

**Motor unit discharge characteristics in response to serotonin receptor blockade in healthy humans**

Author

Prenzler, Michael J

Published

2022-09-06

Thesis Type

Thesis (Masters)

School

School of Pharmacy & Med Sci

DOI

[10.25904/1912/4636](https://doi.org/10.25904/1912/4636)

Rights statement

The author owns the copyright in this thesis, unless stated otherwise.

Downloaded from

<http://hdl.handle.net/10072/418269>

Griffith Research Online

<https://research-repository.griffith.edu.au>

# Motor unit discharge characteristics in response to serotonin receptor blockade in healthy humans.

Michael Prenzler, BExSc, MPhty

Neural Control of Movement laboratory  
Menzies Health Institute Queensland  
Griffith University, Gold Coast

Submitted in partial fulfilment of the requirement for the award of the degree of  
Master of Medical Research

September 2021

## **Abstract**

All motor commands from the brain ultimately synapse on the motoneurons in the spinal cord to regulate the timing and amplitude of muscle contractions. The input-output relationship between command signals and motoneuron activation is relatively simple, whereby increases in the firing rate of the command signal cause a greater output response of the motoneuron. However, a complex parallel neuromodulation system is also present, where brainstem pathways release serotonin, otherwise known as 5-hydroxytryptamine (5-HT) in the central nervous system (CNS) to regulate the gain of motoneuron activity. The purpose of this project was to determine if serotonergic effects associated with muscle activation are dependent on the mode of contraction being performed. Healthy young adults were recruited into this study, where motor unit activity was extracted from high density electromyography (HDEMG) collected from the tibialis anterior during isometric dorsiflexion. Three modes of contractions were assessed: a rapid contraction to 30% maximal voluntary contraction (MVC), a slow ramped contraction to 30% MVC, and a sustained fatiguing contraction at 30% MVC that was held to failure. Each participant was tested under normal conditions (placebo) and a condition where 5-HT<sub>2A</sub> receptors were blocked in the CNS using cyproheptadine. The main finding of the project was that a blockade of 5-HT<sub>2A</sub> receptors suppressed discharge rate of motor units during a fatiguing isometric 30% MVC contraction of the tibialis anterior muscle. In contrast, there were no drug-related differences when examining the effects of 5-HT<sub>2A</sub> antagonism for shorter contraction times that were based on rapid contractions or slow ramped contractions to achieve a steady state. It is likely that the prolonged contraction evoked more release of 5-HT into the CNS compared to the shorter duration contractions, and cyproheptadine reduced the ability of 5-HT to excite motoneurons. This project provides a valuable foundation for future research that assessed pharmacological

intervention and motor function, as well as research that uses HDEMG to assess motor unit activity for submaximal contractions.

# Table of Contents

Abstract.....	2
List of Figures.....	7
List of Tables.....	9
List of Abbreviations.....	10
Acknowledgements.....	11
Statement of originality.....	12
Chapter 1.0 Introduction.....	13
1.1 Background.....	13
1.2 Statement of the problem.....	18
1.3 Purpose of the project.....	19
1.4 Aims and hypotheses.....	20
1.5 Novelty and innovation of the research project.....	20
Chapter 2.0 Literature review.....	22
2.1 Neural activation of muscle.....	22
2.2 Motor unit physiology.....	24
2.3 Ionotropic and neuromodulatory inputs into motoneurons.....	27
2.4 The importance of neuromodulators for muscle activity.....	30
2.5 The serotonergic system and motoneuron function.....	31
2.6 Serotonin and fatigue.....	34
2.7 Pharmacological interventions to alter 5-HT activity in the CNS.....	37

2.8 Technological advances in EMG for assessment of motor function .....	38
2.9 Summary .....	39
Chapter 3.0 Methods .....	41
3.1 Participants.....	41
3.2 Ethical approval .....	42
3.3 Experiment design .....	42
3.4 Drug intervention.....	43
3.5 Instrumentation .....	45
3.5.1 Assessment of ankle torque .....	45
3.5.2 High-Density Electromyography (HDEMG).....	46
3.5.3 Bipolar EMG.....	48
3.6 Experimental protocols .....	48
3.6.1 Maximal voluntary contraction.....	49
3.6.2 Rapid contractions .....	49
3.6.3 Ramped submaximal contractions .....	50
3.6.4 Fatigue-inducing sustained contractions.....	51
3.7 Data analysis .....	52
3.7.1 HDEMG signal preparation .....	52
3.7.2 Extraction of motor unit activity from HDEMG .....	52
3.8 Statistical analysis.....	53
Chapter 4.0 Results .....	55

4.1 Participant characteristics .....	55
4.2 MVC comparison between the placebo and cyproheptadine conditions .....	55
4.3 Motor unit discharge during rapid dorsiflexion contractions .....	56
4.4 Motor unit discharge during unfatigued ramped dorsiflexion contractions.....	59
4.5 Motor unit discharge during fatigue inducing sustained submaximal dorsiflexion.....	61
Chapter 5.0 Discussion .....	64
5.1 Summary of findings.....	64
5.2 Maximal voluntary contraction amplitude was reduced with 5-HT antagonism.....	64
5.3 5-HT antagonism had no effect on rapid or sustained contractions for unfatigued muscle .....	65
5.4 5-HT antagonism reduced motoneuron discharge rate during submaximal fatiguing contractions .....	67
5.5 Physiological considerations.....	69
5.6 Technical considerations.....	70
5.7 Future directions .....	72
5.8 Conclusions.....	72
Chapter 6.0 References .....	74

## List of Figures

<b>Figure 1. 1.</b> A single motoneuron and the discharge rate associated with ionotropic and neuromodulatory inputs. ....	15
<b>Figure 2. 1.</b> Descending pyramidal pathways synapse on lower motoneurons that project to skeletal muscle. Figure sourced from (Gandevia, 2001). 23	23
<b>Figure 2. 2.</b> Discharge rates for two biceps brachii motor units recruited during a triangular ramp contraction. Sourced from Farina et al. (2009).....	25
<b>Figure 2. 3.</b> The input-output function of a given motor pool and associated muscle in response to synaptic current. Sourced from (Heckman et al., 2009). ....	31
<b>Figure 2. 4.</b> Illustration of projections from the raphe nuclei in the brainstem to the spinal cord. Adapted from (Holstege & Kuypers, 1987).....	32
<b>Figure 2. 5.</b> Ramp-hold protocol used to examine cyproheptadine effects on muscle function in people with stroke. Adapted from Murphy (2018).....	36
<b>Figure 3. 1.</b> A schematic representation of the experiment design. 43	43
<b>Figure 3. 2.</b> Experimental setup. ....	46
<b>Figure 3. 3.</b> The three contraction types used in this study all to 30% MVC. ....	51
<b>Figure 4. 1.</b> Representative data for a single participant performing a rapid dorsiflexion during the placebo session (A) and during the cyproheptadine session (B).....	56
<b>Figure 4. 2.</b> Discharge rate of motor units extracted from HDEMG of the tibialis anterior for the performance of rapid contractions to 30% MVC.....	58
<b>Figure 4. 3.</b> Representative data for a single participant performing a slow ramped contraction during the placebo session (A) and during the cyproheptadine session (B). ....	59

**Figure 4. 4.** Discharge rate of motor units extracted from HDEMG of the tibialis anterior for the performance of a steady state phase following a slow ramped contraction to 30% MVC..  
.....60

**Figure 4. 5.** A single participant performing a fatigue-inducing submaximal dorsiflexion of 30% MVC until task failure.....61

**Figure 4. 6.** Representative data for a single participant performing a sustained 30% MVC dorsiflexion until task failure.....62

**Figure 4. 7.** Motor unit discharge rate during the sustained 30% MVC fatiguing contraction.  
.....63

## List of Tables

<b>Table 4. 1.</b> Anthropometric characteristics and peak force during maximal voluntary dorsiflexion contractions.....	55
--	----

## List of Abbreviations

5-HT	Serotonin
ANOVA	Analysis of variance
CNS	Central nervous system
EMG	Electromyography
HDEMG	High-density electromyography
K+	Potassium ion
MUAPS	Motor unit action potentials
MVC	Maximum voluntary contraction
Na+	Sodium ion
PIC	Persistent inward current
PNR	Pulse-to-noise ratio
SCI	Spinal cord injury
SPSS	Statistical Package for the Social Sciences
SSRI	Selective serotonin reuptake inhibitor

## **Acknowledgements**

Firstly, I would like to thank my principal supervisor Justin Kavanagh for his support and guidance throughout this project. Your support has made this transition into research possible and relatively seamless given the many novel challenges of the last year and a half or so.

Thanks to Sean Horan for assisting with the project especially in the early stages and helping me find my feet in the lab. I would also like to extend a substantial thank you to Ben Goodlich, without your expertise and guidance this project would still be in its infancy.

I would like to thank all the participants, without which, this project would not have been possible. Your efforts and involvement are greatly appreciated. Furthermore, I would like to thank my research candidate colleagues for their support throughout this project, you have all made this experience much more enjoyable.

I would finally like to thank my parents, Bernadette and Terrence, and my siblings for their unyielding support, not just through this year and half but for the last 26 years. None of this would have been possible without you.

## **Statement of originality**

*"This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself."*

Michael Prenzler, 4<sup>th</sup> September 2021

# Chapter 1.0 Introduction

## 1.1 Background

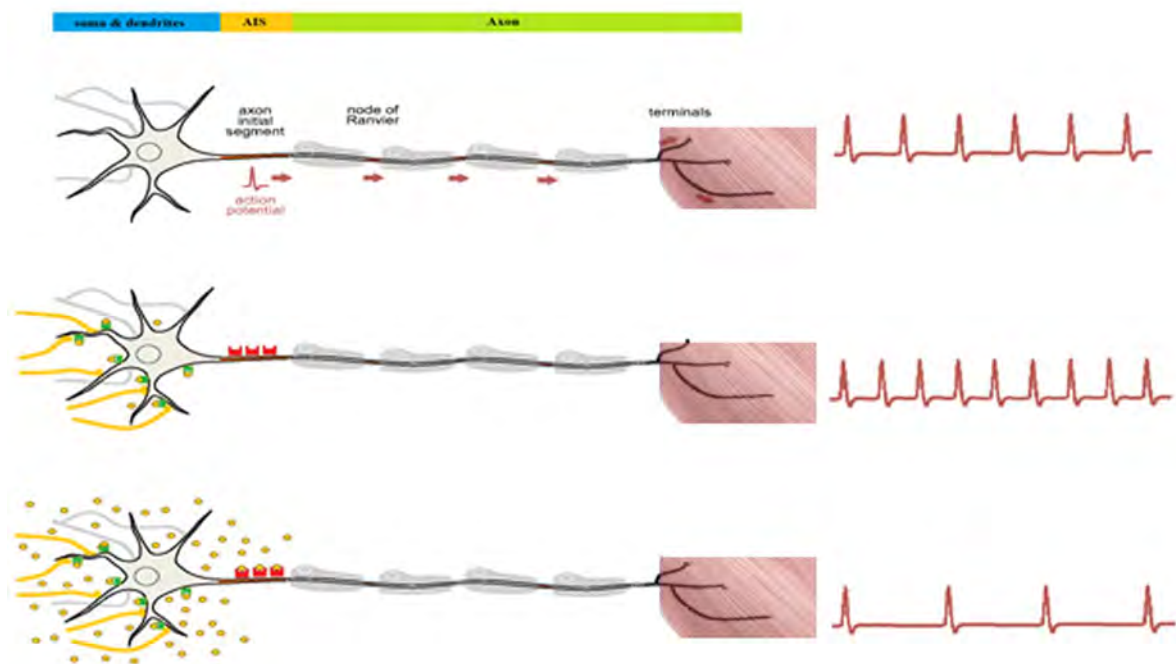
The motor unit forms a junction between the neural output of the CNS and the development of force from a muscle (Sherrington, 1925) and can be described as a neuromechanical transducer (Heckman & Enoka, 2012). The motor unit is made up of a motoneuron and the number of muscle fibres it innervates (Heckman & Enoka, 2012). Overall, an individual muscle contains hundreds of motor units whose motoneuron cell bodies are located in pools within the spinal cord. These motoneuron pools have been suggested to have a level of organization within the spinal cord's ventral horn. The motoneuron pools responsible for proximal musculature tend to be more ventral and lateral than distal musculature. However, anterior musculature motoneuron pools have been suggested to be more lateral than motoneuron pools of posterior musculature (Del Valle & Thomas, 2005). Although there is clear organisation of motoneurons in the spinal cord, the dendrites of each motoneuron extensively overlap throughout the whole ventral horn (Cullheim, Fleshman, Glenn, & Burke, 1987).

Motoneuron excitability is regulated by three forms of synaptic input: ionotropic, neuromodulatory and presynaptic inhibition. At the most basic description, an ionotropic input is the signal from the brain or peripheral sensory nerves to activate the motoneuron. At a deeper level, structures that carry ionotropic effects such as the descending corticospinal pathway or peripheral type Ia fibres deliver action potentials to the dendrites of the motoneuron for subsequent activation (Heckman, Mottram, Quinlan, Theiss, & Schuster, 2009). Although ionotropic inputs can activate motoneurons they cannot facilitate maximal force production from a muscle. In fact, computer simulations have revealed that the descending voluntary drive of ionotropic inputs that initially activates motoneurons may only contribute to 20-30% of maximal force generation in a muscle (Heckman & Enoka, 2012; Heckman, Gorassini, &

Bennett, 2005). The remaining 70-80% of force generation arises from neuromodulatory inputs to the motoneurons. Neuromodulatory inputs control the excitatory state of the motoneuron by modulating its response to ionotropic inputs. Thus, neuromodulation can act as a gain mechanism to enhance the discharge rate of motoneurons to enhance force production. Presynaptic inhibition is a phenomenon where a presynaptic efferent neuron is depressed prior to its synapse to a post synaptic neuron (Wu & Saggau, 1997). Activation of presynaptic inhibition has been suggested to act as a mediator of synaptic depression at neuromuscular junctions in animal models (Redman & Silinsky, 1994). This study was aimed at manipulation of the neuromodulatory role of 5-HT and what role it plays in human motor unit behaviour.

There are two primary neuromodulation systems for spinal motoneurons. They are the norepinephrine system and the serotonergic system. Norepinephrine is a monoamine neurotransmitter and catecholamine heavily involved in the sympathetic nervous system and the norepinephrine system within the CNS (O'Donnell, Zeppenfeld, McConnell, Pena, & Nedergaard, 2012). Serotonin (5-HT) is a monoamine neurotransmitter produced in the raphe nuclei within the brainstem and was the focus of this study. 5-HT pathways project to many areas of the CNS, including inferior to the spinal cord. These projections deliver 5-HT to the required site to activate receptors on the post synaptic cell. There are seven distinct families of 5-HT receptors and 14 different receptors currently known in humans each with varying roles. 5-HT is involved in modulating mood, sleep, temperature, sexual behaviour, voluntary, appetite, gastrointestinal motility, and movement (Berger, Gray, & Roth, 2009). Depending on which receptor is activated the role of 5-HT is excitatory or inhibitory. Specific to the motor system, alpha motoneurons have excitatory 5-HT<sub>2A</sub> receptors on the dendrites and soma, and inhibitory 5-HT<sub>1A</sub> receptors that are exclusively located on the axon initial segment (Figure 1.1). The location of the 5-HT<sub>2A</sub> and the 5-HT<sub>1A</sub> on the neuron are important as this has been

suggested to be the mechanism behind central fatigue; the concentration of 5-HT acts as a trigger, with higher concentrations causing some 5-HT to reach the axon initial segment (Kavanagh, McFarland, & Taylor, 2019; Thorstensen, Taylor, Tucker, & Kavanagh, 2020). Activation of each type of receptor has consequences for movement, whereby serotonergic activation of 5-HT<sub>2A</sub> receptors enhances voluntary activation of a muscle, and activation of 5-HT<sub>1A</sub> receptors inhibits activation of a muscle (Wei et al., 2014).



**Figure 1. 1.** A single motoneuron and the discharge rate associated with ionotropic and neuromodulatory inputs. The top panel highlights ionotropic input, such as activity in the corticospinal pathway, where activation of ACh receptors cause an action potential to be generated from the axon initial segment. The middle panel depicts the addition of 5-HT to the dendrites of a motoneuron, where activation of excitatory 5-HT<sub>2A</sub> receptors on motoneuron dendrites enhances discharge rate. The lower panel illustrates the consequence of prolonged 5-HT release. An over-abundance of 5-HT spills over into the extracellular space to activate inhibitory 5-HT<sub>1A</sub> receptors which are only located on the axon initial segment. This activation of inhibitory receptors causes a direct reduction in motoneuron output.

Animal preparations have revealed that motoneuron discharge depends largely on the concentration of 5-HT at a given spinal motor pool (Perrier & Cotel, 2015; Perrier, Rasmussen, Christensen, & Petersen, 2013). In the adult turtle, a brief (~1 s) stimulation of the dorsolateral funiculus causes a moderate release of 5-HT from the raphe-spinal pathway that facilitates spinal motoneuron output (Cotel, Exley, Cragg, & Perrier, 2013). Under these conditions, 5-HT activates excitatory 5-HT<sub>2A</sub> receptors located on the somato-dendritic compartment of the spinal motoneuron, whereby the activation of these 5-HT<sub>2A</sub> receptors promote self-sustained spinal motoneuronal firing via voltage-gated persistent inward currents (PICs) (Heckman, Hynstrom, & Johnson, 2008). However, further release of 5-HT from the descending raphe system leads to an abundance of 5-HT which in turn causes activation of extra-synaptic 5-HT<sub>1A</sub> receptors; these 5-HT<sub>1A</sub> receptors suppress action potential generation (Cotel et al., 2013). Recently, the activation of extra-synaptic 5-HT<sub>1A</sub> receptors has been implicated in human motor fatigue during tasks requiring strong muscle contraction (Kavanagh et al., 2019; Perrier, 2019), where 5-HT ‘spills over’ from the synaptic cleft to extra-synaptic compartments of the spinal motoneuron after intense 5-HT release from the raphe-spinal pathway (Beliveau et al., 2017; Cotel et al., 2013). In support of this proposition, the pharmacological activation of the 5-HT<sub>1A</sub> receptor with the agonist buspirone decreases spinal motoneuron excitability (D'Amico et al., 2017) and worsens exercise performance (Marvin et al., 1997; Perrier, 2019) in humans. Therefore, central fatigue has been suggested to have a dosage dependent relationship to the concentration of 5-HT released at the synapse due to the location and activation of 5-HT<sub>1A</sub> receptors.

Animal models, using cats and turtles, have shown that contraction intensity has a positive relationship with 5-HT release (Bigland-Ritchie, Johansson, Lippold, Smith, & Woods, 1983; Kavanagh et al., 2019; Veasey, S., Fornal, C., Metzler, C., & Jacobs, B., 1995), using a higher

intensity of running as a trigger for intensity of muscle contraction. Recently, human studies have suggested the same using discreet percentages of MVC (Thorstensen et al., 2020). This then creates a positive feedback loop with, as the increased release of 5-HT creates a higher discharge rate, resulting in an increased discharge rate from motoneurons. Drugs have been discovered that manipulate 5-HT concentrations and its effects for various purposes.

Many drugs have effects on the serotonergic system; a Selective Serotonin Reuptake Inhibitor (SSRI) is one class of drug that manipulates the serotonergic system. SSRIs increase the amount of 5-HT by inhibiting its reuptake after it has been released in the synaptic cleft. Serotonin antagonists such as cyproheptadine compete with 5-HT for their receptors. Cyproheptadine is a first-generation antihistamine with anti-serotonergic properties (Gillman, 1999); it competes with 5-HT for its receptors. Of particular interest is the 5-HT<sub>2A</sub> receptor, because it is both antagonised by cyproheptadine and responsible for the electrical gain to alpha motor neurons. Additionally, the 5-HT<sub>1A</sub> receptor is also antagonised by cyproheptadine, though to a lesser extent than the 5-HT<sub>2A</sub> receptor (Charig, Anderson, Robinson Sen, Nutt, & Cowen, 1986); the 5-HT<sub>1A</sub> receptor has been suggested to play a significant role in central fatigue (Kavanagh et al., 2019).

Electromyography (EMG) is the measurement and representation of the electrical activity within skeletal muscle (Dondelinger, 2010). Historically, surface EMG represented the summation of all electrical activity within a skeletal muscle, unable to separate the individual motor units. High density electromyography (HDEMG) addressed this issue. HDEMG measures electrical activity through an electrode array placed on the skin over a muscle belly. Compared to the two surface electrodes of traditional surface EMG, HDEMG collects electrical activity from up to 64 electrodes with the array all a standardised distance from each other.

