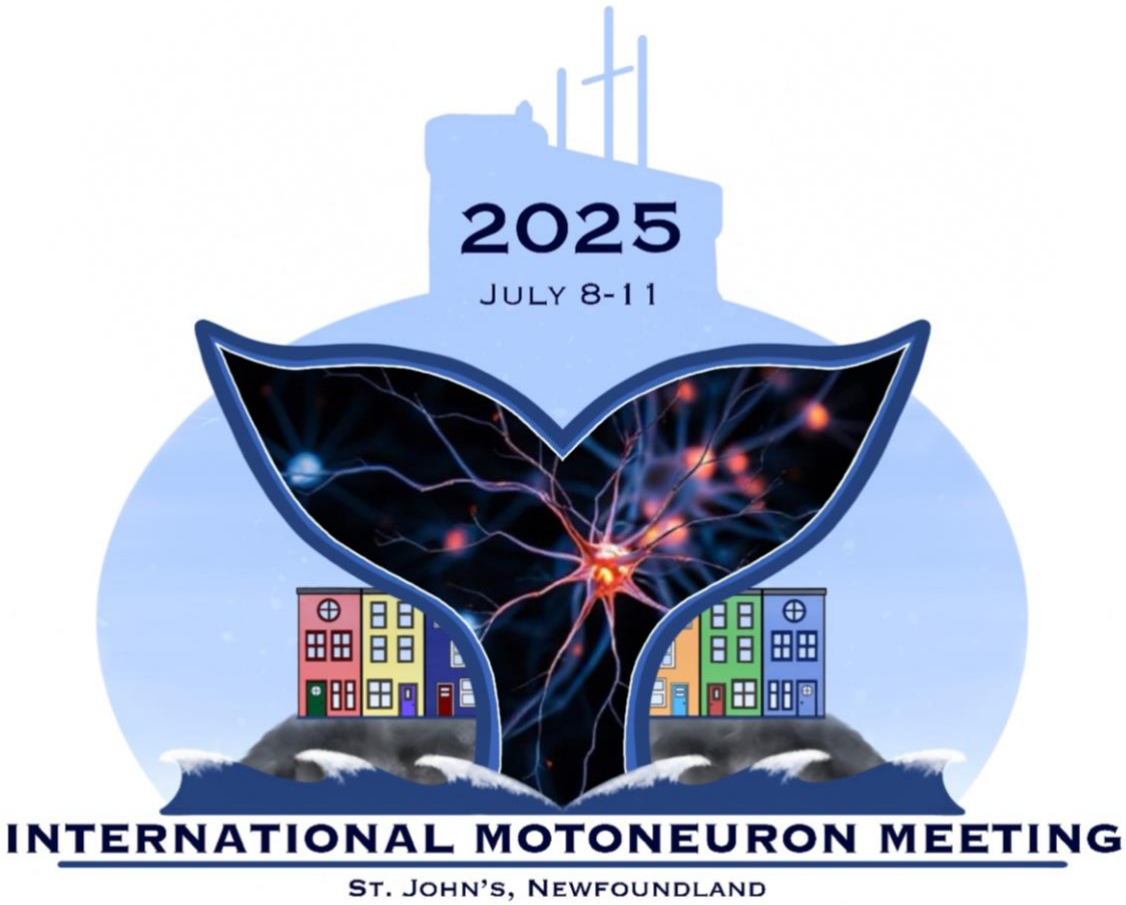

ABSTRACT BOOKLET

International
MOTONEURON SOCIETY

TABLE OF CONTENTS

WELCOME

Dear colleagues and friends,

Welcome to Motoneuron 2025, the 14th International Motoneuron Society Meeting! We are absolutely thrilled to host you in beautiful St. John's, Newfoundland, from Tuesday, July 8 to Friday, July 11, 2025.

This year's programme promises to be both scientifically and socially rich. We have a diverse array of oral sessions, covering topics from basic mechanisms of motoneuron firing and inputs to motoneurons, to ALS and other motor impairments, neuromuscular junctions, and human motor unit behaviour. A dedicated poster social, frequent breaks, and morning networking time provide ample opportunity to exchange ideas and spark collaborations.

Beyond the science, we've planned a vibrant social agenda: including an unforgettable Newfoundland kitchen party at Quidi Vidi Brewery on Thursday and an optional puffin- and whale-watching boat tour to immerse you in Newfoundland's breathtaking natural beauty.

As you gather at the base of Signal Hill, may the warmth of the local culture and the excitement of our scientific community inspire you throughout the week. Whether you're a first-time attendee or a returning veteran, we hope you feel energized by the scientific content, meaningful conversations, and new friendships waiting around every corner.

On behalf of the International Motoneuron Society, welcome to St. John's, where exceptional science, camaraderie, and unforgettable memories await!

With great enthusiasm,

Greg

Chair, Local Organizing Committee

International Motoneuron Society Meeting 2025

ORAL PRESENTATIONS

Session I – ALS 1

1. **Selective, stress-induced motor neuron loss correlates with TDP-43 translocation in vivo**

   ALICIA DUBINSKI (1), MARIAM CHOUGHARI (1), ALA FERDI (1), MELISSA TOUATI (1), SARAH PEYRARD (1), CHRISTINE VANDE VELDE (1)

   1) University of Montreal, Canada

   Neurons must effectively respond to external stress, and defects in stress granule dynamics have been implicated in ALS pathogenesis. Stress granules have been proposed to seed cytoplasmic TDP-43 inclusions, a hallmark of ALS and ~50% of FTD cases. Using an in vivo heat stress paradigm, we previously showed that TDP-43 M337V mice fail to assemble stress granules and exhibit increased TDP-43 translocation. Here, we examined the long-term consequences of this defect. After one month of recurrent stress, RNA granules did not persist in non-transgenic (NTg) or TDP-43 M337V mice, but non-overlapping cytoplasmic TDP-43 and TiaR granules were detected. However, motor neuron loss progressed from 30% to 50% in stressed TDP-43 M337V mice, selectively affecting alpha motor neurons. Notably, a high proportion of these mice developed hyperthermia-induced seizures, despite no exacerbation of motor dysfunction. These findings suggest that defective stress granule assembly is detrimental to motor neuron health and may contribute to broader neurological consequences beyond ALS-related motor decline. Moving forward, we aim to identify the mechanisms underlying hyperthermia-induced seizures and investigate the electrophysiological properties of neurons in these mice.

2. **A Macaque Model of Motor Neurone Disease**

   RACHAEL JONES (1), LUCIANO SAIEVA (1), FABIEN BALEZEAU (1), IAN SCHOFIELD (1), CAROLINE MCCARDLE (1), MARK BAKER (1,2), STUART BAKER (1)

   1) Newcastle University, UK; 2) Royal Victoria Infirmary, UK

   Motor Neurone Disease (MND) is a rapidly progressive and ultimately fatal neurodegenerative disease, characterised by the loss of upper and lower motor neurons. The primary proteinopathy found in approximately 97% of all cases involves cytoplasmic mislocalisation and aggregation of the ubiquitous nuclear protein, TDP-43. Despite the

identification of many implicated genes during the last few decades, our understanding of the mechanisms involved in the onset and propagation of pathology have advanced very little. Translatable advancements have likely been limited due a lack of reliable animal models which accurately recapitulate this complex disorder. No model has been created to date which replicates the progressive motor weakness; characteristic histopathologies; extended pre-symptomatic phase and subsequent rapid deterioration. Rodents have been the dominant species in MND research; however, their anatomy and genetic profile differs fundamentally to humans. Crucially, they lack the direct monosynaptic connection between the upper and lower motor neurons, unique to primates. We have harnessed a novel intersectional genetics approach to induce the overexpression of the human TDP-43 protein in a selective spinal motoneuron population in two Rhesus macaques. Focal overexpression of TDP-43 in a spinal motor pool was sufficient to induce the expression of pathological phosphorylated TDP-43 (pTDP-43) throughout the motoneurons of the cervical spine and corticomotoneuronal cells of the primary motor cortex. The detection of this histopathology in the distant giant cells of Betz in the primary motor cortex is consistent with the pattern of pathology seen in patients, and may indicate the involvement of the corticospinal tract.

3. **Motoneuron recruitment-derecruitment hysteresis is reduces over time in weaker, but not stronger, muscles in people living with ALS**

GABRIEL SIQUEIRA TRAJANO (1), JOAO SALDANHA HENKIN (1), GABRIEL BASTO LIMA DO AMARAL FERNANDES (2), LUCAS BET DA ROSA ORSSATTO (3), PAMELA MCCOMBE (3,4), EHSAN SHANDIZ (3), ROBERT HENDERSON (3,4)

1) Queensland University of Technology, Australia; 2) Southern Cross University, Australia; 3) University of Queensland, Australia; 4) Royal Brisbane Women's Hospital, Australia.

There is a critical need to develop new biomarkers of disease progression for people living with ALS. The investigation of physiological changes within motoneurons is a logical step towards the development of a longitudinal biomarkers of disease progression as changes in motoneuron excitability are closely related to motoneurons degeneration. We monitored changes in motoneurons recruitment-derecruitment hysteresis, which is largely dependent on the magnitude of persistent inward Na+ and Ca2+ currents (PICs), over time together with the progression of the disease. We used high-density EMG (HD-EMG) and paired motor unit analysis to calculate the recruitment-decruitment hysteresis of motor units (ΔF). The ΔF was then used as an estimate of the magnitude of PICs in motoneurons innervating the tibialis anterior (TA). 68 people living with MND were tested 3 times (0, 3, 6 months). They performed dorsiflexion contraction up to 40% of their maximal capacity and HD-EMG signals were recorded from the TA muscle. TA muscles were also classified into stronger and weaker based on their MRC strength scores. ΔFs decreased from the first to the second visit (-16%) and remained lower at the third visit (-20%) in weaker muscles ($p<0.01$). ΔF values did not changed during the three visits in stronger muscles. Weaker muscles had lower ΔFs values compared to stronger muscles at the second (-

22%) and third visit (-26%; $p<0.01$). PICs contribution to motoneuron self-sustained firing, measured as ΔF, is reduced in muscles exhibiting clinical signs of weakness, but not in muscles with normal strength values.

4. **Activity Dependent Changes of ALS Patient-Derived iPSC Motor Neurons**

PAIGE REYNOLDS (1), LAURA DUMAS (1), VICTOR RAFUSE (1), YING ZHANG (1)

1) Dalhousie University, Canada

Amyotrophic Lateral Sclerosis (ALS) is a rapidly progressing fatal neurodegenerative disease. It is characterized by motor neuron degeneration, which leads to the loss of voluntary movement control and premature mortality. Currently, there is no known cure for ALS. Induced pluripotent stem cells (iPSC) derived from ALS patients have become a powerful tool to study the disease mechanism and find new therapeutic strategies. We have established several iPSC lines sourced from both healthy individuals and ALS patients harboring the genetic mutations C9ORF72 or TARDBP. In addition, the introduction of Channelrhodopsin-2 (CHR2), a light-sensitive protein, into these cell lines enables the manipulation of cell activity via light stimulation. Using whole-cell patch clamp, we have systematically characterized motor neurons derived from these hiPSC lines. We revealed an early loss of depolarizing activity in iPSCs bearing ALS mutations when compared to control neurons at the same stage of maturation. To investigate how to alter the maturation and degeneration trajectories of MNs, changing culture conditions and modulation via long-term optogenetic stimulation was explored. Exposing cultures to long-term optogenetic stimulation revealed that ALS iPSCMNs were more reactive to light stimulation over time while the control iPSCMNs remained unchanged. This presents a potential avenue for modulating cellular behavior at varying developmental stages, thereby potentially extending ALS cell survival.

Session II – Movement & Plasticity

1. **Investigating motor unit firing rates during arm cycling in humans**

DANIEL C. BASILE (1), ALYSHA D. WIRA (2), CHARLES L. RICE (1), KEVIN E. POWER (2)

1) The University of Western Ontario, Canada; 2) Memorial University of Newfoundland, Canada

There is good evidence that similar to quadrupeds, humans have spinal central pattern generators (CPGs) capable of generating rhythmic motor outputs. Evaluating motor unit firing rates (MUFR) as a direct output of the spinal motoneuron pool would thereby improve

the understanding of CPG-mediated rhythmic motor outputs in humans. Using arm cycling as a model of locomotor generated activity, the purpose was to determine whether MUFR differed during arm cycling compared to intensity-matched isometric contractions. We hypothesized that MUFR would be greater during arm cycling than isometric contractions, assessed at various working intensities. Young males (n=10) and females (n=4) completed arm cycling bouts and isometric contractions of the biceps brachii. Indwelling fine-wire electrodes were inserted into the biceps brachii to record MUFR during arm cycling with combinations of two power outputs (25 and 50 W) and cadences (30 and 60 RPM), and subsequently compared to intensity-matched isometric contractions. Results indicated that MUFR were significantly higher during arm cycling compared to isometric contractions ($p=0.003$), and MUFR increased with greater cycling intensity ($p<0.001$). Collectively, when compared with isometric contractions in the absence of CPG-mediated activity, enhanced descending drive and greater spinal motoneuron excitability likely facilitated an increase in MUFR during arm cycling. Thus, different neural control strategies are used during a rhythmic locomotor output compared with isometric contractions in humans.

2. **Implications of Modifying Motoneuron Excitability on the Proprioceptive Sense of Force**

KAITLYN SUTTON (1), OLIVIA RYAN (1), GREGORY PEARCEY (1)

1) Memorial University of Newfoundland, Canada

The ability to produce and control meaningful movements is vital to the well-being of humans and relies on the appropriate control of motor units. Motor unit firing is affected by motoneuronal persistent inward currents (PICs), which are facilitated by monoaminergic neuromodulation and heavily contribute to the gain control of motoneuronal output. They are also highly sensitive to inhibition – for instance, inducing Ia reciprocal inhibition through antagonist muscle vibration drastically reduces discharge rate hysteresis (ΔF). To identify any potential force control alterations under conditions of reduced PIC magnitude, we implemented an isometric force reproduction task without visual feedback. Tendon vibration was applied to either the agonist or antagonist muscle during either the first (with visual feedback) or second contraction (without visual feedback) and participants were asked to match perceived effort across contractions, in an attempt to match neural drive to the motor pool. In support of our hypothesis, torque output was significantly increased during the second contraction compared to the first when antagonist, but not agonist vibration was applied in the first contraction. When vibration was applied in the second contraction, the opposite effects were observed. Since the central nervous system must constantly process and respond to new information about movement and limb position coming from proprioceptive receptors during dynamic motor output, it is possible that errors in force reproduction are exacerbated in dynamic conditions. The goal of ongoing thesis work is to explore this phenomenon by quantifying errors in force reproduction due to antagonist vibration under dynamic conditions.

3. **Endurance vs. Strength Training – Differential Changes in Proprioceptive Input from Muscle Spindles To Slow and Fast Motoneurons**

PIOTR KRUTKI, MAJA KRAUZE (1), HANNA DRZYMAŁA-CELICHOWSKA (1), MARCIN BĄCZYK (1)

1) Poznan University of Physical Education, Poland

The aim of this study was to investigate adaptive changes in afferent synaptic transmission from muscle spindles to spinal motoneurons following two forms of physical training: the endurance training (running on a treadmill) or the strength training (voluntary progressive weight-lifting). Each training program was conducted in 15 adult male rats for 5 weeks, and a day after the last training session an acute electrophysiological experiment was performed on each rat under general anesthesia. The respective control groups (n=15) were assigned for each training protocol. Lumbar motoneurons innervating the medial gastrocnemius (MG) or lateral gastrocnemius and soleus (LG-S) muscles were recorded intracellularly. The passive membrane properties were measured and Ia monosynaptic EPSPs were evoked by stimulation of muscle afferents from muscle spindles of synergistic LG-S or MG muscles. In a proportion of motoneurons a sodium channel blocker QX-314 was used to record EPSPs from homonymous muscles. Following the endurance training an increase in amplitudes of heteronymous EPSPs was observed in slow motoneurons only, while weight-lifting training resulted in an increase of EPSP amplitudes in homonymous and heteronymous EPSPs, predominantly in fast motoneurons. To explain differences in the adaptive changes of the Ia synaptic input in various forms of training one should consider changes in membrane properties of motoneurons, altered levels of presynaptic inhibition, different modes of motor unit recruitment and different intensity of muscle spindle activation during particular types of exercise. The study was supported by the National Science Centre (NCN) Grant No. 2022/45/B/NZ7/00102

4. **Well-Behaved Motor Units Rarely Make History – The Challenging Task of Quantifying Female Motor Output Across the Menstrual Cycle**

SOPHIA JENZ (1), PADRAIG SPILLANE (2), MOLLIE O'HANLON (3), ALEX BENEDETTO (1), JAMES A. BEAUCHAMP (4), MELISSA FAJARDO (1), MATHEW PIASECKI (5), CJ HECKMAN (1), PAUL ANSDELL (2), JESSICA PIASECKI (3), GREGORY PEARCEY (6)

1) Northwestern University, USA; 2) Northumbria University, UK; 3) Nottingham Trent University, UK; 4) Carnegie Mellon University, USA; 5) University of Nottingham, UK; 6) Memorial University of Newfoundland, Canada

For years, female motoneurons have been understudied—perhaps in part because they don't behave like males. Females have largely been excluded from HDsEMG studies due to lower motor unit (MU) yield, greater variability, and/or difficulty in analysis. Yet, when

sex is considered, differences in MU discharge rate and recruitment emerge, offering insight into mechanisms underlying sex differences in motor control and neurological disease. Our lab and, more recently, others have shown that estimates of persistent inward currents (PICs) are higher in females during low-intensity contractions, suggesting sex-related differences in neuromodulatory drive or inhibition pattern. Given the effects of female sex hormones on serotonin and norepinephrine signaling in the brain, it remains likely these hormones also influence spinal circuitry—yet this remains largely unexplored. We conducted a multi-site study in eumenorrheic females performing isometric dorsiflexion ramp contractions at 30, 50, and 70% MVC across the menstrual cycle at the early follicular (EF), ovulatory (O) and mid luteal (ML) phases. Across contraction intensities, discharge rates were highest during ML, and nonlinearity in the ascending discharge rate modulation (brace height) increased from O to ML. Modulation in discharge rate hysteresis (ΔF) was intensity-dependent: during EF, ΔF only increased at 70%, while during O and ML ΔF increased from 30 to 50% but not further at 70%. These findings suggest elevated progesterone and estradiol may affect the interactions between neuromodulatory and inhibitory motor commands, which ultimately contribute to the complexity of quantifying female motor output.

5. **Attenuated corticospinal excitation and inhibition during fatigue limit spinal motoneuron firing responses at task failure in sustained submaximal contractions**

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1) University of Birmingham, UK; 2) University of Twente, Netherlands; 3) University of Brescia, Italy

Recent studies on motoneuron adaptations to fatigue have identified a biphasic discharge rate (DR) response, characterized by an initial decline followed by a subsequent increase. However, despite maximal effort, the late increase in DR at task failure remains lower than that observed during high-force contractions. This study investigated 1) corticospinal contributions to this biphasic DR response and 2) the mechanisms limiting DR at task failure. Fourteen participants performed an isometric dorsiflexion at 50% maximum voluntary contraction (MVC) to assess DR in a high-intensity, non-fatigued state. They then sustained a 25%MVC contraction until task failure, with transcranial magnetic stimulation delivered every 3s. Motoneuron firing patterns were tracked, and corticospinal excitatory/inhibitory responses were assessed via peristimulus frequencygrams at 20%, 40%, 60%, 80%, and 100% of endurance time. DR increased significantly after 40% of endurance time (non-linear response), whereas corticospinal excitation and inhibition amplitudes increased linearly throughout the contraction (both $p<.001$) and were unrelated to non-linear DR changes. A significant correlation was found between ΔDR (the difference in DR between 50%MVC and 25%MVC at task failure) and changes in excitation ($r=-0.74$) and inhibition ($r=0.59$) amplitudes from 20% to 100% of endurance time (Δinhibition and Δexcitation). Additionally, both Δinhibition and Δexcitation were associated with time to

failure (r=0.58). These findings suggest that DR dynamics may not be primarily driven by corticospinal mechanisms but rather by peripheral factors underlying neuromuscular fatigue. However, the inability to increase corticospinal excitability and inhibition during sustained submaximal contractions was linked to attenuated DR responses and associated with task failure.

