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

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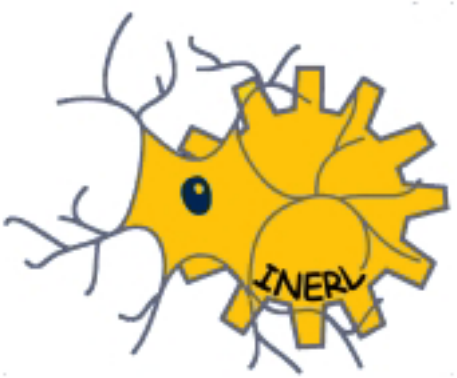
CHANGES IN CORTICAL ACTIVITY IN STROKE SURVIVORS UNDERGOING BOTULINUM TOXIN THERAPY FOR TREATMENT OF FOCAL SPASTICITY



Integrative Neural Engineering and Rehabilitation Laboratory

by

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A Thesis submitted to the Faculty of the Graduate School, Marquette University, in Partial Fulfillment of the Requirements for the Degree of:

Master of Science

Milwaukee, WI
December, 2018

ABSTRACT

CHANGES IN CORTICAL ACTIVITY IN STROKE STURVIVORS UNDERGOING BOTULINUM TOXIN THERAPY FOR TREATMENT OF FOCAL SPASTICITY

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Functional magnetic resonance imaging (fMRI) has provided evidence of neuroplastic changes following rehabilitation in stroke survivors. Botulinum toxin (BoNT) injection therapy has become a common approach to combating spasticity—a motor disorder characterized by a velocity dependent increase in muscle tone. The neurotoxin acts to inhibit muscle contraction, relieving spasticity symptoms with peak effects occurring between 4 and 6 weeks. With decreases in muscle tone, BoNT injections could free arm movement for rehabilitation, creating the opportunity for enhanced control of the upper limb, which would have underpinnings in altered brain activity. Given evidence of cortical activation changes following other stroke rehabilitative methods, it is expected that BoNT therapy would produce similar types of changes. The aim of this study was to quantify changes in task-related activity throughout the brain in stroke survivors undergoing BoNT therapy. Understanding the changes in cortical activity resulting from BoNT injections could help improve rehabilitation methods and predict functional outcome.

Changes in cortical activation in response to BoNT injections have only been documented in a handful of studies. Of these past studies, participant pools tended to include patients with moderate to high functional ability of the affected limb. Although patients receiving BoNT injections often fall within a wide range of severity, BoNT injections have been shown to provide the biggest impact on the highly impaired population. BoNT injections have also been shown to provide greatest effects during the initial injections, and the effects on long-term spasticity treatment are less prevalent in the literature.

In this study, we used a voxel-based approach to quantify neuroplastic effects and capture changes in activity throughout the brain, including regions outside the primary sensorimotor cortices. We recruited a majority of participants that presented with severe spasticity, who were scheduled to receive a round of BoNT injections as part of their standard of care. By assessing the brain activation associated with repeated injections, we obtained insight into BoNT on a long-term basis, as it is traditionally prescribed.

ACKNOWLEDGEMENTS

Kelsey Tynes, B.S.

Thank you to my wonderful committee members Brian Schmit, Allison Hyngstrom, and John McGuire. Many thanks to my advisor Brian Schmit for his endless support and patience throughout this project. I would also like to thank my two mentors Kaleb Vinehout and Miguel Sotelo for their assistance and guidance. Lastly, thanks to my friends and family for the endless support.

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1.1 MOTOR RECOVERY FOLLOWING STROKE: EFFECTS AND SOLUTIONS TO POSTSTROKE SPASTICITY

To most of the world's population, tasks such as bathing, dressing, and exercise come as a standard to any daily routine. To an individual experiencing spasticity, however, each of these tasks could require some effort and at times assistance. Approximately 3 in 5 stroke survivors experience spasticity—of those, only half seek treatment to relieve symptoms (Urban et al., 2010).

Treatments and rehabilitation measures are prescribed depending on severity and comfort level of the patient. A method of spasticity treatment implemented in medical clinics is Botulinum-toxin A (BoNT) injection therapy. These injections are applied to affected spastic limbs and are intended to aide in relaxing the hyperactive muscle. The injections can be used for reasons related to pain management, hygiene, ease of care, and activities of daily living.

Botulinum-toxin A injection therapy has been a technique for managing spasticity symptoms for decades; however, the understanding behind its effects on neuronal activity in the brain is limited. Efforts to understand the underlying effects of BoNT therapy on functional motor recovery and its effects on associated neuronal activity are believed to have the potential to help further develop clinical therapies. A handful of studies have assessed cortical activation of stroke survivors' naïve to BoNT injections, which have showed varying results (Bergfeldt, Jonsson, Bergfeldt, & Julin, 2015; Diserens et al.,

2010; Manganotti et al., 2010; Senkárová, Hlustík, Otruba, Herzig, & Kanovský, 2010; Tomášová et al., 2013; Veverka et al., 2014, 2016, 2012). This study aims to quantify changes in functional activity of stroke survivors undergoing BoNT injection therapy as a standard practice of care, adding to the limited knowledge on the topic. This chapter discusses the causes of spasticity and how BoNT therapy works to counter its disabling effects, and outlines the benefits of fMRI to quantify the effects of BoNT on a neuronal level.

1.1.1 Stroke

Stroke is the fifth leading cause of death in the United States, affecting approximately 795,000 people each year (Benjamin et al., 2018). A stroke can occur by two different means and is categorized by type (Gomes & Wachsman, 2013). An *ischemic* stroke occurs when a clot forms within the brain's blood vessels or travels from elsewhere in the body to form a blockage within the brain's blood supply. When blood supply is cut off, the region is deprived of oxygen and brain cells quickly die. A *hemorrhagic* stroke is the result of a ruptured vessel in the brain, most commonly caused by high blood pressure or an aneurysm (Donnan, Fisher, Macleod, & Davis, 2008). In both cases, the outcome following stroke often results in reduced physical condition and capability.

1.1.2 Spasticity

OVERVIEW

Spasticity is a chronic stretch reflex disorder characterized by increased muscle tone in upper and/or lower extremities, most commonly caused by damage to a portion of the brain or spinal cord. This is a velocity-dependent disorder, which causes increased resistance when a muscle group is passively stretched. It is emphasized that spasticity is merely one component of the upper motor neuron syndrome (UMNS) (Lance et al., 1980). EMG recordings during passive stretch show increased activity with increased velocity, with a delayed effect where the muscle continues to contract even after movement has ended. This suggests the hypertonia occurs by two means: hypertonia elicited by the stretch reflex (spasticity), and hypertonia elicited by muscle contracture (intrinsic hypertonia) (Galiana, Fung, & Kearney, 2005; Dietz &Berger, 1983).

Affecting over 12 million people worldwide, hypertonia can be seen by the physical manifestation of a tight fist, flexed elbow, and an arm pressed against the chest (Nair & Marsden, 2014). These permanent contractions can become painful and hinder overall muscle movement and coordination, causing difficulties with everyday activities (Benjamin et al., 2018).

NEURAL MECHANISMS

Neural Basis of Motor Impairment

Lesions that occur in the gray matter of the primary motor cortex (M1) or damage the white matter fibers comprising the corticospinal tract (CST) are most likely to cause gross motor impairment (Maraka et al., 2014). Infarcts localized to the pons, cerebellum, thalamus, or association areas can often hinder fine motor movements (Darling et al.,

2011). Voluntary motor actions are mediated both by direct and indirect anatomical pathways. Simple motor tasks (single muscle or joint) are largely controlled by pyramidal cells in the M1, which elicit movement at low stimulation thresholds by projecting to motoneurons in the spinal cord. Planning of more intricate patterns is often produced by premotor areas, which project to both the M1 and spinal cord in order to produce the physical movement (Rizzolatti, Fadiga, Gallese, & Fogassi, 1996). Stimulation and damage to these areas have provided extensive evidence supporting their involvement in movement.

Neural Basis of Spasticity

In healthy subjects, the stretch reflex acts to resist the lengthening of a muscle. This process is mediated by excitatory connections between Ia afferent fibers stemming from muscle spindles and alpha-motoneurons innervating the same muscle (Trompetto et al., 2014). By passively stretching a muscle, the firing rate increases in the Ia spindle fiber, leading the Ia fibers to fire and send signals to the alpha motoneurons through monosynaptic pathways, resulting in contraction of the original muscle and relaxation of its antagonist (Figure 1.1) (Pearson & Gordon, 2000).

The exact mechanism behind spasticity's exaggeration of the stretch reflex is still unclear today. The disorder could theoretically be caused by two different factors: increased excitability of Ia afferents stemming from the muscle spindles, or abnormal processing of sensory inputs from muscle spindles creating excessive reflex activation of the alpha-motoneurons (Trompetto et al., 2014).

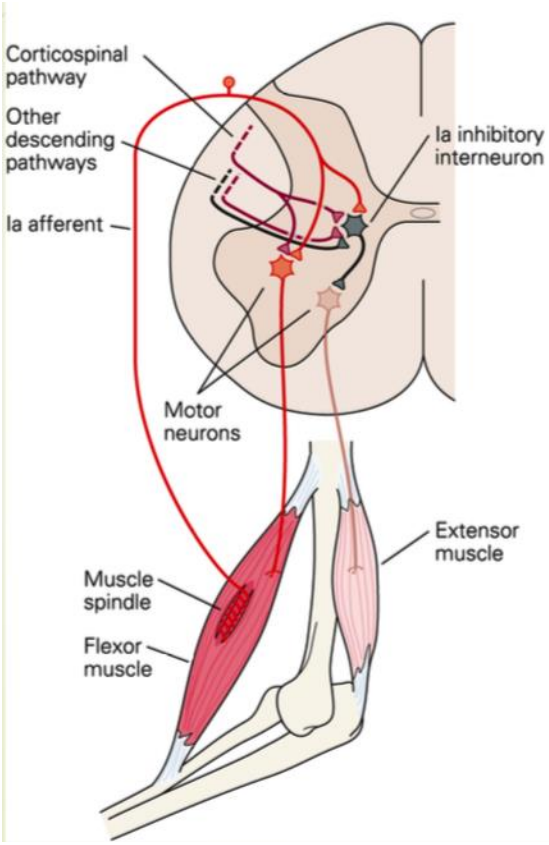


Figure 1.1: Stretch Reflex Pathway. This diagram illustrates the monosynaptic pathways that mediate the stretch reflex, indicating the primary neurons involved (Pearson & Gordon, 2000).

LIMITATION OF FUNCTION AND IMPACT ON THERAPY

Therapeutic interventions become necessary when spasticity symptoms begin to interfere with daily living (Levin & Hui-Chan, 1992). Upper-limb spasticity can often make tasks such as reaching, grasping, and releasing objects difficult (Francisco & McGuire, 2012). Early management of spasticity can often lead to improved motor recovery outcomes (Thompson, Jarrett, Lockley, Marsden, & Stevenson, 2005).

Rehabilitation plans tend to break down to three main categories: improving movement, improving daily life activities, and improving quality of life. Improving movement focuses on unmasking voluntary movements and recovering damaged neural

circuits by practicing motor plans. Improving daily life tends towards getting around, dressing, and personal hygiene. Quality of life focuses on regaining an independent lifestyle and possible reintegration into the workplace (Gracies, Elovic, McGuire, & Simpson, 1997).

1.1.3 Neural Plasticity Following Stroke

While neural plasticity is most promising during developing stages of the brain, changes in structural and functional integrity have been seen in stroke survivors during the course of recovery. Alpha-motoneurons have been known to release growth factors locally following traumatic injury (Weidner, Ner, Salimi, & Tuszynski, 2001). As these neurons grow and spread, they form new synapses between interneurons and the severed motoneurons, creating new pathways to replace the nonfunctional ones (Raineteau & Schwab, 2001). Additionally, alternative descending pathways could be recruited to take over motor drive to compensate for loss of corticospinal pathways. The excitatory pathways descending from the brainstem tend to be less selective, resulting in over-active muscle contractions (Trompetto et al., 2014).

Post-stroke studies have used structural (Schaechter, Perdue, & Wang, 2008; Stinear et al., 2007) and functional (Johansen-Berg et al., 2002; Ward, Brown, Thompson, & Frackowiak, 2003) imaging to document neuroplastic effects in both ipsilesional and contralesional areas of the primary motor cortices. Studies have been advancing into evaluation of the impact of therapeutic interventions on imaging data, which have also shown promising results.

Functional MRI (fMRI) has been used to show a correlation between favorable motor recovery following therapy and increased activity in the contralesional M1 during motor tasks in chronic stages of stroke (Jones et al., 2015). Additional changes in activity have been seen in motor areas of the brain following pedaling activity (Promjunyakul, Schmit, & Schindler-Ivens, 2015), constraint-induced movement therapy (Schaechter et al., 2002), and mirror therapy techniques (Michielsen et al., 2011).

1.2 BOTULINUM TOXIN-A INJECTION THERAPY

Several treatments are suggested for those affected by spasticity including physical therapy, oral medication, injectable neurolytic medications, and surgical procedures. Physical therapy often includes stretches and range of motion exercises, which may be paired with more invasive options such as peripheral nerve blocks or botulinum toxin injections. Botulinum toxin-A injections are a common treatment to relieve spasticity experienced by stroke survivors. The toxin is injected directly into the overactive muscle, and acts by preventing the release of neurotransmitters required to elicit muscle contraction. Patients typically receive injections every 3-4 months to manage symptoms.

1.2.1 History

Botulinum-toxin is produced by the motile bacterium *Clostridium botulinum*; the toxin was first discovered in 1897 after rancid ham caused symptoms of botulism in several guests attending a funeral. Emilie van Ermengem worked as a professor of bacteriology and the University of Ghent, and discovered the agent responsible for the

illnesses, initially naming it *Bacillus botulinum* (Devriese, 1999). In 1946, the toxin had been purified and produced in mass quantities by researcher Edward Schantz. Nearly twenty years later, botulinum toxin injections were found to induce paralysis and atrophy when administered to muscles in a chick embryos (Drachman, 1964).

This finding fueled extensive research into many movement and autonomic disorders, leading to various animal models to assess pain and mobility. The involvement of BoNT in pain modulation was assessed using primarily mouse and rat models, evaluating peripheral inflammatory pain (Cui, Khanijou, Rubino, & Aoki, 2004), visceral pain (Chuang, Yoshimura, Huang, Chiang, & Chancellor, 2004), and neuropathic pain (Bach-Rojecky, Relja, & Lacković, 2005). All results supported the notion that BoNT could vastly reduce pain caused by several neurological conditions. Effects of BoNT on the gastrocnemius muscle of mice showed that significantly decreased gastrocnemius muscle stiffness during passive stretching (Haubruck et al., 2012). A study involving injections to the masseter muscle of rabbits found that EMG activity of the muscle was immediately reduced, demonstrating BoNT's paralytic effects (Park et al., 2015).

Though animal models are continuing to grow in complexity, their initial results motivated further research. Ophthalmologist Alan B. Scott theorized the toxin's muscle-relaxing effects might help treat crossed eyes. His group went on to treat monkeys with BoNT-A for strabismus in the 1960s, and successfully translated the study to humans in 1981 (Scott, 1981). This pioneering study sparked research in therapeutic applications of BoNT, including, but not limited to, cervical dystonia, migraine, and spasticity.

Human trials testing the safety and effectiveness of BoNT-A as a therapeutic agent for managing post-stroke spasticity symptoms began in 1996 when Simpson

demonstrated the effects of three different doses as compared to a placebo group. Evaluated at two, four, and six weeks post-injection, all BoNT groups showed better outcomes than placebo (Simpson et al., 1996). This protocol and those similar have been repeated by many, resulting in FDA approval for Botox® in 2010. The effects of this therapy have been extensively reviewed and the technique is continuously growing in practice.

1.2.2 Cellular Mechanisms

In preparation of muscle contraction, presynaptic neuromuscular nerve endings contain vesicles storing the neurotransmitter Acetylcholine (ACh). The nerve action potential initiates the fusion of the vesicle to the nerve membrane, leading to the release of ACh into the nerve synapse. Here ACh is able to bind to receptors on the muscle, and elicit muscle contraction (Kuo & Ehrlich, 2015). The fusion process between neurotransmitter-containing vesicles and presynaptic nerve membrane is facilitated by a group of proteins forming the SNARE complex (Rossetto, Pirazzini, & Montecucco, 2014).

The BoNT molecule is comprised of two parts: a heavy chain (H-chain) and a light chain (L-chain). The two chains are bound together by a disulfide bond and protected by a protein coating. After injection, the molecule dissociates from the protein and the H-chain binds to receptors on motor and sensory neuron nerve endings. The entire complex is then pulled into the cell by endocytosis (Pickett, 2009). Here, the L-chain is released into the presynaptic cell cytoplasm. In motor neurons, the L-chain acts by cleaving SNAP-25, an essential component of the SNARE complex (Söllner et al.,

1993). Without a functional SNARE complex, the vesicles cannot release ACh, and muscle contraction cannot occur (Figure 1.2). Similarly, in sensory neurons, the L-chain is believed to act on SNAP-25 in a similar fashion. This blocks the release of neuropeptides, inhibiting the sensitization of pain nerves (J. Park & Park, 2017). BoNT is not believed to affect nerve conduction nor the synthesis of ACh, but simply hinders the mechanisms essential for release.

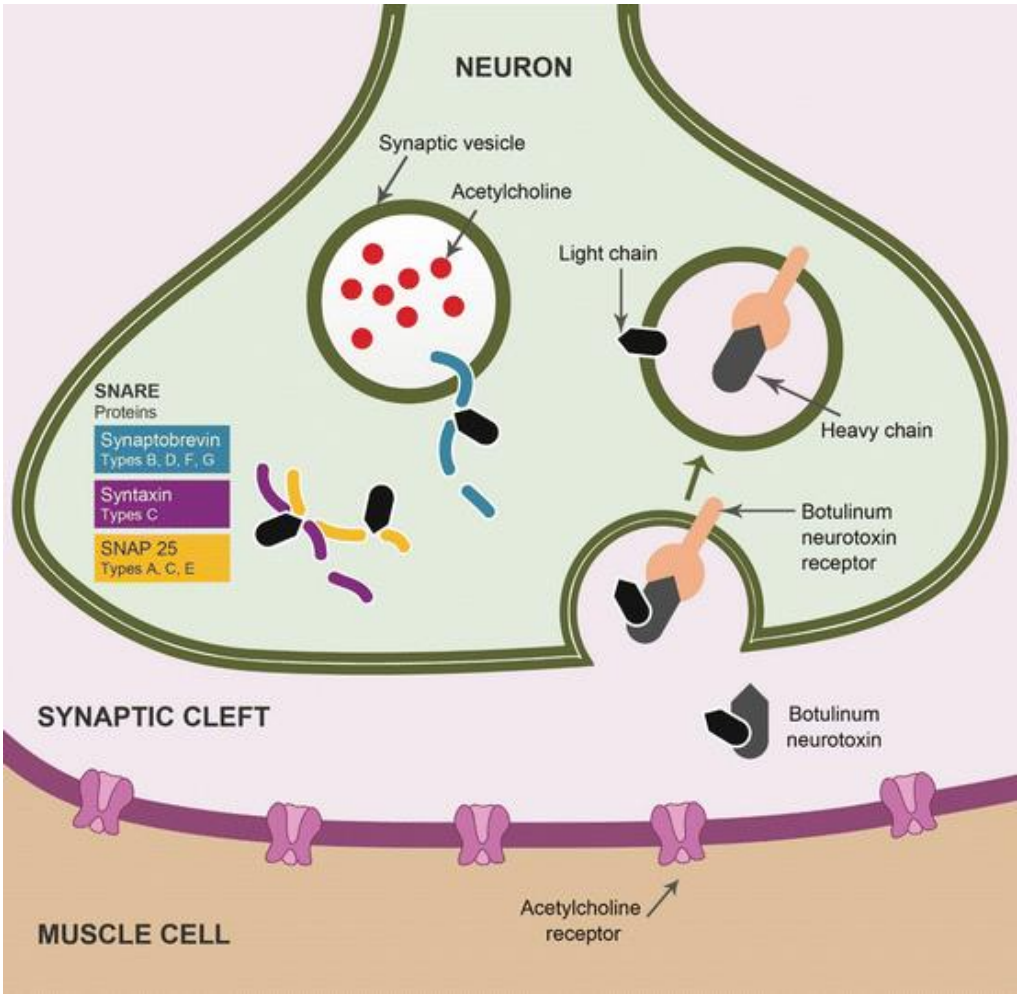


Figure 1.2: Molecular Action of BoNT. The diagram serves as a flowchart starting at the introduction of BoNT to the muscle tissue. Illustrated is the neurotoxin's uptake from the synaptic cleft and release into the presynaptic motor neuron, cleavage of SNAP-25 and inhibition of ACh release (Saravanan, Rajaseger, Eric, & Moochhala, 2015).

1.3 FUGL MEYER ASSESSMENT

The Fugl-Meyer Assessment (FMA) is an index measured specifically in stroke populations to quantify motor impairment (Fugl-Meyer, Jääskö, Leyman, Olsson, & Steglind, 1975). The motor score portion of the test includes evaluations of movement, coordination, and reflexes in both upper and lower extremities. In clinical settings FMA is a common score used to determine impairment severity and predict motor recovery. Scoring is based on a scale of 0 to 2 for each motion, 0 equating to total hemiplegia and 2 indicating normal motor performance.

Motions of the upper extremity motor test include reflex activity, flexor synergy, extensor synergy, movements combining synergies, and movement out of synergy. The wrist is tested for stability, flexion, extension and circumduction in varying combinations of elbow and shoulder positions. The hand is assessed for mass finger flexion and extension, along with various grasping tasks. Coordination and speed of a finger-to-nose task compares affected and unaffected limbs to quantify impairment in dysmetria, tremor, and speed. The upper extremity FM motor test has a maximum score of 66.

The FMA has been shown to have excellent validity and shows concurrence with other accredited stroke motor scores, while providing a more detailed review of motor ability. The scoring system has appraised for its inter- and intra-tester reliability and construct validity (Gladstone, Danells, & Black, 2002). A clear correlate between spasticity severity and decreased FMA scores has been found and confirmed by several researchers (Katz, Rovai, Brait, & Rymer, 1992; Opheim, Danielsson, Alt Murphy, Persson, & Sunnerhagen, 2015).

1.4 MAGNETIC RESONANCE IMAGING (MRI)

1.4.1 MRI Signal

The MRI signal is derived from the precession and relaxation of hydrogen protons in the presence of a large magnetic field. Each hydrogen atom consists of a single proton that, when exposed to a static magnetic field (B_0), aligns with the field and precesses about its own axis at a frequency known as the Larmor frequency. With all protons aligned together, their angular momentums sum to form a net magnetization (M_0) in the direction of B_0. When an external radio-frequency (RF) pulse is administered at the Larmor frequency, these protons are tipped from their low-energy state, now uniform in both phase and direction (Haacke, Brown, Thompson, & Venkatesan, 1999).

Two phenomena are observed as the protons relax back to their static field state. The longitudinal magnetization (M_z) is the portion of M_0 seen parallel to the magnetic field. Following the RF pulse, protons realign with B_0, and M_z approaches its initial value of M_0. In addition, the phase-locked protons also disperse with respect to the x-y plane, contributing to a transverse magnetization (M_{xy}) or the portion of M_0 seen perpendicular to the magnetic field. This is the signal measured by the MR receive coil (Haacke et al., 1999).

M_z regrowth and M_{xy} decay correspond to T1 and T2 relaxation times respectively. These variables are distinctive to each biological material, allowing for unique MRI pulse sequence designs tailored to any given application. T1 weighted images, characterized by the rate at which protons return to equilibrium along the B_0 field, are often used to obtain a high resolution anatomical image to be used in diagnostics. T2 and T2* together describe the transverse relaxation. The T2 signal

describes the decay observed in spin-echo measurements and is related to the spin-spin interactions as the protons dephase. Because the natural T2 relaxation cannot be measured directly, the T2* or effective T2 signal, is measured using gradient-echo sequences and is susceptible to changes in local magnetic fields (i.e. changes due to increased/decreased blood flow). The T2* signal gives rise to additional analyses of MRI results, such as the BOLD contrast method used with fMRI data (Huettel, Song, & McCarthy, 2009).

1.4.2 BOLD Signal

MRI technology is capable of providing much more than anatomical data. The T2* signal has been shown to be sensitive to oxygenation of the blood—the foundation of functional MRI (Ogawa, Lee, Kay, & Tank, 1990). In the presence of a gradient-spin-echo sequence, the paramagnetic properties seen in deoxyhemoglobin provide a natural contrast in the MR image, and is said to be blood oxygen level dependent (BOLD).

BOLD contrast is the most common way to study local changes in brain activation during a particular task. The paramagnetic properties of deoxygenated hemoglobin tend to suppress the MRI signal, while diamagnetic properties of oxygenated hemoglobin do not show this effect (Lindquist, Meng Loh, Atlas, & Wager, 2009).

During the initial moments of performing a mental task, deoxygenated hemoglobin is high in concentration as neuronal activity is consuming ATP. As metabolic demands increase, the vessels dilate to increase blood flow and oxygenated blood is delivered to the active areas in need. This continues for about 4-8 seconds to ensure the active area is supplied with enough oxygen. As demand decreases, blood flow

is reduced while the active area consumes the remaining oxygen, and deoxygenated hemoglobin again becomes high in concentration before returning to resting state conditions (Figure 1.3) (Kim & Ogawa, 2012).

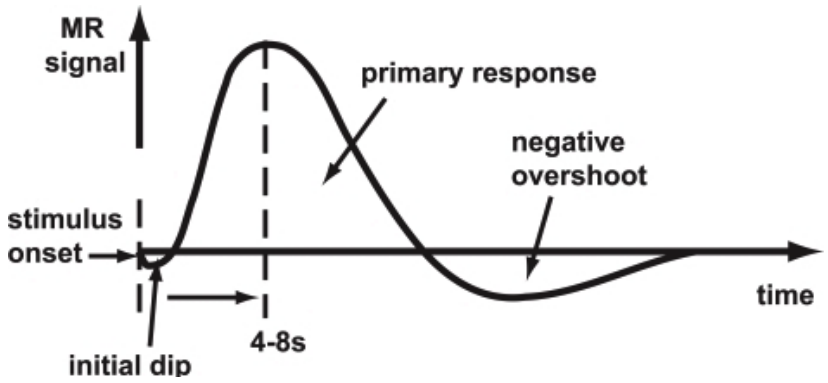


Figure 1.3: HDR Model. The standard model for the hemodynamic response function, illustrating the primary fluctuations over time (initial dip at stimulus onset, primary response window, negative overshoot following the end of mental task) (Kornak, Hall, & Haggard, 2011)

The BOLD technique takes advantage of this varying MRI signal and allows researchers to assess brain activity by measuring areas that follow the pattern characterized by a modeled hemodynamic response function (HRF).

1.5 SPECIFIC AIM

The aim of this study was to determine changes in cortical activation patterns of stroke survivors over the course of a single round of BoNT injections to the upper extremity. These results could produce helpful insight into the effects of BoNT on motor recovery and improve physical therapy interventions to best suit each patient.

CHAPTER 2: CHANGES IN CORTICAL ACTIVITY IN STROKE SURVIVORS UNDERGOING BOTULINUM TOXIN THERAPY FOR TREATMENT OF FOCAL SPASTICITY

2.1 INTRODUCTION

The purpose of this study was to identify changes in brain activation associated with hand movement following botulinum toxin-A (BoNT) injections to the upper extremity as treatment for focal spasticity. Previous studies have documented varying results regarding changes in brain activation in response to finger tapping and flexion/extension of the hand in patients undergoing BoNT therapy. Prior to injection, most studies have reported extensive bilateral activation of primary sensorimotor and supplementary motor areas during movement of the affected hand (Manganotti et al., 2010; Senkárová et al., 2010; Tomášová et al., 2013; Veverka et al., 2012). Activity patterns following BoNT intervention decrease in active volume in primary motor areas, demonstrating a localizing and lateralization effect (Bergfeldt et al., 2015; Manganotti et al., 2010; Tomášová et al., 2013; Veverka et al., 2014). Other studies have shown increases in activity in similar motor areas, along with increased activity in higher-order motor areas such as the cerebellum, thalamus, anterior cingulate gyrus, among others (Diserens et al., 2010; Tomášová et al., 2013; Veverka et al., 2016). These studies have mainly included participants with finger movement ability, even before botulinum injection. In people experiencing more severe impairments, BoNT injections could free arm movement through the reduction of spastic restraint, creating the opportunity for enhanced control of the upper limb (Park et al., 2017). In these cases, botulinum toxin

treatment could result in widespread activation of brain areas as patients access motor control networks for hand movement. In this study, we hypothesized that when BoNT is used to free arm movements, higher-order areas of the brain such as motor planning and sensory association areas would increase in activity during movement of the hand.

Our approach in this study was to examine changes in brain activation using functional magnetic resonance imaging (fMRI) in participants with a wide range of upper extremity impairment. Past studies have documented changes in cortical activity patterns following BoNT injections in post-stroke participants performing sequential finger patterns and flexion/extension of the hand—tasks that require substantial control of the finger muscles (Bergfeldt et al., 2015; Manganotti et al., 2010). In order to determine whether releasing spasticity of the arm using BoNT enhances higher-order brain function, we targeted fMRI measurements in participants with limited hand function prior to injection. An assistive device was created to aid in finger extension while in the scanner and ensure that the task comprised an actual movement. This approach allowed observation of the neurotoxin effect in a severely impaired test group, who have traditionally benefitted most from the injections (Welmer, Holmqvist, & Sommerfeld, 2010).

As BoNT works to relieve spasticity, the muscle relaxes to an extent that allows the patient to practice a wider range of motion. Functional recovery of a spastic limb may be limited by the severity of the stroke, but BoNT injection therapy has proven to be a safe and effective method to manage spasticity symptoms and help patients regain functionality of the impaired limb (Hesse, Brandi-Hesse, Bardeleben, Werner, & Funk, 2001; Lagalla, Danni, Reiter, Ceravolo, & Provinciali, 2000; Slawek, Bogucki, &

Reclawowicz, 2005). The largest improvements are seen in patients experiencing extreme symptoms, characterized by Modified Ashworth Scale (MAS) scores of 3+ (Hurvitz, Conti, & Brown, 2003; Jahangir et al., 2007). With this release of spastic restraint comes improvement in motor control and likely recruitment of high-order motor centers. By resuming use of the otherwise neglected muscle groups, sensorimotor and integration areas might increase activity. Specifically, areas such as the premotor cortex, supplementary motor area, sensory integration regions, and other secondary motor areas are likely recruited following BoNT injection to compensate for losses in primary motor output (Maier, 2002). Although it is unlikely higher order areas completely substitute for the damaged corticospinal tract, adoption of primary motor roles by secondary sensorimotor areas would suggest neuroplastic effects take place in response to improvements in focal spasticity.

In order to discern changes in cortical activity resulting from BoNT injection to the upper-extremity, we utilized a voxel-based analysis of fMRI data obtained from assisted flexion and extension of the fingers in participants with mild to severe impairments of the hand. fMRI measurements were made before injection (W0) and at 6 weeks after injection (W6) in participants with stroke and in age-matched controls at a 6-week interval. Our approach of correlating the HRF with each individual voxel separately was used to capture changes in activity throughout the brain, including regions outside the primary sensorimotor cortices. We hypothesized that BoNT's peripheral effects on the affected limb would allow for improved hand function, causing increases in brain activity in higher order motor control centers.

2.2 METHODS

2.2.1 Participant Population

This study consisted of fMRI measurements of brain activation in participants with chronic stroke undergoing BoNT therapy. All procedures were approved by the Institutional Review Board (IRB) of the Medical College of Wisconsin (MCW). All participants in this study were contacted through an IRB-approved database of the Stroke Rehabilitation Center of Southeast Wisconsin, hosted by the MCW Department of Physical Medicine and Rehabilitation. All participants gave written informed consent to take part in this study and all procedures were conducted in accordance with the Helsinki Declaration of 1975 (as revised in 2000).

Nine people with chronic stroke were enrolled in the study (5 female; aged 58.2 +/- 3.8, range 42-77) with the following inclusion criteria: undergoing botulinum neurotoxin (BoNT) therapy as a part of clinical care; stroke onset more than 6 months prior to the study; wrist/finger impairment; no contraindication to MRI. All participants suffered from distal hemiparesis and spasticity of the upper extremity following stroke and had previously undergone at least one session of BoNT injections. Wrist and finger flexor spasticity was assessed per the Modified Ashworth Scale (MAS) prior to BoNT injection (Bohannon & Smith, 1987). Characteristics of participants in the stroke group are described in Table 2.1, with further details regarding size and location of the lesions illustrated in Figure 2.1. An age-matched control group (3 female; aged 56.4 +/-2.2, range 47-70) was enrolled after being screened for the following criteria: no known neurological or muscular disease and no MRI contraindications.

Table 2.1: Participant Demographics and Clinical Characteristics. MCA = middle cerebral artery; LD = Lower Division; UD = Upper Division; Le = Lenticulostriate; Ip = Intraparenchymal; BG = Basal Ganglia; R = Right; L = Left; Isch = Ischemic; Hem = Hemorragic; Note: information regarding the number of injections and details of physical therapy for participant number 5 was not available due to transfer between centers.