This allows the information collected by HDEMG to be deconstructed and separated into individual motor unit activity algorithmically. This initial separation occurs automatically through software. Following this separation, manual inspection of the data is required for noise reduction and vetting of the motor unit activity to ensure it is physiological in nature and not environmental noise recognised in error by the HDEMG algorithm. HDEMG has been suggested to equivalent to the gold standard measurement of motor unit specific EMG, indwelling electrodes (Del Vecchio et al., 2020; Holobar, Minetto, & Farina, 2014; Martinez-Valdes, Laine, Falla, Mayer, & Farina, 2016). Therefore, HDEMG creates a complex and technically intricate methodology for measurement of motor unit activity that has been shown to be comparable to the gold standard of indwelling electrodes.

## **1.2 Statement of the problem**

Our ability to move is governed by a network of circuits in the brain and motoneurons in the spinal cord that command muscles to contract. The neuromodulator 5-HT plays a fundamental role in regulating motor activity (Kavanagh et al., 2019; Perrier et al., 2013; Perrier, 2019; Politis & Niccolini, 2015), where releasing 5-HT onto motoneurons in the spinal cord can cause a 5-fold increase in firing rate. A lack of 5-HT has profound consequences for movement, with neural models indicating that muscles may only produce 20-30% of maximal force without neuromodulation (Heckman & Enoka, 2012; Wei et al., 2014). Although every human has a serotonergic system, investigations into 5-HT have predominantly been from a benchtop perspective. Given that the existing 5-HT literature have been performed on animal preparations and computer simulations, our understanding of how 5-HT regulates motoneurons in the human spinal cord is almost non-existent.

To date, there have only been a small number of human investigations that have attempted to translate benchtop and simulation studies into a human model. These studies have mostly used

a selective serotonin reuptake inhibitor (SSRI) to enhance the availability of 5-HT in the CNS (often Paroxetine), and then use Transcranial Magnetic Stimulation (TMS) and nerve stimulation to determine how voluntary activation of unfatigued and fatigued muscle is affected by changes in neuromodulation (Celada, 2004; Gerdelat-Mas et al., 2005; Kavanagh et al., 2019). However, TMS manipulates the CNS at a cerebral level and therefore motor units, specifically individual motor units, are not within the focus of these studies. Human in vivo studies of how 5-HT acts as a neuromodulator on motor unit behaviour in submaximal intensities have not been explored at the time of this writing. With the availability of new technologies that can decompose surface EMG signals into individual motor units, we have reached a point we can now translate even more neuromodulation benchtop research into human motor unit research.

### **1.3 Purpose of the project**

The purpose of this project was to determine if serotonergic effects associated with muscle activation are dependent on the mode of contraction being performed. Healthy young adults were recruited into this study, where motor unit activity was extracted from HDEMG data collected from the tibialis anterior during isometric dorsiflexion. Three modes of contractions were assessed: a rapid contraction to 30% MVC, a slow ramped contraction to 30% MVC, and a sustained fatiguing contraction at 30% MVC that was held to failure. Importantly, each participant was tested under normal conditions (placebo) and a condition where 5-HT<sub>2A</sub> receptors were blocked in the CNS (cyproheptadine). Overall, this design allowed the examination of how 5-HT impacts the ability to perform different modes of muscle contraction.

## **1.4 Aims and hypotheses**

This project had three specific aims where HDEMG techniques were used to extract motor unit discharge characteristics during three different types of contractions of the tibialis anterior. The aims were to assess 5-HT effects:

- 1) during rapid dorsiflexion contractions where participants contracted as fast as possible
- 2) during an unfatigued ramped submaximal dorsiflexion of short duration
- 3) during a fatigue inducing sustained submaximal dorsiflexion of long duration

This project had three specific hypotheses that aligned with the aims. It was hypothesised that antagonising 5-HT<sub>2A</sub> receptors with the drug cyproheptadine would

- 1) decrease motor unit discharge rates when compared to placebo during rapid dorsiflexions to 30% MVC
- 2) decrease motor unit discharge rates when compared to placebo during a ramped submaximal dorsiflexion of 30% MVC which was held for 10 s
- 3) decrease motor unit discharge rates when compared to placebo during a submaximal dorsiflexion 30% MVC which was held until 30% MVC could no longer be maintained

## **1.5 Novelty and innovation of the research project**

The most fundamental aspect of the research is to use pharmacology to selectively activate or deactivate 5-HT receptors in healthy human participants. This technically difficult experimental manipulation may allow us to better grasp the importance of the serotonergic system and how muscle activity contributes to, or relies on, 5-HT release in the CNS. Theoretical frameworks, animal preparations, and computer simulation studies have all been

valuable for understanding neurotransmitter activity, as these approaches avoid invasive and potentially harmful procedures from being performed on humans. However, these models cannot represent the complexity of the human nervous system. This project had the potential to take a large step forward in understanding neuromodulation and motor performance. Importantly, it may remove the requirement to base assumptions about how neurotransmitters influence human movement on cell preparations, animal preparations, and computer simulations as this is a novel in vivo study of healthy humans. The intention of this project is to deliver a clearer understanding of how neurotransmitters in the CNS contribute to muscle activation, which could directly inform future research regarding the physiological effects of physical activity.

## **Chapter 2.0 Literature review**

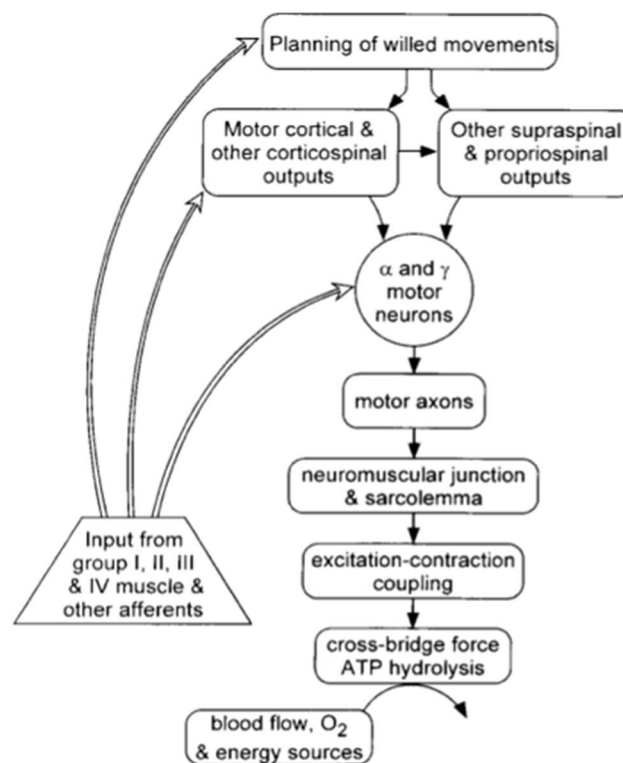
This narrative literature review provides an overview of literature that is pertinent to this project. The review will firstly detail how the CNS activates a muscle, which is followed by a description of how motor units contribute to this process, which is followed by an overview of how neuromodulation may contribute to motor unit activation in unfatigued and fatigued muscle. The final section of this review will summarise how EMG signal processing has evolved in recent years to be a powerful tool in understanding the role that motor units play in health and disease.

### **2.1 Neural activation of muscle**

The CNS operates under a hierarchical organization consisting of three levels – the motor cortex, the brainstem, and the spinal cord. At the lowest level, the spinal cord is the final common pathway for all voluntary movement (via the efferent motoneurons) and will integrate the various proprioceptive and cutaneous afferent signals from the periphery with descending motor commands from higher CNS centres. At the second level, brainstem nuclei can modify motor processes, such as the descending drive to the motoneuron pool, the spinal interneuron activity, or lower motoneuron outputs. The highest level of control is the motor cortex (primary, premotor and supplementary), which can exert a descending control over the lower levels of the hierarchical motor system in an “all-or-nothing” fashion (Scott, 2004). This is due to the motor cortex being the source of voluntary movement and ionotropic inputs from the upper motor neurons to lower motor neurons.

For a skeletal muscle to voluntarily contract, action potentials from the primary motor cortex will exit the cerebrum via descending pyramidal pathways through the medulla and spinal cord (Kuypers, 1964). These pyramidal pathways consist of upper motoneuron bundles, which can

be arranged into functional tracts, such as the corticospinal tract. These upper motoneurons will synapse via brainstem nuclei, and alpha and gamma motoneurons that project through motor axons to skeletal muscles situated across joints. Afferent signals from the periphery also return to the CNS, which are integrated to allow modification of this efferent output via supraspinal and spinal sensorimotor loops (Abbruzzese & Berardelli, 2003; Nielsen, 2004). The premotor and supplementary motor areas will ‘inform’ the motor cortex about the specific motor command (Nachev, Kennard, & Husain, 2008), alongside input from other cortical association areas and the extrapyramidal motor system (consisting of the basal ganglia, cerebellum, vestibular nuclei, and the brainstem nuclei) (Figure 2.1).



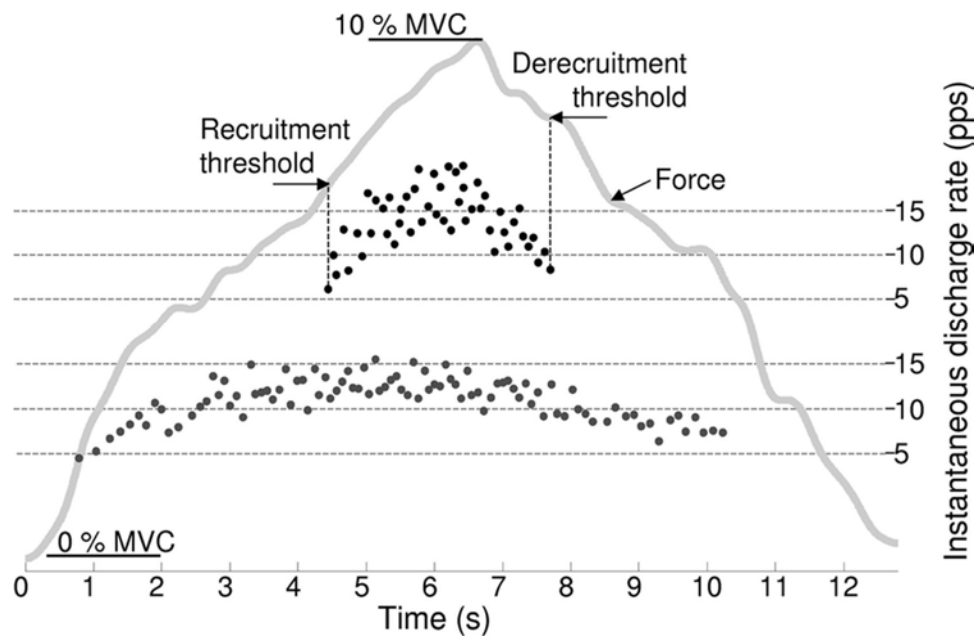
**Figure 2. 1.** Descending pyramidal pathways synapse on lower motoneurons that project to skeletal muscle. At the skeletal muscle, action potentials transmitted along the sarcolemma will lead to contractions via cross bridge interactions (excitation-contraction coupling). Afferent signals from peripheral receptors can also return to the CNS to modify this efferent output at the level of the lower motoneuron directly, at supraspinal sites, or at ‘higher’ CNS centres. Figure sourced from (Gandevia, 2001).

## 2.2 Motor unit physiology

A motor unit is an alpha motoneuron and all the individual muscle fibres it innervates. The collection of alpha motoneurons that innervate a muscle is called a motoneuron pool (Buchthal & Schmalbruch, 1980). The CNS controls the amount of force a muscle produces in two sequential ways. Firstly, the motoneuron firing rate can be increased, and secondly a synergistic motor unit can be recruited when more force is required (Bigland-Ritchie et al., 1983; De Luca & Hostage, 2010; Dorfman, Howard, & McGill, 1990; Heckman & Enoka, 2012) (Figure 2.2). This sequence of events to produce force has been shown to occur consistently in submaximal muscle contractions but may differ when performing explosive muscle contractions. De Luca and Erim (1994) describe the sequence of increasing firing rate followed by gradual recruitment of motor units as force increased in a submaximal ramped contraction. However, Del Vecchio, Casolo, et al. (2019) studied motor unit discharge rate in explosive MVCs, rather than gradual ramped force or submaximal contractions, and describe a rapid discharge rate, far exceeding the required rate for the force requirement, as well as recruitment of motor units prior to force development but decreased as force plateaued. This phenomenon was attributed to the higher recruitment of cortical neurons to increase the rate of force development; this exaggerated recruitment and firing of motor units beyond what is required for a desired force is suggested to be a characteristic of rapid contractions (Del Vecchio, Casolo, et al., 2019).

Descending action potentials from the motor cortex reach the alpha motoneurons within the spinal cord. Once these action potentials reach the threshold potential for the alpha motoneuron, the alpha motoneuron then must propagate the action potential terminating at the muscle fibre; this can then be called an “all or nothing” response to central drive. This is due to the ionotropic inputs from the motor cortex; once threshold voltage is reached action potentials will propagate into the post-synaptic alpha motor neuron. Modulation of this all or nothing response is

important and can increase or decrease the firing rate of the alpha motor neuron (Heckman & Enoka, 2004; Johnson & Heckman, 2014; Wei et al., 2014).



**Figure 2. 2.** Discharge rates for two biceps brachii motor units recruited during a triangular ramp contraction. The force is represented by the gray line, where elbow flexion force was increased to 10% MVC over a 6 s period, and then decreased to a resting state over a 6 s period. The dots correspond to the discharges of the motor units and the y-axis on the right indicates instantaneous discharge rate. Sourced from Farina et al. (2009).

Smaller motor units have smaller motoneurons in comparison to larger motor units which have larger motoneurons. When performing ramped muscle contractions the smaller neurons are activated first due to the size of their soma and dendrite geometry (Enoka & Stuart, 1984; Henneman, 1957). This also provides a mechanism for fine and gross motor control. Muscles requiring fine adjustments in force production are innervated by more alpha motoneurons than muscles that do not require fine modulation of force. This allows finer adjustment of force than a muscle with a smaller motor unit pool; larger motoneurons not only innervate more muscle fibres but due to their axon diameter being larger relative to a motoneuron from a smaller motor unit, they transmit action potentials at a greater speed. An example of this is the difference

between the first dorsal interosseous muscle and the vastus lateralis muscle; the later having less but larger alpha motoneurons despite having greater cross-sectional mass (Thomas, Ross, & Stein, 1986). Motor unit recruitment varies at different levels of MVC for each muscle, for example the first dorsal interosseous muscle will stop recruiting motor units at 50% of MVC instead relying on discharge rate modulation to alter force production. The tibialis anterior continues to recruit motor units until 90% of MVC (Van Cutsem, Feiereisen, Duchateau, & Hainaut, 1997). The tibialis anterior has been used in previous research on motor unit behaviour regarding neuromodulatory input (De Luca & Hostage, 2010; Del Vecchio, Casolo, et al., 2019), and due to its superficial position made an ideal muscle to study. Overall, the size of motor units and their respective motoneuron govern their recruitment threshold due to their soma and dendrite geometry and this provides a mechanism from which fine and gross motor control can be achieved; this is exploited by muscles requiring fine motor control by having a larger motor unit pool of small motor units rather than fewer large motor units.

The action potentials generated by motoneurons are produced by positively charged ions of sodium ( $\text{Na}^+$ ) depolarising the axon of a neuron (Stuart, Spruston, Sakmann, & Häusser, 1997; Vetter, Roth, & Häusser, 2001). For a neuron to depolarise the minimal threshold must be achieved by an excitatory input; the amount of stimulus required to reach this threshold is known as the rheobase, that is the minimal stimulus to trigger an action potential (Stuart et al., 1997). Depolarisation causes the resting negative charge of the axon to become positively charged. This is then corrected by the axon removing positively charged potassium ( $\text{K}^+$ ) to repolarise the cell membrane. This functionally resets the neuron to receive and propagate another action potential (Coombs, Curtis, & Eccles, 1957; Kole et al., 2008). Repolarisation, therefore, maintains homeostasis within the axon. Excitatory inputs will lead to an increased firing rate and therefore an increase in the rate of depolarisation and repolarisation to have a summative effective on the innervated muscle fibres. Summation of these action potentials

from the alpha motor neuron will increase tension in the muscle and in turn increase the force produced by that muscle (Giuliodori & Zuccolilli, 2004; Josephson, Rose, & Knight, 2019). Inhibitory inputs decrease the firing rate of action potentials therefore decreasing the rate of depolarisation and repolarisation needed to maintain homeostasis and decreasing the rate of summation of action potential and in turn muscle force (Kavanagh et al., 2019). PICs are a mechanism by which alpha motor neurons increase their action potential output frequency and therefore summation of action potentials and muscle force respectively (Heckman & Enoka, 2012; Heckman et al., 2009; Johnson & Heckman, 2014; Wei et al., 2014). There are many inputs to alpha motoneurons while some simply propagate action potentials, others modulate the propagation of PICs and the excitability of motoneuron baseline potential, otherwise known as the rheobase (Chen, Ge, Cheng, & Dai, 2019).

### **2.3 Iontropic and neuromodulatory inputs into motoneurons**

There are many structures that have inputs into alpha motor neurons: descending cortical neurons, projections from brainstem nuclei, sensory neurons, and interneurons; interneurons account for most inputs to alpha motor neurons (Heckman & Enoka, 2012; Heckman et al., 2009; Holstege & Kuypers, 1987; Johnson & Heckman, 2014; Loeb, Brown, & Cheng, 1999). The inputs of these structures can also be categorised into three main types, ionotropic, neuromodulatory and presynaptic, depending on their method of interacting with the alpha motor neuron. Iontropic inputs such as the input from upper motor neurons to alpha motor neurons, result in the propagation of action potentials in a one to one fashion (Heckman et al., 2009). Neuromodulatory inputs can be thought of as the adjustment to the sensitivity to the ionotropic inputs, they can be excitatory or inhibitory and often take the form of interneurons, sensory inputs, or brainstem inputs (Heckman et al., 2008; Johnson & Heckman, 2014; Thompson et al., 2019). Presynaptic inputs, as the name implies, are inputs prior to the synapse; they can be thought of as extensions of ionotropic inputs (Heckman et

al., 2009). This is due to presynaptic inputs mediating the upper motor neurons that synapse to alpha motor neurons and form an ionotropic input (Stein, 1995; Wu & Saggau, 1997).

While presynaptic inhibition is an important to recognise as it can affect the action potentials to lower motor neurons (Rudomin, 2002; Stein, 1995; Wu & Saggau, 1997); for this study, ionotropic and neuromodulatory inputs are most relevant as they are the inputs directly influencing alpha motor neurons.

Ionotropic inputs are known of as such because of the receptors they activate on the post-synaptic neuron. These receptors are ligand gated receptors which are activated by a neurotransmitter (Heckman & Enoka, 2004); depending on the pre-synaptic neuron or input this neurotransmitter may be either excitatory or inhibitory. The method of an excitatory input would be to activate a ligand gated receptor which promoted depolarisation through diffusion of  $Na^+$  across the across the neuron's membrane potentially leading to an action potential propagating in the post-synaptic neuron. Opposingly, an inhibitory input would allow diffusion of  $Cl^-$  ions across the membrane. The differing charge of the ions entering the post-synaptic neuron change the overall charge, making it either more or less likely to reach threshold voltage to trigger an action potential. This mechanism also introduces the idea of graded potentials, where the post-synaptic neuron responds to an excitatory input however not enough to reach activation threshold. The activation threshold of a post-synaptic neuron can be modulated by other neurons making it further or less susceptible to excitatory ionotropic inputs, this mechanism is termed neuromodulation (Rekling, Funk, Bayliss, Dong, & Feldman, 2000).

Neuromodulatory inputs synapse on to the post-synaptic neuron or in the context of this study the alpha motor neuron. Neuromodulation changes the activation voltage required to generate an action potential (Cheng, Song, Ge, & Dai, 2021; Johnson & Heckman, 2014).

