in the second session (W6). The control group participated only in the imaging portion of the procedures, with the exception of participants C1 and C5, who did not attend the second session. These controls were used as a comparison to stroke participants. All data were processed with the same analysis, except lesions were identified in stroke participants to aid in registration.
TABLE 1 | Participant demographics and clinical characteristics.

| Participant | Sex | Age (Years) | Stroke type | Lesion location | Time post stroke (Years) | MAS finger/wrist | FM Pre/Post injection | Nth injection | Physical therapy |
|-------------|-----|-------------|-------------|----------------|--------------------------|-----------------|------------------------|--------------|------------------|
| BTX 1       | F   | 48          | Isch        | R MCA-UD       | 4.6                      | 3/3             | 23/26                  | 6            | Prescribed       |
| BTX 2       | M   | 58          | Hem         | L Ip-BG        | 4.4                      | 2/2             | 26/27                  | 13           | Prescribed       |
| BTX 3       | F   | 42          | Hem         | L Ip-BG        | 3.9                      | 3/4             | 19/22                  | 12           | Prescribed       |
| BTX 4       | M   | 77          | Isch        | L MCA-LD, UD, Le | 1.4                    | 3/3             | 20/22                  | 2            | Prescribed       |
| BTX 5       | F   | 67          | Isch        | R MCA-LD, UD   | 1.8                      | 3/2             | 9/9                    | N/A          | N/A              |
| BTX 6       | F   | 60          | Isch        | R MCA-LD, UD, Le | 11.9                   | 1/1             | 23/25                  | 40           | Prescribed       |
| BTX 7       | M   | 69          | Isch        | L Pons         | 1.1                      | 2/1             | 63/83                  | 2            | Not Prescribed   |
| BTX 8       | F   | 48          | Isch        | R MCA-LD, UD   | 8.9                      | 4/4             | 44/47                  | 27           | Home Exercises   |
| BTX 9       | M   | 55          | Isch        | R MCA-LD, UD, Le | 5.1                    | 4/2             | 35/40                  | 17           | Home Exercises   |

MCA, middle cerebral artery; LD, Lower Division; UD, Upper Division; Le, Lenticulostriate; Ip, Intraparenchymal; BG, Basal Ganglia; R, Right; L, Left; Isch, Ischemic; Hem, Hemorrhagic; Note: information regarding the number of injections and details of physical therapy for participant number 5 was not available due to transfer between centers.

TABLE 2 | Therapy dosage.

| Name | BoNT # | DOSE | # MUSCLES |
|------|--------|------|----------|
| BX1  | B6     | 450  | 4 PMj, PMn, LD, TR, BRA, BRD, ECR, FCR, FDP, FPL |
| BX2  | B11    | 350  | 0 PMj, BRA, BRD, PT, FCR, FCU, FDS, FDP, FPL |
| BX3  | B12    | 300  | 0 BIC, BRD, PT, FCR, FCU, FDS, FDP |
| BX4  | D2     | 550  | 4 PMj, LD, PT, FDS, FDP |
| BX5  | B4     | 200  | 0 PMj, BIC, FDS |
| BX6  | B40    | 200  | 0 LS, LD, PMn, PT, ECR, FPB, Lu |
| BX7  | X2     | 125  | 0 LS, BRA, BRD |
| BX8  | B27    | 325  | 0 LD, BRA, BRD, FCR, FCU, FDS, FDP, FPL, FPB, Lu |
| BX9  | B17    | 325  | 0 PMj, BRA, BRD, PT, ECR, ECU, FDS, FDP, FPB, Lu |

Table listing the BoNT dosage and physical therapy sessions for each stroke participant. aTherapy, therapy sessions between BoNT-A treatments; BoNT B, Botulinum Toxin B (OnabotuliumtoxinA); D, Dysport (AbobotulinumtoxinA); X, Xeomin (IncobotulinumtoxinA); PMj, Pect Major; PMn, Pect Minor; LD, Latissimus Dorsi; LS, Levator scap; TR, Triceps; BRA, Brachialis; BRD, Brachioradialis; PT, Pron Teres; Lu-Lumbricals; FCU, Flexor Carpi Ulnaris; FPB, Flexor Pollicis Brevis; BIC, Biceps Brach; FDS, Flexor Digitorum Superficialis; FPL, Flexor Pollicis Longus; ECR, Extensor Carpi Radialis.

