Identifying the potential effect of zolmitriptan on the 1b pathway of Golgi tendon organs in regulating intermuscular inhibition in the extremities to find a link in the mechanism of spasticity

A Thesis

By

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Introduction

Spinal cord injury (SCI) is a condition that leaves the patients with severe deficits in motor ability ranging from a mild lack of muscular control to paralysis. Even partial transection can lead to deficits in motor movement as well as disrupt neural pathways in the spinal cord (Tomaschek et al. 2019). Such disruptions can lead to what is known as spasticity or hyperreflexia, which is uncontrolled muscle tension and contraction that prevents the individual from freely moving the limb or using their muscles. The underlying mechanism for spasticity is still a widely debated topic, but one leading hypothesis is that SCI causes denervation of descending tracts that results in a lack of inhibitory signaling in the spinal cord, which leads to the hyperexcitable motoneurons shown to underly the pathology of hyperreflexia (Thaweerattanasinp et al. 2016; Nardone et al. 2015; Murray et al. 2011).

Serotonin (5-HT) has been found to inhibit afferent information in the spinal cord and therefore provide inhibition to spinal reflexes, specifically tracts delivering 5-HT through the dorsal spinal cord from the raphe nucleus of the brainstem (Skagerberg and Björklund 1985). SCI causing the loss of this 5-HT induced inhibition is what is hypothesized to play a role in spasticity (Nardone et al. 2015). There are a few potential sources of spasticity caused by decreased serotonin signaling. One series of studies suggested that there is a group of 5-HT sensitive interneurons in the deep dorsal horn (DDH) region of the spinal cord that exhibit bursting behavior (Thaweerattanasinp et al. 2016; Thaweerattanasinp et al. 2020). It is hypothesized that these interneurons play a key role in spasticity due to their bursting behavior, and it was found that these interneurons show decreased bursting when administered with the migraine drug zolmitriptan. Zolmitriptan is a selective serotonin reuptake inhibitor (SSRI) that

has recently been shown to have anti-spastic effects (Nardone et al. 2015). This drug is also an agonist for 5-HT specific to the receptor subtype on these interneurons, 5-$HT_{1B/1D}$.

It is therefore hypothesized that these 5-HT deprived interneurons play a role in motoneuron excitability and muscle spasticity through their bursting behavior. However, these studies have mostly been performed with the isolated spinal cord of the rodent model in vitro, so the effects of these 5-HT dependent interneurons on muscle length afferent groups Ia and II have been studied extensively (Nardone et al. 2015). An SSRI has been used in the decerebrate feline model in studying the clasp knife reflex, a symptom of hyperreflexia (Miller et al. 1995), but the effects on the Ib fiber pathway for muscle force feedback are currently unknown.

Sensory feedback from the Ib fibers also integrate with interneurons in the DDH for heterogenic muscle control (Nichols 2018; Jankowska & Edgley 2010). The Ib reflex network is important for the maintenance of gait and posture in the human lower extremities through inhibitory connections that work to increase muscle compliance. That is, this feedback system relaxes the muscles, making them more compliant to passive movement. Deficits in this circuitry caused by SCI have been shown to lead to unbalanced muscle inhibition and excessive compliance about the ankle joint (Niazi et al. 2020; Kajtaz et al. 2021), but the research involving the mechanism of SCI affecting this system is still limited. This inhibitory force feedback circuit integrates in the DDH, where there are also 5-HT deprived bursting interneurons potentially mediating hyperreflexia. Interactions between these neural circuits have yet to be investigated. This increases the importance of this proposed study that will focus on the changes in the function of the force feedback during administration of the SSRI, zolmitriptan.

Literature Review

The papers that make up the primary drive for the proposed study are three papers with the common author Tysseling (2016; 2017; 2020). These studies explore the role of the serotonin descending from the brainstem through the spinal cord and how it relates to spasticity following SCI. The papers from 2016 and 2020 both focus on how the synthetic glutamate agonist N-methyl-D-aspartate (NMDA) and zolmitriptan modulate the interneurons that exhibit bursting firing behavior in the deep dorsal horn region of the spinal cord following acute and chronic spinal cord injury respectively (Thaweerattanasinp et al. 2016; Thaweerattanasinp et al. 2020). These describe how zolmitriptan inhibits the bursting interneurons with serotonin, which normally inhibits their activity and is now in short supply following spinal cord injury, preventing the bursting behavior, indicating a potential cause of spasticity.

The third paper is a study on the excitatory 5-HT receptors of the motoneurons themselves which have been upregulated by the lack of serotonin to be constitutively active, providing another potential cause of spasticity (Tysseling et al. 2017). While the 5-HT receptor subtype on the axons of the motoneurons is known, it was shown that the persistent effect of these receptors on motoneuron activity was not affected by the GABA agonist drug baclofen, which normally dampens neuronal excitability. This indicates constitutive activity of the serotonergic receptors and that their activity is not dependent on a homeostatic mechanism of motoneuron activity. It is worth noting that these experiments show that 5-HT has excitatory receptors along the motoneuron axon that are upregulated by 5-HT loss, but this will not be affected in the proposed study as only the mentioned bursting interneurons will express the receptor subtype specific to zolmitriptan (Thaweerattanasinp et al. 2020). It is still research worth pursuing on how loss of excitatory 5-HT causes hyperreflexia, but it is currently unknown

how to address this issue pharmaceutically (Tysseling et al. 2017). For this reason, the mechanism of the bursting interneurons will be addressed in the current study.

With the main purpose of this study being to investigate the role of descending serotonin in the spinal cord, it is important to have sources that explain the processes involved. It has been characterized that there is not just the dorsal pathway of descending serotonin, but also the intermediate and ventral pathways which may have a role in future studies on serotonin (Skagerberg et al. 1985). Each of these serotonin pathways is accompanied by just as many if not more non-serotonergic tracts, which provides room for future studies on loss of supraspinal control after SCI as well as the possibility of the serotonin loss having effects on other reflex circuits. The background knowledge for this concept is extensively described and compiled in a journal review, *Serotonergic Transmission After Spinal Cord Injury*. This review discusses the role of descending serotonin in inhibiting sensory feedback and how spinal cord injury disrupts this system, leading to spasticity (Nardone et al. 2015). The authors support this with an extensive exploration of the roles of serotonin in the functions of the spinal cord. This paper even includes studies on zolmitriptan as an anti-spastic drug in its section on spasticity and how it is a relatively novel hypothesis that the 5-HT receptor subtypes affected by zolmitriptan may be involved in the inhibition. These studies almost certainly sparked the Tysseling experiments as they are referenced there (Thaweerattanasinp et al. 2016; Tysseling 2017; Thaweerattanasinp et al. 2020). These papers also note that zolmitriptan has no effect on motoneuron properties, therefore serotonergic inhibition can be restored after SCI without affecting motor function (Nardone et al. 2015).

But as noted before, the experiments that have investigated the inhibited response of these neurons to zolmitriptan have yet to be tested on the Ib afferent system. The proposed study

will investigate how zolmitriptan affects the Ib spinal circuitry from the Golgi Tendon Organs (GTOs) in the decerebrate cat model in vivo. The cat is used in this study as it has been extensively shown to exhibit similar Ib afferent circuitry in the hindlimbs to those of humans, which cannot be achieved as well in the rodent model (Duysens et al 2000, Nichols 2018). GTOs are part of the proprioceptive feedback system in muscle tendons. Specifically, they are activated by increases in muscular force and communicate to interneurons in the spinal cord through Ib sensory fibers which then send inhibitory signals to other muscles in the hindlimb. This study will provide insight into the potential connections between the understood spinal circuits of the GTOs of the lower limbs and the bursting interneurons in the deep dorsal horn, which will help expand our knowledge of spinal circuitry and possibly help guide how to address the issue of spasticity as well as other SCI-induced motor deficits caused by the reorganization of this reflex network.

Understanding of the GTO network in the cat model comes primarily from muscle stretch studies. Lyle et al. (2018) performed a study on decerebrate cats and measured the force responses of each leg extensor muscle in combination with each other to measure the inhibition inflicted from one on the other. This provided information on how Ib fibers provide inhibitory feedback globally across the muscles of the hindlimbs to support a system of limb mechanics that is susceptible to change based on the type of motor task. This paper as well as Lyle's previous study (2016) provide the base methodology and data acquisition for the control experiments against which the manipulation of adding zolmitriptan will be compared. An older foundational study for this technique is referenced in many papers like the previously cited one as it uses the same kind of experiment that shows how the stretch reflex and passive properties of a muscle regulate its stiffness and length (Nichols and Houk 1976). The methods for activation

and recording of muscles are also described in substantial detail, and this information provides much of the basis of the methodology for all of the muscle stretch studies described in this review, including the proposed study. To provide additional background information and data on the Ib system, in an in depth review, Nichols (2018) describes how proprioceptive feedback from GTOs and muscle spindles integrates in the central nervous system and regulates limb mechanics. The purpose of this paper is to give an updated review of the role of Ib pathways on limb mechanics as well as force feedback circuitry in the spinal cord.

In order to study the spinal cord and GTO circuitry in this study, where the context is spinal cord injury, there needs to be an understanding of how the circuitry is affected by SCI, especially when the dorsal horn is damaged. Taylor et al. (1997) understood the need to find the causes of hyperreflexia and used a variety of spinal lesions, or injury to a specific region of tracts at one location on the spinal cord, primarily dorsolateral quadrant lesions. They demonstrated evidence of hyperreflexia in all of the dorsal hemisections. This supports one of the causes of spasticity to lie in the dorsal area, which will be the target of zolmitriptan, but this study mainly attributed these increases in muscle resistance to motoneuron hyperexcitability and increased muscle spindle excitation. In a study that combines these concepts of SCI induced circuity changes with the methods to be used, Niazi et al. (2020) investigated how the global inhibitory system described earlier is impacted by a lateral hemisection of the spinal cord. In several experiments, they compared the inhibitory feedback in muscle pairs as done in the previous paper but in groups of control versus acute, subchronic, and chronic SCI. Normally there are three patterns of inhibition seen between two muscles: balanced inhibition or directional bias of one muscle onto the other. In this study, it was found that the muscles in the hindlimb lost these patterns following SCI and exaggerated a single pattern of converging inhibition onto the ankle

extensor muscles, resulting in an abnormally compliant ankle joint. The toe flexor muscle, flexor hallucis longus provided especially increased heterogenic inhibition onto the ankle extensor gastrocnemius. It is because of these results that this specific muscle interaction was chosen as the primary focus for this current study with zolmitriptan in the context of SCI. This paper also provides additional methodology and results that will be compared with my study for the subjects that will have undergone partial SCI, and this research is still ongoing as part of a larger study in collaboration with the University of Louisville, which focuses on the spinal cord injury studies with cats. A more recent study performed the same methods but focused on hyperreflexia from the autogenic circuit of extensor muscles after a lateral hemisection (Kajtaz et al. 2021). This paper helps to provide more information on the reworking of spinal circuitry following SCI, specifically more insight on length feedback that inputs onto the Ia and II interneruons, also in deep dorsal horn region of the spinal cord. In combination with the data gathered on force feedback by Niazi et al. (2020), these studies provide much of the background information on changes in intermuscular inhibition after a partial spinal cord lesion in felines.