Participant	*Sex*	*Age (Years)*	*Stroke Type*	*Time Post Stroke (Years)*	*MAS Finger/Wrist*	*Lesion Side*	*Lesion Location*	*Nth Injection*	*Physical Therapy*
BTX 1	F	48	Isch	4.6	3/3	R	MCA-UD	6	Prescribed
BTX 2	M	58	Hem	4.4	2/2	L	Ip-BG	13	Prescribed
BTX 3	F	42	Hem	3.9	3/4	L	Ip-BG	12	Prescribed
BTX 4	M	77	Isch	1.4	3/3	L	MCA-LD,UD,Le	2	Prescribed
BTX 5	F	67	Isch	1.8	3/2	R	MCA-LD,UD	N/A	N/A
BTX 6	F	60	Isch	11.9	1/1	R	MCA-LD,UD,Le	40	Prescribed
BTX 7	M	69	Isch	1.1	2/1	L	Pons	2	Not Prescribed
BTX 8	F	48	Isch	8.9	4/4	R	MCA-LD,UD	27	Home Exercises
BTX 9	M	55	Isch	5.1	4/2	R	MCA-LD,UD,Le	17	Home Exercises

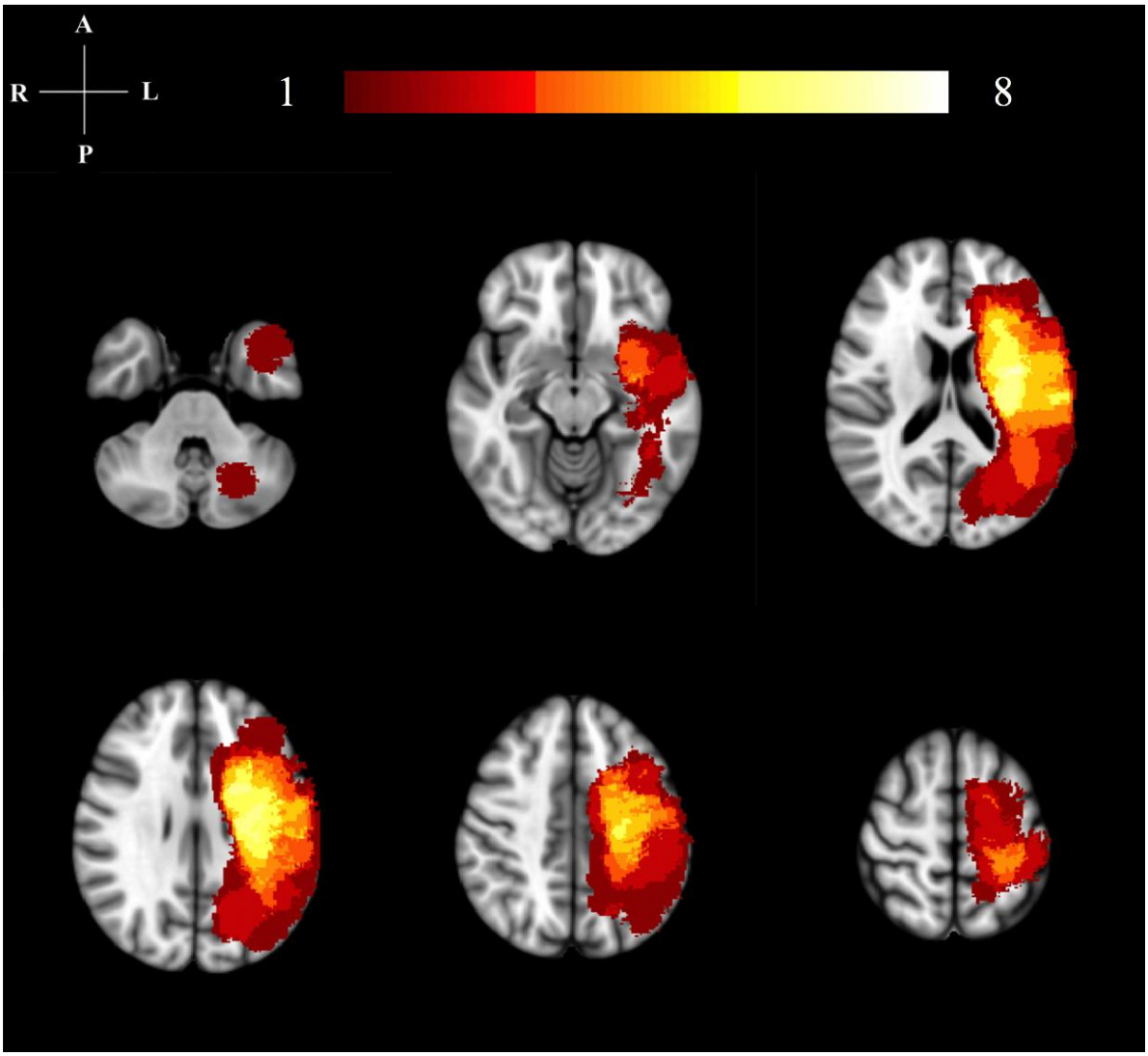


Figure 2.1: Lesion Location Map. This image illustrates lesioned areas of all participants where lesions common among all participants are shown in bright colors (yellow/white) and lesions specific to few participants are shown in dark red. Lesion maps have been overlaid on the Montreal Neurological Institute (MNI) 1mm template and all right hemisphere lesions have been flipped to the left hemisphere for analysis.

2.2.2 Treatment

Using EMG guidance, participants were treated with intramuscular injections of BoNT administered to the affected arm as part of normal clinical treatment. All participants received several injections at numerous locations of the upper extremity, ranging in both dosage and dilution (see Appendix C; injection locations, dilutions, and dosages reference only the treatment for this study; prior injections may have had different locations and/or doses). The prescribed amount varied based on each patient's functional abilities, comfort levels, and physical characteristics. Effects of BoNT treatment were assessed using fMRI measures of brain activity and the upper extremity motor portion of the Fugl-Meyer Assessment (FMA).

2.2.3 Data Collection

This study consisted of two test sessions scheduled six weeks apart. For participants receiving BoNT therapy, each session included an MRI scan and a clinical assessment. At least 3-4 months had passed since the patients' most recent BoNT injections before being enrolled in this study. The first session was conducted 1-4 days before participants received their BoNT injection (W0), and the protocol was repeated six 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.

IMAGING DATA ACQUISITION

All images were collected using a 3.0T GE Discover 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 (FSPRG-EPI) protocol: TE = 3.2 ms, TR = 8.16 ms, FOV = 240 mm, and 156x1 mm slices. Two 6-minute trials were conducted for the fMRI, using a GE's gradient echo planar imaging (GRE-EPI) protocol following the listed parameters: TE = 25 ms, TR = 2000 ms, FOV = 224 mm, Matrix: 64x64 mm, and 41x3.5 mm sagittal slices.

TASK

Due to spasticity, some participants with stroke were unable to fully extend the fingers without assistance. To counteract this issue, a device was created to aid in finger extension and was used by all participants during the task-based fMRI assessment. The device is described and depicted in Figure 2.2. All participants were able to actively flex against the resistive bands while the device acted to passively extend the fingers.

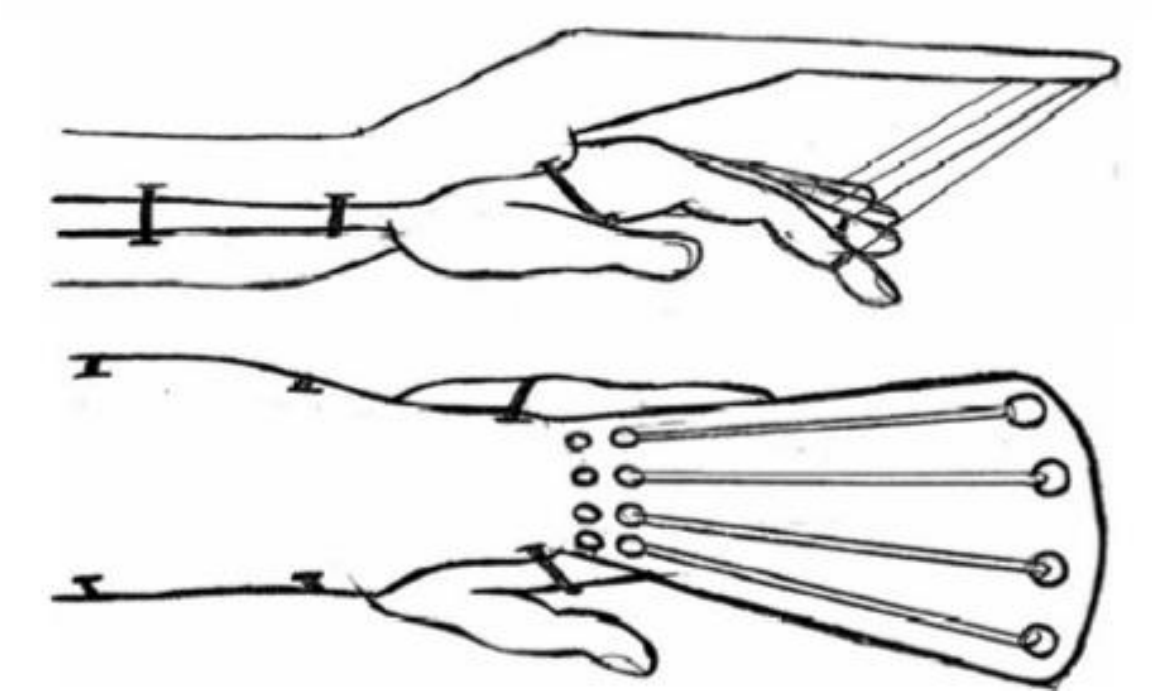


Figure 2.2: Sketch of Assistive Finger Extension Device. This device was created to assist finger extension of stroke participants experiencing severe spasticity. Velcro straps attached to the patient's first four digits and connected to an elastic band (Theraband: 10.7 N resistance at 100% elongation, 15.1 N at 200%). The bands facilitated extension, stretching the fingers up and away from the palm of the hand, and were supported by an outrigger that spans out over the top of the hand. During the task performed in the scanner, the bands produced approximately 5.1 N to the fingers to aide in extension. A plastic brace served to stabilize a participant's forearm and wrist as they are instructed to flex and extend the digits.

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-second intervals for a total of 6 minutes (Figure 2.3 & 2.4). Each participant performed two experimental runs. The two experimental runs underwent image preprocessing separately. The data were then concatenated and trimmed for statistical analysis on a single data set representative of a single session. A detailed description of data processing follows.

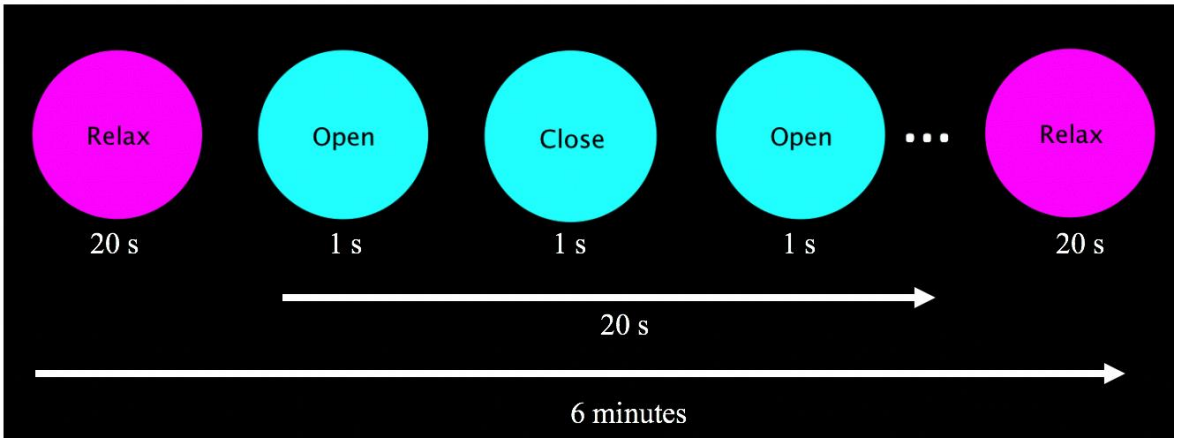


Figure 2.3: Visual Presentation Prompt. The above diagram shows the visual prompt all participants saw within the scanner. The cue was presented on a screen inside the bore and read "Relax" centered in a magenta circle (20 s) and "Open/Close" centered on a cyan circle (20s, 1 Hz); this rest-movement block pair persisted for 9 repetitions.

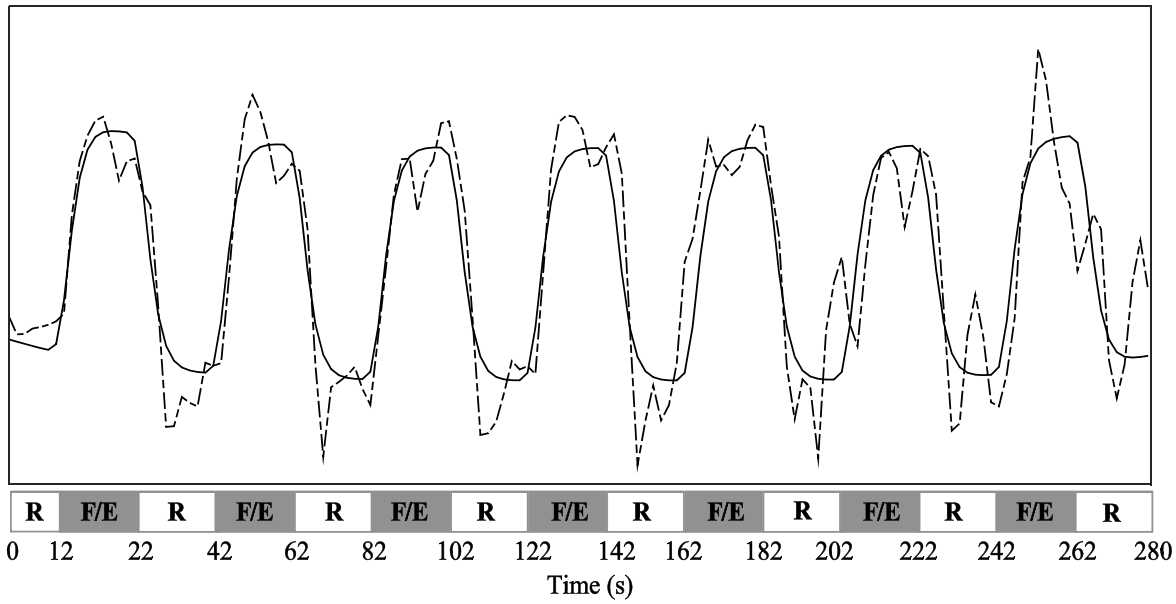


Figure 2.4: Block Design of Task. The above diagram visually represents one trial of the motor task, which consisted of 20 seconds of rest followed by 20 seconds of finger flexion/extension controlled at a rate of 1 Hz. These intervals are denoted above where white blocks represent rest (R) and grey blocks signify flexion/extension (F/E). A model of the canonical hemodynamic response function (Model Data, solid line) was fit to data recorded as a single voxel (Experimental Data, dashed line) over the timecourse of a single trial.

CLINICAL ASSESSMENT

Following the MRI portion of the session, all participants with stroke were assessed using the FMA to quantify impairment of the affected arm. The upper extremity portion of the FMA was conducted before and six weeks after receiving BoNT injections (W0 and W6). The FMA is designed to evaluate movement control, reflex activity, coordination, and muscle strength and was originally designed as a measure of motor impairment for stroke survivors. The upper extremity FMA motor scale specifically assesses movements of the hand, wrist, and upper extremity and totals to 66 points, with each category ranging from 0 (totally impaired) to 2 (no impairment) (Fugl-Meyer et al., 1975).

2.2.4 Data Analysis

Changes in the volumes of brain activation with BoNT treatment in participants with stroke were compared to changes in repeated measures of brain activation in age-matched controls. Activation maps were computed for each participant, along with group average activity maps, and between-session contrasts of each test group were compared. The pipeline for image processing is summarized in Figure 2.5 and a detailed description follows.

DATA PREPROCESSING

Prior to image analysis for the quantification of brain activity, all data underwent several preprocessing steps. After collecting data, DICOM files were converted to NIFTI format using Mricron's *dcm2nii* software. The 4D data sets from the two trials were concatenated and trimmed for final statistical analysis; the first 4 TRs were removed to

account for magnetic stabilization (Diedrichsen & Shadmehr, 2005). Using the Oxford Center for Functional MRI of the Brain Software Library (FSL), participant data were first reoriented to a standard orientation (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012). Correction for intensity differences on the outer edges of the brain was performed using the Advanced Normalization Tools (ANTs) software's *N4BiasFieldCorrection* command (Avants et al., 2009). Using FSL's Brain Extraction Tool (*bet*), the skull and other non-brain matter was removed from both T1-weighted and fMRI images (Jenkinson et al., 2012).

All stroke participants with left-arm hemiparesis (i.e. right hemisphere brain lesions) and control participants that self-identified as right-hand dominant were flipped in the right-left direction to allow for group analysis of movement using the non-dominant hand. The R-based Lesion Identification with Neighborhood Data Analysis (LINDA) program—which requires lesions to be located in the left hemisphere—was used to create lesion masks for all stroke participant T1-weighted images (Pustina et al., 2016). These masks were manually edited as necessary depending on LINDA's accuracy. Masks were overlaid on each participant's anatomical T1 image to assess the precision of the lesion detection. Upon visual inspection, LINDA at times under- or overestimated the size of the lesion. In these cases, the masks were manually expanded (or reduced) to better reflect the lesions' actual pathology. Using FSLView's masking tool, voxels were manually added and removed from the mask to ensure all lesioned areas were included in the mask.

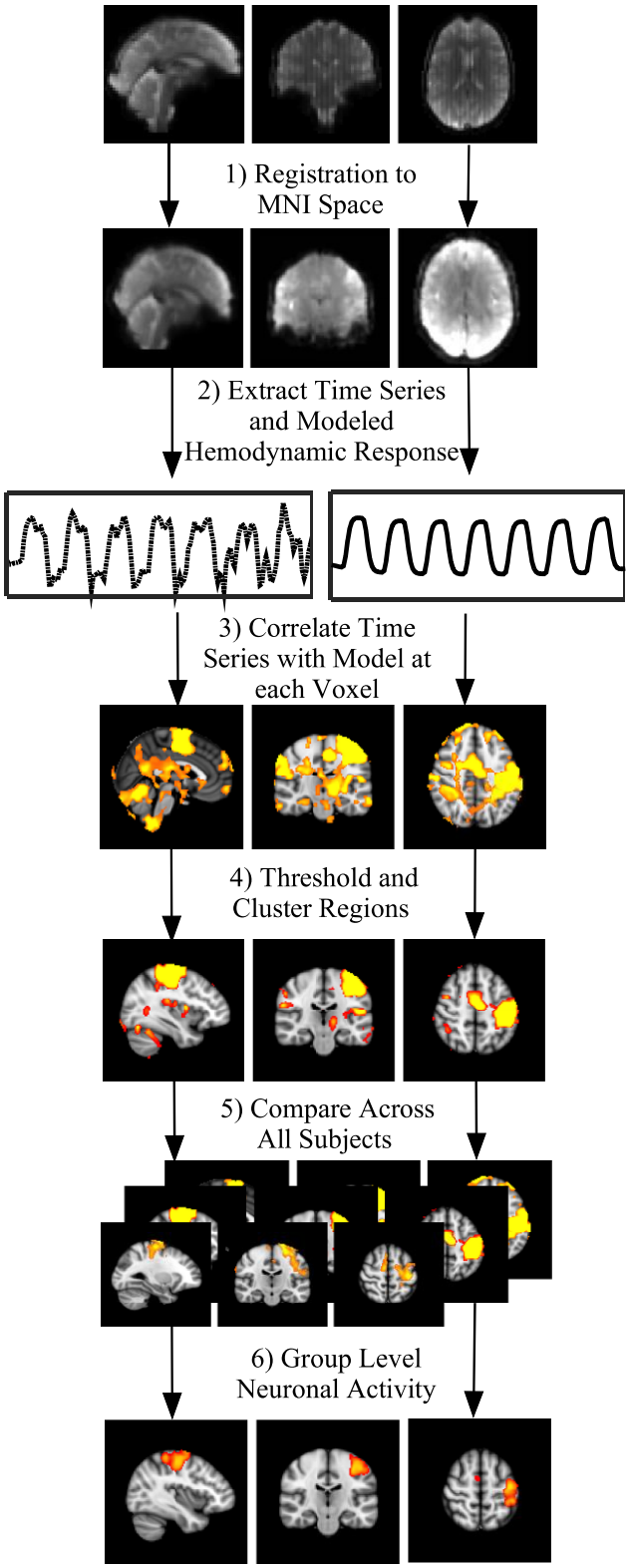


Figure 2.5: Processing Pipeline. The primary steps taken during image analysis in order to arrive at the final results are summarized. The pipeline begins with participant-specific data and results in group average results that were used to visualize between-session and between-group differences.

REGISTRATION

In general, fMRI data were registered to a Montreal Neurological Institute (MNI) standard brain and underwent statistical analysis to identify brain activity during movement of the impaired (non-dominant) hand. For registration, each participant's T1 weighted anatomical image was aligned to a standard MNI 2mm-voxel brain for comparison of images in standard space. Both linear and nonlinear registrations of the T1-weighted image were carried out using the ANTs software. First, linear registration was performed using a rigid algorithm to perform necessary rotations and translations. A second-level linear transformation was done using an affine algorithm to perform shearing and scaling of the image to match the MNI template. Lastly, nonlinear registration was performed using a symmetric-normalization (SyN) algorithm (Avants et al., 2008). This top-performing algorithm is a diffeomorphic registration, which allows for flexible local matching of tissues in the brain to the template (Klein et al., 2009). It is considered symmetric because its outputs allow for transformation from subject-space to standard-space as well as the inverse transformation from standard-space back to subject-space. This process produced 3D warp (nonlinear) and an affine (linear) transformations that were then applied to the 4D functional data over all volumes, resulting in the normalized datasets that were subsequently used to determine neuronal activity in the brain.

DETERMINING NEURONAL ACTIVITY

Neuronal activity was assessed by utilizing statistical maps in which statistical parameters (z-score) were indicative of correlation with the hemodynamic response function on a voxel wise basis. FMRI analyses were carried out using the fMRI Expert

Analysis Tool (FEAT) Version 5.0, a subsection of FSL. First-level FEAT analysis was performed on individual data using a Fixed Effect (FE) analysis. Data were corrected for motion using FMRIB's Linear Image Registration Tool (MCFLIRT). Spatial smoothing was performed on data using a full-width/half-maximum (FWHM) Gaussian kernel of 5 mm. The data were filtered temporally with a 0.01 Hz high pass filter to remove unwanted low-frequency signals (i.e. breathing, heartbeat, drifts within the scanner). Data were also prewhitened using FMRIB's Improved Linear Model (FILM), which uses nonparametric estimations of the time series autocorrelation to prewhiten each voxel's time series to improve estimation efficiency.

A model of the expected hemodynamic response (HDR) was created using FEAT's General Linear Model setup to convolve the binary block design with a Gamma wave (phase = 0s, std. dev. = 3s, mean lag = 6s). Statistical analysis of each voxel's timeseries was performed using the FMRIB's Improved Linear Modeling (*FILM*) to correlate the time series to the model. The activation images (Z-value, Gaussianized T/F statistic (Jenkinson & Woolrich, 2000) went through a cluster-threshold using $Z > 2.3$, resulting in a corrected cluster significance of $p < 0.05$. This first-level analysis created each participant's activity map at each session, where higher z-scores indicated a greater probability of a given voxel being associated with the motor task. These maps were later used to quantify volume of activation and areas that correlated highly with the task.

GROUP MEAN ACTIVITY & CONTRASTS

Group analysis was performed using FMRIB's Local Analysis of Mixed Effects, Stage 1 (FLAME1). A general linear model (GLM) was used to categorize individual participants by participant type (Stroke/Control) and session (W0/W6). The GLM is a

binary matrix meant to assign each data set to one of four groups, with participants as a random factor: control at W0, control at W6, stroke at W0, and stroke at W6. Group mean activation maps were created using nonparametric permutation testing for each group at a threshold $Z > 2.3$ and cluster significance $p < 0.05$. FSL's False Discovery Rate (FDR) was used to correct for multiple comparisons.

Contrasts between the two sessions were calculated for both control and stroke groups, using a two-sample paired t-test. Control and stroke groups were analyzed separately to account for difference in population variance. Significant differences ($Z > 2.3$, $p < 0.05$) found between sessions in stroke participants can thus be attributed to effects resulting from the BoNT injection over time. Contrasts between time points for controls were used as a method of verification and to test for the existence of an interaction effect from the BoNT therapy.

ACTIVE VOLUME AND AVERAGE CORRELATIONS OF REGIONS OF INTEREST

Regions of interest (ROIs) assessed in this study were identified by contrast analysis between W0 and W6 sessions of the stroke group. Clusters showing a significant difference in signal following BoNT injections were broken into individual ROIs based on anatomical structure and function using the Jülich Histological Atlas for regions in cortical and subcortical areas; because cerebellar areas are not included in the Jülich Atlas, the Taliarch Daemon Label Atlas was used to identify regions within the cerebellum. Clusters containing more than one anatomical area were overlaid with the atlas's predefined probability maps of the regions of interest, creating masks where the anatomical area and cluster overlapped.

Binary masks of each cluster-based ROI were created and used as an input to FSL's *featquery* tool. *Featquery* was used to count the number of active voxels within each ROI within that region for each participant-specific dataset. In addition to active volume, the average z-score (correlation to the HRF) was calculated using all voxels contained within the ROI resulting in a representative z-score for the region. These data were used as quantitative measures to assess the impact of BoNT on activation patterns related to movement.

CORRECTION FOR LESIONED BRAIN MATTER

Due to the large number of cortical lesions in the participant pool, group means and contrasts were recalculated while correcting for lesion location. The goal of this approach was to determine if any changes in activation were seen in the common lesioned areas, primarily in the lesioned M1. Participants that were identified as having a lesion in a specific location were excluded from statistical analysis. Using this technique, statistical analyses were performed using information coming only from viable brain tissues that were capable of activation. This also resulted in each voxel having different statistical power because of variations in the number of samples included in each calculation.

2.3 RESULTS

Activity patterns were analyzed from all participants in this study both individually and in groups according to participant type (stroke/control) and session (W0/W6). Main effects were seen comparing stroke and control groups, but no effect was seen comparing sessions alone. No significant difference was observed comparing sessions in the control group. The stroke group, however, showed significant increases in activity from W0 to W6, indicating an interaction effect had taken place in stroke survivors in response to the BoNT intervention.

2.3.1 Imaging: Subject-Specific

Individual participants' activity maps for those in the control group showed consistent patterns of activation in bilateral primary motor (M1) and ipsilateral cerebellum areas at W0 and W6. The activity map associated with non-dominant hand movement in a representative healthy control is shown at W0 and W6 in Figure 2.6. Though this pattern was common among all control participants, activity patterns for participants in the stroke group showed more variability in individual maps. Unlike the control participants, which largely showed similar activity patterns at each session, stroke participants showed differences in activation between W0 and W6. An example of the activity pattern of a representative participant with stroke is illustrated in Figure 2.7. This participant showed widespread brain activation in several areas outside the primary motor cortex that increased from W0 (Figure 2.7a) to W6 (Figure 2.7b). Most stroke participants showed an increase in whole brain active volume with several areas showing increased activity following BoNT injections. This trend excludes participant BTX7,

who showed an overall decrease in whole-brain active volume; it is worthwhile to note this participant's low spasticity rating and high functional ability, a distinctive trait as compared to the other stroke participants.

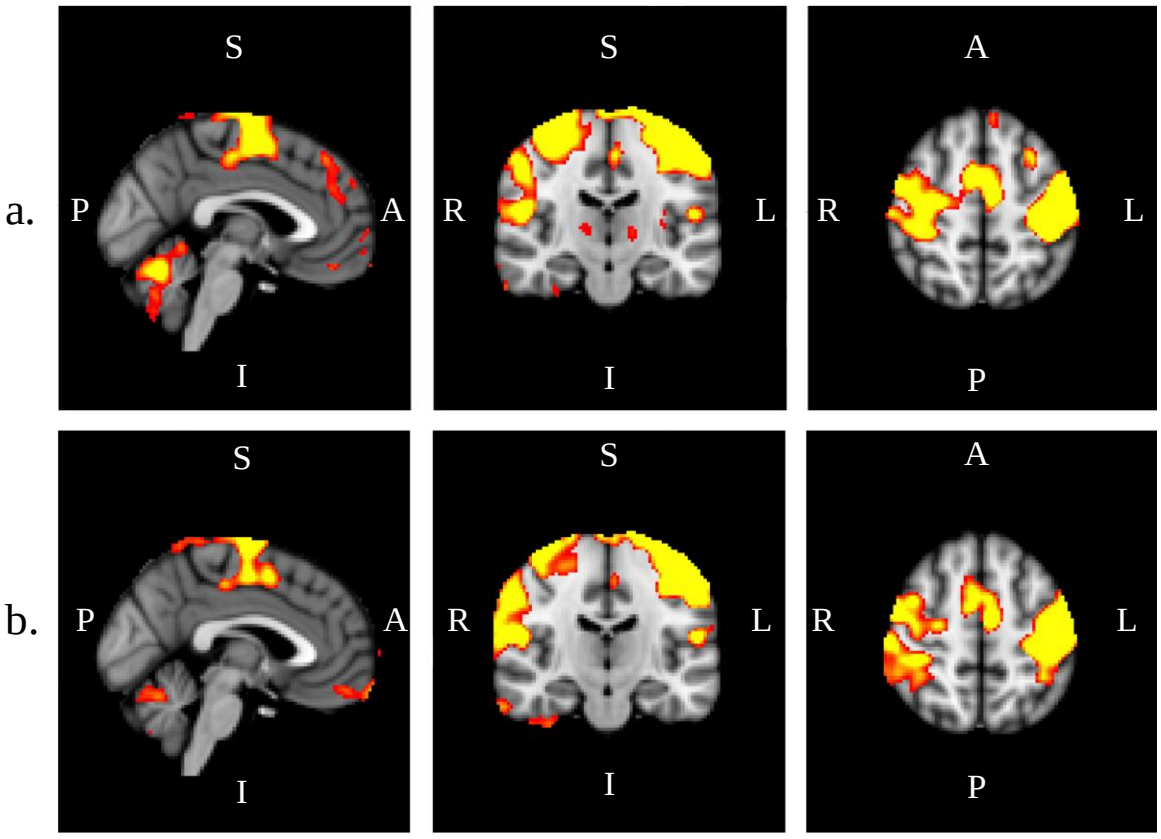


Figure 2.6: Single Control Participant Activation Pattern. The mean BOLD activity associated with task performance is shown for participant C7 at W0 *(a)* and W6 *(b)*. No apparent changes were seen in the control participant data over time.

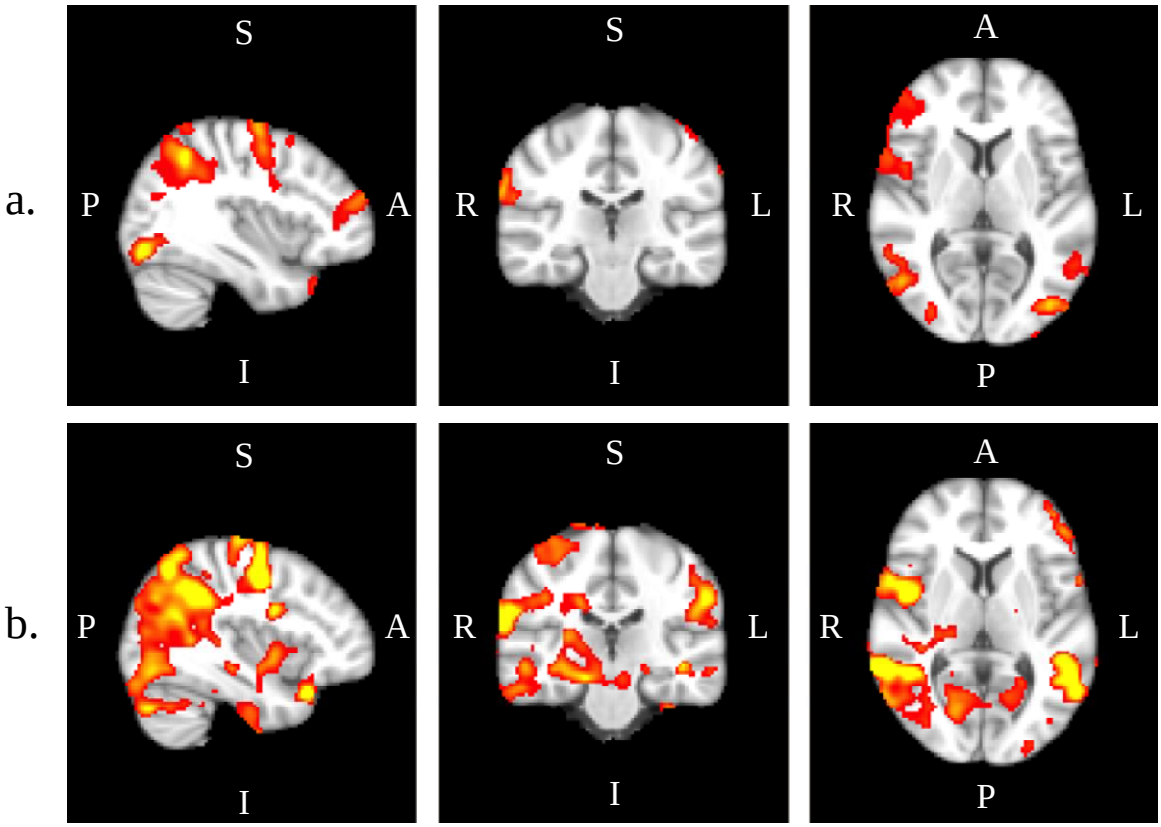


Figure 2.7: Single Stroke Participant Activation Pattern. The mean BOLD activity associated with task performance is shown for participant BTX5 at W0 *(a)* and W6 *(b)*. Signal increased in both volume and average z-score following the BoNT injection therapy.

2.3.2 Imaging: Group Averages

The average activation map for the control group was calculated using all participant data from both W0 and W6 after taking account for repeated measures. This group average of BOLD activity during hand movement for all control participants is shown in Figure 2.8. The contralateral primary motor cortex (M1) and ipsilateral cerebellum appear to be the areas exhibiting the highest volumes of activity. In addition to these primary motor areas, the following areas also showed significant activity: ipsilateral premotor cortex, ipsilateral supplementary motor area, hand portion of the M1, and various areas of the basal ganglia.

Because the individual data maps showed clear differences between W0 and W6 for stroke participants, group average maps were calculated separately for each time point (Figure 2.9). At W0, group results showed minimal activation, including areas limited to the cerebellum, occipital cortex, and small portions of the hand region of M1 (Figure 2.9a). Conversely, at W6 group maps showed widespread activity throughout the whole brain. Areas of activation included the cerebellum and occipital cortex (as seen at W0) as well as areas in the ipsilateral and contralateral primary motor cortex, hand area of M1, and the basal ganglia (Figure 2.9b). Group activity maps for the stroke participants following the lesion-correction analysis showed similar patterns, and are documented in Appendix E. While the W6 group average showed notable increases in primary and secondary motor regions, it is clear that areas outside of the primary motor cortex become engaged as well.