BoNT-A Administration and Clinical Data

Each participant had been treated with BoNT-A 3–4 months prior to the study period as part of the usual care (Table 1). Each participant was prescribed physical therapy following BoNT-A injections; however, only two participants underwent therapy (4 sessions between W0 and W6, for all cases: see Table 2). The dosage and muscles injected (Table 2) were determined by the severity of spasticity and the individual’s goals for the treatment. EMG guidance was used for all injections. The dose of BoNT-A and muscle injected was not adjusted for study purposes. Summary information of the number of BoNT-A injections, muscles injected, and physical therapy sessions are shown in Table 2.

All stroke participants’ paretic arm motor impairment was assessed using the Fugl-Meyer Assessment (FMA) (27) at time-points W0 and W6. The wrist and finger flexor spastic hypertonia and increased muscle tone were assessed using the Modified Ashworth Scale (MAS) (28) at W0. The MAS has moderate test-retest reliability (29) very good interrater reliability (30), and convergent validity with the FMA, EMG response to a ramp stretch, and the pendulum test (31). The FMA has high test-retest and interrater reliability in people with stroke (32–34). The W0 and W6 FMA scores were checked for non-normality using the Anderson-Darling test and compared for significant changes with a paired t-test. The MAS and FMA measurements were compared with activation volume, activation intensity, and functional connectivity measurements. Comparisons with the FMA scores used a Pearson correlation, while those with the MAS used a Spearman correlation. A Pearson correlation was used for the FMA based on the assumption that the FMA data is measured on an interval scale. In contrast, the MAS was assumed to be an ordinal variable and thus, the Spearman correlation was used. The FMA was collected at both timepoints due to its greater clinical relevance and psychometric properties compared to the MAS (35). Characteristics of the participants in the stroke group are described in Table 1, with further details regarding size and location of the lesions illustrated in Figure 1.

Imaging Data Acquisition

All images were collected using a 3.0T GE Discovery MR750 scanner equipped with a 32-channel head receive coil (MR Instruments, Inc.; Distributed by GE Healthcare; frequency: 127.73 MHz; field: 3T). Anatomical 3D images were collected using the following fast spoiled gradient echo planar imaging (FSPR-G-EPI) protocol: Echo Time (TE) = 3.2 ms, Repetition Time (TR) = 8.16 ms, Field of View (FOV) = 240 mm, and 156 × 1 mm slices. Two 6-min trials were conducted for the fMRI, using a GE’s gradient echo planar imaging (GRE-EPI) protocol with the following parameters: TE = 25 ms, TR = 2,000 ms, FOV = 224 mm, Matrix: 64 × 64 mm, and 41 × 3.5 mm sagittal slices.
Due to spasticity, some participants with stroke were unable to fully extend the fingers without assistance. To address this issue, a device was created to aid in finger extension and was used by all participants (stroke and control) during the task-based fMRI assessment. The device is described and depicted in Figure 2A. All participants actively flexed against the resistive bands while the device passively extended the fingers. In effect, while this device allowed for all subjects to complete the task, it also resulted in predominantly active finger flexion while producing passive finger extension. In addition, the device allowed testing within the spastic joint range of motion for the fingers (36). The substantial loss of cortical modulation of stretch reflex threshold (37) is likely to impact the extended finger range of motion, especially for the fingers. Since motor learning can be impaired in the spastic joint range of motion (38), the range of motion used for testing in this study was designed to include a pre-injection spastic range of motion that was relieved by BoNT-A injections.

Participants were scanned while prompted by a visual cue to perform full-hand flexion and extension using the affected (stroke group) or non-dominant (control group) hand. A visual cue was presented in a block paradigm, which alternated rest and hand movement at 20-s intervals for a total of 6 min (Figure 2B). Each participant performed two experimental runs.
**Figure 2**

(A) Sketch of assistive finger extension device. This device assists finger extension of stroke participants with spasticity via elastic bands (Theraband: 10.7 N resistance at 100% elongation, 15.1 N at 200%) that are supported by an outrigger on the dorsal part of the hand. Velcro straps attached to the patient’s first four digits and were connected to an elastic band. A plastic brace served to stabilize the participant’s forearm and wrist as they were instructed to flex and extend the digits.