As shown in past reflex experiments, such as the ones previously mentioned, the primary focus and explanations of SCI symptoms have been centered around increased excitation of the motoneurons due to the excitatory feedback of the muscle spindle afferent pathways. That is, the pathways activated by the autogenic stretch reflex and length change feedback have been shown to be upregulated after SCI, resulting in hyperactivity of the motoneurons (Kajtaz et al. 2021). However, gait and posture dysfunction following SCI necessarily depends on this autogenic excitatory muscle spindle input in combination with heterogenic inhibitory GTO input (Nichols 2018). Therefore, it is essential to better explore the potential contributions of force feedback in motor deficits following SCI, especially in the context of diminished 5-HT input from

supraspinal centers. It has been found that the ankle joint shows increased compliance due to inhibitory convergence on the ankle extensor muscles from unbalanced force feedback after SCI (Niazi et al. 2020, Nichols 2018), but it is not known if this pathology is independent of 5-HT deprivation. In fact, a previous study showed that a 5-$HT_{1B/1D}$ receptor agonist, just like zolmitriptan, used in the decerebrate feline model was able to almost completely reverse clasp knife reflex patterns induced by a temporary cold block on the dorsal spinal cord (Miller et al. 1995). Clasp knife reflex is only one component of SCI-induced reflex pathology, and a cold block is a questionable substitute for spinal cord transection, but the results of this study indicate that this drug may provide stability to overexaggerated or deprived signals in the dorsal spinal cord after injury. By administering the anti-spastic SSRI zolmitriptan, it is hypothesized that the unbalanced force feedback that causes increased ankle compliance will decrease due to the drug increasing inhibitory 5-HT input onto the interneurons of the DDH which, through underling neural connections, will inhibit the increased inhibitory feedback from the Ib pathway.

As the base methods will be the same as the muscle stretch studies previously mentioned, in which the muscles are stretched individually and in pairs, the stretches where only one muscle is pulled will provide information on the stretch reflex as it changes across conditions of spinal cord injury and zolmitriptan use. Because of this, the effects on the length-dependent stretch reflex will be assessed as well as force feedback. Based on the results of previous studies on hyperreflexia following SCI (Nardone et al. 2015; Kajtaz et al. 2021), it is hypothesized that SCI will hyperexcite the stretch reflex compared to the control, while zolmitriptan will increase inhibition of the motoneurons and decrease the response of the stretch reflex compared to the no drug SCI condition.

With the base methods of cat reflex testing understood, it is important to have information on the methodology of drug administration for zolmitriptan, especially as this drug has never been used before in this kind of experimental setup. One study involving the administration of L-Dopa is especially helpful. This paper is valuable for the proposed study as it provides detailed instructions of how to dissolve and give the drug to the cat intravenously (Conway et al. 1988), which is essentially what will be done with zolmitriptan. This report is also just an excellent decerebrate cat drug study to which the data of the proposed study can be compared. As for the dosage required in this study, it is preferred that it be as high as possible without disrupting the cat's vital systems. MacLennan et al. (1998) found that at small doses (1-30 micrograms per kg) there is an effect on hemodynamics, but at 1-1000 micrograms per kg they found no effect on cerebral vasculature, and there was only a decrease in heart rate at the 1000 micrograms per kg. This paper focuses more on the conventional medical use of zolmitriptan as a vasoconstrictor, but this will be the main basis for what dosage of zolmitriptan to provide to the cat as it tells how much can be given without disrupting vitals. The exact amount will be adjusted as necessary, but it is worth making sure to provide a sufficient amount to elicit an effect on the decerebrate cat without prematurely ending the experiment.

Expected Outcomes

The results of this study should provide information on the potential integration of the Ib network in the DDH region of the spinal cord and bursting interneurons in the decerebrate feline model. While the effects of zolmitriptan on the interneurons in the DDH are known, the effects of this mechanism on the GTO reflex network is unknown. It is hypothesized that SCI will hyperexcite the stretch reflex compared to the control, while zolmitriptan will increase intermuscular inhibition of motoneurons and decrease the response of the stretch reflex

compared to the spinal lesion without the drug. It is also possible that there may be no significant effect on the inhibitory reflex network from administration. However, investigating this will still provide valuable information about the circuitry between the DDH interneurons and the GTO reflex pathways. This study will either label the two systems as possibly independent in regard to spasticity and other SCI-related motor deficits or show that the two are in fact intertwined.

It may also be seen if there are 5-$HT_{1B/1D}$ receptors in unexpected areas of this network, which could reveal an unexpected mechanism for the results. This could indicate potential side effects of zolmitriptan or new focuses for future drug intervention. The anti-spasticity drug zolmitriptan will be tested to see how it affects this particular reflex network, the results of which may be applied to future studies of this drug for testing other reflex circuits or for using different agents to test the other descending spinal pathways that are also lost after injury. With the integration of these systems, steps can be taken to move closer to understanding the underlying mechanism of muscle spasticity and the connections that are distorted after SCI.

Methods

All methods were performed with approval by the Institutional Case and Committee (IACUC) at the Georgia Institute of Technology and the University of Louisville. The subjects of the experiments were purpose-bred adult male or female cats ranging from 3-8 kg in weight, all less than a year in age. Chronic spinal cord injuries (SCI) were completed at the University of Louisville on the animals in that study group. These animals along with the control animals underwent terminal mechanographic experimentation at Georgia Tech. The animals that underwent SCI were all female and underwent the terminal experiment at about 7 weeks after SCI (n=2). These animals received a lateral hemisection at the level of T9-T10 via exposing the spinal cord using bilateral laminectomies while anesthetized for surgery under isoflurane. More details of this procedure have been described previously (Kajtaz et al. 2021). The control animal was a male cat that received no prior surgery (n=1). All animals received social housing except during recovery of spinal cord injury.

Terminal Experiment

The methods for the terminal experiment were performed at Georgia Tech and have been well established and described in previous studies in detail (Lyle et al. 2016; Kajtaz et al. 2021). In brief, all animals were anesthetized in an induction chamber with isoflurane and oxygen. Once the animal was under to the point where reflexes were incapacitated, it was then moved to the surgical table while still being given the anesthesia mix through a facial cone. A tracheal intubation was then performed, and breathing was controlled by a ventilator. The gas flow was maintained to keep the animal under deep anesthesia for the remainder of the surgery. At this point, all necessary instruments have been added to the animal to monitor heart rate, oxygen

saturation, carbon dioxide partial pressure, blood pressure, temperature, and electrocardiogram (ECG). A heating pad and lamp were used to maintain appropriate body temperature.

Using blunt dissection, both carotid arteries were isolated, and strings were tied loosely around them so that blood flow to the head could be decreased for decerebration. A cannula was inserted into the external jugular vein for intravenous (IV) administration of saline and drugs.

The animal was then placed into the apparatus for the experiment. The abdomen was supported by a sling, the head was placed in a stereotaxic frame, and the hindlimbs were fixed to the apparatus. The knees were held at a fixed angle using intermedullary pins in the femur and tibia clamped together. The hips were held up by firmly screwed rods, the ankles were securely clamped, and the tail was moved away from the legs.

A select group of muscles were chosen based on well-established patterns of inhibitive intermuscular force feedback between them. That is, each muscle receives signals from the GTOs and not from the monosynaptic stretch pathway of the other muscle. The muscles chosen for this study in all animals were the gastrocnemius (GAS) and flexor hallucis longus (FHL). In a control animal, the rectus femoris (RF) was also chosen to be paired with GAS. Using mechanical dissection, these muscles were carefully isolated from other muscles and connective tissue to minimize mechanical coupling while maintaining their neural and vascular connections. The sartorius and RF or vastus muscle group (VASTI), when not being used in the experiment, were severed to remove any mechanical coupling between the quadriceps muscles as well as to prevent movement at the hip. The VASTI and RF were carefully separated from each other as well as from the sartorius using blunt dissection to minimize mechanical coupling. The patellar ligament was severed, and a steel cable was clamped through a hole drilled into the patella and

connected to RF. This cable was run through a pulley underneath the hindlimb with the other end attached to a tendon clamp.

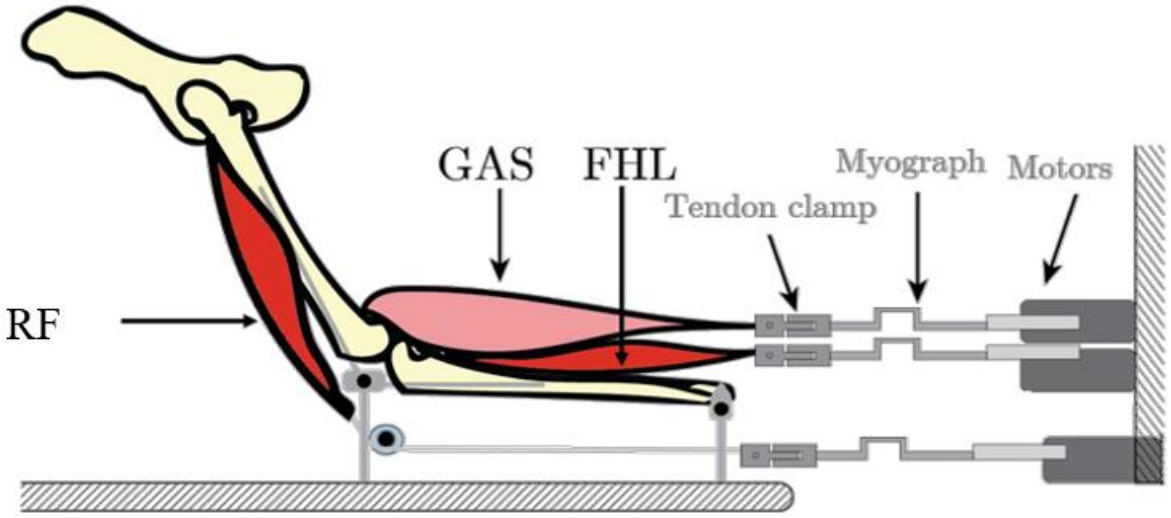


Figure 1. Apparatus of the hindlimb for mechanographic testing. The muscles of the hindlimb were dissected, and then tendons were secured to tendon clamps connected to myographs and linear servomotors (Lyle et al. 2016).

