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PROTOCOL TITLE: Effect of Neural Constraints on Movement in Stroke

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STUDY SUMMARY:

Investigational Agent(s) (Drugs or Devices)	Drugs: Tizanidine, Placebo
IND / IDE / HDE #	Tizanidine is a FDA approved drug, IDE or IND number is not available for either of them.
Indicate Special Population(s)	☐ Children ☐ Children who are wards of the state ☐ Adults Unable to Consent ☐ Cognitively Impaired Adults ☐ Neonates of Uncertain Viability ☐ Pregnant Women ☐ Prisoners (or other detained/paroled individuals) ☐ Students/Employees
Sample Size	64
Funding Source	NIH and PTHMS Departmental research fund
Indicate the type of consent to be obtained	☒Written ☐Verbal/Waiver of Documentation of Informed Consent ☐Waiver of HIPAA Authorization ☐Waiver/Alteration of Consent Process
Site	☐ Lead Site (For A Multiple Site Research Study) ☐ Data Coordinating Center (DCC)
Research Related Radiation Exposure	☐Yes ☒ No
DSMB / DMC / IDMC	☐Yes ☒No

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Prime Award Recipient*	Julius P.A. Dewald, PT, PhD	Jun Yao, PhD

OBJECTIVES:

In the next 40 seconds, someone in the United States will have a stroke[1]. Of the estimated 7.6 million survivors, 40-50% will experience moderate to severe chronic (> 6 months), often life-long, upper extremity impairments that impede activities required for normal daily living[2-4]. Our long-term goal is to alleviate these motor impairments in chronic stroke by providing science-based rehabilitative strategies that leverage underlying pathophysiological mechanisms to empower both pharmacology and physical interventions. These impairments include weakness, hyperactive muscle (hypertonia), and stretch reflexes (spasticity), as well as a loss of independent joint control (e.g., flexion synergy). The flexion synergy is expressed as an abnormal coupling of joint torques such that lifting the arm upward (i.e., shoulder abduction) generates involuntary elbow, wrist, and finger flexion. Our prior work indicates that these impairments result from a loss of corticospinal and corticoreticular motor pathways[5-7].

A unilateral loss of corticospinal tracts forces a greater reliance on weak and indirect motor pathways from the contralesional hemisphere. Furthermore, damage to cortico-reticular tracts from the lesioned hemisphere perturbs the balanced control of reticulospinal projections, potentially allowing for greater activity in the locus coeruleus and increased levels of spinal norepinephrine (NE)[8, 9]. NE is a potent driver of motoneuron excitability, resulting in the amplification of the otherwise weak inputs from indirect pathways and causing strong expression of stretch reflex hyperexcitability and flexion synergy expression. Our previous human and animal research strongly supports this theory but has yet to guide clinical rehabilitative efforts[5, 7, 10-12]. **Consequently, our central hypothesis** is that **norepinephrine-induced increases in <u>spinal motoneuron excitability amplify indirect motor pathways</u>**, which then **drive the primary motor impairments** seen in **stroke survivors.** In support of this hypothesis, we will provide preliminary data that show that decreasing NE by administering tizanidine (TIZ), which acts to reduce locus coeruleus activity, improves reaching ability and hand opening by attenuating motor neuron excitability and reducing contralesional cortical activity. We propose <u>3 specific aims</u>:

Aim 1: Identify the effect of TIZ on spinal motoneuron excitability in chronic hemiparetic stroke. NE markedly increases the excitability of spinal motoneurons, <u>amplifying and prolonging</u> their response to excitatory inputs, and is believed responsible for the persistent muscle activity observed in chronic stroke. Thus, *our working hypothesis* is that TIZ administration will decrease

motoneuron excitability and, consequently, reduce the persistent motoneuron hyperactivity observed in chronic hemiparetic stroke. Changes in motoneuron function will be assessed with novel and validated quantitative metrics derived from high-density surface EMG during isometric elbow flexion tasks before/after administering TIZ or a placebo. *For all three aims, we will use pupillary diameter measurements as an independent method to monitor systemic NE levels.*

Aim 2: Determine whether norepinephrine amplifies the commands of indirect motor pathways. In the last grant cycle, we showed that contralesional cortical activity is linked to flexion synergy expression and greater contralesional corticomuscular connectivity. Since hyperexcitable motoneurons facilitate the use of weak and indirect contralesional pathways, *our working hypothesis* is that TIZ will decrease contralesional corticomuscular connectivity. Furthermore, we expect this to be paralleled by a decreased contralesional but increased lesional motor cortical activity, as reduced motoneuron and brainstem excitability make the use of contralesional motor pathways less effective. To test this hypothesis, we will simultaneously measure EEG/EMG signals, during shoulder abduction loading or hand opening, in individuals with chronic hemiparetic stroke before/after administering TIZ or a placebo.

Aim 3: Evaluate how norepinephrine modulates the expression of flexion synergy and spasticity. We have previously shown that chronic (nine weeks) TIZ administration increases reaching work area, likely from a reduced amplification of the indirect contralesional motor commands that generate flexion synergy. Thus, *our working hypothesis* is that TIZ will attenuate flexion synergy expression and result in improved reaching distance and volitional and electrical stimulation induced hand-opening ability as a function of shoulder abduction loading. We will use robot-induced increases in shoulder abduction loading to modulate flexion synergy expression during reaching and hand opening tasks before/after administering TIZ or a placebo. In addition, we will quantify stretch reflex hyperexcitability during reaching using robot-induced ramp stretches either before movement onset or at the beginning of the movement.

We expect to show that norepinephrine increases spinal motoneuron excitability in chronic stroke (Aim 1) and amplifies the effect of using indirect motor pathways (Aim 2), which together generate multiple chronic motor impairments that impede muscle relaxation, and independent joint control, thus limiting the functional use of the paretic arm (Aim 3). Results from this study will directly inform more effective rehabilitative strategies that pair pharmacology with targeted physical interventions to increase upper limb motor function and improve the quality of life of individuals post hemiparetic stroke. These outcomes will help fulfill the missions of *NICHD* and *NINDS* *which seek to increase knowledge* about the brain and nervous system to optimize abilities for all.

BACKGROUND

A. Significance

This study will systematically investigate the role of NE in post-stroke motor impairments for the first time. Over the past three decades, our research has primarily centered on studying motor function in stroke survivors with moderate to severe impairments. We have dedicated substantial efforts to comprehending the loss of independent joint control, often manifested as abnormal coupling between shoulder abduction and flexion of the elbow, wrist, and fingers (commonly referred to as the flexion synergy). These investigations have covered both static[12-16] and dynamic conditions[10, 17-19], all contributing to uncovering the neural mechanisms underlying post-stroke motor deficits and the successful implementation of targeted science-based physical interventions (i.e., progressive shoulder abduction loading during reaching and FES during grasping[20, 21], respectively).

Previous efforts, both from our lab and other research groups, have largely concentrated on elucidating post-stroke alterations in facilitatory and/or inhibitory inputs from the brain to

motoneurons using various short-lasting neural stimulation methods[22-24]. In parallel with these endeavors, our recent findings have also highlighted the critical role of modulatory neurotransmitters (i.e., neuromodulation) in post-stroke motor impairments[7, 25, 26]. Specifically, norepinephrine (NE) increases spinal motoneuron excitability by inducing persistent inward currents (PICs) which profoundly amplify and prolong excitatory synaptic inputs[27]. This amplification and prolongation provided by PICs are consistent with many motor impairments post-stroke; more excitable motoneurons may engender hyperactive stretch reflexes (i.e., spasticity) and muscle hyperactivity, while also amplifying the weak and indirect commands that generate the loss of independent joint control. However, there are no comprehensive studies designed to systematically investigate the central role of NE in post-stroke motor control, despite the FDA approval of clinical practices aiming to modulate NE, such as vagus nerve stimulation (VNS), in stroke populations. Considering the potentially vast scientific and clinical impact, it is imperative that the specific role of NE in post-stroke motor control is elucidated. This has many potential implications for advancing stroke rehabilitation strategies and may further help inform the relatively low response rates of VNS, where only about 50% of stroke participants experience a clinically meaningful improvement[28, 29].

We aim to determine how a TIZ-induced reduction in NE affects cortical and spinal cord activity, as well as corticospinal communication via descending pathways and their impact on functional motor tasks. Prior studies investigating NE's role in motor control have often focused on either cortical or spinal effects, potentially missing the comprehensive impact of NE on motor control[30]. In the brain, the locus coeruleus (LC) functions as the primary source for NE release. The LC-NE circuitry exhibits extensive bidirectional projections to various brain and spinal cord regions[31, 32], emphasizing the simultaneous influence on cortical, brainstem, and spinal circuitry and its potential role in motor control. Furthermore, LC activity is tightly regulated with both tonic and phasic activity that correlates with attentional cues, and a theorized U-shaped curve where deviations in activity may yield suboptimal outcomes[33].

To comprehensively investigate the role of norepinephrine (NE) in motor function following stroke, we have developed three distinct Aims. Aim 1 will focus on assessing tizanidine (Zanaflex®, TIZ)-induced effects on NE levels in the spinal cord measured via motor unit (MU) excitability, while Aim 2 will examine its influence at the cortical level as measured by corticomuscular communication. Additionally, as part of Aim 3, we will quantify the effect of TIZ-induced changes in NE on functional motor behavior. We will integrate behavioral measures with assessments of MU and cortical activity, along with their communication, while using pupil size as a proxy for changes in NE levels. Furthermore, we will consider other subject-specific biological data, such as impairments, lesion location, age, and sex. This study represents a pioneering effort to provide a more comprehensive understanding of how NE impacts post-stroke motor control.

While we aim to directly uncover the mechanisms that yield post-stroke motor impairments, our results may additionally impact future clinical practice. If our findings demonstrate that TIZ-induced reduction of NE in the central nervous system alleviates post-stroke motor limitations by diminishing motor unit hyperexcitability and enhancing the utilization of the ipsilesional corticospinal tracts (CST), TIZ could potentially serve as a valuable adjunct to physical interventions in post-stroke rehabilitation. TIZ is already an FDA-approved drug for management of spasticity. As research has demonstrated its effectiveness in managing spasticity following a stroke[40, 81], this translation would be relatively straightforward. Moreover, we will gather data from a large sample size, administering individualized doses while monitoring motor unit excitability, cortical activity, pupil size, and motor behavior. These data will help identify key characteristics of the responsive group, determine appropriate dosages, and assess responses in terms of upper limb motor unit excitability levels and pupil diameter size, thereby laying a robust groundwork for future R01 clinical trials.

B. Innovation

We have chosen to administer tizanidine to modulate the level of NE and will be using comprehensive measures to quantify its neuromodulatory effect for the first time. In humans, both

vagus nerve stimulation and drugs can be used to modulate NE levels in the central nervous system (CNS). However, VNS will impact the quality of EEG recordings[34] and cannot be implemented inside an MRI scanner[35]. Therefore, combining VNS with non-invasive imaging methods remains difficult. Conversely, drug-based NE modulation gives us a safe and valid time window (see *Monitoring of NE levels*, *Approach*) to test the neural changes at different dosage levels with various imaging methods. tizanidine is currently prescribed for managing spasticity resulting from various conditions, including multiple sclerosis, spinal cord injury, and stroke. TIZ reduces the release of catecholamine norepinephrine by acting as a central noradrenergic α-2 and imidazoline[36] agonist. Although there are other medications with the potential to lower NE levels, our previous work and preliminary findings have demonstrated the most promising results in reducing the expression of the flexion synergy and motor unit (MU) hyperexcitability post-stroke with TIZ[7, 25].

The single dose of TIZ will be carefully regulated (see *Methods*) to take into account subject-specific dosage effects. We will innovatively monitor TIZ's modulatory effect using the combination of motor unit excitability, cortical activity, pupil diameter, and motor behavior measures. The pupil diameter is a simple and widely employed method for monitoring changes in neuromodulators, primarily ACh (cholinergic) and NE. Its validity has been established through animal studies where optogenetic stimulation of the locus coeruleus leads to pupil dilation that positively correlates with stimulation frequency[37]; and in humans where pupil diameter covaries with LC BOLD activity[38]. We therefore have selected pupil diameter at baseline as an indicator of NE changes induced by TIZ administration, while our other measures, like changes in MU excitability and cortical activity, will reflect motor related alterations caused by TIZ. Through a combination of impairment assessments, functional behavior quantification, MU and cortical activity measures, we will, for the first time, uncover the contributions of NE to post-stroke motor deficits. This will highlight changes from the cortex to the spinal cord and subsequently behavior, with a cohort size sufficiently powered for model-based analysis.