Session III – Cellular Mechanisms of Motoneuron Firing

1. **The dark secrets of experimental measures of motoneuron PICs and self-sustained firing**

DJ BENNETT (1), MA GORASSINI, K HARI (1), T AYANTAYO (1), M ALMOKDAD (1)

1) University of Alberta, Canada

Recent recordings from spinal cord slice preparations in young mice have thrown into question whether persistent inward currents (PICs) contribute to self-sustained firing of motoneurons (hysteresis, delta-I and delta-F), and even whether small motoneurons have PICs at all. Here we retrospectively examine our motoneuron recordings from adult cats, rats and humans, as well as examine new recordings from mice, to resolve this issue. Early on our cat recordings showed that while all sizes of motoneurons exhibit PICs and firing hysteresis, limitations emerged that obscured the PIC actions on firing, including the PIC being synaptically activated subthreshold to firing (like an iceberg lurking below threshold) and inhibition blocking the PICs. When we next turned to recording rat motoneurons, by luck we developed an ultrasharp electrode capable of recording fine dendrites where the PICs are located, though we only now realize this in retrospect. This allowed intracellular dendritic activation to produce self-sustained firing identical to that triggered by synaptic input in the awake rat, ultimately enabled us to both prove that awake rats have self-sustained firing and confirm the validity of our delta-F method of PIC estimation used in humans. We also by luck chose to study specific rat motoneurons with an identical firing frequency range to that in humans. This helped us to show that in humans, like in rats, PICs caused self-sustained firing in all small low threshold units tested, though this required a robust estimation of the synaptic input activating the PIC, an issue that remains a sticking point. Subsequent rat and mouse intracellular recordings confirmed these in vivo results. However, complications have since emerged that affect self-sustained firing, such as the PIC being strongly inhibited by AHP and other SK currents, and the discovery that the PICs have many components, including those mediated by Ca, TRP, and Na channels. We found that the latter Na PIC plays an unexpectedly critical role in sustained firing of any kind, and when large enough can alone cause slow self-sustained firing in human and rodent motoneurons. The Na PIC also turns out to be very sensitive to even slight mechanical damage, making slicing, dimpling or stretching the cord eliminate self-sustained firing, with somatic penetrations where the Na PIC is located particularly sensitive to loss of firing. In summary, adult motoneurons exhibit

PICs and self-sustained firing, though many complicating factors can make these experimentally hard to detect.

2. **Spinal neurons with a biophysical signature consistent with a gamma motoneuron identity emerge during the third week of postnatal development in mice**

SIMON A. SHARPLES (1,2), GARETH B. MILES (1)

1) University of St Andrews, UK; 2) Cincinnati Children's Hospital, USA

The development of functionally distinct motoneuron subtypes is crucial for the emergence of complex motor behaviors during postnatal development. Gamma motoneurons, a specific subtype of spinal neuron, innervate muscle spindles and play a key role in maintaining spindle sensitivity during muscle contractions. Gamma motoneurons are endowed with a distinct biophysical signature that is characterized by a low recruitment threshold and high firing rate, which is essential for supporting complex movements. However, the timing of its establishment during development and the mechanisms underlying this signature remain unclear. We hypothesized that the maturation of this biophysical signature in gamma motoneurons would coincide with the emergence of complex movement during postnatal development. Our findings reveal a cluster of low-threshold, high-firing gain motoneurons with intrinsic properties consistent with gamma motoneurons, emerging in the third week of postnatal development. Additionally, 92% of putative gamma motoneurons exhibited a sodium pump-mediated ultra-slow afterhyperpolarization, a well-established marker of gamma motoneurons. The low recruitment threshold could be attributed to their lower capacitance, higher input resistance and a more hyperpolarized activation voltage of persistent inward currents. In contrast, the higher firing rates in gamma motoneurons were not linked to differences in persistent inward current amplitude, but instead, were likely due to shorter action potentials and smaller medium afterhyperpolarizations. These results suggest that the development of a functional gamma motoneuron identity may be a key process for the emergence of complex motor behavior during the third week of postnatal development in mice.

3. **Modelling Human Motoneuron Firing Patterns Through Parameter Manipulations of Afterhyperpolarization Time Constants and Voltage Thresholds for Persistent Inward Current Activation**

MELISSA E. FAJARDO (1), MATTHIEU K. CHARDON (1, 2), Y. CURTIS WANG (3), MARTA GARCIA (2, 4), & CHARLES J. HECKMAN (1)

1) Northwestern University, USA; 2) Northwestern-Argonne Institute of Science and Engineering (NAISE), USA; 3) California State University, USA; 4) Argonne National Laboratory, USA

Elucidating the neural mechanisms underlying human motor unit firing patterns may be feasible using computer modelling. More than 20 years' worth of animal data were used to create motoneuronal models that produce realistic firing behavior. However, generalizing animal data tuned models to human behavior is challenging. Therefore, there is a need to re-tune intrinsic parameters of current motoneuron models to human motoneurons along different synaptic input combinations. For this study, a 20 motoneuron pool model (Powers et al., 2017 and Chardon et al., 2023) underwent 1755 combinations of various levels of inhibition schemes, neuromodulation as well as two intrinsic parameter manipulations for either afterhyperpolarization time constants or half-activation voltage threshold to activate persistent inward currents that give rise to nonlinear firing characteristics seen in human motor units (±30% of its default published value). A total of 35,100 individual simulations were performed using Argonne National Laboratory's supercomputer, Bebop. Preliminary results show a broad range of motoneuron pool firing behaviors with few combinations displaying reasonable human-like firing behaviors. Parameters setting afterhyperpolarization time constants and half-activation voltage threshold to activate persistent inward currents induce vast effects on model firing patterns. Our primary goal is to illustrate input-output relationships between model inputs and model firing characteristics as well as establishing model and parameter boundaries for the eventual purpose of reverse engineering human motor units of several muscle types.

4. **Resolving motoneuron subcellular dynamics with optical imaging**

KEITH FENRICH (1), VLADIMIR RANCIC (1)

1) University of Alberta, Canada

Using electrophysiological approaches such as single cell recording has been essential to study the input-output properties of spinal motoneurons (MNs) for many decades, as these approaches offer precision in voltage and current recording and high temporal fidelity. However, these approaches have limitations in capturing the spatiotemporal dynamics of different subcellular compartments such as the soma and throughout the dendritic arbor. One way to overcome this limitation is the use of optogenetic reporters, which, for the purposes of this study, is a set of genetically encoded biosensors that allow researchers to record physiochemical changes in live neurons. Our goal is to develop a reliable optical imaging method using optogenetic reporters to record the physiochemical changes in MN subcellular compartments in response to various inputs (e.g., Ia afferents vs. vestibular), in both neonatal and adult spinal cord. One such optogenetic reporter is a genetically encoded calcium indicator (GECI) called GCaMP8s, whose fluorescent brightness increases in the presence of Ca2+. Spinal motoneurons rely on calcium currents for normal function, especially with regards to calcium persistent inward currents (Ca-PIC) that lead to sustained depolarisations and can amplify synaptic inputs to motoneurons. Though previous work indicates a dendritic origin of Ca-PICs, their spatiotemporal activation dynamics remains largely unexplored. Using Hb9creER/+::Igs7GCaMP8s/+

mice in which GCaMP8s is expressed in Hb9+ motoneurons, we aim to test the hypothesis that Ca-PICs originate in the intermediate dendrites and that inputs from different systems (e.g., Ia vs. vestibular) preferentially activate Ca-PICs in specific dendrites.

5. **Double discharges in response to fast onset of synaptic input**

ROBIN ROHLÉN (1), DAVID BENNETT (2), MONICA GORASSINI (2)

1) Umeå University, Sweden; 2) University of Alberta, Canada

Initial doublet discharges are important for quickly producing force due to the catch-like property of muscles, resulting in a non-linear summation of motor unit twitch forces (Burke et al., 1976). The doublet literature regarding healthy humans has focused primarily on repetitive doublets close to the minimal rhythmic firing rate (Bawa and Calancie, 1983) and ballistic contractions with high force levels (Desmedt and Godaux, 1977). However, whether a fast onset of synaptic input at lower force levels is sufficient to produce initial doublets is unclear. We performed a human experiment comprising a slow ramp followed by superimposed fast sinusoidal movements (5-15% MVC-range) at low force levels using the tibialis anterior while recording multichannel surface electromyography. Initial doublets were produced in some higher-threshold units that were phasically active during the superimposed sinusoidal contractions (mean inter-spike interval 9.9 ms). Motor units that were tonically active throughout the contraction did not exhibit doublets. In parallel intracellular recordings of high-threshold sacral motoneurons in adult mice, initial doublets (8-10 ms) were also produced in response to large rectangular current pulses (>3 nA), which allowed the afterdepolarization (AD) in the first action potential to reach the firing threshold. Once the afterhyperpolarization (AHP) was activated after the doublet spike, the size of subsequent afterdepolarizations was reduced, and no further doublets were produced. The outward SK currents activated during the AHP likely counteract the inward voltage-activated Ca+2 currents mediating the AD to restrict doublet discharges to the first action potential, thereby producing a firing pattern that maximizes force production.

Session IV – Brainstem Motor Control in Health and Disease

1. **Introduction to the cranial nerve motor system**

RALPH FREGOSI (1)

1) University of Arizona, USA

The cranial nerve motor system controls head, neck, and visceral muscles through brainstem nuclei, while the spinal motor system controls trunk and limb muscles through anterior horn neurons. Cranial motor nuclei receive more bilateral cortical input than spinal neurons, making cranial muscles less vulnerable to unilateral upper motor neuron lesions.

Cranial systems specialize in precise bilateral coordination for functions like eye movements, facial expression, and tongue control, whereas spinal systems focus on posture and locomotion. This session will emphasize laryngeal and hypoglossal motoneurons, which control breathing and swallowing muscles, to establish foundational knowledge for our session on brainstem motor control in health and disease.

2. **Increased laryngeal activity post-cervical spinal cord injury: acute to chronic models of cervical hemisection**

TERESA PITTS (1)

1) University of Missouri, USA

Laryngeal dysfunction is a common symptom following cervical spinal cord injury (cSCI) and currently there are no effective pharmacological interventions or behavioral treatments. The larynx is the primary valve controlling entrance into the trachea and lungs via opening and closing of the vocal folds. Although not well studied following cSCI, it is understood that laryngeal deficits during swallow often produce aspiration of food/liquid significantly increasing risk of pneumonia. Our previous terminal electrophysiology experiments using freely breathing sodium pentobarbital anesthetized cats with acute hemisections at C3 showed immediate alterations in laryngeal activity. These alteration persisted for 4 hours (testing duration). In a related study, we tested if alterations seen acutely in laryngeal activity persist chronically, and if the 8-OH-DPAT clinical-correlate buspirone (Buspar) would produce similar therapeutic results. Asynchronous vocal fold movement was seen in all animals, defined by unilateral movement delays, and the extent of opening and closing of the glottis became variable. In a subset of animals, oral buspirone (dose) was given with food. One hour later, repeat endoscopic evaluation revealed full resolution of laryngeal function with synchronized movements during normocapnia and hypercapnia. Most importantly, with termination of buspirone dosing laryngeal dysfunction returned. Results indicate that buspirone is a high-priority target for additional investigation as a therapeutic intervention after cSCI. This work was supported by NIH grants HS113169, HL163008, HD110951 and, the Craig H. Neilsen Foundation Pilot Research Grant 546714.

3. **Motor unit recruitment and rate coding strategies in human genioglossus; insights from flow limited breathing in obstructive sleep apnoea**

*PETER G. BURKE (1,2), *BILLY L. LUU (1), TEODORA NEDELKOSKA (2), DANNY J. ECKERT (3), FIONA L. KNAPMAN (1,2), LAURIANE JUGE (1), LYNNE E. BILSTON (1), JANE E. BUTLER (1)

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*indicates shared first authors

The neural control of genioglossus is complex, with distinct patterns of single motor unit (SMU) activity observed during quiet breathing in wakefulness. This study examined SMU activity from the genioglossus muscle during sleep in 11 participants with OSA (apnoea hypopnoea index AHI>10 events/hour/sleep). Forty-four SMUs were recorded with fine-wire electrodes in the genioglossus and epiglottic pressure was measured with a catheter. Participants slept supine with continuous positive airway pressure (CPAP), where 2 tonic and 16 inspiratory units were active. During transient CPAP drops to induce airflow limitation and corresponding negative epiglottic pressure swings, a further 26 phasic inspiratory SMUs were recruited; 10/26 became tonic inspiratory with increasing respiratory stimuli. All inspiratory units increased their activity with increasing negative pharyngeal pressure (original 16 SMUs by1.0±0.9 Hz/-1 cmH2O, mean±SD, range 0.1-3.5 Hz; recruited 26 SMUs by 1.3±1.4 Hz/-1 cmH2O, range 0.2-5.8 Hz). By contrast, tonic unit firing became phasically inhibited during inspiration, and deactivated by airflow limitation. No new tonic units were recruited. These data indicate that during sleep, inspiratory genioglossus SMUs increase their activity via recruitment and rate coding and that a limited subset of inspiratory hypoglossal motor neurons are recruited reflexly to compensate for airflow limitation and maintain airway patency.

4. **Neonatal Riluzole Treatment Blocks Early Motoneuron Hyperexcitability and Dendritic Alteration, and Delays Neuromotor Deficits and Motoneuron Death in hSOD1G93A Mice**

MATTHEW J. FOGARTY (1), REFIK KANJHAN (2), THOMAS KEECH (2), PETER G. NOAKES (2), MARK C. BELLINGHAM (2)

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In hypoglossal motoneurons (XII MNs) of hSOD1G93A cf. wildtype (WT) mice, we previously demonstrated that intrinsic and extrinsic (excessive glutamatergic synaptic activity) hyperexcitability start by the third postnatal day (P3) and persist up to P21-28, shortly before XII MN loss begins. By contrast, others report MN hypoexcitability at later stages of disease in this and other ALS models. To test whether early hyperexcitability/excitotoxic mechanisms contribute to later MN pathology, we reduced hyperexcitability by treating WT and hSOD1G93A mouse pups with riluzole or vehicle (10mg/kg in DMSO/saline by i.p. injection, once daily) for 14 days (P3-17), subsequently assessing XII MN excitability and morphology by whole-cell patch-clamp recording and biocytin filling at P21-25. Riluzole reduced hSOD1G93A XII MN firing and glutamatergic synaptic activity, and decreased XII dendritic expansion and spine density to WT levels. In an identically treated cohort, we assessed neuromotor behavior, from presymptomatic (~P40) through onset (~P60-70) and mid-disease stages (~P90-100). At P90-100, where hSOD1G93A MN loss is well advanced, we then stereologically quantified lumbar MN numbers in sham- and riluzole -treated WT and hSOD1G93A mice where riluzole treatment had ceased at P17. Again, neonatal riluzole treatment preserved neuromotor

behavior and lumbar MN number to WT levels at P90-100. These results show that a brief window of neonatal riluzole treatment when unequivocal hyperexcitability is present will prevent early MN changes and subsequently delay adult neuromotor deficits and MN loss, consistent with a significant contribution of early hyperexcitability to later disease pathogenesis.

5. **Varied Calcium Sensitivity Underlies Functional Specialization of Hypoglossal Motoneurons Innervating the Superior Longitudinalis and Genioglossus Tongue Muscles**

   MICHAEL FRAZURE (1), EMILY FLANIGAN (1), RALPH FREGOSI (1)

   1) University of Arizona, USA

   Hypoglossal motoneurons (XIIMNs) control tongue movement, which must be precisely coordinated for communication, swallowing, and respiration. We investigated the mechanisms underlying functional differences of XIIMNs innervating the superior longitudinalis (SL) and genioglossus (GG) muscles, which retract and protrude the tongue, respectively. We obtained whole-cell patch-clamp recordings from retrogradely-labeled SL and GG XIIMNs obtained from male and female neonatal rats. SL and GG XIIMNs exhibited distinct firing patterns, and SL XIIMNs were more easily excitable than GG XIIMNs. Next, voltage-clamp studies aimed to determine the ionic mechanisms responsible for functional differences between SL and GG XIIMNs. While whole-cell K+ conductance was similar in both populations, SL XIIMNs exhibited a large, sustained Ca2+-sensitive K+ current that was not observed in GG XIIMNs. Subsequent current-clamp studies evaluated the influence of Ca2+-sensitive K+ currents on firing behavior. Bath application of the Ca2+ channel antagonist CdCl2 produced opposite effects on firing behavior in SL and GG XIIMNs. Ca2+ blockade impaired repetitive firing in SL XIIMNs and increased firing frequency in GG XIIMNs. These data indicate that distinct ionic currents contribute to the functional specialization of XIIMNs that control different muscles. Understanding the neuromotor control mechanisms of specific tongue muscles offers a starting point for the development of novel therapeutics for obstructive sleep apnea, dysphagia and related disorders.

Session V – Computational Tools for Motor Unit Identification and Analysis, and Their Translation in Motor Diseases

1. **Low-dimensional Control of Motor Unit Activity During the Acquisition of a New Motor Skill Task**

   HÉLIO V. CABRAL (1), J. GREIG INGLIS (1), ELMIRA POURREZA (1), MILENA A. DOS SANTOS (1), CATERINA COSENTINO (1), FRANCESCO NEGRO (1)

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Spinal motor neuron activity is thought to be governed by low-dimensional control, resulting in a high degree of correlation among motor unit spike train outputs. Consequently, dimensionality reduction techniques, such as factor analysis (FA), have been applied to motor unit discharge rates to identify dominant patterns of covariation, yielding a reduced set of explanatory components that capture key control features. In this study, we used FA to investigate the low-dimensional motor unit control during short-term learning of a new motor skill. Participants performed 15 isometric dorsiflexion contractions at 10% maximal voluntary contraction, following oscillations of a randomly generated target signal for 30 seconds (i.e., learning task). The two trials with the highest and smallest root-mean-square-error between force and target were selected to represent pre-learning and post-learning trials, respectively. For these two selected trials, motor units were decomposed from high-density surface electromyograms acquired from the tibialis anterior muscle, tracked across trials, and their smoothed discharge rates were decomposed into two low-dimensional components using FA. To assess the association between the two motor unit components and target oscillations, cross-correlation was computed between the detrended signals. In both pre- and post-learning trials, the first motor unit component showed significantly higher correlations with target oscillations than the second component. Notably, learning was associated with an increased correlation between the first component and the target, while the correlation between the second component and the target decreased. These preliminary findings suggest that factorization techniques can provide valuable insights into low-dimensional motor unit control during motor learning.

2. **Resting State Activity of Lumbar Spinal Interneurons: Keeping the Motoneurons Warm through Distributed and Functionally Coupled Subpopulations**

MOHAMED H. MOUSA (1), MARTIN ZABACK (1), MICHEL A. LEMAY (1), CHRISTOPHER K. THOMPSON (1)

1) Temple University, USA

Understanding the intrinsic dynamics of spinal networks is critical to unravel the mechanisms underlying motor control and sensory integration. Traditional views of spinal circuitry emphasize their role in reflex pathways or intrinsic oscillatory circuits; however, even in the absence of overt sensory inputs or motor output, spinal interneurons remain highly active. Characterizing the functional connectivity of resting state activity of spinal interneurons will help us better understand how sensorimotor pathways interact with this basal level of activity. To address this, we recorded extracellular spiking activity from the lumbar spinal cord of five spinal-intact decerebrate cats. Two 64-channel microelectrode arrays were used to target the rostral (L3-4) and caudal (L5-7) lumbar spinal cord regions, capturing two to three minutes of tonic spinal interneuron activity at two distinct depths,

corresponding to laminae VI-VII or laminae II–V. From this data we identified the discharge of ~100 spinal interneurons per trial. The average discharge rate ranged from 4 to 35 Hz, with a wide range of CoVISI. The functional connectivity was quantified through a number of analyses including, principal component analysis, factor analysis, agglomerative hierarchical clustering, and general linear modeling. Lastly, we mapped the anatomical location of these functionally connected networks. Our findings reveal multiple distributed subpopulations of functionally coupled spinal interneurons, underscoring the complex organization of spinal circuits even at rest. These results not only enhance our understanding of spinal motor control and sensory processing but also provide a critical baseline for comparisons in injury or disease states.