The GAS and SOL distal insertions were separated from the bone while preserving the bone chip directly attached to the muscle tendon. The FHL was separated from the connective tissue covering it to minimize mechanical coupling. The end of each tendon with the bone chip attached was secured to a tendon clamp that could be connected to a myograph and motor for stretching the muscle and measuring the tension. This setup is shown in Figure 1.

To keep reflexes intact while keeping the animal unconscious and painless, a precollicular decerebration was performed. The skull rostrally to the cribriform plate to the tentorium was removed. The brain was exposed by cutting and peeling back the dura. All brain tissue was removed rostral to a vertical transection made at the anterior margin of the superior colliculus. Bleeding was minimized by placing gelfoam, thrombin, and cotton at the base of the cranium. Anesthesia was titrated down and off over a 30 minute period. Data collection began

approximately 30 minutes to one hour after this, but it was seen through test trials if the animal's reflexes were regained to allow for data collection to begin.

After at least one control trial was obtained, zolmitriptan was prepared to be administered as established previously (MacLennan et al., 1998). 1 milligram of zolmitriptan per kilogram weight of the cat was dissolved in dimethyl sulfoxide (DMSO) and delivered intravenously in two boluses, each about 20 minutes apart to maintain the effect of the drug. This amount of drug was determined in the aforementioned study to be enough to elicit an effect without overdosing the animal or inducing significant changes in blood pressure. Heart rate was monitored during administration to determine that the zolmitriptan was taking effect as it is known to induce tachycardia. At the end of the experiment, the animal was perfused with heparin, then sodium nitrite, then saline and paraformaldehyde for effective euthanasia and fixation of tissue. The fixed tissue was prepared and sent for histological analysis by the research group from the University of Louisville.

Data collection

The protocol for the ramp hold release stretch as well as the equipment and programs used have been well-established and described in previous studies (Lyle et al., 2016). A tendon clamp from each of two muscles on the same hindlimb was connected to a myograph and a linear servomotor. The two muscles were subjected to 2 mm stretches, each comprising of a pull phase lasting 50 ms, a 100 ms hold, and a 50 ms release at a velocity of 0.04 m/s. The muscles were pulled in one of two states. In state 1, the autogenic state, only one muscle was stretched. In state 2, the heterogenic state, both muscles were pulled together. The states were repeated in an alternating fashion at 0.07 Hz to include 40-50 stretches per trial as shown in Figure 2. The muscle stretched during both state 1 and state 2 is the recipient muscle. The muscle stretched

only during state 2 is known as the donor muscle as it modulated the response of the recipient muscle, which was determined through comparing the recipient muscle force response in state 1 versus state 2. Background force, or passive force without stretches, in this paradigm was maintained between 1.7-2.3 N to minimize mechanical influence.

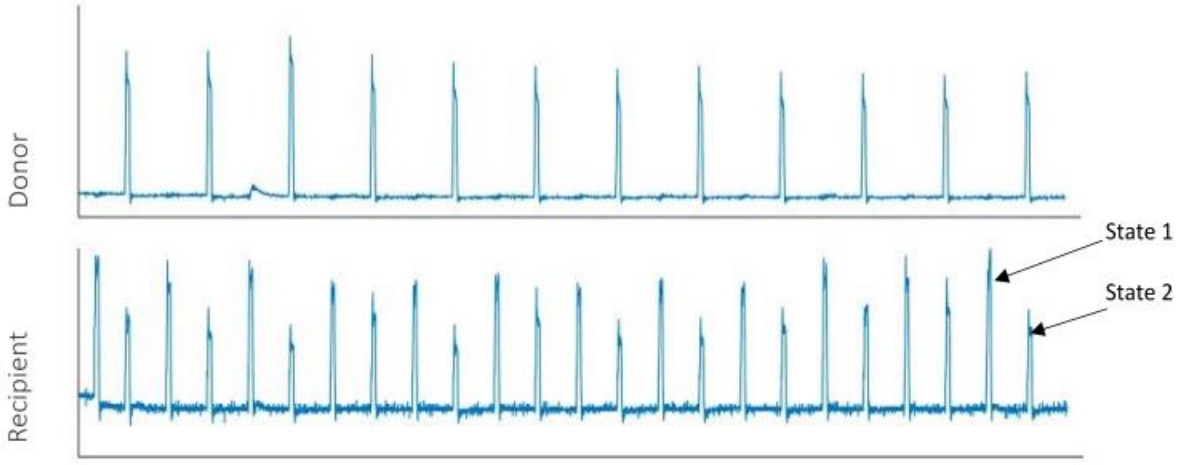


Figure 2. A segment of a trial with force in the y-axis over time in the x-axis. During state 1, only the recipient muscle is stretched in the autogenic state. In state 2, the donor muscle is stretched as well as the recipient muscle I the heterogenic state. Each spike in the figure represents the muscle response as the result of a single stretch.

Data analysis

Background force was determined as the average force 10 ms before each stretch. To account for variations in background force, this was subtracted from the force reading to remove the baseline for data analysis of the muscle force response. Stretches with sudden changes in background force preceding the stretch were eliminated. The state 1 stretches were analyzed to investigate the autogenic stretch response as well as changes in the hold phase of the response. This was measured to account for possible changes in the 1a reflex pathway as a result of zolmitriptan, calculated as a slope of the force response in the 25-50 ms time frame after the onset of the stretch.

The average muscle force in the late stage (110-150 ms after the stretch) was analyzed across states in the recipient response to determine changes in force feedback as a result of the donor muscle. The primary data from each experiment revolves around the measured percent inhibition from the gastrocnemius (GAS) muscle when it is stretched individually in state 1 versus when it is stretched in tandem with the flexor hallucis longus (FHL) in state 2. The magnitude of inhibition was calculated as a percent difference between the measured force of the recipient muscle from state 1 to state 2. The early and middle stages (40-50 ms and 70-110 ms after the stretch respectively) were also analyzed, but their importance was not as high as the late stage for determining intermuscular inhibition based on pre-established methods. Rather, these were added in order to compare the extent of inhibition across the hold phase. The time frames for the stretch and muscle response can be seen in Figure 3.

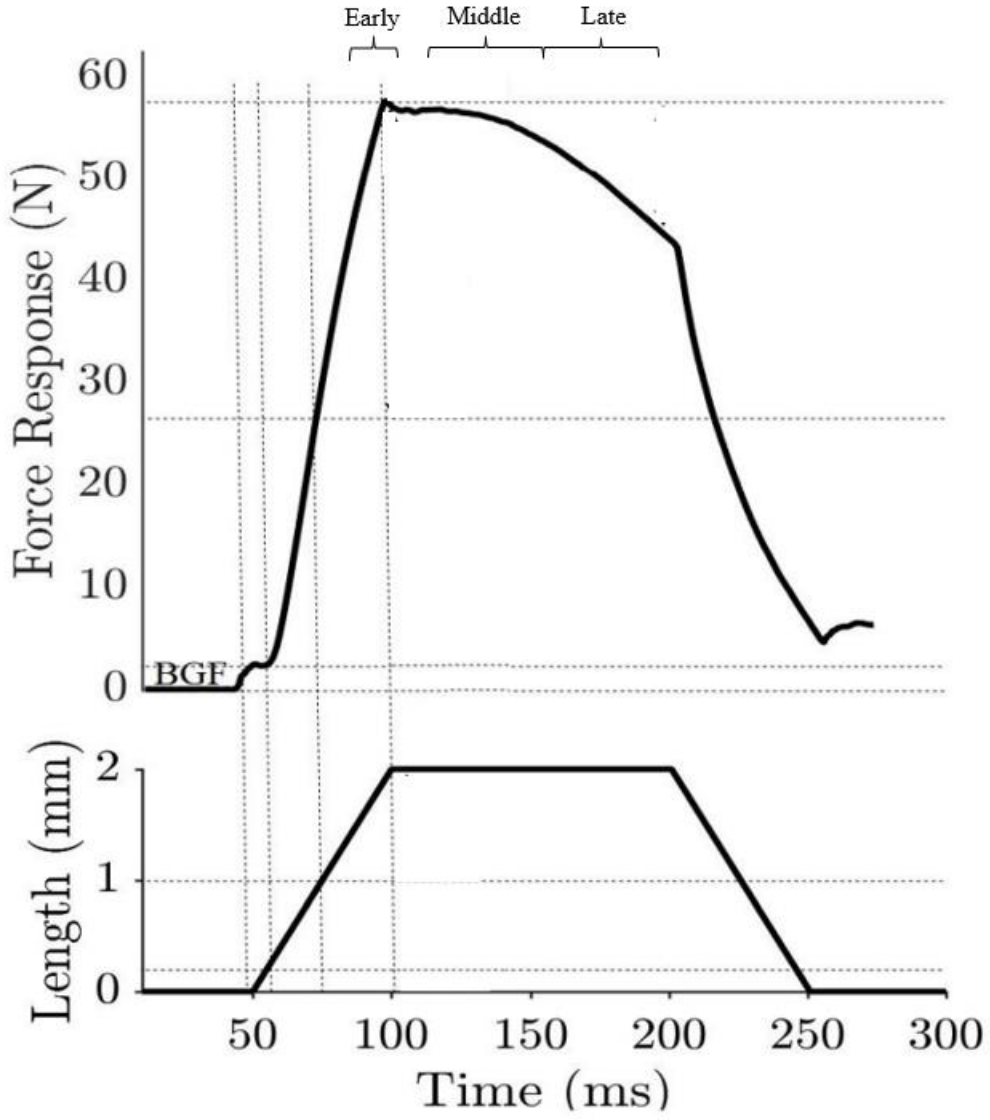


Figure 3. A plot of a single stretch and the typical associated muscle force response simultaneously. The muscle is stretched at the 50 ms time mark for a 50 ms duration, held for 100 ms, and released over 50 ms. This is shown in the graph for muscle length over time in the bottom plot. Background force has been subtracted from the force response. The early stage of the muscle response is marked by 90-100 ms, the middle stage is from 120-160 ms, and the late stage is from 160-200 ms. The early stage encompasses the peak force response while the middle and late stages divide the plateau of the muscle response during the hold phase. Autogenic stretch response is analyzed during the 75-100 ms time frame. Image modified from Kajtaz et al. 2021.

Statistical analysis

The effect of zolmitriptan was investigated by comparing force feedback inhibition, stretch response magnitude, and the slope of the muscle response during the hold phase before and throughout the course of the trials where the drug was administered. A two tailed t-test for significant differences ($p<0.05$) was used to compare if the degree of inhibition was significant for a given trial. The same test was also applied for each of those three dependent variables between the set of data before the drug and a trial during drug administration that had the closest BGF to the trial before the drug, about 10-20 minutes after the first bolus. General trends of these effects were also compared qualitatively between the control and SCI animals.

Results

Data were gathered from three separate experiments: two lateral hemisection animals and one control animal. Results will focus on the intermusclar inhibition, stretch reflex, and background forces of the GAS and FHL muscles over time.