We will use novel high-resolution quantitative measures to assess the hyperexcitability of spinal motoneurons. Previous investigations into the spasticity-reducing effects of TIZ have primarily used the low-resolution Modified Ashworth Scale[39-42], with the impact of TIZ on heightened motoneuron excitability post-stroke yet to be explored. To address this gap, we will use high-density surface EMG arrays to estimate MU firing patterns (Aim 1). Recent advancements in flexible surface array electrodes and sophisticated signal decomposition techniques have enabled the noninvasive and simultaneous recording of multiple motor units[43, 44]. Additionally, we have recently put forth novel metrics to estimate human motor unit discharge patterns and validated these metrics with supercomputer simulations of realistic motoneuron models[45, 46]. This includes the metric of brace height (see Aim 1), which, when combined with traditional approaches (ΔF), will enable us to estimate amplification and prolongation of motor unit activity.

We will employ both validated and innovative methods to quantify cortical activity and corticomuscular communication. Among various non-invasive imaging methods, we have chosen EEG-based measures, since they are easy to be combined with well-controlled motor protocols and provide rich temporal information that is important for reflecting the utilization of corticospinal or cortico-reticulospinal tracts (see Aim 2 Methods). To quantify cortical activity and corticomuscular-communication (CMC), we will utilize subject-specific model-based inverse calculations and wavelet-based coherence methods, as opposed to Fourier-based approaches[47, 48], since wavelet-based coherence methods provide a better fit for non-stationary signals like EEGs and EMGs. Both inverse and coherence methods have been integral to our laboratory's research for over two decades[47]. We anticipate that TIZ administration will result in both tonic changes (which are relevant to the baseline NE level) and phasic changes (which are more relevant to motor task execution)[49, 50]. This study particularly focuses on motor-related changes. As the changes in wavelet coherence are primarily driven by phase-locked activity, any cortical activity changes that do not translate to muscle activation will not lead to a phase-lock change between cortical and muscular activity, and thus will not be captured by the wavelet-based

coherence method[51]. Furthermore, as a complementary approach, we will also examine the cross-frequency CMC, using our newly developed multi-frequency methods[52]. These well-established and innovative methods developed within our group will allow us to investigate TIZ-induced changes in cortical activity and corticomuscular communication.

We will use a newly developed innovative robotic setup to measure motor behavior before and after TIZ administration during functional movements. Using innovative robotics and recently developed approaches, we will determine the effects of reducing NE on functional behaviors like reaching distance, hand opening ability, and the expression of hyperactive stretch reflexes when perturbing the arm during reaching movements[53] (Aim 3). We will use a robotic system based on a combination of admittance and position control with three degrees of freedom to allow for multi-joint planar and vertical movements. The admittance controller allows for free reaching and hand opening under varying levels of shoulder abduction, while the position controller can perturb the arm both before movement and during movements to determine stretch reflex excitability during functional motor tasks. *In summary, the proposed research will utilize highly innovative and high-resolution measures at the forefront of modern signal analysis and robotics to explore human motor function in new and insightful ways. Many of these innovative methods have been specifically developed for this study, placing us in a unique and competitive position to conduct this research.*

RELEVANT PRIOR EXPERIENCE AND GAPS, AS WELL AS PRELIMINARY DATA

AIM 1: Identify the effect of TIZ on spinal motoneuron excitability in chronic hemiparetic stroke.

Introduction

Accumulating evidence points to the role that noradrenergic drive plays in the manifestation of motor impairments in chronic hemiparetic stroke[7, 11, 25]. A stroke-induced lesion of the cortex or internal capsule elicits damage to both corticospinal and cortico-reticular projections, which subsequently perturbs the balanced control of reticulospinal tract (RST) output. Specifically, the pontomedullary reticular formation receives extensive input from the cortex, exhibits many ionotropic projections with contrasting influences on spinal circuitry (e.g., inhibitory dorsomedial RST, excitatory ventromedial RST), and is coupled to monoaminergic spinal cord projections[8]. While it is theorized that the ionotropic RST projections are relied upon for movement post-stroke (see Aim 2), these pathways are relatively weak, and thus heightened monoaminergic drive is likely critical for amplifying these commands[9, 55-57].

Monoamines (e.g., norepinephrine; NE) act on G-protein coupled receptors to facilitate persistent inward currents (PICs) in motoneurons. PICs are excitatory dendritic currents mediated by voltage-sensitive channels that, once facilitated, exert extraordinary actions on motoneurons by amplifying excitatory synaptic inputs as much as 3-5-fold and prolonging inputs for many seconds[27, 58-60]. The amplifying and prolonging effects of PICs align with our observations in individuals with chronic hemiparetic stroke, who not only exhibit disturbances in the recruitment pattern and modulation of motor unit discharge rate but also persistent activity of motor units. These alterations greatly diminish the ability to gradually modulate force and likely contribute to the persistent and hyperactive muscle behavior (i.e., hypertonia) observed in stroke. Thus, by reducing systemic levels of NE, we hypothesize that TIZ will attenuate motoneuron excitability and yield reduced estimates of amplification and prolongation.

Preliminary Results

With a high-density surface EMG array on the biceps (BIC, see *Figure 2*), we asked three individuals with moderate to severe chronic hemiparetic stroke to generate linear isometric triangular ramp contractions up to 30% of their maximum elbow flexion torque (MVT; like the thin black trace in *Figure 4A*). We repeated this for up to 10 ramps both before and 1.5 hours after the administration of TIZ with dosage adjusted for body mass (4, 6, and 8 mg for participants 1, 3, and 2, respectively). For all trials, we decomposed EMG activity into the discharge times of many concurrently active MUs using a convolutive blind source separation algorithm[43, 44], and created smooth continuous estimates of discharge rate. The colored lines in *Figure 4A* indicate the

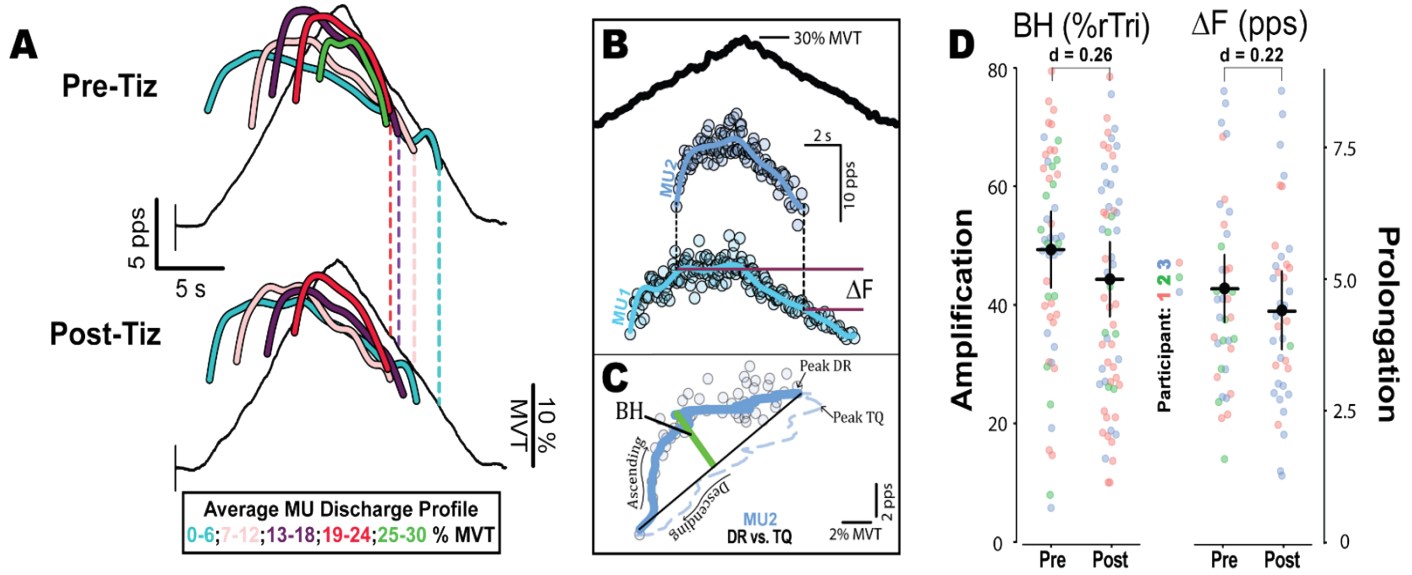


Figure 1: A) Average MU discharge profiles for all participants pre- and post-TIZ administration. MUs are averaged in five cohorts based on the joint torque (black lines) that they were recruited at, showing a more gradual and improved recruitment and de-recruitment following TIZ. Dashed vertical lines indicate average de-recruitment pre-TIZ. We employed ΔF to estimate prolongation and brace height (BH) to estimate amplification, shown in B and C, respectively. These values of ΔF and BH can be observed in D pre- and post-TIZ. Individual dots indicate unique MUs, colored by participant, black horizontal lines indicate marginal means, and

ensemble averages of MU discharge rate during these triangular ramp contractions, separated into five cohorts based on the torque at which MUs were recruited. As shown in *Figure 4A*, pre-TIZ, the expected modulation of MU discharge rate across recruitment thresholds appears disorderly, with higher threshold MUs quickly surpassing the discharge rates of lower threshold MUs. In contrast, MUs post-TIZ follow the gradual recruitment and modulation of discharge expected during linear increases in effort, reflecting an improved ability to gradually recruit and control MUs. Furthermore, the dashed vertical lines indicate the average instance of de-recruitment for each cohort of MUs pre-TIZ, displaying an earlier de-recruitment of lower threshold MUs post-TIZ. This suggests that individuals were able to more effectively de-recruit MUs and terminate the undesirable persistent behavior of MUs observed in chronic stroke. Together this indicates less excitable MUs following TIZ, allowing for more gradual increases in force and a greater ability to volitionally relax muscle. Additionally, we estimated the amplification and prolongation of MU discharge using two validated metrics, brace height (BH) and ΔF, respectively (*Figure 4B-C*; see *Data Collection and Analysis*). We found TIZ to reduce both metrics (*Figure 4D*), supporting our hypothesis and demonstrating the feasibility of the approach.

AIM 2: Determine whether norepinephrine amplifies the commands of indirect motor pathways.

Introduction

At the cortical level, the norepinephrine (NE) level is probably dominated by the activity of the locus coeruleus (LC). LC connects widely to various brain regions, with relatively stronger connections with the prefrontal and primary motor cortices. On the one hand, the continuous activation of LC-NE helps regulate baseline arousal and supports various sensory-motor and behavioral functions[33, 62-64]. On the other hand, recent insights have revealed a more temporary and task-specific form of LC-NE activation, enabling specific brain regions to be modulated[33, 50, 65]. Despite growing attention to LC projections, our understanding of NE's role in modulating cortical activity and correspondingly altering descending motor pathways' utilization during motor tasks remains limited.

Our previous studies have demonstrated an amplification and prolongation in motoneuron unit (MU) activity in the paretic upper limb, potentially attributed to heightened persistent inward currents (PICs) and indicating a probable increase in norepinephrine presence at the spinal cord post-stroke[7, 11, 26, 66]. This increase in MU activity aligns with the presence of spasticity[28] and synergies[12, 67]. In this aim, we further anticipate that this heightened MU excitability might also facilitate the utilization of the cortico-reticulospinal track (cRST), an extensively documented adaptation following a stroke[5, 6, 10, 12]. Ordinarily, the synaptic strength of the cRST is relatively weak[68] , which could restrict its utilization under normal circumstances. However, the postulated post-stroke heightened NE levels, which make MUs more excitable, may result in the cRST becoming more effective, as more excitable MUs necessitate less central drive to initiate firing. As a side effect, due to the branched nature of the cRST, utilizing this pathway might enhance the expression of the flexion synergy[12, 67], in line with synergy observations in this cohort. Encouraging preliminary results indicate that TIZ administration reduces the amplification of MU firing at the spinal cord (as shown in Aim 1), potentially diminishing the efficacy of indirect cRST motor pathways. TIZ administration may also reduce NE levels at the cortical level, as evidenced by our preliminary results of changes in pupil size (see *Figure 3*). This dual effect (i.e., reduced MU and cortical excitability) could decrease the effectiveness of the cRST and prompt the cortex to rely more on the remaining CST from the affected sensorimotor cortices to activate targeted motor neurons.