(B) Visual presentation prompt. The visual prompt was presented on a screen inside the bore and read “Relax” centered in a magenta circle (20 s) and “Open/Closed” centered on a cyan circle (20 s, 1 Hz) for 6 min.

**Data Pre-processing**

The first four TRs were removed from each fMRI trial, and both trials were concatenated. Advanced Normalization Tools (ANTS) software (N4BiasFieldCorrection) corrected for bias field inhomogeneities in both anatomical and functional MR images. The skull and other non-brain matter were removed from both anatomical and functional MR images using the FMRIB Software Library (FSL) Brain Extraction Tool (bet). Anatomical and functional MR images were flipped in the right-left direction for stroke participants with left hemiparesis and control participants self-identified as right-hand dominant, to standardize brain activation to the left hemisphere. In total, 5 stroke participants and 4 control participants were flipped. Registration to the Montreal Neurological Institute (MNI)
FIGURE 3 | Connectivity pipeline. Registration (1A) and task regression (1B) were performed on the data collected for this study. Using the Smith et al. (44) data, an ICA (2A) and a clustering algorithm were performed to get 149 subcomponents, shown in red circles (2B). From these 149 subcomponents, task ROI (2C) were defined by correlating the HRF with the mean timeseries of our study’s data. For these task ROI, shown with red circles the global connectivity (3) strength, shown with brown lines, was calculated.

152-subject average brain was performed using ANTs for all images, and a non-linear warp was applied to the functional MR images. Lesion masks obtained from the Lesion Identification with Neighborhood Data Analysis (LINDA) algorithm (42) aided in image registration for stroke participants. Lesion masks and registration were visually inspected for accuracy.

Activity Analysis
FMRI analyses were carried out using the fMRI Expert Analysis Tool (FEAT) Version 5.0, in FSL. First-level FEAT analysis was performed on individual data using a Fixed Effect (FE) analysis (43), which included motion correction, spatial smoothing using a full-width/ half-maximum (FWHM) 5 mm Gaussian kernel, temporal high pass filter (0.01 Hz) on the BOLD signal, and prewhitening. A modeled hemodynamic response (HDR) was created by convolving the binary block design with a gamma wave (phase = 0s, std. dev. = 3s, mean lag = 6s), each voxel’s timeseries was correlated to the time series model, and the resulting activation images were clustered and thresholded at a Z-value > 2.3 (p < 0.05).

Group analysis was performed using a general linear model (GLM) which categorized individual participants by their group (Stroke/Control) and session (W0/W6). Group mean activation maps were created using non-parametric permutation testing (10,000 permutations) for each group at a threshold Z > 2.3 and cluster significance p < 0.05 with false discovery rate correction.

Regions of Interest for Activation
Within the stroke group, the activation images at time-point W6 were compared to the activation images at time-point W0, and the voxels where W6 was >W0 defined our volume of interest. These regions were further subdivided using the Jülich Histological Atlas for supratentorial regions and the Taliaarch
Daemon Label Atlas for cerebellar regions, and the number of active voxels and the intensity of each region was recorded.

Regions of Interest for Connectivity
For the connectivity analysis, ROIs were defined using publicly available data. First the ICA of these control data in both resting state and task-based functional connectivity was determined (44) (Figure 3.2A). These processes resulted in 149 sub-components (Figure 3.2B), in which only the gray matter volume was included within region of interest masks for the connectivity analysis. Utilizing these ROIs defined by functional connectivity instead of a priori ROIs allows for detection of the full functional connectivity network for a given region.

Using this study’s task-based fMRI data, task ROIs were identified by correlating the hemodynamic response function (HRF) with the mean timeseries of each ROI as defined above (Figure 3.2C) [see Vinehout et al. (25) for additional details]. Briefly, subcomponents that were correlated with the HRF ($r > 0.2$) were considered task regions of interest (task ROIs). This was done across all stroke and control participants so one set of task-ROIs was defined for all participants in this study. See Table 3 for descriptions of the resulting ROIs. These task-ROIs corresponded to the ROIs involved in the finger flexion/extension task. This seed-based functional connectivity approach allows targeted assessment of motor pathways (45–47).