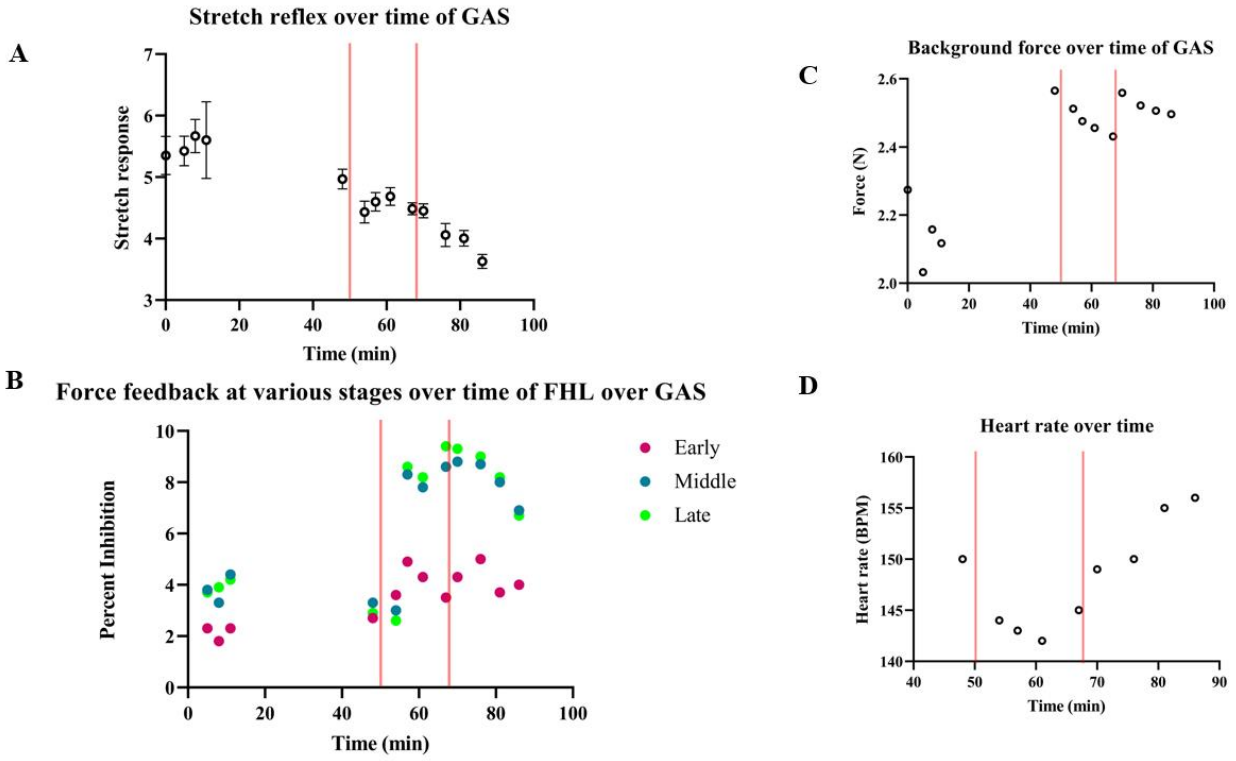


Figure 4. Summary of stretch reflex and force feedback data of gastrocnemius affected by FHL in a second lateral hemisection preparation. (A-C) All measurements were the same with the exception of time being measured in trials rather than specific units. Data from trials at least an hour before the drug was administered were also included to better highlight how its effect deviated the baseline. (B) In all trials for inhibition measured in late stage, $p<0.005$. (D) Heart rate was measured to find any possible relationship between it and zolmitriptan administration.

The second lateral hemisection experiment had much more consistent results. Background force was very stable throughout, standard deviation in stretch response was minimal, and late stage inhibition for all trials was statistically significant ($p<0.005$) (Figure 4A-

C). Unlike the first experiment, there seemed to be no significant change in the magnitude of the stretch reflex from zolmitriptan (Fig. 4A), but there was an undeniable and consistent increased baseline in late and middle stage inhibition from FHL onto GAS shortly after the drug was given (Fig. 4B, 5). There seemed to be no remarkable change in heart rate over time, although it did generally increase over time, possibly suggesting an effect from the drug (Fig. 4D). The autonomic stretch reflex as well as heart rate remaining unaffected were unexpected and may otherwise indicate zolmitriptan having no real influence on the feline preparation.

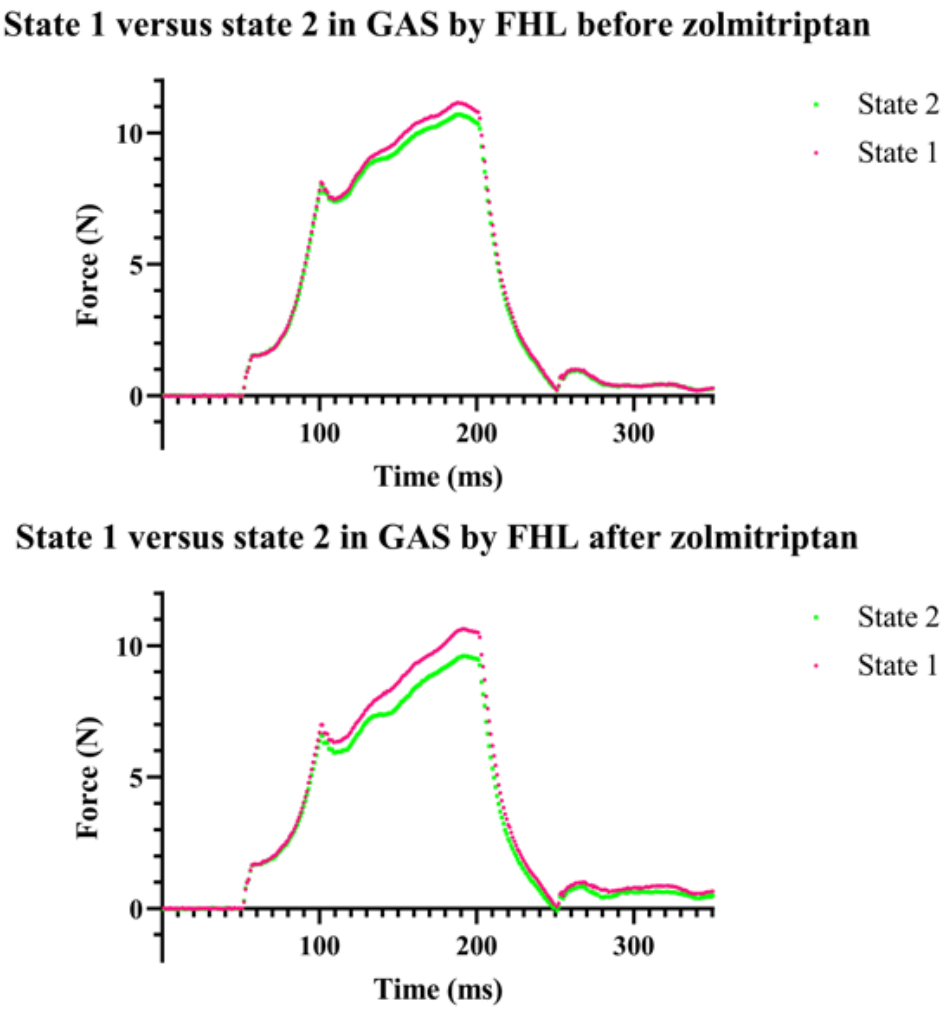


Figure 5. Comparison of state 1 versus state 2 force response in GAS with FHL donor muscle before and after drug delivery. The top graph is the average response from 45 minutes before the first bolus, and the bottom graph is from 17 minutes after. Here, the increase in middle and late stage inhibition is visualized from the pattern seen in Figure 4B.

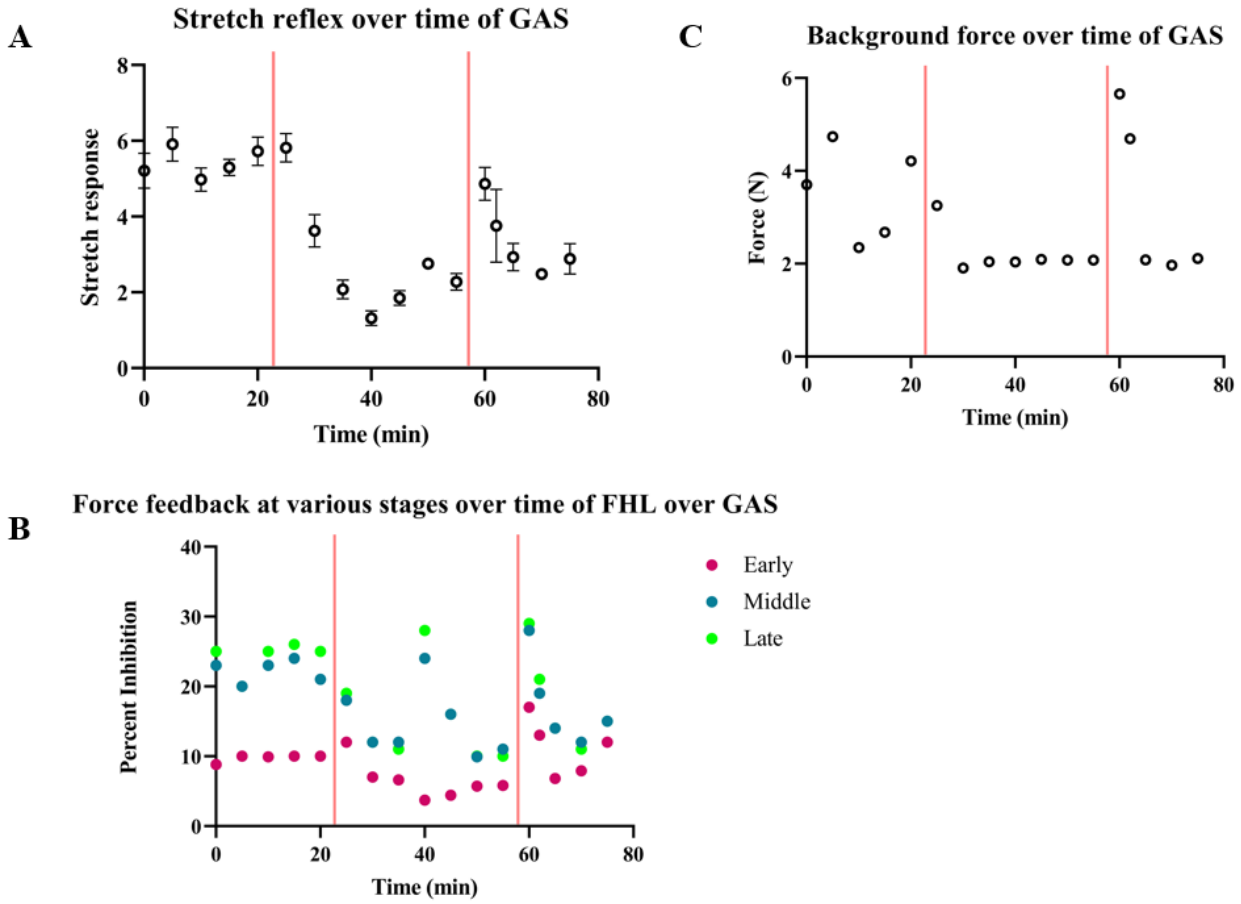


Figure 6. Summary of stretch reflex and force feedback data of gastrocnemius affected by FHL in a lateral hemisection preparation. Red arrows indicate the time points when zolmitriptan was delivered into the bloodstream. (A) Stretch response is measured as the average slope of the muscle response between 25 and 50 ms. Standard deviation for each trial. (B) Force feedback is measured as the percent difference between the state 1 and state 2 response of the recipient muscle, a positive value indicating inhibition, a decreased response in force as a result of activation of the donor muscle. A value is given for each trial for the early, middle, and late stages of the muscle response (refer to Figure 3). In all trials for inhibition measured in late stage, $p<0.005$. (C) Background force is given to provide context as both the stretch reflex and force feedback systems are dependent on this.