In accordance with this framework, we hypothesize that reduced NE following TIZ administration in individuals with chronic stroke will prevent the amplification of weak cRST projections from the non-lesioned hemisphere. This should promote greater reliance on remaining CST projections from the lesioned hemisphere and be observed as a shift in sensorimotor activity towards the ipsilesional side, and greater corticomuscular connectivity between the lesioned hemisphere and the paretic limb. To test this hypothesis, we will measure EEG and EMG during carefully designed shoulder and hand tasks; a shoulder task that likely depends on both CST and cRST, and a hand opening task with greater CST dependence[69]. Quantifying cortical activity and corticomuscular coherence will allow us to evaluate the use of descending motor pathways before and after TIZ administration.

Preliminary Results

Four preliminary experiments were conducted involving two participants with chronic hemiparetic stroke, each tested and retested with their paretic upper extremity (UE). Both participants exhibited moderate-severe impairments in their paretic arm (FMA= 26/66 and 43/66). During the preliminary experiment, participants generated and maintained 40% of their maximum shoulder abduction (SABD) before and after the TIZ administration, while EEG, EMG, force/moment, and pupil size data were collected. EEG and EMG data were divided into one-second epochs to compute corticomuscular connectivity (CMC) using wavelet coherence. This method was chosen because it has been proven to be sensitive to synchronized EEG-EMG activities at the same frequency component[51]. Furthermore, we quantified the beta-band coherence by averaging all the significant coherence values within the beta band (13-30 Hz)

across the entire time window. We focused on the beta band because CMC in this band has been shown to be related to corticospinal drive[70].

We calculated the laterality index (LI) and CMC between EEG of the lesioned hemisphere and the IDL in the beta band during an SABD hold. The LI describes the ratio of cortical activity in the regions of interest between the ipsilesional (I) vs. contralesional (C) side as (I-C)/(I+C). A more positive LI corresponds to a greater ratio of power being utilized in the ipsilesional hemisphere. As observed in *Figure 5A*, three out of four experiments show an increase in LI post-TIZ. This increase can be interpreted as a shift in cortical activity from the contralesional hemisphere to the ipsilesional hemisphere. Corticomuscular connectivity (CMC) reveals functional connections between the cortex and muscles[71]. As shown in *Figure 5B*, the first three experiments demonstrated an increase in beta band CMC, suggesting an increased use of CSTs[72, 73]. These preliminary results demonstrate that the administration of TIZ increased 1) the utilization of the ipsilesional hemisphere, and 2) the beta band connectivity between the lesioned hemisphere and the agonist muscle of the paretic arm. These results demonstrate the feasibility of our approaches and support our hypothesis, suggesting the post-TIZ recruitment of fast CST projections that are located closer to the lesion and yet remain functional.

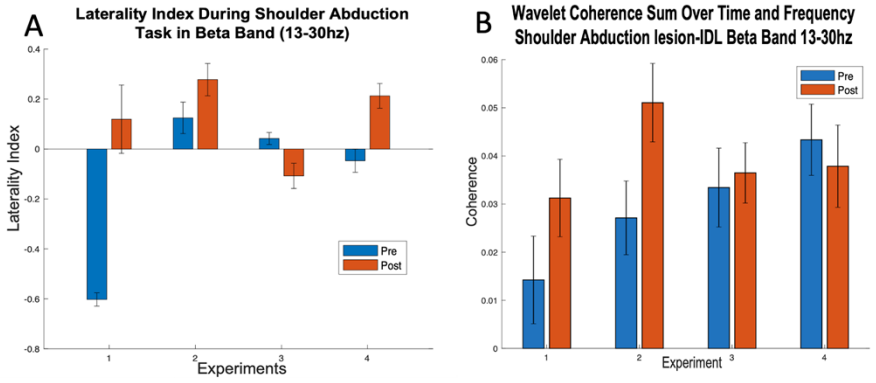


Figure 2: A) The Laterality index (LI) in the 100 ms window before the onset of EMG in the beta band during each of the 4 experiments. B) The corticomuscular coherence in the beta band while holding 40% SABD task for the 4 experiments.

AIM 3: Evaluate how norepinephrine modulates the expression of the flexion synergy and spasticity.

Introduction

In this aim we will explore whether the TIZ-induced change in norepinephrine levels leads to observable alterations in functional motor behavior. The flexion synergy, a notable post-stroke motor impairment, significantly impacts reaching[7] and hand-opening[77] abilities while lifting the paretic arm against a load (i.e., shoulder abduction or SABD). As TIZ administration may enhance the utilization of the corticospinal tract and diminish the reliance on cortico-reticulospinal tracts (as postulated in Aim 2), this drug intervention could potentially mitigate the expression of the flexion synergy and, consequently, enhance reaching and hand-opening abilities during SABD loading. This investigation is essential, since Aim 2 employs isometric measures to augment the CMC analysis, while the objective of Aim 3 is to incorporate dynamic measures thereby providing a more functionally relevant evaluation of the effects of reducing NE levels.

Another prevalent post-stroke motor deficit is the abnormally increased stretch reflex, commonly referred to as spasticity. Recent studies have demonstrated hyperexcitability in the biceps when rapidly extending the elbow, both before and during ballistic reaching with the NACT-3D robot in individuals with stroke with the paretic arm supported[78], a phenomenon not observed in control participants[53]. This hyperexcitable biceps may be linked to increased motoneuron excitability (as demonstrated in Aim 1) and/or a reduction in reciprocal inhibition. In line with this, recent research on spasticity-related motor impairments in spinal cord injury has suggested that losses of noradrenergic pathways disrupt the typical modulation of reciprocal and recurrent inhibition, impacting stretch reflex excitability[79]. As postulated in Aim 1, the TIZ-induced changes

in NE will reduce motoneuron excitability, which in turn may influence reciprocal and recurrent inhibition. We will test the hypothesis that a TIZ-induced reduction in NE will lead to a decrease in stretch reflex responses, as measured by reduced EMG activity due to fast stretches. We also have the ability to separate out the short-latency (25 – 75 ms post perturbation) versus long-latency (75 – 125 ms) stretch responses as the former measures the changes in spinal excitability (see Aim 1) and the latter changes in spinal and cortical excitability (see Aims 1 & 2) following the administration of TIZ.

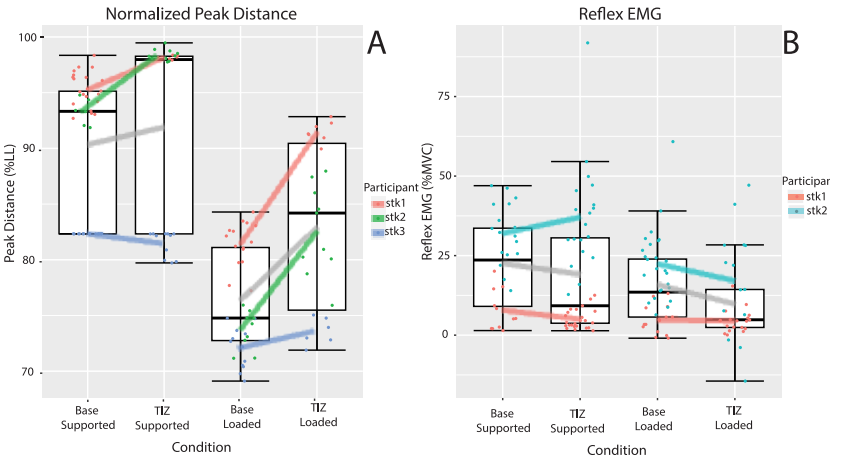


Figure 3: A) Normalized peak reaching distance for 3 participants (represented by colored dots). B) Normalized reflexive EMG for 2 participants. In both A and B, the thick grey lines indicate changes in the group mean in both the *supported* and *loaded* condition following TIZ, with colored lines indicating participant means. Results in both A and B are split by drug condition (*base* and *TIZ*) and loading condition (*supported* and *loaded*).

Moreover, due to the expression of the flexion synergy in the post-stroke population, the stretch-induced activation of the biceps is expected to increase when lifting the arm against an SABD load before reaching (as demonstrated in the previous cycle of this grant[77]). Due to the potential post-TIZ reduction in flexion synergy, the stretch reflex may exhibit no or reduced correlation with an increased SABD load. Therefore, for the first time, we will apply stretch perturbations both before and during movements during SABD loading. In short, this aim will provide quantitative evidence about how TIZ administration will impact functional motor behavior in this cohort of moderately to severely impaired individuals with hemiparetic stroke.

Preliminary Results

We measured peak reaching distance and reflex excitability in three participants with chronic hemiparetic stroke using the NACT-3D in haptic mode while providing full limb support or at set levels of SABD loading. During reaching trials, participants were instructed to move the paretic limb from a starting position located 5 cm in front of the sternum to a target position 5 cm beyond their maximal reaching distance, based on limb segment lengths. Peak reaching distance was defined as the hand position that was furthest away from the starting position in the forward direction (parallel to the sagittal plane) during a reaching movement and normalized to maximal reaching ability based on upper limb segment lengths. Participants performed an average of 6 reaches per trial for the *supported* condition (where the hand rested on a virtual solid surface generated by the NACT-3D), and 4 reaches per trial in the *loaded* condition (where the NACT-3D robot applies a vertical force equal to 50% of the participant's maximal SABD force) (total of 34 trials). Following TIZ administration, the peak reaching distance increased for most participants, especially under the *loaded* condition, with increased improvements in the *supported* condition (see *Figure 6A*).

Reflex excitability was measured with the same setup and drug/loading conditions, for two participants with chronic hemiparetic stroke. For this task, participants were asked to first move toward and keep their hand in a starting position corresponding to 45° shoulder flexion and 135° elbow flexion. A series of perturbations were applied, which extended the elbow at a rate of 270 °/s for 50 ms. Participants were asked to relax their arm and not resist the perturbations. Surface EMG data was recorded from the biceps brachii of the paretic arm. Reflexive EMG was calculated by taking the average EMG voltage during the short-latency reflex window (25 – 75 ms following perturbation onset) and subtracting the average pre-activation EMG voltage (100 – 0 ms prior to perturbation onset). This difference was then normalized to 100% maximal voluntary contraction (MVC) EMG collected in separate trials. An average of 5 perturbations were applied per trial

across 23 trials. As shown in *Figure 6B*, TIZ administration reduced the reflexive EMG for both the *supported* and *loaded* conditions.

Overall, all preliminary results as shown in *Figure 6* support the feasibility and the hypotheses of this aim, i.e., single dose TIZ can increase reaching distance and reduce reflex excitability, likely due to a NE-related reduction in MU excitability and an increase in CST use (see Aims 1 and 2, respectively).

STUDY ENDPOINTS

AIM 1: Identify the effect of TIZ on spinal motoneuron excitability in chronic hemiparetic stroke.

Hypothesis: We hypothesize a decrease in neuromodulation following tizanidine administration, indicating less amplification from Persistent Inward Currents PICs.

Type	Name	Time Frame	Description
Primary	Brace Height	pre-post administration of TIZ	Brace height quantifies the amplification in motor unit discharge rate generated by PICs. Brace height is sensitive to the level of neuromodulatory drive received by motoneurons.
Secondary	ΔF	pre-post administration of TIZ	ΔF quantifies hysteresis (i.e., prolongation, or persistence) in motor unit firing induced by PICs. ΔF is less selective to the level of neuromodulatory drive, but also accounts for inhibitory synaptic inputs to motoneurons.

Hypothesis: TIZ will A) decrease the proportion of biceps brachii motor units demonstrating sustained firing (SSF) patterns during TVR and B) decrease the magnitude of elbow flexion torque generated in response to Tonic Vibration Reflexes (TVR).

Type	**Name**	**Time Frame**	**Description**
Primary	TVR-MU	pre-post administration of TIZ	(TVRs) evoke motor unit firing by stimulating Ia afferent neurons and indirectly assess spinal motoneuron excitability.

AIM 2: Determine whether norepinephrine amplifies the commands of indirect motor pathways.

Hypothesis: We hypothesize an increased beta-band cortico-muscular coherence (CMC) following tizanidine administration especially with shoulder abduction (as opposed to without), indicating the increased use of corticospinal tract.

Type	Name	Time Frame	Brief Description
Primary	Cortico-muscular-coherence in beta band	pre-post administration of TIZ	Cortico-muscular coherence (CMC) is a commonly used method to measure the synchronized activity between cortex and muscles. Specifically, beta-band CMC has

			been reported to be linked to the use of corticospinal tract.
Secondary	Cortico-muscular-coherence in alpha band	pre-post administration of TIZ	Alpha-band CMC has been reported to be linked to the use of cortico-reticulospinal tract. A decreased use of cortico-reticulospinal tract will indirectly reflect an increased use of corticospinal tract, when the required motor task is still performed successfully.
Secondary	Cortical Laterality Index (LI)	pre-post administration of TIZ	LI measures the relatively higher (close to 1) or lower (close to -1) cortical activity between the lesioned vs non-lesioned motor cortices. An increase in LI indicates a shift of cortical activity towards the lesioned motor cortices, and thus reflecting an increased use of corticospinal tract.