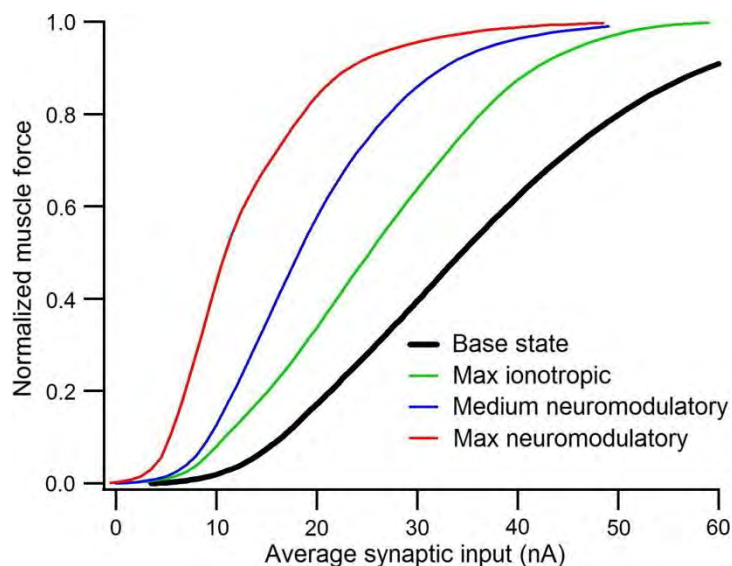
Additionally, 5-HT as a neuromodulator promotes two essential components of PICs within the alpha motor neuron, the Calcium PIC (CaPIC) and the Sodium PIC (NaPIC) (Heckman & Enoka, 2012; Heckman et al., 2005; Wei et al., 2014). This is achieved through a signalling cascade that results in the opening of  $\text{Na}^+$  channels to promote PIC development (Cheng et al., 2021; Heckman & Enoka, 2012; Johnson & Heckman, 2014). Both the NaPIC and the CaPIC are methods of inducing a PIC by opening  $\text{Na}^+$  and  $\text{Ca}^+$  channels respectively (Heckman & Enoka, 2012). Therefore, not only do neuromodulatory inputs decrease the activation voltage of the post-synaptic neuron they also promote PICs and in turn voltage gain. Though it is important to note that neuromodulatory inputs can also be inhibitory; the action of these inputs is dependent on the neurotransmitter released and the receptor activated on the post synaptic neuron. In the case of this study, 5-HT is the neurotransmitter and the receptors needs for consideration are the  $5\text{-HT}_{2A}$  receptor and the  $5\text{-HT}_{1A}$  receptor. Both receptors are present on the alpha motor neuron and have antagonistic actions. The signalling pathway for the  $5\text{-HT}_{2A}$  receptor differs to that of the  $5\text{-HT}_{1A}$  receptor from the point of their second messenger as they are both G-protein coupled receptors, they have many similarities up to this point.  $5\text{-HT}_{2A}$  receptors activate phospholipase C to generate phospholipid-derived second messengers. This leads to the cascade causing excitatory or inhibitory effects for  $5\text{-HT}_{2A}$  and  $5\text{-HT}_{1A}$  receptors respectively. The connection between PICs and 5-HT have been shown in rat with severed spinal cords having little to no PIC activity (Heckman & Enoka, 2012). This suggests 5-HT is integral to alpha motor neuron function and action potential summation (Giuliodori & Zuccolilli, 2004; Harvey, Li, Li, & Bennett, 2006; Murray et al., 2010).

Presynaptic inhibition from afferent sources work to inhibit descending action potentials, thereby decreasing the excitation of lower motoneurons (Wu & Saggau, 1997). The inhibition can occur at the level of the spinal cord with the dorsal horn or centrally from motor output centres or motor planning centres (Figure 2.3). Presynaptic inhibition occurs at higher levels within the CNS to mitigate the release of excitatory neurotransmitters and disrupt the transmission of action potentials (Eccles, Schmidt, & Willis, 1963; Stein, 1995). There are many neurotransmitters associated with presynaptic inhibition of motoneurons including adenosine and 5-HT (Rongen et al., 1996; Singer, Bellingham, & Berger, 1996).

#### **2.4 The importance of neuromodulators for muscle activity**

Neuromodulatory inputs regulate the overall excitability of neurons, and these inputs have been suggested to assist in a 'gain control' system for ionotropic inputs (Johnson & Heckman, 2014; Wei et al., 2014). Thus, it is important to note that neuromodulatory inputs can considerably amplify skeletal muscle force for a given level of synaptic input. Simulations based on the properties of the motoneuron pool and motor units of the cat medial gastrocnemius muscle have revealed the importance of descending neuromodulatory input on the input-output gain of a motoneuron (Figure 2.3). As neuromodulatory input increases, the overall slope (input-output relationship) substantially increases (Heckman, Lee, & Brownstone, 2003; Thompson et al., 2019). This relationship between the neuromodulatory input to lower motor neurons and muscle force indicates that by releasing 5-HT onto motoneurons, there should be less reliance on command signals from upper motor neurons to dictate the amount of force that a muscle generates. PICs underly this firing behaviour and the relationship between muscle force and neuromodulatory input. PICs are located on the dendrites of the motoneuron and markedly amplify synaptic input (up to fivefold or more) depending on the level of monoaminergic input (Enoka & Duchateau, 2017; Oya, Riek, & Cresswell, 2009); as PICs can be the result of both

5-HT and norepinephrine inputs. The distinctive difference between these two inputs is 5-HT is proportional to motor output and norepinephrine input will vary with arousal (Aston-Jones, Rajkowski, & Cohen, 2000; Heckman & Enoka, 2012; Jacobs, Martín-Cora, & Fornal, 2002). Therefore, increases in serotonergic activity should align with increases in motor activity, PIC activity and increases in motoneuron excitability (Heckman et al., 2003; Kavanagh et al., 2019; Perrier & Delgado-Lezama, 2005).

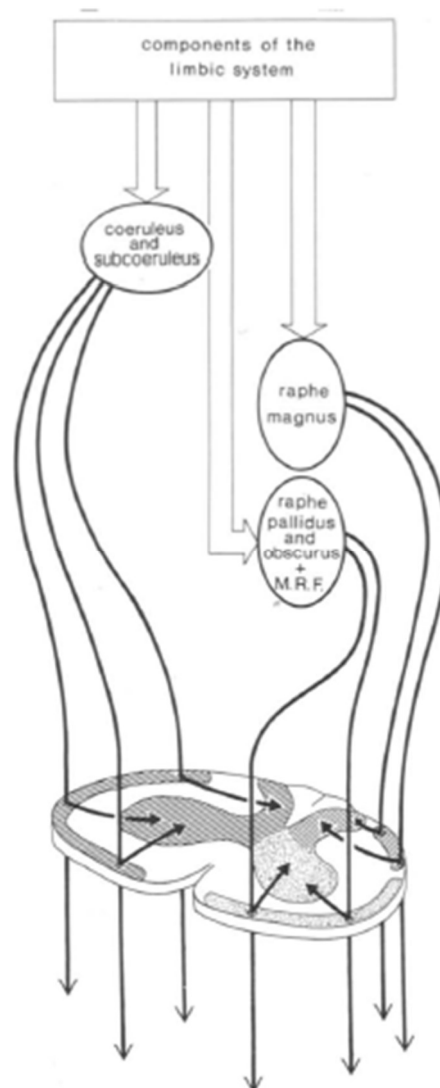


**Figure 2. 3.** The input-output function of a given motor pool and associated muscle in response to synaptic current. Neuromodulatory inputs (e.g. 5-HT and norepinephrine) to the spinal motoneurons increase muscle force for the same absolute synaptic input when compared to simulated conditions that are absent from neuromodulation. This figure is based on computer simulations of acquired data for the feline gastrocnemius. Sourced from (Heckman et al., 2009).

## 2.5 The serotonergic system and motoneuron function

5-HT has various roles throughout the body, where approximately 10% of 5-HT located in the CNS (Kim & Camilleri, 2000). 5-HT is produced in the CNS by the raphe nuclei located in the brainstem. Within the CNS, 5-HT has diverse roles including motor, memory, and it is the predominate neurotransmitter regulating mood (Berger et al., 2009). The raphe nuclei have

descending axons located largely in the ventral and lateral funiculi, these project to all grey matter laminae (Holstege & Kuypers, 1987) (Figure 2.4). This is largely due to the significant presence of motor tracts in the ventral and lateral funiculus of the spinal cord (Bowker, Westlund, Sullivan, & Coulter, 1982; Bowker, Westlund, Sullivan, Wilber, & Coulter, 1983; Holstege & Kuypers, 1987; Törk, 1990); other projections reflect the diverse nature of 5-HT. 5-HT has complex roles in motor function which is conveyed by the receptors it has and their roles of excitatory and inhibitory receptors.



**Figure 2. 4.** Illustration of projections from the raphe nuclei in the brainstem to the spinal cord. The projections are shown to be incorporated into every spinal cord lamina. This reflects the diverse role of 5-HT within the CNS. Adapted from (Holstege & Kuypers, 1987).

5-HT has seven known receptor families and fourteen subtypes (Beliveau et al., 2017; Berger et al., 2009; Daubert & Condron, 2010). Each of these receptor families has different actions, most are excitatory with only two being inhibitory (5-HT<sub>1</sub> and 5-HT<sub>5</sub>); 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> are the receptors of interest in this study. During physical activity 5-HT is released to increase the excitability of motoneurons, during prolonged motor unit activity the amount of extra synaptic 5-HT increases (Kavanagh et al., 2019; Young, 2007). 5-HT<sub>2A</sub> receptors are g-protein coupled receptors they are membrane bound to the efferent alpha motoneuron. 5-HT<sub>2A</sub> receptors have excitatory potential for the alpha motoneuron within the spinal cord (Harvey et al., 2006; Heckman & Enoka, 2012; Heckman et al., 2005; Wei et al., 2014). Endogenous 5-HT activates the resultant excitatory signalling cascade ultimately leads to the alpha motoneuron being in an excited state. Harvey et al. (2006) described this signalling cascade leading to opening Na<sup>+</sup> channels to facilitate a PIC. This excited state facilitates voltage sensitive changes within the motoneuron (Wei et al., 2014), in turn promoting voltage gain potential.

Animal models have shown 5-HT plays an important role in motor function. Chen et al. (2019) showed that exercise sensitises spinal interneurons of mice to 5-HT, providing enhanced modulation of motor units. Furthermore, Chen et al. (2019) suggested that membrane induced changes of interneurons and motoneurons are mediated by 5-HT, including a decrease of the afterhyperpolarisation voltage of interneurons and induction of the hyperpolarisation voltage threshold of spinal interneurons. Husch, Dietz, Hong, and Harris-Warrick (2015), supports 5-HT effect on spinal interneurons, also in mice, also stating that 5-HT effects are more pronounced in adult mice when compared to neonatal mice. Animal studies have also suggested that lack of 5-HT is responsible for early muscle wasting in sepsis by not promoting motoneuron excitability, though it is unclear what contribution lack of mobility versus sepsis plays in this (Nardelli, Powers, Cope, & Rich, 2017). Harvey et al. (2006) suggested that 5HT<sub>2A</sub>

receptors are responsible for motor unit dysfunction causing spasticity in spinal cord injured rats due to the upregulation of 5-HT<sub>2A</sub> receptors on motoneurons as a sequelae of spinal cord injury causing hypersensitivity to 5-HT. Murray et al. (2010) expanded on this by blocking 5-HT<sub>2C</sub> receptors and decreasing spasticity in spinal cord injured rats. This depicts the role of 5-HT<sub>2A</sub> receptors in motor control, due to the loss of 5-HT in the spinal cord the motoneurons became hypersensitive ultimately leading to uncontrolled spasms due to the increased excitability of motoneurons. In summary, animal models have been used to show the extensive effects of 5-HT and its receptors on motoneuron function and the interneurons synapsing to them; these effects largely promote motoneuron and interneuron excitability.

Physical activity has been shown to increase the prevalence of 5-HT in the spinal cord, this is due to the key role it plays in modulating motoneuron behaviour. Historically, it was unclear if this was due to the release of 5-HT as a neuromodulator or more 5-HT was being produced (Young, 2007). Rueter and Jacobs (1996) suggested that 5-HT release and production are both increased during exercise. In addition, Chaouloff, Laude, Guezennec, and Elghozi (1986) suggest that the precursor of 5-HT, tryptophan has an increased concentration in the CNS that persists after exercise. Therefore, as motor units are used during voluntary contractions 5-HT is not only released in larger amounts it is also produced in larger amounts. The presence of 5-HT<sub>1A</sub> receptors on alpha motoneurons suggests that 5-HT has a role in inhibiting motoneurons, playing a role in central fatigue.

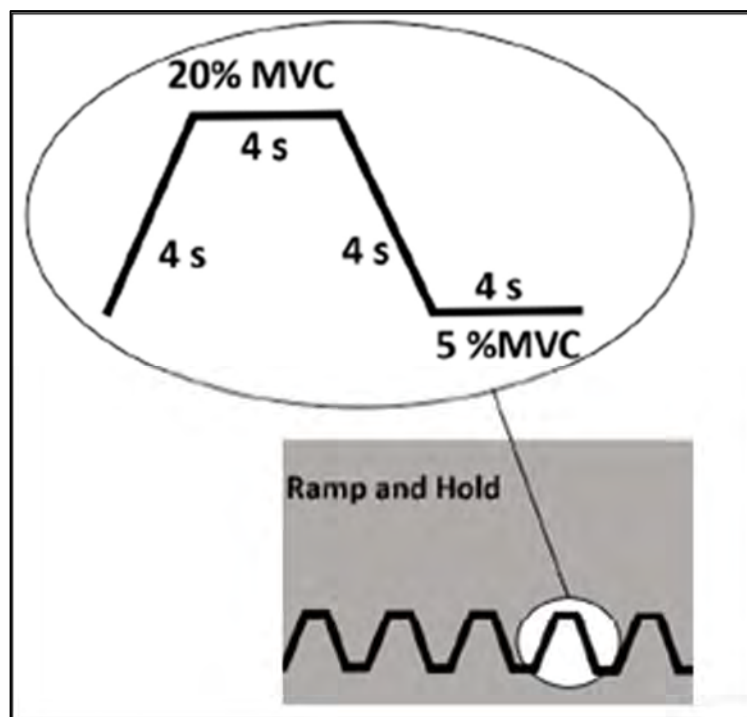
## **2.6 Serotonin and fatigue**

An interesting feature of 5-HT within the CNS is that it can play a dual role in motor function whereby the presence of 5-HT does not always align with enhanced muscle activity. There have been studies showing that 5-HT has an inhibitory effect on motoneurons leading to central fatigue that directly refuted the evidence of 5-HT being excitatory and others offering possible

relationships between the 5-HT<sub>2A</sub> and 5-HT<sub>1A</sub> receptors (Cotel et al., 2013; Kavanagh et al., 2019; Perrier, 2019; Thorstensen et al., 2020). Cotel et al. (2013) indicated that increasing 5-HT increased central fatigue in turtles by reducing the amount of action potentials being produced by motoneurons; they also found that for a period the action potentials increased but no physiological premise behind this was offered by the authors. Kavanagh et al. (2019), cleared the historical uncertainty and described a possible physiological mechanism behind central fatigue in humans suggesting that 5-HT is both excitatory and inhibitory in different concentrations in the CNS. This is due to the location of the excitatory and inhibitory receptors on the postsynaptic motoneuron. During physical activity, the levels of 5-HT increases causing excess 5-HT to spill out of the synaptic cleft where it can reach the inhibitory (5-HT<sub>1A</sub>) receptors located on the axon initial segment of the motoneuron. Caperuto, Dos Santos, Mello, and Costa Rosa (2009) supported this in rats, where exhaustive exercise was shown to increase concentrations of 5-HT within the CNS and reduce endurance capacity. This is further supported in studies where activation of the 5-HT<sub>1A</sub> receptor reduced exercise performance in humans (Marvin et al., 1997) and shown to reduce motoneuron excitability in turtles (D'Amico et al., 2017). Therefore, 5-HT was suggested to be both excitatory and inhibitory dependent on the amount of 5-HT spill over from the synaptic cleft; this is due to the location of the 5-HT<sub>2A</sub> and 5-HT<sub>1A</sub> receptors, the latter of which is located away from the direct activation of 5-HT on the axon initial segment.

Persistent inward currents PICs form the primary role of voltage gain in the motoneuron (Heckman et al., 2005). Once attached to the 5-HT<sub>1A</sub> receptor, Na<sup>+</sup> channels on the neuron responsible for the genesis of PICs are inhibited (Cotel et al., 2013). The motoneuron is still able to produce action potentials; however, the frequency is greatly reduced. This provides a negative feedback loop for motoneuron excitability. Cyproheptadine, a drug with strong anti-serotonergic effects, blocks the excitatory 5-HT<sub>2A</sub> receptors. In an unpublished Doctoral

Dissertation, Murphy (2018) suggests there was no difference in recruitment of motor units in stroke and healthy elderly people during sustained isometric contraction with and without cyproheptadine. There was a significant difference in both stroke and healthy aged-matched participants with de-recruitment of motor units with cyproheptadine in a ramp-hold pattern; therefore, motor units were de-recruited at a higher given torque with cyproheptadine regardless of group (Figure 2.5). While this is not evidence to be relied on due to using the different population and not being a peer reviewed paper, it does provide a conceptual framework (Figure 2.5). Due to the novelty of this study, a higher standard of evidence for this segment of a methodological concept was not available at the time of writing.



**Figure 2. 5.** Ramp-hold protocol used to examine cyproheptadine effects on muscle function in people with stroke. A concept outline for the experimental protocol in the current project. Time is on the x-axis and the percentage of MVC on the y-axis. Adapted from Murphy (2018).

## 2.7 Pharmacological interventions to alter 5-HT activity in the CNS

There are no human-approved exogenous 5-HT<sub>2A</sub> agonists. However, several experiments have verified that excitatory motor effects observed with the SSRI paroxetine administration arise from activation of the 5-HT<sub>2A</sub> receptor. Use of an SSRI removes the need to create a 5-HT agonist as SSRI drugs simply inhibit the uptake of 5-HT, leaving more of it present to act on receptors for longer; opposed to creating an agonist that will activate a receptor in the absence of 5-HT (i.e., an agonist). Paroxetine is an ideal drug to study motor performance as it causes an accumulation of naturally occurring 5-HT in the CNS (Thomas, Nelson, & Johnson, 1987; Wilson & Maughan, 1992). It also has a low affinity for adrenergic, dopaminergic, histaminergic, and muscarinic receptors, and uptake inhibition ratio of 5-HT to norepinephrine is amongst the highest of SSRIs (Bourin, Chue, & Guillon, 2001), this is important as interacting with other neurotransmitters would create confounding variables. However, a key feature of using SSRIs to alter CNS 5-HT concentration is that it is difficult to control what 5-HT receptors are activated. An SSRI will enhance the availability of circulating 5-HT which can be accepted by any 5-HT receptor that needs it. Cyproheptadine, a 5-HT<sub>2A</sub> antagonist, is a potent 5-HT<sub>2A</sub> antagonist, and it will be administered in the current project to cause a receptor blockade of the 5-HT<sub>2A</sub> receptor (Gillman, 1999). It also has low affinity for adrenergic and dopaminergic receptors, and although moderate antihistaminergic and weak peripheral anticholinergic effects can occur (Gillman, 1999; Rashid et al., 2003); Honrubia et al. (1997) suggests that these coincidental effects will not affect the measurements in this study due to the high affinity cyproheptadine has for the desired receptors 5-HT<sub>2A</sub>. In summary, an antagonist such as cyproheptadine provides more specific control over the influence of 5-HT receptors compared to SSRI that create an abundance of 5-HT present in the synaptic cleft and can also spill over extrasynaptically, binding with other receptors not located in the synaptic cleft.

## **2.8 Technological advances in EMG for assessment of motor function**

Electromyography (EMG) is the represented summation of all electrical signals propagated from motor units. EMG achieves this by measuring and evaluating the electrical activity in muscles. The term EMG is also given the resultant recording of this activity, that is, EMG is both the technique and measurement. There are several different techniques that use EMG for varying purposes and applications, these are separated based on the electrodes used; surface, needle and fine wire electrodes were historically used (Dondelinger, 2010), High-Density Electromyography (HDEMG) is a more recent electrode style with additional benefits (Martinez-Valdes et al., 2016). Surface EMG involves the application of surface electrodes to the skin to measure underlying muscle activity. This has the limitations of being nonspecific to individual motor units and having to measure the electrical activity through the superficial soft tissue of the body which act as a low pass filter for electrical activity (Petrofsky, 2008); the upside of surface EMG is that it is a low risk, non-invasive procedure and is cheaper than some other techniques. The gold standard of EMG for studying individual motor units is indwelling electrodes as these bypasses many of the limitations of surface EMG as it measures directly from a muscle fibre of a motor unit (Dondelinger, 2010; Juel, 2019; Rubin, 2019). HDEMG, somewhat, has the best of both previously mentioned techniques with some additional considerations. HDEMG can measure electrical activity from individual motor units, is non-invasive and highly reliable compared to indwelling electrodes (Del Vecchio et al., 2020; Holobar et al., 2014). In summary, surface EMG is a non-invasive method to measure the electrical activity of the muscle but is unable to measure the activity of motor units, fine wire electrodes solve this issue by being able to measure the activity of an individual motor unit but is invasive and due to the precise nature of the electrode difficult to perform repeated measures, HDEMG is non-invasive, able to measure individual motor unit activity, and reliably repeat these measurements.