Functional Connectivity Analysis
After identification of task-ROIs, the HRF of the flexion/extension task was regressed out of the task-based fMRI data. The HRF was removed for task-based functional connectivity to reduce the effect that brain activations have on spurious connectivity measurements (48, 49). For each participant and task, a mean fMRI time series was computed for each of the 18 identified task ROIs (see Table 3 for location of task-ROIs). Pearson correlation coefficients were computed on all pairwise combinations of this mean time series for the 18 task ROIs; Fisher-Z transformations were applied to the Pearson correlation coefficients. These values provided a measure of global connectivity that represented the strength of functional connections between task ROIs (Figure 3.3). The FSL randomize (41) non-parametric permutation test with Bonferroni correction for multiple comparisons was used for comparisons between W0 and W6. These measures provided insight into the strength of the functional connections among task ROIs.

Correlation With Clinical Impairment
Correlations were performed between activation volume, activation intensity, functional connectivity, and clinical measurements, using a Spearman correlation for MAS and a Pearson correlation for FMA. These correlations were performed for W0 and W6 measurements, corrected for multiple comparisons with a False Discovery Rate.

### Table 3 | Eighteen functional connectivity ROIs.

| ROI # | Main region of interest | Size (Voxels) | ROI (X,Y,Z) |
|-------|-------------------------|--------------|-------------|
| 1     | Right Limbic Lobe and Cingulate Gyrus (BA 24) | 1062 | 47, 57, 50 |
| 2     | Right Limbic Lobe and Cingulate Gyrus (BA 6) | 332 | 55, 35, 20 |
| 3     | Left Parietal Lobe and Inferior Parietal Lobule (BA 40) | 7588 | 25, 50, 63 |
| 4     | Left Parietal Lobe and Postcentral Gyrus (BA 3) | 3039 | 50, 64, 66 |
| 5     | Right Frontal Lobe and Superior Frontal Gyrus (BA 6) | 1598 | 41, 64, 67 |
| 6     | Right Frontal Lobe and Precentral Gyrus (BA 4) | 4011 | 72, 60, 50 |
| 7     | Right Frontal Lobe and Precentral Gyrus (BA 6) | 3766 | 18, 59, 49 |
| 8     | Right Frontal Lobe/Posterior of Cerebellum | 1879 | 54, 30, 22 |
| 9     | Left Parietal Lobe and Postcentral Gyrus (BA 3) | 4279 | 38, 52, 69 |
| 10    | Left Frontal Lobe and Medial Frontal Gyrus (BA 6) | 4867 | 27, 44, 57 |
| 11    | Right Posterior/Anterior Lobe of Cerebellum. (PACb) | 3873 | 59, 28, 28 |
| 12    | Right Anterior/Posterior Lobe of Cerebellum (APCb) | 5197 | 49, 34, 21 |
| 13    | Right Anterior/Posterior Lobe of Cerebellum (PACb) | 5252 | 69, 53, 43 |
| 14    | Left Parietal Lobe and Inferior Parietal Lobule (BA 40) | 4187 | 27, 44, 57 |
| 15    | Left Frontal Lobe and Middle Frontal Gyrus (BA 6) | 4867 | 27, 44, 57 |
| 16    | Left Frontal Lobe and Precentral Gyrus (BA 6) | 301 | 30, 43, 69 |
| 17    | Left Parietal Lobe and Postcentral Gyrus (BA 3) | 8012 | 58, 42, 26 |
| 18    | Right Anterior Lobe of Cerebellum (ACb) | 4894 | 49, 27, 28 |

Table listing all the task ROI derived from the group ICA of controls. The number of voxels correspond to the size of each ROI in 2mm standard MNI space. X, Y, Z coordinates refer to standard MNI space. Percentages listed are for each gray matter region that encompassed more than 5% of a given ROI. Names provided are based on the labels of the Talairach Atlas. BA, Brodmann’s Area.

RESULTS

Reduced Motor Impairment Following BoNT-A Therapy
Stroke participants showed a significant increase in FMA motor scores following injections of BoNT-A to the affected arm ($p = 0.004; \bar{df} = 8; t$-stat = $-3.9194$; paired $t$-test) with a mean increase of 2.1 ± 1.62 (Table 1). Four of nine participants showed improvement in wrist function, and three showed improvements in finger extension. Those that improved in finger extension had the highest MAS scores prior to injection. Other areas of improvement (mass finger extension, forearm pronation/supination, shoulder flexion, and abduction) varied between participants.