AIM 3: Evaluate how norepinephrine modulates the expression of the flexion synergy and spasticity.

Hypothesis 3.1: We hypothesize that reaching distance and hand opening ability area will decrease less under SABD load following tizanidine administration, indicating a reduced expression of the flexion synergy.

Type	Name	Time Frame	Description
Primary	Difference in reaching distance under SABD load	Pre / post administration of TIZ	The difference in reaching distance under SABD load measures the strength of flexion synergy.
Secondary	Difference in Hand hexagon area under SABD load	Pre / post administration of TIZ	Hand hexagon area (HHA), i.e. the polygon created by connecting the five fingers of the hand with the base of the palm together in a plane, measures volitional and electrical stimulation hand opening abilities. The difference in HHA under SABD load also measures the strength of flexion synergy, but more distally. We hypothesize that the difference under load will become less following tizanidine administration thus hand opening will improve volitionally and with electrical stimulation of the wrist/finger extensors.

Hypothesis 3.2: We hypothesize that a perturbation prior to ballistic reaching will induce a smaller increase in short-latency reflexive EMG activity following tizanidine administration, indicating a reduction in motor neuron excitability. This may result from changes in spinal

motoneuron excitability (short latency) and/or a reduction in brainstem (long latency) excitability.

Primary	Reflexive EMG activity prior to movement	Pre / post administration of TIZ	Reflexive EMG activity will be calculated as the difference between perturbation-induced EMG amplitude and baseline EMG, averaged during three-time windows 1) before the perturbation -100- 0 ms pre-perturbation; 2) 25 – 75 ms (short-latency stretch reflex) and 2) 75 – 125 ms (long latency stretch reflex) after the perturbation onset. Perturbations occur at rest before the onset of a regular ballistic reaching trial. EMG is then normalized by the maximal EMG of the corresponding muscle.

Hypothesis 3.3: We hypothesize that following tizanidine administration, antagonist EMG activity during the onset of ballistic reaching, both before and following a perturbation, will decrease. This indicates an increased influence of reciprocal inhibition on motor neuron excitability due to an increased use of the corticospinal tract.

Primary	Reflexive EMG activity during movement	Pre / post administration of TIZ	We will calculate the reflexive EMG activity as in Hypothesis 3.2, but with the perturbation occurring during the onset of a ballistic reach.

STUDY INTERVENTION(S) / INVESTIGATIONAL AGENT(S):

The experiment uses a two-arm, cross-over, double-blinded, pre-test-post-test, randomized controlled design where each stroke participant will receive a single dose of either tizanidine or Placebo. We intend for tizanidine to reduce participant's norepinephrine, and then quantify the change in upper limb activity.

Tizanidine

Tizanidine (TIZ) (Zanaflex®) is a centrally acting noradrenergic α-2 agonist and a ligand of I3 (non-I1/I2) imidazoline receptors. It is currently indicated for the management of spasticity. TIZ acts on inhibitory α2 noradrenergic auto-receptors within descending monoaminergic bulbospinal fibers, thereby inhibiting the bulbospinal release of norepinephrine. TIZ also acts on imidazoline receptors (I3) within the brainstem to modulate the activity of noradrenergic neurons within the bulbospinal pathways that originate in the locus coeruleus. Both α2 and I3 receptor proteins have both been found in the spinal cord, but pharmacological evidence suggests that TIZ acts through supraspinal mechanisms to reduce the release of NE onto spinal motoneurons[28].

A single dose administration study found TIZ to be generally well tolerated in individuals at an 8 mg dosing and when taken with food elicits a time to peak plasma of 1.41 and 3.00 hours for the tablet and capsule formulations, respectively[29, 30]. Furthermore, dosages of 8 mg have been found tolerable in previous studies in our lab. Specifically, prior work from our lab in individuals with chronic hemiparetic stroke has shown that chronic administration of TIZ (8 mg, 3 times daily) was tolerable by participants for up to 4 weeks and increased an individual's reaching ability[12]. Based on these findings, 8 mg TIZ is expected to prove tolerable in the healthy population recruited for

this study and up to 8 mg of TIZ will be administered as a noradrenergic probe to decrease the relative amount of NE acting on spinal motoneurons.

TIZ is an FDA-approved drug already used in the stroke population[39, 40, 81]. TIZ fits under criteria of investigational new drug exemption. It is lawfully marketed in the United States. This investigation is not intended to be reported to the FDA in support of a new indication or to change the labeling of TIZ. The study is not intended to support a change in advertising for the drug. All routes of administration and dosages are on-label, and the use of a single dose in the geriatric population and the stroke population does not increase the risk associated with the use of the drugs. This investigation will be in compliance with requirements for review by an IRB and requirements for informed consent and is not intended to promote or commercialize the drugs.

Placebo*:* One lactose pill will also be administered as a placebo (PLB) for individuals under the control condition.

The Investigational Drug Services at Northwestern Memorial Hospital will store, dispense, and control our doses of TIZ and PLB. Additionally, they will collaborate with our study physician and research coordinator to maintain the blinded portion of our study. The cohort will be separated into two groups, TIZ first or PLB first. We will try to maintain similar averages of FMA between the two groups. They will be added to the group where the addition of their FMA results in a smaller difference in average FMA between groups. This serves to ensure we are not biasing our results by having more mild or severely impaired participants starting with one drug over the other.

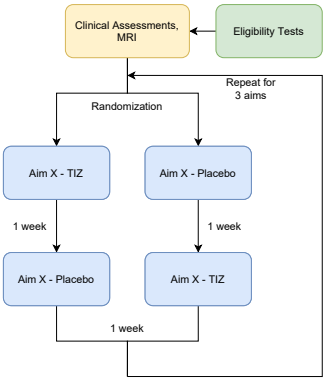


Table 1: Crossover experimental design. After confirming eligibility and performing clinical and imaging assessments, participants are randomly assigned to an aim and either TIZ or placebo. Participants then complete the same aim with the other drug condition, followed by another random aim until all 3 aims are completed. All sessions will be scheduled 1 week apart.

PROCEDURES INVOLVED:

Overall Experimental Design

Crossover Design

After confirming eligibility, all enrolled participants will be scheduled for one anatomic brain MRI data collection and 6 arm/hand experiments: 2 for each aim, one with TIZ and the other with placebo. All experiments take place on separate days at least one week apart (see *Figure 1*). During each of the experimental sessions, the participant will complete the protocol both before and 1.5 hours after administration of either TIZ or a placebo. The order of using TIZ or a placebo will be double-blinded and randomized within stratified FMA scores to ensure balanced groups for receiving TIZ or placebo during the first session. The total time from enrollment to completion of participants is expected to be 8-12 weeks, during which participants will be instructed to keep performing routine daily activities.

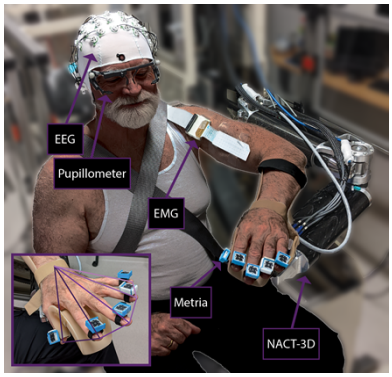


Figure 5: Experimental setup. For each aim, data will be collected using a combination of the devices shown here: NACT-3D robotic platform, finger tracking, OT-BIO EMG system for muscle activity, CORE pupillometer, and BrainVision EEG system for cortical activity. Inset: hand opening area is quantified by Hand Hexagon Area obtained from markers on each finger and the palm.

Common Experimental Device

For all aims, the experimental protocol will be carried out using a custom haptic robotic device: the New Arm Coordination Training in 3D (NACT-3D, see *Figure 2***Error! Reference source not found.**). The NACT-3D is a custom-built two-link planar robot with 3 degrees of freedom (DOF): two for movement on a horizontal plane, and one for movement perpendicular to the plane. It is instrumented with a 6 DOF force/torque sensor (MCS10-005-6C, HBK, Darmstadt, Germany), 3 DOF accelerometer on the endpoint, and encoders for tracking the

position of the proximal and distal links and the orientation of the participant's forearm. Participants are seated in a Biodex chair (Biodex Medical Systems, Shirley, NY) which is adjusted to the participant's physical dimensions. The participant's forearm is connected to the NACT-3D using a custom-sized orthosis that also immobilizes the wrist but allows free hand opening. For Aims 1 and 2, the NACT-3D will be used as a static, isometric setup with the endpoint configured to match the desired participant's elbow and shoulder joint angles, making use of the force/torque sensor to perform the necessary tasks for motor unit decomposition and EEG recordings. For Aim 3, the NACT-3D will be set to haptic mode to quantify reaching ability at various vertical loads requiring shoulder abduction effort and use position control mode to elicit stretch reflexes at precise points of a reaching movement away or toward the body. In both the static and dynamic scenarios, vertical loads imposed on the endpoint thus requiring shoulder abduction to balance, will be used to study the role and expression of the flexion synergy in motor control.

Administration of TIZ and Monitoring of NE Level

The single dose of TIZ (usually 4-8 mg per experiment) will be determined by Dr. Urday, the neurologist on the team, based on the subject-specific weight and other health-related conditions before each experiment. After finishing the first round of designated data collection for a session's Aim, a random selection of either TIZ or a placebo will be administered orally with lunch. Throughout the second round, the effects of the administered drug will be monitored by taking hourly blood pressure and heart rate measurements. Results will be discussed with Dr. Urday and confirmed to be safe before proceeding with the protocol. This TIZ/placebo protocol has already been used in 11 healthy control and 6 stroke participants for various research purposes, and no adverse events have been reported. Notably, though a different protocol and study, we found TIZ to exert no effects on participant strength in a prior study with prolonged TIZ administration[7].

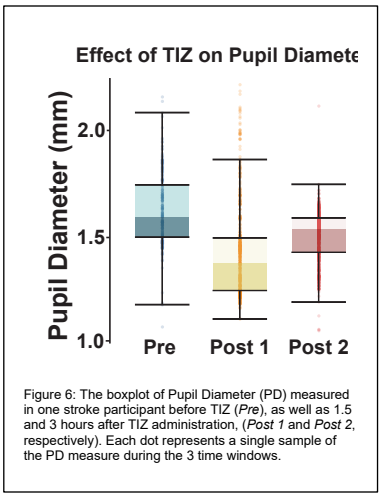


Figure 6: The boxplot of Pupil Diameter (PD) measured in one stroke participant before TIZ (*Pre*), as well as 1.5 and 3 hours after TIZ administration, (*Post 1* and *Post 2*, respectively). Each dot represents a single sample of the PD measure during the 3 time windows.

Pupil diameter has been widely used as a proxy measure of NE in both animal and human studies[38, 54]. We will measure pupil diameter every hour with pupil tracking glasses (Pupil Labs, Berlin, see *Figure 2* and *Figure 3*) that record a participant's pupil diameter for 20 seconds at a 120 Hz sampling rate in a lab with a controlled light system. During these 20 seconds, participants are instructed to relax and maintain a steady gaze at a grey cross displayed on a black screen placed ~1.5 meters in front of them. Preliminary data in *Figure 3* shows typical pupil diameter results before the use of TIZ (*Pre*), 1.5 hours (*Post 1*), and 3 hours (*Post 2*) after TIZ administration. As shown, TIZ administration significantly reduced ($p<0.05$) the pupil diameter at 1.5 hours after TIZ. This effect was observed to diminish at the 3-hour mark following the single dose ($p>0.05$), although the average pupil diameter remained lower than pre-TIZ levels.

AIM 1: Identify the effect of TIZ on spinal motoneuron excitability in chronic hemiparetic stroke.

Experimental Setup and Protocol

Participants will be seated in a Biodex chair, with shoulder and waist restraints to prevent movement. Their paretic arm will be secured in a custom orthosis and rigidly coupled to the NACT-3D robotic device (see *Figure 2*). The paretic limb will be fully supported and fixed at 85° of shoulder abduction, 40° of shoulder flexion, and 90° of elbow flexion. We will collect high-density surface electromyography with multi-channel electrode grids, placed atop the skin overlying the biceps brachii (BIC), anterior deltoid (ADL), intermediate deltoid (IDL), the long head of triceps brachii (TRI), and the flexor carpi radialis (FCR). Each array has 64 gold electrodes organized in

a 13x5 grid with an 8mm inter-electrode distance (GR08MM1305, OT Bioelettronica, Inc., Turin, IT).