3. **Motor Unit Reflex Probability Distribution to Determine Spinal Motoneuron Adaptation**

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The variability in reflex amplitude among a heterogeneous population of motor units (MUs) is influenced by a nonlinear combination of factors such as MU size, discharge rate and membrane noise. This uncertainty limits the use of MU reflexes as reliable measures of excitability. We hypothesized that this variability could be mitigated by analyzing the probability distribution of a large MU sample. To test this, we combined high-density surface and intramuscular EMG decomposition to increase MU sampling during H-reflex measurements. We recorded MU reflex data from the tibialis anterior and triceps surae during isometric contractions at 10% and 20% of MVC. Additionally, we simulated a pool of 200 motoneurons with a predefined soma size distribution. Reflex amplitude was determined from the cumulative sum of the PSTH and PSF for both experimental and simulated data, and probability density functions were compared across conditions. Results showed that the shape parameters of the cumulative distribution function (CDF) of reflex amplitudes measured from PSF were sensitive to differences in MU pool characteristics (e.g., tibialis anterior vs. soleus). Simulations revealed a significant correlation ($\rho > 0.5$) between CDF shape and predefined MU size distribution at higher contraction levels. These findings suggest that a probabilistic model based on a large MU population can reliably estimate spinal cord adaptation. This approach offers a valuable tool for studying neuromuscular adaptations and pathological changes.

4. **Sources of Common Inputs to Triceps Surae Motor Nuclei: Insights from a Multiscale Neuromuscular Model**

ELIANE CREPALDI RODRIGUES (1), LEONARDO ABDALA ELIAS (1)

1) University of Campinas, Brazil

Common motor unit (MU) oscillations significantly influence force variability in isometric contractions due to correlated inputs shared by many motoneurons in a motor nucleus. However, the sources of these correlated inputs remain unknown. We conducted computer simulations using a multiscale neuromuscular model to evaluate the roles of spinal and supraspinal pathways in producing common MU oscillations across different frequency bands. The base model encompassed three motor nuclei innervating the triceps surae muscles, and stochastic point processes represented the descending commands. Each motor nucleus had a population of two-compartment spiking motoneuron models. We evaluated three model structures: 1) the open-loop system (base model), 2) additional proprioceptive feedback from Ia muscle spindle afferents to spinal motoneurons, and 3) the complete closed-loop model with proprioceptive spinal feedback and delayed feedback of muscle force modulating descending commands (mimicking a visuomotor task). Common modulation of MU activity was assessed using coherence analysis between compound spike trains. Simulation results showed that the open-loop model could produce common MU oscillations up to 50Hz, depending on the regularity and connectivity of descending commands. Including proprioceptive feedback from Ia afferents increased MU coherence across all frequency bands. The dynamic fusimotor drive had a larger influence on delta band (<5Hz) oscillations, while the force feedback loop did not significantly alter delta band oscillations but increased alpha band (5-15Hz) oscillations with higher feedback gains. In conclusion, common MU oscillations are influenced by the properties of descending commands and spinal and supraspinal feedback loops, which may vary with task demands.

5. **Heterogeneity of Pathophysiological Voluntary Motor Commands in People with Multiple Sclerosis**

LAURA MCPHERSON (1), KEITH LOHSE (1), SKYLER SIMON (1), DANIEL FREE (1), DREW BEAUCHAMP (2), ROBERT NAISMITH (1), ANNE CROSS (1)

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Multiple sclerosis (MS) is a progressive inflammatory neurodegenerative disease that degrades communication between the brain and spinal alpha-motoneurons. These voluntary motor commands contain excitatory, inhibitory, and neuromodulatory components that must be appropriately balanced for skilled motor control. Unlike other populations, in MS we have no knowledge about how voluntary motor commands are disrupted or how they relate to motor deficits. In part, this is because MS clinical symptoms vary so much across patients, making systematic research of neurophysiological correlates of motor deficits difficult. Here, we characterized voluntary motor commands in MS, related them to clinical symptoms, and determined how they changed over time. We

enrolled a heterogeneous sample of 57 participants with MS with a range of sensorimotor symptoms and disability and 38 age-/sex-matched controls. We characterized the voluntary motor command from motor unit population discharge using a reverse engineering paradigm. We recorded motor unit discharge from tibialis anterior and soleus during isometric dorsiflexion/plantarflexion triangle contractions. We calculated 16 variables from our reverse engineering paradigm based on temporal and geometric patterns of the motor unit discharge relative to the contraction profile. Participants in the MS group varied from controls in different ways, particularly for variables reflecting neuromodulatory and inhibitory inputs to the motoneuron pool. We will present these findings, their associations with motor function, and our preliminary findings from longitudinal measurements. Characterizing voluntary motor commands at the level of the spinal motoneuron provides rich, multidimensional data that shows promise for explicating neural mechanisms of motor heterogeneity in MS.

Session VI – Acute Intermittent Hypoxia and Other Therapeutics

1. **Adenosinergic Regulation of Intermittent Hypoxia-Induced Phrenic Motor Plasticity**

ALEXANDRIA B. MARCIANTE (1), GORDON S. MITCHELL (1)

1) University of Florida, USA

Acute intermittent hypoxia (AIH) elicits phrenic long-term facilitation (pLTF), a commonly studied form of respiratory motor plasticity. AIH-induced pLTF is the most commonly studied form of respiratory motor plasticity and presents as a persistent increase in phrenic nerve activity after AIH. This form of plasticity is evoked when hypoxia increases the activity of brainstem raphe neurons, releasing the neurochemical serotonin within the phrenic motor nucleus of the cervical spinal cord. At the same time, the slower development of spinal tissue hypoxia during hypoxic episodes triggers adenosine formation near phrenic motor neurons, constraining serotonin-induced plasticity. Years of study of these fundamental mechanisms has inspired clinical trials of moderate AIH as a treatment to elicit plasticity, and improve breathing and other non-respiratory motor function in people living with spinal cord injury, ALS, MS and stroke. In all completed trials to date, functional improvements are pronounced in ~60% of subjects, but nearly 40% are “low responders”. Our central hypothesis is that factors shifting the spinal serotonin/adenosine balance regulate the extent of functional recovery; specifically that spinal levels of hypoxia-evoked and basal adenosine determine the magnitude and mechanism of respiratory motor plasticity. We performed a metanalysis of pLTF and spinal adenosine data collected from male and female young (3-6 month), middle-aged (12-15 month), and aged (22-24 month) Sprague-Dawley rats focusing on factors that increase spinal adenosine levels including: 1) aging; 2) systemic and neuroinflammation; 3) time-of-day in which AIH is delivered; 4) details of the AIH protocol (duration of hypoxic

episodes); 5) severity of hypoxia within episodes; and 6) APOE4 alleles. We found a significant, sigmoidal relationship between spinal adenosine levels and pLTF magnitude in AIH protocols consisting of either shorter (15x1 min; p=0.020) or longer (3x5 min; p=0.009) hypoxic episodes. We found a sigmoidal relationship between basal spinal adenosine levels and 15x1 AIH-induced pLTF is more robust than for 3x5 AIH-induced pLTF, which is likely due to minimal accumulation of hypoxia-evoked spinal adenosine during shorter (versus longer) hypoxic episodes. Blocking spinal adenosine 2A receptors prior to either AIH protocol, significantly elevates pLTF across all groups regardless of spinal adenosine levels. Understanding how specific factors affect basal and hypoxia-evoked adenosine and its regulation of AIH-induced phrenic motor plasticity has translated to unanesthetized rodents in preliminary studies and may help identify individuals most/least likely to respond to treatment, revealing new targets for precision interventions of AIH.

2. **Mild Intermittent Hypoxia, Respiratory Plasticity and Sleep Apnea**

JASON H. MATEIKA (1,2)

1) Wayne State University, USA; 2) John D. Dingell VA Medical Center, USA

During this invited talk, evidence to demonstrate that exposure to mild intermittent hypoxia initiates respiratory plasticity (i.e. progressive augmentation of ventilation and long-term facilitation of ventilation and upper airway muscle activity) in humans will be presented. In addition, the impact that (i) mild intermittent hypoxia protocol parameters (carbon dioxide level, hypoxic episode number, duration and intensity) (ii) time of day (iii) carotid chemoreflex sensitivity and (iv) obstructive sleep apnea severity has on the magnitude of respiratory plasticity will be addressed. Thereafter, the possibility that mild intermittent hypoxia may serve as an adjunctive therapeutic modality to treat sleep apnea and its co-morbidities will be addressed. Specifically, evidence that repeated daily exposure to mild intermittent hypoxia enhances the magnitude of long-term facilitation of upper airway muscle activity in individuals with obstructive sleep apnea will be presented. Data will also be presented to show that initiating this form of plasticity may lead to reductions in the severity of sleep apnea, or reductions in the therapeutic positive airway pressure used to treat sleep apnea coupled to improved treatment adherence. Lastly, the impact of reducing apnea severity or improving treatment adherence on one negative outcome measure (i.e. blood pressure) linked to obstructive sleep apnea will be briefly addressed.

3. **Intermittent Hypoxia Induced Plasticity for Motor Recovery and Neurorehabilitation**

MILAP SANDHU (1), ARAVIND NEHRUJEE (1), GAIL FORREST (2), MONICA PEREZ (1), STEVE KIRSHBLUM (2), GUOGEN SHAN (3), WILLIAM Z RYMER (1)

1) Shirley Ryan AbilityLab and Northwestern University, USA; 2) Kessler Foundation and Rutgers University, USA; 3) University of Florida, USA

Acute intermittent hypoxia (AIH) involves brief, repeated exposures to reduced oxygen levels and has been shown to induce spinal plasticity and enhance motor function in the animal models. Clinical research has explored the potential of intermittent hypoxia protocols in individuals with neurological deficits, demonstrating encouraging results across multiple conditions. However, important questions remain regarding its clinical utility, optimal implementation, and mechanisms of action. This session will discuss a recent multi-site clinical trial examining the use of daily AIH combined with task-specific training to improve upper-extremity function in individuals with spinal cord injury. This will be followed by a discussion of parallel studies in persons with multiple sclerosis, where preliminary data shows improvement in motor function but also highlights variability in individual responses. Finally, ongoing efforts to identify physiological predictors of AIH responsiveness will be reviewed. This work aims to identify biomarkers that may distinguish responders from non-responders. A biomarker-driven approach will enable us to personalize protocols, optimize dosing strategies, and enhance clinical efficacy of AIH.

4. **The Impact of Anodal and Cathodal TsDCS on Passive Membrane and Firing Properties of Spinal Motoneurons in SOD1 G93A Mice**

BARTOSZ WASICKI (1), PIOTR ZAWISTOWSKI (1), MARCIN BĄCZYK (1)

1) Poznan University of Physical Education, Poland

In Amyotrophic Lateral Sclerosis (ALS), the electrophysiological profile of spinal motoneurons (MNs) undergoes significant alterations, marking the hallmark for their degeneration. Transcutaneous spinal direct current stimulation (tsDCS) is a neuromodulation method that evokes long-term neuroplasticity in MNs. We have recently demonstrated that chronic tsDCS alters spinal MN synaptic excitation levels in a polarity-dependent manner. Here, we expand our investigations to determine if spinal MN firing properties are also affected by chronic tsDCS. Presympthomatic SOD1 G93A mice were exposed to 60 μA Anodal, Cathodal, or Sham tsDCS at p35-p40, and in vivo intracellular recordings of MNs were performed at p45-p50. A significant reduction in the cells' plateau input resistance and firing gain was observed following anodal tsDCS. Conversely, cathodal tsDCS did not change MNs input resistance but increased its membrane time constant and reduced the rheobase and Ion current. Surprisingly, both anodal and cathodal tsDCS increased the MNs SAG ratio. The polarization-dependent changes in the electrophysiological profile of MNs significantly influenced their population behavior. After anodal polarization, a notably larger proportion of MNs were able to reach a primary range of firing in response to depolarizing ramps of current. In contrast, following cathodal polarization, more cells could generate action potentials during ramp current injection but without reaching the primary firing range. These results raise the issue of quality versus quantity in MNs management in ALS. This research was supported by Polish National Science Centre grants 2019/35/B/NZ4/02058 and 2022/04/Y/NZ4/00117.

5. **Added transcutaneous spinal stimulation to locomotor training does not improve walking in spinal cord injury: an international double-blinded randomised sham-controlled trial**

ELIZABETH A. BYE (1,2), CLAIRE L. BOSWELL-RUYS (1,2,3), MARTIN E. HÉROUX (1,2), EUAN J. MCCAUGHEY (1,4), ZOË J DJAJADIKARTA (1), BONSAN B LEE (1,2,3), MONICA A PEREZ (6,7), GABRIELLE A MENDOZA (6,7), MARIEL PURCELL (4), CLAIRE LINCOLN (4), JULIAN TAYLOR (8,9,10), MARTA RÍOS-LEÓN (8,10), GAVIN WILLIAMS (11,12), MATT KUNDEVSKI (11,13), JOANNA DIONG (1,5), PETER HUMBURG (1,2), JANE E. BUTLER (1,2), SIMON C. GANDEVIA (1,2,3), EWALK COLLABORATION GROUP†.

E. BYE and C. BOSWELL-RUYS share first authorship.
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ABSTRACT TEXT WILL BE MADE AVAILABLE DURING THE MEETING

Session VII – Computational Approaches to Study Motoneurons

1. **A Population Model of Motor Units – What's Missing?**

ANDREW J FUGLEVAND (1)

1) University of Arizona, USA

This talk will provide an overview of a widely used model that predicts motor unit activity and that can be used to simulate muscle force and associated electromyographic signals. As it stands, this model has three main components: 1) a simple algorithm to predict firing rates in a population of motor neurons under different levels of synaptic drive, 2) a framework that simulates the forces produced by individual motor units having different mechanical properties and in response to varying rates of spiking input, and 3) a method to simulate the distinct electrical signals generated by active motor units as detected by surface or intramuscular electrodes. Some examples of applications of the model to address a variety of problems will be presented. Importantly, limitations of the model (and

some attempts to address them) will be discussed. Furthermore, and most crucially, aspects of motor unit physiology for which experimental insight is weak or missing - and that should be included in such a model - will be outlined.

2. **Estimating mechanisms of gain control from motor unit discharge patterns across contraction intensities**

JAKOB SKARABOT (1), JAMES ANDREW BEAUCHAMP (2), GREGORY PEARCEY (3)

1) Loughborough University, UK; 2) Carnegie Mellon University, USA; 3) Memorial University of Newfoundland, Canada

The transduction of motor commands by motor units (MUs) into mechanical actions of muscle fibres is a non-linear transfer function due to combinations of ionotropic (excitatory/inhibitory) and metabotropic (neuromodulatory) inputs. However, how motor commands are modified to scale motor output (i.e., gain control) is speculative despite its critical role in the neural control of movement. To address this question, we investigated the relative contribution of neuromodulation and the pattern of inhibition to changes in MU discharge patterns with contraction force. In human experiments, we demonstrated that discharge rate hysteresis increases and MU discharge patterns become more linear with increasing force output. This behaviour was consistent across several muscles (tibialis anterior, vastus lateralis and medialis), and was apparent regardless of contraction duration and rate of force increase. To appreciate the physiological mechanisms gleaned from experimental human data, we used biophysical models of spinal motoneurons to replicate the experimentally observed MU discharge patterns in silico. We demonstrated a sharply restricted solution space, where the change in MU discharge patterns as a function of contraction force can only be recreated by increased neuromodulation and a shift in inhibitory commands to a pattern that is more reciprocal to excitation (i.e. push-pull excitation-inhibition synaptic control). This unique orchestration of motor commands to support increases in contraction force might explain the results of our subsequent experiments whereby the differences in MU discharge patterns between young and ageing adults, and between trained and untrained individuals appear dependent on contraction force.

3. **In Silico Framework for Exploring the Input-Output Dynamics of Spinal Motoneurons**

PRAKARSH YADAV (1), MELISSA FAJARDO (2), MATTHIEU CHARDON (2), CJ HECKMAN (2), DOUG WEBER (1), JAMES A BEAUCHAMP (1)

1) Carnegie Mellon University, USA; 2) Northwestern University, USA

Estimating synaptic inputs to spinal motoneurons and the intrinsic properties shaping their input-output relationships is crucial for understanding motor control and informing

pathological states. However, the vast parameter space governing motoneuron outputs is intractable for naive methods of optimization which require exploration of large parts of the parameter space. In this work, we present an iterative approach for in silico reverse engineering experimental motoneuron firing patterns into putative inputs to spinal motoneurons. Our method updates excitatory, neuromodulatory, and inhibitory inputs to motoneurons by independently minimizing the error between experimental and simulated motoneuron firing characteristics (e.g., CST, ΔF, and geometrics estimates). This parameter exploration scheme results in simulated motoneuron firings which closely reproduce the experimental data with single motoneuron accuracy, without the need to explore and simulate the entire input parameter space. The presented approach makes the reverse engineering of motoneuron input-output relationships computationally efficient and more amenable to the introduction of new motoneuron properties and parameters. We validate the presented approach for various ground truth datasets, including simulated motoneurons and human motor unit behavior with pharmacological perturbations. We were able to reproduce motoneuron firing characteristics with low error and with orders of magnitude fewer simulations necessary than a naive search over the parameter space. We will introduce this framework and outline future plans to incorporate “self-learning” capabilities that leverage large datasets, enabling adaptive refinement through continual use.

4. **The recruitment portrait: A time-series estimate of motor unit recruitment**

MARTIN ZABACK (1), MOHAMED H. MOUSA (1), CHRISTOPHER K. THOMPSON (1)

1) Temple University, USA

The force produced by a muscle is regulated by the number of active motor units (MUs) and their firing rate. While the influence of rate coding on muscle force has been extensively investigated, the influence of recruitment has received less attention. We present a time-series estimate of the number of active MUs to provide insight into how changes in MU recruitment influence the control of muscle force. Four young adults completed a series of single- and dual-legged standing balance trials. High-density surface EMG was recorded from the soleus (SOL) and medial gastrocnemius (MG) and decomposed into the spike times of individual MUs. Binary on-off values denoting periods when each MU was repetitively firing (interspike intervals (ISI) < 400 ms) were summed within each muscle, creating a time-series of the number of active MUs (recruitment portrait). Variability in MU recruitment, calculated as the path length of each recruitment portrait, was highly correlated with global estimates of MU intermittency, but less so with firing rate variability. Recruitment variability differed between muscles dependent on task, with SOL demonstrating less variable recruitment than MG during dual-, but not single-legged standing. Computer simulations of motor pool activation and resulting EMG were performed to validate the application of the recruitment portrait. These simulations revealed the recruitment portrait provides a better-optimized fit to the synaptic drive than the composite spike train. Since changes in motor unit firing rate and recruitment are often

incongruent, the recruitment portrait offers insight into a fundamental, yet underappreciated process underlying motor control.

5. **A Simple but Effective Model of Axonal Excitability. Modelling the Node of Ranvier and its Adjacent Internode**

JAMES (TIM) HOWELLS (1,2), HUGH BOSTOCK (1,3)

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The Bostock model of a human motor axon is a structurally simple model consisting of two compartments, a node and an internode, which are connected via the 'Barrett-Barrett' pathways through and under the myelin sheath.
This model is well suited to modelling axonal excitability studies, which are usually performed by stimulating superficial nerve with electrodes that are several times larger than the internodal distance (ie approximately a space-clamped setup).
In contrast, the representation of ion channels is more detailed with representation of transient and persistent Na+ channels at the node, fast and slow K+ channels at the node and internode, ohmic 'leak' channels and the Na+/K+ pump at both the node and internode.