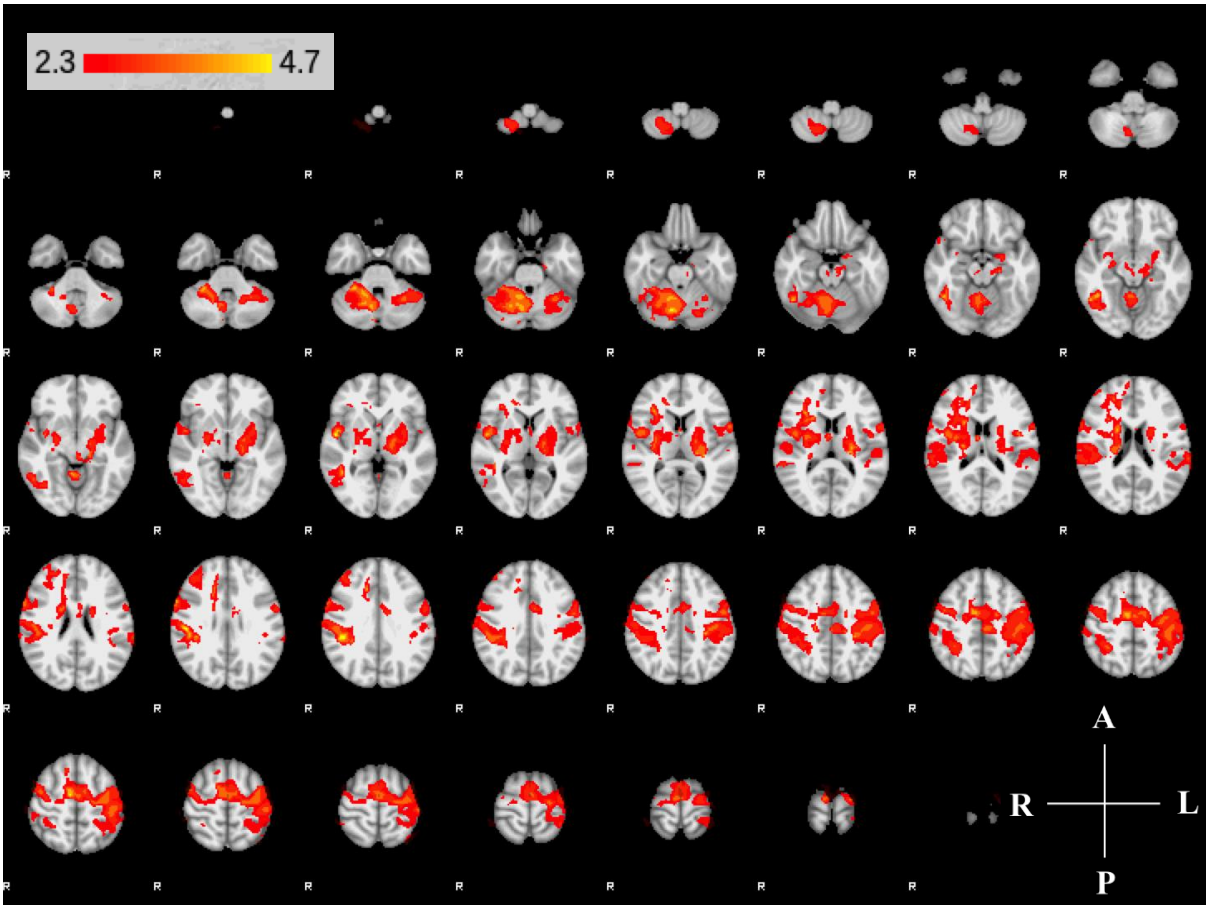
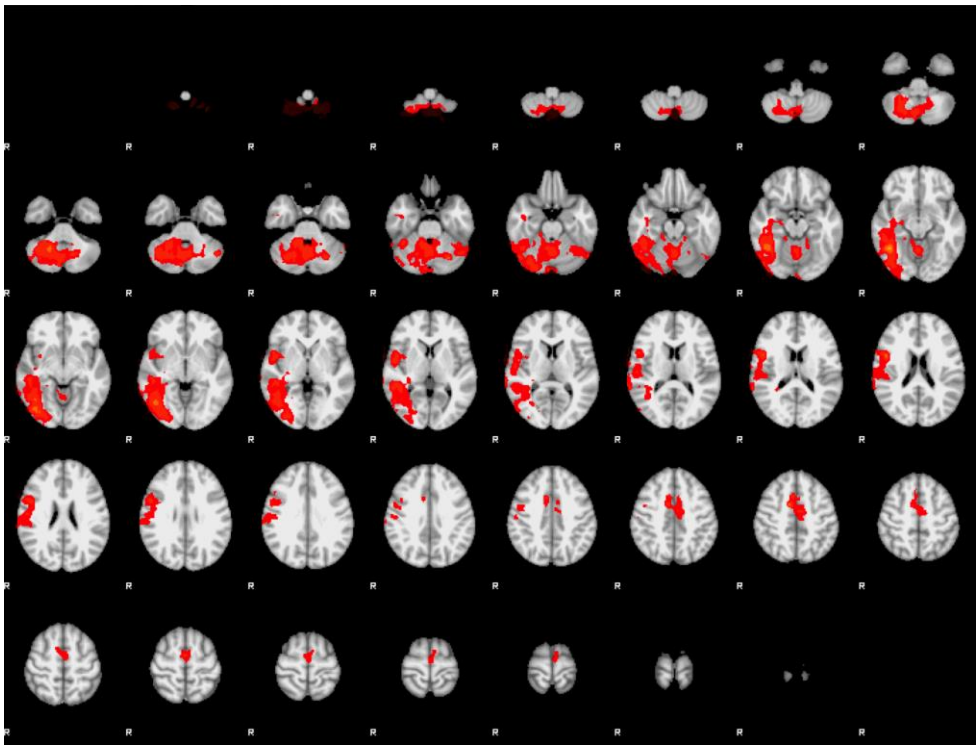


Figure 2.8: Group Activity Maps of Control Participants. The above panel shows slices of the MNI template overlaid with z-statistic ($Z > 2.3$) maps of the averaged control group activity during non-dominant hand movement. Activity maps indicate volumes in which there was significant ($p < 0.05$) levels of activity across the group. The right hemisphere of the brain is displayed on the left.

a.



b.

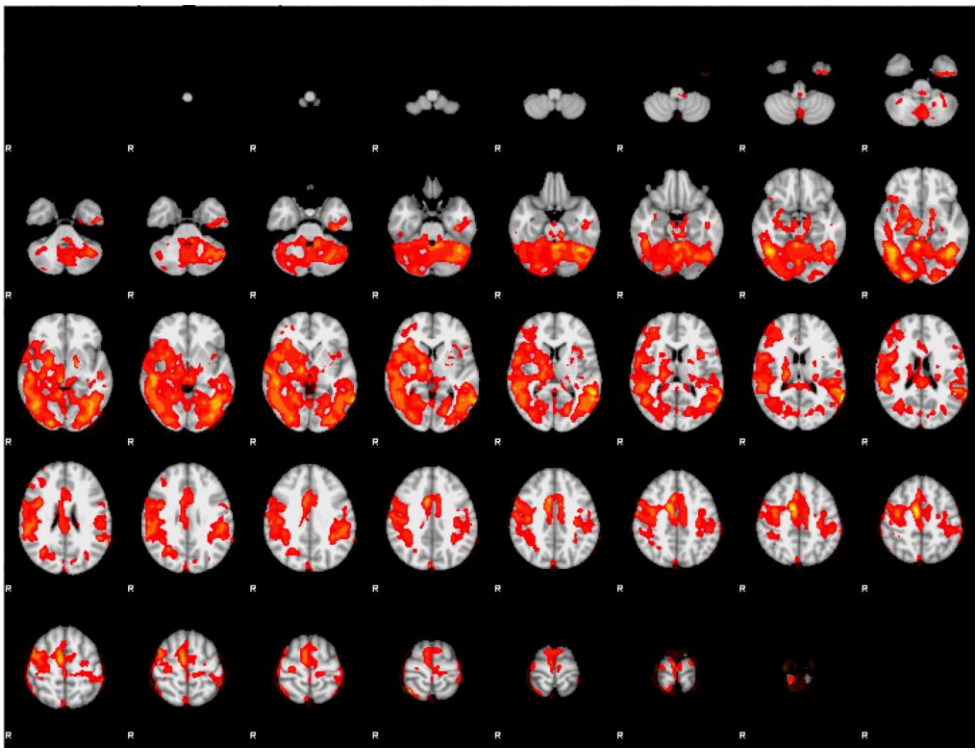


Figure 2.9: Group Activity Maps of Stroke Participants. Each panel shows axial slices of the MNI template overlaid with z-statistic maps of the averaged stroke group *(a)* before BoNT injection and *(b)* six weeks after injection. Activity maps indicate volumes in which there was significant ($p < 0.05$) levels of activity across the group. The right side of the brain is displayed on the left.

2.3.3 Imaging: Contrasts

Whole brain comparison of BOLD signal in the stroke group at W0 and W6 revealed significant differences in cortical activation following BoNT intervention. A significant increase in BOLD activity was seen in several regions including premotor, cerebellar, and sensory integration areas following BoNT injections ($P < 0.05$; paired t-test) (Figure 2.10a). These increases in activation appeared in clusters that spread across several anatomical and functional areas (Figure 2.10b). The voxel-based approach revealed three significant, connected clusters of voxels, from which five anatomical and functional regions of interest were identified: 1) ipsilateral premotor cortex (PMC-R), 2) ipsilateral cingulate gyrus (CG-R), 3) ipsilateral thalamus (Th-R), 4) superior cerebellum (S-CB), and 5) somatosensory and visual integration areas (Sens-IA). These regions are further are described in Table 2.2 and illustrated in Figure 2.11.

Following lesion correction, no significant difference was seen in the contralateral M1, which was originally hypothesized. Small areas of increased activation were identified throughout the contralateral hemisphere that did not reach a significant value. These increases in activity following lesion correction are illustrated in Appendix E.

a.

b.

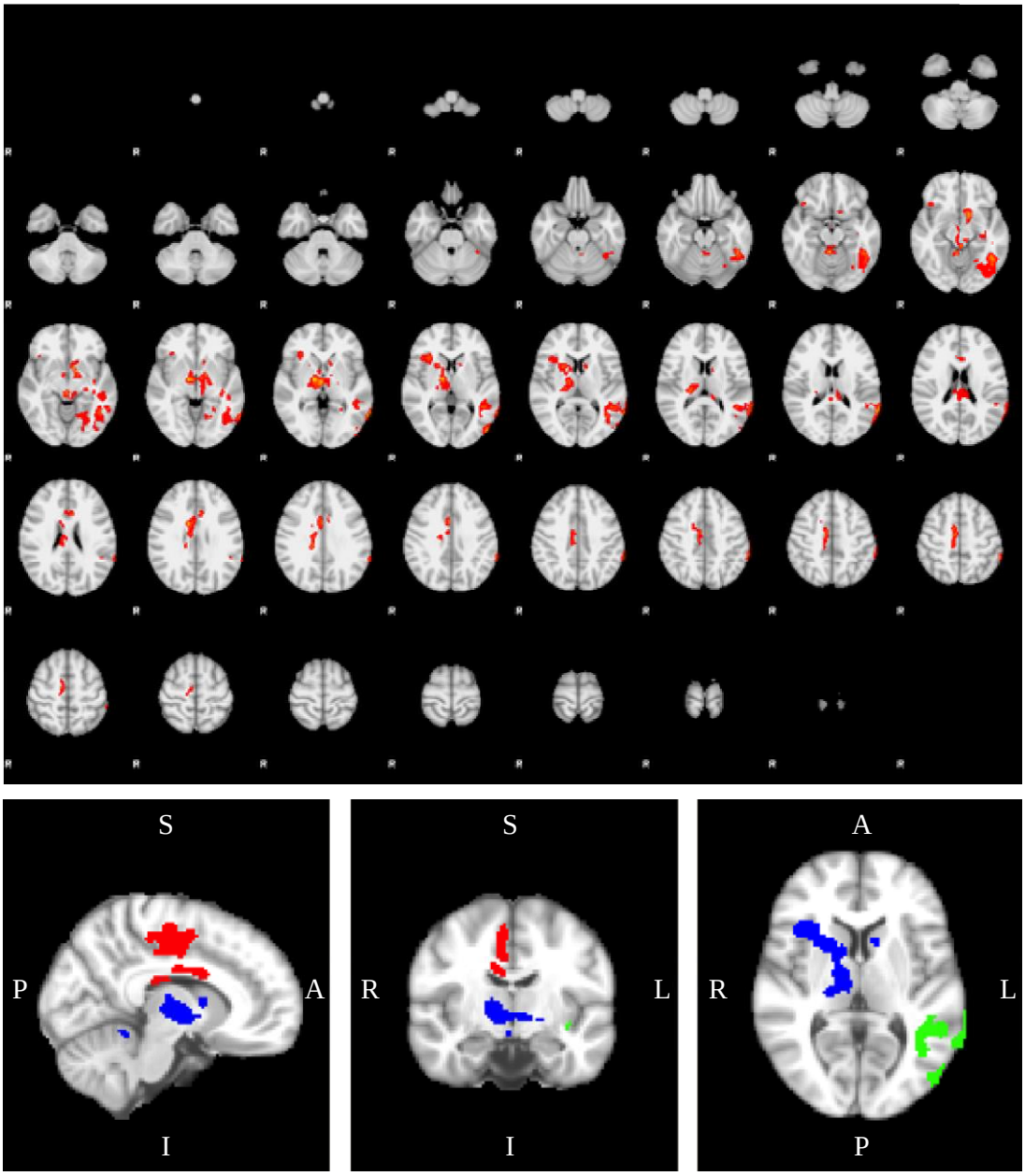


Figure 2.10: Contrast between Stroke Participants Before and After BoNT Intervention. Significantly activated voxels ($Z > 2.3$, $p < 0.05$) are overlaid on a standard MNI brain *(a)* illustrating areas that showed a significant increase in activity following the BoNT intervention. These active areas were observed in three clusters *(b)* indicated by red, blue, and green volumes.

Table 2.2: ROI Characteristics

Main Region of Interest	*Abbreviation*	*Size (Voxels)*	*Region Description*
Premotor Cortex	PMC-R	441	64% GM Premotor Cortex BA6 R 14% GM Primary Motor Cortex BA4a R
Cingulate Gyrus	CG	758	40% WM Cingulum R 18% WM Callosal Body
Right Thalamus	Th-R	855	78% Right Thalamus 19% Right Cerebral, WM
Superior Cerebellum	S-CB	298	Right Cerebellum Anterior Lobe Cerebellar Lingual
Sensory Integration Area	Sens-IA	2680	55% Inferior Temporal Gyrus, temporo-occipital part; 14% Temporal Occipital Fusiform Cortex 7% Lateral Occipital Cortex, inferior division 3% Occipital Fusiform Gyrus

Note: Probabilities describing each ROI's anatomical makeup were determined using Jülich Histological Atlas for cortical ROIs and the Taliarch Daemon Label Atlas for at the voxel of greatest z-score overlaid onto a 2 mm-MNI brain.

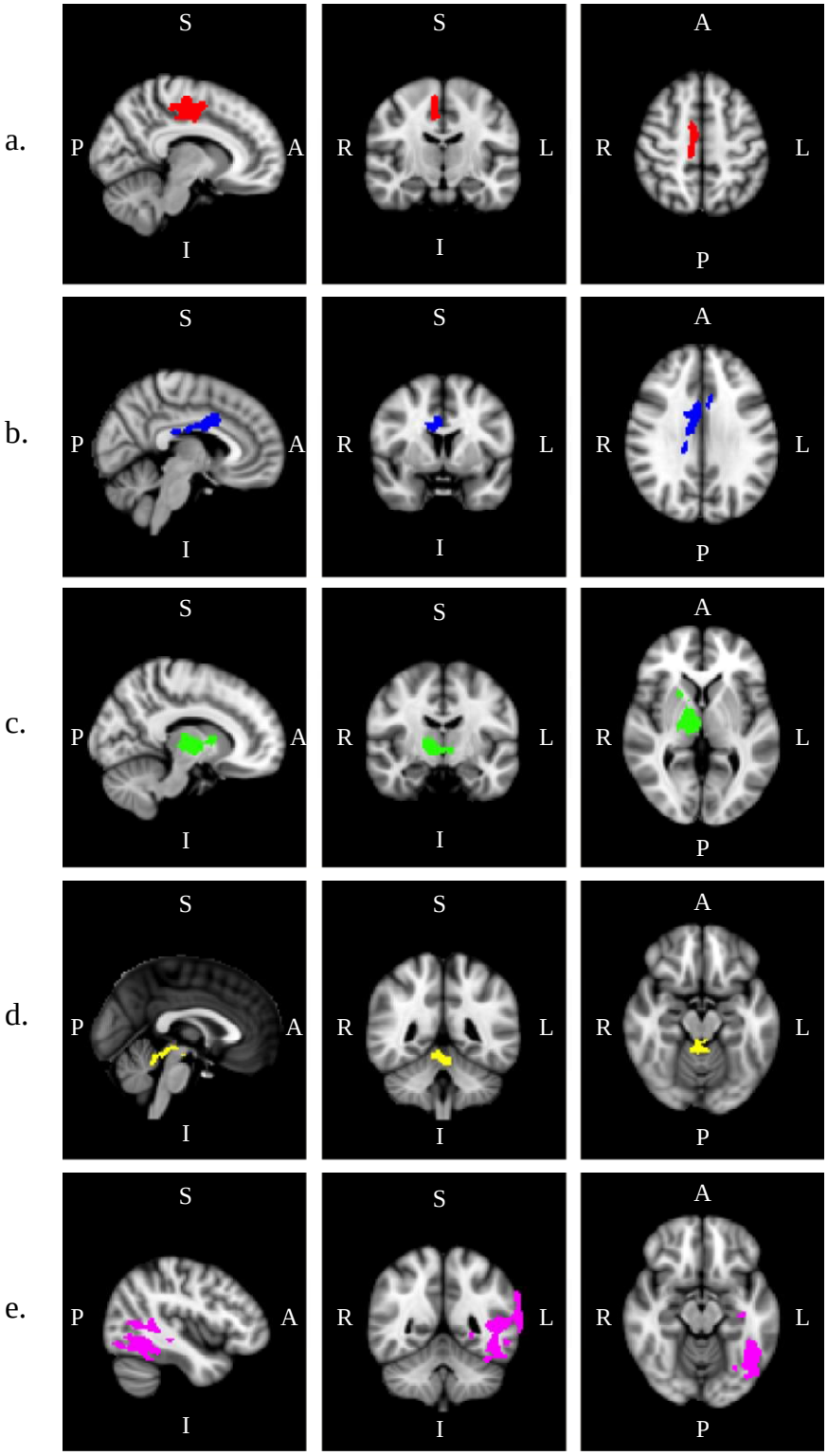


Figure 2.11: Defining Regions of Interest. Using the clusters identified in Figure 2.5, regions of interest were identified and further segmented into regions of interest utilizing the Jülich Histological Atlas for cortical regions and the Taliarch Daemon Label Atlas for cerebellar regions. *(a)* PMC-R *(b)* CG-R *(c)* Th-R *(d)* S-CB *(e)* Sens-IA.

2.3.4 Activation Volume and Correlation Analysis

The five data-defined ROIs were used as masks to identify the number of active voxels and average z-score in the given volume for the stroke group at W0 and W6 (Figure 2.12 & 2.13). Participants with stroke showed increased activity in the PMC-R region by 40.5%, CG-R by 38.7%, Th-R by 37.3%, S-CB by 46.7%, and Sens-IA by 39.2% of total ROI volume ($p < 0.05$). The average z-score within each ROI also increased following BoNT injections; analysis showed significant increases in z-scores of 0.87 to 3.43 in the PMC-R, 0.70 to 2.77 in the CG-R, 0.80 to 3.14 in the Th-R, 0.91 to 3.28 in the S-CB, and 1.17 to 3.78 in the Sens-IA respectively ($p < 0.05$).

The stroke group showed higher volume of activation in all mentioned ROIs at W6 than controls at either time point ($p < 0.05$). Controls showed greater volume of activation in the contralateral M1compared to stroke participants at W0 ($p < 0.05$), but showed no significant difference at W6. No significant difference was found between sessions for control participants. There were no areas that showed significantly decreased activity following BoNT injections.

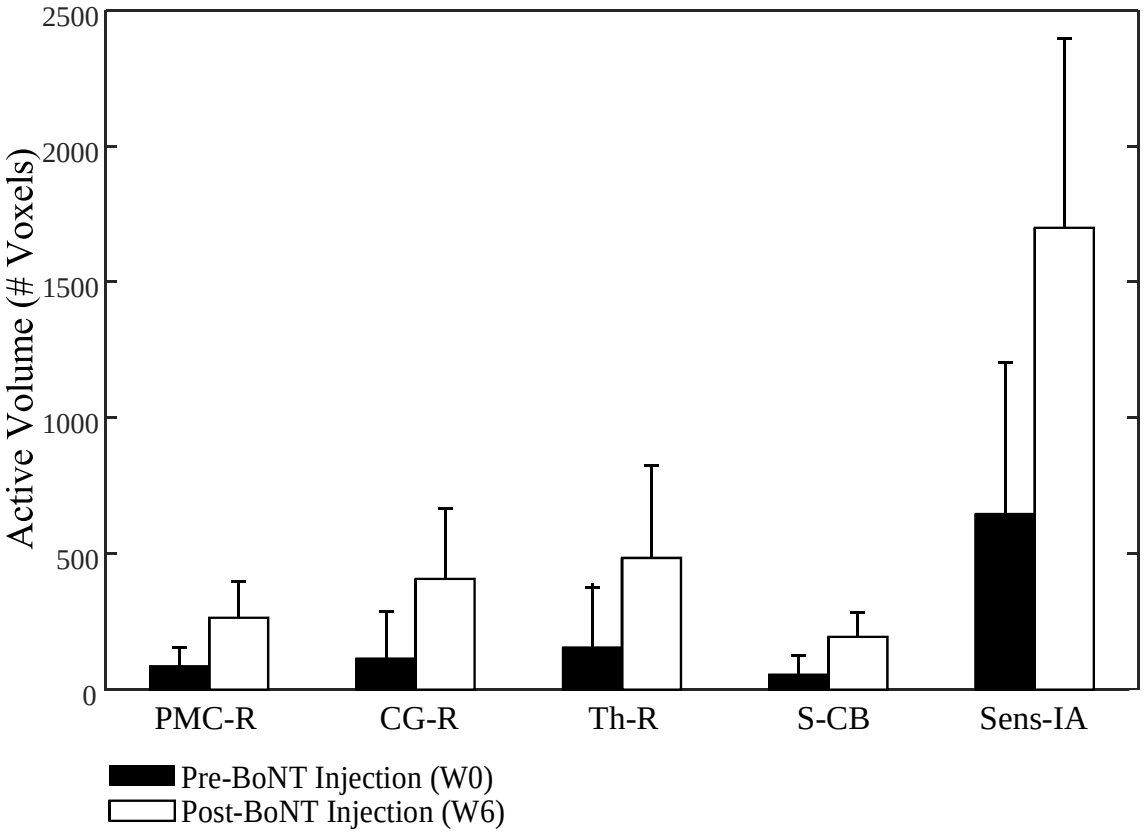


Figure 2.12: Changes in Activation Volume Following BoNT Injection Therapy. All areas of interest show a significant difference in active volume between sessions. Control group results showed no difference between sessions, and are not included in this figure.

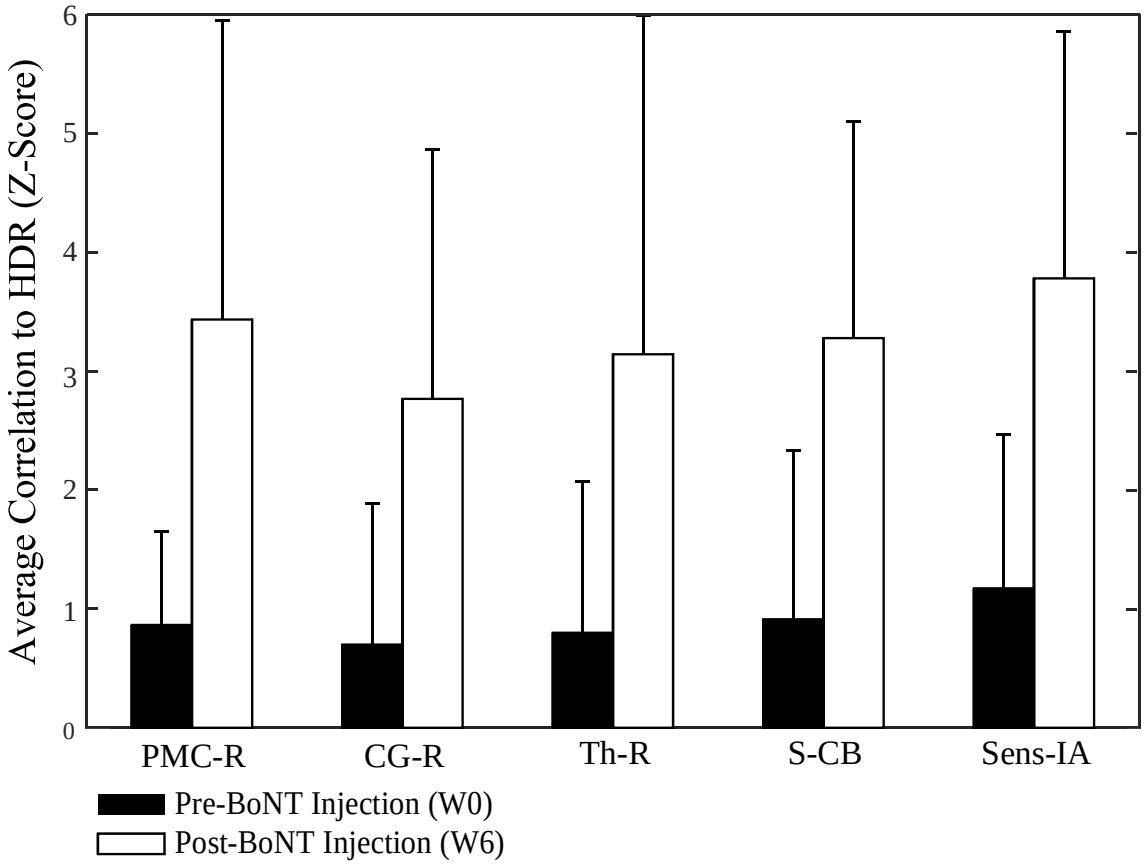


Figure 2.13: Changes in Average Z-Score Following BoNT Injection Therapy. All areas of interest show a significant difference in average intensity between sessions. Control group results showed no difference between sessions.

2.3.5 Clinical Assessment

Participants showed a significant increase in FM motor scores following injections of BoNT to the affected arm ($p < 0.01$; paired t-test). Nearly all participants receiving BoNT showed an increase in FM-UE scores following injection therapy (Table 2.3), showing a mean increase of 1.8 +/- 0.2 points increase. Four of nine participants showed improvement of wrist function, and three showed improvements in finger extension. Those that improved in finger extension had the highest MAS scores prior to injection.

Correlations were performed between activation volume, activation intensity, and clinical measurements (MAS, FMA). General trends between the initial MAS scores and activation volume at W6 were identified, although they did not reach a level of statistical significance. These included minor correlations between initial MAS scores and the changes in Sens-IA ROI volume ($r = 0.53$, $p < 0.142$) and average z-score ($r = 0.54$, $p < 0.135$). Additionally, initial MAS scores were correlated with active volume in the ipsilateral hemisphere ($r = 0.51$, $p < 0.166$). Patient BTX7 showed substantially higher functional ability in the affected limb as compared to other stroke participants, indicated by physical characteristics, initial MAS score, and FMA scores at both W0 and W6. It is important to note that this participant alone showed decreases in cortical activity following BoNT intervention.

Table 2.3: **Fugl-Meyer Scores**

Patient No.	FM (W0)	FM (W6)
BTX 1	23	26
BTX 2	26	27
BTX 3	19	22
BTX 4	20	22
BTX 5	9	9
BTX 6	23	25
BTX 7	63	63
BTX 8	44	47
BTX 9	35	40

2.4 DISCUSSION

In this study, we found evidence of the effects of BoNT on higher-order brain activation using fMRI. Following BoNT intervention, an interaction effect was seen in significant increases in the BOLD signal in the contralesional premotor cortex (PMC-R), cingulate gyrus (CG-R), and motor thalamus (Th-R), ipsilesional sensory integration regions (Sens-IA), and bilateral superior cerebellum (S-CB). These regions of interest showed increased activity, characterized by both larger volume of activation and greater correlation to the HDR. The results suggest that in people with severe spasticity, BoNT can enable activation of higher motor centers, possibly associated with renewed access to motor planning and control of movement.

2.4.1 Activation Patterns in Control and Stroke Groups

CONTROL

Traditionally, cortical motor function is reliant on the supplementary motor area to plan movements and the primary motor cortex to execute said plan (Ghez, Hening, & Gordon, 1991). Additionally, somatosensory areas interpret sensory information to assess the performance as compared to the plan, and adapt accordingly (Fetz, Finocchio, Baker, & Soso, 1980; Flament & Hore, 1988). These findings have been verified using fMRI, indicated by BOLD-induced signals produced during movement (Meier, Aflalo, Kastner, & Graziano, 2008; Olman, Pickett, Schallmo, & Kimberley, 2012).

In the present study, analysis of control subjects showed significant activation of the contralateral primary motor (M1), supplementary motor (SMA), cingulate motor (CMA), premotor, and somatosensory areas (S1), as well as the ipsilateral cerebellum.

Ipsilateral premotor and primary S1 were also activated, though to a lesser degree than the equivalent contralateral regions.

Our results of activation patterns for the control group were consistent with previous studies, which vary in task protocol (Meier et al., 2008; Olman et al., 2012). Reproducible activation patterns are observed in the contralateral primary, supplementary, and premotor areas as well as the ipsilateral cerebellum during repetitive hand movements (i.e. finger tapping, finger sequence patterns, whole-hand flexion/extension) (Yoo et al., 2005). Additionally, EEG/fMRI shows higher order motor areas (SMA and CMA) are activated slightly before primary motor areas, and are believed to trigger actual motor movement by releasing inhibition of the primary motor cortex (Ball et al., 1999). Engagement of the primary somatosensory cortex (along with previously established motor areas) has also been shown during executed movement, an area of activation not seen in imagined movement (Lotze et al., 1999). Similar activation patterns have been seen among passive and active movements of the hand, with passive movements engaging the same regions but less volume, which is thought to be due to the removal of motor drive (Boldyreva et al., 2014).

The motion of making a fist combines the simple task of single finger flexion and has been shown to produce nearly identical activation patterns. Comparing movements of the same joint which involve different muscles (i.e. flexion/extension v. abduction/adduction of a single finger) shows a large overlap of the hand/fingers sites on the primary motor and somatosensory cortices (Lotze et al., 2000). Although voluntary movement of the hand is classically thought to be controlled by the contralateral cortex, ipsilateral motor areas might also be involved. Engagement of the ipsilateral M1 during

unilateral hand tasks remains controversial, as bilateral activation is inconsistently observed in fMRI studies. Previous findings suggest a positive correlation between the volume of activation in ipsilateral motor areas and the precision required for the task, complexity of the task, and duration of the movement (Buetefisch et al., 2014; Verstynen, 2004; Newton, Sunderland, & Gowland, 2005; Bernard et al., 2002).

STROKE

Severity of motor impairment has an impact on functional activity patterns related to hand movement in stroke survivors. Stroke survivors with recovered motor function show activity patterns similar to those produced by healthy controls, often exhibiting less localization and more active volume (Cramer et al., 1997; Nair et al., 2007). Movement of the unimpaired hand elicits nearly identical patterns to those seen in controls; however, in stroke survivors with motor impairments, movement of the affected hand activates areas of the motor cortex that are spatially distinct from those produced during movement of the unaffected hand (Cramer et al., 1999). Study participants exhibiting severe deficits often recruit motor areas outside the primary motor cortices including supplementary motor areas, cingulate motor areas, premotor cortices, and cerebellar regions (Ward et al., 2003). Poor motor outcome in chronic stroke is associated with changes in interhemispheric balance, shifting cortical activation to the non-lesioned hemisphere to provide compensation for areas damaged by the infarct (Cramer & Crafton, 2006). These foundational findings have fueled fMRI research to determine neural correlates of motor recovery following stroke and to design therapies to facilitate these neuroplastic changes (Buetefisch, 2015; Johansen-Berg et al., 2002; Jones et al., 2015; Michielsen et al., 2011; Rehme, Fink, Von Cramon, & Grefkes, 2011; Schaechter et al., 2002).

COMPARISON BETWEEN CONTROL AND STROKE

The tasks chosen for movement-based fMRI analysis in both control and stroke related studies show a wide variety of complexity and involvement, all of which produce near-identical activation patterns. Comparing activation maps between the present and past studies, both control and stroke groups show more extensive contralateral somatosensory activation compared to prior studies (Schaechter et al., 2008; Cramer et al., 1997). It is possible that this larger area of activation is due to the increased sensory stimulus elicited by the assistive device. Congruency between current and previous findings in control participants regarding the involvement of all contralateral motor areas, as well as ipsilateral cerebellar areas provides further validation of our imaging methods.

Activation in control participants' ipsilateral motor areas could be explained by the long blocks of movement and the target of achieving a fully formed fist and extended hand (Newton et al., 2005). Similar results are to be expected when comparing motor tasks ranging from single-joint finger tapping to mass flexion and extension of the hand due to the similarity of the movement tasks (Lotze et al., 2000); contrasts seen between control and stroke groups might be due to the difficulty of the present study's task (Verstynen, 2004). In controls, minor recruitment of ipsilateral secondary motor areas may be a result of the uniqueness of a task comprised of assisted extension and resisted flexion, even if the movement is not considered challenging. Furthermore, higher order motor areas recruited by stroke subjects with large impairments may be due to the additional effort and planning required to perform a seemingly simple motor task (Bernard et al., 2002).