For the purposes of this aim, the NACT-3D will keep the participant's arm stationary (i.e., isometric). At the onset of each experiment, participants will be asked to generate maximum voluntary isometric elbow flexion contractions, with the maximum voluntary torque (MVT) that is generated used to normalize subsequent contractions. Participants will then be asked to generate 10 isometric elbow flexion ramp contractions. Each ramp contraction will start from rest and consist of a 10-second linear increase to 30% MVT and a 10-second decrease back to rest (i.e., 3% MVT/s rise and decay speeds), with 60 s rest between ramps. Participants will receive real-time visual feedback of their torque and an idealized target ramp.

Additionally, a handheld vibrator with force sensor integration will be placed over the muscle belly of the biceps brachii to evoke TVR for a duration of 5 seconds. The TVR response will be recorded under the following conditions: relaxed, voluntary 15% MVT elbow flexion, and shoulder abduction that results in synergistically driven, involuntary, 15% MVT elbow flexion. Participants will complete 10 trials of each condition in randomized order with adequate rest in between.

The whole data collection protocol requires 35-45 mins, which will be repeated 1.5 hours after oral administration of tizanidine or placebo.

AIM 2: Determine whether norepinephrine amplifies the commands of indirect motor pathways.

Experimental Setup and Protocol

The experimental setup, including the isometric use of the NACT-3D robot, the arm configuration, and the display is the same as that used in Aim 1, except for the following three components. First, instead of using the surface array EMG electrodes, we will use bipolar EMG electrodes on the anterior deltoid (ADL), intermediate deltoid (IDL), biceps brachii (BIC), the extensor digitorum communis (EDC), and the flexor carpi radialis (FCR). Second, we will use a 64-channel EEG system to collect scalp EEG data and 2 extra-channels for electrooculogram (EOG) data measurement. Electrode gel will be used to reduce the impedance of all electrodes to less than 5 kΩ. Third, we will use a markerless motion capture system to capture hand and arm kinematics. Six FLIR Blackfly S GigE cameras (Teledyne, Ontario, CA) will be synchronized together and will acquire data at 30 fps [12]. The cameras will be calibrated prior to data acquisition. PosePipe will be used to manage the videos and track the upper limb during movement [12]. We will use existing whole-body model algorithms [12] adapted to focus on the hand, arm, and torso. The output of these algorithms is the kinematic data for the 12 finger joints (3 per finger) and 2 thumb joints.

At the beginning of each session, we will measure the maximum voluntary SABD torque (MVT) and maximal EMGs for all involved muscles, for subsequent normalization. Each participant will then perform a randomly selected single task, either 1) a maximal hand open (OPEN) or 2) a 30% of SABD MVT (SABD) for 10 seconds. This single task will then be followed by a dual task that requires a maximal hand open while holding 30% SABD torque (OPEN+SABD) for 5 seconds. In total, each participant will perform 7 trials with OPEN first (i.e., OPEN-then-'OPEN+SABD') and 7 trials with the SABD first (i.e. SABD-then-'OPEN+SABD'), with the order of these tasks randomized. Throughout the OPEN phase, participants will see a hand-opening picture with the filling of the hand indicating time. During the SABD phase, participants will receive visual feedback of their SABD torque and an idealized target trace consisting of a two-second ramp up to 30% SABD followed by a 10-second hold at the target torque. During the dual task phase, participants will receive both the hand picture and the SABD bar. Participants will be reminded to avoid muscle activity unrelated to the task (e.g., in the face, jaw, eyes) while performing each trial. A one-minute

break time will be provided between trials to avoid fatigue. In total, this data collection protocol lasts about 20-25 minutes, and will be repeated 1.5 hours after administration of TIZ or placebo.

AIM 3: Evaluate how norepinephrine modulates the expression of the flexion synergy and spasticity.

Experimental Setup and Protocol

The experimental setup will be the same as that described in Aim 1 and 2, with the main difference being the use of the haptic and perturbation features of the NACT-3D to allow for reaching movements.

For reaching tasks, the participant will see a starting position indicated on a screen, placed 5 cm in front of the sternum parallel to the sagittal plane, and a target position that is 5 cm beyond their maximal reaching distance (based on limb segment lengths). During each trial, while in the robotic setup, the participant will be instructed to move to and maintain the starting position for 3 seconds, then slowly reach, as far as possible, outward to the target position and hold that position for 3 seconds before moving back to the starting position. These reaches will be performed slowly to avoid any velocity-related influences of spasticity. The reaches will be performed with two different shoulder loading conditions: fully supported (*supported)* and at 50% of their maximal SABD torque (*loaded*). During the *loaded* conditions, participants are instructed to lift their paretic arm above the haptic surface and reach for the target without touching the surface. In total, 15 reaches will be collected per loading (*supported*/*loaded*) condition, with adequate breaks to avoid fatigue.

For hand opening tasks, the participant's hand will be placed as close to the target position described above as possible, within their maximal reaching distance. The arm will either be supported by the haptic surface or subjected to the aforementioned shoulder abduction load while maintaining the arm above the haptic surface. While maintaining this position, the participant is asked to volitionally open the hand as much as they can, spreading and stretching the fingers to span the largest possible area with the fingertips and hand opening will be generated by stimulating wrist and finger extensor muscles using the Zynex Medical E-Wave Stimulator (510(k) FDA cleared within the USA, from Englewood, CO USA). Optical markers (Metria system, see Figure 2) are placed on the wrist, palm, and distal phalanges, with cameras placed at key positions around the participant to obtain visual coverage for all possible hand orientations; see Figure 2. Also, markerless based kinematics will be implemented using an array of cameras and artificial intelligence (AI) if marker-based kinematics is too dificult to implement. In total, 5 trials will be collected per loading (supported/loaded) condition, with adequate breaks to avoid fatigue.

For stretch reflex tasks, we will first ask the participant to reach from the starting position toward the target position three times as quickly as possible, with the positions being the same as in the reaching trials described above. Data in these trials will be used to determine the participant's maximal voluntary movement velocity. In subsequent trials, participants will be instructed to start at the starting position and hold there for 3 seconds, then to reach as quickly as possible towards the target, while on the haptic surface or lifting against an SABD load. During random trials, the NACT-3D will perturb the tested arm at one of two timepoints: either prior to movement, or during movement when the instantaneous reaching speed exceeds 5% of the maximal velocity. This perturbation will rapidly extend the elbow at a rate of 270 °/s over a 50 ms period (with a 15 ms ramp-up to the target velocity, a 20 ms plateau phase, and a 15 ms ramp-down to 0 °/s), and finally, a 250 ms movement-block during which the participant's velocity is forced at 0 °/s by the robot. Trials end after the movement block is over, or when the participant completes the ballistic reach. For each loading condition, trials will be collected until 5 unperturbed reaches, 5 perturbations prior to movement onset, and 5 perturbations at movement onset have been collected. The occurrence of the perturbations will be randomized, with adequate breaks to avoid fatigue.

The total duration for these 3 protocols (slow reaching, hand opening, and ballistic reaching + stretch reflex) is about 25 mins. These protocols will be repeated at 1.5 hours after TIZ or placebo administration.

SHARING RESULTS WITH PARTICIPANTS

In normal cases, we will not share study results or individual participant results to persons that do not belong to this study. However, in special cases, including but not limited to, that we found doubtable readings from a participant's brain image, or clinical tests, we will share these results with the participant. With participant's agreement, we may also share related information with participant's primary care physician.

STUDY TIMELINES

Duration of an individual's participation: Each participant will be enrolled in this study until they have completed the two experiments (PLB or TIZ, randomized) for each protocol outlined in this proposal. Experiments will be scheduled flexible to the participant and research team's time. It is mandatory that the experiments are scheduled at least a week apart which allows for sufficient wash out of the drug.

Duration of the whole study: All pilot experiments have been designed. In the first 6 months of the grant, we will recruit and train the new team members, like new research assistants and research physical therapists, using the developed experimental protocols and other human subjects-related training. During the preparation phase, we will also conduct the final tests and integration of the devices. Recruitment will commence during this period and subsequently recruitment, data collection, and data processing of Aims 1, 2, and 3 will occur in parallel as much as is feasible. Where possible, experimental sessions for these aims will be combined. We aim to start recruitment within 6 months and collect and process data immediately after IRB approval. After the first 5 sets of data have been collected, a review of the quality of the collected data will be performed, and any final major revisions to the protocols and hardware will be made. After the review is complete, we aim to recruit and collect data from 16 participants per year for the following 4 years, for a total of 64 participants. While making progress, we will publish our preliminary results in various conferences and results in professional journals. During the final year of the

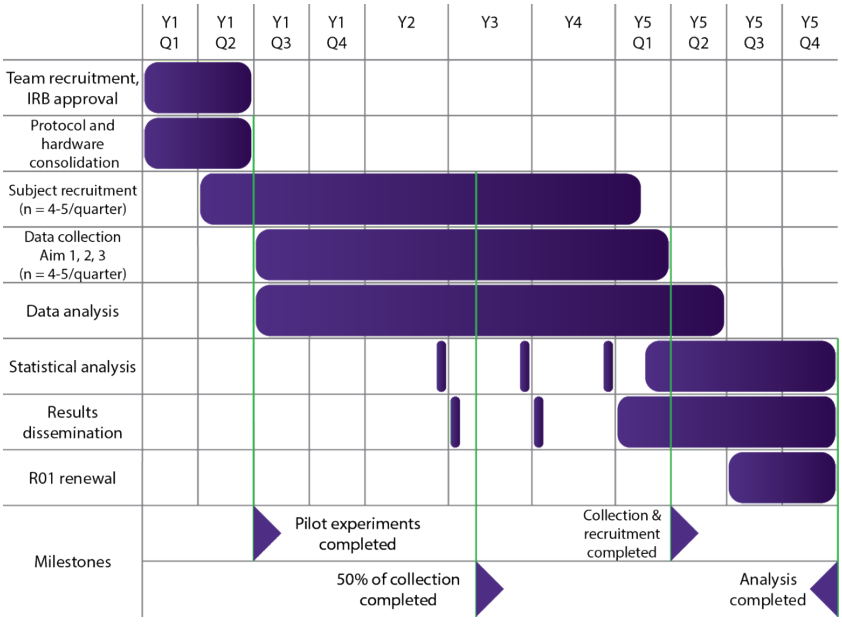


Table 2. The study timeline.

grant, synthesized data analysis will be completed, and the overall results will be used for publications to disseminate the findings from the various aims. An overview of the timeline is given in table X.

INCLUSION AND EXCLUSION CRITERIA

The following inclusion criteria will be applied to the stroke participants: 1) History of unilateral supratentorial ischemic stroke that occurred at least six months prior to enrollment; 2) Age between 18-80; 3) Paresis confined to one side, with substantial motor impairment of the upper limb and some residual voluntary movement (Upper Extremity Fugl-Meyer Assessment in the range of 10-50/66); 4) Ability to communicate, understand, and provide informed consent; 5) Ability to elevate their limb against gravity up to at least 75 degrees of shoulder flexion and to generate active elbow extension; 6) MRI compatible; 7) Intact skin on the hemiparetic arm; 8) Ability to sit for three hours.

Exclusion criteria are: 1) Motor or sensory impairment in the non-affected limb (FMA<66, filament >3.6); 2) Any brainstem and/or cerebellar lesion; 3) untreated cardiovascular disease; 4) History of neurologic disorder other than stroke that affects the arms; 5) Any acute or chronic painful condition in the upper extremities or spine, indicated by a score ≥5 on a 10-point visual analog scale; 6) Current use of a pacemaker; 7) History of seizure disorder; 8) Chemo denervation: botulinum toxin injection to any portion of the paretic upper extremity within the last 6 months, or phenol/alcohol injections <12 months before participation; 9) Flexion contractures larger than 30 degrees in the elbow, wrist, metacarpophalangeal joints (MCP) and interphalangeal joints (IP) after stretching for 15 minutes; 10) Current participation in any experimental rehabilitation or drug studies; 11) Individuals with any known contraindications to tizanidine or currently taking tizanidine or other pharmaceutical agents to control spasticity; 12) concurrent use of medications known to suppress central nervous system activity; 13) pregnant women or women who are nursing;

Additionally, each participant will be asked to provide a list of their current medications and a medical screening questionnaire will be sent to their primary physician. Each participant's list of medications will be reviewed for possible interactions with the study drug and, at the study physician's advise, will be excluded from the study or asked to withhold medications when applicable. Specifically, those taking medications that are known CYP1A2 inhibitors, oral contraceptives, central nervous system depressants, α-2 adrenergic agonists, anti-hypertensives will be excluded if the medications cannot be held (e.g. the day of the study or taken in the evening instead of the morning). Additional medication interactions will be screened using UpToDate, Connor RF (Ed), Wolters Kluwer.