HDEMG shares many attributes to standard surface EMG, in that it involves non-invasively applying electrodes to the skin to measure underlying electrical activity of the muscles. Where it differs is the application of an electrode array with standardised distances between each electrode compared to the two electrode and a ground used in standard surface EMG (Del Vecchio et al., 2020). The array of electrodes allows for extra information collection and ultimately analysis of matching waveforms of electrical activity across the array indicative of motor unit action potentials (MUAPs). Holobar and Farina (2014) described using algorithmic decomposition of surface EMG stating the method allows detailed and accurate identification of individual motor unit activity. This accuracy relies on a Pulse-to-Noise Ratio (PNR) >30db to maximise accuracy of the decomposition. Martinez-Valdes et al. (2016) examined the reliability to accurately assess motor unit behaviour and concluded that it was able to accurately determine motor unit activity in longitudinal interventional studies. A high PNR ratio was used as a benchmark (>30db) when determining reliability.

## **2.9 Summary**

To summarise, neural activation of muscle is complex and hierarchical, with the motor cortex, brainstem and spinal cord all playing significant roles due to different levels control and modulation of signals. On a smaller scale, neural activation of muscle can be better understood by looking at activation of a motor unit. Motor units are the smallest functional unit within a muscle, due to them being innervated by a single alpha motor neuron coming from the spinal cord. This organisation is important as it provides structure for fine motor control and often muscles required in fine motor tasks have a larger number of motor units with fewer muscle fibres in each to allow for finer modulation of force when compared to motor units with more muscle fibres as force would increase much faster. Compounding this is the size of the alpha motor neuron; larger motor units have larger innervating motor neurons allowing for faster transmission of action potentials and faster summation of these action potentials in turn

increasing the capacity for rapid force development. To control the force development of muscles, multiple neural inputs go into the alpha motor neurons. Ionotropic and neuromodulatory inputs act directly on the alpha motor neuron within the spinal cord. Ionotropic inputs act through opening ion-channels to the post-synaptic neuron while neuromodulatory inputs adjust the sensitivity of the post synaptic neuron to activation by ionotropic inputs. An important neuromodulatory input are serotonergic neurons, 5-HT receptors, 5-HT<sub>2A</sub> and 5-HT<sub>1A</sub>, have antagonising roles on the alpha motor neuron. 5-HT<sub>2A</sub> being excitatory, in that it lowers the threshold voltage needed for an action potential to fire as well as promoting PICs within the alpha motor neuron and 5-HT<sub>1A</sub> being inhibitory, by increasing the threshold voltage and close ion channels needed for the productions of PICs. 5-HT<sub>2A</sub> and 5-HT<sub>1A</sub> form a negative feedback loop by virtue of their located on the alpha motor neuron, with 5-HT<sub>2A</sub> being within the synaptic cleft and 5-HT<sub>1A</sub> being located on the axon initial segment and therefore not being further away from the release of 5-HT from the serotonergic neuron. The 5-HT<sub>1A</sub> receptor has also been suggested as a potential mechanism for central fatigue due to its inhibitory nature. The release of 5-HT is relative to the amount of physical activity performed, therefore longer contraction times and higher contraction frequency increase the amount of effect of 5-HT on the alpha motor neuron. The magnitude of the 5-HT can be examined by introduction of a 5-HT blockade in the form of cyproheptadine, due to its strong affinity and antagonism for 5-HT<sub>2A</sub> receptors. HDEMG is an ideal and somewhat novel method of measuring individual motor unit activity and firing rates and provides a method of comparing the same motor units over multiple assessments, for example, in a control and experimental within subject's study design.

## Chapter 3.0 Methods

### 3.1 Participants

The target population were young, apparently healthy, neurologically normal individuals with no recent history of musculoskeletal injury. From this target population, a convenience sample of recreationally active right-footed males and females ( $n = 8$ , age  $28.31 \pm 6.41$  years, 1 female) were recruited into this study. Participants were sourced from the *Griffith University* community by placing flyers on notice boards throughout the Gold Coast campus. Important to note that recruitment of participants was negatively impacted by the Covid-19 pandemic, which effected both retention of participants and recruitment of them; this is the reason for the heavily one-sided gender mix of the sample as well as the low number of participants. Participants were screened using a modified medical history questionnaire that identified contraindications and safety concerns regarding the drug cyproheptadine, and the muscle contraction task used in this study. Each participant was naïve to the experimental aims and hypotheses.

To be eligible for the study, participants needed to be aged between 20 and 60 years old, were free of neurological and liver disorders, had no implanted medical devices (such as a pacemaker), and were not pregnant. This age range was chosen due for fitting the target population and generally having less of the co-morbidities that would exclude them from the study (Kim, Kisseleva, & Brenner, 2015). With regards to the administration of cyproheptadine, individuals with overactive thyroids, closed angle glaucoma, high blood pressure, or bladder dysfunction were not permitted to enrol into the study. Furthermore, the potential participants could not be taking cyproheptadine or any other medications at the time of testing and were not allergic to cyproheptadine or any of its ingredients. Participants were asked to refrain from any CNS stimulants or depressants such as caffeine, alcohol or moderate

to high intensity exercise for six hours prior to testing. Experimental testing was performed at least two hours post food intake, to control for any postprandial effects secondary to carbohydrate intake or the catecholamine releasing properties attributed to the consumption of dietary amines.

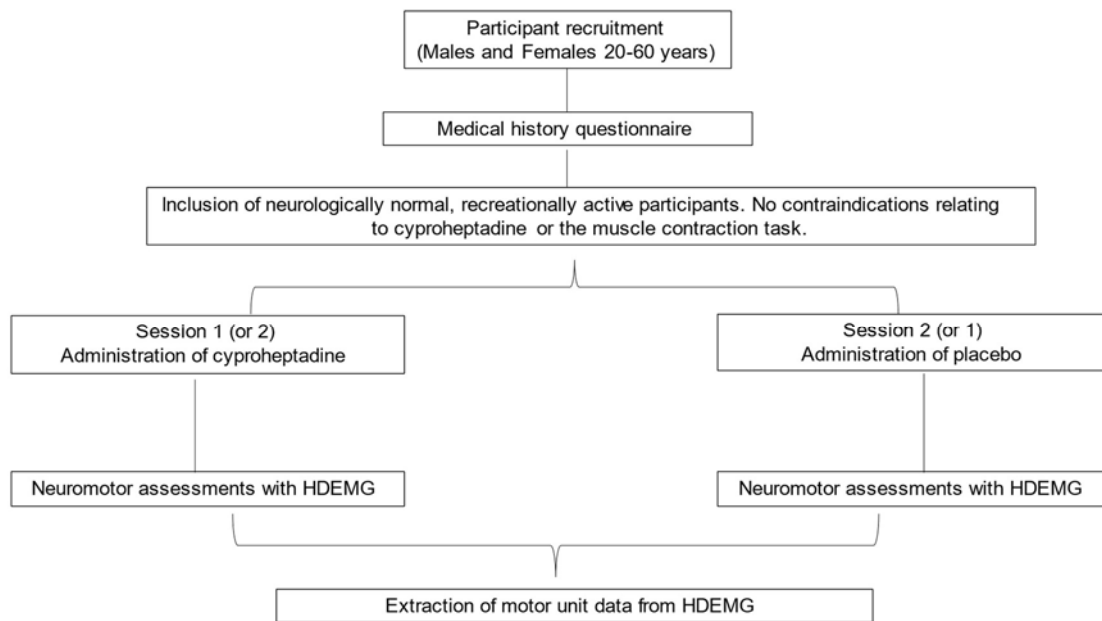
### **3.2 Ethical approval**

All the participants recruited into the study provided written informed consent to participate. Data was de-identified after experimental testing had finished so that data analysis was blinded. All experimental procedures followed guidelines outlined in the *Griffith University Research Ethics Manual*, and the *National Statement on Ethical Conduct in Research Involving Humans*. This project was approved by the *Griffith University Human Research Ethics Committee* (GU Ref No: 2020/264) and was performed in accordance with the *Declaration of Helsinki*.

### **3.3 Experiment design**

This study employed a human, double-blind, single-dose, placebo-controlled design (with a two-way crossover). A ‘within subject’ arrangement required each participant to undertake two experimental sessions on separate days at the *Griffith University* neurophysiology laboratory. A placebo or cyproheptadine capsule was administered to the participant at each session, where a counterbalanced order was determined by an independent researcher. Each testing session consisted of identical neuromotor tasks that assessed muscle activity in the right leg. These testing sessions were separated by a period of one week. All but one participant completed both sessions. All testing (data collection) was performed from midday ( $\pm$  one hour) and lasted approximately two hours. An extended effort was made to maintain a similar testing start time and experiment duration within participants (at both testing dates) to help control for any potential confounding circadian effects. For example, changes in habitual ‘sleepiness’ may have influenced maximal exertion (Van Cutsem et al., 1997) and norepinephrine level and

cognitive arousal levels (Heckman & Enoka, 2012). Hence, the implementation of a consistent testing time meant comparisons between conditions were made during the same time of the day, and any differences could be attributed to planned interventions (Figure 3.1).



**Figure 3. 1.** A schematic representation of the experiment design. Recruited participants were between 20 and 60 years old, and health status was determined prior to the first testing session via a self-reported medical history questionnaire. Each participant ingested a placebo or a cyproheptadine capsule at two testing sessions on separate weeks. The order of capsule administration was counterbalanced throughout the cohort to avoid order effects. That is, half of the participants ingested the placebo in their first session and the other half ingested cyproheptadine in their first session. The two testing sessions comprised an identical battery of tests that examined muscle activity in the right leg with HDEMG. Motor unit extraction from the HDEMG signals occurred offline after all data was collected.

### 3.4 Drug intervention

A single 8 mg dose of *Periactin* (cyproheptadine) was administered orally. This titration provided a safe and effective way of assessing acute serotonergic changes in healthy individuals. An absolute dose was selected over a relative dose (e.g., a dose standardised to

bodyweight), as the administration of multiple capsules was deemed unfeasible and impractical. While the use of an absolute dosage may play a role in the pharmacotherapeutics of cyproheptadine, 4-8mg initial dosage I will recognised a safe amount of the drug (Graudins, Stearman, & Chan, 1998). The peak plasma concentration of cyproheptadine was estimated to be between 1 and 4 hours (Gunja, Collins, & Graudins, 2004; Mendes et al., 2012), differing from the standard of ~4 hours due to the tablets being crushed to maintain blindness; so, the time of neuromotor assessment was 2 hours after administration. Importantly, pilot testing revealed that this was the time period where effects on voluntary movement commenced. Therefore, it was assumed that the drug had crossed the blood-brain barrier and was acting on CNS receptors at approximately 2 hours post-ingestion.

The recommended therapeutic dose of cyproheptadine is 4-20 mg per day in adults, although it may be increased up to and above 32 mg in order to control clinically specific symptoms. Within Australia, cyproheptadine has several approved indications, including acute and chronic allergies, pruritus, migraine, and other vascular headache. Cyproheptadine blocks the ligand-mediated activation of the 5-HT<sub>2A</sub> receptor through competitive antagonism, where it binds with high-affinity to the A, B and C receptor subtypes (Boess & Martin, 1994; Honrubia et al., 1997). It has been demonstrated that oral cyproheptadine suppresses human reflex responses between 30 mins and ~5 hrs post administration (D'Amico et al., 2013; Wei et al., 2014).

Both the cyproheptadine and placebo were compounded into identical opaque gelatin capsules to facilitate blinding. The capsules were premade and left prepared for the researcher collecting data to administer too the participant, this way the researcher and the participant were blinded to the condition of the participant each testing session. Participants were asked to report any side effects on both testing dates and were followed up with a phone call 24 hours post-drug administration. Within this study, there were no reported adverse effects secondary to

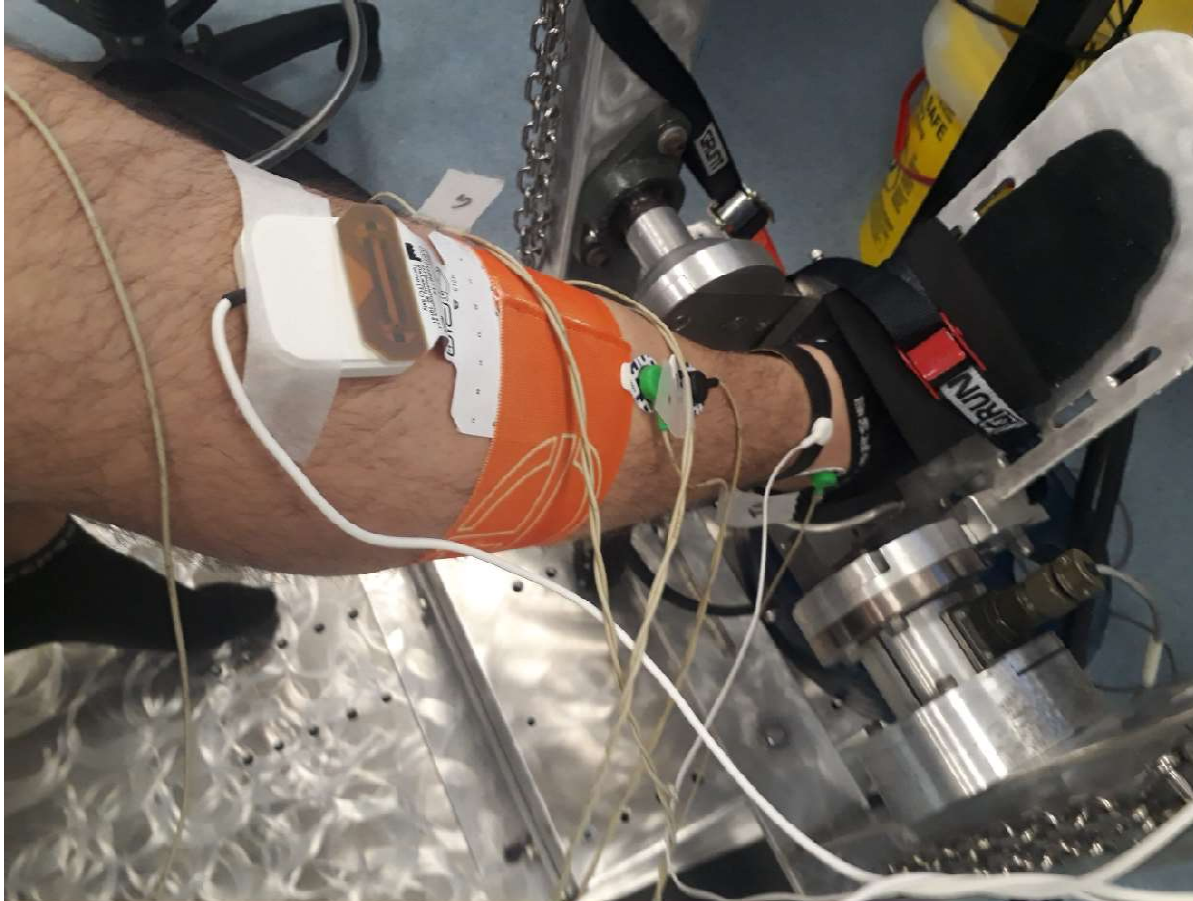
cyproheptadine use, although some participants felt ‘unusually tired’ immediately after the testing session. The safe use of cyproheptadine (brand name: *Periactin*) in this experiment coincided with the available Consumer Medicine Information and Product Information documents.

### **3.5 Instrumentation**

#### *3.5.1 Assessment of ankle torque*

Participants sat upright in a therapy chair with their hip and ankle positioned at 90° in the sagittal plane, and their right foot was secured into a custom-designed torque transducer with a non-compliant, ratchet type binding (Figure 3.2). The custom designed torque transducer incorporated a commercially available torque sensor (Model 2110; Honeywell International Inc., Charlotte, NC, USA) which was capable of measuring isometric dorsiflexion torques and isometric torques. It was noted during pilot testing that when the knee is in 0° of flexion, knee extensor activity would contribute to torque measurements during maximal effort dorsiflexor movements. Through a series of pilot EMG studies sampling from the vastus lateralis muscle on the test leg to monitor knee extensor activity, it was determined that placing the participant into 15° of knee flexion effectively minimised the influence of the knee extensor muscles on maximal dorsiflexion tasks. Due to the length of the adjustable seat participants were on, knee flexor activity was limited physically due to the seat preventing knee flexion; therefore, knee flexor activity did not have to be monitored as a confounding factor. Prior to all testing, the foot plate and torque sensor were calibrated using several known masses across a range of magnitudes. Torque was sampled at 2000 Hz using a Power 1401 interface with Spike2 software (version 7, Cambridge Electronic Design Ltd., UK). Feedback for the torque signal

was provided using Spike2 software and displayed on a computer monitor positioned approximately 1 metre from the participants eyes.



**Figure 3. 2.** Experimental setup. All measurements were performed on a single leg. The right foot was firmly fixed into a torque transducer which was capable of examining isometric dorsiflexion and plantarflexion contractions. HDEMG was obtained from the tibialis anterior of the right leg, and muscle activity of the vastus lateralis was continually monitored to ensure that knee extension did not contribute to the torque being detected by the ankle transducer.

### *3.5.2 High-Density Electromyography (HDEMG)*

HDEMG was recorded during submaximal dorsiflexion contractions using a semi-disposable 64-channel electrode grid (8x8) with a 10-mm inter-electrode distance (OTBioelettronica,

Torino, Italy). Following standard skin preparation for EMG data collection, the optimal position of the electrode grid was determined. This is achieved by identifying the right tibialis anterior muscle belly, which was identified via palpation. Electrodes were then fixed to the middle of the muscle belly using a bi-adhesive, perforated foam layer and conductive paste (SpesMedica, Battipaglia, Italy). A strap that contained the ground electrode (OTBIOelettronica, Torino, Italy) was positioned over the ankle malleoli. The HDEMG signals will be recorded in monopolar mode and converted to digital signal by a 16-bit wireless amplifier (Sessantaquattro, OTBIOelettronica, Torino, Italy) using OTBioLab+ software (version 1.3.0., OTBIOelettronica, Torino, Italy). HDEMG signals were monitored during data collection to assess signal quality and background noise.

Tibialis anterior typically has  $120 \pm 50$  motor units (Boe, Dalton, Harwood, Doherty, & Rice, 2009), half of which are recruited by 20% MVC, and an upper limit of recruitment of approximately 90% MVC (Van Cutsem et al., 1997). As a superficial muscle, signals produced by tibialis anterior are likely to undergo less physiological low pass filtering from adipose tissue compared to deeper muscles, this consideration would help with accurate data collection. Furthermore, motor unit activity sampled from tibialis anterior has been reported extensively within the literature (Boe et al., 2009; Feiereisen, Duchateau, & Hainaut, 1997; Stephenson & Maluf, 2011; Van Cutsem et al., 1997), including during rapid contractions (Del Vecchio, Negro, et al., 2019; Desmedt & Godaux, 1977; Van Cutsem, Duchateau, & Hainaut, 1998), and during sustained contractions (Miller et al., 2019; Murphy, 2018; Van Cutsem et al., 1997). When using blind source separation techniques to decompose HDEMG recordings into individual motor units, the tibialis anterior muscle consistently yields highly accurate (Pulse to noise ratio  $>30$  dB) motor units across a range of contraction intensities (Del Vecchio et al., 2020). This includes an average yield of over 10 motor units at contraction intensities  $\geq 70\%$  MVC. The high yield of accurate motor units irrespective of contraction intensity further

reinforces the use of tibialis anterior as an ideal muscle to study when using HDEMG and blind source separation techniques.