Session VIII – Motor Impairments 1

1. **Disordered Postnatal Maturation of Hindlimb Motor Units in a Mouse Model of DYT-TOR1A**

KIM DHILLON (1), FILIPE NASCIMENTO (1), M. GÖRKEM ÖZYURT (1), LEIRE GARRIDO PEREZ (1), MARCO BEATO (1), AMANDA M. POCRATSKY (1), ROBERT M. BROWNSTONE (1)

1) University College London, UK

DYT-TOR1A, the most common genetic form of dystonia, is defined by early onset disorganised movement and abnormal posturing. We previously demonstrated that a biallelic conditional knockout (cKO) of Tor1a restricted to the mouse spinal cord produces a model recapitulating the severe form of the human condition, exhibiting a dystonic phenotype that progresses in severity throughout postnatal development. Previously, we reported physiological changes in cKO lumbar motoneurons: they appeared smaller, with reduced capacitance, increased input resistance, and diminished afferent-evoked excitatory postsynaptic potentials. Furthering this analysis within the context of the

postnatal phenotype onset, we now report changes in caudal lumbar motoneurons from postnatal day 1 (P1)-P21 in cKO mice. We have found that L4-L5 motoneurons are anatomically smaller and appear to decrease in number as postnatal age increases. We also observe an overall decrease in sensory afferent and cholinergic input to motoneurons, indicating altered circuit connectivity. Motoneuron intrinsic properties are also altered: targeted patch-clamp recordings of P1-P21 labelled TA motoneurons show reduced whole-cell capacitance and input conductance, lower rheobase, and a shift towards predominantly immediate-firing phenotypes, potentially reflecting a shift towards slow-type motoneurons. In studying motoneuron postsynaptic partners, we found that P1, P7, and P18 distal hindlimb muscles had decreased cross-sectional area, as well as a progressive shift to slower fibre types as identified by embryonic and mature myosin heavy chain expression. Together, these data reveal changes in both motoneurons and their muscles, raising the question of whether disordered postnatal motor unit maturation contributes to the dystonic phenotype.

2. **Primary afferent depolarization and hyperreflexia in cerebral palsy**

E. MENA AVILA (1), E. J. REEDICH (1), L. T. GENRY (1), M. A. GORASSINI (2), D. J. BENNETT (2), K. A. QUINLAN (1)

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Cerebral palsy (CP) is the most prevalent physical disability in children, arising from perinatal insults that cause permanent motor deficits. Damage to the cortex and corticospinal tracts (CSTs) is believed to disinhibit spinal motoneurons, leading to hyperreflexia, though the exact pathways are unknown. The rabbit model of CP based on prenatal hypoxia-ischemia (HI) replicates key features of spasticity and dystonia, including hypertonia and hyperreflexia (namely reflex irradiation). In the spinal cord, primary afferent depolarization (PAD) modulates sensory input, mediated by GAD2+ GABAergic interneurons (GABApre). CST projections normally influence these neurons, but a weakened CST in CP may allow aberrant nociceptive drive, leading to excessive PAD and increased Ia afferent conduction and reflex irradiation. To investigate spinal circuit dysfunction, HI was induced in pregnant New Zealand White rabbits via transient uterine artery occlusion. Spinal cord physiology was examined in neonatal rabbits (P1–5) using midsagittal hemisected preparations. Reflex activity was assessed by stimulating ventral and dorsal roots while recording monosynaptic reflexes (MSR) and dorsal root potentials (DRP). Preliminary findings reveal enhanced low-threshold DRP spread and increased MSR irradiation in HI rabbits, indicating hyperexcitable spinal circuits. These results suggest that CP-related motor dysfunction extends beyond cortical injury, implicating maladaptive plasticity within spinal sensory-motor networks. This study underscores the need for in-depth pharmacological and electrophysiological analysis to unravel CP pathophysiology in the spinal cord. Understanding these spinal alterations could open new therapeutic avenues for mitigating CP-associated motor impairments at the level of spinal circuits, not just the brain.

3. **Hyperactive Homeostatic Plasticity Disrupts Motoneuron Excitability in ALS**

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Motoneuron excitability is altered in the SOD1G93A (mSOD1) mouse model of ALS, with both increases and decreases observed throughout disease progression. We hypothesized that motoneuron homeostatic plasticity is dysregulated in ALS, leading to an increased 'gain' of compensatory mechanisms. This would cause overcompensation and lead to oscillatory instability during early disease stages, potentially contributing to motoneuron ensuing degeneration. To evaluate the homeostatic response of presymptomatic mSOD1 motoneurons (P30–P40), we administered a 10-day treatment of riluzole via drinking water. Riluzole, known to suppress excitability, was used to evoke a compensatory increase in motoneuron excitability. Following treatment, the sacrocaudal spinal cord was extracted and maintained ex vivo for excitability assessment. We recorded individual motoneurons intracellularly using sharp glass electrodes and subjected them to various depolarizing current patterns to measure the gain of their frequency-current (F-I) relationship. Data from mSOD1 mice were compared to age-matched Wild-type (WT) controls undergoing the same treatment protocol. Our data demonstrated that chronic riluzole treatment at clinically relevant doses induced a significant increase in the F-I gain of mSOD1 motoneurons, whereas WT motoneurons showed no change. Notably, there was no difference in the response of WT and mSOD1 motoneurons when riluzole was reintroduced in vitro. These findings provide direct evidence of hyperactive homeostatic plasticity in motoneurons of this ALS model. Moreover, our results challenge the prevailing notion that riluzole's therapeutic benefits stem solely from excitability suppression. Instead, they suggest an alternative mechanism of action with broader implications for ALS pathophysiology.

4. **Synaptic Excitation-transcription Uncoupling in Motoneurons May Play an Essential Role in Amyotrophic Lateral Sclerosis (ALS) Pathophysiology**

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In a previous work, we provided anatomical and functional evidence indicating that Ia

synapses to spinal motoneurons are impaired at a presymptomatic age in mSOD1 mice (1). In this study, we explored the consequences of this impairment on the synaptic excitation-transcription coupling. Whereas Ia synaptic density and structure of presynaptic boutons are preserved, the post-synaptic side displays multiple alterations: expressions of AMPA and NMDA subunit receptors, metabotropic mGluR5 receptor as well as anchoring proteins are substantially reduced. These anatomical alterations translate into physiological impairment since Ia EPSPs are also reduced in mSOD1 motoneurons compared with WT. We then ask whether these dysfunctions have an impact on the activity-dependent signaling and transcription. To explore this issue, we set up an original protocol combining physiological activation of the Ia synapse and immunostaining assays of phosphorylated transcription factors. We discovered that, in sharp contrast with WT mice, the Ia synaptic excitation is uncoupled from transcription in mSOD1 motoneurons. We further show that enhancing Ia EPSP with Ampakine does not restore the coupling. However, and most interestingly, when the PKA activity is enhanced using Rolipram, a selective inhibitor of cAMP-specific phosphodiesterase-4, the coupling between Ia synaptic activity and transcription is restored. Further experiments are ongoing to investigate the consequences of restoring the synaptic excitation-transcription coupling on ALS cellular disease markers. Supported by: Polish National Science Centre 2019/35/B/NZ4/02058 and 2022/04/Y/NZ4/00117; Join Deutsche Forschungsgemeinschaft/Agence Nationale de la Recherche “SynaptALS” project (ANR-20-CE92-0029-01, DFG 545426613 / DFG 446067541). (1) Bączyk, Ouali Alami and al., J. Exp. Med., 2020, 3;217(8):e20191734.

5. **NeuroMap 3D: Precision Mapping of Motoneuron Architecture and Protein Expression**

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Structural analysis of motoneuron somas and their associated proteins via immunohistochemistry (IHC) remains tedious and subjective, requiring costly software or adapted 2D manual methods that lack reproducibility and analytical rigor. Yet, neurodegenerative disease and aging research demands precise structural comparisons to elucidate mechanisms driving neuronal degeneration. To address this need, we developed a novel algorithm that automates repetitive and subjective IHC analysis tasks, enabling thorough, objective, blinded, order-agnostic, and reproducible 3D batch analysis. With no manual tracing, the algorithm produces 3D cartesian reconstructions of motoneuron somas from 60x IHC images of mouse lumbar spinal tissue. From these reconstructions, it measures soma volumes and 3D surface area and efficiently quantitates net protein expression and macro-cluster size of somatic proteins. We validated this new algorithm by comparing its measurements against manual measurements and across multiple algorithm users to confirm accuracy and reproducibility of results. Our sensitivity analysis characterizes the impact of analysis settings on the algorithm measurements, ensuring reliability of results. This novel, customizable tool

enables efficient and high-fidelity 3D motoneuron analysis, replacing tedious, qualitative, cell-by-cell manual tuning with automatic threshold adaptation and quantified batch settings. For the first time, we attain reproducible results with quantifiable accuracy, exhaustive sampling, and a high degree of objectivity. In summary, our revolutionary soma and protein analyzer streamlines immunohistochemistry analysis with improved workflow and never-before-seen rigor and reproducibility of results. Automated features include setting tracking, setting sensitivity analysis, iterative batch processing, cell-by-cell threshold tuning, and statistics-software-friendly excel table outputs. Further customizations are available for other proteins/neurons.

Session IX – ALS 2

1. **Cellular vulnerability and prion-like spread in amyotrophic lateral sclerosis: Update**

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Amyotrophic lateral sclerosis (ALS) has two alternate explanations—a network disease of upper and lower motor neurons (UMNs and LMNs) or a focal disease that begins as a point source and spreads through the UMN and LMN network. The idea that ALS begins as a point source and spreads neuroanatomically provides a framework to imagine many aspects of disease at many levels. The onset is focal muscle deficit at virtually any somatic region (disease epicenter). The neurons innervating this somatic region are upper and lower motor neurons (disease hypocenters) which are networked together and organized somatotopically at two levels of the CNS. Over time, neurodegeneration propagates at highly variable progression rates (disease kinetics). Deconstructing spatial and temporal aspects of disease allows individualization of phenotypes, progression, prognostication, measurement, and biomarker correlations. Respiratory failure involves motor neurons arising in medulla, cervical, and thoracic spinal cord innervating accessory, diaphragmatic, intercostal, and abdominal muscles, (and poorly understood upper motor neuron projections) and causes fatal hypoventilation, ironically arresting further neurodegeneration in other CNS regions at a point in time. Because neurodegeneration propagates neuroanatomically, neuronal death at the cellular level is desynchronized in different neurons side-by-side and in different CNS regions, and neuropathology thus may provide a unique window into dynamic aspects of degeneration. Propagation likely has multiple mechanisms (not unlike cancer): contiguous spread, transsynaptic, axonal, retrograde, antegrade, or through CSF pathways. Cell-to-cell propagation is underscored by the signature protein TDP-43, which has prion-like and liquid-liquid phase separation properties. Ironically, TDP-43 neuropathology may itself not be a reliable biomarker of disease “stage” since after motor neurons degenerate, aggregated TDP-43 protein may be removed by microglia and thus appear and then disappear in anatomic regions. In

stating ALS is a disease of upper and lower motor neurons, our very language mixes clinical, anatomical, and pathological terms, noting that the diagnosis “ALS” is in fact neuropathological not clinical.

2. **MMI as a prodromal syndrome in ALS: frequency and relevance to phenoconversion**

MICHAEL BENATAR (1), JOANNE WUU (1)

1) University of Miami, USA

Amyotrophic lateral sclerosis, when viewed as a biological entity rather than a clinical syndrome, evolves along a continuum, with the initial clinically silent phase eventually evolving into clinically manifest disease. Since motor neuron degeneration is incremental and cumulative over time, it stands to reason that the clinical syndrome of ALS is probably preceded by a prodromal state characterized by minor motor abnormalities that are initially insufficient to permit a diagnosis of ALS. This prodromal period, however, is usually missed, given the invariably long delays between symptom onset and diagnostic evaluation. Pre-Symptomatic Familial ALS (Pre-fALS), a longitudinal natural history and biomarker study of unaffected carriers of any ALS-associated pathogenic variant, offers a unique opportunity to observe what is typically unseen. Indeed, through Pre-fALS we have observed evidence of a prodromal state that we have termed mild motor impairment (MMI) in all genetic forms of disease studied to date. We have also developed and proposed formal research criteria for MMI and shown that MMI is associated with a higher short-term of ALS phenoconversion.

3. **Spinal microcircuit adaptations in early-stage ALS: bridging insights from mice to humans**

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In neurological disease, early-stage circuit adaption ensures relatively normal behaviour. Such an example is amyotrophic lateral sclerosis (ALS), in which motor weakness manifests only when more than 50% of the motoneurons have died. Local circuits in the spinal cord are fundamental for motor behaviour, and it has long been hypothesized that compensatory alterations in motoneurons and pre-motor circuits may help to sustain physiological motor output in the face of substantial neuronal loss. In ALS mice carrying humanized mutated genes (SOD1G93A and FUSΔ14/Δ14) we have found that alterations in motor and sensory spinal microcircuits are some of the earliest changes occurring long before onset of motor weakness. Interestingly, some of these alterations are not monotonic but instead multiphasic, indicating that microcircuits exhibit remarkable homeostatic flexibility to ensure behaviour is maintained during progressive

neurodegeneration. Given the shared functional homology between mouse and human spinal cord circuits, we sought to investigate whether similar microcircuit alterations occur in the early stages of ALS in humans. We have refined high-density surface electromyography (HDsEMG) in the study of inhibitory spinal microcircuits in humans, enabling the sampling of multiple motor units per subject, and the assessment of subject-specific microcircuit dynamics. We are currently employing HDsEMG to map the temporal evolution of inhibitory in ALS, from clinically silent to late stage periods. With this integrative mouse-human approach, we aim to understand microcircuit resilience and breakdown in ALS, and leverage insights in the improvement of diagnosis, defining early therapeutic windows and establishing novel neurophysiological biomarkers.

4. **Electrophysiological Biomarkers of ALS that may Precede or Follow Motoneuron Degeneration Before the Occurrence of Muscle Weakness.**

MONICA GORASSINI (1), ALEXANDRA YACYSHYN (1), JENNIFER DUCHCHERER (1), KELVIN JONES (1), SIYU DU (1), BABAK AFSHARIPOUR (1), WENDY JOHNSTON (1), HELIO DA VEIGA CABRAL (2), FRANCESCO NEGRO (2)

1) University of Alberta, Canada; 2) University of Brescia, Italy

Tibialis anterior (TA) motoneurons of a SOD1 participant (minimal upper limb symptoms with normal CSF and blood neurofilament light chain levels), and participants diagnosed with sporadic ALS but with normal TA muscle strength, exhibit involuntary low frequency firing (5-10 Hz), with low variability (SD > 0.5 Hz) that is likely mediated by the repetitive activation of an enhanced persistent inward sodium current (NaPIC). In the SOD1 mouse model, NaPICs are elevated within the first week after birth, long before any spinal motoneuron degeneration occurs. Thus, enhanced NaPIC-mediated firing in ALS patients may represent altered spinal motoneuron function before any degeneration occurs. In addition, sprouting of motor axons from intact (likely small) motoneurons to dennervated muscle fibres occurs after the degeneration of large motoneurons but before any clinical weakness of the muscle is apparent. Thus, a spatially altered or expanded motor endplate innervation zone that develops before any clinical motor weakness manifests may provide a presymptomatic biomarker of spinal (lower) motoneuron degeneration. I will present initial results mapping the motor axon innervation zone in the biceps muscle from high density surface EMG in ALS participants with and without elbow flexion weakness. Finally, degeneration of large corticomotoneuron axons in ALS may occur before muscle weakness manifests due to compensation from other descending tracts. I will show early results using beta band coherence of cumulative motor unit spike trains that activation of spinal motoneurons by corticomotoneuronal axons might also be reduced before TA muscle weakness develops.

5. **Evaluating the Utility of Nerve Excitability and CMAP Scan Biomarkers in ALS: Diagnostic, Prognostic, and Predictive Insights**

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Biomarkers in amyotrophic lateral sclerosis (ALS) require careful validation for specific clinical purposes: diagnosis, prognosis, monitoring progression, and predicting or confirming responses to therapy. Recent consensus guidelines (2020) validated nerve excitability testing (NET; TROND protocol) for quantifying lower motor neuron pathology. Our previous systematic review (2023) identified four excitability indices from NET as potential diagnostic biomarkers in ALS patients without overt muscle atrophy. A subsequent prospective diagnostic accuracy study (2023) combining these indices with motor unit number estimation (MUNE) derived from compound muscle action potential (CMAP) scans (MScanFit algorithm) achieved robust diagnostic performance (AUC=0.85), correctly identifying 73% of clinically equivocal ALS patients at their initial visit, thus reducing diagnostic delay. Further advancements in 2024 introduced principal component analysis-based pattern recognition of NET data, revealing four distinct ALS subgroups, suggesting heterogeneous underlying pathophysiological mechanisms. Our ongoing longitudinal study investigates 24 ALS/progressive muscular atrophy patients assessed quarterly over one year. Utilizing individualized survival predictions (ENCALS model) for staging, we address critical questions: 1) Can the previously validated diagnostic Risk Score serve as a biomarker of ALS progression? 2) Does subgroup classification stability over time reflect consistent underlying pathophysiology or dynamic changes in mechanistic burdens? 3) Can we develop predictive biomarkers identifying patients likely to respond to therapies targeting neuromuscular junction maintenance, particularly those with limited neuromuscular resilience? These analyses aim to refine biomarker utility, enhancing clinical decisions from diagnosis through targeted therapeutic interventions in ALS.

Session X – Neuromuscular Junctions in Health and Disease/Injury

1. **Dysmorphic Neuromuscular Junctions in Spastic Cerebral Palsy**

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Cerebral palsy (CP) arises when a non-progressive lesion to the developing brain results in motor system impairment and lifelong physical disability. CP is the most common severe neuromotor condition arising in childhood. Spastic CP, the most prevalent type, is associated with movement deficits, high muscle tone, skeletal muscle changes, and musculoskeletal deformation. Clinical experience indicates that CP patients have altered sensitivities to pharmaceutical agents targeting neuromuscular junctions (NMJs), and in

IRB-approved studies, our team has found that children with CP harbor highly dysmorphic NMJs. In particular, we have identified significant mis-localization of synaptic versus post-synaptic proteins in subpopulations of NMJs from muscles of CP patients. To investigate these differences, our group used laser capture microdissection and quantitative RT-PCR to evaluate gene expression in the post-synaptic myonuclei of dysmorphic CP NMJs, non-dysmorphic CP NMJs, and normal control NMJs. Our analysis revealed altered expression of several genes that play key roles in NMJ structure and function, including several components of the junctional basal lamina. We found differential expression of NMJ enriched genes in both non-dysmorphic and highly dysmorphic CP NMJs including significant upregulation of LAMB2, LAMA5, P2X2, and MUSK while GADD45B and NCAM1 were found to be significantly down-regulated in CP. The expression of additional genes was significantly altered in the highly dysmorphic CP NMJs with a general trend toward down regulation. The differential expression patterns of NMJ genes in CP indicate that CP muscle harbors a mosaic of NMJs with altered molecular regulation that are not seen in controls.

2. **Motor Unit Development in a Rabbit Model of Cerebral Palsy**

EMILY REEDICH (1), ELVIA MENA AVILA (1), LANDON GENRY (1), ELIAN GONZALEZ SANCHEZ (1), BRENDAN MOLINE (1), CASSANDRA KRAMER (1), REBECCA MANUEL (1), KATHARINA QUINLAN (1), MARIN MANUEL (1)

1) University of Rhode Island, USA

Cerebral palsy (CP) is the most common motor disability in children, occurring in 1:500 live births. The mechanisms through which CP-causative injuries like hypoxia-ischemia (HI) cause motor deficits remain unresolved. Motor unit development occurs in the perinatal period when CP-causative injuries occur, and depends on motoneuron activity, which is increased in the HI rabbit model of CP. We hypothesize that prenatal HI injury dysregulates neuromuscular junction maturation and disrupts motor unit development. To test our hypothesis, we are using immunofluorescence to track the emergence of mono-innervation at the neuromuscular junction, an anatomical hallmark of maturity. This process is driven by the developmental switch from synchronous to asynchronous motoneuron activity. We are therefore analyzing the synchronicity of motor unit activity recorded in awake rabbits across neonatal development using Myomatrix array electrodes. In addition, we are using immunostaining to label type I, IIa, IIx, and IIb myofibers and are evaluating differences in fiber type distributions of sham-operated control and HI rabbit muscles in order to assess their force-generating capacity and fatigability. Our preliminary data suggests that neuromuscular junctions undergo delayed maturation in HI rabbit skeletal muscle and indicates that in the third postnatal week when poly-neuronal innervation is eliminated, HI muscle has a slower, weaker, and less fatigable fiber type profile than that of typically developing rabbits. This may reflect chronic, low frequency motor unit activity consistent with CP. Overall, this work suggests aberrant

motor unit development after prenatal HI injury contributes to motor dysfunction in the rabbit model of CP.