VULNERABLE POPULATIONS

We will not recruit pregnant woman, neonates, neonates of uncertain viability, prisoners, children, or adults with impaired decision making.

PARTICIPANT POPULATION(S)

Accrual Number:	Category/Group:	Consented:	Enrolled:

	(Adults/Children Special/Vulnerable Populations)	Maximum Number to be Consented or Reviewed/Collected/Screened	Number to Complete the Study or Needed to Address the Research Question
Local	64/0/0	1000/80/600	64
Study-wide	64/0/0	1000/80/600	64
Total:	64/0/0	1000/80/600	64

RECRUITMENT METHODS:

Stroke participants will be recruited from the Clinical Research Registry maintained by the Shirley Ryan Ability Lab. Additionally, recruitment flyers will be placed around the university and may be shared online through departmental social media. Research team will also contract individuals who signed up for participating studies in the PTHMS department before and agreed to be contacted for research purposes. Potential participants may also be referred to the research team by clinicians, other researchers, and current or previous participants. Research team may also utilize Northwestern Medicine Health Care's electronic health record (NMHC EHR) and enterprise data warehouse (EDW) report for direct participant recruitment. Use of the EDW will involve working with the EDW analytical team to identify individuals that meet the study's inclusion/exclusion criteria. The study team will contact these individuals via telephone or email. Research team may also use Epic to send an approved email to eligible individuals, notifying them to log on to their MyChart portal to learn about the study.

Research team may also collaborate with other researchers and labs to organize open house events where flyers and study information will be distributed, disseminated, and presented to potential participants interested in joining studies for individuals with stroke. The study flyers distributed at these open house events may be created by the marketing department from SRALab.

Study may also use the OpenClinica Recruit (formerly BuildClinical) platform for recruitment. OpenClinica Recruit is a data-driven platform that helps academic researchers recruit participants for research studies more efficiently using social media, software, and machine learning. It utilizes study-specific advertisements to engage participants on digital platforms such as Facebook, Google, WebMD, etc. and redirect them to a study-specific landing page should they click it. OpenClinica's Secure Socket Layer (SSL) software encrypts all inputted information and keeps participant information private and HIPAA compliant. The platform's backend servers are stored in the US at some of the most secure data centers in the world.

Research assistants, research coordinator, PhD students, research physical therapists, and study physicians (Dr. Urday) are responsible for calling and screening potential participants. REDCap database (Research Electronic Data Capture, Vanderbilt University) will be enlisted to manage subject recruitment and data.

COMPENSATION FOR PARTICIPATION IN RESEARCH ACTIVITIES

Participants will receive $50 for the consenting and screening visit, which determines eligibility and enrollment in the study. This visit will last approximately 2 hours. In the event that the consenting and screening visit lasts an hour or less, participants will receive $25.

After enrollment, participants will be scheduled for future visits. For these visits, they will be compensated using one of the following two methods, whichever results in lower total payment per visit:

1. $150 per experimental visit (typically lasting about 6 hours) for participation in one experimental session of the study. If a visit exceeds 6 hours, the participant will receive an additional $20 per hour for the extra time. Time will be rounded up if the additional duration is 30 minutes or over the hour mark. For example, if the visit is 6 hours and 45 minutes, the participant will be paid $150 for the first 6 hours and $20 for the additional 45 minutes. If the duration is 29 minutes or less over the hour mark, time will be rounded down to the nearest hour. For example, if the visit is 6 hours and 20 minutes, the participant would be compensated $150.
2. $25 per hour for the total duration of the visit. Time will be rounded up if the duration is 30 minutes or over the hour mark. For example, if a visit is 2 hours and 45 minutes, the participant will be paid for 3 hours. If the duration is 29 minutes or less over the hour mark, the time will be rounded down to the nearest hour. For example, if a visit is 2 hours and 20 minutes, the participant will be paid for 2 hours.

Reimbursement for public transportation will be added to the $150 per visit or $25 per hour compensation. If the participant would like to use Lyft, they will be asked to contact the research team so a Lyft can be ordered for them to bring them to lab. At the end of the visit, another Lyft will be ordered by the research team for them to depart lab.

If a participant drives to or is driven to and dropped off at the lab by a family member or friend, one parking voucher will be provided per visit. Participants are responsible for their own gas expenses. In special circumstances—such as severe weather events—the study team may cover the cost of a private rideshare service vehicle ordered by the participant (e.g., Lyft or Uber), at the discretion of the Principal Investigator (PI).

For experimental visit days which are scheduled for 6 hours (typically 9am to 3pm), participants will be encouraged to bring a pre-packaged lunch from home. In the event that the participant did not bring lunch with them, lunch will be covered for the participants, up to $25 (with tax).

Communication-related expenses (e.g., phone calls) will not be reimbursed.

Reimbursement and payment will be submitted to the accounting department on a weekly basis. Typically, the accounting department will mail a participant a check 4 weeks after receiving the payment request. Money will be paid by check via standard mail approximately 3-4 weeks following each of the visits. The Accounting Services at Northwestern University will be given a participant's name, address, and Social Security Number in order to issue a check for the study participation. Study payments are considered taxable income and reportable to the IRS. A Form 1099 will be sent to a participant if their total payments are $600 or more in a calendar year.

WITHDRAWAL OF PARTICIPANTS:

Participants can withdraw from the study at any moment during the course of the experimental protocol. Participant safety concerns may result in involuntary termination. Examples of such are rare but include inconsistent and unsafe operation of the robotic protocol despite adequate instruction and practice and inadequate physical endurance/cognitive constitution to complete individual assessments (indicated by symptomatic hyperventilation, vasovagal response, anxiety).

RISKS TO PARTICIPANTS:

The measurements made in this study are noninvasive and the risks are small. Participants will be made aware of all risks in detail (see below). Participants are allowed to withdraw from the study at any time.

Muscle Soreness - Reaching movements: The primary risks of the proposed work are muscle soreness. Muscle soreness is a normal response to resistance exercise and may occur 24-48 hours after the experiment. Additionally, although the movements of a participant's arm will not exceed their elbow's normal range of motion, repeated motions may cause minimal temporary discomfort. This potential discomfort will be addressed by stretching or massaging of the arm prior to and following the experiment.

Skin Irritation: Skin irritation may occur because of the placement of surface EMG and/or EEG electrodes. The possibility of irritation will be minimized by cleaning the skin with alcohol before and after the application of the EMG electrodes. There is a similar possibility of scalp irritation due to the use of EEG electrodes which will be minimized by prompt removal of the electrodes/gel following the experiment. Additionally, to secure the EEG electrodes on the scalp, a cap with a strap around the chin will be used, which may lead to potential discomfort. We will place cotton or foam underneath the strap to minimize this side effect. There is no known increase in risk in performing EMG or EEG recording on individuals with stroke.

Drug Probes: The medications used in this study may produce side effects (see details below) that should be reported immediately to the study monitor. If, at any point, the participant or the researchers notice any adverse effects because of drug ingestion, participants will be withdrawn from the experiment and monitored closely by trained study personnel. Additionally, participants will be encouraged to contact the study team with any questions or concerns about potential side effects after the experiment to ensure that participant safety is paramount. The participants' heart rate and blood pressure will be documented routinely during each experimental session.

Potential side effects for *tizanidine (TIZ)* are as follows*:

- TIZ may cause hypotension (low blood pressure), syncope (fainting due to low blood pressure), dizziness, bradycardia (slow heart rate), dry mouth, asthenia (weakness/fatigue), constipation, vomiting, blurry vision, flu-like symptoms, nervousness, dyskinesia (abnormal body movement), hepatotoxicity (liver injury), sedation (drowsiness), rhinitis/pharyngitis (inflammation of nose/throat), hallucinations or psychosis (found in 3% of patients studied), hypersensitivity reaction (allergic reaction that could include rash, itching, trouble breathing), anaphylaxis (life-threatening allergic reaction)

- There are less common (<3%) reactions of Steven Johnson Syndrome (rare reaction with fever, rash, blisters on mouth), allergic reaction to the medication, seizure, depression, ventricular tachycardia (heart arrhythmia), tremors, arthralgia (aching joints), that were reported by people who were taking tizanidine, which may or may not be due to the medication.
- There is a risk in individuals with liver or kidney problems, so individuals with liver and kidney problems may be asked to refrain from participating in the study.

** Of note, these side effects are documented with prolonged administration, whereas this study employs only a single dose.*

The study physician will discuss the potential of lactose side effects with lactose intolerant participants during the initial screening visit. Research staff will ask about and document lactose intolerant status during phone screening.

Potential side effects of lactose are as follows:

- Abdominal pain and bloating
- Excessive flatus
- Watery stool following the ingestion of foods containing lactose[80]

Drug holding

- Participant's daily medications are taken into account for inclusion/exclusion and whether their participation is safe to enroll in this study. Study physician, Dr. Urday, may deem a participant safe to enroll but advise holding a medication (e.g. on the day of the study or taken in the evening rather than the morning)
- This is usually in the case of blood pressure or sedating medications, so symptoms of increased or decreased blood pressure or drowsiness are not compounded on experiment day.
- Potential risks include change to blood pressure which study team will monitor throughout the study day to ensure it stays in safe range.

Taking MRI: Participants may be asked to complete an MRI scan at the Center for Translational Imaging at the Feinberg School of Medicine at Northwestern University, located at 710 N. Fairbanks Ct., Lower Concourse Chicago, Illinois 60611.

MRIs use strong magnets when creating images, so metal in a participant's body that's attracted to magnets can alter the images or even cause metal objects to move. To ensure the MRI is safe, participants will need to fill out a screening form about any metal in their body and answer questions about their medical history that impact or could lead to harm if they have an MRI prior to starting this study. They will change into gowns to ensure no metal is on their clothing, be visually inspected for any metal, and go through sensitive metal detectors. They will be asked to tell the researchers if they have:

- Metal fragments in their eyes or face
- Implants like pacemakers, defibrillators, cochlear implants, or nerve stimulators
- Had surgery on their brain's blood vessels or heart valves
- Claustrophobia (fear of small spaces)
- Body piercings or tattoos

If they have metal in their body, they may not be able to have the research MRI unless the metal is determined to be MRI-safe.

Before the MRI, participants will be given instructions, such as lying very still on a table, and that their head will be positioned in a special holder with a foam cushion. The table will slide into the MRI scanner, and the scan will take around 20 minutes.

Participants will be asked to let the researchers or MRI technicians know if they feel uncomfortable or if they need to stop the MRI procedure. During the MRI procedure, some people feel fatigued, uncomfortable, or claustrophobic in the scanner. Therefore, they can terminate the MRI scan at any time by pushing the emergency squeeze ball and by communicating with the research team using the 2-way sound system that includes headphones. Participants will be given earplugs or headphones to protect their hearing. These headphones will also significantly reduce the scanner noise. They will be able to communicate with the MRI technologist through an intercom during the MRI scan for any reason. Finally, there are 2 digital cameras located inside the magnet room (MRA Inc, Pittsburgh, PA). They provide continuous monitoring of the whole room and a close-up view of the participant for safety purposes.

Risk to privacy and/or confidentiality: This study involves the use of a participant's identifiable, personal information:

- All information in a medical record
- Results of physical examinations
- Medical history
- Lab tests, or certain health information indicating or relating to a particular condition as well diaries and questionnaires
- Records about study medication or drugs
- Records about study devices
- Billing information
- Mental Health information

There is a chance that a loss of confidentiality could occur. The researchers have procedures in place to lessen the possibility of this happening.

Other risk: In addition to these risks, this research may hurt a participant in ways that are unknown. These may be a minor inconvenience or may be so severe as to cause death.

POTENTIAL BENEFITS TO PARTICIPANTS

We cannot promise any direct benefits to participants or others from taking part in this research.

DATA MANAGEMENT AND CONFIDENTIALITY

Subject Selection and Power Analysis

We will recruit 64 moderately to severely impaired individuals (based on the Fugl-Meyer Motor Assessment (FMA)), each to complete all experiments for the three aims. The sample size (N = 64) has been determined based on our preliminary results for each aim. Briefly, for each primary measure of a specific aim, we initially assessed the normality of the measurements. If the data

followed a normal distribution, the power analysis relied on a paired t-test to examine the difference in the primary measure (e.g., Brace Height for Aim 1) from zero before and after TIZ administration. In cases where the distribution exhibited a significant deviation from normality, we employed a Wilcoxon signed rank-sum test, a non-parametric equivalent of the paired-test. The power analysis aimed to establish the required sample size to achieve over 80% power at a significance level (alpha) of 0.05. This power analysis was replicated for each hypothesis across various aims, and the maximum required sample size (i.e., N = 52 for aim 3) was adopted to ensure sufficient power for all aims. An allowance for up to 20% attrition was factored in, resulting in the final sample size of 64 (please refer to the *Statistical Design and Power* section in the Human Subject and Clinical Trial document).