### *3.5.3 Bipolar EMG*

Ankle dorsiflexion can be a difficult task to complete in absolute isolation, particularly during prolonged contractions and high intensity contractions. To account for this, bipolar surface EMG was obtained from antagonistic and synergistic muscles using 24 mm Ag/AgCl electrodes (Kendall ARBO; Cardinal Health, Dublin OH, USA). Specifically, bipolar EMG will be sampled from medial gastrocnemius, soleus and vastus lateralis. These signals will be monitored during testing to ensure feedback can be provided to minimise compensatory movements during dorsiflexion tasks (e.g., knee extension). Electrodes were aligned parallel to the underlying muscles fibres, with an inter-electrode distance of 24 mm. EMG signals were differentially amplified (x 1000) by a NL844 pre-amplifier, and bandpass filtered (10 Hz - 500 Hz) by a NL135 Low Pass Filter and NL144 High Pass Filter (all Digitimer Ltd., UK). All surface EMG were sampled at 2000 Hz via a Power 1401 interface with Spike2 software (version 7, Cambridge Electronic Design Ltd., UK).

## **3.6 Experimental protocols**

Participants attended two testing sessions that were separated by a week. Each testing session consisted of the same dorsiflexion protocols, where four types of muscle contractions were performed. These were maximal voluntary contractions, rapid contractions, ramped submaximal contractions, and fatigue inducing sustained contractions. HDEMG was obtained from the tibialis anterior throughout all contractions.

### *3.6.1 Maximal voluntary contraction*

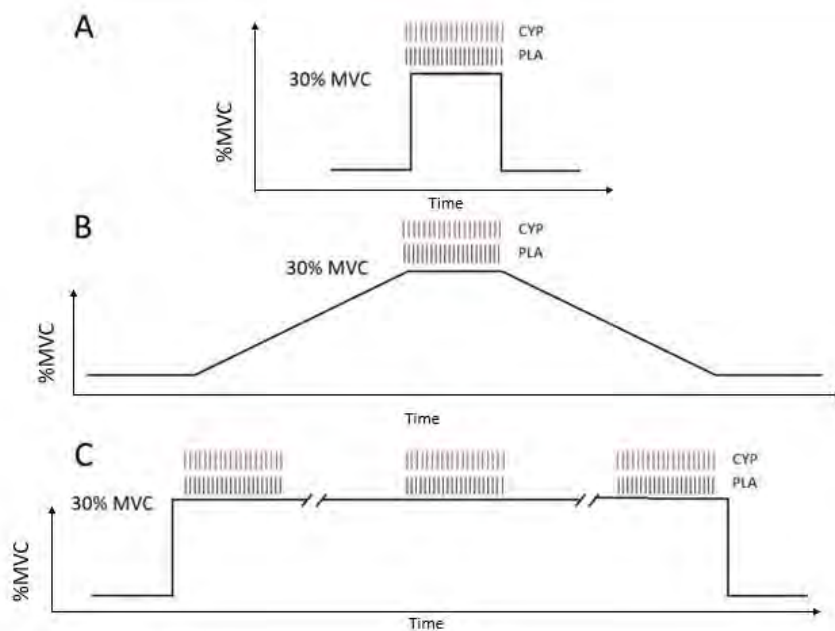
At the commencement of the contraction protocols, it was critical to measure the MVC torque for the individual participant. This not only determines if the drug affected the maximal torque generating capacity of the person, but it was also used to set the percentage of MVC that participants would be targeting for each remaining type of contraction. Three dorsiflexion MVCs were performed with a rest period of 2 min to ensure that fatigue did not accumulate. The trial that included the greatest amplitude of torque was designated as the participants MVC, this was repeated on both the placebo and cyproheptadine sessions. Participants were continually encouraged to only contract the tibialis anterior when dorsiflexing the ankle. EMG of the knee extensor was also monitored during this period to ensure that muscles of the upper leg were not contributing to torques generated about the ankle.

### *3.6.2 Rapid contractions*

Participants performed a contraction protocol which comprised a series of rapid isometric contractions at 30% MVC. The target torque was presented as a target window on the y-axis, where a tolerance of  $\pm 5\%$  MVC. Given that the rapid isometric contractions occurred for only a brief period of time, a 'decomposition calibration' contraction was performed before the rapid contraction. This consisted of performing a 30% MVC dorsiflexion for 20 s so that the decomposition filters had enough data to identify genuine MUAPs in the tibialis HDEMG signal. This calibration contraction was followed  $\sim 5$ s later by the rapid contraction. For the rapid contraction participants were instructed to dorsiflex 'as fast as possible', so their level of torque entered the target window, and then maintain the contraction for 5 seconds. This was repeated for 3 repetitions with 60 seconds rest between repetitions. The rapid isometric contractions were initiated with an auditory countdown to ensure they aware the ramp was initiated and the participants didn't over-react by contracting beyond the  $30\% \pm 5\%$  MVC.

### *3.6.3 Ramped submaximal contractions*

Participants performed a ramped, trapezoidal contraction protocol with a time to reach 30% MVC of 20 seconds. The slow ramping contraction was chosen as it creates a substantially different task to achieve a steady state contraction compared to the rapid contraction. That is, the ramped submaximal contraction achieved steady-state through gradual muscle activation. Del Vecchio, Negro, et al. (2019) suggests that during rapid contractions firing rates are exaggerated to meet rapid development of force needed; a ramped contraction should provide insight into the behaviour of motor units slower development of force. The plateau for each trapezoidal ramp was 5 seconds long for all and each ramp was completed a minimum of three times to allow familiarisation. The ramp that most accurately matched the force trace would be used for analysis; a rest period of 60 seconds was in place between each contraction. A tolerance of  $\pm 5\%$  MVC was permitted in successful trials of each ramp. Participants were instructed to follow the coloured ramp with their force line on a screen placed directly in front of them (Figure 3.3). Guidelines were superimposed on to the force trace the participant was looking at, only successful traces were included in the analysis of data.



**Figure 3.3.** The three contraction types used in this study all to 30% MVC. A. Rapid contraction lasting five seconds. B. A Ramped contraction lasting a total of 45 seconds; 20 second ramp up and down on either side of a five second plateau at 30% MVC. Only the five second plateau was analysed. C. The time to fatigue contraction. Three segments of this contraction were analysed to assess motor unit activity across the duration of the contraction; each segment was ten seconds in duration.

### 3.6.4 Fatigue-inducing sustained contractions

Participants performed a time to fatigue protocol after 5 minutes of rest after the ramped submaximal contractions. Participants were asked to maintain a 30% MVC dorsiflexion contraction for as long as possible. A tolerance of  $\pm 5\%$  MVC was permitted before the contraction was terminated. In particular, if the participant dropped below the tolerance window for more than 5 consecutive seconds the contraction was terminated. Participants received verbal encouragement to maintain the contraction as long as possible.

### **3.7 Data analysis**

#### *3.7.1 HDEMG signal preparation*

Offline analysis was performed using the DEMUSE tool, which is a custom designed Matlab script designed by Professor Ales Holobar (Holobar & Zazula, 2007), and is widely considered the gold-standard software for extracting motor unit activity from HDEMG signals. Prior to extraction, the individual channels from the matrix electrode were band pass filtered from 20–500 Hz using a second-order Butterworth filter. A differential high-pass filter was then selectively applied to HDEMG data to emphasise the differences in MUAPs waveform shapes. A notch filter was applied to manage line interference, and spatial filters were applied to leverage row and/or column-wise signal differentiation of the HDEMG grid channels. Ideally, a minimal amount of filtering was applied during signal preparation, as each additional application of a filter resulted in the loss of data for the decomposition algorithm. Therefore, the application of these filters was justified by specific considerations, such as subject anthropometry (e.g. adipose tissue) and background electrical noise.

#### *3.7.2 Extraction of motor unit activity from HDEMG*

HDEMG signals were decomposed into individual MUAPs by convolutive blind source separation. This method has been validated against fine-wire indwelling electrode EMG recordings for a variety of contraction intensities across a range of muscles and offers high accuracy in the identification of motor unit spike trains (Del Vecchio, Negro, Felici, & Farina, 2017; Holobar et al., 2014; Negro, Muceli, Castronovo, Holobar, & Farina, 2016). This iterative process of blind source separation typically converges after 40-50 runs of the decomposition algorithm, after which there are diminishing returns on further runs. In order to ensure convergence, HDEMG signals collected during each study were decomposed over a minimum of 60 runs of the decomposition algorithm. Motor unit decomposition accuracy was

assessed using PNR (Holobar et al., 2014), whereby a higher PNR is indicative of better accuracy. Motor units showing pulse-to-noise ratios  $< 30\text{dB}$  were discarded from the analysis (Holobar et al., 2014). Decomposed motor units were also visually inspected to ensure that only HDEMG MUAPs with comparative characteristics to fine wire EMG MUAPs were considered for analysis.

### **3.8 Statistical analysis**

All statistical analyses were performed using IBM Statistical Package for the Social Sciences (SPSS) for windows. The skewness and kurtosis of the dependent variables were assessed prior to analysis. For the rapid contractions and the ramped submaximal contractions, one-way ANOVA was used to determine if 5-HT antagonism (cyproheptadine vs placebo) had an effect on motor unit discharge rate. This approach was used to examine the average of motor units extracted during the task, and for motor units that were matched between testing sessions. For the sustained fatiguing contraction, a two-way repeated-measure ANOVA was used to assess if motor unit discharge rates were altered by 5-HT antagonism (cyproheptadine vs placebo) and the segment of the contraction (first 10%, middle 10%, and final 10% of sustained contraction). If a significant main effect of segment was identified, post-hoc analyses with Bonferroni corrections were subsequently performed. Important to note that these comparisons were made with matched motor units found in both conditions and “unmatched” motor units, that is, all motor units found in either condition regardless of being matched or not (Del Vecchio, Casolo, et al., 2019). For the time to fatigue and MVC analyses, t-tests were performed two-sided significance was used for both analyses. Furthermore, Cohen’s  $d$  was used post-hoc to determine effect sizes of the comparisons. The level of significance set for all statistical analyses in this study was  $p < 0.05$ .

An a priori power analysis was conducted using G\*Power version 3.1.9.7 (Faul, Erdfelder, Lang, & Buchner, 2007) to establish sample size required to test the study hypotheses sufficiently. The G\*Power results indicated the sample size needed to achieve 80% power for detecting a medium effect, at a significance criterion of  $\alpha = .05$ , was  $N = 34$  for ANOVA: repeated measures, within factors. Therefore, the obtained sample size of  $N = 7$  is not adequate to test the study hypothesis with this level of power. Further recruitment was hampered by the Covid-19 pandemic to reach the desired power level of the sample.

## Chapter 4.0 Results

### 4.1 Participant characteristics

Eight participants (7 male) were recruited. One participant did not complete the second session and was excluded from the results (Table 4.1). All participants were right leg dominant. All participants tolerated the drug intervention; no adverse events were reported throughout the study.

**Table 4. 1.** Anthropometric characteristics and peak force during maximal voluntary dorsiflexion contractions

	Placebo	Cyproheptadine
Gender	6 male/1 female	
Age (yrs)	28.43 ± 6.8	
Height (m)	1.81 ± 0.08	
Weight (kg)	80 ± 6.78	
Body mass index (kg/m <sup>2</sup> )	24.5 ± 2.1	
MVC force amplitude (N.m)	175.2 ± 43.1	158.9 ± 47.9

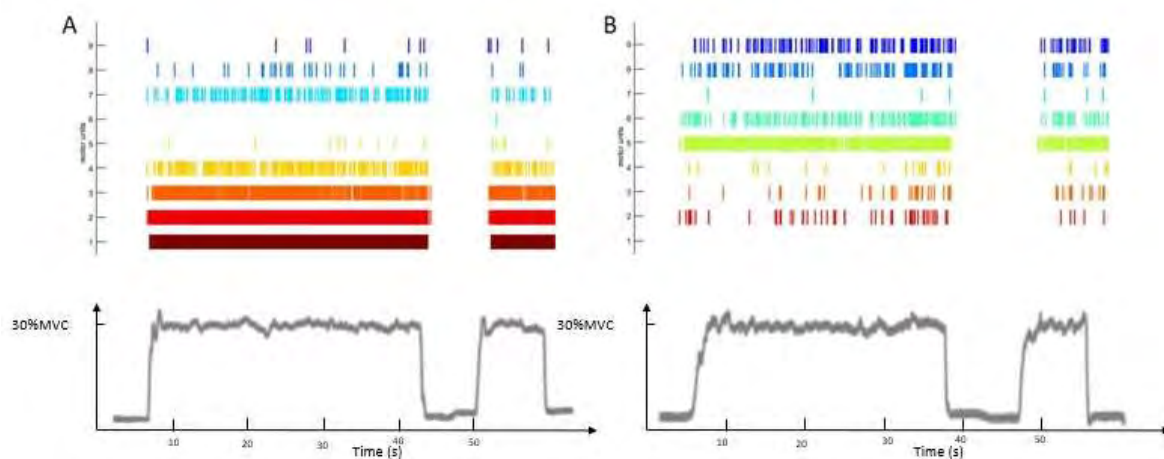
MVC, maximal voluntary contraction; sEMG, surface EMG for tibialis anterior. MVC is presented as group means ± SD ( $n = 7$  participants)

### 4.2 MVC comparison between the placebo and cyproheptadine conditions

As can be seen in table 4.1, there is a noticeable difference in the MVC between the two conditions. The difference between placebo and cyproheptadine was found to be significant through a t-test for the placebo ( $T(6) = 9.9$ ,  $p = <0.001$ ,  $d = 3.76$ ), and the cyproheptadine ( $T(6) = 8.1$ ,  $p = <0.001$ ,  $d = 3.07$ ) conditions.

### 4.3 Motor unit discharge during rapid dorsiflexion contractions

As can be seen in the representative force data of Figure 4.1, data was able to be successfully collected during rapid dorsiflexion contractions in both the placebo and cyproheptadine conditions. However, the decomposition and subsequent tracking of tibialis anterior motor units between sessions was more challenging. In particular, the ability for the decomposition algorithm to consistently identify motor unit discharges between sessions was limited for this small length of data (i.e., rapid contractions). This was the case even with the inclusion of a calibration contraction, which is recommended for small data sets.

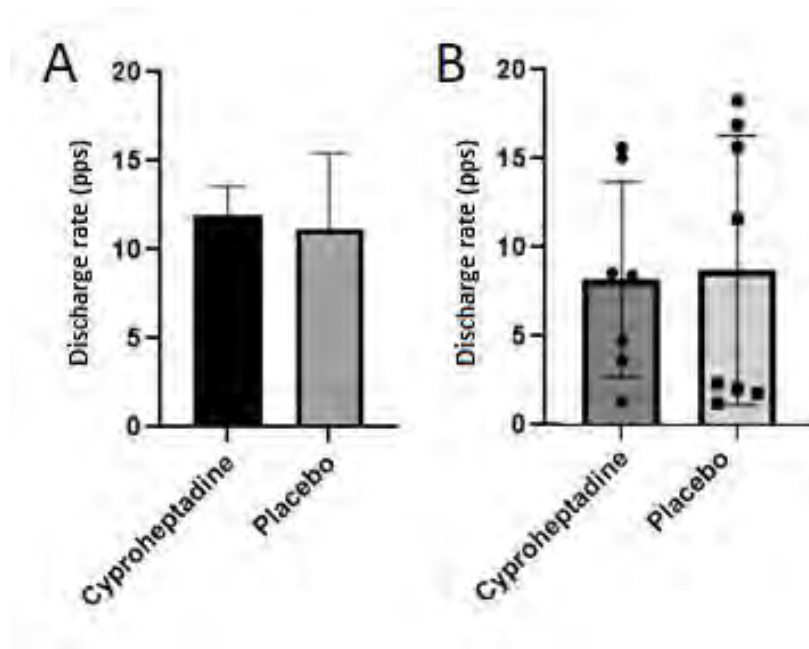


**Figure 4. 1.** Representative data for a single participant performing a rapid dorsiflexion during the placebo session (A) and during the cyproheptadine session (B). The lower trace represents force when performing the calibration contraction and then the rapid contraction to 30% MVC. The upper traces indicate the instances of motor unit discharges for each of the motor units identified in the tibialis anterior HD EMG decomposition. Only discharges from matched motor units are included in this data where matched motor units are shown as the same colour and same position in the placebo panel and the cyproheptadine panel.

For the representative data example that is presented, the algorithm was unable to successfully identify the same repetitively discharging motor units in the cyproheptadine session that were present in the placebo session. Although it might be expected that antagonism of the 5-HT<sub>2A</sub>

could reduce motoneuron excitability so that discharges are unable to be detected, the current result appears to be technical rather than physiological. This is because for many other trials the algorithm was unable to consistently identify the same repetitively discharging motor units in the placebo session that were present in the cyproheptadine session. There was no consistent pattern for which session was problematic in decomposition, and there was not an obvious cause for this lack of consistency. Overall, motor units were collected from every participant. In total, 63 motor units were collected and analysed, of which 28 were matched. All motor units were then assessed against the PNR criteria prior to statistical analysis. Previous studies suggest this methodology to be sound (Del Vecchio, Casolo, et al., 2019; Del Vecchio et al., 2020), though this study differs in the both the duration and intensity of contraction which may have affected results.

The extracted tibialis anterior motor unit data were assessed in two ways. The first was for motor unit data that were unmatched, where all extracted motor units were pooled for the placebo condition and for the cyproheptadine condition (Del Vecchio, Casolo, et al., 2019). For unmatched data, there was no significant differences in motor unit discharge rate between the cyproheptadine and the placebo conditions when performing rapid contractions ( $F(1,42) = 0.199$ ,  $p = 0.658$ ,  $d = 0.005$ , Figure 4.2A). Both the placebo and the cyproheptadine condition had a mean discharge rate  $>10$  Hz when motor units were unmatched. There was a large amount of overlap in discharge rate between the cyproheptadine and placebo condition, where the placebo condition exhibited the largest range.

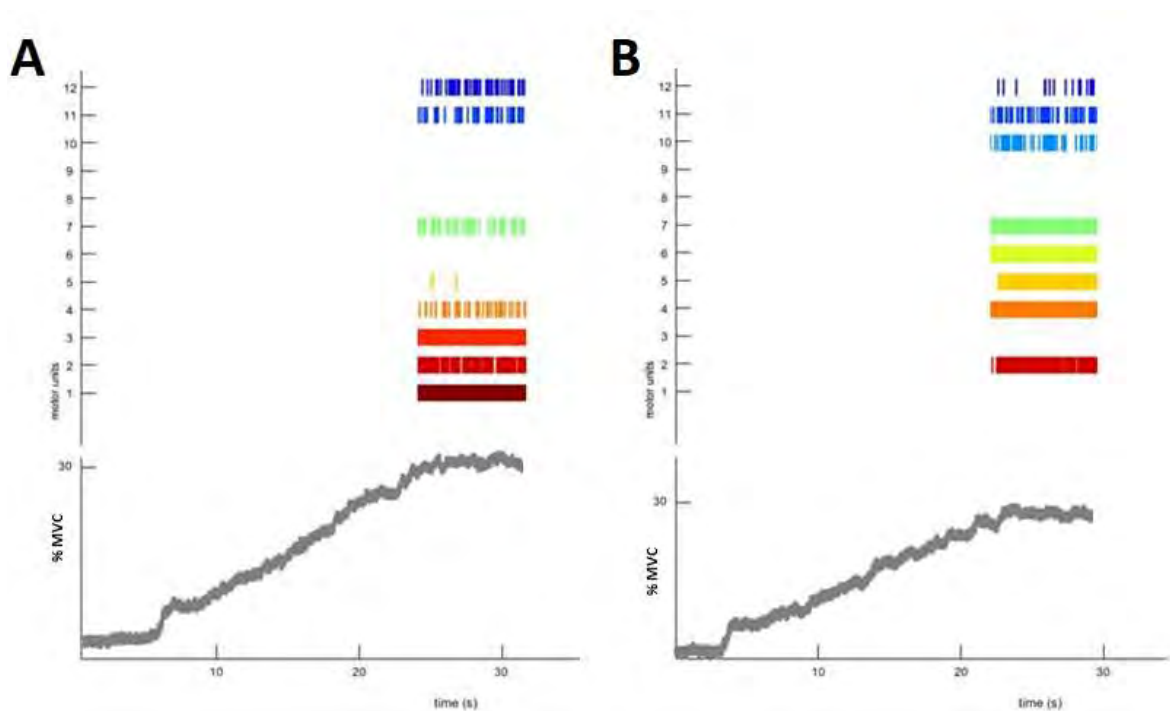


**Figure 4. 2.** Discharge rate of motor units extracted from HDEMG of the tibialis anterior for the performance of rapid contractions to 30% MVC. Following extraction of motor units, unmatched motor units were grouped and compared between the cyproheptadine and placebo condition (A). Motor units were also tracked between testing sessions, where motor units that were detected in both the cyproheptadine and placebo sessions were compared (B). Data are presented as the mean discharge rate (pulses-per-second) and error bars represent the standard deviation of the mean. Individual motor unit discharge data are presented with the matched motor unit data.