3. **Neuromuscular Junction as Therapeutic Target in ALS**

ELSA TREMBLAY (1,2,3), DANIELLE ARBOUR (1,2,3), JOANNE VALLÉE (1), ROBERTA PIOVESANA (1,2,3), EMINE MECHICHI (1), RICHARD ROBITAILLE (1,2,3)

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Neuromuscular junction (NMJ) denervation is an early pathological event in amyotrophic lateral sclerosis (ALS) causing motor deficits and paralysis. Glial cells at the NMJ ensure a balance between maintenance and repair via the activation of their muscarinic receptors. In ALS mouse models, glial cells show an aberrant, early and persistent muscarinic hyper-activation at innervated and denervated NMJs. Hence, we tested if the excessive muscarinic activation prevents glial cells from ensuring NMJ stability and repair in ALS. From symptoms onset, SOD1G37R mice received daily oral administration of darifenacin, a type 3 muscarinic acetylcholine receptor antagonist that reduced glial muscarinic activation. The treatment improved locomotion and survival due to enhanced muscular and NMJ functions. These improvements were supported by enhanced NMJ innervation, glial repair signs and better survival of lumbar motor neurons. These preclinical data show that neuromuscular glial muscarinic hyperactivity contributes to the neuromuscular component of the pathology and could lead to new therapeutic strategies targeting the NMJ in ALS.

4. **Muscular Swedish mutant APP in neuromuscular junction maintenance and Alzheimer's disease development**

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Alzheimer's disease (AD) is a progressively degenerative brain disorder. Recent research has indicated that peripheral tissues or organs, including muscles, undergo early changes during the preclinical phase of AD, such as muscle weakness. This weakness may eventually develop into severe sarcopenia in the later stages of the disease. However, the relationship between muscle weakness and AD, as well as the underlying mechanisms, remains largely unexplored. In this study, we present evidence of early neuromuscular junction (NMJ) deficits in mice expressing the Swedish mutant APP specifically in skeletal muscles (TgAPPsweHSA-Cre). These deficits include impaired neuromuscular transmission, as measured by compound muscle action potential (CMAP), a reduced

frequency of miniature end-plate potentials (mEPP), and decreased nerve terminal innervation at NMJs. Notably, these NMJ deficits were age-dependent, with more pronounced effects observed at presynaptic terminals and in soleus muscles, which are enriched in slow muscle fibers. We are currently investigating the molecular mechanisms driving these changes and their potential role in AD progression.

5. **Neuromuscular Junction Transmission Failure in Sarcopenia**

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Sarcopenia, the pathological age-related decline in muscle mass and function, imposes significant personal, societal, and economic burdens. Its pathogenesis is multifactorial, but growing evidence underscores the critical role of neuromuscular dysfunction. The neuromuscular junction (NMJ), the final link between motoneurons and skeletal muscle, is essential for translating neural signals into effective muscle contraction. Age-related structural NMJ alterations, such as endplate fragmentation, denervation, and reinnervation, have been extensively documented in rodent models, though human studies remain inconsistent. However, beyond structural changes, the functional integrity of the NMJ in aging and sarcopenia has received comparatively less attention. Surprisingly, ex vivo synaptic recordings of endplate potentials and currents suggest preserved or even enhanced neurotransmission in aged models, raising questions about compensatory adaptations. However, recent in vivo electrophysiological studies in both mouse and rat models have demonstrated NMJ transmission failure, with single-fiber electromyography and nerve-stimulated force assessments revealing impaired synaptic fidelity and reduced safety margins. These deficits correlate strongly with motoneuron dysfunction, reduced muscle contractility, and increased fatigability, suggesting NMJ failure as a potential driver of sarcopenia. This presentation will examine emerging evidence from rodent and human studies linking NMJ dysfunction to sarcopenia, explore the cellular and molecular mechanisms contributing to failed transmission, and discuss a promising therapeutic approach aimed at preserving NMJ function to counteract age-related muscle decline. Targeting NMJ dysfunction could offer a novel strategy for maintaining motor function and reducing the impact of sarcopenia in aging populations.

Session XI – Neural Constraints That Govern Motor Unit Activity in Humans

1. **Neuromodulation increases the flexibility of motoneuron control**

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The classic view of motoneuron control is that of a rigid control: motoneurons that receive a common synaptic input are constrained in their activity by their size. Recent studies, however, suggest that motoneuron control may be more flexible: the recruitment/derecruitment order and correlations between pairs of motoneurons from the same pool can change both during intracortical microstimulation (Marshall et al., Nature Neuroscience, 2022) and under volitional control (Bräcklein et al., eLife, 2022). Here, we sought to understand the neural mechanisms underlying these apparent violations of rigid motoneuron control. We hypothesized that such flexibility would originate from persistent inward currents (PICs). We developed a biophysical motoneuron pool model incorporating common synaptic, neuromodulatory, and inhibitory inputs. After calibrating it with large-scale motoneuron recordings (~130 per subject), the model matched experimental data well ($R^2 = 0.89$). In Bräcklein et al.'s cursor control task participants used two motoneurons from the same pool to control a two-dimensional cursor. Thus, this was simulated with the aim of decorrelating the activity of the two motoneurons. We found that PICs were both necessary and sufficient to achieve this decorrelation, enabling deviations from rigid control. However, flexibility only emerged in motoneuron pairs with differing biophysical properties, as these differences allowed distinct responses to neuromodulation—consistent with experimental findings. Moreover, analyzing and simulating motoneuron activities over multiple days, we observed a gradual improvement in the ability to decorrelate, suggesting either an increased ability to regulate motoneuron flexibility or a better control of force to leverage on the differences between motoneurons.

2. **Precise Decoding of Spared Neural Control in the Paralyzed Human Hand Using Multiple Muscle Implants**

DEVON ROHLF (1), RAUL SIMPETRU (1), DANIELA SOUZA DE OLIVEIRA (1), DANIEL FENZEL & KYRILL HOFFMANN (1), MARTIN REGENSBURGER (2), MATTHIAS PONFICK (3), ALESSANDRO DEL VECCHIO (1)

1) Friedrich-Alexander-Universität, Germany; 2) Universitätsklinikum Erlangen, Germany; 3) Querschnittzentrum Rummelsberg, Germany

Spinal cord injuries (SCI) affect millions worldwide, disrupting neural signal flow and muscle control below the injury. Despite impairments, it has been demonstrated that some preserved activity remains, despite no discernible voluntary movement. Compared to surface electromyography (EMG), which is prone to signal attenuation and muscular crosstalk, intramuscular EMG (iEMG) enables more precise, localized measurements with minimal filtering. However, iEMG also poses challenges in implant site selection and signal selectivity. To enable better understanding of the neural control of movement in SCI through iEMG, we developed a multichannel fine-wire electrode that allows multiple channels to be recorded through a single needle puncture. To overcome the current

limitation of iEMG decomposition, we developed a new software program, MyoLytica, which offers single-channel decomposition techniques in a user-extensible framework, to identify motor units in low-density applications. Preliminary findings from SCI patients reveal surprising aspects of spastic motor units, including modulated firing during dynamic tasks. With real-time motor unit feedback, we found that these SCI patients can effectively learn to control motor unit activity proportionally, however, several motor units show persistent tonic firing and an inability to de-recruit the active motor units, potentially caused by active Persistent Inward Currents (PICs). These results also highlight the promise of intramuscular-driven proportional control for neural interfacing. Future improvements to electrode design and signal processing algorithms may further refine detection of subtle motor unit activity. Ultimately, these advances may lead to more effective rehabilitation strategies for SCI and other motor impairments of the central or peripheral nervous system.

3. **Behavioural and Neural Constraints on Motor Control**

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The human motor system enables a vast repertoire of movements by coordinating motor units—each comprising an alpha motor neuron and its innervated muscle fibers. A fundamental question remains: how does the brain coordinate spinal motor neuron activity to meet varied behavioral demands? Traditionally, Henneman's size principle and biomechanical constraints suggest that neural commands recruit fixed pools of motor units, reducing the number of independent signals required for movement. However, recent evidence challenges this view, revealing greater flexibility (Marshall et al., 2022; Bräcklein et al., 2022). This raises the question: what principles govern motor unit control? To investigate this, we recorded motor unit spiking activity using high-density surface and intramuscular electromyography (HD-EMG) from the flexor carpi ulnaris (FCU) and first dorsal interosseous (FDI) muscles in healthy participants performing isometric force tasks. In one condition (rate trial), participants tracked patterned profiles by modulating the discharge rate of a single motor unit. In another (force trial), they reproduced the force exerted during the rate trial—this time by generating force rather than controlling an individual motor unit's discharge rate. This allowed us to compare motor unit population activity across two conditions matched for behavior but differing in task demands. Additionally, we altered wrist posture to assess how muscle length influences motor unit behavior. Our findings will shed light on how the central nervous system orchestrates motor unit activity to generate movement. Understanding these principles may provide crucial insights into neuromuscular function and rehabilitation strategies for motor impairments.

4. **Decoding the Bilateral Force Deficit During Dorsiflexion: Insights from Motor Unit Activity and the Off-Direction Force**

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The aim of our study was to evaluate the magnitude of bilateral deficit, in maximal voluntary contraction (MVC) force and rate of force development (RFD) for the dorsiflexors and the underlying differences in the discharge characteristics of motor units (MUs) in tibialis anterior. Additionally, off-direction (foot adduction) forces were recorded. A two-way ANOVA assessed force production differences between unilateral and bilateral contractions, and linear mixed models analyzed motor unit (MU) discharge characteristics. MVC was 20% less in bilateral compared with unilateral contractions. A deficit in force during bilateral contractions was observed at four time points (F50, F100, F150, Fmax) during each contraction, with decreases of 23%, 19%, 28%, and 24%, respectively, in the bilateral condition. There were no significant differences in either discharge rates (bilateral: 65.6±27.1 pps, unilateral: 63.4±29.8 pps, p=0.71) or recruitment speed (bilateral: 16.3±8.8 ms, unilateral: 16.8±7.8 ms, p=0.56) of the identified Mus (358). Moreover, there were no differences (p=0.795) in maximal discharge rate for the 80 MUs that were matched across tasks. The reductions in MVC and RFD were not associated with differences in MU discharge rates. However, the neuromechanical delay was significantly shorter in unilateral (43±10 ms) than bilateral contractions (49±13 ms). Critically, the off-direction forces were greater during bilateral than unilateral contractions ($p<0.05$). Our findings indicate that the bilateral deficit observed for the dorsiflexor muscles is attributable to differences in task performance rather than the neural drive to tibialis anterior during the unilateral and bilateral contractions.

5. **Structured higher-dimensional common synaptic inputs dictate violations in size principle**

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Understanding the changes in motor unit (MU) control and the amount of shared neural inputs to different pools of spinal motor neurons still remains a challenge. Some studies report a fixed control of spinal MUs whilst others show potential violations in their orderly recruitment. Here, we hypothesise that potential violations of the size principle are rooted in distinct voluntary drives. Using high-density intramuscular EMG electrode arrays

implanted in the first dorsal interosseus (FDI) and flexor digitorum superficialis & profundus (FDS/FDP) of five healthy participants, we investigated the characteristics of MU recruitment during isometric index finger abduction and index finger flexion at equal levels of global FDI muscle activation. Most FDI motor units were active during both abduction and flexion, with only few MUs showing direction-specific activation. FDI MUs showed consistent recruitment thresholds across separate trials at same force directions (Pearson R of MU recruitment thresholds: R_abd1/abd2 = 0.93, R_flex1/flex2 = 0.95, avg. change in FDI relative EMG recruitment threshold: ΔRT_FDI 12.3 % in abduction, 10.8 % in flexion). However, between abduction and flexion, we found a fixed number of MUs that significantly changed their position in the recruitment order, while others remained consistent (R_abd/flex = 0.26, ΔRT_FDI= 38.6 % across all MUs identified in both directions). For subsequent analysis, we hypothesize stronger shared neural input in FDI MUs within index finger abduction compared to flexion, due to groups of FDI MUs receiving shared neural input with MUs from the FDS and FDP during index finger flexion.

Session XII – Inputs to Motoneurons

1. **Spinal and cortical premotor control of primate hand muscles in precision grip**

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Skilled hand movements represent a key evolutionary advance in primates. Hand control relies on phylogenetically distinct corticospinal pathways: the “newer” direct corticomotoneuronal (CM) pathways and the “older” indirect corticospinal pathways mediated by spinal premotor interneurons (PreM-INs). Despite their critical roles, the functional distinctions between these pathways remain poorly understood. In experiments with behaving non-human primates, we found that CM cells precisely fine-tune the activity of individual finger muscles, while PreM-INs drive gross muscle activity by coordinating synergistic muscle groups. Additionally, our findings reveal that gross muscle activity can be automatically modulated by adjusting the gain of the spinal reflex loop that involves PreM-INs, which function as a closed positive feedback system.

2. **Motor Cortical Influence During Ethological Motor Behavior**

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When and how the primary motor cortex (M1) drives muscle activity during natural motor

behavior remains poorly understood, as many species-typical behaviors persist following disturbance to M1 output. This ambiguity stems in large part from a historical focus on experiments in which subjects perform highly constrained, experimenter-defined movements that become stereotyped with repeated performance. To address M1's influence on muscles during ethological behavior, we developed a paradigm in which mice perform a naturalistic climbing behavior to negotiate an unpredictable terrain. By combining rapid optogenetic inactivation, electromyography, and a novel statistical framework for quantifying inactivation effects, we measured M1's direct influence on muscles during climbing. We found that M1 inactivation only affects individual muscles at a subset of muscle activity states, that the effects on muscles vary in magnitude across that subset, and that the pattern of effects across muscle activity states itself varies across muscles. In contrast to existing hypotheses, this reveals that during naturalistic climbing, M1 instructs muscle activity patterns. We also found that this influence involves selective activation of physiological flexors, and smaller, bidirectional effects on their corresponding antagonists. Recording M1 activity with Neuropixels indicated that M1's instructive influence relies on a subset of its activity patterns that is distinct from those to which functional significance has previously been attributed. Our recent results show that this instructive influence on muscle activity patterns is present during many naturalistic behaviors, suggesting that the persistence of species-typical behaviors following cortical activity disturbance has masked a broad M1 involvement in steering ethological motor behavior.

3. **Sensory Expectations Shape Neural Population Dynamics in Motor Circuits**

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The neural basis of movement preparation has been extensively studied during self-initiated actions where motor cortical activity during preparation shows a lawful relationship to the parameters of the subsequent action. However, movements are regularly triggered and constantly corrected based on sensory inputs caused by disturbances to the body or environment. Since such disturbances are often predictable and since preparing for disturbances would make movements better, we hypothesized that expectations about sensory inputs also influence preparatory activity in motor circuits. Here we show that when humans and monkeys are probabilistically cued about the direction of a future mechanical perturbation, they incorporate sensory expectations into their movement preparation and improve their corrective responses. Using high-density neural recordings, we establish that sensory expectations are widespread across the brain, including the motor cortical areas involved in preparing self-initiated actions. The geometry of these preparatory signals in the neural population state is simple, scaling directly with the probability of each perturbation direction. After perturbation onset, a

condition-independent perturbation signal shifts the neural state leading to rapid responses that initially reflect sensory expectations. Based on neural networks coupled to a biomechanical model of the arm, we show that this neural geometry emerges through training, but only when the incoming sensory information indicating perturbation direction coincides with – or is preceded by – a condition-independent signal indicating that a perturbation has occurred. Thus, motor circuit dynamics are shaped by future sensory inputs, providing clear empirical support for the idea that movement is governed by the sophisticated manipulation of sensory feedback.

4. **Spinal motoneuron interconnectivity forms circuits that facilitate forceful tasks**

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Previous evidence showed that spinal motoneuron collaterals form synaptic contacts with other motoneurons. We have recently shown that putative fast motoneurons receive 10-fold stronger recurrent excitation than their putative slow counterparts. This finding raises the following questions: 1) are these connections dominantly between fast motoneurons and/or are there synaptic connections involving slow motoneurons? 2) what is the distribution of motoneuron-motoneuron connectivity? and 3) what is the function of these synaptic connections? We performed paired patch-clamp recordings from motoneurons labelled from either gastrocnemius (ankle extensor) or tibialis anterior (ankle flexor) muscles, as well as from unlabelled motoneurons innervating hip/proximal leg muscles. Within individual motor nuclei, there was a predominance of connections between fast motoneurons and only rarely were slow motoneurons identified as a synaptic partner. We also found direct connections between antagonist pools with similar synaptic connectivity as that observed within nuclei: connectivity was observed predominantly between fast motoneurons. Moreover, we have also detected recurrent excitatory connections between motoneurons which innervate proximal and distal leg muscles, showing synaptic connectivity across joints. Given that these connections were blocked by glutamate antagonists, we next genetically removed vGluT2 from cholinergic neurons, and found, using patch-clamp, that recurrent excitation is halved. Behavioural testing revealed clear deficits, including in grip strength. Collectively, our results indicate the importance of motoneuron recurrent connectivity as a facilitator in behaviours requiring high force production.

5. **Behavior of Large Populations of Motor Units: What Does it Reveal About the Neural Circuits Involved in Movement Generation?**

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The dimensionality of motor unit behavior is often assumed to reflect the number of independent neural commands received by motor units. To test this assumption, we applied factor analysis to the spiking activity of a large populations of motor units from the vastus lateralis (VL) and gastrocnemius medialis (GM) muscles. Our findings revealed that GM motor unit activity was effectively captured by a single latent factor, whereas three latent factors were needed to capture the behavior of VL motor units, defining a multidimensional manifold. We then assessed motor unit flexibility, defined as deviations from the strictly monotonous recruitment predicted by Henneman's size principle. First, we quantified the flexibility of entire motor unit pools during isometric force-control tasks (sinusoidal contractions at various frequencies). Second, we used an online control paradigm to evaluate participants' ability to independently control individual motor units. Our results showed no evidence of greater flexibility in the VL compared to the GM, suggesting that a multidimensional manifold does not necessarily imply the presence of distinct, controllable neural commands. Based on a simulation model, we proposed that multidimensionality can emerge from spinal circuits even with a single descending input and identified recurrent inhibition as a potential underlying mechanism. To investigate this, we developed a novel, stimulation-free method to assess recurrent inhibition leveraging the probability of motor units firing relative to one another's spike time. Preliminary findings indicate that recurrent inhibition is organized differently across muscle groups and contraction intensities, thereby shaping specific behavior of motor unit populations.

Session XIII – Motor Impairments 2

1. **Intermuscular differences in motor unit firing parameters to predict muscle strength after spinal cord injury**

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There is substantial functional heterogeneity within the spinal cord injured (SCI) population, yet we know little about underlying mechanisms. Motor unit (MU) firing behavior after SCI may reveal physiological mechanisms that contribute to this heterogeneity. We assessed MU behavior in cervical SCI patients across four different muscles (biceps brachii (n=20), triceps brachii (n=20), vastus lateralis (VL) (n= 9), and vastus medialis (VM) (n=9)) to determine parameters that might predict muscle strength.