Our findings in differences in control and stroke participant activation patterns associated with hand movement are consistent with previous results. Stroke participants

tend to have much more variety in cerebral activation patterns, particularly when compared to neurologically intact control subjects (Ward et al., 2003; Cramer et al., 1997, 1999). Comparing activation of the contralateral M1 between groups, controls showed significantly higher volume and magnitude of activation in the primary motor cortex ($p < 0.025$) than BoNT participants, even following the therapy. This is likely due to the control group's ability to perform the task fully, as movement amplitude and M1 activation in fMRI have been positively correlated (Waldvogel, van Gelderen, Ishii, & Hallett, 1999). Although this increase was not significant following contrast analysis, it is possible that this trend might become more apparent with a larger sample size.

2.4.2 Motor Areas of Activation Unique to Stroke

Activation patterns related to hand motor tasks in stroke participants have been examined with regards to motor recovery and therapy outcomes. Reports vary greatly in study design but show consistently more disperse activity patterns in stroke populations as compared to neurologically intact populations (Cramer et al., 1997, 1999; Schaechter et al., 2002; Ward et al., 2003;). Studies on early post-stroke motor recovery have demonstrated correlations between the contralesional primary motor cortex (M1) and behavioral recovery, associating high levels of contralesional activation with poor recovery and inability to fully perform the task (Rehme et al., 2011; Calautti et al., 2007; Buetefisch et al., 2015; Marshall et al., 2009; Johansen-Berg et al., 2002). Given the highly impaired participants in the current study, high levels of contralesional involvement—as seen in both pre- and post-BoNT fMRI data—would be expected. The present study demonstrates a general trend indicating initial MAS scores may be

correlated with ipsilateral hemisphere activity; this implies that patients experiencing severe spasticity require more extensive engagement of ipsilateral structures for compensation. The overall consensus on the effects of therapy as indicated by fMRI activity follows a trend of lateralization and localization to the contralateral hemisphere, generally indicating decreases in overall brain activity. This finding has been reported in constraint-induced (Johansen-Berg et al., 2002), mirror (Michielsen et al., 2011), and physical rehabilitative therapies (Cauraugh & Summers, 2005; Schaechter et al., 2002). Reports on increased activity of higher-order motor areas following therapeutic interventions, however, remain minimal.

The first group to assess BOLD signal neural correlates associated with the impact of BoNT injection therapy did not assign a rigorous physical therapy protocol along with the study (Manganotti et al., 2010). Eight chronic stroke survivors performed sequential finger tapping during an fMRI; following BoNT injections, activation decreases in both ipsilateral and contralateral motor areas, showing a clear lateralization to the affected hemisphere. As it is traditionally recommended, most studies assess the effects of BoNT in combination with physiotherapy. Diserens et al. (2010) found increases in activity in contralesional secondary motor areas and the ipsilesional somatosensory cortex following repetitive arm cycling over a 3 month period. Following this initial study, more fMRI findings have been discovered by combining BoNT injections with daily (~1 hr.) rehabilitation training tailored to each participant, with highly variable results (Senkárová et al., 2010; Veverka et al., 2012, 2014, 2016). Their initial 4-subject study showed decreases in the posterior cingulate and precuneus regions (Senkárová et al., 2010). A subsequent study included 14 study participants and extensive

activity increases are seen within the contralesional thalamus and bilateral cerebellum while decreases are seen bilaterally in primary, supplementary, and premotor cortices following BoNT (Veverka et al., 2012, 2014, 2016). Bergfeldt et al. (2015) also conducted a 6-person study, and documented bilateral decreases in M1, making note that ipsilateral M1 activity is more greatly reduced. These studies all identify changes in activity of several primary motor areas following BoNT intervention; however, the inconsistency in findings surrounding involvement of areas outside the primary motor cortex suggests further investigation is needed.

In the current study, group results showed increases in contralateral primary motor cortex and bilateral cerebellar activity following BoNT injections (Figure 2.9b), congruent with several findings from previous studies involving cerebral changes due to BoNT and combined therapies (Tomášová et al., 2013). Stroke survivors tend to have greater variety in activation patterns, often including several areas outside the primary motor cortex in both the affected and unaffected hemisphere. Cross-session analysis revealed three clusters in which BOLD activity significantly increased after BoNT treatment; these three clusters were broken into five main regions of interest: the contralesional premotor cortex (PMC-R), cingulate gyrus (CG-R), contralesional thalamus (Th-R), bilateral superior cerebellum (S-CB), and ipsilesional sensory-integration areas (Sens-IA). Each of these ROIs has been proven to play a supporting role in motor planning and execution.

Increases in cortical activity after BoNT may be explained by a number of factors. First, due to the severity of contractures and muscle weakness in most participants, it is possible the task was unable to be performed even with the assistance of the finger-

extension device. The release of spasticity by BoNT injections may have allowed for greater magnitude of the hand movement within the scanner, which could translate to increases in activity in motor areas. This increase in activity could also be a result of neuroplastic events occurring as individuals incorporate the affected arm into daily use, as BoNT works to gradually release spasticity over six weeks. Additionally, previous studies identified similar activation patterns among passive and active movements, where passive movement produced a weaker response due to the removal of motor control (Bernard et al., 2002); this finding is congruent with the hypothesis that BoNT injections allow for increased motor control and explains why these areas of activation would increase in volume.

Increased activity in higher-level motor areas could also be a result of repeated injections. Documentation of the cortical activation changes associated with BoNT have primarily focused on first-time injections; because long-term BoNT injection therapy itself tends to exhibit a plateau-effect, it is likely that activity patterns change accordingly. The present study assesses these changes following repeat injections, wherein no present literature is available for comparison.

Additionally, prior studies have included intense rehabilitative therapy regimens for all participants receiving BoNT injections (Diserens et al., 2010; Veverka et al., 2012, 2014, 2016; Bergfeldt et al., 2015). Similar activation patterns are shown in studies assessing cortical activation changes following motor recovery due to physical therapy methods regardless of the presence of BoNT injections; it is likely that these training and therapy methods are largely responsible for the general trend of localization and lateralization to the contralateral M1 seen in prior studies (Schaechter et al., 2002;

Johansen-Berg et al., 2002; Ward et al., 2003). Physical therapy regimens were not controlled in the present study, which may explain why this pattern was not seen in the fMRI results.

Too often in fMRI studies, regions of interest are often predetermined based on predicted outcome, identifying premotor and primary motor cortices as major targets. This tendency can often cause less-obvious patterns to be overlooked when observing group-average activity maps; to address this issue, significance testing on the contrast between sessions can filter out areas of non-significant changes in activity. The present study's data analysis was performed using a voxel-based approach to identify involvement of higher order motor areas that might be engaged following BoNT intervention. By analyzing each voxel independently, clusters directly associated with the task can be identified across the whole brain. The use of a contrast approach allows for detection of significant differences in activity between sessions, presumably the broader motor areas involved in motor planning and execution that are thought to adapt to compensate for lost primary control.

The individual data-defined regions of interest have all been related to roles involved in motor control by functional and structural connections shared between the ipsilateral premotor cortex (Calautti et al., 2007; Johansen-Berg et al., 2002), cingulate gyrus (Dum & Strick, 1991; MacDonald et al., 2000; Barbas, Henion, & Dermon, 1991; Montaron & Buser, 1988), motor thalamus (Bosch-Bouju et al., 2013; Vitek et al., 1994; Middleton & Strick, 2000; Mushiake & Strick, 1993), superior cerebellum (Mottolese, Szathmari, Beuriat, Sirigu, & Desmurget, 2015; Timmann et al., 2008), and sensory integration areas. It is possible that the regions together act as a new internal model for

hand movement, developed post-stroke due to disuse of the affected arm. Furthermore, higher order motor control structures are required to compensate for the loss of primary motor cortical drive.

2.4.3 Effects of BoNT Therapy

BoNT therapy is a safe and effective method for managing symptoms of spasticity and has become a frontline treatment for focal or multifocal spasticity (Elovic et al., 2008; Hesse, Reiter, Konrad, & Jahnke, 1998; McCrory et al., 2009; Shaw et al., 2011; Sheean, 2006). Best outcomes are seen in patients when action is taken during early stages of symptoms, preventing soft tissue shortening from limb immobility (Kaji et al., 2010; Smith, Ellis, White, & Moore, 2000). Injections of BoNT are effective in improving rehabilitation outcomes, resulting in better mobility as compared to groups receiving a placebo (Gracies et al., 1997; Marciniak et al., 2012; Rosales et al., 2012).

Use of BoNT injections to treat spasticity in participants of the current study helped reduce spasticity and slightly decrease motor impairment, as indicated by a significant increase in FM motor scores ($p < 0.01$). The components in which scores improved were often related to improved wrist strength, as seen in 4 out of 9 patients. Other areas of improvement (mass finger extension, forearm pronation/supination, shoulder flexion and abduction) varied between participants, most likely impacted by injection location and dose.

Prior BoNT injections in participants of the current study might have influenced the results. Prior studies assessing the effects of BoNT injections use participants naïve to the therapy, documenting greater improvement following first-time injection (Hesse et

al., 1998, 2001; Hurvitz et al., 2003). It is possible that the small increase in FM score after the injections in the current study, though significant, could be limited by improvements that already occurred following prior injections. Patients appear to experience a greater change in clinical scores following their first injection, while follow-up injections serve primarily to maintain the spasticity reduction. This model would show a plateau effect in clinical scores when documenting long-term use.

Some have concluded that BoNT injections provide best results when administered shortly after symptoms occur (Kaji et al., 2010; Smith et al., 2000). The minor improvements seen in this study's FM scores could be a result of the timeframe in which patients received their first injection following the appearance of spastic symptoms. If the study group dealt with symptoms for substantial time period before seeking treatment, it would follow that their improvements following BoNT would be less pronounced. Because the onset of these symptoms is not specifically recorded, this hypothesis cannot be tested.

The efficacy of BoNT to improve peripheral motor impairment was seen in changes in cortical activation following treatment. It is possible that the increased activation seen in contralateral primary motor areas was elicited from greater magnitude of movement within the scanner; however, this increase in activity was not significant following the subject-paired contrast analysis across timepoints (W0 v. W6). While ability to perform the task may have improved slightly following BoNT injections, it does not compare to that of the controls, which showed significantly greater activation.

2.4.4 Study Limitations

The study enrolled a small sample, and it is possible that including more participants would provide more areas of significant difference. Despite the low number of participants, results showed consistent patterns among each group. One limitation to the present study is the lack of measurement of hand movement while in the scanner. Quantification of increased movement or lack thereof would have proven helpful in drawing final conclusions on the effects of BoNT injections. A real-time measurement of hand movement would have the potential to control for mirror movements that are often seen in stroke survivors trying to perform tasks with a significantly impaired limb (Ejaz et al., 2018; Nelles, Cramer, Schaechter, Kaplan, & Finklestein, 1998; Ohtsuka, Matsuzawa, Ishii, & Shimizu, 2015). It is possible that activity seen in ipsilateral motor areas may have resulted from mirror movements of the unaffected hand, though it is unlikely because mirror movements would have been present both before and after BoNT injection.

Assessments of improvements in spasticity was limited by the documentation of the MAS only prior to the injection; a follow-up measure to quantify improvements in spasticity would add to the interpretation of the BoNT effects. Likewise, the FMA is a measure of motor impairment rather than functional ability. Additional tests such as the Wolf Motor Function Test (WMFT) and the Box and Blocks test would have added beneficial information regarding any changes in functional ability following BoNT injections (Mathiowetz, Volland, Kashman, & Weber, 1985; Wolf et al., 2001). All participants performed physical therapy exercises tailored to their specific needs. Physical therapy treatment plans, duration, and frequency were not controlled, but

assumed to remain constant over the six-week period of involvement in the study. Neither injection site nor dose were controlled for, as each participant showed varying levels of spasticity. The total number of injections received ranged from 2 to 50+ injections over the participants' lifetimes but did not show any correlation with effect of the injection.

2.5 CONCLUSION

This study showed differences between control and stroke brain activation patterns in response to an assisted hand flexion/extension task that were generally consistent with previous reports. The results also showed an interaction effect due to BoNT treatment, demonstrating increased cortical activation in higher-order motor areas following BoNT injection therapy in highly impaired stroke participants performing hand-motor tasks. It is likely that these areas of increased activation play a compensatory role in highly impaired participants and allowed for the production of movements regained through the release of spasticity.

CHAPTER 3: CONCLUSIONS AND FUTURE DIRECTIONS

3.1 LONGITUDINAL EFFECTS OF BOTULINUM TOXIN INJECTIONS: CLINICAL, FUNCTIONAL, AND FMRI OUTCOMES

A current limitation to the present study was the inability to assess long-term longitudinal effects of BoNT injections on relieving spasticity. Injections show the greatest improvements in patients who seek treatment shortly after the onset of symptoms (Burbaud et al., 1996; Mohammadi, Abdoulrahmani Balouch, Dengler, & Kollewe, 2010). Evidence has shown BoNT therapy to exhibit the greatest effects following first-time injection, where repeated injections act to maintain the initial improvement (Gordon et al., 2004). It would follow that these follow-up injections, which are intended to maintain symptoms, would elicit different activity patterns at different timepoints, as indicated by fMRI. The present study observed these patterns, showing results that understandably varied from past studies that looked into first-time injections only.

3.1.1 Long-term Effects of BoNT Injections on Functional Ability

While long-term use of BoNT injections as a method of treatment for post-stroke spasticity has been concluded to be safe and effective, the impact on functional outcome remains unclear. Longitudinal studies have evaluated kinematic improvements in spasticity following treatment, quantified by changes in MAS scores (Bhakta, Cozens, Chamberlain, & Bamford, 2000; Hare et al., 2009; Richardson et al., 2000; Shaw et al., 2011). Likewise, studies have concluded BoNT injections are a contributing factor to improvements in patient's self-assessed quality of life, pain, and hygiene (Caty,

Detrembleur, Bleyenheuft, Deltombe, & Lejeune, 2009; Elovic et al., 2008; Lim, Koh, & Paik, 2008). Past findings of improved clinical scores and overall quality of life have been consistent; however, no general consensus has been agreed upon regarding functional outcomes due to BoNT injections.

Often, studies associate improvements in clinical scores with improvements in everyday function; however, it is difficult to make direct translations between the two without a standard objective functional scale. Some have reported improvements in functional outcomes, reporting patient ability to attain prespecified goals and improved gait patterns following BoNT injections (McCrory et al., 2009; Sheean, Lannin, Turner-Stokes, Rawicki, & Snow, 2010). Conversely, several studies have reported improvements quantified by decreased muscle tone, while observing no apparent functional benefit from the injection therapy (Bensmail, Robertson, Fermanian, & Roby-Brami, 2010; Fridman et al., 2010; Turner-Stokes et al., 2010). It is possible that the improvements seen in function are responses to first-time injections, while long-term BoNT injections do not elicit additional improvements. More likely, the improvements in function are due to therapy paired with the BoNT injections, using the neurotoxin as an aide to maximize the effects of the training. These conflicting results suggest that there are several factors that affect how any given patient will improve due to BoNT injections (i.e. time since onset, number of injections, dose).

The minimal evidence behind BoNT effects on function with regards to dexterity, active movement, and goal-driven movement has led some to suggest spasticity is not the direct cause of functional deficits seen post-stroke (Stinear et al., 2007). The reason behind the loss in function may be due to muscle weakness rather than increased muscle

tone caused by spasticity. With BoNT injections acting to minimize contraction of the targeted muscle, this should provide the patient opportunity to strengthen the antagonist muscle. Perhaps weight training and other therapies in combination with BoNT injections would provide the best functional outcomes in stroke survivors experiencing motor deficits.

Researchers have not yet determined the root cause of spasticity. It may be a result of increased excitability of afferent neurons from muscle fibers themselves, an abnormal effect in processing this sensory information, or some other unknown mechanism. As therapies to combat this excessive reflex action develop further, we gather more insight into the effects of this spastic restraint. Brain imaging studies have the potential to provide understanding of where the signal pathway is failing. If sufficient signal is generated from cortical levels, it's possible that this signal could be harnessed to control an assistive device or exoskeleton to further support functionality.

3.1.2 Changes in Activity Patterns Corresponding to Improved Spasticity

Though the impact of BoNT therapy on functional motor outcome remains unclear, improvements in clinical assessment following injections has been documented consistently. An understanding of underlying neural reorganization has the potential to further improve therapies and subsequently motor outcome following stroke. A handful of studies have been performed to assess the effects of BoNT and release of spastic restraint on activation patterns (Bhakta et al., 2000; Manganotti et al., 2010; Tomášová et al., 2013; Veverka et al., 2012, 2014, 2016). Findings across these studies show

commonalities in the effects of primary motor areas, but show wide variation in broader motor and sensory areas impacted by the therapy.

Of these studies on task-related brain activation, most have observed only highly functional patients that were naïve to BoNT injections prior to the beginning of the study (Bhakta et al., 2000; Manganotti et al., 2010; Tomášová et al., 2013; Veverka et al., 2012, 2014, 2016). The present study found evidence of changes in cortical activity associated with improved clinical assessments in patients receiving repeated injections. These findings provide insight into how activity patterns change due to the long-term effects of BoNT as it is traditionally prescribed in 3-4 month blocks. The primary purpose of these repeated injections is to maintain a reduced severity of initial spastic symptoms, where the greatest improvement is seen in earlier treatments. Just as the muscle adjusts to these repeated injections, it would follow that cortical activity patterns would adapt accordingly. It is possible that the plateau-trend seen in spasticity improvement could be paralleled in activation patterns. Following a group of stroke patients undergoing BoNT therapy for a longitudinal study would allow us to test this hypothesis.

3.1.3 Future Directions

In future studies, it would be worthwhile to follow patients from first-time injections through several rounds of the injection therapy, ideally over a 1-year period, including patients' first 5 BoNT treatments. Assessments of spasticity severity (MAS), functional outcome (WMFT, Box and Blocks), and cortical activity would be reported directly before and 6 weeks after each injection. In addition, subjects could be recruited into groups presenting mild, moderate, and severe symptoms of spasticity to allow for

additional analyses on the effects of impairment level on activity patterns. The assistive device used in the present study would be adapted to include optical fiber sensors to measure movement throughout the task-related scans. The proposed modifications would allow for a better understanding of the long-term longitudinal effects of BoNT on cortical activity and how they come about.

3.2 CHANGES IN CONNECTIVITY FOLLOWING MOTOR RECOVERY

3.2.1 Functional and Effective Connectivity using Resting State and Task-Based Analysis

Further analysis of the fMRI data collected in this study has the potential to reveal resting-state and task-related network connectivity changes associated with BoNT therapy. Connectivity as a whole is categorized into two types—resting state and task-based. (Friston & Büchel, 2003). Task-based data can be analyzed to determine task-based functional connectivity or the correlations between spatially remote neurophysiological events (Perkel, Gerstein, & Moore, 1967).

Although an ongoing debate over the use of task-based versus resting state connectivity analyses persists, both are continually used to assess connectivity parameters neural networks. The aim of task-related connectivity studies is to accentuate components associated with the task (Friston et al., 1994). These tasks may include working memory, motor movements, feedback systems, among others. Studies aimed to address the overlap of resting state and active networks have been growing in popularity, most of which observe high correspondence between the two (Fair et al., 2007; Fox et al., 2005; Greicius, Krasnow, Reiss, & Menon, 2003). Smith et al. (2009) concluded all networks utilized during a task are “continuously and dynamically active, even when at

rest". While the networks themselves may be active at rest and during task, it has been shown that the strength of these networks and the strength of the connections to these networks might be modulated with task (Cole et al., 2013). Therefore, future directions might benefit from using both task-based and resting state analysis on these data.

Correlations identified by functional connectivity can arise from various factors, including common stimulus inputs and synaptic connections to areas of direct activation. Effective connectivity is another method of measuring connectivity within the brain, which quantifies the influence one neuronal system exerts over another, giving a direction to the connectivity measurement rather than connection strength alone (Perkel, Gerstein, & Moore, 1967). Functional and effective connectivity are both dynamically dependent on activity patterns; however, effective connectivity aims to separate the shared activation response to a stimulus from those induced by synaptic connections between two areas (Aertsen, Gerstein, Habib, & Palm, 1989). While the common inputs from other brain areas may manifest as functional connectivity, effective connectivity integrates a model to account for exertion over one neuronal system on another, which acts to discount additional influence (Friston et al., 2011). Functional connectivity, in contrast, can be used to explore several functional systems; resting state analysis—unbiased regarding task performance and the *a priori* assumptions made by effective connectivity approaches—can provide intrinsic brain connectivity (Rehme & Grefkes, 2013).

3.2.2 Changes in Connectivity After Stroke

Network connectivity changes following stroke have been studied extensively using both resting state and task-related approaches to compare effects of the trauma to neurologically intact participants, noting most importantly the connection between ipsilateral and contralateral M1 (Biswal, Zerrin Yetkin, Haughton, & Hyde, 1995). Grefkes et al. (2008) assessed resting state and task-related connectivity in subacute stroke survivors and compared findings to that of age-matched controls. Resting state analysis showed decreased intrinsic coupling between ipsilesional M1 and SMA and interhemispheric connections between SMAs. Additionally, effective connectivity related to movement of the impaired arm resulted in a decreased connection from ipsilateral to contralateral M1s, the strength of which correlated with deficit severity (Grefkes et al., 2008). Another study by Carter et al. (2010) assessed behavioral changes in motor impairment and their correlation with resting state inter- and intra-hemispheric connections to primary motor areas. Similar to the Grefkes's group, results found the integrity of inter-hemispheric somatomotor network connections correlated with impairment, while their analysis focused on intra-network correlations yielded no effect (Carter et al., 2010).

A subsequent resting-state study followed acute stroke patients, concluding on longitudinal effects in functional connectivity based on results at 0, 1, 3, and 6 months post-stroke (Park et al., 2011). The group verified the reduced connection between ipsilesional and contralesional M1, showing the greatest deficit 1 month following the stroke. Additionally, increases in activity of the ipsilesional M1 with the cerebellum, thalamus, middle frontal gyrus, and posterior parietal cortex were documented. Another

study assessing functional connectivity in acute recovery administered resting-state fMRIs at < 24 hours, 7 days, and 90 days after onset in participants with and without motor deficits (Golestani, Tymchuk, Demchuk, & Goodyear, 2013). Significant decreases in connectivity inter-hemispheric M1s were seen within 24 hours in patients exhibiting motor impairments. Unlike previous studies, these results demonstrated that inter-hemispheric deficits between motor areas are not affected by deficits outside of motor ability. Following several other verifying studies, a general pattern of reduced connectivity between interhemispheric cortical motor areas has been accepted. These results are promising in predicting motor recovery following stroke, with implications for improving rehabilitation methods to promote the strengthening of these network connections.

3.2.3 Changes in Connectivity Following Therapeutic Intervention

Using the foundational knowledge of functional network connectivity following stroke, steps were taken to assess the effects of rehabilitation. James et al. (2009) presented results regarding acute stroke patients and the effects of a 3-week upper extremity rehabilitation program on connectivity. All participants showed improvements following therapy along with increased effective connectivity of the affected premotor cortex on unaffected premotor areas (James et al., 2009). A follow-up study assessed changes in the connectivity among primary, supplementary, and premotor areas during imagined and executed motor tasks following mental practice and 60 hours of physical therapy. The intervention showed improvements in regional connectivity among motor areas during both tasks (Bajaj, Butler, Drake, & Dhamala, 2015).

An additional study looked into the patterns resting state functional connectivity of the primary motor cortex of the affected hemisphere after four weeks of robot-assisted bilateral arm therapy (Fan et al., 2015). This study found increases in connections between ipsilateral and contralateral M1 following intervention, the magnitude of which correlated with motor and functional recovery. Similarly, Li et al. (2017) recently assessed the effects of conventional antiplatelet aggregation drug treatments with and without additional acupuncture therapy (conventional group and acupuncture group) on resting state connectivity. Compared to the conventional group, acupuncture patients showed increased functional connectivity between contralesional and ipsilesional motor regions. In all patients, a correlation between improvements in neurological deficit and functional connectivity between bilateral M1s was found (Li et al., 2017). These findings regarding rehabilitation techniques consistently find increases in the connection between interhemispheric motor areas, particularly in the primary motor cortex. Results show a neuroplastic effect influenced by all different therapies, suggesting that functional and effective connectivity may serve as a biomarker for recovery following stroke.

Although connectivity analysis has not been documented regarding BoNT injections neither as a standalone therapy nor in combination with other physical therapy methods, it could produce the same effects on M1-to-M1 connectivity as seen in previous studies. There is also reason to believe underlying connectivity differences exists based on the functional segregation in activity patterns seen in the present study. We might expect that global networks related with the task would increase following BoNT intervention, provided the increase in activity of high-order motor areas and their link to M1 in motor planning pathways.

3.3 TEMPORARY NERVE BLOCKS

In addition to BoNT injections, phenol nerve blocks are a peripheral method for reducing spasticity. The blocks have shown to be effective in improving range of motion in joints limited by muscle contracture, alleviating painful spasms, and allowing for strengthening in antagonists of blocked (Copp, Harris, & Keenan, 1970; Keenan, 1988; Khalili & Betts, 1967). Phenol nerve blocks show immediate onset of action, which requires patients to adapt quickly; when administered to lower extremities, this may interfere with gait (Bakheit, 2012). In addition, long-term use of phenol nerve blocks could lead to permanent sensory loss (Botte, Abrams, & Bodine-Fowler, 1995). These indicate two primary reasons why BoNT injections have become the more prevalent peripheral intervention.

Though BoNT remains the primary protocol for focal spastic intervention, phenol nerve blocks could provide additional information to help understand how these local anesthetics impact cortical activity. Due to the peripheral nerve block's immediate effect, physicians may be able to determine how a patient may react to BoNT injections that utilize similar inhibition methods to prevent muscle contraction. It would be beneficial to perform fMRI and clinical (MAS) as well as functional (FMA, Wolf Motor Function Test, Box and Blocks Test, etc.) assessments before injection of the peripheral nerve block and again shortly after administering the block. This would allow a better understanding of how peripheral treatment methods work to alter brain activity. Hypothetically, if increases in range of movement elicited no change in activation patterns, differences seen over time due to BoNT injections are likely due to neuroplastic effects caused by gradually practicing movements with the newly improved limb.

Likewise, if significant changes are indeed elicited by the sudden improvement in motor ability following peripheral nerve blocks, it is likely the increased magnitude of motor movement that causes undamaged areas involved in motor movement to become more or less engaged.

3.4 IMPLICATIONS OF FMRI IN CLINICAL APPLICATIONS

FMRI is but one of several functional imaging modalities. The technique depends upon blood oxygenation levels (BOLD signal) related to metabolic action during cognitive exertion. Other methods use electrical activity, magnetic fluctuations, or positron emitting isotopes to quantify functional activity. Using fMRI in clinical applications provides several advantages over other approaches; high spatial resolution, ability to observe neuronal activity in deep brain structures, no radiation, and noninvasive administration are a few major benefits. An understanding of the pathophysiology associated with any diseased population is essential to determine proper rehabilitation methods.

Several years of research utilizing fMRI has led to the identification of functional activity and connectivity patterns associated with different diseases of the brain. The understanding of how neural activity is affected following traumatic brain injury is valuable in designing therapies to combat debilitating consequences. Furthermore, fMRI technology has proven beneficial in assessing the effects of therapies, providing more information of underlying effects on the central nervous system; this knowledge is useful for improving therapies to achieve optimal results.

Both activation and connectivity studies have produced informative correlations with motor ability. In fMRI activity studies, impairment severity has been related to contralesional motor recruitment; following therapeutic interventions, those that show improvements in motor assessment have decreased contralesional activation and increased activity in ipsilesional primary motor areas. This trend is translated to connectivity results, with patients demonstrating increased connectivity between interhemispheric motor areas. Therapeutic interventions can be developed or revised to target this connection, focusing on neurorehabilitation tactics to exercise these regions and strengthen associated networks.

As studies investigating the effects of BoNT injection therapy progress, we will gain a better understanding of the causal relationship between improved motor movements and changes in neuronal activity. Exploration of other peripheral therapies in focal spasticity management may determine the origin of the motor impairment so often experienced by stroke survivors. Clarification will guide therapies to focus on either peripheral or central approaches to the problem. A major question remains unanswered: Does focal spasticity stem from cortical signal suppression or the muscles inability to respond to the signal? Further investigation using fMRI technology could hold the key to the answer, providing a doorway to new and improved rehabilitation methods.

BIBLIOGRAPHY

Aertsen, A. M., Gerstein, G. L., Habib, M. K., & Palm, G. (1989). Dynamics of neuronal firing correlation: modulation of "effective connectivity." *Journal of Neurophysiology*. https://doi.org/10.1152/jn.1989.61.5.900

Avants, B. B., Epstein, C. L., Grossman, M., & Gee, J. C. (2008). Symmetric diffeomorphic image registration with cross-correlation: Evaluating automated labeling of elderly and neurodegenerative brain. *Medical Image Analysis*. https://doi.org/10.1016/j.media.2007.06.004

Avants, B. B., Tustison, N., & Song, G. (2009). Advanced Normalization Tools (ANTS). *Insight Journal*. https://doi.org/http://hdl.handle.net/10380/3113

Bach-Rojecky, L., Relja, M., & Lacković, Z. (2005). Botulinum toxin type A in experimental neuropathic pain. *Journal of Neural Transmission Vienna Austria 1996*. https://doi.org/10.1007/s00702-004-0265-1

Bajaj, S., Butler, A. J., Drake, D., & Dhamala, M. (2015). Brain effective connectivity during motor-imagery and execution following stroke and rehabilitation. *NeuroImage: Clinical*. https://doi.org/10.1016/j.nicl.2015.06.006

Bakheit, A. M. O. (2012). The pharmacological management of post-stroke muscle spasticity. *Drugs and Aging*. https://doi.org/10.1007/s40266-012-0034-z

Ball, T., Schreiber, A., Feige, B., Wagner, M., Lücking, C. H., & Kristeva-Feige, R. (1999). The role of higher-order motor areas in voluntary movement as revealed by high-resolution EEG and fMRI. *NeuroImage*. https://doi.org/10.1006/nimg.1999.0507

Benjamin, E. J., Virani, S. S., Callaway, C. W., Chang, A. R., Cheng, S., Chiuve, S. E., & Muntner, P. (2018). Heart Disease and Stroke Statistics—2018 Update: A Report From the American Heart Association. *Circulation*. https://doi.org/10.1161/CIR.0000000000000558

Bensmail, D., Robertson, J. V. G., Fermanian, C., & Roby-Brami, A. (2010). Botulinum toxin to treat upper-limb spasticity in hemiparetic patients: Analysis of function and kinematics of reaching movements. *Neurorehabilitation and Neural Repair*. https://doi.org/10.1177/1545968309347682

Bergfeldt, U., Jonsson, T., Bergfeldt, L., & Julin, P. (2015). Cortical activation changes and improved motor function in stroke patients after focal spasticity therapy--an interventional study applying repeated fMRI. *BMC Neurology*. https://doi.org/10.1186/s12883-015-0306-4

Bernard, R. A., Goran, D. A., Sakai, S. T., Carr, T. H., McFarlane, D., Nordell, B., & Potchen, E. J. (2002). Cortical activation during rhythmic hand movements performed under three types of control: An fMRI study. *Cognitive, Affective and Behavioral Neuroscience*. https://doi.org/10.3758/CABN.2.3.271

Bhakta, B. B., Cozens, J. A., Chamberlain, M. A., & Bamford, J. M. (2000). Impact of botulinum toxin type A on disability and carer burden due to arm spasticity after stroke: A randomised double blind placebo controlled trial. *Journal of Neurology Neurosurgery and Psychiatry*. https://doi.org/10.1136/jnnp.69.2.217

Biswal, B., Zerrin Yetkin, F., Haughton, V. M., & Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echoplanar mri. *Magnetic Resonance in Medicine*. https://doi.org/10.1002/mrm.1910340409

Bohannon, R. W., & Smith, M. B. (1987). Inter rater reliability of a modified Ashworth Scale of muscle spasticity. *Phys Ther*. https://doi.org/10.1007/978-1-4471-5451-8_105

Boldyreva, G. N., Sharova, E. V, Zhavoronkova, L. A., Cheliapina, M. V, Dubrovskaia, L. P., Simonova, O. A., & Kornienko, V. N. (2014). [EEG and fMRI reactions of a healthy brain at active and passive movements by a leading hand]. *Zh Vyssh Nerv Deiat Im I P Pavlova*. https://doi.org/10.7868/S0044467714050049

Bosch-Bouju, C., Hyland, B. I., & Parr-Brownlie, L. C. (2013). Motor thalamus integration of cortical, cerebellar and basal ganglia information: implications for normal and parkinsonian conditions. *Frontiers in Computational Neuroscience*. https://doi.org/10.3389/fncom.2013.00163

Botte, M. J., Abrams, R. A., & Bodine-Fowler, S. C. (1995). Treatment of acquired muscle spasticity using phenol peripheral nerve blocks. *Orthopedics*.