Changes in Brain Activity Patterns Following BoNT-A Therapy
We found expected activity patterns and no significant difference between W0 and W6 sessions for controls. Across sessions, controls consistently and significantly activated bilateral primary
FIGURE 4 | Group activity maps of control participants. The figure shows slices of the MNI template overlaid with z-statistic (Z > 2.3) maps of W0 and W6 average control group activity during non-dominant hand movement. Activity maps indicate volumes in which there was significant (p < 0.05) levels of activity across the group. The right hemisphere of the brain is displayed on the left. Right hemisphere is ipsilateral to the movement arm. No differences were detected between W0 and W6.

motor (M1) and ipsilateral cerebellar areas at W0 and W6 (Figure 4) during right-hand movement. In addition to these regions, the ipsilateral premotor and supplementary motor area, bilateral hand portion of the M1, the thalamus and the putamen showed significant task-related activity. Although control participant’s activity maps were similar across sessions, stroke participants showed differences between W0 and W6 (Figure 5). At W6 there was more widespread and bilateral activation in the stroke group compared with W0; whereas activation before injection was restricted to the contralesional hemisphere, activation increased in both hemispheres after injection (Figure 5). Activation maps yielded p-values for each voxel; these maps were threshold with p < 0.05 to assess significance. Significant differences (p < 0.05, df = 8; z > 2.3; paired z-test) between W6 and W0 in the stroke group included: (1) contralesional premotor cortex (PMC-R), (2) contralesional cingulate gyrus (CG-R), (3) contralesional thalamus (Th-R), (4) somatosensory and visual integration areas (Sens-IA), and (5) superior cerebellum (S-CB). These regions of activation are further described in Table 4 and illustrated in Figure 5.

The five identified regions of activation were subsequently used as masks to identify the number of active voxels in the given volume for the stroke group at W0 and W6 (Figure 6). Participants with stroke showed a significantly increased number of active voxels in all five regions following BoNT-A injections.

Changes in Brain Connectivity Following BoNT-A Therapy

Interestingly, the connectivity analysis did not show significant differences between W0 and W6 in either stroke or control groups after multiple-comparison corrections. Trends of increased functional connectivity after BoNT-A were observed across the 18 task-related ROIs; however, these trends were not significant once corrected for multiple comparisons. The largest connectivity changes were observed in three nodes,
FIGURE 5 | Stroke differences during BoNT-A injections. The first two columns show significant ($p < 0.05$) levels of activity across the stroke group for W0 (left) and W6 (right). Columns 3 and 4 show the coronal (left) and axial (right) view of significant increase in activity following the BoNT-A intervention, displayed as five regions of interests. These areas are: (a) right premotor cortex (b) right cingulate gyrus (c) right thalamus (d) sensory integration area (e) superior cerebellum. The physical coordinates of the axial slices are shown to the left or right of axial images, and the coronal views are denoted by the yellow horizontal lines.
the bilateral premotor/motor cortices and the insula of the non-lesioned hemisphere (Figure 7 and Table 3), which showed small p-values but were not statistically significant when the correction for multiple comparisons was applied. In contrast, the control group did not demonstrate trends for increased functional connectivity between W0 and W6. See Figure 7 for visual depiction of trends in stroke participants for these 3 nodes.
Clinical Correlations With Activity and Connectivity Values

Correlations were performed between activation volume, activation intensity, functional connectivity, and clinical measurements (MAS and FMA). The correlations between the MAS (W0) and activation volume, activation intensity and functional connectivity were not significant when corrected for multiple comparisons. There were trends between these measurements that had small $p$-values before multiple comparison correction. A total of 52 correlations had an uncorrected $p < 0.05$. Three correlations had uncorrected $p < 0.01$. These trends were W0 functional connectivity measurements that correlated with W0 FMA scores and W6 functional connectivity measurements that correlated with W6 FMA scores, as summarized in Figure 8. At W0, connections between the contralesional anterior cerebellum and the contralesional posterior cerebellum were moderate and positively correlated ($R = 0.83$ and $R = 0.81$) with W0 FMA scores. At W6, contralesional and ipsilesional premotor areas were moderate and positively ($R = 0.83$) correlated with the FMA scores.