AIM 1: Identify the effect of TIZ on spinal motoneuron excitability in chronic hemiparetic stroke.

Data Collection and Analysis

Elbow flexion torque will be quantified via a Jacobian based algorithm from the forces and moments measured by the force/torque sensor. EMG signals will be collected with a Quattrocento signal amplifier (OT Bioelettronica, Inc., Turin, IT; input range: 33mVpp), amplified (x150), band-pass filtered (10-500Hz), and sampled at 2048 Hz. The EMG activity during each trial will be decomposed into individual discharge instances of concurrently active MUs using convolutive blind source separation[43, 44]. Continuous estimates of MU discharge rate will then be generated with non-linear regression with the following metrics. (See *Figure 4B-C*)

Brace Height (BH): To estimate the amplification of MU discharge rate related to PICs, we will use Brace Height (green line *Figure 4C*). Brace Height is quantified as the magnitude of the largest orthogonal line between a theoretical linear increase in discharge rate and the observed discharge rate from MU recruitment to peak discharge. Notably, BH is quantified on MU discharge rate as a function of the joint torque, or behavior, that is performed during a contraction. Recent work from our group has validated this metric as a sensitive quantifier of PIC magnitude[45, 46]. Brace Height will be our primary metric.

ΔF: To estimate the prolongation of MU discharge rate related to PICs, we will use Delta-F (ΔF). ΔF is a commonly employed metric and represents the hysteresis of a higher threshold MU with respect to the discharge rate of a lower threshold unit. ΔF for a given MU (test unit, MU2 in *Figure 4B*) is quantified as the change in discharge rate of a lower threshold MU (MU1 in *Figure 4B*) between the recruitment and de-recruitment instance of the test unit[61].

TVR-MU: To estimate the excitability of the biceps motor unit: Decomposing MU from TVR trials where participants arm is relaxed, voluntary EF, and synergy EF. Assess and quantify the sustained firing of biceps motor unit in response to vibration.

Statistical Analysis

We will use a linear mixed model to predict changes in BH and ΔF with fixed effects of drug administration (pre, post), drug (TIZ, placebo), and their interaction. Participants will be treated as random effects. Exploratory analysis of race, gender, age, and impairment level will be performed and employed as covariates, when necessary, as demographic factors may influence baseline motoneuron excitability. To ensure the validity of model fitting, the assumptions of linearity and normal, homoscedastic residual distributions will be inspected. Statistical significance will be set at type I error rate (alpha) = 0.05.

AIM 2: Determine whether norepinephrine amplifies the commands of indirect motor pathways.

Experimental Setup and Protocol

The experimental setup, including the isometric use of the NACT-3D robot, the arm configuration, and the display is the same as that used in Aim 1, except for the following three components. First, instead of using the surface array EMG electrodes, we will use bipolar EMG electrodes on the anterior deltoid (ADL), intermediate deltoid (IDL), biceps brachii (BIC), the extensor digitorum communis (EDC), and the flexor carpi radialis (FCR). Second, we will use a 64-channel EEG system to collect scalp EEG data and 2 extra-channels for electrooculogram (EOG) data measurement. Electrode gel will be used to reduce the impedance of all electrodes to less than 5 kΩ. Third, we will use a TrakSTAR motion capture system (see *Figure 2*; Northern Digital, Inc, Waterloo, Canada) to quantify hand opening. This system uses electromagnetic tracking to locate finger position in space.

At the beginning of each session, we will measure the maximum voluntary SABD torque (MVT) and maximal EMGs for all involved muscles, for subsequent normalization. Each participant will then perform a randomly selected single task, either 1) a maximal hand open (OPEN) or 2) a 30% of SABD MVT (SABD) for 10 seconds. This single task will then be followed by a dual task that requires a maximal hand open while holding 30% SABD torque (OPEN+SABD) for 5 seconds. In total, each participant will perform 7 trials with OPEN first (i.e., OPEN-then-'OPEN+SABD') and 7 trials with the SABD first (i.e. SABD-then-'OPEN+SABD'), with the order of these tasks randomized. Throughout the OPEN phase, participants will see a hand-opening picture with the filling of the hand indicating time. During the SABD phase, participants will receive visual feedback of their SABD torque and an idealized target trace consisting of a two-second ramp up to 30% SABD followed by a 10-second hold at the target torque. During the dual task phase, participants will receive both the hand picture and the SABD bar. Participants will be reminded to avoid muscle activity unrelated to the task (e.g., in the face, jaw, eyes) while performing each trial. A one-minute break time will be provided between trials to avoid fatigue. In total, this data collection protocol lasts about 20-25 minutes, and will be repeated 1.5 hours after administration of TIZ or placebo.

Data Collection and Analysis

All EEG, EMG and EOG data will be collected using the BrainVision system (Brain Vision, LLC, NC, USA) with a 2500 Hz sampling rate. Furthermore, EEG electrode positions will be digitized (KRIOS, NDI, Canada). Force and moment data will be collected at 1000 Hz with the force/torque sensor. Pupil diameter data will be collected by pupil tracking glasses (Pupil Labs, Berlin) at 120 Hz. In addition, we will collect MRI scans at Northwestern University's Center for Translation Imaging on a 3 Tesla Siemens Prisma scanner with a 64-channel head coil in a different session. Structural T1-weighted scans will be acquired using an MP-RAGE sequence (TR = 2.3s, TE = 2.94ms, FOV = 256x256mm^2), producing an isotropic voxel resolution of 1x1x1 mm^3. Visual inspection of acquired images will be performed immediately following the data acquisition to guarantee no artifacts and a stable head position. MRI data will be backed up on the Northwestern University Research Imaging Processing System (NURIPS), a web-based data repository and processing system. EEG, EMG, EOG, MRI, and other participant data collected during experimental sessions will be stored on FSMResFiles, a centralized data storage platform exclusively for research use.

MRI data processing: MRI data will be imported to CURRY software (Compumedics Neuroscan Ltd., Charlotte, NC) to build a subject-specific boundary element method (BEM) model as used previously[47, 48]. The use of the subject-specific BEM model will enhance seed location estimation.

EEG Data pre-processing: EEG data will be imported into SPM12 software (SPM12, Centre for Human Neuroimaging, www.fil.ion.ucl.ac.uk/spm/). This data will first be inspected to remove trials with eye or muscle movement artifacts and undergo a bandpass filter (5-50 Hz). For the remaining artifact-free trials, the onset of EMG will be detected offline to align EEG signals in

individual trials. We will then project the artifact-free trials from the channel space to the cortical sources. Such inverse calculation will use the generalized inverse of the lead-field matrix based on a subject-specific BEM model and an equivalent current dipole for each of the regions of interest (ROIs), including bilateral primary sensorimotor cortices (SM1), premotor area (PM), combined pre-supplemental and supplementary motor areas (SMA), and the medial and anterior prefrontal cortex (PFC). The selection of these source areas is based on recent human cRST and CST pathways[75]. The EEG data at the source level will be further used for corticomuscular coherence and cortical activity analysis.

We will compute Corticomuscular coherence (CMC) as the primary measure of descending track usage. EMG data will undergo a bandpass filter (5–200 Hz) and notch filters to remove 60 Hz power line voltage and its harmonics. Activity of each ROI (the inverse results of EEG) and EMG data will be segmented into one-second epochs. We will then calculate the wavelet coherence between pairs of cortical source EEG (8 sources in total) and muscle EMG. We choose wavelet coherence (WTC) as opposed to regular Fourier Transform based coherence as WTC is more sensitive to non-stationary signals, such as physiological signals measured by EEG and EMG. This wavelet coherence matrix will then be averaged in a specific frequency range across all the holding time to obtain the CMC value for one pair of cortical ROI and muscle, which will be used as our primary measure for this aim. We are particularly interested in the alpha (8-12 Hz) and beta (13-30 Hz) bands.

We will compute the laterality index (LI) as the primary measure of shift in cortical activity. First, we will calculate the cortical activity ratio (CAR) as the strength of activity in a particular ROI divided by the total strength of activity observed across all ROIs[21]. We will check the CAR in the alpha (8-12 Hz) and beta (13-30 Hz) bands for each task (SABD and OPEN). Using CAR, we can calculate the laterality index (LI) by calculating the ratio of cortical activity as (ipsilesional – contralesional) / (ipsilesional + contralesional). We will check LI together with CAR when we need to understand whether the shift is due to changes from a particular hemisphere or from both hemispheres.

Shoulder and elbow torque couple: We will calculate the shoulder and elbow torque using the force/torque measurements from the NACT-3D and the participant's arm length measures. We will calculate the ratio of elbow torque to shoulder torque to measure the spontaneous torque coupling at these two joints.

Statistical analysis: A linear mixed effects model will be used to determine whether the drug administration (pre, post), drug use (TIZ and placebo), motor tasks (SABD, OPEN, SABD+OPEN), and their interaction explain the measured changes in each pair of CMC (= outcome) in the alpha and beta bands, separately. Furthermore, a linear mixed model will be used to determine whether the drug administration, drug use, motor tasks, and their interaction explain the measured changes in LI (= outcome). As a secondary measure, at each of the ROIs (bilateral SM1, PM, PFC, SMA for a total of 8 ROIs), we will use a linear mixed model to check whether each factor, as listed above, and their interactions explain the changes in CAR (= outcome). Our proposed model will detect and adjust for the potential differences caused by impairment level, hand opening, torque coupling, age, gender, and race. Demographic factors may influence the outcome metrics irrespective of drug condition, and task performance (hand opening, torque coupling) may confound interpretation since the task pre-/post-TIZ may change. For all linear mixed effects models, we will check if the model assumptions are met. Statistical significance will be set at type I error rate (alpha) of 0.05, with Bonferroni correction for multiple comparisons.

AIM 3: Evaluate how norepinephrine modulates the expression of the flexion synergy and spasticity.

Experimental Setup and Protocol

The experimental setup will be the same as that described in Aim 1 and 2, with the main difference being the use of the haptic and perturbation features of the NACT-3D to allow for reaching movements.

For reaching tasks, the participant will see a starting position indicated on a screen, placed 5 cm in front of the sternum parallel to the sagittal plane, and a target position that is 5 cm beyond their maximal reaching distance (based on limb segment lengths). During each trial, while in the robotic setup, the participant will be instructed to move to and maintain the starting position for 3 seconds, then slowly reach, as far as possible, outward to the target position and hold that position for 3 seconds before moving back to the starting position. These reaches will be performed slowly to avoid any velocity-related influences of spasticity. The reaches will be performed with two different shoulder loading conditions: fully supported (*supported)* and at 50% of their maximal SABD torque (*loaded*). During the *loaded* conditions, participants are instructed to lift their paretic arm above the haptic surface and reach for the target without touching the surface. In total, 15 reaches will be collected per loading (*supported*/*loaded*) condition, with adequate breaks to avoid fatigue.

For hand opening tasks, the participant's hand will be placed as close to the target position described above as possible, within their maximal reaching distance. The arm will either be supported by the haptic surface or subjected to the aforementioned shoulder abduction load while maintaining the arm above the haptic surface. While maintaining this position, the participant is asked to open the hand as much as they can, spreading and stretching the fingers to span the largest possible area with the fingertips. Markerless motion capture system with six FLIR Blackfly S GigE cameras will locate the wrist, palm, and distal phalanges, with cameras placed at key positions around the participant to obtain visual coverage for all possible hand orientations; see *Figure 2*. In total, 5 trials will be collected per loading (*supported*/*loaded*) condition, with adequate breaks to avoid fatigue.

For stretch reflex tasks, we will first ask the participant to reach from the starting position toward the target position three times as quickly as possible, with the positions being the same as in the reaching trials described above. Data in these trials will be used to determine the participant's maximal voluntary movement velocity. In subsequent trials, participants will be instructed to start at the starting position and hold there for 3 seconds, then to reach as quickly as possible towards the target, while on the haptic surface or lifting against an SABD load. During random trials, the NACT-3D will perturb the tested arm at one of two timepoints: either prior to movement, or during movement when the instantaneous reaching speed exceeds 5% of the maximal velocity. This perturbation will rapidly extend the elbow at a rate of 270 °/s over a 50 ms period (with a 15 ms ramp-up to the target velocity, a 20 ms plateau phase, and a 15 ms ramp-down to 0 °/s), and finally, a 250 ms movement-block during which the participant's velocity is forced at 0 °/s by the robot. Trials end after the movement block is over, or when the participant completes the ballistic reach. For each loading condition, trials will be collected until 5 unperturbed reaches, 5 perturbations prior to movement onset, and 5 perturbations at movement onset have been collected. The occurrence of the perturbations will be randomized, with adequate breaks to avoid fatigue.