The second way that motor units were assessed was via matched motor unit pairs between the cyproheptadine and placebo sessions. The comparison between the two conditions with matched motor units yielded similar results to the unmatched analysis. For matched data, there was no significant differences in motor unit discharge rate between the cyproheptadine and the placebo conditions when performing rapid contractions ( $F(1,12) = 0.170$ ,  $p = 0.899$ ,  $d = 0.001$ , Figure 4.2B). The range of discharges within the matched motor units was similar to the pooled motor unit data where the placebo condition showed a larger range compared to the cyproheptadine condition.

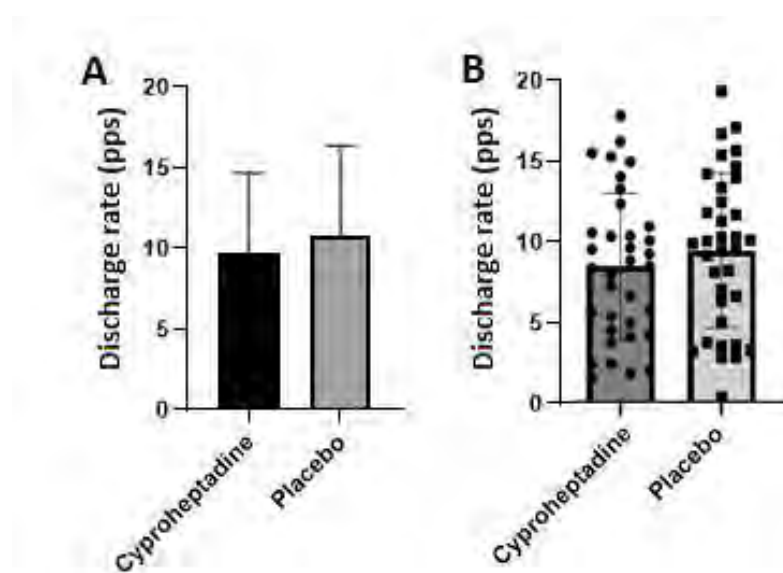
#### 4.4 Motor unit discharge during unfatigued ramped dorsiflexion contractions

As can be seen in the representative force data of Figure 4.1, participants were able to successfully increase their dorsiflexion force in a slow and controlled manner, and then maintain the steady state contraction for a period of 5 s. This was consistent for the placebo and cyproheptadine sessions. A key feature of the slow ramped contraction was that there was more consistency in identifying motor unit discharges within each session, and between each session, compared to the rapid contractions. Motor units were successfully collected and analysed from every participant; a total of 85 motor units were collected and analysed, with 70 of them being matched motor units.



**Figure 4. 3.** Representative data for a single participant performing a slow ramped contraction during the placebo session (A) and during the cyproheptadine session (B). The lower trace represents force when performing the dorsiflexion to 30% MVC. The upper traces indicate the instances of motor unit discharges for each of the motor units identified in the tibialis anterior HD EMG decomposition. Only discharges from matched motor units are included in this data where matched motor units are shown as the same colour and same position in the placebo panel and the cyproheptadine panel.

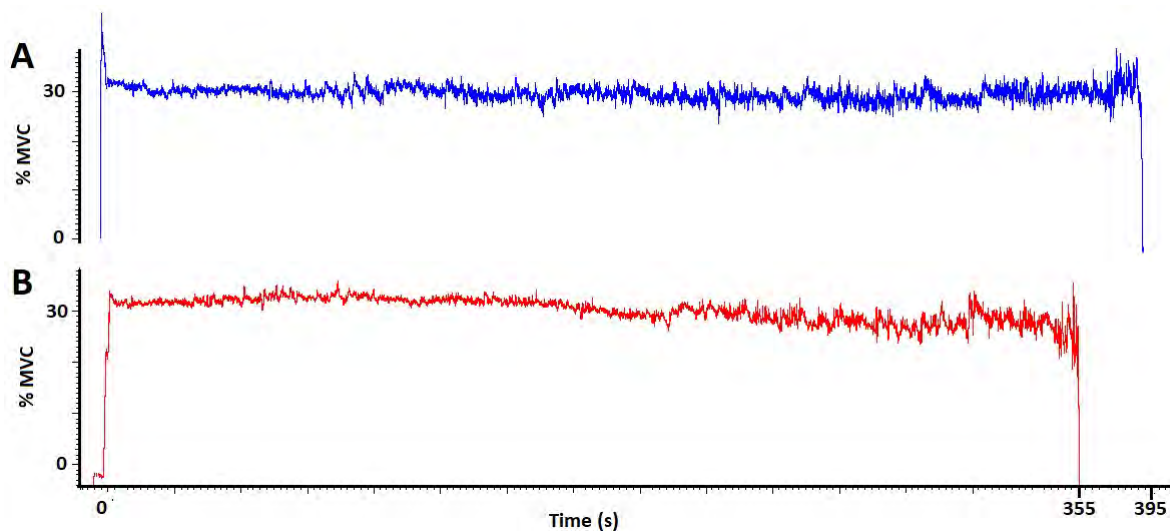
Once again, the extracted tibialis anterior motor unit data were assessed in two ways. For unmatched data, there was no significant differences in motor unit discharge rate between the cyproheptadine and the placebo conditions when performing steady-state contractions ( $F(1,64) = 1.271$ ,  $p = 0.264$ ,  $d = 0.19$ , Figure 4.4A). This was consistent with the match motor unit data, where no significant differences in motor unit discharge rate between the cyproheptadine and the placebo conditions when performing steady-state contractions ( $F(1,72) = 0.805$ ,  $p = 0.373$ ,  $d = 0.011$ , Figure 4.4B).



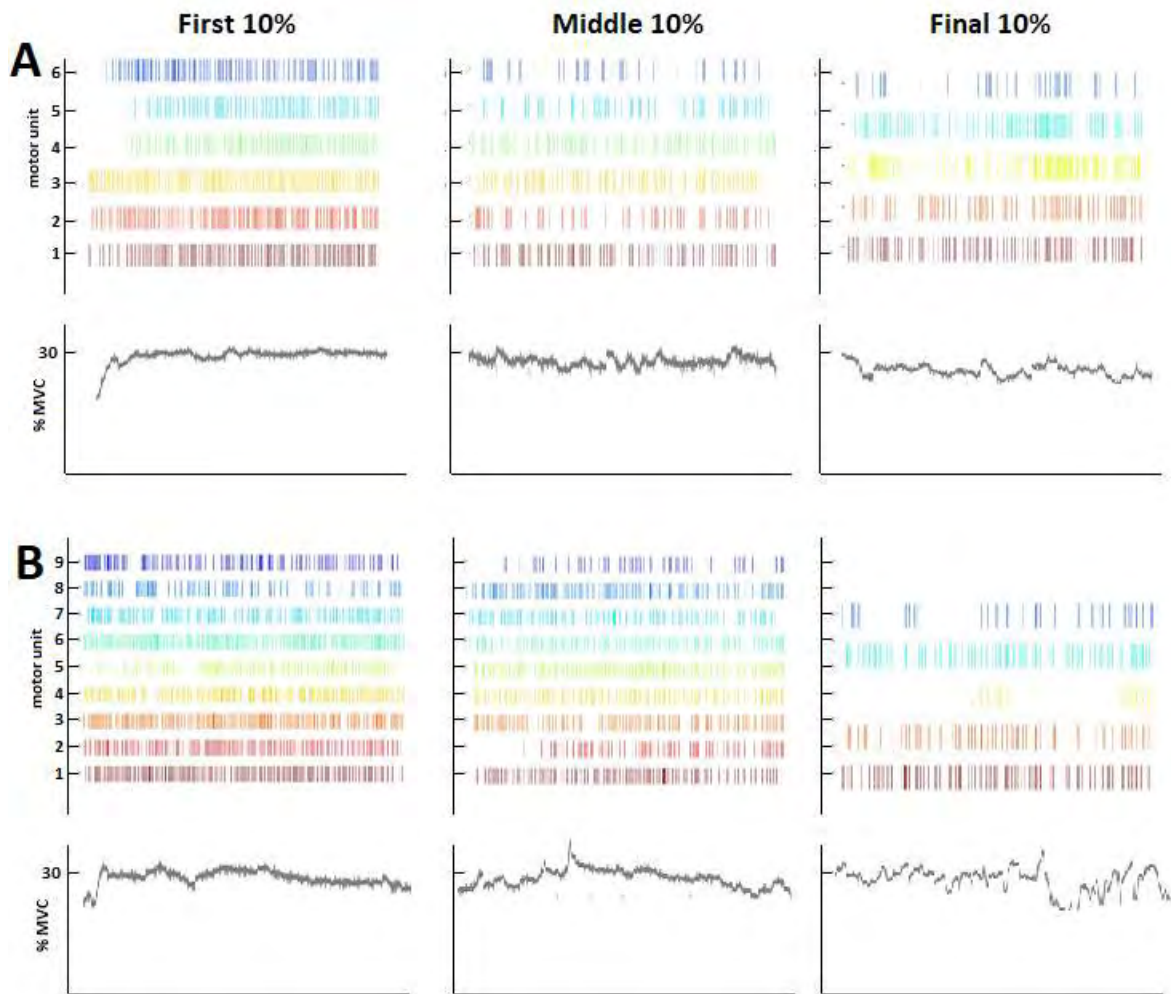
**Figure 4. 4.** Discharge rate of motor units extracted from HDEMG of the tibialis anterior for the performance of a steady state phase following a slow ramped contraction to 30% MVC. Following extraction of motor units, unmatched motor units were grouped and compared between the cyproheptadine and placebos condition (A). Motor units were also tracked between testing sessions, where motor units that were detected in both the cyproheptadine and placebo sessions where compared (B). Data are presented as the mean discharge rate (pulses-per-second) and error bars represent the standard deviation of the mean. Individual motor unit discharge data are presented with the matched motor unit data.

#### 4.5 Motor unit discharge during fatigue inducing sustained submaximal dorsiflexion

All participants performed a submaximal dorsiflexion of 30% MVC to induce fatigue in the tibialis anterior. Motor units were collected and analysed from every participant and section of the fatigue protocol. In total, 96, 77 and 53 motor units were collected and analysed from segment 1, 2 and 3 respectively. This contraction was performed until participants were no longer able to maintain an intensity of 30% MVC (Figure 4.5A and 4.5B), thus achieving the criterion of fatigue which was being unable to perform the task. The time-to-task failure for the placebo condition was  $624.6 \pm 363.1$  s ( $T(6) = 4.213$ ,  $p = 0.006$ ,  $d = 1.59$ ), whereas the time-to-task failure for the cyproheptadine condition was  $602.3 \pm 102.9$  s ( $T(6) = 14.339$ ,  $p < 0.001$ ,  $d = 5.42$ ). Therefore, significance has been shown in both conditions, though considerably more so in the cyproheptadine condition; the presence of cyproheptadine appears to affect the time of fatigue.



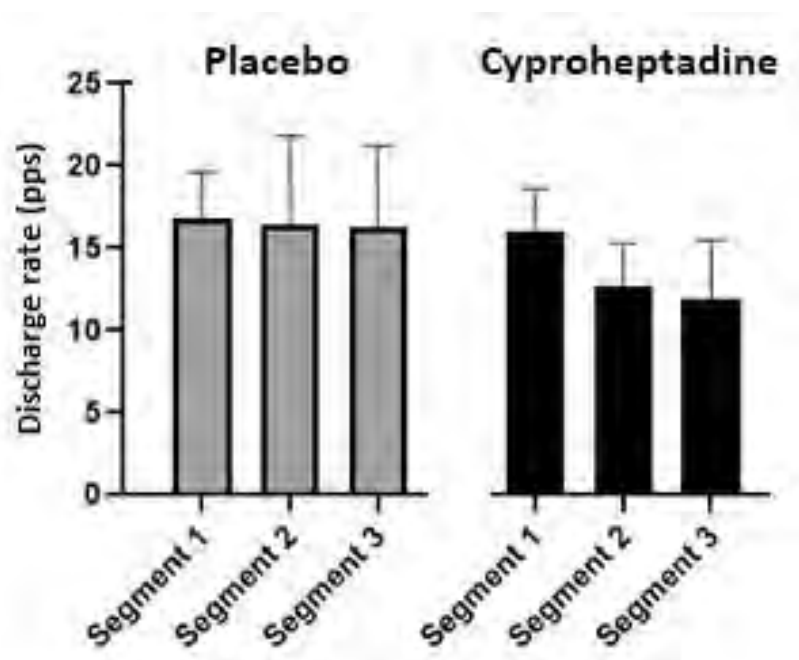
**Figure 4. 5.** A single participant performing a fatigue-inducing submaximal dorsiflexion of 30% MVC until task failure. Force data is presented for the fatiguing contraction of the placebo session (A) and the cyproheptadine sessions (B). The criteria for task failure was an inability to remain within the tolerance window of  $30\% \pm 5\%$  MVC for 5 consecutive seconds.



**Figure 4. 6.** Representative data for a single participant performing a sustained 30% MVC dorsiflexion until task failure. Data are presented for the placebo session (A) and the cyproheptadine session (B). The left columns represent the first 10% of the duration of the contraction, the middle panels represent the middle 10% of the duration of the contraction, and the right panels represent the final 10% of the contraction before task failure. The lower trace in each panel represents force when performing the 30% MVC dorsiflexion. The upper traces indicate the instances of motor unit discharges for each of the motor units identified in the tibialis anterior HD EMG decomposition.

For the sustained fatigue-inducing contraction it was not possible to track motor units between sessions. Therefore, motor units were unmatched and all extracted motor units were pooled for the placebo condition and for the cyproheptadine condition. Two way ANOVA revealed that there was a significant effect of 5-HT antagonism on averaged motor unit discharge rates throughout the fatiguing contraction ( $F(1,219) = 13.643, p < 0.001, d = 0.059$ , Figure 4.7),

where the cyproheptadine condition caused significant reduction in discharge rate compared to the placebo condition. There was also a main effect of contraction segment identified ( $F(2,219) = 3.328, p = 0.038, d = 0.029$ ), where post hoc analyses revealed that discharge rate during the middle 10% and final 10% of the contraction task were lower than the initial 10% of the contraction task. No drug by contraction segment interaction effect was identified for motor unit discharge rate.



**Figure 4. 7.** Motor unit discharge rate during the sustained 30% MVC fatiguing contraction. Data are presented for three segments of the contraction, where segment 1 represents the first 10% of the contraction, segment 2 represents the middle 10% of the contraction, and segment 3 represents the final 10% of the contraction. Data are presented as the mean discharge rate (pulses-per-second) and error bars represent the standard deviation of the mean.

## **Chapter 5.0 Discussion**

### **5.1 Summary of findings**

The purpose of this project was to determine if serotonergic effects associated with muscle activation are dependent on the mode of contraction being performed. Healthy young adults were recruited into this study, where motor unit activity was extracted from HDEMG data collected from the tibialis anterior during isometric dorsiflexion. The main finding of the project was that a blockade of 5-HT<sub>2A</sub> receptors significantly suppresses discharge rate of motor units during a fatiguing isometric 30% MVC contraction of the tibialis anterior muscle. In contrast, there were no notable differences when examining the effects of 5-HT<sub>2A</sub> antagonism for shorter contraction times that were based on rapid contractions or slow ramped contractions to achieve a steady state. This project provides baseline evidence for the function of 5-HT on motor unit function in humans and will serve as a foundation for future 5-HT experiments in humans.

### **5.2 Maximal voluntary contraction amplitude was reduced with 5-HT antagonism**

All motor commands from the brain will ultimately synapse on the motoneuron in the spinal cord to regulate the timing and amplitude of muscle contractions. The input-output relationship between command signals and motoneuron activation is relatively simple, whereby increases in the firing rate of the command signal will cause a greater output response of the motoneuron. However, a complex parallel neuromodulation system is also present, where brainstem pathways will release 5-HT into the CNS during voluntary contractions. The impact of the serotonergic system is not viewed as trivial, as approximately 1500 serotonergic fibres synapse on each motoneuron (Alvarez et al., 1998), and acceptance of this neuromodulator by 5-HT<sub>2A</sub> receptors on the motoneuron is suggested to cause a 5-fold increase in the ability

generate muscle force (Barry, Pascoe, Jesunathadas, & Enoka, 2007; Enoka & Duchateau, 2017; Miller et al., 2019; Moritz, Barry, Pascoe, & Enoka, 2005; Oya et al., 2009). In the current project, 5-HT<sub>2A</sub> antagonism compromised the ability to generate maximal force in the dorsiflexor muscles. Therefore, it is evident that serotonergic effects are critical to the performance of strong contractions, and in particular, MVCs where voluntary drive to the motoneuron pool maximally used. This supports an emerging line of research that suggests 5-HT effects are scaled to contraction intensity, where the greatest effects will be seen during large contraction intensities (Thorstensen et al., 2020; Wei et al., 2014).

### **5.3 5-HT antagonism had no effect on rapid or sustained contractions for unfatigued muscle**

No drug effects were identified for the rapid contraction task, which indicates that 5-HT antagonism does not influence discharge rate during fast submaximal movements. This was reflected in both the unmatched and the matched motor unit analyses. The main explanation for this finding lies in the nature of 5-HT release in the CNS. It is well-known that 5-HT is synthesised in the raphe nuclei of the brainstem (Berger et al., 2009; Perrier & Delgado-Lezama, 2005), where the raphe-spinal pathway is responsible for 5-HT release onto motoneurons (Holstege & Kuypers, 1987). However, it is unknown how fast the raphe-spinal pathway is able to release 5-HT onto motoneurons. Given that the raphe-spinal system operates in parallel to motor pathways travelling from the brain to the muscle, it is possible that only a feedforward mechanism could cause a level of 5-HT release so fast that motoneuron discharge would be altered during rapid contractions. Of the human data available, SSRIs have been used to enhance availability of 5-HT in the CNS during the performance of brief maximal contractions (Kavanagh et al., 2019). F-waves were used to assess motoneuron excitability, and it was reported that 2 seconds of maximal contraction was able to alter F-waves, but they

returned to baseline within 30 seconds. Therefore, it is plausible that the serotonergic system plays a role in brief maximal efforts. However, the current project supports other investigations that suggests that the serotonergic system cannot act fast enough to play a role in rapid submaximal efforts or short contraction durations (Johnson, Edwards, Van Tongeren, & Bawa, 2004; Perrier, Rasmussen, Jørgensen, & Berg, 2018).

The second contraction protocol that was employed in this project examined tibialis anterior motor unit discharge rate during steady-state dorsiflexion contractions. An important feature of the experimental design was that the steady state contraction was performed after a very gradual increase in force to 30% MVC, which is a considerably different task compared to the rapid dorsiflexion in the first protocol. Once again, there were no significant differences in discharge rate identified between the cyproheptadine and placebo conditions. This was reflected in both the pooled motor unit data and matched motor unit data. Enoka and Duchateau (2017) described the effect of discharge rate of motor units being an important component of force development which has been supported by many other studies (Barry et al., 2007; Moritz et al., 2005; Oya et al., 2009).

The absence of 5-HT effects for the slow ramped contraction was somewhat surprising, as slow ramped contractions are the preferred mode of contraction to evoke persistent inward current activity (Enoka & Duchateau, 2017). The facilitatory effects of 5-HT on motoneurons are caused by PICs on motoneuron dendrites, and the overall consequence of PIC activity is generating a strong response in discharge rate (Eckert & Lux, 1976; Enoka & Duchateau, 2017; Harvey et al., 2006; Johnson & Heckman, 2014). Dendritic PICs amplify ionotropic input significantly, and promote a sustained depolarization of motoneurons, where the degree of PIC facilitation in motoneurons is in proportion to the level of neuromodulatory drive from the brainstem (Heckman et al., 2003; Heckman et al., 2008; Heckman et al., 2009). It is important to highlight that a sustained depolarizing current is needed to activate dendritic PICs, such as

sustained ionotropic input from the corticospinal system that bring motoneurons above their voltage threshold for discharge. Thus, it is surprising that 5-HT antagonism did not affect discharge rate during the slow ramped contraction in this project.