DISCUSSION

In this study, we found preliminary evidence of the effects of BoNT-A on higher-order brain activation using fMRI. Following BoNT-A, significant increases in the BOLD signal in the stroke group were observed in the contralesional premotor cortex...
Increased Volume of Activation in Higher-Order Brain Regions After BoNT-A Therapy

Our functional activation results showed that BoNT-A therapy increased functional activity in the ipsilesional and contralateral hemispheres during unilateral paretic finger flexion-extension, suggesting BoNT-A therapy promotes neural reorganization. The activation patterns for the control group (Figure 4) were consistent with previous studies, showing activation in the contralateral motor areas in addition to the ipsilateral cerebellum (50–52). Interestingly, the control group also had activation of ipsilateral (right hemisphere) motor areas and bilateral subcortical regions, which have been associated with task precision and movement duration (53–56). In the stroke group, BoNT-A therapy increased the volume of activation in both hemispheres. Prior to injection (W0), stroke participants had activity associated with wrist flexion mainly in the contralesional hemisphere (Figure 5). After injection (W6), the volume of activation encompassed bilateral motor areas, subcortical regions including the thalamus, and the cerebellum, similar to the control group.
There are a limited number of studies on the effects of BoNT-A therapy on brain activation. Contrary to our results, reduced brain activation is observed after BoNT-A therapy for imagined movements (57), finger tapping (19), and mass finger flexion (17). In particular, Manganotti et al. (17) observed decreased BOLD activation following BoNT-A injection therapy, localizing in the ipsilesional motor cortex and contralesional cerebellum. There are two key methodological differences in our study and these prior studies. First, our protocol used a device that passively extended the fingers, which facilitated finger movement through the full range; prior studies involved paced isotonic contractions, which were constrained by an orthosis allowing for 30° range of motion. The second key difference is our protocol aimed to observe the effects of BoNT-A injections on brain activity during the normal course of stroke rehabilitation.

The increase in brain activation following BoNT-A injection therapy in stroke participants might be due to an increased neural drive to flex the fingers through the full range of motion, or, at least in some participants, because physical therapy contributes to the increase in activation. In a similar BoNT-A and fMRI study, Diserens et al. (21) found bilateral increases in BOLD activation in motor regions following BoNT-A injections during passive arm movement, and the BOLD activity increased further following paced repetitive passive movements of the plegic hand and BoNT-A injections. Additionally, Veverka et al. (20) found increased volume of activation in the bilateral cerebellum, contralesional sensorimotor cortex, and the contralesional occipital cortex during passive wrist movements. Thus, reducing spastic hypertonia may increase the afferent feedback from hand movement (20, 21) and allow for greater improvements during physiotherapy (58–60), resulting in increased brain activation.

Another possible reason we saw differences in activation in bilateral brain regions compared to prior studies might be differences in the stroke participant pre-injection function. The participants in our study had heterogeneity of their baseline Fugl-Meyer scores ranging from 9 to 63. The inclusion of high impairment participants is reflected in these participants with lower Fugl-Meyer scores. Stroke survivors have increased activation, particularly in the contralesional hemisphere, during hand movements (13, 61–64). Additionally, it has been reported that therapy increases activity in the ipsilesional hemisphere, lateralizing and localizing activity during paretic hand movement (65–68). Our results included an increased ipsilesional activation following BoNT-A injection therapy, which has been associated with improved motor recovery (69, 70). However, our results also showed increased activation in the contralesional side. It has been suggested that hyper-excitability of the contralesional hemisphere is detrimental to motor recovery following stroke due to interhemispheric inhibition (71). However, recent findings suggest that well-recovered patients have increased contralesional motor activity, which may play a supportive role during rehabilitation (71, 72). This trend might be more apparent in study participants with severe motor deficits (13, 14, 66).

Functional Connectivity in Motor-Related Regions After BoNT-A Therapy

While there were no significant differences in connectivity between W0 and W6 for the stroke or control participants, there was a trend of increased connectivity in the stroke group at W6. A larger sample size might have been able to parse out these differences. Changes in functional connectivity after treatment can be significant (73–75). During W0 we saw correlations between regions of the right cerebellum and FMA score; during W6 we saw correlations in the connectivity between the contralesional and ipsilesional premotor areas and FMA score. Interestingly the areas that overlapped between functional connectivity and activation were areas in which functional connectivity correlated with the FMA score. This further highlights the importance of the cerebellum and contralesional and ipsilesional premotor areas in recovery. The W6 correlations between contralesional and ipsilesional premotor areas suggest that these connections might be more clinically relevant as people with stroke recover. Others have highlighted the clinical importance of these interhemispheric connections (76).