Age-matched, non-injured participants were used as a control group. Maximal voluntary contractions (MVCs) were used as a measure of muscle strength and to normalize submaximal isometric ramp contractions (30% MVC) across participants. High-density surface electromyograms were obtained during elbow flexion, elbow extension, and knee extension, and blind source separation was used to identify spike times of the MUs. Firing rate hysteresis was quantified using the paired-MU analysis technique (ΔF) and normalized to account for differences in firing rate modulation between the SCI and control groups. Preliminary analysis has revealed a positive association between normalized ΔF and MVC in the triceps and VL of SCI participants. This association was absent in the biceps and VM, and across muscles in the control group. Conversely, the modulation amplitude of firing rates was positively associated with MVC in biceps and VM, but not in the triceps or VL of SCI participants. These results reveal intermuscular heterogeneity of MU firing behavior which may promote functional recovery after SCI. Additional analyses are on-going.

2. **Leveraging residual motor unit activity after spinal cord injury**

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Spinal cord injury (SCI) disrupts motor control, yet residual muscle activity often persists below the lesion level. Understanding this spared motor unit activity could enhance neural interfaces for assistive devices. In this study, we analyzed motor unit discharge patterns using high-density electromyography (HDsEMG) in eight individuals with chronic motor-complete SCI and twelve healthy controls during cyclic hand tasks involving finger flexion and extension. Despite impaired voluntary control in SCI, with a greater presence of non-modulated motor units, non-negative matrix factorization revealed similar patterns of motor unit activity between SCI and control groups. This suggests that spared motor activity is sufficient to decode movement intent and could be leveraged for movement restoration. To explore this potential, we tested the feasibility for assistive device control by mapping motor unit activity to command a supernumerary robotic sixth finger. This approach was tested by three participants from the SCI group, and with just a few minutes of training they were able to proportionally control the sixth finger for grasping different objects. As a final step, we investigated whether selecting only the most strongly modulated motor units could improve neural interface reliability. This offline analysis aimed to refine control strategies by focusing on task-relevant motor units while minimizing variability from less consistent ones. Our findings suggest that spared motor activity in SCI individuals can be leveraged for intuitive neural interfaces. By refining motor unit selection, we aim to improve the robustness of neural interfaces for assistive grasping, enhancing functional independence for individuals with SCI.

3. **Effect of joint position on alpha-motoneuron persistent inward currents in humans with and without spinal cord injury**

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In agreement with recent findings, our previous study in healthy individuals demonstrated that alpha-motoneuron persistent inward currents (PICs) are modulated by joint position and that synaptic excitation-inhibition patterns likely underlies this modulation. Given that individuals with spinal cord injury (SCI) exhibit a dysregulation of synaptic inhibition, their ability to regulate PICs according to joint position may be compromised, potentially contributing to position-dependent motor impairments such as spasticity. To investigate this, we conducted a study to determine whether individuals with incomplete SCI can appropriately modulate their PICs according to joint position during voluntary contraction. We included 18 individuals with incomplete SCI and 18 control participants. Participants performed isometric plantar flexion contractions at 40% of their maximum voluntary contraction at 0° (neutral) and 20° of plantar flexion. Surface electromyography signals were recorded from the gastrocnemius medialis and soleus muscles and decomposed into motor unit spike trains. Paired motor unit analysis was used to calculate ΔF, a non-invasive estimate of PIC magnitude. Preliminary results showed that ΔF was significantly reduced in individuals with SCI, by approximately 38% in the gastrocnemius medialis and 28% in the soleus. However, ΔF was not different between the two ankle positions tested in both groups. Our findings reveal a reduced contribution of PICs to alpha-motoneuron discharge rates in individuals with SCI during voluntary contraction, which likely contributes to paresis. While previous studies support dysregulation of inhibition in individuals with SCI, our results show that this does not inherently disrupt the control of PICs in response to joint position.

4. **Novel two-hit mouse model of neonatal hypoxic ischemic encephalopathy results in developmental delays, long-term motor deficits, and decreased brain volume**

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Hypoxic ischemic encephalopathy (HIE) is one of the most serious causes of neurological deficits in children born at term. HIE can lead to diffuse brain injury often impacting white matter and deep brain regions, resulting in lifelong motor, cognitive, and memory deficits. Maternal inflammation is a significant risk factor for HIE. Our model pairs maternal immune

activation (MIA) via the administration of lipopolysaccharide (LPS) on gestational day 18 with a progressive hypoxia to 0% oxygen for 8 minutes on postnatal day 6 (P6). Our model of HIE produces predominantly motor developmental delay in young mice. In adulthood, HIE animals exhibited weaker grip strength and gait changes, similar to the pattern of deficits in cerebral palsy. On P7 brains from HIE animals demonstrated smaller overall volume on P7. Bulk and single cell RNAseq demonstrate upregulation in inflammatory genes in monocytes. Cluster analysis reveals two microglia subpopoulations involved in neurogenesis and axonogenesis that may be responsible for changes to the brain after HIE. These findings support our novel two-hit HIE model as a clinically-relevant tool to explore the underlying mechanisms of pathology in neonatal HIE, particularly with regard to motor impairment.

5. **Persistent Inward Current Decline Following Electrically-induced Muscle Cramps**

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Muscle cramps, affecting 30–50% of healthy individuals, pose a significant clinical challenge with unclear pathophysiology (Schwellnus, 2007). While dehydration theories historically dominate, emerging evidence implicates a dysregulation of spinal motoneuron excitability (Minetto et al., 2011), potentially mediated by persistent inward currents (PICs). The aim of this study was to estimate acute PIC modulation following electrically-induced cramps. Thirty healthy young adults (25 ± 6 years) performed triangular voluntary contractions (0–20% of maximal voluntary plantar flexion) before and immediately after successive tetanic stimulations of the gastrocnemius medialis (GM) to induce a muscle cramp. Participants were categorized into crampers (n=15) or controls (n=15) based on cramp induction success. High-density electromyography was used to estimate PICs in GM and soleus (SOL) muscles via paired motor unit analysis (ΔF). Plantar flexor neuromuscular function (e.g., activation level, contractile properties, H:M ratio) was also evaluated before and after the cramp protocol. Among 361 tracked motor units (GM: 265; SOL: 96), ΔF increased significantly in controls (GM: +0.61 Hz; SOL: +0.66 Hz; $p<0.05$) but decreased in crampers (GM: −1.07 Hz; SOL: −0.87 Hz; $p<0.05$). The distinct modulation of PIC estimates in the two groups supports the hypothesis that PICs potentially contribute to cramp occurrence in healthy young adults and that cramp induction may be followed by a regulatory mechanism, such as an increased synaptic inhibition.

1. **Sex differences in motor unit firing properties in young, middle-aged and old adults**

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Aging is associated with neuromuscular decline and recent evidence suggests that sex may influence motor unit (MU) adaptations. This study examined sex differences in MU firing properties across young (YG: 26±2 years; N=10, 5 females), middle-aged (MA: 58±3 years; N= 46, 29 females), and old (OLD: 77±4 years; N=35, 11 females) adults. High-density electromyography (HDEMG) was recorded from the vastus lateralis during trapezoidal isometric contractions (2% MVC/s) up to 30% and 50% MVC. MU discharge rates were analysed during both the increasing and plateau phases of contraction. The main findings revealed that OLD adults had significantly lower mean firing rates than YG and MA at both force levels (OLD: 9.3±1.5 pps vs. YG and MA: 10.3±1.6 pps at 30% MVC, $p<0.001$). Sex differences were prominent in YG and MA, with females exhibiting higher firing rates than males (YG: +11.7%, MA: +8.9%, $p<0.001$), but this difference was not observed in OLD ($p=0.60$). Additionally, firing rate modulation was quantified as the firing rate increase between the recruitment and the target torque (50% MVC). This modulation was reduced with age (YG: 5.9±2.5 Δpps, MA: 5.3±2.6 Δpps, OLD: 3.7±2.5 Δpps), and females showed a more pronounced age-related decline (MA vs OLD) in early-recruited MUs. These results demonstrate sex-specific trajectories in neuromuscular aging, potentially due to hormonal, biomechanical, and muscle quality factors. The findings emphasize the need to consider sex differences when studying age-related changes in motor control and muscle function, as they may have implications for developing targeted interventions against sarcopenia.

2. **Modulation of discharge rate for matched motor units in the peronei muscles differs between foot abduction and ankle plantar flexion**

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Our aim was to compare the discharge characteristics of matched motor units in Peroneus Longus (PL) and Brevis (PB) during foot abduction and ankle plantar flexion. Forces applied at submaximal targets are less steady during foot abduction. Seven males

performed isometric contractions at 20 and 40 % of maximal voluntary contraction (MVC) force and high-density electromyographic signals were acquired from the two muscles. The discharge characteristics were quantified as mean discharge rate, coefficient of variation for interspike interval (CoV for ISI), and standard deviation of the filtered cumulative spike train (SD of fCST) during submaximal contractions at 20 and 40% MVC force. The filters for MUs identified during abduction were used to find the same MUs during plantar flexion. ANOVA results indicated a significant main effect of action on mean discharge rate ($p<0.05$), with greater mean discharge rate during abduction (11.5 ± 2.2 pps collapsed across muscles) than plantar flexion (10.9 ± 2.0 pps collapsed across muscles). Although there were significant interactions for both CoV for ISI (abduction: 0.15 ± 0.07, plantar flexion: 0.15 ± 0.07 collapsed across muscles) and SD of fCST (abduction: 0.0023 ± 0.015, plantar flexion: 0.0025 ± 0.0031 collapsed across muscles), pairwise comparisons did not identify any individual differences ($p>0.05$). These results indicate that the strength of the net synaptic input (mean discharge rate) to the involved motor neurons differed during the two actions, but there were no differences in the variability of either synaptic noise (CoV for ISI) or neural drive (SD of fCST).

3. **Synaptic Dysfunction Alters Motoneuron Output in ALS**

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive motoneuron (MN) degeneration. While previous studies have focused on intrinsic changes in MN excitability, network-level contributions remain less understood. In this study, we investigate synaptic dysfunction in ALS using animal models and assess its impact on MN output. Our findings show that synaptic alterations significantly influence MN activity. Together, our results reveal an underlying mechanism of abnormal motoneuron output in ALS.

4. **Neuromuscular Effects of Cerebral Palsy**

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Cerebral palsy (CP) is a neurodevelopmental disorder characterized by lifelong motor impairments that result from early brain injury. While the impact of upper motor neuron damage in CP is well established, the effects on neuromuscular junction (NMJ) function and peripheral motor transmission remain underexplored. This study investigates how CP disrupts NMJ integrity and neuromuscular signaling, utilizing tissue samples from CP

patients requiring surgery as well as rodent models. Existing analyses suggest that NMJ morphology and function are disrupted in CP, with evidence of synaptic pruning defects, altered receptor distribution, and impaired neuromuscular signaling. However, direct histological evidence of these phenomena in human CP patients remains limited.To address this gap, our investigation focuses on histopathological analyses of NMJ structures in both rodent models and human tissue samples from pediatric surgical cases at Nemours Children's Hospital. Through examination of neuromuscular tissue from young patients with CP, this project clarifies how NMJ dysfunction contributes to motor deficits and informs targeted therapeutic strategies. We hypothesize that CP induces distinct histological changes at the NMJ, such as synaptic structural abnormalities and disrupted neuromuscular signaling, which contribute to motor dysfunction. A deeper understanding of NMJ alterations in CP will provide novel insights into disease mechanisms and potential interventions aimed at improving neuromuscular function.

5. **Posterior Root-Muscle Reflex Recruitment Across the Lifespan**

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The Hoffman (H)-reflex is a well-characterized reflex evoked by stimulating large diameter Ia afferents in the peripheral nerve, which monosynaptically activate motoneurons. The resulting motor response is recorded from the homonymous muscle using electromyography. The posterior root-muscle (PRM) reflex is evoked by stimulating large diameter afferents in the dorsal root through electrodes on or adjacent to the spine. The PRM reflex is a compound of the H-reflex and cutaneous reflexes. H-reflex excitability, often characterized by the threshold required to evoke a response, is reduced with age and increased in neuropathic pain. It is unknown how the PRM reflex changes with age or after neural injury. Here, we seek to characterize PRM reflex recruitment across different age groups. We aim to compare “typical” PRM reflex recruitment with recruitment after neural injury. PRM reflexes were evoked using a 1 ms-long monophasic, cathodic pulse delivered through electrodes lateral to the T12-L1 vertebrae. PRM reflex recruitment was obtained from the soleus muscle in 21 neurologically-intact participants (11 female; 23-49 years). Thresholds were 35±9.9 μC for ages 20-29 (n=14), 40±19 μC for ages 30-39 (n=5), and 42±7.8 μC for ages 40-49 (n=2). Linear regression of the thresholds over age was R=0.20. Additional reflex thresholds per decade are expected to strengthen this correlation. Prior work reported thresholds from amputees aged 38-58 to be 59.5±3.8 μC, demonstrating reduced spinal excitability compared to intact individuals. Characterizing how PRM reflex recruitment changes with age enables better understanding of how spinal cord excitability changes with aging and after neural injury.

6. **Influence of the time of day on the contribution of the neuromodulatory system to spinal motor neuron activity.**

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Persistent inward currents (PICs), which regulate spinal motor neuron excitability, are influenced by neuromodulatory inputs, particularly monoamines (e.g., serotonin). Since monoamine secretion follows circadian rhythms, motor neuron excitability may fluctuate throughout the day. This study aimed to determine whether PICs and motor unit discharge characteristics, estimated during submaximal isometric dorsiflexion tasks, vary at different times of the day. Nineteen participants (3 females; 26.6 ± 5.5 years) completed triangular tasks at 20% and 40% of maximal voluntary torque (MVT) in the morning (7:00–8:30 a.m.) and evening (5:00–6:30 p.m.) on separate days. Four grids of high-density surface EMG electrodes were placed over the tibialis anterior. The signals were decomposed into motor unit spiking activity. Two key preliminary findings emerged. First, motor unit peak discharge rates were significantly higher in the evening compared to the morning under the same relative torque (i.e., time-specific 20–40% MVT), and ΔF values were significantly higher in the evening under both relative and absolute (i.e., identical torque-matched task targets across sessions) conditions. Second, complementary geometrical metrics, which help distinguish the effects of neuromodulation from inhibition, revealed that the acceleration slope of motor unit discharge (associated with inhibition-neuromodulation dynamics) was significantly steeper in the evening, whereas brace height, a marker of neuromodulation, remained unchanged. Together, our preliminary findings suggest that excitatory-inhibitory inputs to motor neurons may shift from morning to evening without significant changes in overall neuromodulatory drive. Thus, the circadian modulation of monoamine secretion throughout the day may not directly influence spinal motor neuron behavior.

7. **A new device to remotely monitor the motor activity of rodents 24/7 in the animal facility.**

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Animal experiments aiming at estimating the motor abilities of rodents suffer several flaws: i) the animals are stressed by being tested out of their usual context (even if habituation protocol soften this issue, they are still in an unusual environment when tested) ; ii) the interaction with the zootechnicians/researcher is not a neutral interaction; for example, the manipulator' sex can modulate the physiological response of the animals (Georgiou et al., 2022) ; iii) a daily test lasting 20 min still only represent 1.4% of a day, leaving a lot of time for unobserved events to occur. We designed a device to monitor the activity of the animals in their husbandry cages. Following several authors (Carlsen et al., 2019; Gourrame et

al., 2023; Try & Gebhard, 2023), we record the vibrations elicited by the movements of the animal. This recording is transmitted wirelessly to a central computer that collects, sorts and aggregates all the data and send them to a storage facility for further analysis. This architecture allows to record continuously for a duration that can extend to months if necessary to monitor the evolution of the health status of the animals. Our system is cheap and robust to short interruptions for animal care. Using video recording and deep-learning, we are now attempting to link vibrations patterns to specific animal behaviors.

8. **tDCS Did Not Affect Estimates of Persistent Inward Current in Isometric Contractions**

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A possible effect of transcranial direct current stimulation (tDCS) on the excitability of the central nervous system is the indirect activation of monoaminergic pathways through the stimulation of cutaneous afferents on the scalp. We hypothesize that this tDCS-induced monoaminergic activation would increase the amplitude of motoneuron persistent inward currents (PICs), thereby enhancing motor control. To test this hypothesis, we employed a sham-controlled protocol to assess the effects of tDCS in modulating the amplitude of PICs during isometric contractions of a hand muscle. The study involved 18 healthy participants, randomly assigned to two groups (N=9): 1) active, who received anodal tDCS over the motor cortex (C3 region), and 2) sham, who received placebo stimulation. Each participant attended five consecutive daily tDCS sessions. Active tDCS consisted of 20 minutes of therapy with a 2 mA current. The sham stimulation was applied for 30 seconds and then suppressed. Each participant performed an isometric force control task involving the first dorsal interosseous (FDI) muscle during the first and fifth sessions, following triangular-shaped force profiles with peaks at 10%MVC and 30%MVC. High-density EMG was recorded from the FDI and decomposed to extract motor unit activity. PIC amplitude was estimated by the Delta_F calculated for all possible pairs of motor units. Irrespective of the peak contraction intensity, Delta_F values did not significantly differ between active and sham groups, either after a single session (acute) or five sessions (chronic). Also, the motor unit's peak firing rate did not change in the evaluated conditions.

9. **Specificity of Static Stretches on Force Production and Motor Unit Discharge Characteristics in Hamstring Muscles**

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We investigated the influence of static stretching on maximal force, force steadiness, and motor unit (MU) discharge characteristics of the semitendinosus (ST) and biceps femoris (BF). Fourteen young, healthy participants completed a randomized, counterbalanced, within-subject design. Before and after stretching, participants performed steady isometric contractions at 10% and 40% of maximum voluntary contraction (MVC) force. The knee-flexion task was performed at a 30° knee joint angle with the hip in a neutral position, and the hip-extension task was conducted with the hip neutral and the knee fully extended (180°). Passive knee- and hip-based static stretching comprised of five 90-s trials. High-density EMG recordings were decomposed using blind-source-separation algorithm to identify 832 MUs in ST and 864 in BF. MVC force decreased significantly after stretching (hip extension: −10.5% ± 2.3%, knee flexion: -7.1% ± 4.3% $p < 0.01$) and force steadiness (coefficient of variation for force) worsened after stretching during hip extension after hip-based stretches (5.3% ± 3.2%, $p < 0.01$) and during knee flexion after knee-based stretches (6.1% ± 2.9%, $p < 0.01$). There were significant increases in mean discharge rate (BF: 7.8 ± 4.3 % and ST: 8.6 ± 5.2 %, collapsed across target forces), coefficient of variation for interspike interval (BF: 9.4 ± 5.4 % and ST: 8.9 ± 6.1 %), and SD of the filtered spike train (BF: 7.9 ± 6.3 % and ST: 6.8 ± 5.3 %) muscles. The adjustments in motor unit activity were greatest during tasks in which the stretches were imposed.

10. **Can Humans Voluntarily Separate Neural Inputs to Synergistic Muscles?**

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Voluntary movement relies on the nervous system’s ability to coordinate groups of muscles efficiently, often by projecting shared synaptic inputs to motor neurons of synergistic muscles. This principle suggests that muscles such as the vastus medialis (VM) and vastus lateralis (VL) are largely driven by common input. (Laine, et al. 2015). Recent studies using high-density surface EMG and factor analysis during isometric contractions have revealed that some motor units within VM and VL receive partially independent inputs, suggesting a more flexible control strategy than previously believed. However, it remains unclear whether humans can consciously access this flexibility to dissociate neural drive to synergistic muscles. (Del Vecchio, et al. 2023). We developed a neurofeedback setup combining multiple intramuscular EMG electrodes implanted in VM and VL with real-time visual feedback of RMS amplitude or motor unit activity. Participants were instructed to explore strategies to selectively activate one muscle while maintaining isometric conditions. Our results show that participants could volitionally recruit distinct motor unit populations in VM, but struggled to achieve the same for VL. Offline decomposition of the intramuscular EMG confirmed that certain motor units were uniquely recruited during VM-focused strategies, suggesting task-dependent modulation of input at the motor unit level. These findings provide direct evidence that voluntary modulation of neural input to synergistic muscles is possible and that task strategy can shape motor unit recruitment. This challenges the idea of fixed synergies and highlights a flexible, task-

dependent structure in neuromuscular control. Our findings may inform approaches in motor learning, rehabilitation, and refinement of synergy-based control models.