#### **5.4 5-HT antagonism reduced motoneuron discharge rate during submaximal fatiguing contractions**

The main finding in this project was that 5-HT antagonism compromised the ability to performed prolonged submaximal dorsiflexion contractions, which ultimately lead to an exacerbation of fatigue during the cyproheptadine session. This finding was in agreement with the third hypothesis of the study and reinforces the viewpoint that 5-HT plays a critical role in motor performance and fatigability (Cotel et al., 2013; Kavanagh et al., 2019; Parise, Bosman, Boecker, Barry, & Tarnopolsky, 2001). Although the origin of fatigue is not able to be determined with HDEMG-based motor unit analysis, it is highly likely that the contraction protocol caused a significant amount of central fatigue (Thorstensen et al., 2020). This is because there are no 5-HT receptors on muscle fibres that could contribute to peripheral fatigue (Cotel et al., 2013; Davis & Bailey, 1997; Kavanagh et al., 2019; Meeusen, Watson, Hasegawa, Roelands, & Piacentini, 2006). The use of cyproheptadine can only affect the central component of fatigue as the 5-HT<sub>2A</sub> receptor subtype is only present in the CNS.

The typical role of 5-HT is to enhance motor output, because once released onto motoneurons excitatory 5HT<sub>2A</sub> receptors are activated which enhance motoneuron firing. Early studies used feline spinal preparations to reveal a spinal role for 5-HT in muscle activation, where the activity of the descending raphe-spinal system corresponds to the speed of treadmill walking (Jacobs & Fornal, 1997; Jacobs et al., 2002; Veasey, S. C., Fornal, C., Metzler, C., & Jacobs, B. L., 1995). During faster locomotor speeds, the activity of single raphe-spinal neurons in cats

is greater than that of the same raphe neurons at lower speeds, suggesting that 5-HT release corresponds to the intensity of voluntary movement. While there is some evidence that the amount of serotonergic drive to motoneurons in the spinal cord may be scaled to the level of motor activity in swimming lamprey (Christenson, Franck, & Grillner, 1989), treadmill walking cats (Fornal, Martín-Cora, & Jacobs, 2006), and freely walking rats (Gerin, Legrand, & Privat, 1994), these studies have been highly invasive and not translated to humans. The third protocol in this study aligned these viewpoints, by predicting that prolonged physical activity (i.e. the fatiguing submaximal contraction) would cause prolonged release of 5-HT into the CNS. Thus, 5-HT<sub>2A</sub> antagonism would reduce excitability of motoneurons and prevent motor units from discharging.

It is important to note that there are two motor unit mechanisms responsible for the generation of force in a muscle: the discharge rate of motor units and the recruitment/derecruitment of additional motor units (Baudry, Rudroff, Pierpoint, & Enoka, 2009; Feiereisen et al., 1997; Miller et al., 2019; Scott, 2004). It was not a feature of the current project to examine motor unit recruitment, as the goal from the onset of the project was to track motor units and their discharge characteristics across sessions. Therefore, it cannot be discounted that motor unit recruitment did not contribute to the time-to-fatigue results. Interestingly, the role of motor unit recruitment is still not clear when it comes to fatiguing submaximal contractions. However, the majority of experiments report that as a muscle is progressively more fatigued, the ability to maintain a constant force is accomplished, at least in part, by the recruitment of additional motor units within the muscle (Adam & De Luca, 2003; Carpentier, Duchateau, & Hainaut, 2001; Farina et al., 2009; Garland, Enoka, Serrano, & Robinson, 1994). This additional recruitment is driven by changes to membrane potentials, where motor unit threshold for activation progressively declines as the muscle fatigue progressively develops (Dorfman et al., 1990; Garland et al., 1994). Nonetheless, with the particular decomposition techniques that

were used in the current project, it is clear that the effects of 5-HT antagonism are most dominant during fatiguing contractions regardless of activity in other motor units.

## **5.5 Physiological considerations**

The framework for this research is novel as our understanding of cellular mechanisms that underpin motor function has been largely based on non-human models. Indeed, theoretical frameworks, animal preparations, and computer simulation studies have all been valuable for understanding movement, as these approaches avoid invasive and potentially harmful procedures from being performed on humans. At the other end of the spectrum, cellular mechanisms of motor function have also been revealed by studying the acute and chronic effects of medications on pathological populations such as Parkinson's disease, Multiple Sclerosis, and Motor Neuron Disease. The most fundamental aspect of this project's research design is to use pharmacology to selectively block 5-HT receptors in healthy human participants. This technically difficult experimental manipulation allows us to better grasp the importance of the serotonergic system and how muscle activity contributes to, or relies on, 5-HT release in the CNS. Nevertheless, investigation of recruitment thresholds would give a clearer picture of the motor unit behaviour with 5-HT<sub>2A</sub> receptor antagonism. It is important to note that the results of this study should not be generalised to all contractions, as this project only focussed on 3 types of contraction that were all performed at 30% MVC. Therefore, the results are not all-encompassing and should not be generalised to all rapid, all steady state, and all fatiguing contractions. Instead, the data in this project can provide a platform for future research using each type of contraction.

The absolute dosage of cyproheptadine may have implications between participants. The biodistribution of the participants varied; however, the dosage of the cyproheptadine did not. While this may cause issues with the significance of the effect of the cyproheptadine, it is considered a safe dosage for eliciting change in the serotonergic system (Graudins et al., 1998). Furthermore, due to the therapeutic usage of cyproheptadine being largely for allergies and headaches, there isn't a relative therapeutic dosage available.

Another discussion point is surrounding the measurement of MVC at each session. The reason for this is the prediction that ingestion of cyproheptadine will decrease the MVC of the participant and therefore the submaximal 30%MVC will not be the same between conditions and therefore introduce a confounding factor into the methodology. This was balanced with the procedure of the study, as alternatively, keeping a standard 30%MVC across both conditions would have theoretically meant the cyproheptadine conditions would measure at a higher percentage of MVC, further confounding the findings. This is due to findings of previous research suggesting that at higher intensities of contraction firing rate and recruitment of motor units increase (Del Vecchio, Negro, Felici, & Farina, 2018; Del Vecchio, Negro, et al., 2019). This is a consideration that would need further investigation into the methodology; particularly around motor unit behaviour with MVCs.

## **5.6 Technical considerations**

Although the procedures for HDEMG data collection were relatively simple, the subsequent processing of HDEMG data was extremely complex and time consuming. As stated in the results section, the use of standard personal computers to process data was challenging, and impossible in some circumstances such as the prolonged fatiguing contraction. The vast

majority of HDEMG-based motor unit studies have been for ramp-based sustained submaximal contraction. So, it is not surprising that the decomposition algorithms handled the second protocol in the current study the best. Future research using very rapid, or very long, HDEMG data sets will need to focus on appropriate decomposition filters and data analysis techniques to obtain better yield of motor units.

The decomposition algorithm was successful in identifying motor units in the HDEMG of the fatiguing tibialis anterior. However, similar to the rapid contractions, the ability to track these motor units between sessions was challenging. It is well understood that motor units will substitute in a sustained contraction, that is rotate in and out of use throughout the contraction, this consideration also makes it much less likely to track individual motor unit activity in segments of the sustained contraction. Figure 4.6 provides representative data for a subject performing the fatigue task, and it can be seen that different motor units were identified in each contraction. For this example, 6 motor units were present for the entire duration of the contraction in the placebo session, whereas 9 units were present for the entire duration of the contraction in the cyproheptadine session. From a signal processing perspective, it is not surprising that motor units could not be tracked between sessions. The decomposition algorithm was challenged to identify the same motor unit across the long contraction task, so there was only a small possibility that those small number of units could also be identified in a second long contraction task. From a technological perspective, it was discovered that the processing power of a standard personal computer was also insufficient to decompose multiple long data sets, and specialised high-end computing is necessary to undertake large projects.

## 5.7 Future directions

Understanding 5-HT function with an *in vivo* model will be a leap forward in our knowledge of how neurons are regulated in the CNS. The vast majority of human 5-HT research has been directed towards non-mechanistic (clinical) descriptions of depression and anxiety. The experiments in this Thesis that examine circuits in the spinal cord will for the first time provide mechanistic insight to how 5-HT affects motoneurons in the intact human CNS. This project may also have future applications to spinal cord injury (SCI). Most SCIs spare bridges of intact neural tissue that contain descending fibres still connected to interneurons and motoneurons below the site of injury. These projections remain functionally silent early in the injury response, but typically regain some function in the weeks following the injury. Thus, the remaining intact motor pathways in SCI may still be available to undergo neuromodulation. In recent years pharmacological intervention has been proposed as a critical intervention for SCI. However, the evidence for this therapy in humans has been poorly constructed. The data presented in this Thesis may provide further insight to how 5-HT modifying drugs impact the CNS when performing specific types of muscle contractions.

## 5.8 Conclusions

Investigations into 5-HT have predominantly been from a benchtop perspective, so our understanding of how 5-HT assists in activating muscle in an intact body is fragmented. The remaining 5-HT experiments have been performed on immobilised animals, euthanised animal preparations, or via computer simulations, so our understanding of how 5-HT is linked to muscle activity in humans is almost non-existent. The current study represents a human study that investigated how 5-HT contributes to different modes of muscle contractions. This project revealed that a blockade of 5-HT<sub>2A</sub> receptors produces a significant suppression in discharge rate of motor units during a fatiguing isometric 30% MVC contraction of the tibialis anterior

muscle. In contrast, 5-HT<sub>2A</sub> antagonism for shorter duration contractions, such as a rapid contraction and a slow ramped contractions to a steady-state of 30% MVC, had no effect on discharge rate of motor units. Overall, this project provides a valuable foundation for future research that assessed pharmacological intervention and motor function, as well as research that uses HDEMG to assess motor unit activity for a variety of submaximal contraction types.

## Chapter 6.0 References

- Abbruzzese, G., & Berardelli, A. (2003). Sensorimotor integration in movement disorders. *Movement Disorders, 18*(3), 231-240.
- Adam, A., & De Luca, C. J. (2003). Recruitment order of motor units in human vastus lateralis muscle is maintained during fatiguing contractions. *Journal of Neurophysiology, 90*(5), 2919-2927.
- Alvarez, F. J., Pearson, J. C., Harrington, D., Dewey, D., Torbeck, L., & Fyffe, R. E. (1998). Distribution of 5-hydroxytryptamine-immunoreactive boutons on  $\alpha$ -motoneurons in the lumbar spinal cord of adult cats. *Journal of Comparative Neurology, 393*(1), 69-83.
- Aston-Jones, G., Rajkowski, J., & Cohen, J. (2000). Locus coeruleus and regulation of behavioral flexibility and attention. In *Progress in Brain Research* (Vol. 126, pp. 165-182): Elsevier.
- Barry, B. K., Pascoe, M. A., Jesunathadas, M., & Enoka, R. M. (2007). Rate coding is compressed but variability is unaltered for motor units in a hand muscle of old adults. *Journal of Neurophysiology, 97*(5), 3206-3218.
- Baudry, S., Rudroff, T., Pierpoint, L. A., & Enoka, R. M. (2009). Load Type Influences Motor Unit Recruitment in Biceps Brachii During a Sustained Contraction. *Journal of Neurophysiology, 102*(3), 1725-1735.
- Beliveau, V., Ganz, M., Feng, L., Ozenne, B., Højgaard, L., Fisher, P. M., Svarer, C., Greve, D. N., & Knudsen, G. M. (2017). A High-Resolution In Vivo Atlas of the Human Brain's Serotonin System. *Journal of Neuroscience, 37*(1), 120-128. Retrieved from <https://dx.doi.org/10.1523/jneurosci.2830-16.2016>
- Berger, M., Gray, J. A., & Roth, B. L. (2009). The expanded biology of serotonin. *Annual Review of Medicine, 60*, 355-366.

- Bigland-Ritchie, B., Johansson, R., Lippold, O. C. t., Smith, S., & Woods, J. J. (1983). Changes in motoneurone firing rates during sustained maximal voluntary contractions. *The Journal of Physiology*, *340*(1), 335-346.
- Boe, S., Dalton, B., Harwood, B., Doherty, T., & Rice, C. (2009). Inter-rater reliability of motor unit number estimates and quantitative motor unit analysis in the tibialis anterior muscle. *Clinical Neurophysiology*, *120*(5), 947-952.
- Boess, F., & Martin, I. (1994). Molecular biology of 5-HT receptors. *Neuropharmacology*, *33*(3-4), 275-317.
- Bourin, M., Chue, P., & Guillon, Y. (2001). Paroxetine: A Review. *CNS Drug Reviews*, *7*(1), 25-47. doi:10.1111/j.1527-3458.2001.tb00189.x
- Bowker, R., Westlund, K., Sullivan, M., & Coulter, J. (1982). Organization of descending serotonergic projections to the spinal cord. In *Descending Pathways to the Spinal Cord* (Vol. 57, pp. 239-265): Elsevier Amsterdam.
- Bowker, R., Westlund, K., Sullivan, M., Wilber, J., & Coulter, J. (1983). Descending serotonergic, peptidergic and cholinergic pathways from the raphe nuclei: a multiple transmitter complex. *Brain Research*, *288*(1-2), 33-48.
- Buchthal, F., & Schmalbruch, H. (1980). Motor unit of mammalian muscle. *Physiological Reviews*, *60*(1), 90-142.
- Caperuto, E., Dos Santos, R., Mello, M., & Costa Rosa, L. (2009). Effect of endurance training on hypothalamic serotonin concentration and performance. *Clinical and Experimental Pharmacology and Physiology*, *36*(2), 189-191. doi:10.1111/j.1440-1681.2008.05111.x
- Carpentier, A., Duchateau, J., & Hainaut, K. (2001). Motor unit behaviour and contractile changes during fatigue in the human first dorsal interosseus. *The Journal of Physiology*, *534*(3), 903-912.

- Celada, P., Puig, M. V., Amargós-Bosch, M., Adell, A., & Artigas, F. (2004). The therapeutic role of 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors in depression.
- Chaouloff, F., Laude, D., Guezenec, Y., & Elghozi, J. (1986). Motor activity increases tryptophan, 5-hydroxyindoleacetic acid, and homovanillic acid in ventricular cerebrospinal fluid of the conscious rat. *Journal of Neurochemistry*, *46*(4), 1313-1316.
- Charig, E. M., Anderson, I. M., Robinson Sen, J. M., Nutt, D. J., & Cowen, P. J. (1986). L-Tryptophan and prolactin release: Evidence for interaction between 5-HT<sub>1</sub> and 5-HT<sub>2</sub> receptors. *Human Psychopharmacology: Clinical and Experimental*, *1*(2), 93-97.  
doi:10.1002/hup.470010206
- Chen, K., Ge, R., Cheng, Y., & Dai, Y. (2019). Three-week treadmill training changes the electrophysiological properties of spinal interneurons in the mice. *Experimental Brain Research*, *237*(11), 2925-2938. doi:10.1007/s00221-019-05647-3
- Cheng, Y., Song, N., Ge, R., & Dai, Y. (2021). Serotonergic Modulation of Persistent Inward Currents in Serotonergic Neurons of Medulla in ePet-EYFP Mice. *Frontiers in Neural Circuits*, *15*. doi:10.3389/fncir.2021.657445
- Christenson, J., Franck, J., & Grillner, S. (1989). Increase in endogenous 5-hydroxytryptamine levels modulates the central network underlying locomotion in the lamprey spinal cord. *Neuroscience Letters*, *100*(1-3), 188-192.
- Coombs, J. S., Curtis, D. R., & Eccles, J. C. (1957). The generation of impulses in motoneurons. *The Journal of Physiology*, *139*(2), 232-249.  
doi:10.1113/jphysiol.1957.sp005888
- Cotel, F., Exley, R., Cragg, S. J., & Perrier, J.-F. (2013). Serotonin spillover onto the axon initial segment of motoneurons induces central fatigue by inhibiting action potential initiation. *Proceedings of the National Academy of Sciences*, *110*(12), 4774-4779.

- Cullheim, S., Fleshman, J. W., Glenn, L. L., & Burke, R. E. (1987). Three-Dimensional architecture of dendritic trees in type-identified  $\alpha$ -motoneurons. *The Journal of Comparative Neurology*, 255(1), 82-96. doi:10.1002/cne.902550107
- D'Amico, J. M., Butler, A. A., Héroux, M. E., Cotel, F., Perrier, J.-F. M., Butler, J. E., Gandevia, S. C., & Taylor, J. L. (2017). Human motoneurone excitability is depressed by activation of serotonin 1A receptors with buspirone. *The Journal of Physiology*, 595(5), 1763-1773. doi:10.1113/jp273200
- D'Amico, J. M., Murray, K. C., Li, Y., Chan, K. M., Finlay, M. G., Bennett, D. J., & Gorassini, M. A. (2013). Constitutively active 5-HT<sub>2</sub>/ $\alpha$ 1 receptors facilitate muscle spasms after human spinal cord injury. *Journal of Neurophysiology*, 109(6), 1473-1484.
- Daubert, E. A., & Condron, B. G. (2010). Serotonin: a regulator of neuronal morphology and circuitry. *Trends in Neurosciences*, 33(9), 424-434. doi:10.1016/j.tins.2010.05.005
- Davis, J. M., & Bailey, S. P. (1997). Possible mechanisms of central nervous system fatigue during exercise. *Medicine and Science in Sports and Exercise*, 29(1), 45-57. doi:10.1097/00005768-199701000-00008
- De Luca, C. J., & Erim, Z. (1994). Common drive of motor units in regulation of muscle force. *Trends in Neurosciences*, 17(7), 299-305.
- De Luca, C. J., & Hostage, E. C. (2010). Relationship between firing rate and recruitment threshold of motoneurons in voluntary isometric contractions. *Journal of Neurophysiology*, 104(2), 1034-1046.
- Del Valle, A., & Thomas, C. K. (2005). Firing rates of motor units during strong dynamic contractions. *Muscle & Nerve*, 32(3), 316-325. doi:10.1002/mus.20371
- Del Vecchio, A., Casolo, A., Negro, F., Scorcelletti, M., Bazzucchi, I., Enoka, R., Felici, F., & Farina, D. (2019). The increase in muscle force after 4 weeks of strength training is

- mediated by adaptations in motor unit recruitment and rate coding. *The Journal of Physiology*, 597(7), 1873-1887. doi:10.1113/jp277250
- Del Vecchio, A., Holobar, A., Falla, D., Felici, F., Enoka, R., & Farina, D. (2020). Tutorial: Analysis of motor unit discharge characteristics from high-density surface EMG signals. *Journal of Electromyography and Kinesiology*, 102426.
- Del Vecchio, A., Negro, F., Felici, F., & Farina, D. (2017). Associations between motor unit action potential parameters and surface EMG features. *Journal of Applied Physiology*, 123(4), 835-843.
- Del Vecchio, A., Negro, F., Felici, F., & Farina, D. (2018). Distribution of muscle fibre conduction velocity for representative samples of motor units in the full recruitment range of the tibialis anterior muscle. *Acta Physiologica*, 222(2), e12930.
- Del Vecchio, A., Negro, F., Holobar, A., Casolo, A., Folland, J. P., Felici, F., & Farina, D. (2019). You are as fast as your motor neurons: speed of recruitment and maximal discharge of motor neurons determine the maximal rate of force development in humans. *The Journal of Physiology*, 597(9), 2445-2456.
- Desmedt, J. E., & Godaux, E. (1977). Ballistic contractions in man: characteristic recruitment pattern of single motor units of the tibialis anterior muscle. *The Journal of Physiology*, 264(3), 673-693.
- Dondelinger, R. M. (2010). Electromyography--an overview. *Biomedical Instrumentation and Technology*, 44(2), 128-131.
- Dorfman, L. J., Howard, J. E., & McGill, K. C. (1990). Triphasic behavioral response of motor units to submaximal fatiguing exercise. *Muscle & Nerve*, 13(7), 621-628.
- Eccles, J. C., Schmidt, R., & Willis, W. D. (1963). Pharmacological studies on presynaptic inhibition. *The Journal of Physiology*, 168(3), 500-530. doi:10.1113/jphysiol.1963.sp007205

- Eckert, R., & Lux, H. D. (1976). A voltage-sensitive persistent calcium conductance in neuronal somata of Helix. *The Journal of Physiology*, 254(1), 129-151. doi:10.1113/jphysiol.1976.sp011225
- Enoka, R. M., & Duchateau, J. (2017). Rate Coding and the Control of Muscle Force. *Cold Spring Harbor Perspectives in Medicine*, 7(10), a029702. doi:10.1101/cshperspect.a029702
- Enoka, R. M., & Stuart, D. G. (1984). Henneman's 'size principle': current issues. *Trends in Neurosciences*, 7(7), 226-228.
- Farina, D., Holobar, A., Gazzoni, M., Zazula, D., Merletti, R., & Enoka, R. M. (2009). Adjustments differ among low-threshold motor units during intermittent, isometric contractions. *Journal of Neurophysiology*, 101(1), 350-359.
- Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G\*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, 39(2), 175-191. doi:10.3758/bf03193146
- Feiereisen, P., Duchateau, J., & Hainaut, K. (1997). Motor unit recruitment order during voluntary and electrically induced contractions in the tibialis anterior. *Experimental Brain Research*, 114(1), 117-123.
- Fornal, C. A., Martín-Cora, F. J., & Jacobs, B. L. (2006). "Fatigue" of medullary but not mesencephalic raphe serotonergic neurons during locomotion in cats. *Brain Research*, 1072(1), 55-61.
- Gandevia, S. C. (2001). Spinal and supraspinal factors in human muscle fatigue. *Physiological Reviews*.
- Garland, S., Enoka, R., Serrano, L., & Robinson, G. (1994). Behavior of motor units in human biceps brachii during a submaximal fatiguing contraction. *Journal of Applied Physiology*, 76(6), 2411-2419.