In the first lateral hemisection experiment, the stretch reflex seemed to decrease while inhibition seemed to also decrease but with a sudden and uncharacteristic increase after drug administration (Figure 6). Despite these changes across the course of the experiment, within trials, the data was very consistent. Late-stage inhibition was significant ($p<0.005$) in all trials of this experiment, and standard deviation for stretch response was minimal, indicating nonrandom results from this experiment. It is difficult to tell if there is any pattern of inhibition in any of the stages (Figure 6D), but it is seen in Figure 7 that the shape of the force response changed to be less uniform, especially in the stretch phase and that there is slightly more emphasis on late stage inhibition.

Background force was not entirely consistent either and was notably above the intended baseline in a few random trials (Figure 6C). The spikes in background force seem appropriately to coincide with spikes in the force feedback and stretch reflex data around the 60 minute mark, but the spike in middle and late stage inhibition at 40 minutes does not have any associated background force spike, and this took place 15 minutes after the first drug bolus. When viewing the changes in background force compared to stretch response, the pattern for both seem to be mostly similar over time.

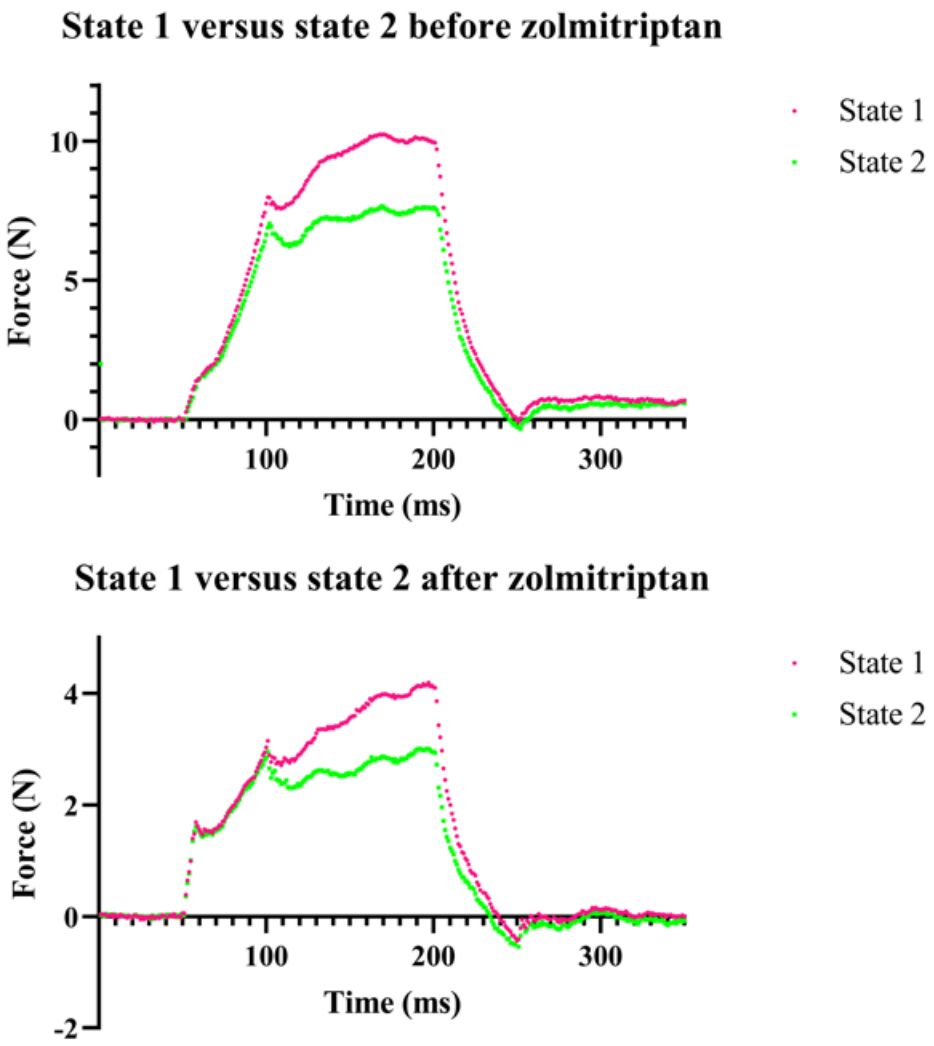


Figure 7. Comparison of state 1 versus state 2 force response in GAS with FHL donor muscle before and after drug delivery. The top graph is the average response from 12 minutes before the first bolus, and the bottom graph is from 18 minutes after. Early stage and late inhibition seems to have decreased, and the muscle response at the later trial has a much lower and less consistent force output, especially in the initial stretch phase.

In addition, the results of this particular experiment may be invalid due to an incorrect use of methods. Zolmitriptan was attempted to be dissolved in water rather than DMSO prior to delivery. DMSO was not available but may have been necessary because zolmitriptan is only sparingly soluble in water, preventing its full or appropriate pharmacology. This increases the

risk that these results were due to chance, and the dramatic fluctuations in the data in Figure 6 may be due to uneven or delayed drug uptake.

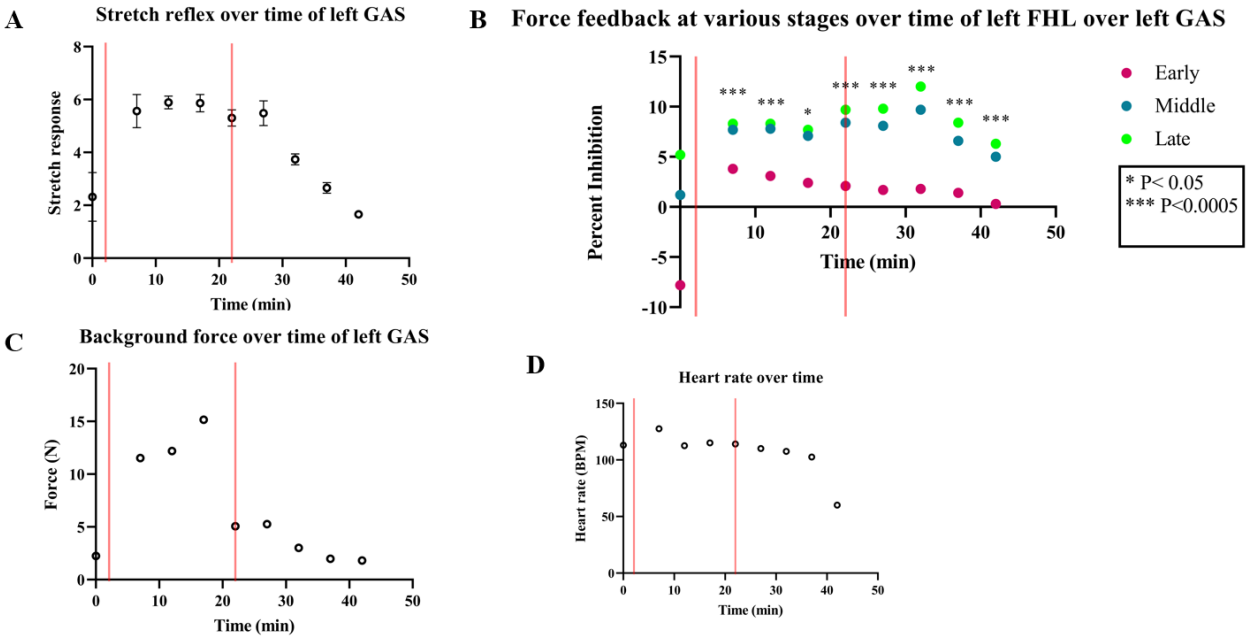
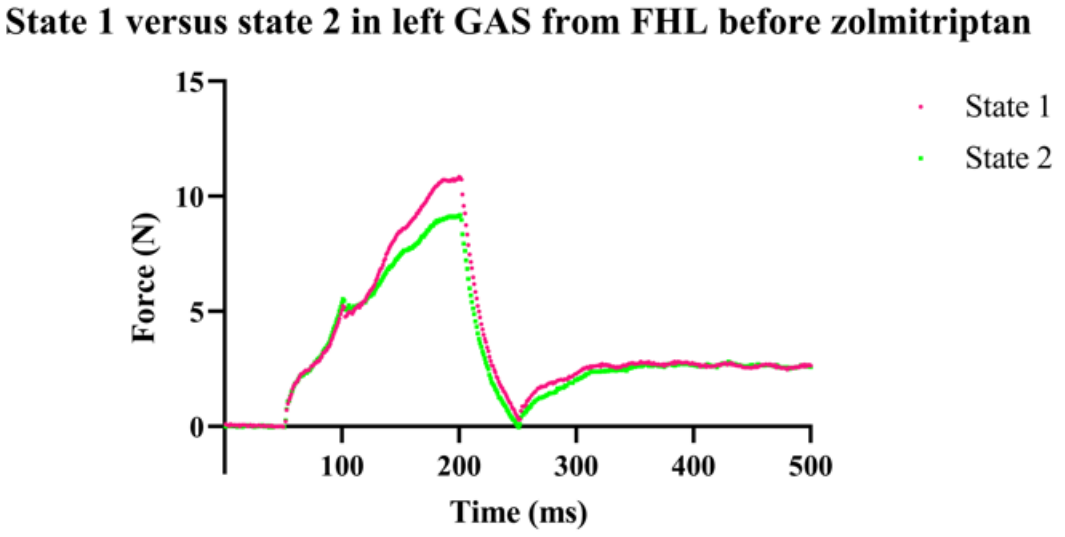


Figure 8. Summary of stretch reflex and force feedback data of gastrocnemius affected by FHL in a control preparation. (A-D) All data was measured and displayed in the same manner as in the previous figures. (B) Significance is indicated for late stage inhibition in each trial with asterisks.