Buetefisch, C. M. (2015). Role of the contralesional hemisphere in post-stroke recovery of upper extremity motor function. *Frontiers in Neurology*. https://doi.org/10.3389/fneur.2015.00214

Buetefisch, C. M., Revill, K. P., Shuster, L., Hines, B., & Parsons, M. (2014). Motor demand-dependent activation of ipsilateral motor cortex. *Journal of Neurophysiology*. https://doi.org/10.1152/jn.00110.2014

Burbaud, P., Wiart, L., Dubos, J. L., Gaujard, E., Debelleix, X., Joseph, P. A., & Lagueny, A. (1996). A randomised, double blind, placebo controlled trial of botulinum toxin in the treatment of spastic foot in hemiparetic patients. *Journal of Neurology Neurosurgery and Psychiatry*. https://doi.org/10.1136/jnnp.61.3.265

Calautti, C., Naccarato, M., Jones, P. S., Sharma, N., Day, D. D., Carpenter, A. T., & Baron, J. C. (2007). The relationship between motor deficit and hemisphere activation balance after stroke: A 3T fMRI study. *NeuroImage*. https://doi.org/10.1016/j.neuroimage.2006.08.026

Carter, A. R., Astafiev, S. V, Lang, C. E., Connor, L. T., Strube, M. J., Pope, D. L. W., & Corbetta, M. (2010). Resting Inter-hemispheric fMRI Connectiviyt Predicts Performance after Stroke. *Annals of Neurology*. https://doi.org/10.1002/ana.21905.Resting

Caty, G. D., Detrembleur, C., Bleyenheuft, C., Deltombe, T., & Lejeune, T. M. (2009). Effect of upper limb botulinum toxin injections on impairment, activity, participation, and quality of life among stroke patients. *Stroke; a Journal of Cerebral Circulation*. https://doi.org/10.1161/STROKEAHA.108.544346

Cauraugh, J. H., & Summers, J. J. (2005). Neural plasticity and bilateral movements: A rehabilitation approach for chronic stroke. *Progress in Neurobiology*. https://doi.org/10.1016/j.pneurobio.2005.04.001

Chuang, Y. C., Yoshimura, N., Huang, C. C., Chiang, P. H., & Chancellor, M. B. (2004). Intravesical botulinum toxin a administration produces analgesia against acetic acid induced bladder pain responses in rats. *Journal of Urology*. https://doi.org/10.1097/01.ju.0000137844.77524.97

Cole, M. W., Reynolds, J. R., Power, J. D., Repovs, G., Anticevic, A., & Braver, T. S. (2013). Multi-task connectivity reveals flexible hubs for adaptive task control. *Nature Neuroscience*. https://doi.org/10.1038/nn.3470

Copp, E. P., Harris, R., & Keenan, J. (1970). Peripheral Nerve Block and Motor Point Block with Phenol in the Management of Spasticity. *Journal of the Royal Society of Medicine*. https://doi.org/10.1177/003591577006300932

Cramer, S. C., & Crafton, K. R. (2006). Somatotopy and movement representation sites following cortical stroke. *Experimental Brain Research*. https://doi.org/10.1007/s00221-005-0082-2

Cramer, S. C., Finklestein, S. P., Schaechter, J. D., Bush, G., & Rosen, B. R. (1999). Activation of Distinct Motor Cortex Regions During Ipsilateral and Contralateral Finger Movements. *Journal of Neurophysiology*. https://doi.org/10.1152/jn.1999.81.1.383

Cramer, S. C., Nelles, G., Benson, R. R., Kaplan, J. D., Parker, R. A., Kwong, K. K., & Rosen, B. R. (1997). A Functional MRI Study of Subjects Recovered From Hemiparetic Stroke. *Stroke*. https://doi.org/10.1161/01.STR.28.12.2518

Cui, M., Khanijou, S., Rubino, J., & Aoki, K. R. (2004). Subcutaneous administration of botulinum toxin a reduces formalin-induced pain. *Pain*. https://doi.org/10.1016/j.pain.2003.10.008

Darling, W.G., Pizzimenti, M.A., & Morecraft, R.J. (2011). Functional recovery following motor cortex lesions in non-human primates: experimental implications for human stroke patients. *Journal of Integrative Neuroscience.* https://doi.org/10.1142/S0219635211002737

Devriese, P. P. (1999). On the Discovery of Clostridium botulinum. *Journal of the History of the Neurosciences*. https://doi.org/10.1076/jhin.8.1.43.1774

Diedrichsen, J., & Shadmehr, R. (2005). Detecting and adjusting for artifacts in fMRI time series data. *NeuroImage*. https://doi.org/10.1016/j.neuroimage.2005.04.039

Dietz, V., & Berger, W. (1983). Normal and impaired regulation of muscle stiffness in gait: A new hypothesis about muscle hypertonia. *Experimental Neurology*. https://doi.org/10.1016/0014-4886(83)90032-8

Diserens, K., Ruegg, D., Kleiser, R., Hyde, S., Perret, N., Vuadens, P., & Seitz, R. J. (2010). Effect of repetitive arm cycling following botulinum toxin injection for poststroke spasticity: Evidence from fMRI. *Neurorehabilitation and Neural Repair*. https://doi.org/10.1177/1545968310372138

Donnan, G. A., Fisher, M., Macleod, M., & Davis, S. M. (2008). Stroke. *Lancet*. https://doi.org/10.1016/S0140-6736(08)60694-7

Drachman, D. B. (1964). Atrophy of skeletal muscle in chick embryos treated with botulinum toxin. *Science*. https://doi.org/10.1126/science.145.3633.719

Dum, R. P., & Strick, P. L. (1991). The origin of corticospinal projections from the premotor areas in the frontal lobe. *Journal of Neuroscience*. https://doi.org/S0022510X0200268X [pii]

Ejaz, N., Xu, J., Branscheidt, M., Hertler, B., Schambra, H., Widmer, M., & Diedrichsen, J. (2018). Evidence for a subcortical origin of mirror movements after stroke: A longitudinal study. *Brain*. https://doi.org/10.1093/brain/awx384

Elovic, E. P., Brashear, A., Kaelin, D., Liu, J., Millis, S. R., Barron, R., & Turkel, C. (2008). Repeated Treatments With Botulinum Toxin Type A Produce Sustained Decreases in the Limitations Associated With Focal Upper-Limb Poststroke Spasticity for Caregivers and Patients. *Archives of Physical Medicine and Rehabilitation*. https://doi.org/10.1016/j.apmr.2008.01.007

Fair, D. A., Dosenbach, N. U. F., Church, J. A., Cohen, A. L., Brahmbhatt, S., Miezin, F. M., & Schlaggar, B. L. (2007). Development of distinct control networks through segregation and integration. *Proceedings of the National Academy of Sciences*. https://doi.org/10.1073/pnas.0705843104

Fan, Y., Wu, C., Liu, H., Lin, K., Wai, Y., & Chen, Y. (2015). Neuroplastic changes in resting-state functional connectivity after stroke rehabilitation. *Frontiers in Human Neuroscience*. https://doi.org/10.3389/fnhum.2015.00546

Fetz, E. E., Finocchio, D. V, Baker, M. A., & Soso, M. J. (1980). Sensory and motor responses of precentral cortex cells during comparable passive and active joint movements. *Journal of Neurophysiology*. https://doi.org/10.1152/jn.1980.43.4.1070

Flament, D., & Hore, J. (1988). Relations of motor cortex neural discharge to kinematics of passive and active elbow movements in the monkey. *Journal of Neurophysiology*. https://doi.org/10.1152/jn.1988.60.4.1268

Fox, M. D., Snyder, A. Z., Vincent, J. L., Corbetta, M., Van Essen, D. C., & Raichle, M. E. (2005). The Human Brain Is Intrinsically Organized into Dynamic, Anticorrelated Functional The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Source: Proceedings of the National Academy of Sciences of the United States of America*.

Francisco, G. E., & McGuire, J. R. (2012). Poststroke spasticity management. *Stroke*. https://doi.org/10.1161/STROKEAHA.111.639831

Fridman, E. A., Crespo, M., Argüello, S. G., Degue, L., Villarreal, M., Bohlhalter, S., & Hallett, M. (2010). Kinematic improvement following Botulinum Toxin-A injection in upper-limb spasticity due to stroke. *Journal of Neurology, Neurosurgery and Psychiatry*. https://doi.org/10.1136/jnnp.2009.188052

Friston, K., & Büchel, C. (2003). Functional Connectivity: Eigenimages and Multivariate Analyses. In *Human Brain Function: Second Edition*. https://doi.org/10.1016/B978-012264841-0/50051-2

Friston, K. J. (2011). Functional and Effective Connectivity: A Review. *Brain Connectivity*. https://doi.org/10.1089/brain.2011.0008

Friston, K. J., Worsley, K. J., Frackowiak, R. S. J., Mazziotta, J. C., & Evans, A. C. (1994). Assessing the significance of focal activations using their spatial extent. *Human Brain Mapping*. https://doi.org/10.1002/hbm.460010306

Fugl-Meyer, A. R., Jääskö, 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*. https://doi.org/10.1038/35081184

Galiana, L., Fung, J., & Kearney, R. (2005). Identification of intrinsic and reflex ankle stiffness components in stroke patients. *Experimental Brain Research*. https://doi.org/10.1007/s00221-005-2320-z

Ghez, C., Hening, W., & Gordon, J. (1991). Organization of voluntary movement. *Current Opinion in Neurobiology*. https://doi.org/10.1016/S0959-4388(05)80046-7

Gladstone, D. J., Danells, C. J., & Black, S. E. (2002). The Fugl-Meyer Assessment of Motor Recovery after Stroke: A Critical Review of Its Measurement Properties. *Neurorehabilitation and Neural Repair*. https://doi.org/10.1177/154596802401105171

Golestani, A. M., Tymchuk, S., Demchuk, A., & Goodyear, B. G. (2013). Longitudinal evaluation of resting-state fMRI after acute stroke with hemiparesis. *Neurorehabilitation and Neural Repair*. https://doi.org/10.1177/1545968312457827

Gomes, J., & Wachsman, A. M. (2013). Types of strokes. In *Handbook of Clinical Nutrition and Stroke*. https://doi.org/10.1007/978-1-62703-380-0_2

Gordon, M. F., Brashear, A., Elovic, E., Kassicieh, D., Marciniak, C., Liu, J., & Turkel, C. (2004). Repeated dosing of botulinum toxin type A for upper limb spasticity following stroke. *Neurology*. https://doi.org/10.1212/01.WNL.0000144349.95487.91

Gracies, J. M., Elovic, E., McGuire, J., & Simpson, D. M. (1997). Traditional pharmacological treatments for spasticity. Part I: Local treatments. *Muscle Nerve Suppl*.

Grefkes, C., Nowak, D. A., Eickhoff, S. B., Dafotakis, M., Küst, J., Karbe, H., & Fink, G.R. (2008). Cortical connectivity after subcortical stroke assessed with functional magnetic resonance imaging. *Annals of Neurology*. https://doi.org/10.1002/ana.21228

Greicius, M. D., Krasnow, B., Reiss, A. L., & Menon, V. (2003). Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.0135058100

Haacke, E. M., Brown, R. W., Thompson, M. R., & Venkatesan, R. (1999). *Magnetic Resonance Imaging: Physical Principles and Sequence Design*. *Book*. https://doi.org/10.1063/1.3554697

Hare, J. M., Traverse, J. H., Henry, T. D., Dib, N., Strumpf, R. K., Schulman, S. P., & Sherman, W. (2009). A Randomized, Double-Blind, Placebo-Controlled, Dose-Escalation Study of Intravenous Adult Human Mesenchymal Stem Cells (Prochymal) After Acute Myocardial Infarction. *Journal of the American College of Cardiology*. https://doi.org/10.1016/j.jacc.2009.06.055

Haubruck, P., Mannava, S., Plate, J. F., Callahan, M. F., Wiggins, W. F., Schmidmaier, G., & Smith, T. L. (2012). Botulinum neurotoxin a injections influence stretching of the gastrocnemius muscle-tendon unit in an animal model. *Toxins*. https://doi.org/10.3 3 90/toxins4080605

Hesse, S., Brandi-Hesse, B., Bardeleben, A., Werner, C., & Funk, M. (2001). Botulinum toxin A treatment of adult upper and lower limb spasticity. *Drugs & Aging*.

Hesse, S., Reiter, F., Konrad, M., & Jahnke, M. T. (1998). Botulinum toxin type A and short-term electrical stimulation in the treatment of upper limb flexor spasticity after stroke: a randomized, double-blind, placebo-controlled trial. *Clinical Rehabilitation*. https://doi.org/10.1191/026921598668275996

Huettel, S., Song, A., & McCarthy, G. (2009). MR Contrast Mechanisms and Pulse Sequences. *Functional Magnetic Resonance Imaging*.

Hurvitz, E. A., Conti, G. E., & Brown, S. H. (2003). Changes in movement characteristics of the spastic upper extremity after botulinum toxin injection. *Archives of Physical Medicine and Rehabilitation*. https://doi.org/10.1053/apmr.2003.50001

Jahangir, A. W., Tan, H. J., Norlinah, M. I., Nafisah, W. Y., Ramesh, S., Hamidon, B. B., & Raymond, A. A. (2007). Intramuscular injection of botulinum toxin for the treatment of wrist and finger spasticity after stroke. *Medical Journal of Malaysia*. https://doi.org/10.1056/NEJMoa011892

James, G.A., Lu, Z.-L., VanMeter, J. W., Sathian, K., Hu, X. P., & Butler, A. J. (2009). Changes in Resting State Effective Connectivity in the Motor Network Following Rehabilitation of Upper Extremity Poststroke Paresis. *Topics in Stroke Rehabilitation*. https://doi.org/10.1310/tsr1604-270

Jenkinson, M., Beckmann, C. F., Behrens, T. E. J., Woolrich, M. W., & Smith, S. M. (2012). FSL. *NeuroImage*. https://doi.org/10.1016/j.neuroimage.2011.09.015

Johansen-Berg, H., Dawes, H., Guy, C., Smith, S. M., Wade, D. T., & Matthews, P. M. (2002). Correlation between motor improvements and altered fMRI activity after rehabilitative therapy. *Brain*. https://doi.org/10.1093/brain/awf282

Johansen-Berg, H., Rushworth, M. F. S., Bogdanovic, M. D., Kischka, U., Wimalaratna, S., & Matthews, P. M. (2002). The role of ipsilateral premotor cortex in hand movement after stroke. *Proceedings of the National Academy of Sciences*. https://doi.org/10.1073/pnas.222536799

Jones, T. A., Adkins, D. L., Adeoye, O., Hornung, R., Khatri, P., Kleindorfer, D., & Li, T. (2015). Motor System Reorganization After Stroke: Stimulating and Training Toward Perfection. *Physiology (Bethesda, Md.)*.

Kaji, R., Osako, Y., Suyama, K., Maeda, T., Uechi, Y., & Iwasaki, M. (2010). Botulinum toxin type A in post-stroke lower limb spasticity: A multicenter, double-blind, placebo-controlled trial. *Journal of Neurology*. https://doi.org/10.1007/s00415-010-5526-3

Katz, R. T., Rovai, G. P., Brait, C., & Rymer, W. Z. (1992). Objective quantification of spastic hypertonia: Correlation with clinical findings. *Archives of Physical Medicine and Rehabilitation*. https://doi.org/10.1016/0003-9993(92)90007-J

Keenan, M. A. (1988). Management of the spastic upper extremity in the neurologically impaired adult. *Clinical Orthopaedics & Related Research*.

Khalili, A. A., & Betts, H. B. (1967). Peripheral Nerve Block With Phenol in the Management of Spasticity. *JAMA*. https://doi.org/10.1001/jama.1967.03120260051008

Kim, S. G., & Ogawa, S. (2012). Biophysical and physiological origins of blood oxygenation level-dependent fMRI signals. *Journal of Cerebral Blood Flow and Metabolism*. https://doi.org/10.1038/jcbfm.2012.23

Klein, A., Andersson, J., Ardekani, B. A., Ashburner, J., Avants, B., Chiang, M. C., & Parsey, R. V. (2009). Evaluation of 14 nonlinear deformation algorithms applied to human brain MRI registration. *NeuroImage*. https://doi.org/10.1016/j.neuroimage.2008.12.037

Kornak, J., Hall, D. A., & Haggard, M. P. (2011). Spatially extended FMRI signal response to stimulus in non-functionally relevant regions of the human brain. *The Open Neuroimaging Journal*. https://doi.org/10.2174/1874440001105010024

Kuo, I. Y., & Ehrlich, B. E. (2015). Signaling in muscle contraction. *Cold Spring Harbor Perspectives in Biology*. https://doi.org/10.1101/cshperspect.a006023

Lagalla, G., Danni, M., Reiter, F., Ceravolo, M. G., & Provinciali, L. (2000). Post-stroke spasticity management with repeated botulinum toxin injections in the upper limb. *American Journal of Physical Medicine and Rehabilitation*. https://doi.org/10.1097/00002060-200007000-00010

Lance, J.W., Feldman R.G., & Young R.R. (1980). *Spasticity, disordered motor control*. *Chicago Year book Medical*. https://doi.org/10.1136/jnnp.44.10.961

Levin, M.F., & Hui-Chan, C.W.Y. (1992). Relief of hemiparetic spasticity by TENS is associated with improvement in reflex and voluntary motor functions. *Electroencephalography and Clinical Neurophysiology/ Evoked Potentials*. https://doi.org/10.1016/0168-5597(92)90079-Q

Li, Y., Wang, Y., Liao, C., Huang, W., & Wu, P. (2017). Longitudinal Brain Functional Connectivity Changes of the Cortical Motor-Related Network in Subcortical Stroke Patients with Acupuncture Treatment. *Neural Plasticity*. https://doi.org/10.1155/2017/5816263

Lim, J.Y., Koh, J.H., & Paik, N.J. (2008). Intramuscular botulinum toxin-A reduces hemiplegic shoulder pain: a randomized, double-blind, comparative study versus intraarticular triamcinolone acetonide. *Stroke; a Journal of Cerebral Circulation*. https://doi.org/10.1161/STROKEAHA.107.484048

Lindquist, M.A., Meng Loh, J., Atlas, L.Y., & Wager, T.D. (2009). Modeling the hemodynamic response function in fMRI: efficiency, bias and mis-modeling. *NeuroImage*. https://doi.org/10.1016/j.neuroimage.2008.10.065

Lotze, M., Erb, M., Flor, H., Huelsmann, E., Godde, B., & Grodd, W. (2000). fMRI evaluation of somatotopic representation in human primary motor cortex. *NeuroImage*. https://doi.org/10.1006/nimg.2000.0556

Lotze, M., Montoya, P., Erb, M., Hülsmann, E., Flor, H., Klose, U., & Grodd, W. (1999). Activation of cortical and cerebellar motor areas during executed and imagined hand movements: An fMRI study. *Journal of Cognitive Neuroscience*. https://doi.org/10.1162/089892999563553

MacDonald, A. W. (2000). Dissociating the Role of the Dorsolateral Prefrontal and Anterior Cingulate Cortex in Cognitive Control. *Science*. https://doi.org/10.1126/science.288.5472.1835

Maier, M. A. (2002). Differences in the Corticospinal Projection from Primary Motor Cortex and Supplementary Motor Area to Macaque Upper Limb Motoneurons: An Anatomical and Electrophysiological Study. *Cerebral Cortex*. https://doi.org/10.1093/cercor/12.3.281

Manganotti, P., Acler, M., Formaggio, E., Avesani, M., Milanese, F., Baraldo, A., & Fiaschi, A. (2010). Changes in cerebral activity after decreased upper-limb hypertonus: An EMG-fMRI study. *Magnetic Resonance Imaging*. https://doi.org/10.1016/j.mri.2009.12.023

Maraka, S., Jiang, Q., Jafari-Khouzani, K., Li, L., Malik, S., Hamidian, H., & Mitsias, P. D. (2014). Degree of corticospinal tract damage correlates with motor function after stroke. *Annals of Clinical and Translational Neurology*. https://doi.org/10.1002/acn3.132

Marciniak, C. M., Harvey, R. L., Gagnon, C. M., Duraski, S. A., Denby, F. A., McCarty, S., & Fierstein, K. M. (2012). Does botulinum toxin type a decrease pain and lessen disability in hemiplegic survivors of stroke with shoulder pain and spasticity? A randomized, double-blind, placebo-controlled trial. *American Journal of Physical Medicine and Rehabilitation*. https://doi.org/10.1097/PHM.0b013e31826ecb02

Marshall, R. S., Zarahn, E., Alon, L., Minzer, B., Lazar, R. M., & Krakauer, J. W. (2009). Early imaging correlates of subsequent motor recovery after stroke. *Annals of Neurology*. https://doi.org/10.1002/ana.21636

Mathiowetz, V., Volland, G., Kashman, N., & Weber, K. (1985). Adult norms for the Box and Block Test of manual dexterity. *The American Journal of Occupational Therapy. : Official Publication of the American Occupational Therapy Association*. https://doi.org/10.5014/ajot.39.6.386

McCrory, P., Turner-Stokes, L., Baguley, I. J., De Graaff, S., Katrak, P., Sandanam, J., & Hughes, A. (2009). Botulinum toxin a for treatment of upper limb spasticity following stroke: A multi-centre randomized placebo-controlled study of the effects on quality of life and other person-centred outcomes. *Journal of Rehabilitation Medicine*. https://doi.org/10.2340/16501977-0366

Meier, J. D., Aflalo, T. N., Kastner, S., & Graziano, M. S. A. (2008). Complex organization of human primary motor cortex: a high-resolution fMRI study. *Journal of Neurophysiology*. https://doi.org/10.1152/jn.90531.2008

Michielsen, M. E., Selles, R.W., Van Der Geest, J.N., Eckhardt, M., Yavuzer, G., Stam, H.J., & Bussmann, J.B.J. (2011). Motor recovery and cortical reorganization after mirror therapy in chronic stroke patients: A phase II randomized controlled trial. *Neurorehabilitation and Neural Repair*. https://doi.org/10.1177/1545968310385127

Middleton, F.A., & Strick, P.L. (2000). Basal ganglia and cerebellar loops: Motor and cognitive circuits. In *Brain Research Reviews*. https://doi.org/10.1016/S0165-0173(99)00040-5

Mohammadi, B., Abdoulrahmani Balouch, S., Dengler, R., & Kollewe, K. (2010). Long-term treatment of spasticity with botulinum toxin type A: an analysis of 1221 treatments in 137 patients. *Neurological Research*. https://doi.org/10.1179/016164109X12478302362734

Mottolese, C., Szathmari, A., Beuriat, P. A., Sirigu, A., & Desmurget, M. (2015). Sensorimotor mapping of the human cerebellum during pineal region surgery. *Neurochirurgie*. https://doi.org/10.1016/j.neuchi.2013.05.004

Mushiake, H., & Strick, P. L. (1993). Preferential activity of dentate neurons during limb movements guided by vision. *Journal of Neurophysiology*. https://doi.org/10.1152/jn.1993.70.6.2660

Nair, D. G., Hutchinson, S., Fregni, F., Alexander, M., Pascual-Leone, A., & Schlaug, G. (2007). Imaging correlates of motor recovery from cerebral infarction and their physiological significance in well-recovered patients. *NeuroImage*. https://doi.org/10.1016/j.neuroimage.2006.09.010

Nair, K.P.S., & Marsden, J. (2014). The management of spasticity in adults. *BMJ (Clinical Research Ed.)*. https://doi.org/10.1136/BMJ.G4737

Nelles, G., Cramer, S. C., Schaechter, J. D., Kaplan, J. D., & Finklestein, S. P. (1998). Quantitative assessment of mirror movements after stroke. *Stroke*. https://doi.org/10.1161/01.STR.29.6.1182

Newton, J. M., Sunderland, A., & Gowland, P. A. (2005). fMRI signal decreases in ipsilateral primary motor cortex during unilateral hand movements are related to duration and side of movement. *NeuroImage*. https://doi.org/10.1016/j.neuroimage.2004.10.003

Ogawa, S., Lee, T. M., Kay, A. R., & Tank, D. W. (1990). Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.87.24.9868

Ohtsuka, H., Matsuzawa, D., Ishii, D., & Shimizu, E. (2015). Longitudinal Follow-Up of Mirror Movements after Stroke: A Case Study. *Case Reports in Neurological Medicine*. https://doi.org/10.1155/2015/354134

Olman, C. A., Pickett, K. A., Schallmo, M. P., & Kimberley, T. J. (2012). Selective BOLD responses to individual finger movement measured with fMRI at 3T. *Human Brain Mapping*. https://doi.org/10.1002/hbm.21310

Opheim, A., Danielsson, A., Alt Murphy, M., Persson, H. C., & Sunnerhagen, K. S. (2015). Early prediction of long-term upper limb spasticity after stroke: part of the SALGOT study. *Neurology*. https://doi.org/10.1212/WNL.0000000000001908

Park, C., Chang, W. H., Ohn, S. H., Kim, S. T., Bang, O. Y., Pascual-Leone, A., & Kim, Y.-H. (2011). Longitudinal changes of resting-state functional connectivity during motor recovery after stroke. *Stroke; a Journal of Cerebral Circulation*. https://doi.org/10.1161/STROKEAHA.110.596155

Park, J., & Park, H. (2017). Botulinum Toxin for the Treatment of Neuropathic Pain. *Toxins*. https://doi.org/10.3390/toxins9090260

Park, S.Y., Park, Y.W., Ji, Y.J., Park, S.W., & Kim, S.G. (2015). Effects of a botulinum toxin type A injection on the masseter muscle: An animal model study. *Maxillofacial Plastic and Reconstructive Surgery*. https://doi.org/10.1186/s40902-015-0010-8

Pearson, K., & Gordon, J. (2000). Spinal Reflexes. In *Principles of Neural Science*.

Perkel, D. H., Gerstein, G. L., & Moore, G. P. (1967). Neuronal Spike Trains and Stochastic Point Processes: I. The Single Spike Train. *Biophysical Journal*. https://doi.org/10.1016/S0006-3495(67)86596-2

Pickett, A. (2009). Dysport®: Pharmacological properties and factors that influence toxin action. *Toxicon*. https://doi.org/10.1016/j.toxicon.2009.03.020

Promjunyakul, N., Schmit, B.D., & Schindler-Ivens, S.M. (2015). A novel fMRI paradigm suggests that pedaling-related brain activation is altered after stroke. *Frontiers in Human Neuroscience*. https://doi.org/10.3389/fnhum.2015.00324

Pustina, D., Coslett, H. B., Turkeltaub, P. E., Tustison, N., Schwartz, M. F., & Avants, B. (2016). Automated segmentation of chronic stroke lesions using LINDA: Lesion identification with neighborhood data analysis. *Human Brain Mapping*. https://doi.org/10.1002/hbm.23110

Raineteau, O., & Schwab, M. E. (2001). Plasticity of motor systems after incomplete spinal cord injury. *Nature Reviews Neuroscience*. https://doi.org/10.1038/35067570

Rehme, A. K., Fink, G. R., Von Cramon, D. Y., & Grefkes, C. (2011). The role of the contralesional motor cortex for motor recovery in the early days after stroke assessed with longitudinal fMRI. *Cerebral Cortex*. https://doi.org/10.1093/cercor/bhq140

Rehme, A. K., & Grefkes, C. (2013). Cerebral network disorders after stroke: Evidence from imaging-based connectivity analyses of active and resting brain states in humans. *Journal of Physiology*. https://doi.org/10.1113/jphysiol.2012.243469

Richardson, D., Sheean, G., Werring, D., Desai, M., Edwards, S., Greenwood, R., & Thompson, A. (2000). Evaluating the role of botulinum toxin in the management of focal hypertonia in adults. *Journal of Neurology Neurosurgery and Psychiatry*. https://doi.org/10.1136/jnnp.69.4.499

Rizzolatti, G., Fadiga, L., Gallese, V., & Fogassi, L. (1996). Premotor cortex and the recognition of motor actions. *Cognitive Brain Research*. https://doi.org/10.1016/0926-6410(95)00038-0

Rosales, R. L., Kong, K. H., Goh, K. J., Kumthornthip, W., Mok, V. C. T., Delgado-De Los Santos, M. M., & Wong, K. S. L. (2012). Botulinum toxin injection for hypertonicity of the upper extremity within 12 weeks after stroke: A randomized controlled trial. *Neurorehabilitation and Neural Repair*. https://doi.org/10.1177/1545968311430824

Rossetto, O., Pirazzini, M., & Montecucco, C. (2014). Botulinum neurotoxins: Genetic, structural and mechanistic insights. *Nature Reviews Microbiology*. https://doi.org/10.1038/nrmicro3295

Saravanan, P., Rajaseger, G., Eric, Y. P.-H., & Moochhala, S. (2015). Botulinum Toxin: Present Knowledge and Threats BT - Biological Toxins and Bioterrorism. In P. Gopalakrishnakone, M. Balali-Mood, L. Llewellyn, & B. R. Singh (Eds.) (pp. 29–42). Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-94-007-5869-8_3

Schaechter, J. D., Kraft, E., Hilliard, T. S., Dijkhuizen, R. M., Benner, T., Finklestein, S. P., & Cramer, S. C. (2002). Motor Recovery and Cortical Reorganization after Constraint-Induced Movement Therapy in Stroke Patients: A Preliminary Study. *Neurorehabilitation and Neural Repair*. https://doi.org/10.1177/154596830201600403

Schaechter, J. D., Perdue, K. L., & Wang, R. (2008). Structural damage to the corticospinal tract correlates with bilateral sensorimotor cortex reorganization in stroke patients. *NeuroImage*. https://doi.org/10.1016/j.neuroimage.2007.09.071

Scott, A. B. (1981). Botulinum toxin injection of eye muscles to correct strabismus. *Transactions of the American Ophthalmological Society*.

Senkárová, Z., Hlustík, P., Otruba, P., Herzig, R., & Kanovský, P. (2010). Modulation of cortical activity in patients suffering from upper arm spasticity following stroke and treated with botulinum toxin A: an fMRI study. *Journal of Neuroimaging : Official Journal of the American Society of Neuroimaging*. https://doi.org/10.1111/j.1552-6569.2009.00375.x

Shaw, L. C., Price, C. I. M., Van Wijck, F. M. J., Shackley, P., Steen, N., Barnes, M. P., & Rodgers, H. (2011). Botulinum toxin for the upper limb after stroke (BoTULS) trial: Effect on impairment, activity limitation, and pain. *Stroke*. https://doi.org/10.1161/STROKEAHA.110.582197

Sheean, G. (2006). Botulinum toxin treatment of adult spasticity - A benefit-risk assessment. *DRUG SAFETY*. https://doi.org/10.2165/00002018-200629010-00003

Sheean, G., Lannin, N. A., Turner-Stokes, L., Rawicki, B., & Snow, B. J. (2010). Botulinum toxin assessment, intervention and after-care for upper limb hypertonicity in adults: International consensus statement. *European Journal of Neurology*. https://doi.org/10.1111/j.1468-1331.2010.03129.x

Simpson, D. M., Alexander, D. N., O'Brien, C. F., Tagliati, M., Aswad, A. S., Leon, J. M., & Monaghan, E. P. (1996). Botulinum toxin type A in the treatment of upper extremity spasticity: a randomized, double-blind, placebo-controlled trial. *Neurology*. https://doi.org/10.1212/WNL.46.5.1306

Slawek, J., Bogucki, A., & Reclawowicz, D. (2005). Botulinum toxin type A for upper limb spasticity following stroke: An open-label study with individualised, flexible injection regimens. *Neurological Sciences*. https://doi.org/10.1007/s10072-005-0379-8

Smith, S. J., Ellis, E., White, S., & Moore, A. P. (2000). A double-blind placebo-controlled study of botulinum toxin in upper limb spasticity after stroke or head injury. *Clinical Rehabilitation*. https://doi.org/10.1191/026921500666642221

Söllner, T., Whiteheart, S. W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P., & Rothman, J. E. (1993). SNAP receptors implicated in vesicle targeting and fusion. *Nature*. https://doi.org/10.1038/362318a0

Stinear, C. M., Barber, P. A., Smale, P. R., Coxon, J. P., Fleming, M. K., & Byblow, W. D. (2007). Functional potential in chronic stroke patients depends on corticospinal tract integrity. *Brain*. https://doi.org/10.1093/brain/awl333

Thompson, A. J., Jarrett, L., Lockley, L., Marsden, J., & Stevenson, V. L. (2005). Clinical management of spasticity. *Journal of Neurology, Neurosurgery and Psychiatry*. https://doi.org/10.1136/jnnp.2004.035972

Timmann, D., Brandauer, B., Hermsdörfer, J., Ilg, W., Konczak, J., Gerwig, M., & Schoch, B. (2008). Lesion-symptom mapping of the human cerebellum. *Cerebellum (London, England)*. https://doi.org/10.1007/s12311-008-0066-4

Tomášová, Z., Hluštík, P., Král, M., Otruba, P., Herzig, R., Krobot, A., & Kaňovský, P. (2013). Cortical activation changes in patients suffering from post-stroke arm spasticity and treated with botulinum toxin A. *Journal of Neuroimaging*. https://doi.org/10.1111/j.1552-6569.2011.00682.x

Trompetto, C., Marinelli, L., Mori, L., Pelosin, E., Currà, A., Molfetta, L., & Abbruzzese, G. (2014). Pathophysiology of spasticity: Implications for neurorehabilitation. *BioMed Research International*. https://doi.org/10.1155/2014/354906

Turner-Stokes, L., Baguley, I. J., De Graaff, S., Katrak, P., Davies, L., McCrory, P., & Hughes, A. (2010). Goal attainment scaling in the evaluation of treatment of upper limb spasticity with botulinum toxin: A secondary analysis from a double-blind placebo-controlled randomized clinical trial. *Journal of Rehabilitation Medicine*. https://doi.org/10.2340/16501977-0474

Urban, P. P., Wolf, T., Uebele, M., Marx, J. J., Vogt, T., Stoeter, P., & Wissel, J. (2010). Occurence and clinical predictors of spasticity after ischemic stroke. *Stroke*. https://doi.org/10.1161/STROKEAHA.110.581991

Verstynen, T. (2004). Ipsilateral Motor Cortex Activity During Unimanual Hand Movements Relates to Task Complexity. *Journal of Neurophysiology*. https://doi.org/10.1152/jn.00720.2004

Veverka, T., Hluštík, P., Hok, P., Otruba, P., Tüdös, Z., Zapletalová, J., & Kaňovský, P. (2014). Cortical activity modulation by botulinum toxin type A in patients with post-stroke arm spasticity: Real and imagined hand movement. *Journal of the Neurological Sciences*. https://doi.org/10.1016/j.jns.2014.09.009

Veverka, T., Hluštík, P., Hok, P., Otruba, P., Zapletalová, J., Tüdös, Z., & Kaňovský, P. (2016). Sensorimotor modulation by botulinum toxin A in post-stroke arm spasticity: Passive hand movement. *Journal of the Neurological Sciences*. https://doi.org/10.1016/j.jns.2015.12.049

Veverka, T., Hluštík, P., Tomášová, Z., Hok, P., Otruba, P., Král, M., & Kaňovský, P. (2012). BoNT-A related changes of cortical activity in patients suffering from severe hand paralysis with arm spasticity following ischemic stroke. *Journal of the Neurological Sciences*. https://doi.org/10.1016/j.jns.2012.05.008

Vitek, J. L., Ashe, J., DeLong, M. R., & Alexander, G. E. (1994). Physiologic properties and somatotopic organization of the primate motor thalamus. *Journal of Neurophysiology*. https://doi.org/10.1152/jn.1994.71.4.1498