Study Limitations

The study enrollment was small, and it is possible that including more participants would have provided more areas of significant activity and connectivity differences with BoNT-A therapy. However, despite the low sample size, there were consistent patterns within the stroke group. A larger sample size might have provided significant functional connectivity results after correction for multiple comparisons, verifying the observed trends. This sample included a heterogeneous group of stroke patients. Arm therapy decreases brain activation in stroke survivors with high baseline function and increases brain activity in those with low initial function (77), suggesting that the variability of stroke severity in our test group might have limited the statistical significance of group comparisons. In the future, a larger sample size could be collected to assess how stroke severity affects changes in brain activation following BoNT-A therapy.

Another limitation to the present study is the lack of measurement of hand movement while in the scanner. Movement of the target had was observed visually in all participants in the current study during fMRI measurements; however, a real-time measurement of hand movement would provide a measure of the change in movement between W0 and W6, which could have impacted brain activity. The hand apparatus helped to normalize movement across participants and sessions, although differences in movement range were still possible. In addition, measurements of movements would allow for control of mirror movements that are often seen in stroke survivors trying to perform tasks with a significantly impaired limb (78–80). It is possible that activity seen in contralesional motor areas may have resulted from mirror movements of the unaffected hand, though it is unlikely because mirror movements were not observed in the orientation sessions.

Assessments of improvements in spasticity were limited to the MAS and FMA prior to the injection. In addition, MAS is
a measurement of spastic muscle tone and does not distinguish active and passive components of hypertonia. A follow-up measure to quantify improvements in spastic hypertonia and impairment would have added to the interpretation of the BoNT-A effects. Additional tests of hand function would have added information on functional ability following BoNT-A therapy. We identified a significant increase of 2.1 FMA points across the group, which is a small increase in the upper extremity FMA, compared to the reported 3.2 minimal detectable change in individual upper extremity score (34). While we saw significant changes in FMA scores pre and post BoNT-A injection (Table 1), these changes were below minimal clinically important differences for this scale (81). Assessments that directly measure the functional goals targeted by the BoNT-A therapy might be more meaningful for future work.

Physical therapy was recommended to patients participating in this study as part of their standard clinical care, with exercises tailored to their specific needs. Physical therapy treatment plans, duration, and frequency were not controlled, but assumed to remain constant over the 6-week period of involvement in the study. For a number of practical reasons, participation in physical therapy sessions was limited (see Table 1 for therapy recommendations and Table 2 for the number of therapy sessions completed by each participant). Thus, the contribution of therapy to the changes in activity and connectivity following BoNT-A injections remains unclear. Injection site and BoNT-A dose were determined by the severity of spasticity and clinical need of each participant. The number of BoNT-A injections that each participant received prior to the study period ranged from 2 to 40. None of these variables correlated with the study outcomes. However, because this study did not include participants after their initial BoNT-A injections the results may not have captured the most significant improvements. Greater improvements in function have been reported following the initial BoNT-A injection (82–84) and subsequent injections are needed to maintain those improvements.

CONCLUSION

This study showed the effects BoNT-A injection therapy on motor impairment and neuroplasticity. BoNT-A injection therapy produced a significant increase in contralesional activation in stroke survivors after therapy. Additionally, there was a trend of increased interhemispheric and intrahemispheric functional connectivity, most notably to motor/premotor nodes. These neuroplastic changes correlated with motor impairment and limb spasticity; ipsilesional functional connectivity measurements were correlated with the Fugl-Meyer scores, and ipsilesional activation measurements were correlated with the Modified Ashworth Scale. These results suggest that neuroplastic effects take place in response to improvements in focal spasticity and highlight the importance of brain activity and connectivity patterns in rehabilitation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical College of Wisconsin: PRO00027569. The patients/participants provided their written informed consent to participate in this study.

DISCLOSURE

The presented work has not been published prior, although this work constitutes a portion of the Master of Science thesis of KT.

AUTHOR CONTRIBUTIONS

BS, KT, and AH contributed to the study conception and design. MS and KT contributed to the material preparation and data collection. Analyses were performed by KT and KV. The first draft of the manuscript was written by KT and KV. All authors commented on previous versions of the manuscript, read and approved the final manuscript.

FUNDING

Funding was provided by the Strategic Fund, a component of the Advancing a Healthier Wisconsin endowment at the Medical College of Wisconsin.

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