- Gerdelat-Mas, A., Loubinoux, I., Tombari, D., Rascol, O., Chollet, F., & Simonetta-Moreau, M. (2005). Chronic administration of selective serotonin reuptake inhibitor (SSRI) paroxetine modulates human motor cortex excitability in healthy subjects. *Neuroimage*, 27(2), 314-322.
- Gerin, C., Legrand, A., & Privat, A. (1994). Study of 5-HT release with a chronically implanted microdialysis probe in the ventral horn of the spinal cord of unrestrained rats during exercise on a treadmill. *Journal of Neuroscience Methods*, 52(2), 129-141.
- Gillman, P. K. (1999). The serotonin syndrome and its treatment. *Journal of Psychopharmacology*, 13(1), 100-109. doi:10.1177/026988119901300111
- Giuliodori, M. J., & Zuccolilli, G. (2004). Post synaptic potential summation and action potential initiation: function following form. *Advances in Physiology Education*, 28(2), 79-80. doi:10.1152/advan.00051.2003
- Graudins, A., Stearman, A., & Chan, B. (1998). Treatment of the serotonin syndrome with cyproheptadine. *The Journal of Emergency Medicine*, 16(4), 615-619. doi:[https://doi.org/10.1016/S0736-4679\(98\)00057-2](https://doi.org/10.1016/S0736-4679(98)00057-2)
- Gunja, N., Collins, M., & Graudins, A. (2004). A Comparison of the Pharmacokinetics of Oral and Sublingual Cyproheptadine. *Journal of Toxicology: Clinical Toxicology*, 42(1), 79-83. doi:10.1081/clt-120028749
- Harvey, P. J., Li, X., Li, Y., & Bennett, D. J. (2006). 5-HT<sub>2</sub> receptor activation facilitates a persistent sodium current and repetitive firing in spinal motoneurons of rats with and without chronic spinal cord injury. *Journal of Neurophysiology*, 96(3), 1158-1170.
- Heckman, C., Lee, R. H., & Brownstone, R. M. (2003). Hyperexcitable dendrites in motoneurons and their neuromodulatory control during motor behavior. *Trends in Neurosciences*, 26(12), 688-695.

- Heckman, C. J., & Enoka, R. M. (2004). Physiology of the motor neuron and the motor unit. In A. Eisen (Ed.), *Handbook of Clinical Neurophysiology* (Vol. 4, pp. 119-147): Elsevier.
- Heckman, C. J., & Enoka, R. M. (2012). Motor unit. *Comprehensive Physiology*, 2(4), 2629-2682. doi:10.1002/cphy.c100087
- Heckman, C. J., Gorassini, M. A., & Bennett, D. J. (2005). Persistent inward currents in motoneuron dendrites: Implications for motor output. *Muscle & Nerve*, 31(2), 135-156. doi:10.1002/mus.20261
- Heckman, C. J., Hyngstrom, A. S., & Johnson, M. D. (2008). Active properties of motoneurone dendrites: diffuse descending neuromodulation, focused local inhibition. *The Journal of Physiology*, 586(5), 1225-1231. doi:10.1113/jphysiol.2007.145078
- Heckman, C. J., Mottram, C., Quinlan, K., Theiss, R., & Schuster, J. (2009). Motoneuron excitability: The importance of neuromodulatory inputs. *Clinical Neurophysiology*, 120(12), 2040-2054. doi:10.1016/j.clinph.2009.08.009
- Henneman, E. (1957). Relation between size of neurons and their susceptibility to discharge. *Science*, 126(3287), 1345-1347.
- Holobar, A., & Farina, D. (2014). Blind source identification from the multichannel surface electromyogram. *Physiological Measurement*, 35(7), R143.
- Holobar, A., Minetto, M. A., & Farina, D. (2014). Accurate identification of motor unit discharge patterns from high-density surface EMG and validation with a novel signal-based performance metric. *Journal of Neural Engineering*, 11(1), 016008.
- Holobar, A., & Zazula, D. (2007). Multichannel Blind Source Separation Using Convolution Kernel Compensation. *IEEE Transactions on Signal Processing*, 55(9), 4487-4496. doi:10.1109/tsp.2007.896108

- Holstege, J., & Kuypers, H. (1987). Brainstem projections to spinal motoneurons: an update. *Neuroscience*, 23(3), 809-821.
- Honrubia, M. A., Rodriguez, J., Dominguez, R., Lozoya, E., Manaut, F., Seijas, J. A., Villaverde, M. C., Calleja, J. M., Cadavid, M. I., & Maayani, S. (1997). Synthesis, affinity at 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> serotonin receptors and structure-activity relationships of a series of cyproheptadine analogues. *Chemical and Pharmaceutical Bulletin*, 45(5), 842-848.
- Husch, A., Dietz, S. B., Hong, D. N., & Harris-Warrick, R. M. (2015). Adult spinal V2a interneurons show increased excitability and serotonin-dependent bistability. *Journal of Neurophysiology*, 113(1522-1598 (Electronic)).
- Jacobs, B. L., & Fornal, C. A. (1997). Serotonin and motor activity. *Current Opinion in Neurobiology*, 7(6), 820-825.
- Jacobs, B. L., Martín-Cora, F. J., & Fornal, C. A. (2002). Activity of medullary serotonergic neurons in freely moving animals. *Brain Research Reviews*, 40(1-3), 45-52.
- Johnson, K. V. B., Edwards, S. C., Van Tongeren, C., & Bawa, P. (2004). Properties of human motor units after prolonged activity at a constant firing rate. *Experimental Brain Research*, 154(4), 479-487. doi:10.1007/s00221-003-1678-z
- Johnson, M. D., & Heckman, C. J. (2014). Gain control mechanisms in spinal motoneurons. *Frontiers in Neural Circuits*, 8, 81.
- Josephson, M. D., Rose, W. C., & Knight, C. A. (2019). Evidence of bilinearity in the relationship between rate of neuromuscular excitation and rate of force development. *Journal of Electromyography and Kinesiology*, 49, 102355. doi:10.1016/j.jelekin.2019.102355
- Juel, V. C. (2019). Single fiber electromyography. *Handbook of Clinical Neurology*, 160, 303-310. doi:10.1016/b978-0-444-64032-1.00019-9

- Kavanagh, J. J., McFarland, A. J., & Taylor, J. L. (2019). Enhanced availability of serotonin increases activation of unfatigued muscle but exacerbates central fatigue during prolonged sustained contractions. *The Journal of Physiology*, 597(1), 319-332. doi:10.1113/JP277148
- Kim, D.-Y., & Camilleri, M. (2000). Serotonin: a mediator of the brain-gut connection. *The American Journal of Gastroenterology*, 95(10), 2698.
- Kim, I. H., Kisseleva, T., & Brenner, D. A. (2015). Aging and liver disease. *Current Opinion in Gastroenterology*, 31(3), 184-191. doi:10.1097/mog.0000000000000176
- Kole, M. H. P., Ilschner, S. U., Kampa, B. M., Williams, S. R., Ruben, P. C., & Stuart, G. J. (2008). Action potential generation requires a high sodium channel density in the axon initial segment. *Nature Neuroscience*, 11(2), 178-186. doi:10.1038/nn2040
- Kuypers, H. G. (1964). The descending pathways to the spinal cord, their anatomy and function. In *Progress in Brain Research* (Vol. 11, pp. 178-202): Elsevier.
- Loeb, G. E., Brown, I. E., & Cheng, E. J. (1999). A hierarchical foundation for models of sensorimotor control. *Experimental Brain Research*, 126(1), 1-18. doi:10.1007/s002210050712
- Martinez-Valdes, E., Laine, C., Falla, D., Mayer, F., & Farina, D. (2016). High-density surface electromyography provides reliable estimates of motor unit behavior. *Clinical Neurophysiology*, 127(6), 2534-2541.
- Marvin, G., Sharma, A., Aston, W., Field, C., Kendall, M., & Jones, D. (1997). The effects of buspirone on perceived exertion and time to fatigue in man. *Experimental Physiology*, 82(6), 1057-1060. doi:10.1113/expphysiol.1997.sp004080
- Meeusen, R., Watson, P., Hasegawa, H., Roelands, B., & Piacentini, M. F. (2006). Central fatigue: the serotonin hypothesis and beyond. *Sports Medicine*, 36(10), 881-909. doi:10.2165/00007256-200636100-00006

- Mendes, G. D., Arruda, A., Chen, L. S., de Almeida Magalhães, J. C., Alkharfy, K. M., & De Nucci, G. (2012). Quantification of cyproheptadine in human plasma by high-performance liquid chromatography coupled to electrospray tandem mass spectrometry in a bioequivalence study. *Biomedical Chromatography*, *26*(1), 129-136.
- Miller, J. D., Lund, C. J., Gingrich, M. D., Shtul, K. L., Wray, M. E., & Herda, T. J. (2019). The effect of rate of torque development on motor unit recruitment and firing rates during isometric voluntary trapezoidal contractions. *Experimental Brain Research*, *237*(10), 2653-2664. doi:10.1007/s00221-019-05612-0
- Moritz, C. T., Barry, B. K., Pascoe, M. A., & Enoka, R. M. (2005). Discharge rate variability influences the variation in force fluctuations across the working range of a hand muscle. *Journal of Neurophysiology*, *93*(5), 2449-2459.
- Murphy, S. A. (2018). *Mechanisms of Impaired Motor Unit Firing Behavior in the Vastus Lateralis Muscle after Stroke*. Marquette University,
- Murray, K. C., Nakae, A., Stephens, M. J., Rank, M., D'amico, J., Harvey, P. J., Li, X., Harris, R. L. W., Ballou, E. W., & Anelli, R. (2010). Recovery of motoneuron and locomotor function after spinal cord injury depends on constitutive activity in 5-HT<sub>2C</sub> receptors. *Nature Medicine*, *16*(6), 694.
- Nachev, P., Kennard, C., & Husain, M. (2008). Functional role of the supplementary and pre-supplementary motor areas. *Nature Reviews Neuroscience*, *9*(11), 856-869.
- Nardelli, P., Powers, R., Cope, T. C., & Rich, M. M. (2017). Increasing motor neuron excitability to treat weakness in sepsis. *Annals of Neurology*, *82*(6), 961-971.
- Negro, F., Muceli, S., Castronovo, A. M., Holobar, A., & Farina, D. (2016). Multi-channel intramuscular and surface EMG decomposition by convolutive blind source separation. *Journal of Neural Engineering*, *13*(2), 026027.

- Nielsen, J. B. (2004). Sensorimotor integration at spinal level as a basis for muscle coordination during voluntary movement in humans. *Journal of Applied Physiology*, *96*(5), 1961-1967.
- O'Donnell, J., Zeppenfeld, D., McConnell, E., Pena, S., & Nedergaard, M. (2012). Norepinephrine: A Neuromodulator That Boosts the Function of Multiple Cell Types to Optimize CNS Performance. *Neurochemical Research*, *37*(11), 2496-2512. doi:10.1007/s11064-012-0818-x
- Oya, T., Riek, S., & Cresswell, A. G. (2009). Recruitment and rate coding organisation for soleus motor units across entire range of voluntary isometric plantar flexions. *The Journal of Physiology*, *587*(19), 4737-4748.
- Parise, G., Bosman, M. J., Boecker, D. R., Barry, M. J., & Tarnopolsky, M. A. (2001). Selective serotonin reuptake inhibitors: Their effect on high-intensity exercise performance. *Archives of Physical Medicine and Rehabilitation*, *82*(7), 867-871.
- Perrier, J.-F., & Cotel, F. (2015). Serotonergic modulation of spinal motor control. *Current Opinion in Neurobiology*, *33*, 1-7.
- Perrier, J.-F., Rasmussen, H. B., Christensen, R. K., & Petersen, A. V. (2013). Modulation of the intrinsic properties of motoneurons by serotonin. *Current Pharmaceutical Design*, *19*(24), 4371-4384.
- Perrier, J.-F., Rasmussen, H. B., Jørgensen, L. K., & Berg, R. W. (2018). Intense Activity of the Raphe Spinal Pathway Depresses Motor Activity via a Serotonin Dependent Mechanism. *Frontiers in Neural Circuits*, *11*. doi:10.3389/fncir.2017.00111
- Perrier, J. F. (2019). If serotonin does not exhaust you, it makes you stronger. *The Journal of Physiology*, *597*(1), 5-6. doi:10.1113/JP277317
- Perrier, J. F., & Delgado-Lezama, R. (2005). Synaptic release of serotonin induced by stimulation of the raphe nucleus promotes plateau potentials in spinal motoneurons of

- the adult turtle. *Journal of Neuroscience*, 25(35), 7993-7999.  
doi:10.1523/jneurosci.1957-05.2005
- Petrofsky, J. (2008). The effect of the subcutaneous fat on the transfer of current through skin and into muscle. *Medical Engineering & Physics*, 30(9), 1168-1176.
- Politis, M., & Niccolini, F. (2015). Serotonin in Parkinson's disease. *Behavioural Brain Research*, 277, 136-145. doi:10.1016/j.bbr.2014.07.037
- Rashid, M., Muntasir, H. A., Watanabe, M., Nakazawa, M., Ozaki, M., & Nagatomo, T. (2003). Assessment of Affinities and Dissociation Potencies of Several 5-HT<sub>2</sub> Antagonists to and from M<sub>2</sub> Muscarinic Receptor in Rat Heart Membranes. *Biological and Pharmaceutical Bulletin*, 26(8), 1184-1187. doi:10.1248/bpb.26.1184
- Redman, R. S., & Silinsky, E. M. (1994). ATP released together with acetylcholine as the mediator of neuromuscular depression at frog motor nerve endings. *The Journal of Physiology*, 477(1), 117-127. doi:10.1113/jphysiol.1994.sp020176
- Rekling, J. C., Funk, G. D., Bayliss, D. A., Dong, X.-W., & Feldman, J. L. (2000). Synaptic Control of Motoneuronal Excitability. *Physiological Reviews*, 80(2), 767-852. doi:10.1152/physrev.2000.80.2.767
- Rongen, G. A., Lenders, J. W., Lambrou, J., Willemsen, J. J., Van Belle, H., Thien, T., & Smits, P. (1996). Presynaptic inhibition of norepinephrine release from sympathetic nerve endings by endogenous adenosine. *Hypertension*, 27(4), 933-938.
- Rubin, D. I. (2019). Needle electromyography: Basic concepts. *Handbook of Clinical Neurology*, 160, 243-256. doi:10.1016/b978-0-444-64032-1.00016-3
- Rudomin, P. (2002). Selectivity of the central control of sensory information in the mammalian spinal cord. In *Sensorimotor control of movement and posture* (pp. 157-170): Springer.

- Rueter, L. E., & Jacobs, B. L. (1996). A microdialysis examination of serotonin release in the rat forebrain induced by behavioral/environmental manipulations. *Brain Research*, 739(1-2), 57-69.
- Scott, S. H. (2004). Optimal feedback control and the neural basis of volitional motor control. *Nature Reviews Neuroscience*, 5(7), 532-545.
- Sherrington, C. S. (1925). Remarks on some aspects of reflex inhibition. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character*, 97(686), 519-545. doi:10.1098/rspb.1925.0017
- Singer, J. H., Bellingham, M. C., & Berger, A. J. (1996). Presynaptic inhibition of glutamatergic synaptic transmission to rat motoneurons by serotonin. *Journal of Neurophysiology*, 76(2), 799-807.
- Stein, R. B. (1995). Presynaptic inhibition in humans. *Progress in Neurobiology*, 47(6), 533-544.
- Stephenson, J. L., & Maluf, K. S. (2011). Dependence of the paired motor unit analysis on motor unit discharge characteristics in the human tibialis anterior muscle. *Journal of Neuroscience Methods*, 198(1), 84-92.
- Stuart, G., Spruston, N., Sakmann, B., & Häusser, M. (1997). Action potential initiation and backpropagation in neurons of the mammalian CNS. *Trends in Neurosciences*, 20(3), 125-131. doi:https://doi.org/10.1016/S0166-2236(96)10075-8
- Thomas, C. K., Ross, B. H., & Stein, R. B. (1986). Motor-unit recruitment in human first dorsal interosseous muscle for static contractions in three different directions. *Journal of Neurophysiology*, 55(5), 1017-1029. doi:10.1152/jn.1986.55.5.1017
- Thomas, D. R., Nelson, D. R., & Johnson, A. M. (1987). Biochemical effects of the antidepressant paroxetine, a specific 5-hydroxytryptamine uptake inhibitor. *Psychopharmacology*, 93(2). doi:10.1007/bf00179933

- Thompson, C. K., Johnson, M. D., Negro, F., Mcpherson, L. M., Farina, D., & Heckman, C. J. (2019). Exogenous neuromodulation of spinal neurons induces beta-band coherence during self-sustained discharge of hind limb motor unit populations. *Journal of Applied Physiology*, *127*(4), 1034-1041.
- Thorstensen, J. R., Taylor, J. L., Tucker, M. G., & Kavanagh, J. J. (2020). Enhanced serotonin availability amplifies fatigue perception and modulates the TMS-induced silent period during sustained low-intensity elbow flexions. *The Journal of Physiology*, *598*(13), 2685-2701. doi:10.1113/jp279347
- Törk, I. (1990). Anatomy of the Serotonergic System. *Annals of the New York Academy of Sciences*, *600*(1 The Neurophar), 9-34. doi:10.1111/j.1749-6632.1990.tb16870.x
- Van Cutsem, M., Duchateau, J., & Hainaut, K. (1998). Changes in single motor unit behaviour contribute to the increase in contraction speed after dynamic training in humans. *The Journal of Physiology*, *513*(1), 295-305.
- Van Cutsem, M., Feiereisen, P., Duchateau, J., & Hainaut, K. (1997). Mechanical properties and behaviour of motor units in the tibialis anterior during voluntary contractions. *Canadian Journal of Applied Physiology*, *22*(6), 585-597.
- Veasey, S., Fornal, C., Metzler, C., & Jacobs, B. (1995). Response of serotonergic caudal raphe neurons in relation to specific motor activities in freely moving cats. *Journal of Neuroscience*, *15*(7), 5346-5359. doi:10.1523/jneurosci.15-07-05346.1995
- Veasey, S. C., Fornal, C., Metzler, C., & Jacobs, B. L. (1995). Response of serotonergic caudal raphe neurons in relation to specific motor activities in freely moving cats. *Journal of Neuroscience*, *15*(7), 5346-5359.
- Vetter, P., Roth, A., & Häusser, M. (2001). Propagation of Action Potentials in Dendrites Depends on Dendritic Morphology. *Journal of Neurophysiology*, *85*(2), 926-937. doi:10.1152/jn.2001.85.2.926

- Wei, K., Glaser, J. I., Deng, L., Thompson, C. K., Stevenson, I. H., Wang, Q., Hornby, T. G., Heckman, C. J., & Kording, K. P. (2014). Serotonin affects movement gain control in the spinal cord. *Journal of Neuroscience*, *34*(38), 12690-12700.
- Wilson, W., & Maughan, R. (1992). Evidence for a possible role of 5-hydroxytryptamine in the genesis of fatigue in man: administration of paroxetine, a 5-HT re-uptake inhibitor, reduces the capacity to perform prolonged exercise. *Experimental Physiology*, *77*(6), 921-924. doi:10.1113/expphysiol.1992.sp003660
- Wu, L.-G., & Saggau, P. (1997). Presynaptic inhibition of elicited neurotransmitter release. *Trends in Neurosciences*, *20*(5), 204-212.
- Young, S. N. (2007). How to increase serotonin in the human brain without drugs. *Journal of Psychiatry & Neuroscience*, *32*(6), 394.