Waldvogel, D., van Gelderen, P., Ishii, K., & Hallett, M. (1999). The effect of movement amplitude on activation in functional magnetic resonance imaging studies. *J Cereb Blood Flow Metab*. https://doi.org/10.1097/00004647-199911000-00004

Ward, N. S., Brown, M. M., Thompson, A. J., & Frackowiak, R. S. J. (2003). Neural correlates of outcome after stroke: A cross-sectional fMRI study. *Brain*. https://doi.org/10.1093/brain/awg145

Weidner, N., Ner, a, Salimi, N., & Tuszynski, M. H. (2001). Spontaneous corticospinal axonal plasticity and functional recovery after adult central nervous system injury. *Proceedings of the National Academy of Sciences of the United States of America*. https://doi.org/10.1073/pnas.051626798

Welmer, A. K., Widén Holmqvist, L., & Sommerfeld, D. K. (2010). Location and severity of spasticity in the first 1-2 weeks and at 3 and 18 months after stroke. *European Journal of Neurology*. https://doi.org/10.1111/j.1468-1331.2009.02915.x

Wolf, S. L., Catlin, P. A., Ellis, M., Archer, A. L., Morgan, B., & Piacentino, A. (2001). Assessing Wolf Motor Function Test as outcome measure for research in patients after stroke. *Stroke*. https://doi.org/10.1161/01.STR.32.7.1635

Yoo, S.S., Wei, X., Dickey, C.C., Guttmann, C.R.G., & Panych, L.P. (2005). Long-Term Reproducibility Analysis of fMRI using Hand Motor Task. *International Journal of Neuroscience.* https:/doi.org/10.1080/00207450490512650

APPENDICES

APPENDIX A: Statistical Analysis of BOLD Signal

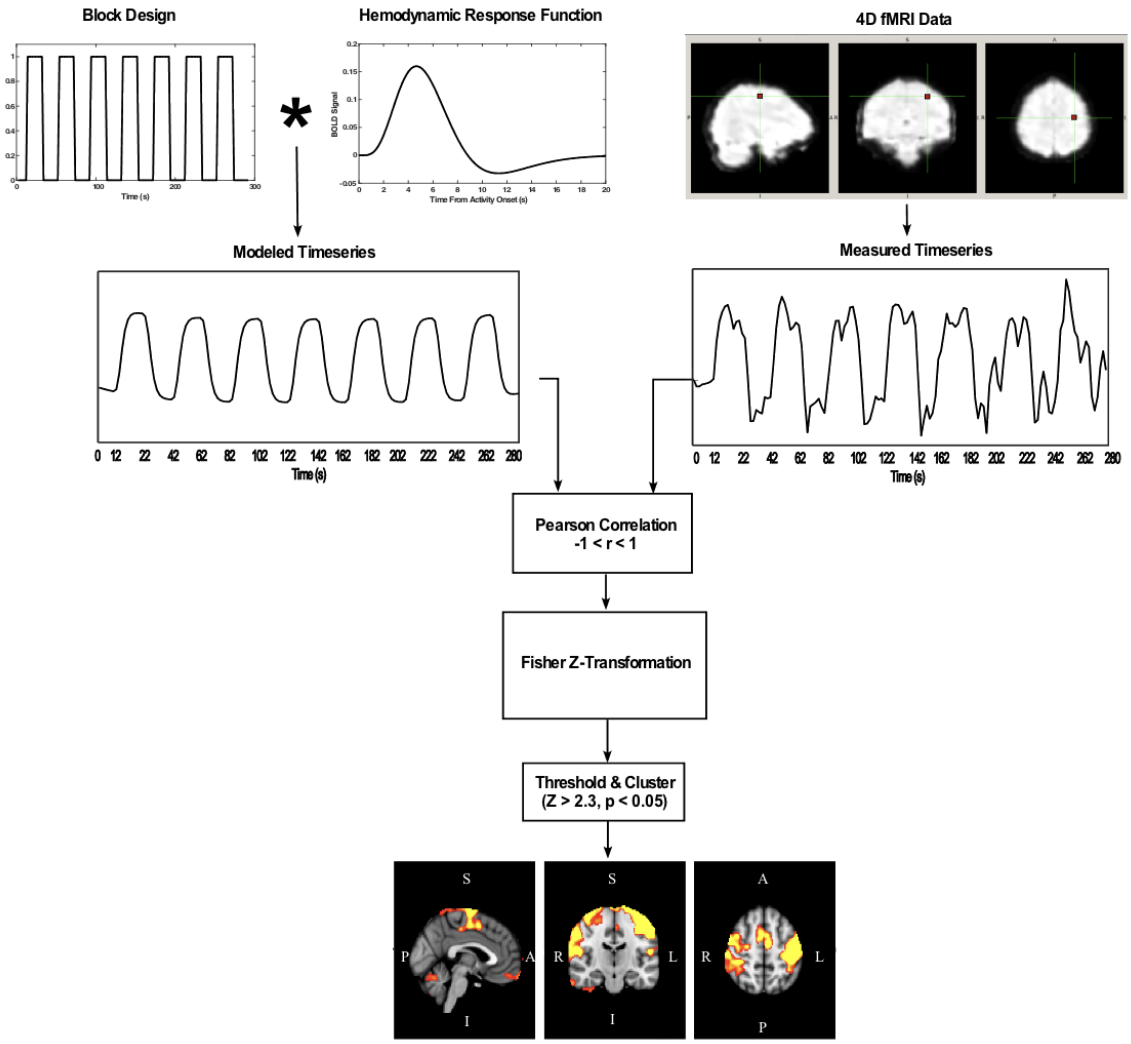


Figure A1: Diagram of Statistical Analysis Pipeline.

APPENDIX B: Fugl-Meyer Scoring Breakdown

Items are scored on a 3-point ordinal scale:
0 = cannot perform
1 = performs partially
2 = performs fully

A. UPPER EXTREMITY, sitting position					
I. Reflex activity			**none**	**can be elicited**	
Flexors: biceps and finger flexors			0	2	
Extensors: triceps			0	2	
		Subtotal I (max 4)			
II. Volitional movement within synergies, without gravitational help			**none**	**partial**	**full**
Flexor synergy: Hand from contralateral knee to ipsilateral ear. From extensor synergy (shoulder adduction/ internal rotation, elbow extension, forearm pronation) to flexor synergy (shoulder abduction/ external rotation, elbow flexion, forearm supination). **Extensor synergy:** Hand from ipsilateral ear to the contralateral knee	Shoulder	retraction	0	1	2
		elevation	0	1	2
		abduction (90°)	0	1	2
		external rotation	0	1	2
	Elbow	flexion	0	1	2
	Forearm	supination	0	1	2
	Shoulder	adduction/internal rotation	0	1	2
	Elbow	extension	0	1	2
	Forearm	pronation	0	1	2
		Subtotal II (max 18)			
III. Volitional movement mixing synergies, without compensation			**none**	**partial**	**full**
Hand to lumbar spine	cannot be performed, hand in front of SIAS		0		
	hand behind of SIAS (without compensation)			1	
	hand to lumbar spine (without compensation)				2
Shoulder flexion 0°-90° elbow at 0° pronation-supination 0°	immediate abduction or elbow flexion		0		
	abduction or elbow flexion during movement			1	
	complete flexion 90°, maintains 0° in elbow				2
Pronation-supination elbow at 90° shoulder at 0°	no pronation/supination, starting position impossible		0		
	limited pronation/supination, maintains position			1	
	complete pronation/supination, maintains position				2
		Subtotal III (max 6)			
IV. Volitional movement with little or no synergy			**none**	**partial**	**full**
Shoulder abduction 0 - 90° elbow at 0° forearm pronated	immediate supination or elbow flexion		0		
	supination or elbow flexion during movement			1	
	abduction 90°, maintains extension and pronation				2
Shoulder flexion 90°- 180° elbow at 0° pronation-supination 0°	immediate abduction or elbow flexion		0		
	abduction or elbow flexion during movement			1	
	complete flexion, maintains 0° in elbow				2
Pronation/supination elbow at 0° shoulder at 30°-90° flexion	no pronation/supination, starting position impossible		0		
	limited pronation/supination, maintains extension			1	
	full pronation/supination, maintains elbow extension				2
		Subtotal IV (max 6)			
V. Normal reflex activity evaluated only if full score of 6 points achieved on part IV					
biceps, triceps, finger flexors	0 points on part IV or 2 of 3 reflexes markedly hyperactive		0		
	1 reflex markedly hyperactive or at least 2 reflexes lively			1	
	maximum of 1 reflex lively, none hyperactive				2
		Subtotal V (max 2)			
		Total A (max 36)			

B. WRIST support may be provided at the elbow to take or hold the position, no support at wrist, check the passive range of motion prior testing		**none**	**partial**	**full**
Stability at 15° dorsiflexion elbow at 90°, forearm pronated shoulder at 0°	less than 15° active dorsiflexion dorsiflexion 15°, no resistance is taken maintains position against resistance	0	1	2
Repeated dorsifexion / volar flexion elbow at 90°, forearm pronated shoulder at 0°, slight finger flexion	cannot perform volitionally limited active range of motion full active range of motion, smoothly	0	1	2
Stability at 15° dorsiflexion elbow at 0°, forearm pronated slight shoulder flexion/abduction	less than 15° active dorsiflexion dorsiflexion 15°, no resistance is taken maintains position against resistance	0	1	2
Repeated dorsifexion / volar flexion elbow at 0°, forearm pronated slight shoulder flexion/abduction	cannot perform volitionally limited active range of motion full active range of motion, smoothly	0	1	2
Circumduction	cannot perform volitionally jerky movement or incomplete complete and smooth circumduction	0	1	2
	Total B (max 10)			

C. HAND support may be provided at the elbow to keep 90° flexion, no support at the wrist, compare with unaffected hand, the objects are interposed, active grasp		**none**	**partial**	**full**
Mass flexion from full active or passive extension		0	1	2
Mass extension from full active or passive flexion		0	1	2
GRASP				
A – flexion in PIP and DIP (digits II-V) **extension in MCP II-V**	cannot be performed can hold position but weak maintains position against resistance	0	1	2
B – thumb adduction 1-st CMC, MCP, IP at 0°, scrap of paper between thumb and 2-nd MCP joint	cannot be performed can hold paper but not against tug can hold paper against a tug	0	1	2
C - opposition pulpa of the thumb against the pulpa of 2-nd finger, pencil, tug upward	cannot be performed can hold pencil but not against tug can hold pencil against a tug	0	1	2
D – cylinder grip cylinder shaped object (small can) tug upward, opposition in digits I and II	cannot be performed can hold cylinder but not against tug can hold cylinder against a tug	0	1	2
E – spherical grip fingers in abduction/flexion, thumb opposed, tennis ball	cannot be performed can hold ball but not against tug can hold ball against a tug	0	1	2
	Total C (max 14)			

D. COORDINATION/SPEED after one trial with both arms, blind-folded, tip of the index finger from knee to nose, 5 times as fast as possible		**marked**	**slight**	**none**
Tremor		0	1	2
Dysmetria	pronounced or unsystematic slight and systematic no dysmetria	0	1	2
		> 5s	**2 - 5s**	**< 1s**
Time	more than 5 seconds slower than unaffected side 2-5 seconds slower than unaffected side maximum difference of1 second between sides	0	1	2
	Total D (max 6)			

TOTAL A-D (max 66)	

APPENDIX C: BoNT Doses and Locations

Patient No.	***Muscle***	***Dilution***	***Dosage***
BTX 1	Pec Major (2 sites)	2:1	50 units
	Pec Minor (1 site)	2:1	50 units
	Latisimus Dorsi (3 sites)	2:1	50 units
	Triceps (3 sites)	2:1	75 units
	Brachialis (3 sites)	2:1	75 units
	Brachioradialis (1 site)	2:1	25 units
	ECR (1 site)	2:1	25 units
	FCR (1 site)	2:1	20 units
	FDP (1 site)	2:1	30 units
	FDL (1 site)	2:1	50 units
BTX2	R SC (1 site)	4:1	25 units
	Pec Major (3 sites)	4:1	75 units
	Brachialis (3 sites)	4:1	75 units
	Brachioradialis (2 sites)	4:1	25 units
	Pron Teres (2 sites)	4:1	25 units
	FCR (1 site)	4:1	25 units
	FCU (1 site)	4:1	25 units
	FDS (1 site)	4:1	25 units
	FDP (1 site)	4:1	25 units
	FPL (1 site)	4:1	25 units
BTX3	Biceps Brachii (2 sites)	4:1	50 units
	Brachioradialis (1 site)	4:1	25 units
	Pron Teres (1 site)	4:1	25 units
	FCR (2 sites)	2:1	50 units
	FCU (2 sites)	2:1	50 units
	FDS (2 sites)	4:1	50 units
	FDP (2 sites)	4:1	50 units
BTX4	Pec Major (4 sites)	2cc:500u	200 units
	Lat Dorsi (2 sites)	2cc:500u	100 units
	Pron Teres (2 sites)	2cc:500u	100 units
	FDS (1 site)	2cc:500u	75 units
	FDP (1 site)	2cc:500u	75 units

BTX5	Pec Major	?	75 units
	Biceps Brachii	?	25 units
	FDS, Digit 2	?	25 units
	FDS, Digit 3	?	25 units
	FDS, Digit 4	?	25 units
	FDS, Digit 5	?	25 units
	Biceps Femoris	?	100 units
	Semimem	?	50 units
	Semitend	?	50 units
BTX6	Lev Scap (1 site)	4:1	25 units
	Lat Dorsi (8 sites)	8:1	75 units
	Lateral Scap CT (10 sites)	8:1	25 units
	Pec Minor (2 sites)	4:1	25 units
	Pron Teres (1 site)	4:1	10 units
	ECR (1 site)	4:1	15 units
	FPB (1 site)	4:1	10 units
BTX7	Vastus Lat (2 sites)	2:1	50 units
	Lat Hamstring (2 sites)	2:1	50 units
	Med Hamstring (3 sites)	2:1	75 units
	Med Gastroc (2 sites)	2:1	50 units
	Lat Gastroc (2 sites)	2:1	50 units
	Lat Dorsi (2 sites)	2:1	50 units
	Brachialis (2 sites)	2:1	50 units
	Brachioradialis (1 site)	2:1	25 units
BTX8	Lat Dorsi (2 sites)	4:1	50 units
	Brachialis (2 sites)	4:1	30 units
	Brachioradialis (1 site)	4:1	20 units
	FCR (1 site)	4:1	25 units
	FCU (1 site)	4:1	25 units
	FDS (2 sites)	4:1	50 units
	FDP (2 sites)	4:1	50 units
	FPL (1 site)	4:1	25 units
	FPB (1 site)	4:1	25 units
	Lumbricals (3 sites)	4:1	25 units
	Vastus Lat (4 sites)	4:1	100 units

BTX9	Pec Major (2 sites)	2:1	25 units
	Brachialis (3 sites)	2:1	75 units
	Brachioradialis (1 site)	2:1	25 units
	Pron Teres (1 site)	2:1	25 units
	ECR (1 site)	2:1	25 units
	ECU (1 site)	2:1	25 units
	FDS (1 site)	2:1	25 units
	FDP (1 site)	2:1	25 units
	FPB (1 site)	2:1	25 units
	Lumbricals (4 sites)	2:1	50 units

APPENDIX D: Individual Participant Activity Maps

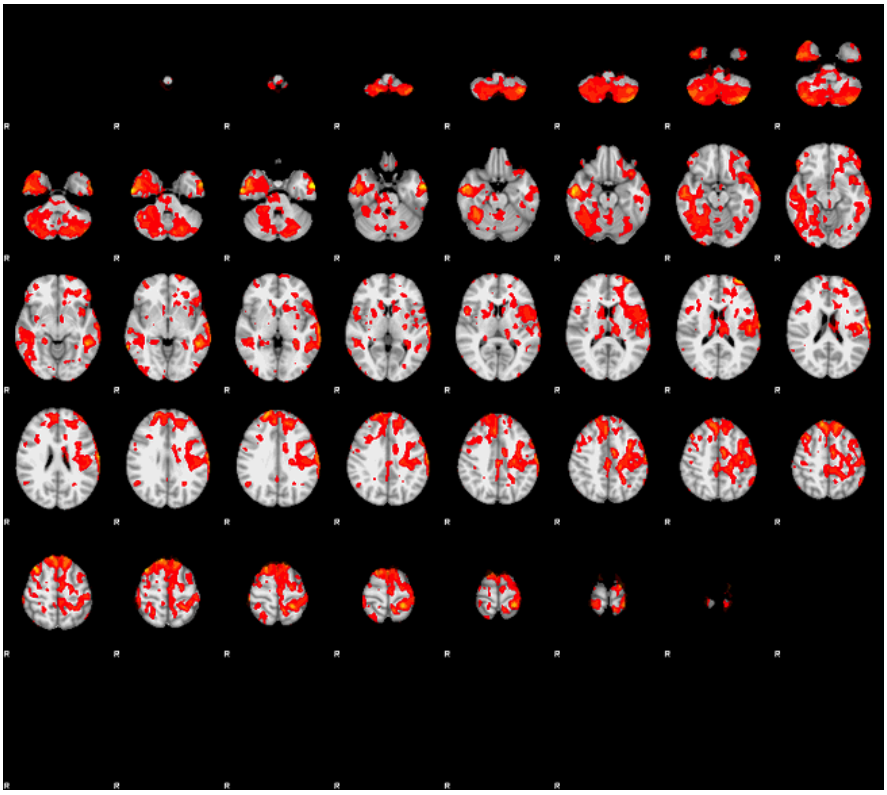


Figure D1: Individual activity map for participant BTX 1 at W0. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

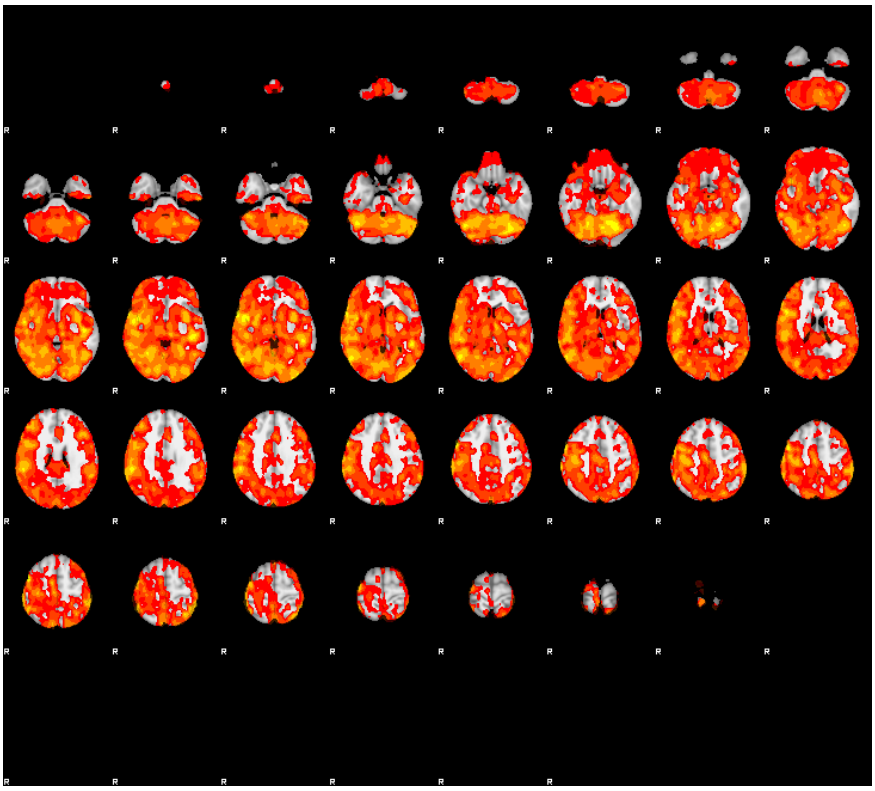


Figure D2: Individual activity map for participant BTX 1 at W6. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

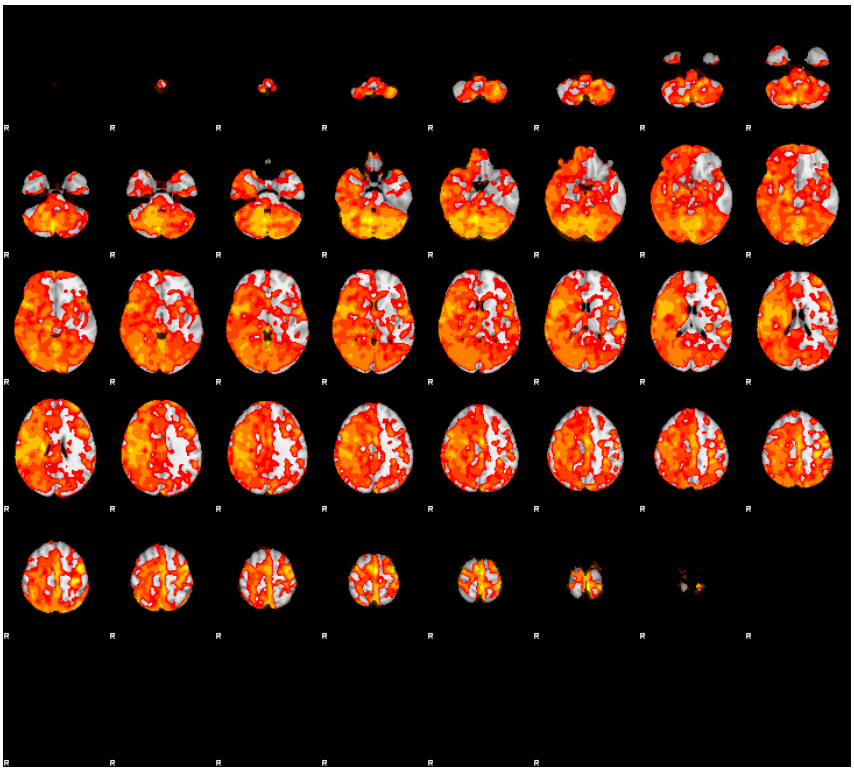


Figure D3: Individual activity map for participant BTX 2 at W0. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

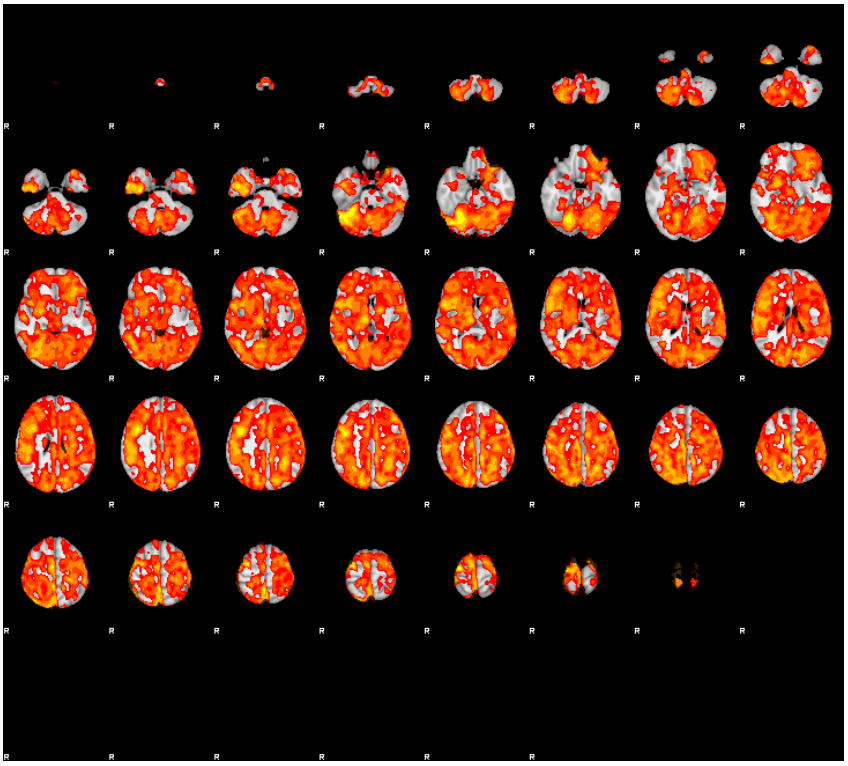


Figure D4: Individual activity map for participant BTX 2 at W6. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

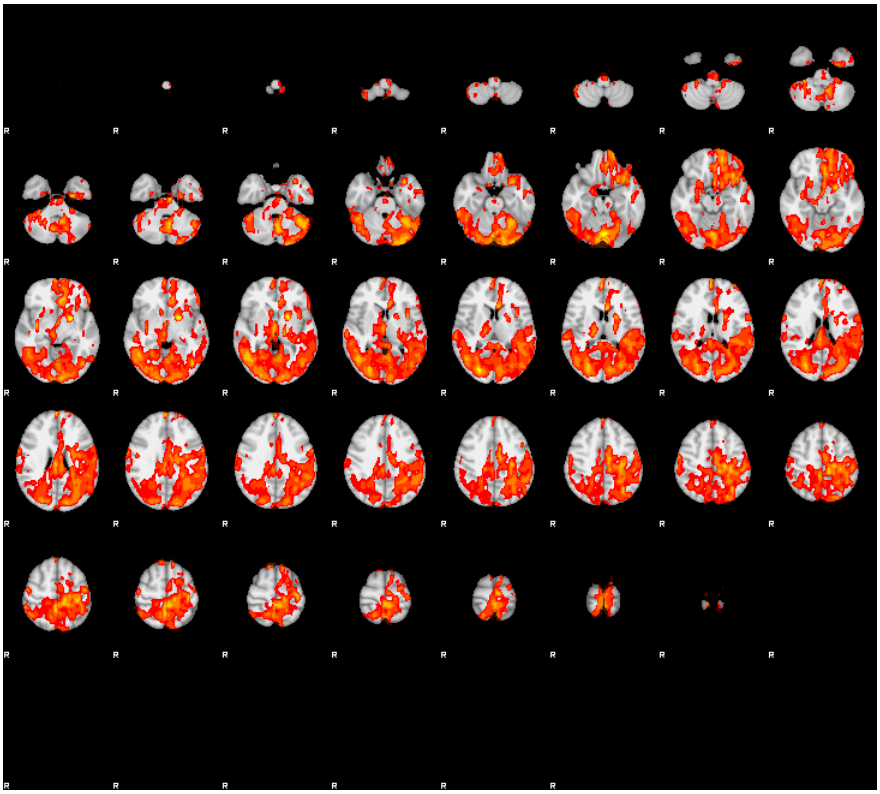


Figure D5: Individual activity map for participant BTX 3 at W0. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

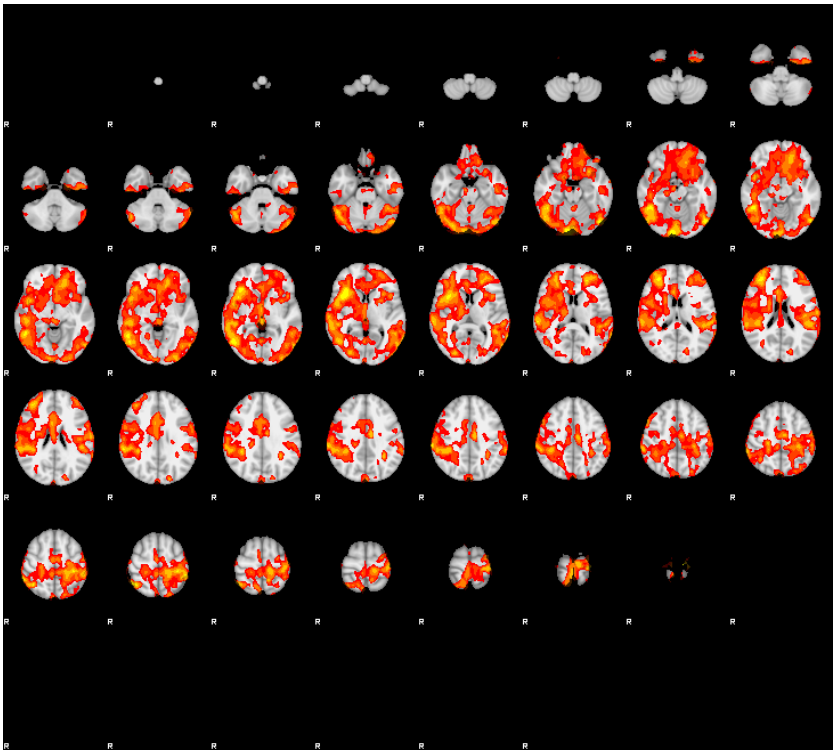


Figure D6: Individual activity map for participant BTX 3 at W6. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

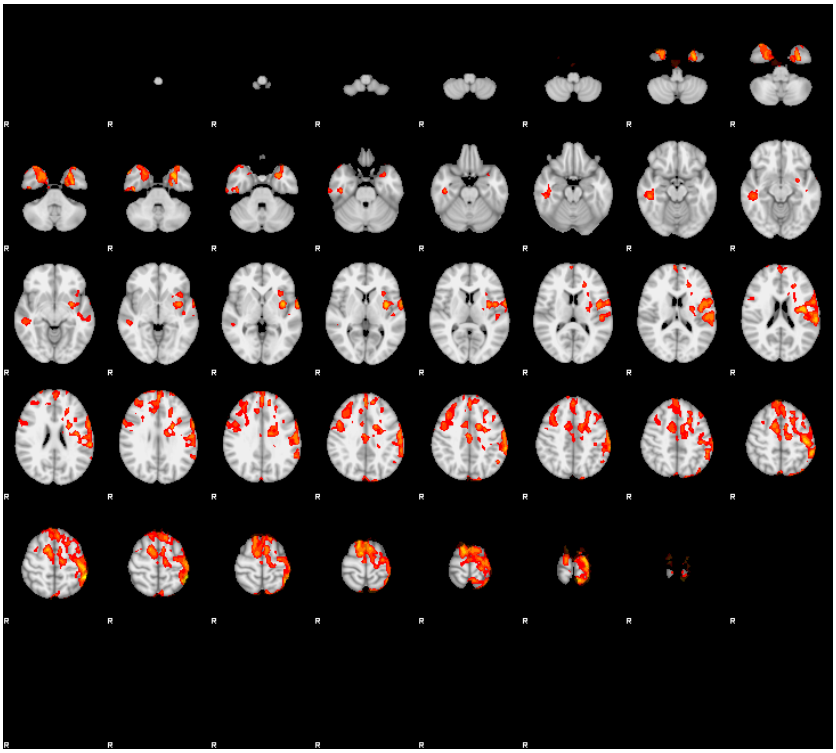


Figure D7: Individual activity map for participant BTX 4 at W0. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

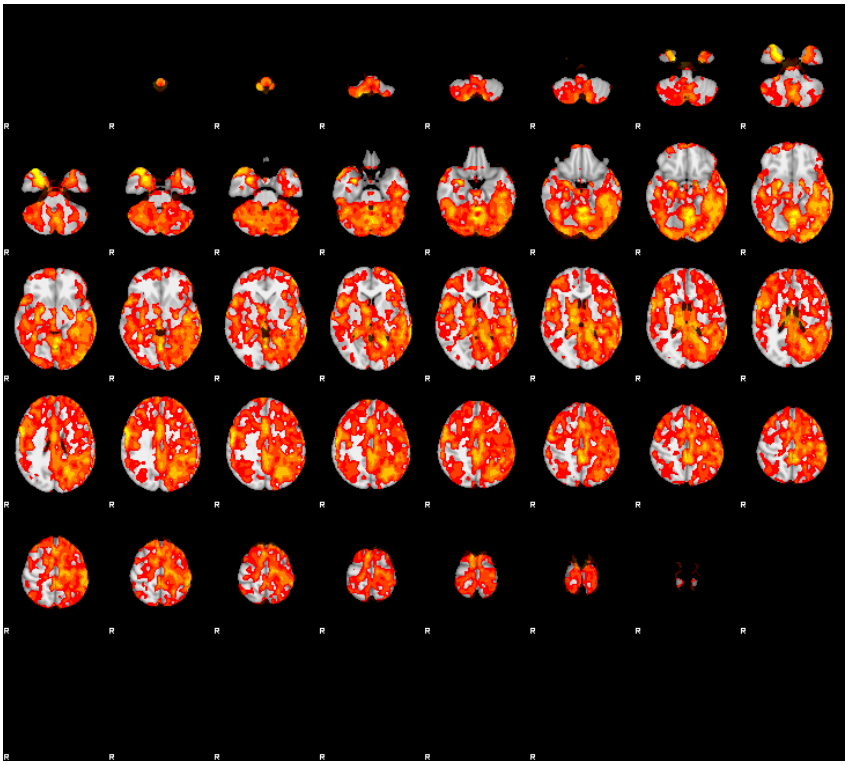


Figure D8: Individual activity map for participant BTX 4 at W6. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

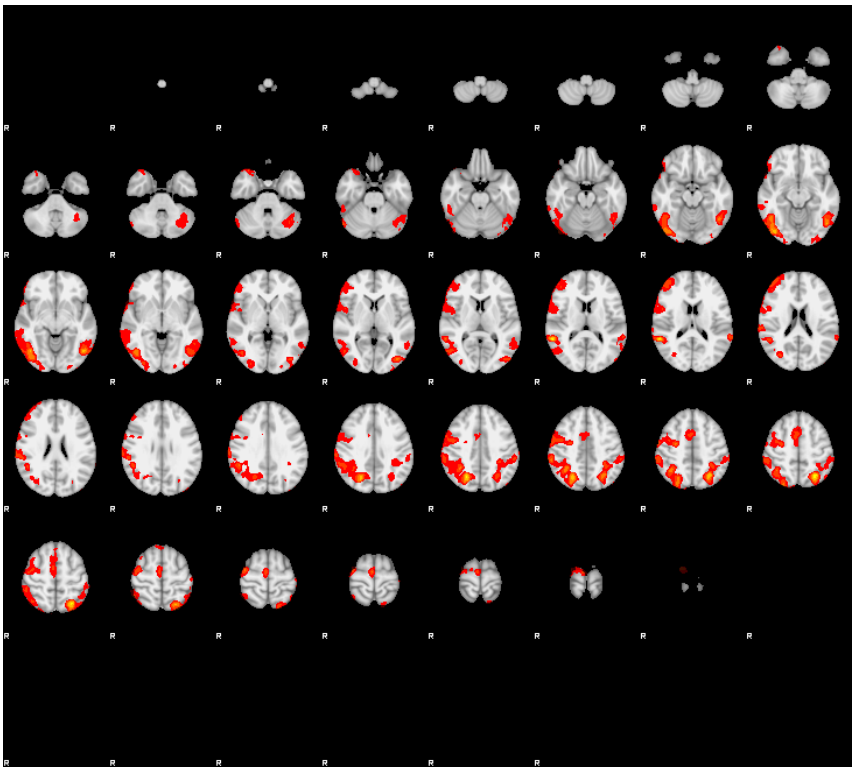


Figure D9: Individual activity map for participant BTX 5 at W0. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