In the control preparation, both legs were used during zolmitriptan delivery with the right leg being used to investigate the influence of rectus femoris as the donor onto gastrocnemius as the recipient while the left leg was used to measure FHL onto GAS as in the previous experiments. After the delivery of the first bolus of zolmitriptan, the background force in the left leg jumped to about 5-7 times the desired value, which appeared to induce an associated jump in stretch response and inhibition, but these changes persisted even when background force dropped closer to baseline (Figure 8A-C). Inhibition of FHL onto GAS seemed to increase over time and was significant ($p<0.05$) for all trials except for the control trial before drug delivery, and the

standard deviation for stretch reflex was also relatively wider. After zolmitriptan, the distribution of inhibition throughout the hold phase seemed to become more balanced with an increased early and middle stage inhibition (Figure 9). This instability was characteristic of this preparation's baseline and will be discussed later in this section. Drug delivery did not seem to correlate with any heart rate changes other than a possible slight decrease with two boluses, but the magnitude of this is relatively small until the drop in vitals at the end of the experiment (Fig. 8D).



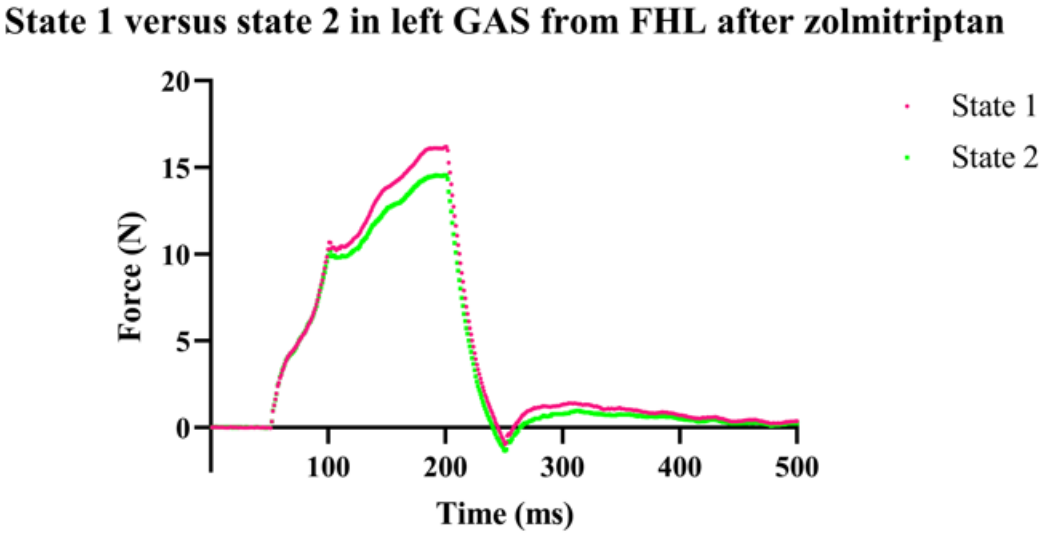


Figure 9. Comparison of state 1 versus state 2 force response in GAS with FHL donor muscle before and after drug delivery. The top graph is the average response from 2 minutes before the first bolus, and the bottom graph is from 22 minutes after. Force and inhibition seem to have increased overall with a more balanced distribution of early, middle, and late stage inhibition compared to before.

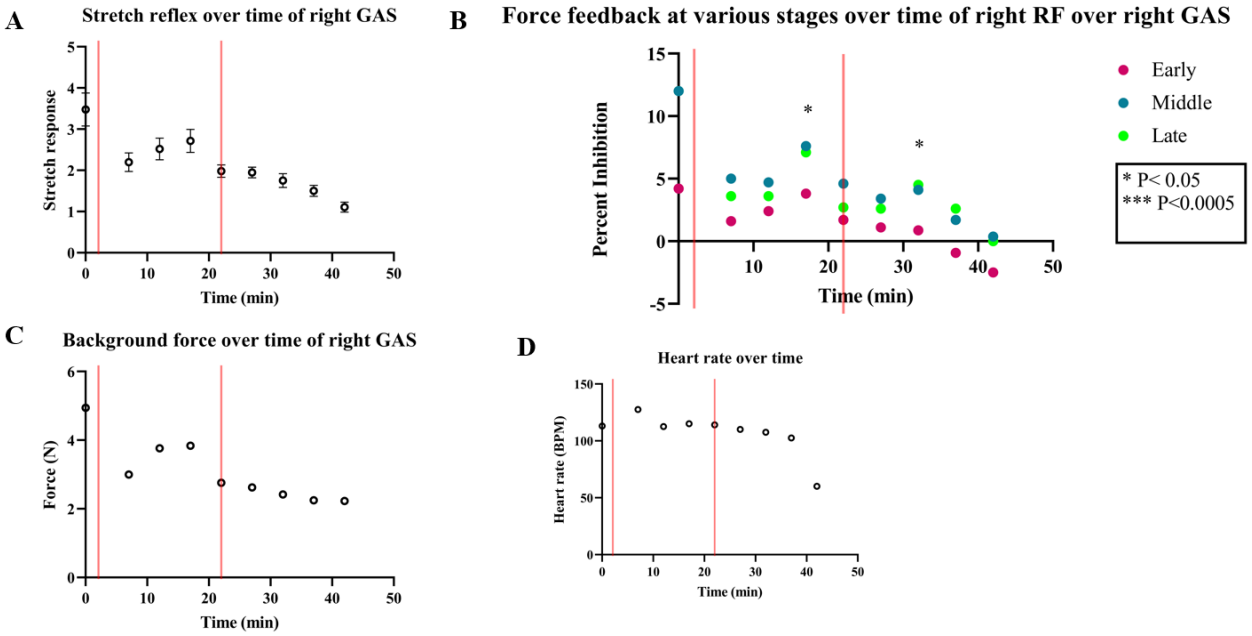


Figure 10. Summary of stretch reflex and force feedback data of gastrocnemius affected by rectus femoris in a control preparation. (A-D) All data was measured and displayed in the same manner as in the previous figures. (B) Significance is indicated for each trial for inhibition measured during the late stage.

In the right leg, background force was much more stable, and both stretch response and inhibition appeared to follow the slightly declining trend in background force with no effect from zolmitriptan (Figure 10A-C). Of note, inhibition from RF onto GAS was not statistically significant as $p<0.05$ for only two of the trials (Fig. 10B). The observed decline in inhibition seems to be time dependent and may not be due to zolmitriptan (Fig. 10, 11).

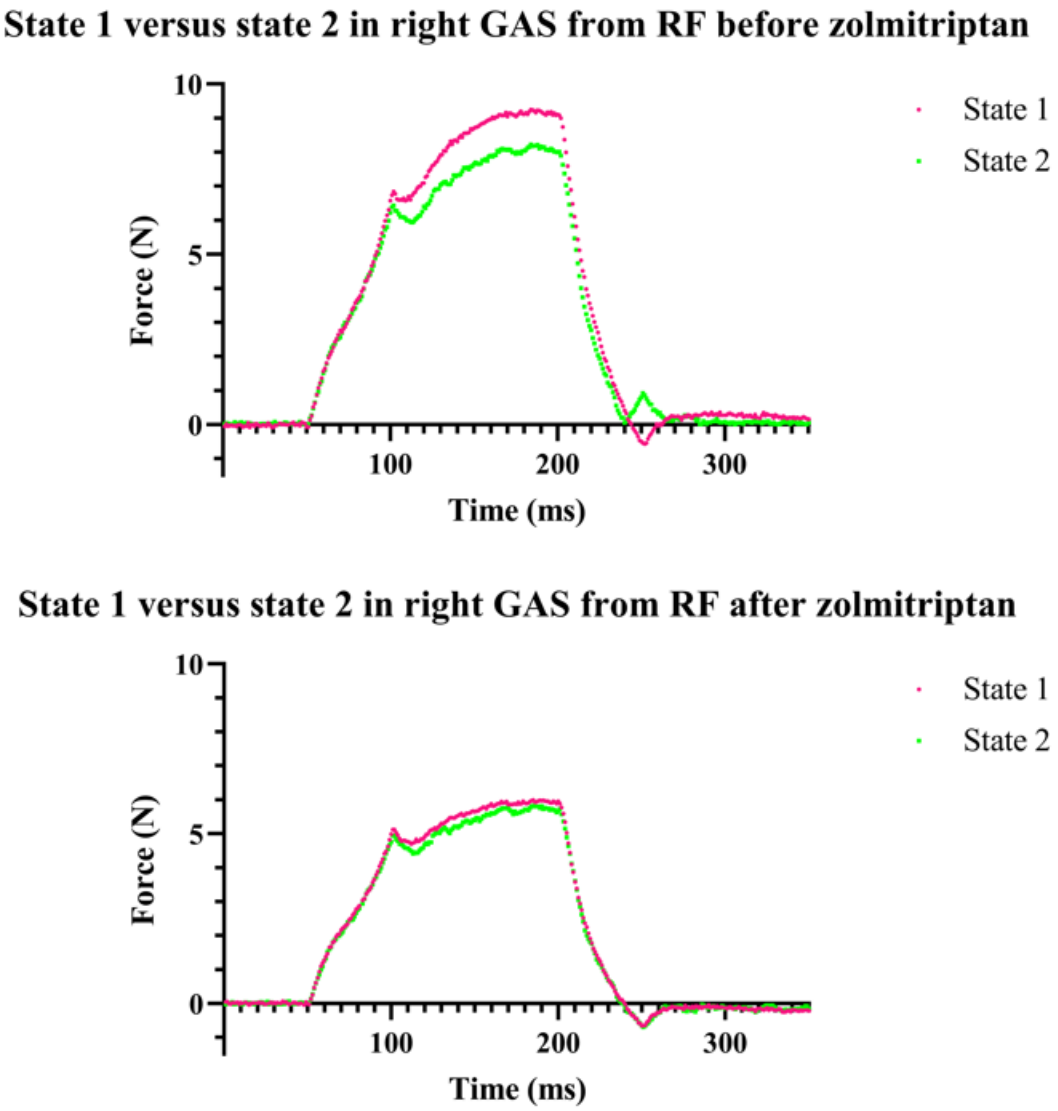


Figure 11. Comparison of state 1 versus state 2 force response in GAS with RF donor muscle before and after drug delivery. The top graph is the average response from 2 minutes before the first bolus, and the bottom graph is from 10 minutes after. All inhibition and overall force seemed to diminish after the drug, but this seems to be time dependent and not statistically significant in the context of Figure 10B.