The total duration for these 3 protocols (slow reaching, hand opening, and ballistic reaching + stretch reflex) is about 25 mins. These protocols will be repeated at 1.5 hours after TIZ or placebo administration.

Data Collection and Analysis

EMG data will be collected with the BrainVision system (Brain Vision, LLC, NC, USA) with a 2500 Hz sampling rate. Force/torque, and kinematic data will be collected at 1000 Hz by the NACT-3D system.

Reaching ability: The position of the participant's hand and shoulder and elbow joint angles will be calculated based on filtered encoder measurements in the robotic system. Reaching distance will be normalized to limb length and calculated as the most distant point in the forward direction reached by the participant's hand during a reaching motion.

Hand opening ability: Image processing and analysis software will be used to determine the position of the 5 fingertips and palm in space, based on the recorded position of the optical markers. Estimated positions for these landmarks will be filtered and a hexagonal polygon (see *Figure 2*) will be drawn between the landmarks at each timepoint[77]. The area of the hexagon ("hand hexagon area" or HHA) will be normalized to the length of the finger segments and size of the palm and used as a measure for hand opening ability.

Reflex excitability: EMG recordings will be taken from the BIC, TRI, ADL and IDL of the paretic arm using the average of the post-processed data from the EMG electrodes. EMG data will be normalized to 100% MVC, collected during separate trials. Reflexive EMG will be evaluated during the Short-Latency Reflex (SLR) window from 25 to 75 ms post perturbation onset. During perturbed trials, the onset occurs at one of two time points: either prior to movement (*prior)*, or during movement when the instantaneous reaching speed exceeds 5% of the maximal velocity (*onset*). During unperturbed trials, muscle activity is evaluated at the same timepoints, but no perturbation occurs when the conditions are met. The difference in EMG between the perturbed and unperturbed time windows will be taken as the reflexive muscle activity following a stretch perturbation.

Statistical Analysis

Separately for reaching distance and hand opening area, both after normalization, a Linear Mixed Effects Model will be used to estimate the influence of the drug-administration (*pre, post*), drug-use (*TIZ, placebo*), loading condition (*supported*, *loaded*) and their interactions on distance/area, with participant as a random factor. For reflexive muscle activity, a Linear Mixed Effects Model will be used to estimate the influence of drug-administration (*pre, post*), drug-use (*TIZ, placebo*), loading condition (*supported*, *loaded*), time window (*prior*, *onset*) and their interactions on reflexive EMG, with participant as a random factor. Our proposed model will account for potential differences caused by age, gender, and race. For all LME models, we will check for the model assumptions. Statistical significance will be set at type I error rate (alpha) of 0.05.

PROVISIONS TO MONITOR THE DATA TO ENSURE THE SAFETY OF PARTICIPANTS

Frequency of Data Review for this study

The frequency of data review for this study differs according to the type of data and can be summarized in the following Data Review Table:

Data Review Table

Data type	Frequency of review	Reviewer
Subject accrual (including compliance with protocol enrollment criteria)	Quarterly	PIs
Status of enrolled subjects, as of date of reporting	Quarterly	PIs
Adherence data regarding study visits and intervention	Quarterly	PIs
AEs and rates (including out of range values)	Quarterly	PIs
SAEs	Per occurrence	PIs, NIH
Evidence to support the proposed hypotheses	One time when half of the data (N=32) were collected	PIs, statisticians,

PROVISIONS TO PROTECT THE PRIVACY INTERESTS OF PARTICIPANTS

Involvement in this research study may result in a loss of privacy, since persons other than the investigator and research team might view study records. Unless required by law, only the following people can review study records and they are required to keep personal information confidential:

- Authorized members of the Northwestern University workforce, who may need to see information, such as administrative staff members from the Office for Research, Office for Research Integrity and members of the Institutional Review Board (a committee which is responsible for the ethical oversight of the study),
- Representatives of the study sponsor, the National Institute of Health
- Representatives of Food and Drug Administration (FDA), and Office for Human Research Protections (OHRP)
- Registries or other research-related databases: the results of the participant's examinations will be kept in a central computer or data registry at the NU Department of Physical Therapy and Human Movement Sciences. These results will be stored by research identifier code for privacy of records and the participant's records will only be accessed by the investigators listed for this study.

The results of this study may also be used for local and regional scientific and healthcare conference presentations, as well as peer-reviewed scientific and medical journal papers. If individual results are discussed, participant identities will be protected by using a study code number rather than a name or other identifying information

The sponsor, monitors, auditors, the IRB, the Northwestern University Office for Research Integrity, the US Office of Research Integrity (ORI), the US Office for the Protection of Human Research Protections (OHRP), the US Food and Drug Administration (FDA) may be granted direct access to medical records to conduct and oversee the research.

Steps that will be taken to protect participants' privacy interests

During this study, medical history and clinical assessments will be performed for eligibility checking. However, all of the materials collected are for research purposes only, and data will be kept in strict confidence. Access to these files will be limited to authorized research team members. All data generated in this study will be identified with an identification code unique to the subject. Confidentiality will be ensured by use of these identification codes. No information will be given to any unauthorized person without permission from the subject, except: 1) if necessary to protect participant's rights or welfare (for example, if they are injured and need emergency care or when the Institutional Review Board monitors the research or consent process); or 2) if required by law.

A database will be used to store/manage all the electronic data. This database will be secured with password protection. The informatics manager will receive only coded information that is entered into the database under those identification numbers. Electronic communication with outside collaborators will involve only unidentifiable information. All information published on papers or presented at conferences will be identified as a number without identification of subject personal information.

Subject's feeling at ease

Subjects who choose to be in this study will be informed that they have the right to be treated with respect, including respect for the decision whether or not to continue or stop participating in the study. All examinations and procedures will be thoroughly explained to each subject to ensure subjects feel at ease with the questions, examinations, and procedures involved. Subjects will be

informed that if at any point they feel uncomfortable about answering any questions on any questionnaires or surveys, they will not be required to answer that question. Subjects will be informed that they are free to choose to stop being in the study at any time, and that choosing not to be in this study or to stop being in this study will not result in any penalty or loss of benefit to which they are entitled. Specifically, the choice not to be in this study will not negatively affect the right to any present or future medical treatment.

Subject's medical record access

The research staff from Northwestern University will obtain a release from the patient, if researchers need to access medical records of the subject.

COMPENSATION FOR RESEARCH-RELATED INJURY

In the event of research-related injury, subjects will not be compensated for medical care required because of an untoward outcome resulting from participation in the research study. Subjects should seek medical treatment through his or her doctor or treatment center of choice. As stated in the consent form:
"If you become ill or are injured as a result of this study (medications, devices or procedures), you should seek medical treatment through your doctor or treatment center of choice. You should promptly tell the research team about any illness or injury (See contact information under "**Who can I talk to?"**).

The hospital [Clinical affiliates, including but not limited to the Shirley Ryan AbilityLab (SRALAB), Northwestern Medical Group (NMG), Northwestern Memorial Hospital (NMH), Northwestern Lake Forest Hospital (NLFH), Northwestern University, researchers, study physician] will not pay for medical care required because of a bad outcome resulting from your participation in this research study."

ECONOMIC BURDEN TO PARTICIPANTS:

There will be no significant costs to participants for any research procedures. Participants will receive $50 for the consenting and screening visit, which determines eligibility and enrollment in the study. This visit will last approximately 2 hours. In the event that the consenting and screening visit lasts an hour or less, participants will receive $25.

After enrollment, participants will be scheduled for future visits. For these visits, they will be compensated using one of the following two methods, whichever results in lower total payment per visit:

1. $150 per experimental visit for participation in one session of the study (typically lasting about 6 hours). If a visit exceeds 6 hours, participants will receive an additional $20 per hour for the extra time,
   or
2. $25 per hour for the total duration of the visit.

Participants are encouraged to bring a quick, pre-prepared or packaged lunch. We also provide snacks and water during the visit. In the event that a participant did not bring lunch with them, lunch will be covered up to $20.

If a participant drives to or is driven to and dropped off at the lab by a family member or friend, one parking voucher will be provided per visit. Participants are responsible for their own gas expenses. In special circumstances—such as severe weather events—the study team may cover the cost of a private rideshare service (e.g., Lyft or Uber), at the discretion of the Principal Investigator (PI).

Communication-related expenses (e.g., phone calls) will not be reimbursed.

CONSENT PROCESS

The consenting process will occur in person at Northwestern University Physical Therapy and Human Movement Sciences Department at 645 N Michigan Ave. Recruitment for this study will take place over the phone or virtually and will schedule potential participants to come in person to further learn about the study, meet study personals, and go through the consenting process. Principal investigator will oversee training of authorized study personnel who will consent the participant. The consenting process will last approximately 15 minutes with additional time for questions or demonstrating protocols to ensure potential participants fully understand the study. We will minimize undue influence and coercion by explicitly asking potential participants if they would like to participate after we have thoroughly discussed consent forms. We will inform them that they are free to withdraw from the study at any time with no penalty to them. Consent will be documented in writing using Template HRP-592. A copy of the signed consent form will be given to the participant, and the original will be kept, locked, in the PI's office. The consent form will also be uploaded to Northwestern's Study Tracker.

PROTECTED HEALTH INFORMATION (PHI AND HIPAA)

When choosing to take part in this study, subjects are giving us the permission to use his/her personal health information that includes health information in the medical records and information that can identify him/her. Health information we may collect and use for this research includes:

- Name
- Street Address
- Telephone number
- Date of Birth
- Information from a physical examination including only: blood pressure reading, upper extremity range of motion, strength, and functional movement assessment.
- Medical record related to stroke, and any potential conditions that may impact eligibility for MRI scan.
- Social Security Number - needed for the Accounts Payable Department at Northwestern University in order to issue the study stipend and for the medical records department of most hospitals to identify the medical record file.
- 3D movement analysis of the arm during reaching/grasping tasks.

QUALIFICATIONS TO CONDUCT RESEARCH AND RESOURCES AVAILABLE

Recruitment feasibility: We will recruit 64 moderately to severely impaired individuals (based on the Fugl-Meyer Motor Assessment (FMA)), each to complete all experiments for the three aims. Individuals will be recruited from the Clinical Research Registry or the New Normal. Clinical Research Registry is managed by Shirley Ryan AbilityLab and Northwestern University and contains more than 1200 individuals with stroke. We will also recruit from other platforms, like the

New Normal, a Chicago-based matchmaking platform. These research registries demonstrating the feasibility of recruitment.

Time allotment: Drs. Dewald and Yao each will spend 20% effort to conducting and completing research outlined in this protocol. They will oversee post docs, PhD students, and research assistants who have completed necessary training and approved to complete procedures outlined in the document. Additionally, the study physician, Dr. Sebastian Urday, will be consulted whether participant inclusion is safe based on medical history and medications and will write prescription for a recommended dose of tizanidine. A trained physical therapist will conduct the Fugl-Meyer Assessment on potential participants to determine if their paretic upper limb impairment fits within the inclusion criteria.
Describe your facilities: Research will take place in the laboratories at the Physical Therapy and Human Movement Department at Northwestern University. Participants will be greeted by front desk support staff and able to sit in a waiting room before proceeding to experiment laboratory space. These experiments will take place across three labs which contain all necessary equipment outlined in protocol. Additionally, each research environment includes height adjustable tables and chairs to ensure safe movement throughout

Research Team: Multiple PIs oversee the research outlined in this protocol and include Dr. Jules Dewald and Dr. Jun Yao. Dr. Jules Dewald is a Professor and the Chairperson of the Department of Physical Therapy and Human Movement Sciences (PTHMS), as well as a Professor of Biomedical Engineering at Northwestern University. With over 25 years of experience in utilizing various neuroimaging tools and robotic technologies, he is a well-established researcher in movement and rehabilitation sciences. The PI, Dr. Yao has about 20-year experience in exploring the neuromechanisms underlying the motor control. She has led a couple of research projects funded by the NIH and other agencies. Dr. Yao is an expert in EEG-based neural imaging. She has also accumulated experience in experimental design for collecting and processing biomechanical data.

The Co-investigators listed in the grant (Dr. Ana Maria Acosta, Dr. CJ Heckman, Dr. Sebastian Urday) are well-established investigators in their fields.

All the members in the research team have valid CITI training certificates. They have all read the IRB. We have weekly meetings to make sure everyone knows their specific duties.

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