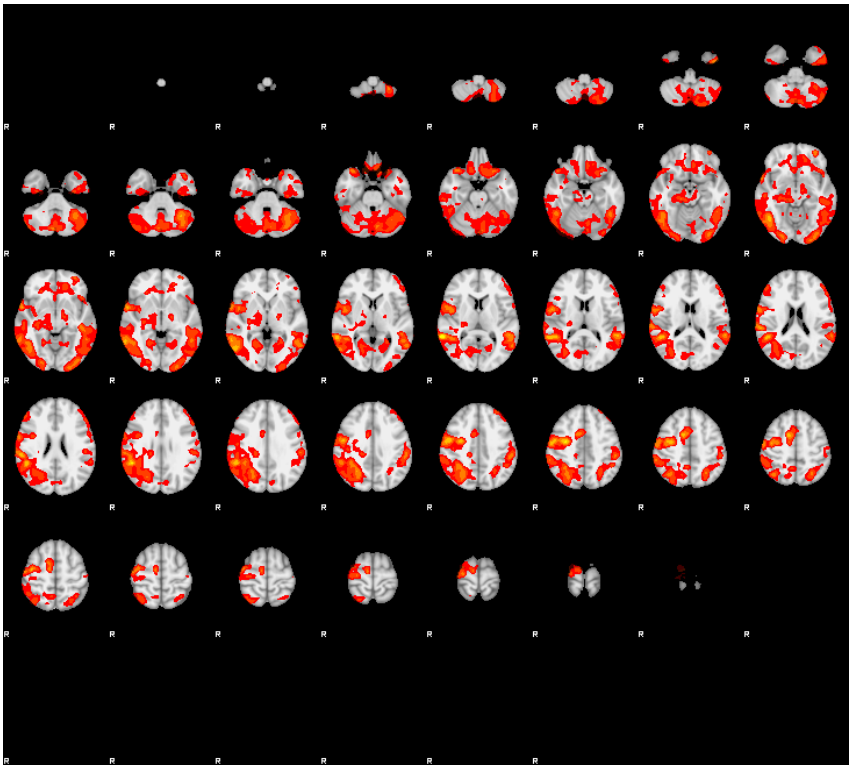


Figure D10: Individual activity map for participant BTX 5 at W6. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

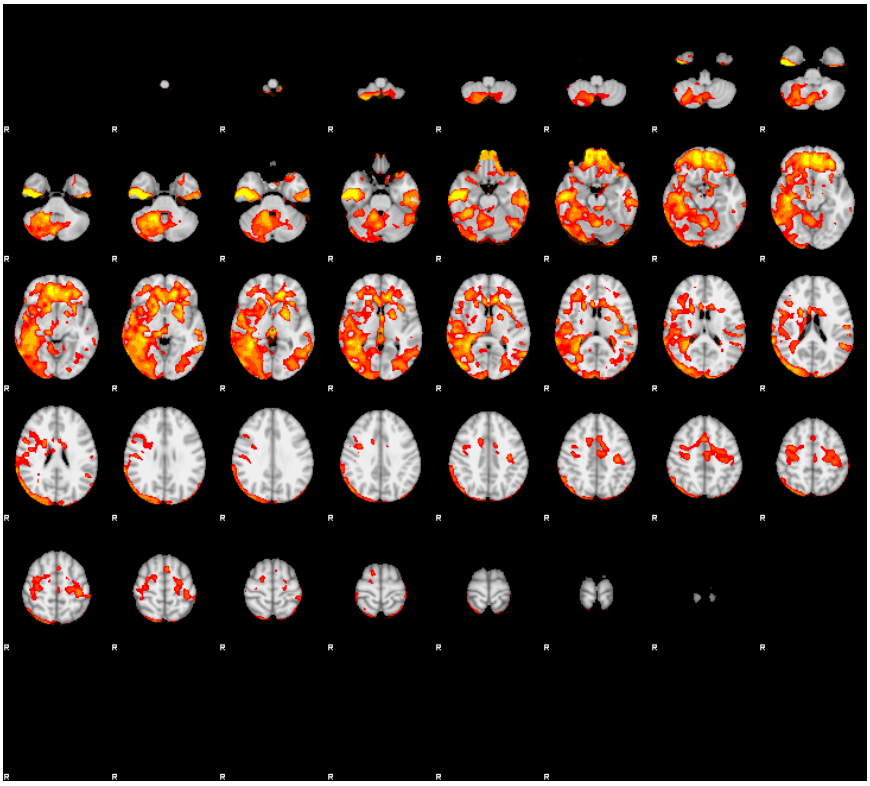


Figure D11: Individual activity map for participant BTX 6 at W0. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

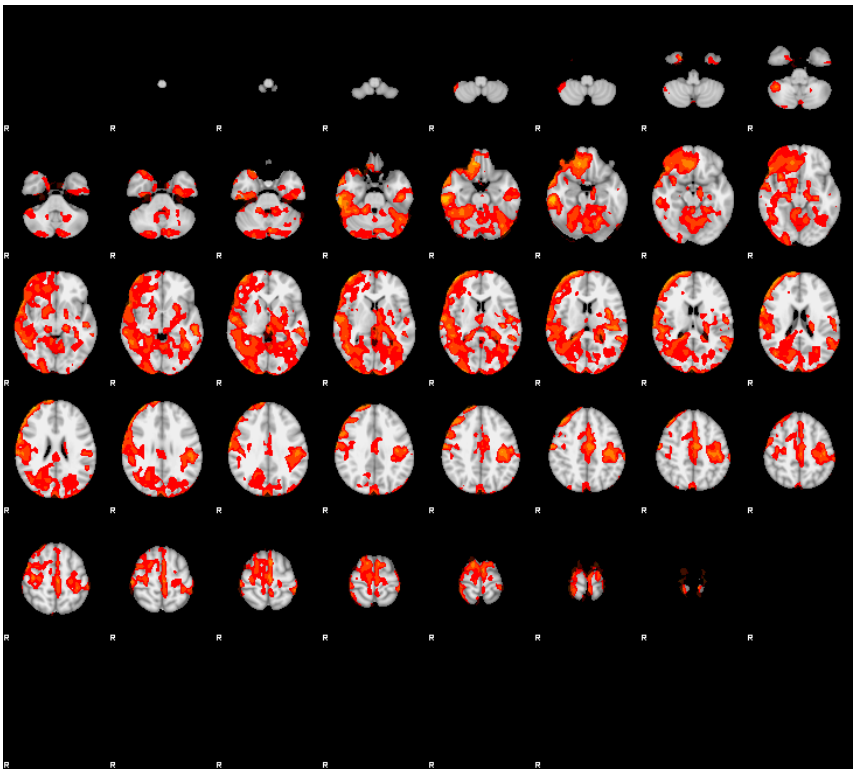


Figure D12: Individual activity map for participant BTX 6 at W6. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

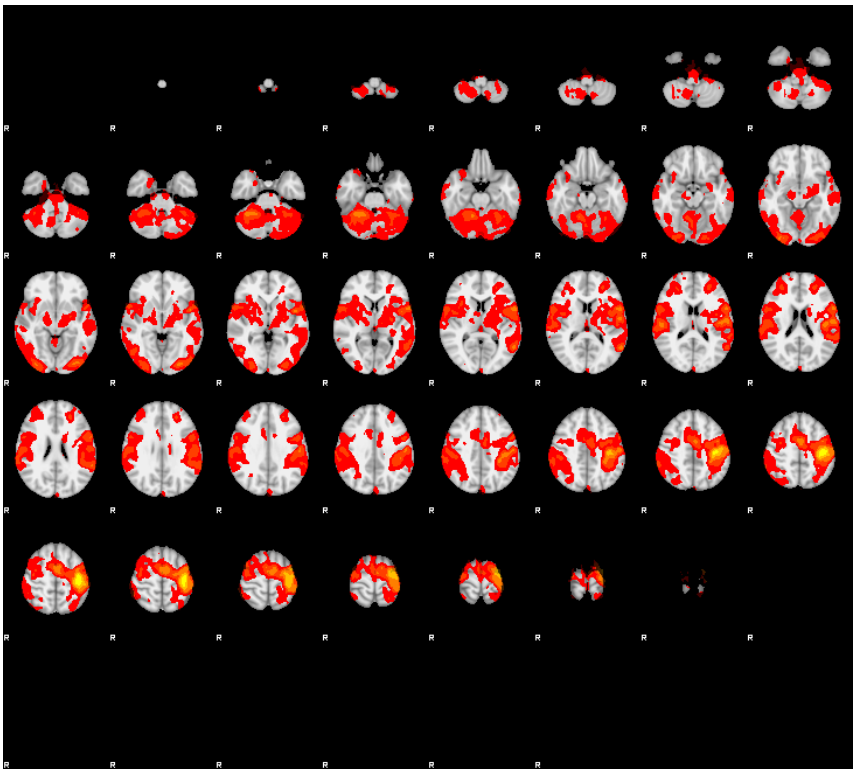


Figure D13: Individual activity map for participant BTX 7 at W0. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

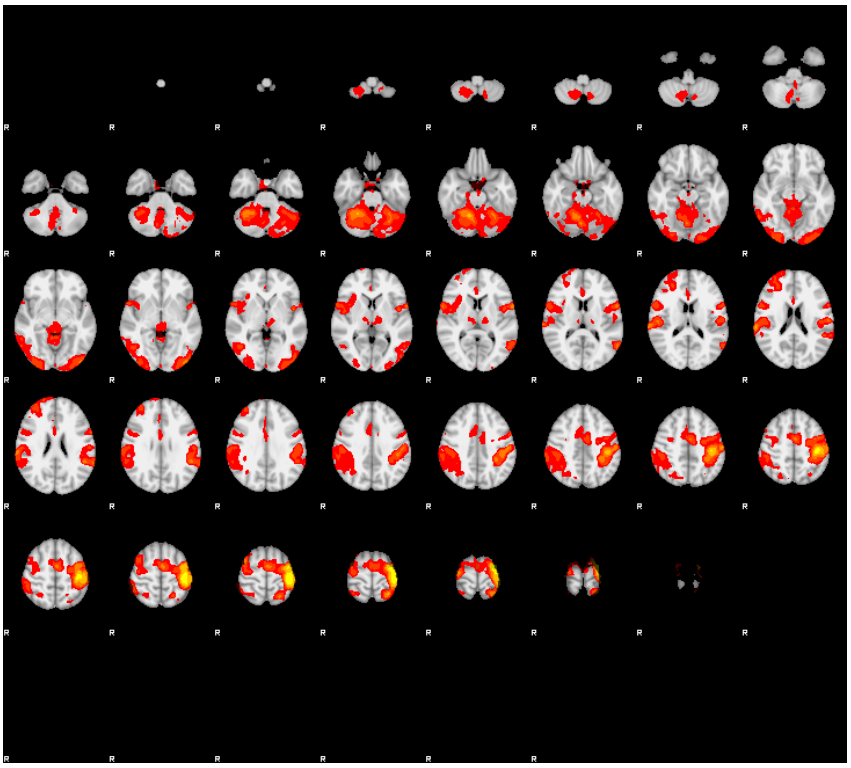


Figure D14: Individual activity map for participant BTX 7 at W6. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

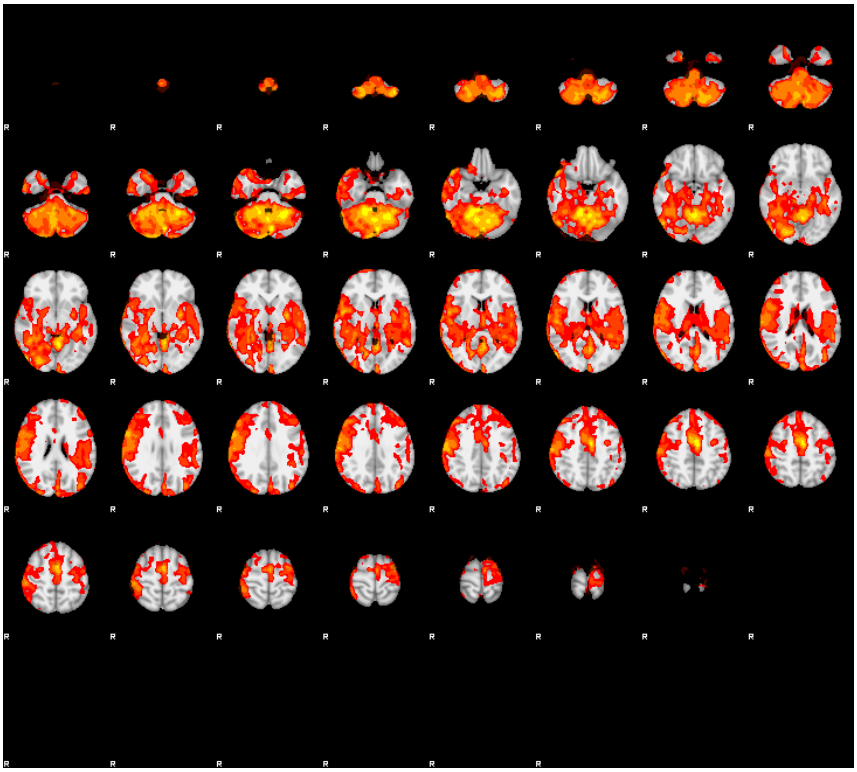


Figure D15: Individual activity map for participant BTX 8 at W0. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

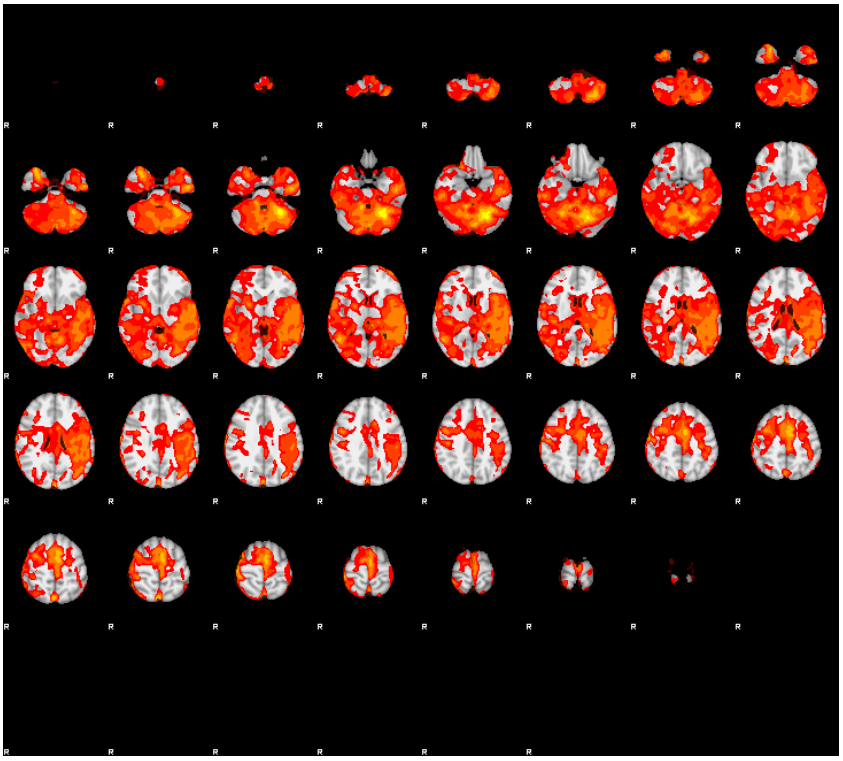


Figure D16: Individual activity map for participant BTX 8 at W6. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

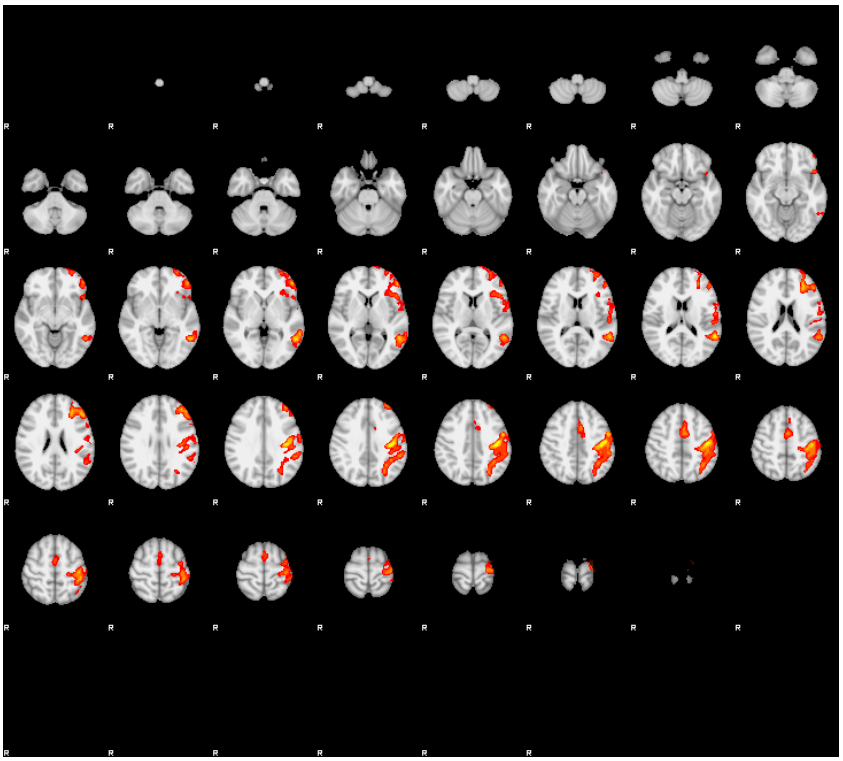


Figure D17: Individual activity map for participant BTX 9 at W0. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

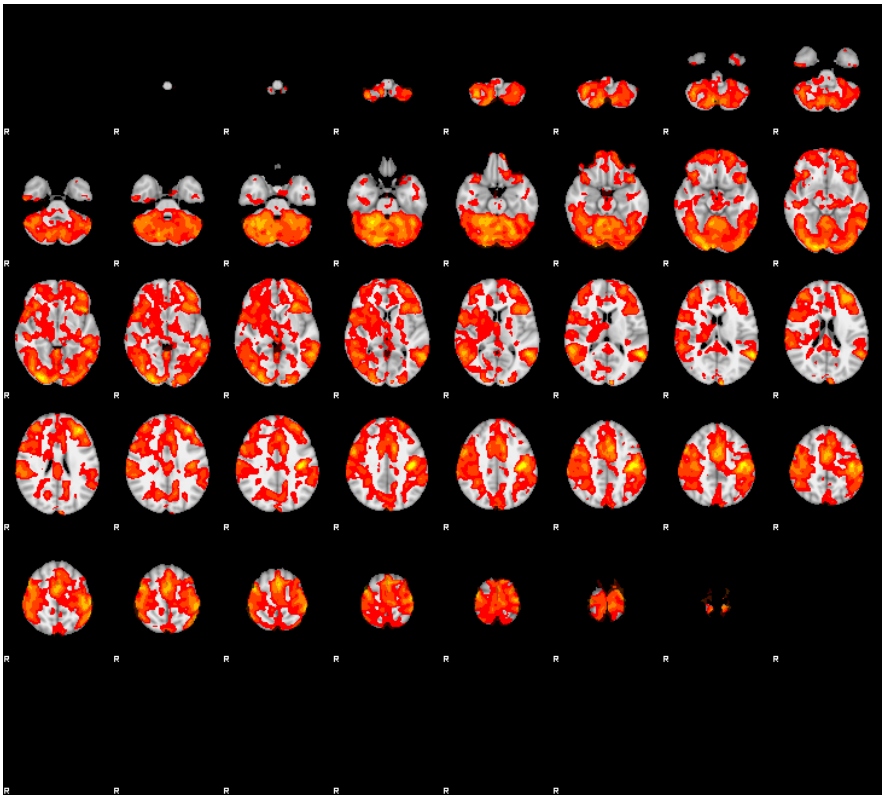


Figure D18: Individual activity map for participant BTX 9 at W6. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

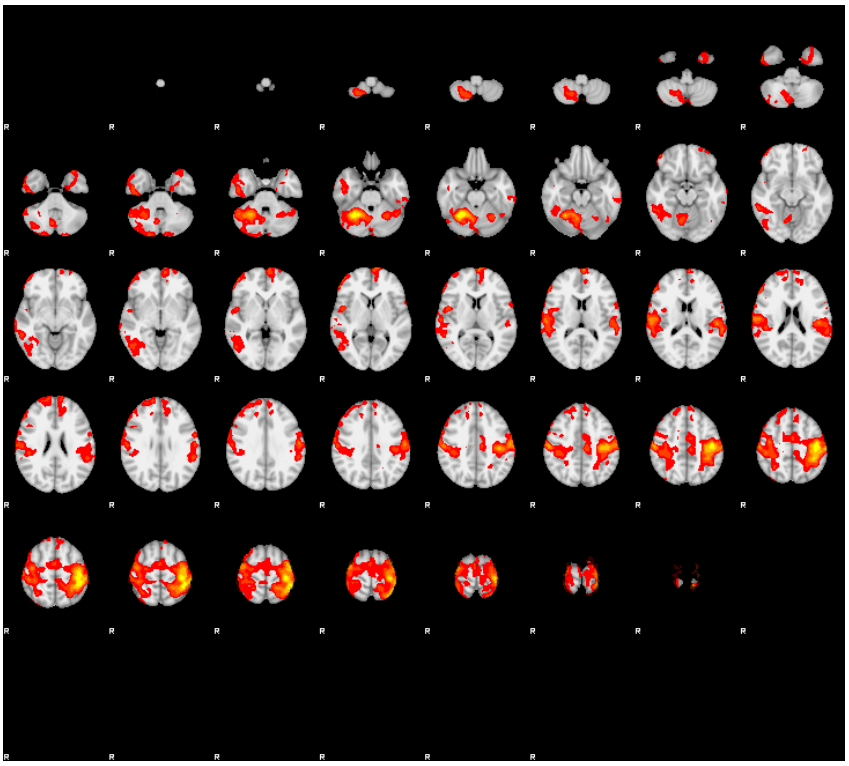


Figure D19: Individual activity map for participant C1 at W6. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

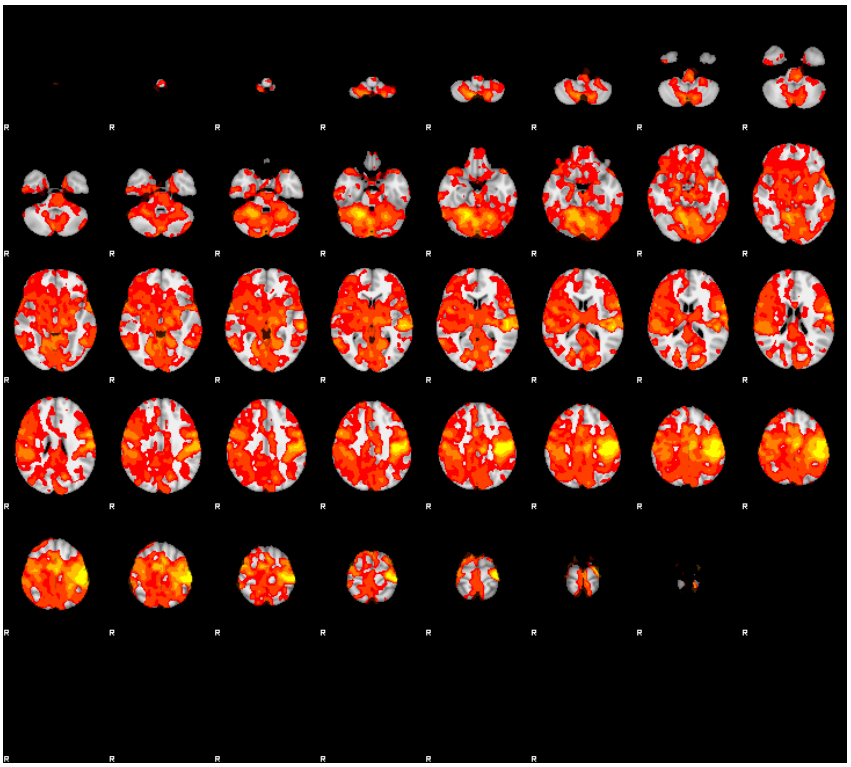


Figure D20: Individual activity map for participant C2 at W0. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

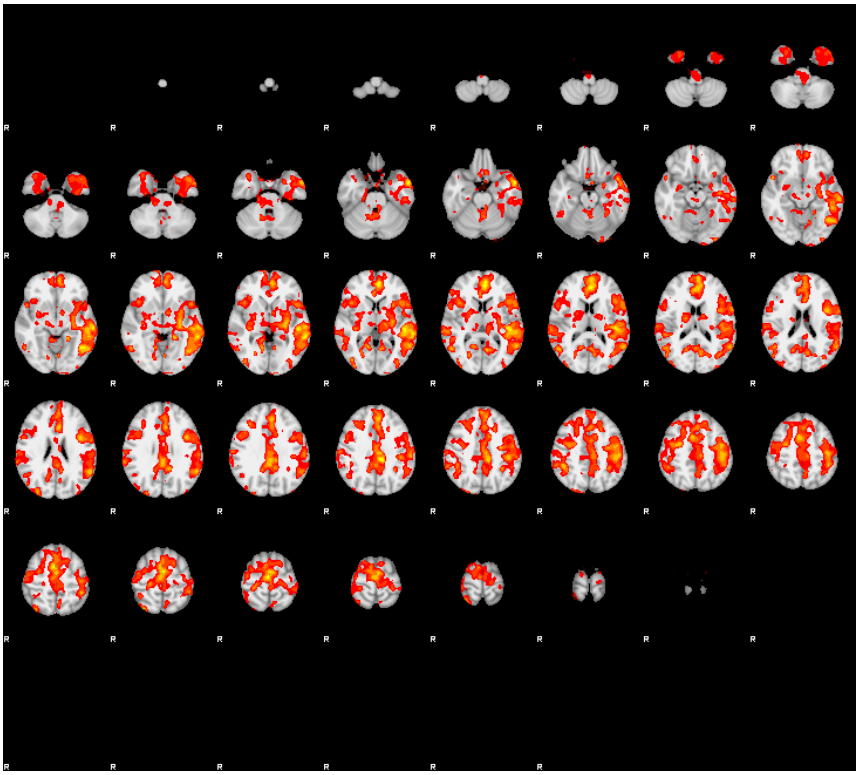


Figure D21: Individual activity map for participant C2 at W6. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

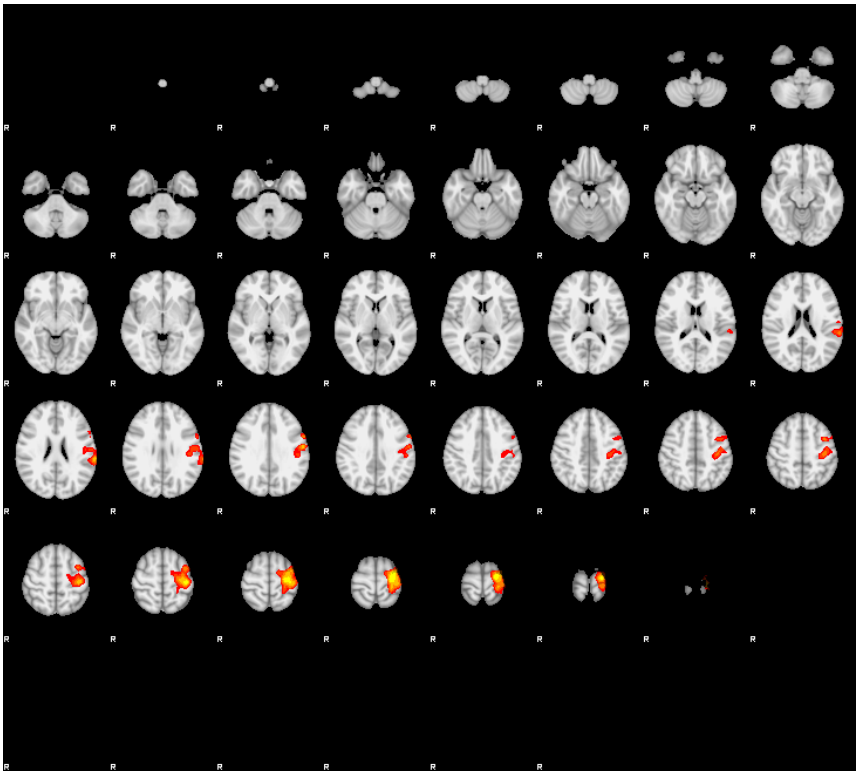


Figure D22: Individual activity map for participant C3 at W0. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

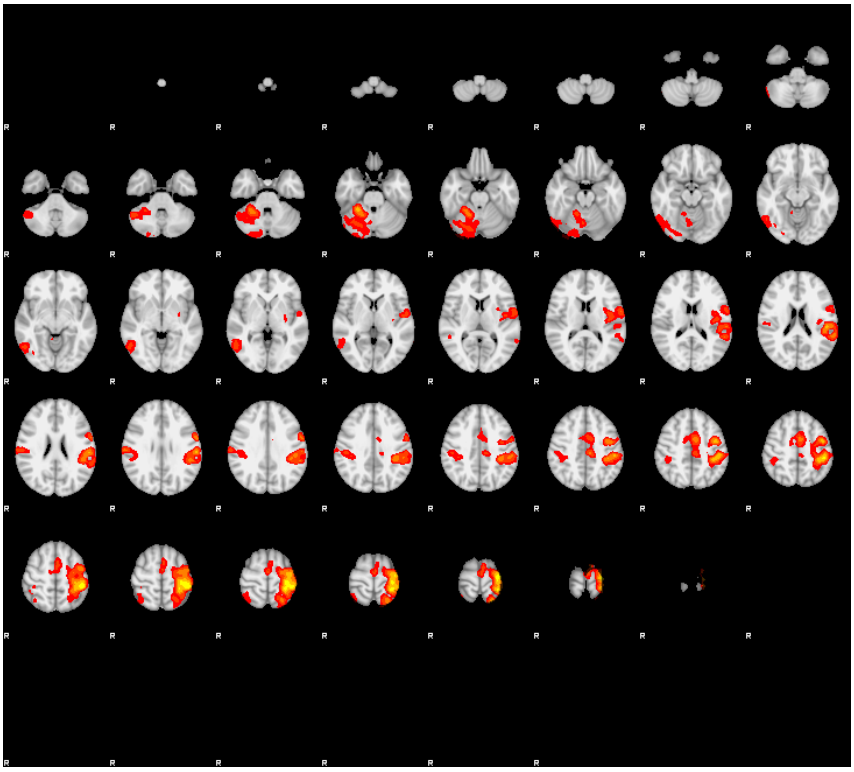


Figure D23: Individual activity map for participant C3 at W6. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

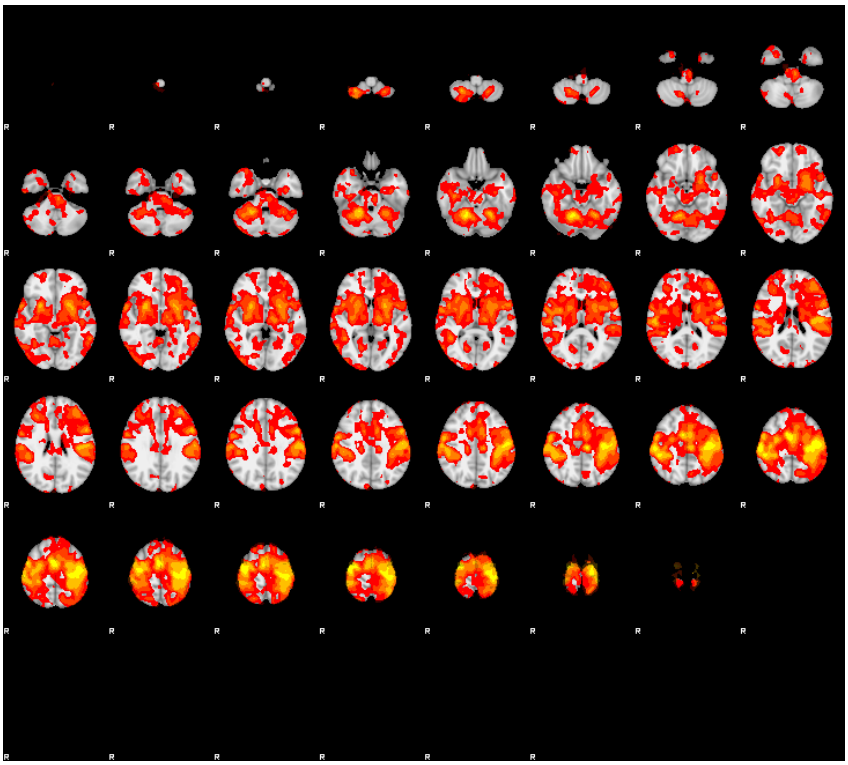


Figure D24: Individual activity map for participant C4 at W0. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