In this experiment, consistent data was much more difficult to obtain. In both hindlimbs, there were dramatic oscillations in background force, undesired changes in muscle response shape, and an overall less consistent force response than expected. However, very quickly after zolmitriptan was delivered, these problems vanished entirely (Figure 12; Figure 13).

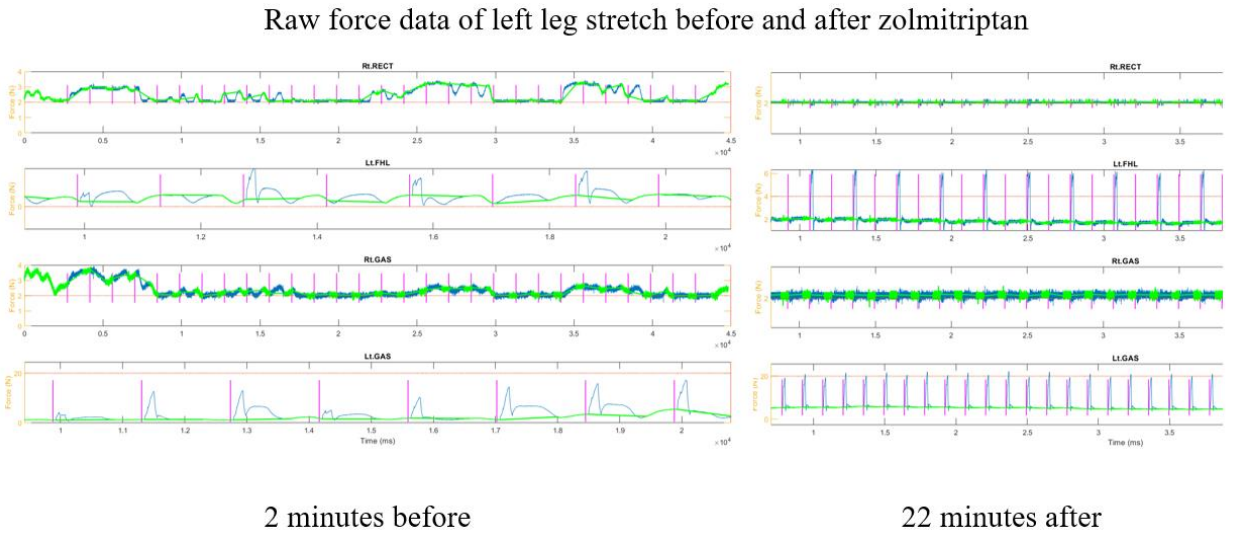


Figure 12. Left gastrocnemius muscle response shape changes after zolmitriptan. The second row depicts the muscle force measured from the left FHL. The fourth row depicts muscle force measured from the left GAS. These two rows have been zoomed in to focus on the irregular response curves. The left depicts a trial two minutes before zolmitriptan was delivered, and the right depicts a trial 22 minutes after. Muscle response shape and background force become significantly more regular. The first row is the force from right RF, and the third row is the force from right GAS, but these muscles were not stretched during these trials and are included to show the resolved fluctuations in background force.

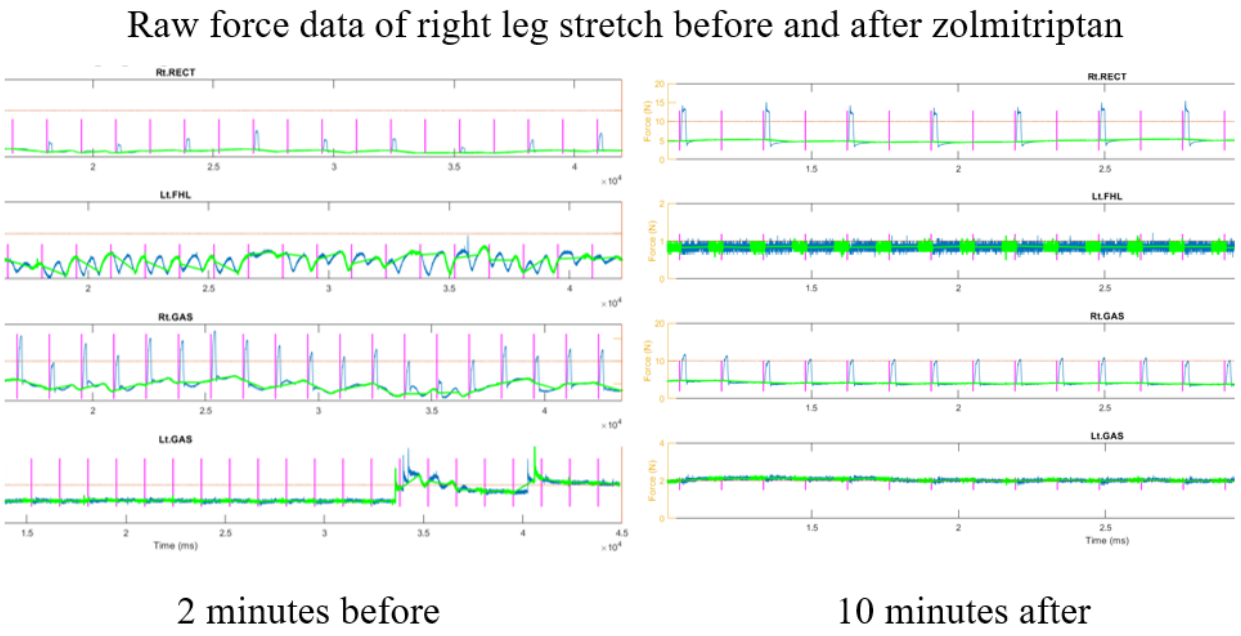


Figure 13. Right gastrocnemius muscle response shape changes after zolmitriptan. The first row depicts the muscle force measured from the right RF. The third row depicts muscle force measured from the right GAS. The left depicts a trial two minutes before zolmitriptan was delivered, and the right depicts a trial 10 minutes after. Muscle response shape and background force become significantly more regular. The left sided muscles are not active during these trials, but their force responses are shown to provide context of the oscillatory background force seen before the drug.

This brought into question how zolmitriptan affects the shape of the muscle stretch response curve. It was already shown that zolmitriptan increases inhibition in the late and middle stages significantly with occasionally an increase in the early stages of the muscle response (Fig. 4B, 5, 8B, 9), but it was worth pursuing if it causes any changes in the baseline shape of the state 1 force response.

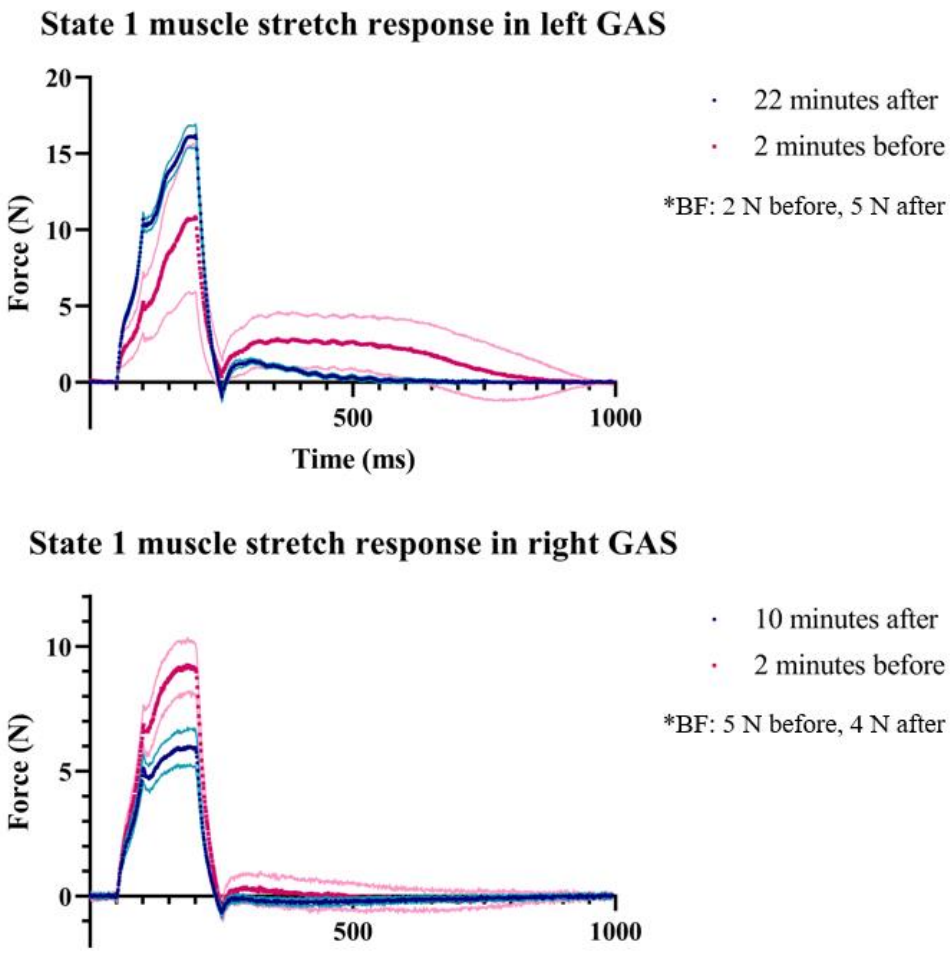


Figure 14. Average state 1 muscle stretch response in each left and right GAS in a control preparation. Each curve is the average force pattern during a single muscle stretch of the indicated time points relative to drug administration. Background force is given to provide context as the force response is dependent on this, and it is subtracted from these curves on the graph. Different time points after drug delivery were chosen for each leg in order to maintain the most consistent background forces for comparison. Standard deviation is given in lines of fainter colors than the mean, and this appears to decrease after drug delivery.

In the control preparation, there was not much difference in the shape induced by zolmitriptan except for that in the right GAS, the middle and late stages seemed to decrease relatively to the early stage to create a more level force plateau (Figure 14). Notably, the initial shape of the force response is much more variable with wide error lines compared to after the

drug, suggesting an increase in consistency. The differences in magnitude are not important for the left gastrocnemius as background forces were not very comparable before and after, and the magnitude is dependent on background force. In the right gastrocnemius, there was a substantial decrease in magnitude after drug delivery despite comparable background forces.

In the lateral hemisections, background force was the same in all trials, so the magnitude of the force response curves has greater importance. The first experiment showed a significant change in force shape after drug delivery as well as a sharp decrease in magnitude, but as stated before, the validity of this experiment is in question (Fig. 15B). The second experiment, which had the most stable results overall, also shows no change in the shape or magnitude of the force response curve from before or after zolmitriptan (Fig. 15A). Standard error is also much lower in Figure 15A than 15B, but the responses in these trials are overall consistent.