11. **NeurOne: High-performance Motor Unit-Computer Interface for the Paralyzed**

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Spinal cord injury (SCI) often leads to the loss of voluntary muscle control, profoundly impacting motor function and independence. While motor-and-sensory complete cervical SCI is traditionally associated with irreversible paralysis, recent evidence suggests that spared motor neurons below the injury retain functional cortical input. However, the extent to which individuals with SCI can harness this residual connectivity for meaningful motor control remains unclear. Here, we show that individuals with motor complete cervical SCI can learn to modulate the activity of spared motor neurons to intuitively control a digital neuromuscular system. Using NeurOne, a software platform that enables high-speed motor unit feedback and physiological twitch-based control, three participants (4 visits) were trained to regulate motor unit recruitment and discharge rates to control a cursor matching a target force level. Participants achieved precise modulation of motor unit output across different target intensities, demonstrating stable and proportional control (correlation r: r1=0.909±0.028, r2=0.866±0.034, r3=0.860±0.072; root-mean-square error RMSE: RMSE1=0.231±0.031, RMSE2=0.280±0.081, RMSE3=0.228±0.042). The motor output of NeurOne was surprisingly stable in a similar way as healthy subjects modulated the muscle force output recorded by a dynamometer (coefficient of variation CoV: CoVNeurOne=0.066±0.030 and CoVForce=0.037±0.005). These findings challenge the prevailing notion that motor complete SCI precludes voluntary neuromuscular control and reveal that spared motor pathways can support fine-tuned neural control strategies. By leveraging this preserved function, NeurOne provides a pathway for intuitive brain-computer interaction that could enhance neurorehabilitation and assistive technologies for individuals with tetraplegia.

12. **Ephaptic Coupling in Human Peripheral Motor Axons**

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Ephaptic (“to touch”) coupling occurs when an electric field in one nerve depolarizes an

adjacent nerve, especially in unmyelinated nerves where voltage activated ion channels are exposed. This phenomenon, although theorized to play a role in a variety of demyelinating disorders like Multiple Sclerosis, has only been demonstrated in animal studies. For example, stimulating sensory afferents in one peripheral nerve, can depolarize an adjacent peripheral sensory nerve in the dorsal column. In this study, we tested in humans if motor axons from one peripheral nerve can trigger action potentials in motor axons from another peripheral nerve that are both contained within the same peripheral nerve trunk. The common peroneal nerve (CPN) and the tibial nerve (TN) to the soleus muscle lie side by side in the main TN trunk. Percutaneous electrical stimulation was used to evoke antidromic action potentials in the CPN to determine if we could ephaptically activate orthodromic action potentials in the TN to the soleus muscle. Indeed, direct motor responses, M-waves, were evoked in the soleus muscle following CPN stimulation at a latency that would suggest ephaptic activation of the soleus motor axons in the main TN trunk. In addition, short latency M-waves were also evoked in the abductor hallucis muscle in the foot, which also contains motor axons in the main TN trunk, ruling out recording crosstalk. This study, for the first time, shows that synchronous activation of myelinated motor axons can ephaptically couple adjacent motor axons in the human.

13. **Giving the Motoneurons in Your Legs a Helping Hand – Concurrent Handgrip Contractions to Facilitate Motoneuron Excitability in the Legs**

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Estimates of persistent inward currents (PICs) in the lower limb are increased following handgrip contractions due to diffuse serotonergic projections throughout the central nervous system. However, since serotonin reuptake can occur quickly after contractions cease, we investigated whether motor unit firing patterns were sensitive to contraction intensity while dual tasking. High-density surface electromyography and blind source separation algorithms were used to record and decompose individual motor unit spike trains from the tibialis anterior (TA). Participants (n=14) performed triangle-shaped dorsiflexion contractions to a peak of 25% and 50% of their maximal voluntary contraction (MVC), whilst simultaneously performing a handgrip contraction of 20% or 60% of their handgrip MVC, as well as control trials without handgrip at each intensity. Discharge rate (DR) hysteresis was using the paired motor unit analysis technique (ΔF), which estimates the magnitude of PICs. DRs were higher during 50% compared to 25% contractions (ΔDR=7.65pps, $p<0.0001$). Across conditions, DR increased during the high-grip task (ΔDR=0.589pps) compared to control ($p=0.02$), but not compared to the low-grip task ($p=0.13$). ΔF also increased with contraction intensity (change in ΔF=1.63pps, $p<0.0001$) and both handgrip conditions relative to the control (change in ΔF from control to: low-grip=0.679pps; $p=0.0037$; and high-grip=0.962pps; $p<0.0001$), however there was no significant difference between the two handgrip conditions (high-grip=5.92pps; low-

grip=5.64pps; p = 0.3255). These findings suggest that while concurrent RVCs do affect excitability of leg motoneurons, intensity-dependent modulation is dampened, which suggests saturation of the interlimb effects during concurrent activity.

14. **The effect of acute normobaric hypoxia on H-reflex responses while standing**

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Postural sway increases when humans experience hypoxia, likely owing to alterations in sensorimotor function. Yet, the exact mechanisms are unknown. Muscle spindles, stretch sensitive sensory receptors, project onto alpha-motoneurons via Ia and II afferents and evoke reflexes important for standing. We aimed to determine if normobaric hypoxia influences the effectiveness of Ia afferents to excite alpha-motoneurons, using the H-reflex technique. Soleus surface electromyography was sampled from fourteen participants (25±7y, 6 females) while they stood quietly breathing normoxic (fraction of inspired oxygen [FIO2]=0.21) or hypoxic (FIO2=0.13) air from a facemask system. The maximal compound muscle action potential (Mmax) as well as maximal (Hmax) and one-half (½-Hmax) H-reflex amplitudes were evoked via tibial nerve stimulation at the popliteal fossa. Seven stimuli (five ½-Hmax and two Mmax) were delivered in a randomized order with one stimulation occurring every ten s (60-s trials). Stimuli were delivered at five timepoints (pre-hypoxia; 5, 30, and 60min of hypoxia; and 10-min post-hypoxia). To account for peripheral excitability, the ½-Hmax was normalized to the Mmax at a given timepoint. A time effect was detected for H/M area ($p<0.001$) and Mmax area ($p<0.001$). The Mmax area increased ($p=0.039$) from Pre (7.0±2.5mV·ms) to 60min (7.6±2.2mV·ms) of hypoxia. However, no post-hoc differences were found for H/M area ($p\geq0.110$) or other Mmax timepoint comparisons ($p\geq0.127$). These results indicate motoneuron excitability is not altered by acute normobaric hypoxia when assessed via the H-reflex. Thus, previously reported hypoxia-related decrements in postural sway may not be attributed to Ia afferent projections onto soleus motoneurons.

15. **Restricting Blood but not Gains: Blood Flow Restriction to Enhance Motor Unit Activity**

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Blood flow restriction (BFR) training has become a popular modality to enhance resistance

training outcomes in both athletic and clinical settings. BFR alters electromyographic (EMG) activity, but how BFR affects motor unit (MU) recruitment and firing rates units is relatively unexplored. To address this, we had participants perform isometric dorsiflexion contractions while 128-channels of high-density surface EMG (HDsEMG) were recorded from the tibialis anterior. Participants were divided in two; half completed the control protocol first; and the other half began with the BFR protocol. In both protocols, they first performed two maximal voluntary contractions (MVC), which were followed by 10 isometric contractions with a 5-second ascending portion, a 3-second hold at 50% MVC, and a 5-second descending portion. During the BFR protocol, a wireless BFR cuff was applied and inflated to 200 mmHg. After completion of the first protocol, 5-minutes of rest was given before the start of the second protocol. Preliminary results indicate that BFR caused a significant increase in motor unit firing rate (18.53 $\pm$ 0.251pps for control and 20.49 $\pm$ 0.250pps for BFR), and a tendency to recruit new units throughout the contractions. These results suggest that BFR stresses the neuromuscular environment, inducing a greater training stimulus at the same force output.

16. **Functional Current Mapping: An Efficient Minimal Neural State Space Realization for Guided Global Minimization**

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Parameter estimation in conductance-based models often utilizes global minimization algorithms over pre-established ion channel kinetics. Global minimization within this framework is limited by computational complexity and relies on the existence of an acceptable solution within the solution space. An acceptable solution is defined as an extremum of the objective function of sufficient coherence with experimental results. To tackle both issues we propose a novel method for state-space generation: Functional Current Mapping (FCM). FCM aims to generate a minimal neural state space realization in the Hodgkin Huxley formalization that assures the existence of an acceptable solution. The method focuses on mapping geometric features of experimental voltage trace data to typical Hodgkin Huxley kinetic descriptions. The kinetic descriptions are then passed into our selected global minimization algorithm: Parallel Tempering Markov chain Monte Carlo (MCMC). To test this method, we selected in vivo patch-clamp recording of mouse RORβ interneurons. Using FCM we produced three custom ion-channel kinetic models consisting of two sodium and a single potassium channel, resulting in a combined five state model. The model was able to reproduce key experimental features such as: dynamic spiking voltage threshold and a transient firing rate, which cannot be captured in other computational efficient modeling techniques such as the FitzHugh-Nagumo integration

and fire model. These results indicate a promising place for FCM within the global minimization pipeline for neural parameter estimation.

17. **Distribution of Motor Unit Modes Across Different Levels of Synaptic Inputs in Vastus Medialis and Vastus Lateralis Using Implanted Electrodes**

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Motor neurons receive common and independent inputs. At low forces the level of common input with respect to independent input is low, while with increasing force the amount of common input with respect to independent input is significantly higher. Previous papers showed that there may be two or more neural factors (motor unit modes) that control the vastii muscles. Here, we hypothesize that if multiple distinct inputs exist, they become more evident at high rather than low force levels. To investigate this, we implanted multiple bipolar intramuscular electrodes in the vastus medialis (VM) and vastus lateralis (VL) muscles to record motor unit (MU) activity. Participants performed multiple isometric contractions at different force levels, ranging from 5 to 30% of maximal voluntary contraction (MVC). Factor analysis and correlation-based clustering were applied to the merged MU pool of VL and VM to identify different motor unit modes. Preliminary results indicate that motor unit modes become more distinct with increasing force levels. At 30% MVC, we could clearly differentiate two motor unit modes. Both factor analysis and correlation-based clustering yielded similar and more stable results across trials, reinforcing the robustness of our findings. These results suggest that higher force levels are needed to noticeably differentiate motor unit modes between synergistic muscles and that the vastii receive two independent common inputs.

18. **Corticospinal Transmission to Spinal Motor Neurons During Repeated Transcranial Magnetic Stimulation at Different Frequencies**

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Repetitive transcranial magnetic stimulation (rTMS) is widely used in clinical and research settings, particularly for its potential in treating neurological disorders. While the effects of rTMS over the primary motor cortex (M1) have been broadly inferred from surface electromyograms (EMG), it remains unclear how cortical entrainment at different rTMS frequencies is transmitted to spinal motor neurons. This study addressed this gap by decomposing high-density surface EMG (HDsEMG) recorded during rTMS at varying

frequencies and intensities. Participants performed low-level isometric thumb flexion while HDsEMG was recorded from thenar muscles. Following a 30-s contraction without stimulation, rTMS was applied over the contralateral M1 for 30s at three intensities (50%, 60%, and 70% of resting motor threshold) and five frequencies (5, 10, 20, 30, and 50 Hz) in a randomized order. Motor unit spike trains were decomposed from HDsEMGs recorded before stimulation, and the obtained separation vectors were reapplied during the rTMS period. Corticospinal transmission was assessed by computing coherence between rTMS input and the cumulative spike train (CST) output, comparing it against shuffled CST input-output coupling. Our results demonstrated input-output coupling at the stimulus frequency only for rTMS ≥ 20 Hz, with greater coupling observed at 70% intensity. The relationship between intensity and input-output coupling was linear but restricted to rTMS frequencies ≥ 20 Hz. These preliminary findings suggest that rTMS-induced beta and gamma entrainment is evident in spinal motor neuron activity during voluntary isometric contractions, whereas corticospinal alpha oscillations (5–15 Hz) are not transmitted effectively to spinal motor neurons.

19. **Contraction intensity-dependent modulation of motor unit behaviour during rapid contractions**

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Unlike in isometric tasks with controlled increase in force output, high-force rapid contractions that maximise rate of force development (RFD) are accompanied by high initial motor unit (MU) discharge rate followed by a decay. However, it is unclear if such MU behaviour is dependent solely on the fast dynamics of synaptic input or whether it is also influenced by the extent of excitation. Furthermore, whilst slow isometric tasks in humans commonly display a negative relationship between MU discharge rate and their recruitment threshold, it is yet unclear whether fast onset of synaptic input disrupts this strategy. Here, we identified tibialis anterior MUs from multichannel EMG recordings during two unilateral isometric tasks at 40% and 80% of maximal voluntary force (MVF): rapid contractions aimed to maximise RFD, and controlled ramp contractions (10 and 40%MVF/s). MU behaviour was tracked across all contractions to facilitate identification of MU recruitment thresholds unimpeded by detection delays. We found that rapid contractions with high RFD were underpinned by faster MU recruitment speeds and greater peak MU discharge rates compared to ramp contractions, though the latter were intensity-dependent (80>40% MVF). The negative relationship between peak MU discharge rate and recruitment threshold observed during ramp contractions was preserved during rapid contractions regardless of contraction level ($r \leq -0.21$). These findings suggest that MU behaviour adapts to the dynamics of synaptic input, but the organisation of MU control remains stable, ensuring effective force modulation during different contraction tasks and intensities.

20. **The Influence of Single Degree-of-Freedom Tasks on Deltoid Motor Unit Behaviour**

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The complex movements of the shoulder require deltoid activation to adapt to both task and musculoskeletal alterations. Changes in muscle length have been reported to influence motor unit (MU) behaviour and common synaptic inputs to the alpha motoneurons. In this study, we evaluated MU discharge behaviour and MU-MU coupling (coherence) during single degree-of-freedom isometric contractions while positioning the deltoid at different muscle lengths. Subjects performed isometric contractions in two directions (lateral shoulder abduction and shoulder flexion) at 5%, 10%, 20% and 30% of a maximal voluntary contraction. Tasks were performed with the deltoid positioned at two angles resulting in alterations to the muscle length: shortened (10° abduction/flexion) and lengthened (60° abduction/flexion). High-density surface electromyograms recorded from the deltoid using two 64-electrode grids were decomposed into MU discharge times where MU mean discharge rate (MUDR), MU discharge rate variability (CoV-ISI) and MU-MU coherence within the delta, alpha and beta bands were calculated. Preliminary results of this study illuminate the effect of altering movement direction (abduction vs flexion) on MUDR and MU-MU coherence. Additionally, according to previous reports, changes in deltoid muscle length influences both the CoV-ISI and MU-MU coherence which is force dependent. Therefore, altering muscle length and shoulder angle will result in changes in commonality between MU spike trains which have significant implications on the neural strategies utilized to perform shoulder tasks. These changes may have meaningful repercussions in clinical populations when prescribing rehabilitation therapy.

21. **An In Vitro Model System to Study Neuromuscular Junction Formation and Function Using Human iPSC Derived Motor Neurons**

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by neuromuscular junction (NMJ) dysfunction and motor neuron death. Multiple in vitro model systems using induced pluripotent stem cells (iPSCs) from ALS patients have been used to study the pathophysiology of motor neurons during disease progression in culture. However, few in vitro studies have examined NMJ function. It is important to study NMJ function in ALS as its dysfunction is an early pathognomonic characteristic of the disease. To develop a simple in vitro model system to examine NMJ pathophysiology, and to determine how neuronal activity affects NMJ function, we cultured induced pluripotent stem cell-derived motor neurons (iPSCMNs) with a C9orf72 mutation, or control iPSCMNs,

with chick myofibers. All iPSCMNs were previously transduced to express channelrhopsin2 (ChR2). Co-cultures were chronically light activated at 0.025 Hz for up to 3 weeks or left unstimulated. To date, our results show muscle fibres visually contracted in all co-culture conditions when exposed to a pulse of blue light indicating that they all formed functional NMJs. However, iPSCMNs with the C9orf72 mutation were less likely to form anatomically visible NMJs compared to control iPSCMNs even though they induced more acetylcholine receptor clustering. Chronic light stimulation increased muscle fiber size and the conversion of fast to slow muscle fiber phenotypes compared to the non-stimulated cultures. These results indicate that this simple iPSCMN/muscle fiber co-culture system can be used to examine how NMJ formation and function varies between genetically different iPSCMNs and how increased neuronal activity affects these processes.

22. **The Indicator of Synaptic Input Used to Quantify Onset-Offset Hysteresis from Multiple Motor Units Affects Its Sensitivity to Neuromodulation and Synaptic Excitation-Inhibition Coupling**

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Motoneuronal persistent inward currents (PICs) are facilitated by neuromodulatory inputs and suppressed by inhibitory synaptic inputs. The PIC amplification and prolongation of excitatory synaptic input introduce onset-offset hysteresis in motoneuron discharge that is often used to estimate PIC magnitude. In humans, the paired motor unit (MU) analysis technique, which is used to quantify onset-offset hysteresis, has been growing rapidly with the wide adoption of high-density electromyography. When identifying multiple MUs simultaneously, several lower-threshold (reporter) units exist for each higher-threshold (test) unit. The proposed approaches for addressing this challenge include calculating ΔF for all possible MU pairs (pairwise), averaging test-unit ΔF values for multiple reporter units (unitwise), and constructing a single reporter unit from multiple low-threshold MUs (composite). To investigate the sensitivity of the three ΔF approaches to the mediators of PICs, we simulated 459 motor pools of 20 motoneurons with known neuromodulation and inhibition patterns. We employed a recruitment threshold distribution and excitation pattern to replicate those observed in human recordings during a triangular contraction to 30% maximal torque (101 participants, 246 contractions, 4392 MUs). Data suggest that the composite approach exhibits lower ΔF values and reduced sensitivity to neuromodulation and inhibition patterns compared to pairwise and unitwise approaches. In human data, ΔF values were also lower with the composite compared to the unit- and pairwise approaches; notably, the calculations were only feasible in 86/246 contractions compared to 239, respectively. These data highlight sensitivity and feasibility considerations for the commonly used approaches to quantify onset-offset hysteresis.

23. **Investigating Lower Motor Neuron Function and Dysfunction In Vivo Using Axonal Excitability Techniques and the TROND Protocol**

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Axonal excitability techniques have been deployed to examine the underlying mechanisms of pathology in a wide range of lower motor neuron disorders. Axonal excitability testing is easy to perform and very reproducible. Testing and tracking the threshold for activation of a single motor unit (or a fraction of a CMAP) provides a simple analogue of membrane potential. The excitability of the axonal membrane is very sensitive to membrane potential, ion channel function and the structural integrity of the axo-glial interface and provides much more information than conventional nerve conduction studies. Axonal excitability testing is non-invasive and is a bit like an in vivo analogue of the current, voltage and patch clamp recordings pioneered by Hodgkin and Huxley. The TROND protocol was introduced to provide a rapid assessment of membrane potential and ion channel function and has been widely used to study the mechanisms of neuropathology. I'll give an introduction to the technique and TROND protocol with some examples of its use to probe membrane potential, ion channel changes and disruption of the paranodal seal in disease.

24. **Associations Between Neural and Peripheral Properties of the Motor Units from High-Density Intramuscular and Surface Electromyography in Hand and Leg Muscles**

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Motor units (MUs) are recruited based on size, with larger neurons innervating larger muscle fibers, thereby linking recruitment to MU action potential (MUAP) properties, such as amplitude. The distribution of muscle fiber innervation numbers and other physiological properties of motor units follows an exponential pattern, where fewer motor units control a large number of muscle fibers. However, studies using surface electromyography (sEMG) report linear correlations between recruitment threshold (RT) and action potential features, despite the fact that the distribution of muscle fiber innervation numbers in several muscles appears to follow an exponential trend. Here we examine the relation between MUAP features and RT using 16-channel intramuscular EMG (iEMG) and high-density sEMG. We recorded from the vastus lateralis (VL) and first dorsal interosseous (FDI) during ramp contractions up to 90% MVC, performed by two participants per muscle. We extracted MU firings from iEMG and used them for spike-triggered averaging in sEMG.