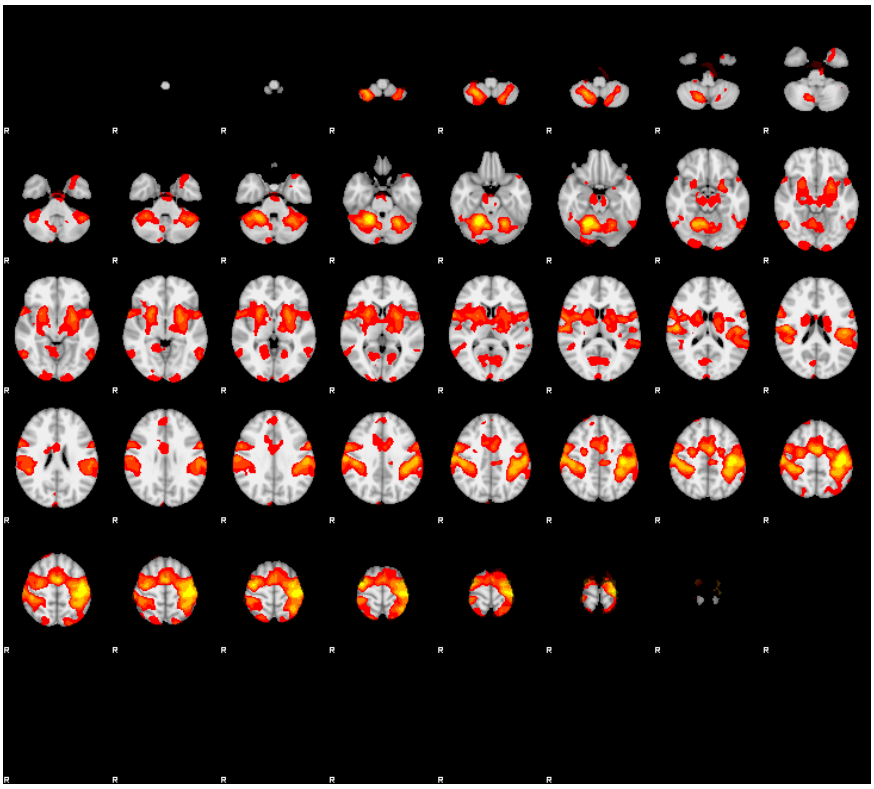


Figure D25: Individual activity map for participant C4 at W6. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

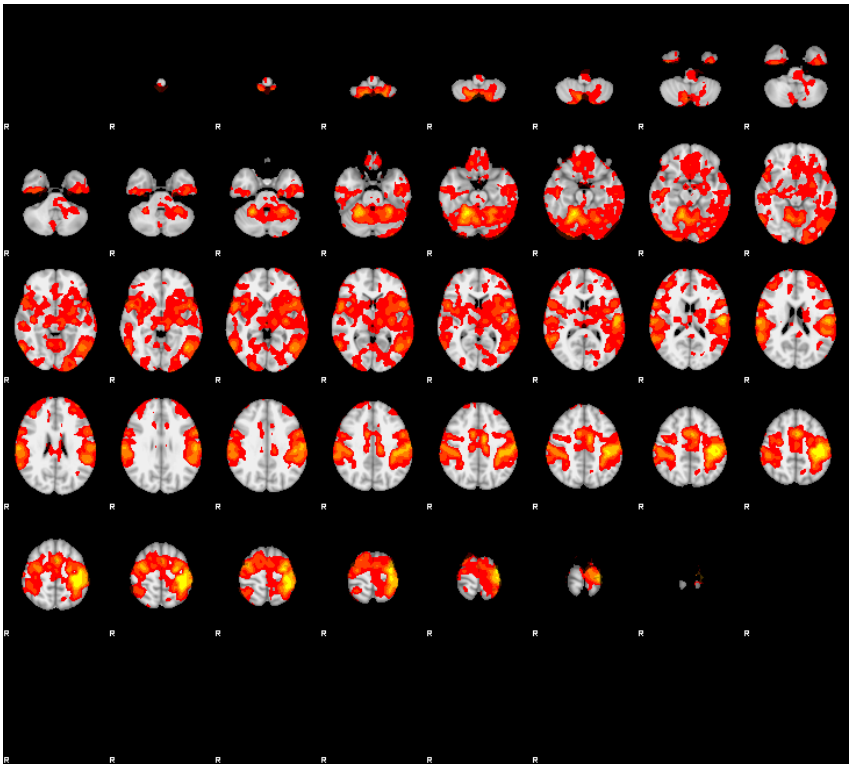


Figure D26: Individual activity map for participant C5 at W0. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

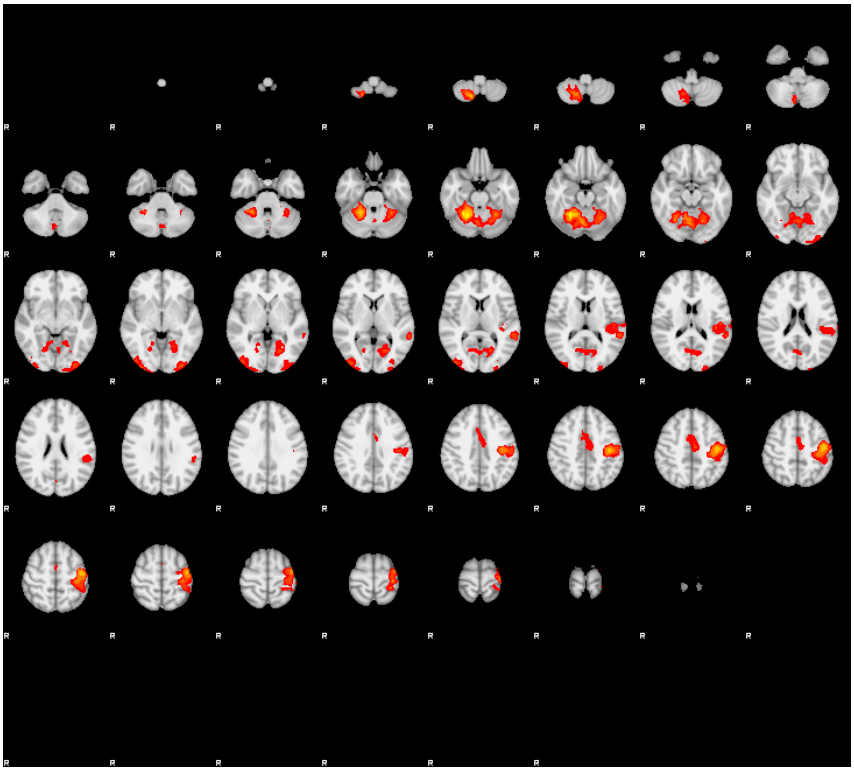


Figure D27: Individual activity map for participant C6 at W0. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

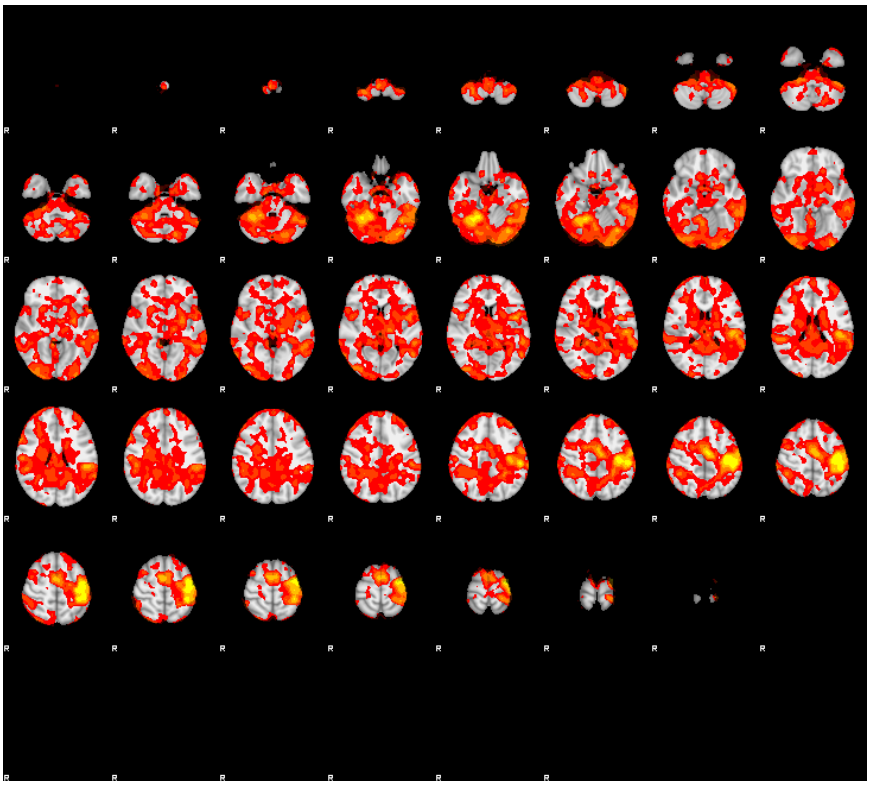


Figure D28: Individual activity map for participant C6 at W6. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

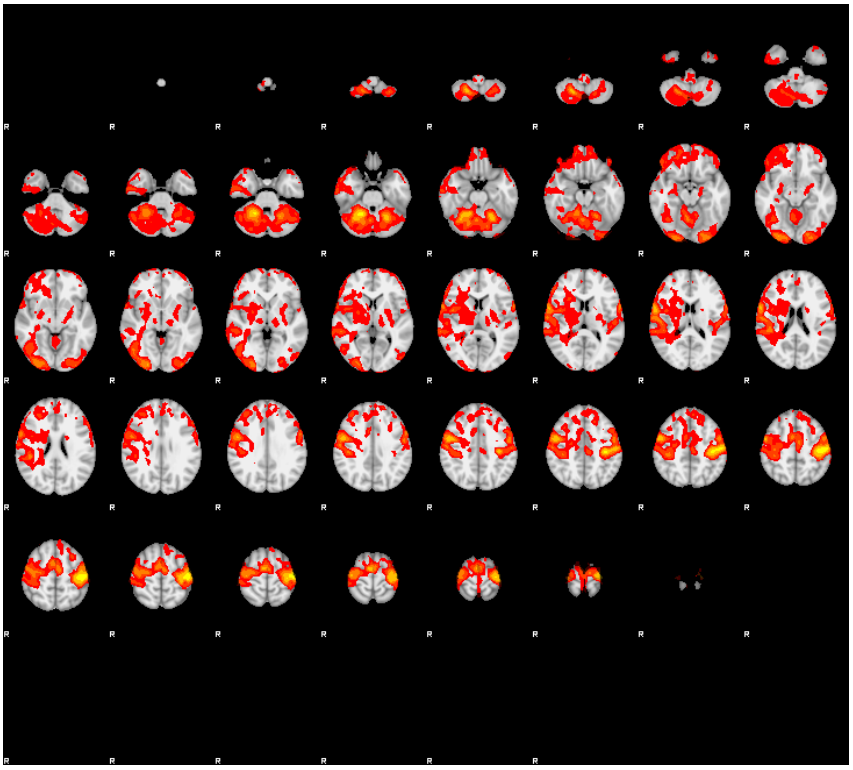


Figure D29: Individual activity map for participant C7 at W0. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

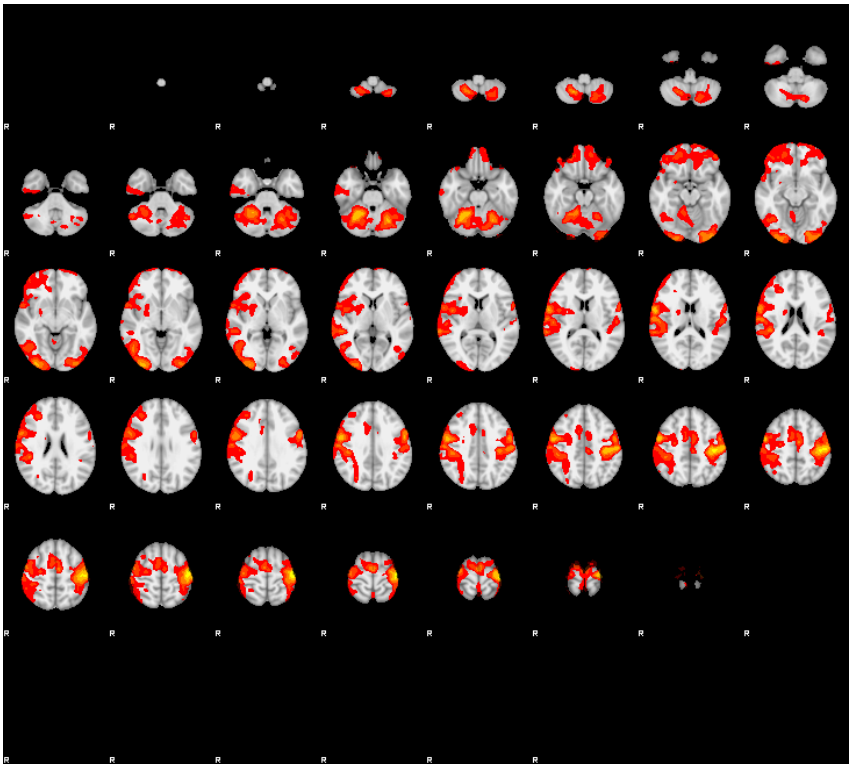


Figure D30: Individual activity map for participant C7 at W6. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

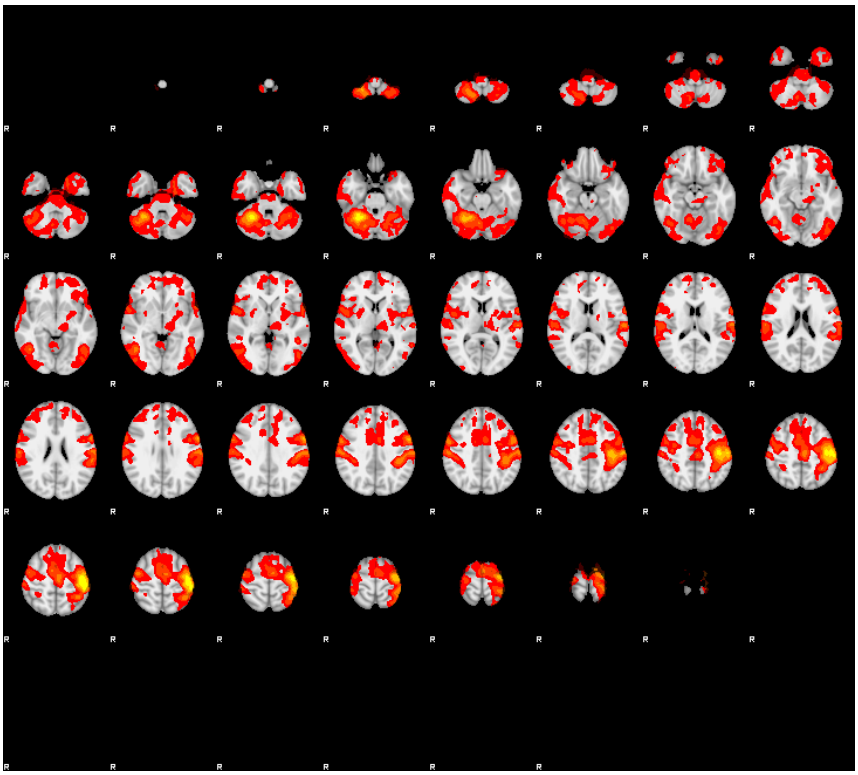


Figure D31: Individual activity map for participant C8 at W0. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

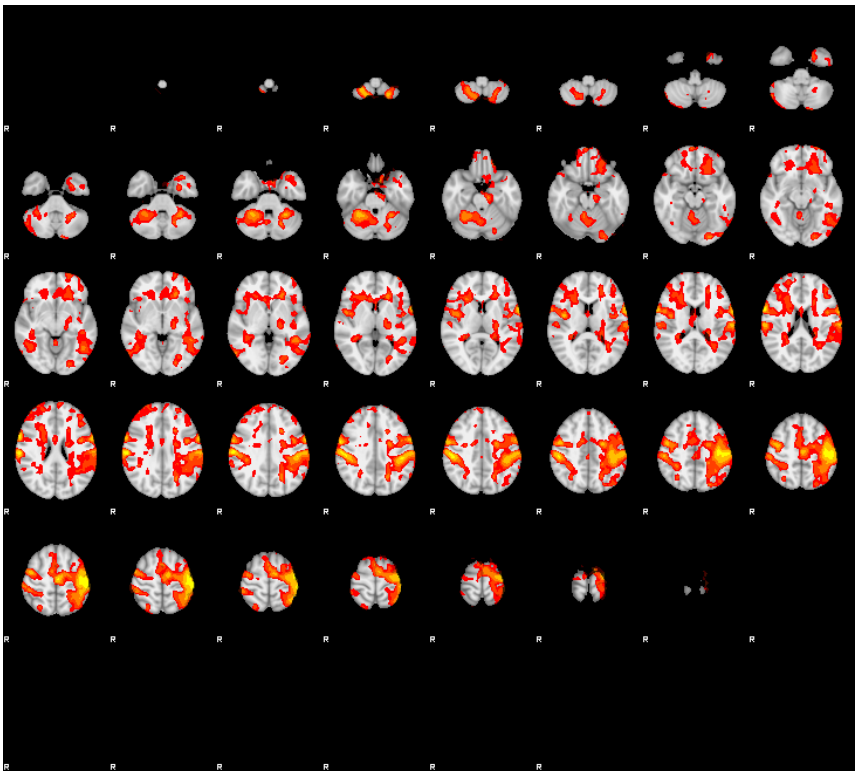


Figure D32: Individual activity map for participant C8 at W6. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

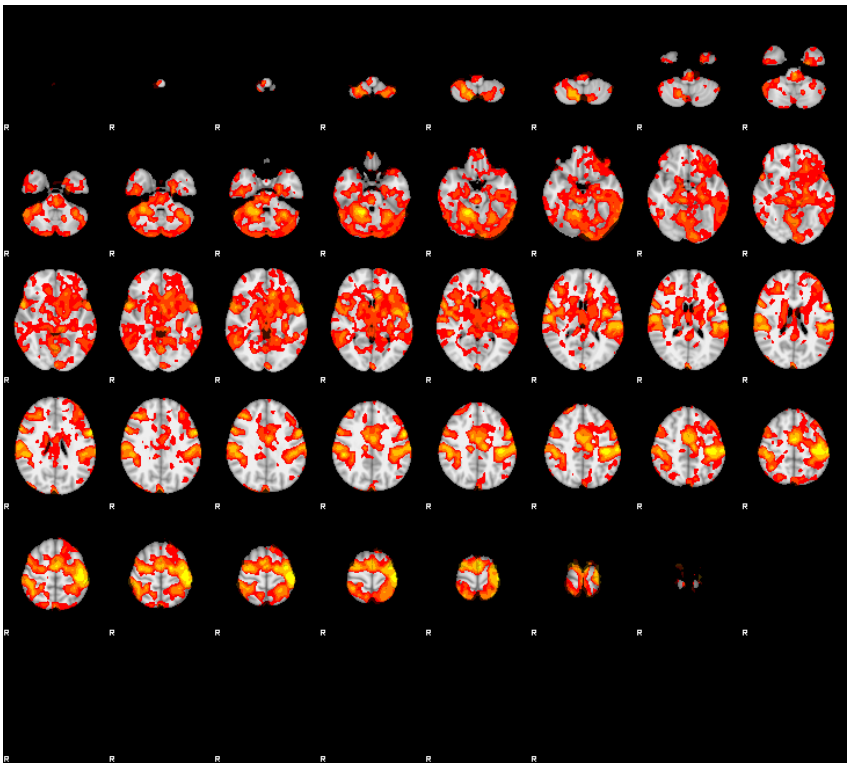


Figure D33: Individual activity map for participant C9 at W0. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

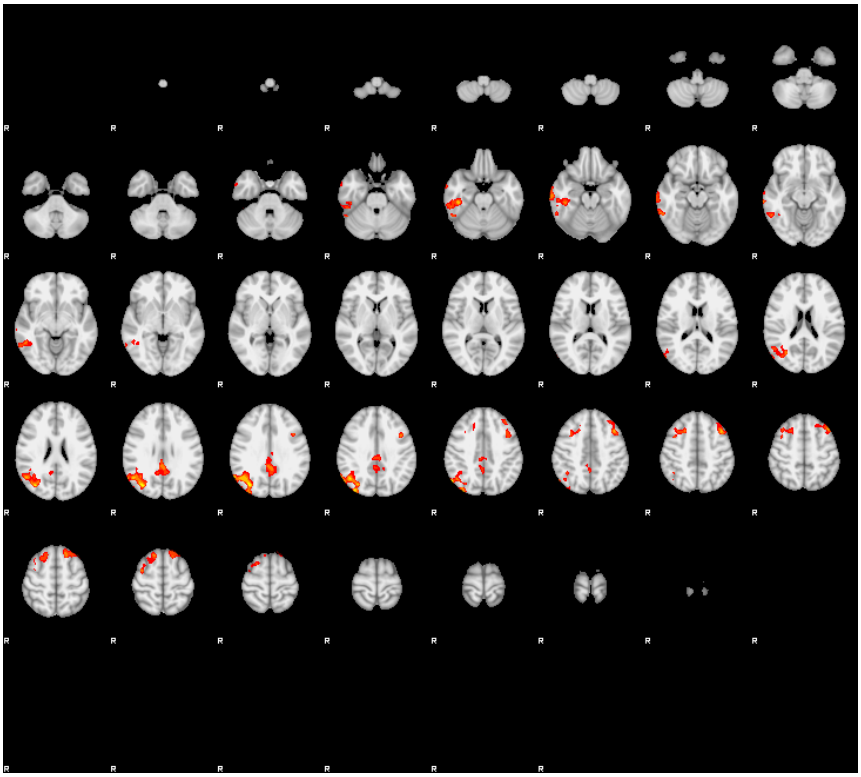


Figure D34: Individual activity map for participant C9 at W6. The mean BOLD activity is shown in red and yellow areas to indicate activity corresponding with the task.

APPENDIX E: Lesion Correction Results

Neuronal activity was assessed by utilizing statistical maps to indicate areas of correlation with the hemodynamic response. FMRI analyses were carried out using FSL's fMRI Expert Analysis Tool (FEAT) as mentioned in the Methods section of Chapter 2, with additional steps taken to remove lesioned areas from the statistical analysis. To determine the group level activity maps, all lesioned voxels were identified for all individual participants. At each voxel, a list of all participants with non-lesioned brain matter is created and stored. For all voxels, all unique combinations are counted and recorded. The FEAT analysis is performed for all combinations, to ensure no lesioned brain areas will impact the final group map results. At every voxel, the combination is read and the corresponding z-statistic is pulled from the same voxel in that combination's FEAT run. This method produced final z-statistic maps that include only participants with non-lesioned brain matter in any given voxel.

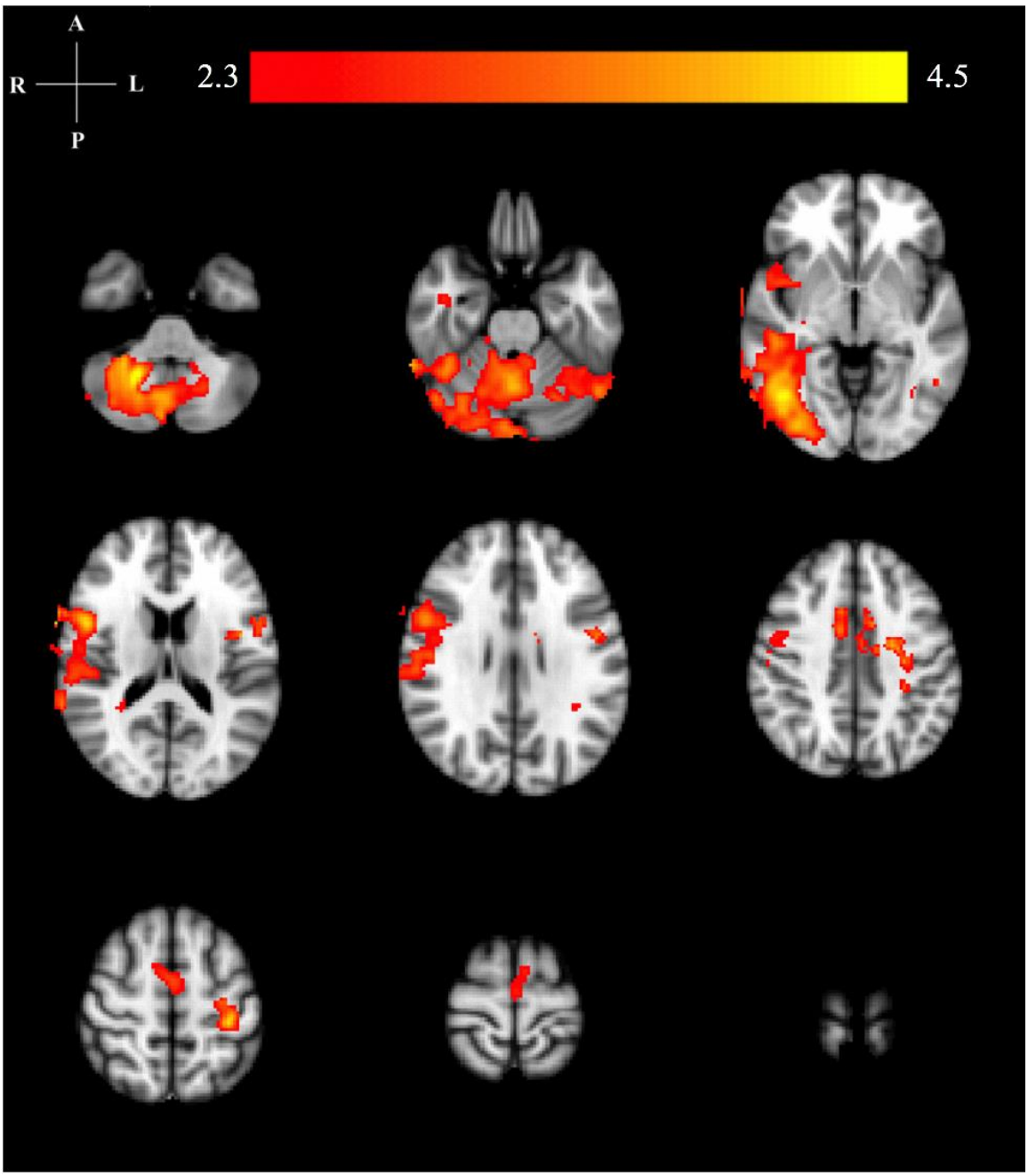


Figure E1: Lesion-Corrected Group Activity Maps of Stroke Participants at W0. Each panel shows axial slices of the MNI template overlaid with z-statistic maps of the averaged stroke group before BoNT injection while accounting for lesion location. Activity maps indicate volumes in which there was significant ($p < 0.05$) levels of activity across the group. The right side of the brain is displayed on the left.

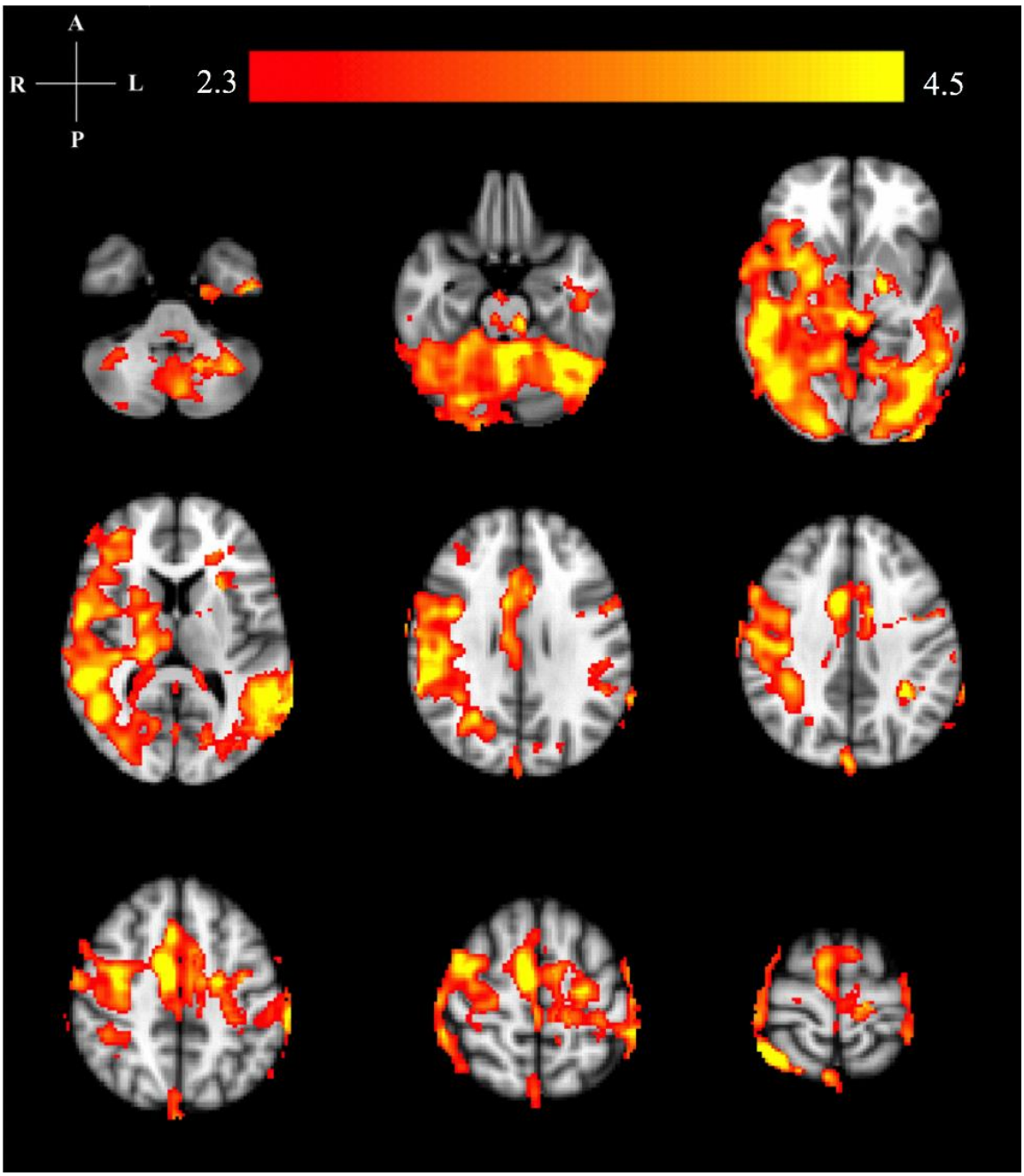


Figure E2: Lesion-Corrected Group Activity Maps of Stroke Participants at W6. Each panel shows axial slices of the MNI template overlaid with z-statistic maps of the averaged stroke group after BoNT injection while accounting for lesion location. Activity maps indicate volumes in which there was significant ($p < 0.05$) levels of activity across the group. The right side of the brain is displayed on the left.

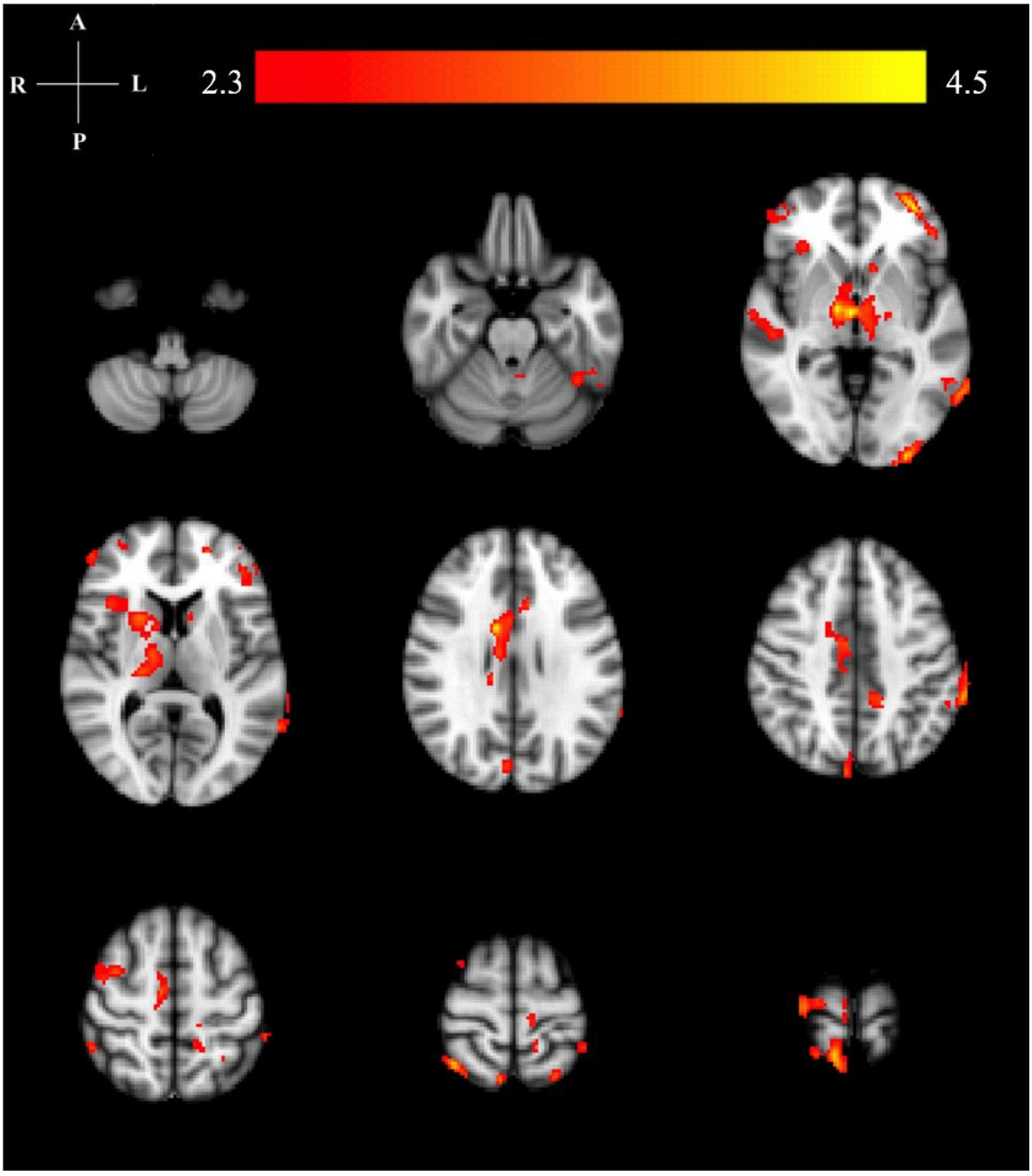


Figure E3: Lesion Corrected Contrast between Stroke Participants Before and After BoNT Intervention. Significantly activated voxels ($Z > 2.3$, $p < 0.05$) are overlaid on a standard MNI brain *(a)*, illustrating areas that showed a significant increase in activity following the BoNT intervention. These active areas were observed in three clusters *(b)* indicated by red, blue, and green volumes.