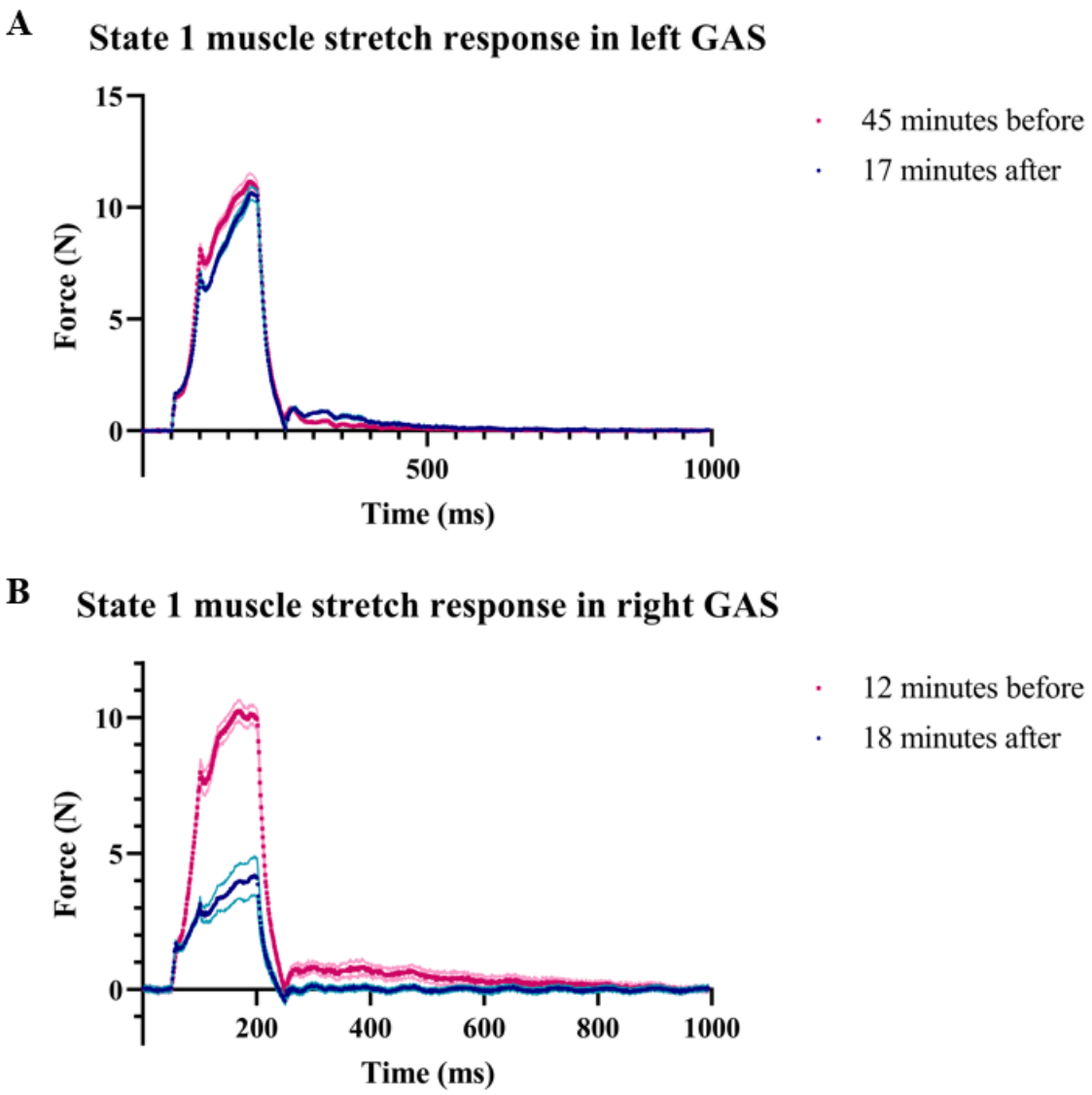


Figure 15. Average state 1 muscle stretch response in each left and right GAS in two separate lateral hemisection preparations. (B) is from the same preparation as in Figure 4 while (A) was taken from the preparation in Figure 6. Each curve is the average force pattern during a single muscle stretch of the indicated time points relative to drug administration. Background force is subtracted from these curves on the graph, measuring about 2 N in all trials. Standard error is shown as faint lines alongside their respective means.

Discussion

Force feedback

Inhibition from force feedback was primarily evaluated at the late stage of the stretch. This was the percentage decrease in muscle tension when stretched simultaneously with another muscle, and this value varied at baseline across all three subjects. In the first lateral hemisection animal, there was a large magnitude of inhibition around the control trials that decreased to about half its magnitude with drug delivery and fluctuated significantly. Meanwhile in the other lateral hemisection animal, there was very little inhibition at baseline but sharply rose and stayed at about double its magnitude with drug delivery. The control animal had a similar trend in inhibition relative to the second lateral hemisection though more progressive. In all cases, around 10% appeared to be the new baseline for inhibition of gastrocnemius (GAS) onto flexor hallucis longus (FHL). The effects of a lateral hemisection have been shown to have variable effects on inhibition across individuals, described more as a reorganization in force feedback circuitry as opposed to a generalized increase or decrease in inhibition (Niazi et al. 2020). It could be then suggested from this current study that zolmitriptan has some corrective influence over inhibition from GAS onto FHL, but more individual experiments would be needed to confirm this from both control and lateral hemisection animals.

Stretch reflex

An increase in the magnitude of the stretch reflex, such as caused by spinal cord injury (SCI) has been long thought to be a cause of hyperreflexia as an increase in stretch reflex induces exaggerated muscle stiffness (Nardone et al. 2015; Kajtaz et al. 2021). Zolmitriptan has also been shown to function as an anti-spastic drug, although its mechanism involving the stretch

reflex is a source of debate. In this study, it was found that there was no consistent effect on the stretch reflex by zolmitriptan. It decreased substantially in the first lateral hemisection animal, but this correlated with the measured background force that was not controlled well in a few of the trials. There was no observable or significant change in this response related to zolmitriptan administration. This suggests that the mechanism for zolmitriptan or the bursting interneurons seen in the deep dorsal horn may not involve the autogenic stretch reflex pathway, and this pathway may not be a major contributor to hyperreflexia. At least, this pathway may not be largely affected by zolmitriptan if there is a serotonergic mechanism. Based on this, it seems that more research is needed to determine if the autogenic stretch pathway is a contributing factor to the bursting behavior of interneurons and associated motoneuron hyperexcitability.

Force stabilizer

Each of these three preparations had starkly different datasets and effects, which was why the results of all three were described in detail. It was uncertain what effects zolmitriptan would have on the decerebrate feline, so the nature of this study was highly exploratory, which was why much of the data analysis in the latter half of this section was unexpected. It was worth studying the changes in oscillation and muscle response curve shape as these may be related to spasticity. The corrections seen in the control preparation support zolmitriptan's influence against hyperreflexia as a potential force stabilizer as well as showing real effects that could lead to formulating more mechanisms of its action.

In the control animal, the drug had an immediate and significant impact on the stability of the background force during the trials. Notably, the background force oscillated uncharacteristically with an amplitude large enough to disrupt some force response spikes. This occurred in all four muscles and completely resolved to a constant background force after

zolmitriptan, although this was at a much higher level in the right leg. There were no significant changes to the shape of the average muscle response curve after zolmitriptan in any of the conditions, however, the consistency of the force response increased, likely secondary to the diminished oscillations. This indicates that zolmitriptan does not likely have any effect on persistent inward currents (PICs) that maintain the activation of motoneurons during the hold phase (Tysseling et al. 2017).

PICs regulate the flow of sodium into the axon of the neuron, keeping the neuron depolarized and has been a possible candidate for contributing to hyperreflexia (Nardone et al. 2015; Tysseling et al. 2017). PICs have also been shown to have serotonergic input and upregulation of serotonin receptors following SCI, but it was not expected that this particular selective serotonin reuptake inhibitor (SSRI) would have any effect on the PICs of the alpha motoneurons, so this is supported by the findings on this from this study. This does not necessarily suggest that zolmitriptan has no effect on the clasp knife reflex, characterized by the abnormal decrease of muscle tension during the stretch hold phase. There did not appear to be any inhibition of force during the hold phase after drug delivery, therefore there is no evidence in this study to support or refute the finding that 5-HT1B/1D agonist attenuates clasp knife reflex (Miller et al. 1995).

The suppression of the oscillations in background force may possibly be from zolmitriptan inhibiting bursting interneurons in the deep dorsal horn, which would support the implications of the previous study (Thaweerattanasinp et al. 2020). However, there is not enough evidence to confirm this connection as it only appears that zolmitriptan treated hyperreflexia pathology, which is known (Nardone et al. 2015).

Limitations and future directions

The main limitation of this study was the lack of subjects. There were only three animals used, one control and two lateral hemisections with a total of four legs and individual GAS muscles studied. In addition, the results of the first lateral hemisection may not be reliable or comparable to the other experiments as the zolmitriptan was delivered using saline rather than DMSO, which dissolves zolmitriptan far more readily. The fact that the results of this study were incredibly varied shows that future experiments of both control and SCI animals using zolmitriptan will need to be performed in the same manner in order to draw a more definite conclusion on its effects on the feline hindlimb, with and without SCI.

Another major limitation was the lack of readily available literature and expertise on the appropriate bolus size of zolmitriptan for a feline. There was clearly an effect from the drug on the data, but the measures used to indicate if it was really taking effect, such as heart rate and blood pressure, did not seem to be relatable indicators. Therefore, there was not much to tell if the changes in the data collection were caused by the drug delivery other than the attempts made to control all other aspects. Data normally changes over the course of the experiment, such as with neural feedback and vitals, as is the nature of this kind of terminal experiment, so it is possible that some of the changes interpreted to be from zolmitriptan were actually caused by the animal's fluctuating physiology, but these signs were generally more obvious and occurred towards the very end of the experiment.

Additionally, the force feedback data was mostly obtained from the interaction of the donor FHL onto the recipient GAS with some data from the control animal of rectus femoris (RF) onto GAS. The stretch reflex data was analyzed only for GAS. The interaction chosen for this study was just one of the more notable examples of intermuscular communication for

regulating stiffness, especially in the context of SCI (Niazi et al. 2020). That is, one of the more significant rearrangements of spinal cord circuitry following injury is that the direction of intermuscular inhibition changes to focus on the ankle extensors. This creates excessive ankle compliance, and the increased inhibition of GAS, a major ankle extensor, from the FHL is a major contributor to this. However, there are numerous other muscular interactions that have been studied extensively that may be affected by SSRIs (Nichols. 2018). With more data on the role of 5-HT on intermuscular inhibition, the circuitry in the deep dorsal horn and its role in reflex and supraspinal integration would be better understood, helping to map the mechanism of hyperreflexia and hopefully develop more precise treatments to the various illnesses caused by damage to the spinal cord.

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