Preliminary results suggest that MUAP amplitude strongly correlates with RT, following an exponential pattern across both sEMG and iEMG. Applying iEMG-derived MU spikes to sEMG reveals stronger correlations than previously reported with sEMG decomposition. The coefficient of determination was higher for sEMG MUAP amplitude (VL: R^2=0.94±0.07, #MU=16±8; FDI: R^2=0.75±0.22, #MU=34±2) than for iEMG (VL: R^2=0.79±0.11; FDI: R^2=0.68±0.11), indicating a stronger dependence on surface-recorded signals. These preliminary findings indicate that MUAP features are strongly linked to RT, highlighting their role in recruitment dynamics. Volume conduction may enhance these correlations in sEMG, offering insights for refining neuromuscular models and improving EMG interpretation.

25. **Neural Basis for the Production of Facial Expressions**

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Both human and non-human primates use facial expressions to communicate their emotions and intentions. The primate amygdala, by virtue of its vast connectivity to sensory association and prefrontal cortices, is involved in the evaluation of facial expressions made by conspecifics. Efferent projections from the amygdala, in turn, may instigate activity in a set of brain structures that culminates in the coordinated engagement of face motor nuclei producing facial expressions. These motor pathways, however, are unknown. In preliminary studies, we have used high density electrodes to record population neural activity simultaneously in the amygdala and lateral primary motor cortex while awake macaques made natural facial expressions in response to emotionally relevant stimuli. We also recorded from these structures while the monkey made voluntary facial actions, such as pursing the lips toward juice tubes or during operantly conditioned mouth opening. Thus far, we have found distinct neural states in the amygdala that precede and are linked to the production of different emotional but not voluntary facial expressions. Conversely, we observed little organized activity in face motor cortex during emotional expressions but clear-cut neural states associated with different voluntary actions. These findings (together with classical clinical observations) imply a non-cortical pathway for the production of facial expressions.

26. **Exploring Sex Differences in Motor Unit Discharge During an Absolute Dorsiflexion Torque Output Across Joint Angles**

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Daily living requires the production of fixed forces (i.e., moving absolute loads). Females are typically weaker and rely on exerting greater forces relative to their maximal voluntary contraction (MVC) compared to males. At moderate relative forces, steadiness is improved compared to low forces and this could diminish sex-related differences if compared at the same absolute force output. This study explored torque steadiness and motor unit (MU) discharge properties between sexes at XYZ of dorsiflexion at three joint angles (80, 95, 110 degrees). It was hypothesized that; 1) torque steadiness and coefficient of variation of interspike interval (CVISI) of the MU discharge would not differ between sexes; and 2) MU discharge rate (MUDR) would be greater in females compared to males. 20 young adults (10 of each sex, 23.5±4.37years) held isometric dorsiflexion contractions for 20-seconds and preliminary data are presented for 10 participants (5 of each sex). The males (19.7±7.6% MVC) exerted lower relative torque than the females (27.5±4.2% MVC) across the joint angles tested. Torque steadiness, MUDR and CVISI did not differ between sexes ($p \geq 0.44$) but there was a significant interaction between sex and joint angle for MUDR. In females, MUDR did not differ between 80 and 95 degrees (~14pps), whereas, in males, MUDR was higher at 80 (14pps) than 95 degrees (12.6pps) ($p=0.000023$). At absolute loads, when relative torque is higher in females, torque steadiness and MU discharge does not significantly differ between the sexes, but males are more responsive to muscle length-dependent MUDR modulation.

27. **Unlocking the Role of Dopaminergic Medication in Modulating Force Control and Motor Unit Properties in Parkinson's Disease**

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Parkinson's disease (PD), a progressive neurologic disorder, is characterized by motor symptoms including tremor, rigidity and bradykinesia, which can respond to dopaminergic treatment. Treatment benefit is varied, and patients can exhibit "ON-OFF" treatment response fluctuations. It is unclear whether or how dopaminergic treatment influences motor unit (MU) behavior (a key contributor to motor output) and force control during ON-OFF fluctuations. This study investigates biceps brachii MU properties and elbow flexor force steadiness (FS) in participants both ON and OFF dopaminergic medication. Sixteen people with PD participated in two separate sessions; ON and OFF dopaminergic medication. Isometric elbow flexion 1.5 sec ramp up and down contractions with a 25 sec plateau were held constant at 5, 10, 15, and 25% of MVC and the least steady 10 sec was assessed using the coefficient of variation (CV) of force. 64-channel high-density surface EMG was recorded and decomposed into contributions of individual MUs. MU recruitment threshold (RT), MU discharge rate (MUDR), and the CV of the interspike interval (CVISI), were quantified. During the ON phase, CV of force (10%:3.1±1.3; 15%:2.3±1.0) and CVISI (10%:18.9±11.5; 15%:16.9±7.4) were significantly lower (10%:3.8±1.8; 15%:3.0±1.2) and less variable (10%:22.0±13.2; 15%:21.1±11.7; $p<0.05$) than OFF phase. Statistical

significance was not observed in MUDR and RT at any of the force levels ($p>0.05$). Dopaminergic treatment enhances force control in PD by reducing the variability of motor unit discharge, supporting its role in motor control.

28. **Muscle Strength is Associated with Estimates of Motor Neurone Persistent Inward Currents in Amyotrophic Lateral Sclerosis**

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterised by muscle weakness that progressively spreads across multiple body regions, leading to paralysis and, generally, death from respiratory failure. Despite the potential benefits of monitoring motoneuron behaviour to understand disease progression and improve treatment strategies, there are currently no reliable neurophysiological biomarkers of motoneuron degeneration. High-density surface electromyography (HDs-EMG) during voluntary contractions can be used to estimate the contribution of persistent inward currents (PICs) to motoneuron firing, based on the recruitment–de-recruitment hysteresis (ΔF) calculated with the paired-motor unit analysis. Scales of muscle strength are used as a proxy of disease progression to determine the ALS staging rather than relying solely on "time from diagnosis" due to individual rates of progression. Thus, we aimed to determine the association between ΔF and the Medical Research Council (MRC) Scale for muscle strength. We assessed 77 individuals at different ALS stages. MRC Scale determined tibialis anterior strength while HDs-EMG obtained motor unit data to calculate ΔFs. Participants performed two 30-s isometric triangular-shaped contractions up to 40% of their maximal EMG amplitude on each leg, with a 64-channel high-density electrode grid placed on the tibialis anterior. Robust linear mixed model indicated that for each one-score reduction in MRC, ΔF changes by 0.27 units (95%CI: 0.052 to 0.492, $t = 2.43$). No association was found between MRC and peak discharge rate or brace height. ΔFs were associated with MRC scores, suggesting a reduction in motoneuron recruitment-de-recruitment hysteresis accompanying muscle weakness and disease progression.

29. **Combining Transcutaneous Spinal Cord Stimulation and Neuromuscular Electrical Stimulation: Are Two Techniques Better Than One?**

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Neuromuscular electrical stimulation (NMES) is delivered through the skin over nerves or muscles. NMES can produce contractions by activating motor axons, resulting in an M-wave, or sensory axons, resulting in an H-reflex. NMES can help restore function for people with a spinal cord injury (SCI), but relatively small contractions and rapid contraction fatigability limit its clinical application. Transcutaneous spinal cord stimulation (tSCS) is delivered non-invasively through the skin of the back to target the spinal cord. tSCS is thought to facilitate voluntary movements in individuals with SCI by increasing spinal excitability, thereby "boosting" weak voluntary signals from the brain. The goal of my research was to examine the effect of combining tSCS with NMES (tSCS+NMES) on contraction amplitude and spinal excitability. We predicted that tSCS+NMES would generate larger contractions and H-reflexes than NMES alone. NMES was delivered to the tibial nerve at 20Hz (7s "on" 10s "off") in 12 participants with no history of neuromuscular injury/disease. NMES was delivered alone and paired with tSCS at three intensities (0.6x-, 0.8x-, and 1.0x-spinal-evoked response threshold) and two frequencies (30Hz and 100Hz). Contraction amplitudes and soleus H-reflexes were compared between NMES alone and tSCS+NMES trials. Contraction amplitudes (i.e., average area under the curve) across the group were not significantly different with tSCS "on" or "off" regardless of tSCS intensity or frequency. H-reflexes analyses are in progress. These results suggest that tSCS+NMES is not better than NMES alone and raises questions about the extent to which tSCS "boosts" voluntary contractions by increasing spinal excitability.

30. **Transcutaneous spinal cord stimulation begins exciting soleus motor neurons when delivered at 70% resting threshold during plantar flexion contractions**

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Transcutaneous spinal cord stimulation (tSCS) can improve voluntary movement for people experiencing paralysis. During tSCS, pulses of electrical stimulation are delivered over the spinal cord, targeting afferents in the dorsal roots. tSCS intensity is often set relative to threshold for evoking a short-latency excitatory response (SER) in relaxed muscles, thought to reflect the depolarisation of dorsal root afferents. However, tSCS is typically delivered below this "resting" threshold, making it difficult to know if it actually recruits any dorsal root afferents. The purpose of this study was to determine the intensity, below resting SER threshold, at which tSCS begins recruiting dorsal root afferents and influencing the soleus motor pool. 13 participants held a small soleus contraction while tSCS was delivered at 0.3 Hz over the lumbar enlargement. In separate trials, 100 pulses were delivered at intensities between 50%-100% resting SER threshold. In an additional trial, tSCS was delivered 10 cm left of the tSCS electrode at 100% SER threshold. tSCS delivered at 60% SER threshold and below, or 10 cm off the midline, did not evoke any response in soleus in most participants. tSCS delivered at 70% SER threshold and above,

however, did produce an SER and this was absent during lateral stimulation. The lower SER threshold during the contraction than at rest supports the reflexive origin for the SER and suggests that tSCS begins recruiting dorsal root afferents when delivered at 70% of resting threshold. We propose that tSCS delivered below this intensity is not “spinal cord” stimulation.

31. **Multi-Timescale Neural Adaptation Underlying Long-Term Musculoskeletal Reorganization**

ROLAND PHILIPP (1,2), YUKI HARA (3), NAOHITO OHTA (1,2), NAOKI UCHIDA (1,2), TOMOMICHI OYA (1,4), TETSURO FUNATO (2), KAZUHIKO SEKI (1)

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This study investigates the mechanisms by which the central nervous system (CNS) adapts to long-term musculoskeletal system alterations. A tendon transfer model in non-human primates was employed, surgically relocating finger flexor (FDS) and extensor (EDC) muscles. This allowed for the investigation of how the CNS modifies its strategy for controlling finger movements in response to these musculoskeletal changes. Muscle activities during grasping tasks were measured to track the adaptive processes. Two months following the surgical procedure, the monkeys demonstrated a significant recovery of grasping function, despite the initial disruption caused by the tendon transfer. Our findings indicate a two-phase adaptation process. The initial phase focuses on enabling functionality with the newly transferred muscles, allowing the monkeys to regain a degree of grasping ability. However, this initial solution is not the final stage of adaptation. In the subsequent phase, the CNS refines its control strategy, abolishing the previously enabled function and restoring a more efficient and sufficient control strategy that closely resembles the original pre-surgery state. These results highlight a multi-phase CNS adaptation process with distinct time constants in response to sudden bodily changes, offering potential insights into understanding and treating movement disorders. These results offer potential insights into the underlying mechanisms of movement disorders and could pave the way for novel therapeutic approaches. By understanding how the CNS adapts to internal bodily changes, we may be able to develop targeted interventions that facilitate and optimize the recovery of motor function in individuals with movement impairments.

32. **Cutaneous information is processed differently than muscle proprioception by the cuneate nucleus**

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Cutaneous and muscle proprioceptive mechanoreceptors provide critical sensory information to the primary somatosensory cortex (SI) for voluntary hand function in primates. The thalamus relays sensory signals from the spinal cord to SI—but the less-known cuneate nucleus (CN) of the brainstem, in fact, critically relays information from the dorsal root ganglion to the thalamus. Given that the CN is known to modulate haptic signals in the cat, we investigated if it also modulates upper limb cutaneous and muscle proprioceptive signals in primates. To test for differential and contextual modulation in the cuneate nucleus on the way to SI during voluntary wrist flexion and extension movements. We recorded stimulation-evoked local field potentials (SEPs) from the cuneate nucleus (8 electrodes) and the SI (32 electrodes) during 1 ms cuff-electrode stimulation pulses to the sensory (i) deep radial nerve (DR, mostly muscle proprioceptive) and (ii) superficial radial nerve (SR, cutaneous) in during voluntary wrist flexion and extension (Kubota et.al. (2024)). The average SEPs have distinct shapes across subregions of CN and SI, and across tasks. During SR stimulation (cutaneous), with a larger initial negative peak in SEPs, compared to DR stimulation (muscle proprioceptive). Cross-correlations between CN and SI are lower for DR stimuli and tend to peak after cross-correlations for SR stimuli during both flexion and extension. Lower correlations for DR may suggest proprioception is more weakly represented in SI compared to tactile information. This may help explain the subconscious nature of muscle proprioception. Funding support: NSF US-Japan CRCNS to FVC. JSPS 26120003, 26250013, 23H05488, and 24K21313 to KS.

33. **Assessing the Robustness of the CMAP Scan Algorithm (MScanFit) for Motor Unit Number Estimation**

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Motor Unit Number Estimation (MUNE) techniques have evolved significantly since first described by McComas in 1971. The Compound Muscle Action Potential (CMAP) scan, introduced in 2007, represents the latest advancement in MUNE methodologies. The MScanFit algorithm, proposed by Bostock in 2016, analyzes CMAP scans to estimate both the number and size of motor units. While extensively applied, particularly in amyotrophic lateral sclerosis (ALS) research, MScanFit's accuracy has yet to be independently replicated and validated across diverse pathophysiological conditions. Our study independently recreated Bostock's generative computational model based on his original publication to validate and extend its findings using simulated CMAP scans. Using a repeated-measures design, we systematically varied three physiological factors in these simulations: motor unit degeneration (random or selective), reinnervation pattern (random, selective, distributive), and neuromuscular resilience (0%, 20%, or 60% collateral sprouting). Accuracy of motor unit number and size estimates was evaluated across

progressive degeneration from 160 to 5 motor units. Estimation statistics revealed that MUNE accuracy was unaffected by the type of degeneration or neuromuscular resilience. However, accuracy varied significantly with reinnervation patterns and improved as the disease progressed and fewer motor units remained. Our findings highlight that while MScanFit has inherent limitations, particularly in early disease stages or with varied reinnervation strategies, it remains a valuable tool for tracking disease progression. These results support the utility of CMAP scan-derived MUNE as a response biomarker, sensitive to therapeutic interventions during advanced stages of neuromuscular degeneration.

34. **Electrophysiological Characterisation of Spinal Microcircuits Controlling Urinary Function**

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Bladder voiding is controlled by the coordinated actions of supraspinal and local spinal circuits. Voiding is initiated at the brain stem level (Barrington's nucleus) and coordinated primarily by lumbosacral spinal circuits involving autonomic and somatic sphincter motoneurons, and autonomic motoneurons innervating the bladder wall smooth muscles. As the bladder is filled, sphincter motoneurons increase their output to ensure urine storage. In response to bladder pressure, voiding starts voluntarily or reflexively with coordinated bladder contraction concomitant with sphincter relaxation often manifesting as bursts. However, several experimental limitations have prevented progress in understanding this physiology: 1) the muscles involved in model organisms are small and poorly accessible; 2) spinal circuits are often studied at juvenile ages when micturition control is far from fully developed. We optimised a method in mice to inject both the sphincter muscle and intramural ganglia of the bladder to label autonomic and somatic motoneurons and obtain whole-cell patch clamp electrophysiology recordings of identified pelvic adult motoneurons in vitro. Moreover, we optimised high-density EMG recordings (using myomatrix electrodes) from sphincter muscles during bladder filling and voiding to study motor unit functional properties. The definition of these properties in normal micturition could set the stage for the identification of new targets to mitigate urinary dysfunction.

35. **Possible investigation of motoneuron discharge characteristics in presymptomatic amyotrophic lateral sclerosis**

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Amyotrophic lateral sclerosis (ALS) is a rare disease of unknown etiology and without established effective treatment. One of the factors complicating research is the extremely long presymptomatic period of ALS. Fortunately, recent studies of the MN discharge properties in patients with early symptomatic stages of ALS have revealed a prodromal period of mild impairment of the motor system. During this period, the several measures of MN excitability are increased, including shortening of AHP duration. Notably, AHP duration of a MN is correlated with the twitch duration of muscle fibers innervated by this MN. At the last meeting in Bordeaux, I presented a hypothetical evolution of the MN AHP during the course of ALS. This hypothesis was consistent with the results of animal experiments showing a gradual change in muscle fiber type associated with a change in the MN discharge properties during the process of denervation and reinnervation. Unfortunately, this hypothesis is based on rather sparse experimental data. I suppose that verification of this hypothesis in a larger cohort of patients could expand our knowledge of the presymptomatic course of ALS and facilitate the identification of groups of patients who could be effectively treated. I believe that such verification could be carried out in many clinics around the world after agreement on a common research protocol. I intend to present an outline of such a protocol during the IMNS meeting.

36. **Motor Unit Behavior in Individuals With and Without Clonus Following Spinal Cord Injury**

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Ankle clonus is a common involuntary motor behavior following Spinal Cord Injury (SCI), characterized by rhythmic muscle contractions and joint oscillations. Clonus has been primarily attributed to increased spinal reflex excitability and reduced descending inhibition after SCI, but the exact mechanisms are not well understood. It has been suggested, but not confirm in humans, that the recovery of motoneuron Persistent Inward Currents (PICs) may contribute to the development of clonus. To explore this, we collected High-Density Surface Electromyograms from Tibialis Anterior (TA) and Soleus muscles in participants with SCI (5 with clonus, 5 without clonus) and 5 non- injured participants. Each participant performed triangular submaximal isometric dorsiflexion and plantarflexion contractions to a peak of 30% of their maximum voluntary contraction, and blind source separation algorithms were used to identify motor unit (MU) spike times. We quantified firing rate hysteresis using the paired-MU analysis technique (ΔF) to estimate PIC amplitude. Preliminary analyses show peak firing rates were higher in both muscles for No-Clonus and Non-Injured participants compared to Clonus participants. For SCI participants without clonus, ΔF values were significantly larger in the TA (4.13 ± 0.502 pps) compared to the soleus (1.90 ± 0.504 pps, p=0.007), but there were no significant differences between muscles (Sol=1.69 ± 0.482, TA=3.00 ± 0.463 pps, p=0.10) in SCI participants

with clonus. These results suggest that altered intrinsic motoneuron properties across motor pools after SCI could contribute to the development of ankle clonus in some individuals. Participant recruitment and analysis is ongoing and will be presented.

37. **Altered Motor Unit Behaviour and Torque Steadiness in Athletes with Patellofemoral Pain during Single- and Multi-Joint Exercises**

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This study aimed to evaluate differences in vasti muscle motor unit (MU) firing between athletes with patellofemoral pain (PFP) and asymptomatic controls during submaximal isometric knee extension contractions performed during single-joint (knee extension) and multi-joint (leg press) exercises. Ten athletes with PFP and ten asymptomatic controls performed contractions ranging from 10% to 70% of maximal voluntary isometric contraction (MVIC). High-density surface electromyography was used to assess MU discharge properties of the vastus medialis (VM) and vastus lateralis (VL), and torque steadiness was quantified using the coefficient of variation of torque. Neural drive to the vasti muscles was similar between groups; however, athletes with PFP demonstrated significantly reduced torque steadiness during single-joint compared to multi-joint exercises, accompanied by higher pain intensity and increased MU discharge rate variability in the vasti muscles, particularly of the VL at higher torque levels (50-70%MVIC). Additionally, MU firing-torque relationships revealed compensatory recruitment strategies in athletes with PFP, indicated by significantly lower cross-correlation values during multi-joint exercises compared to the asymptomatic controls. Moreover, the increased MU firing-torque relationship observed during single-joint exercises in athletes with PFP was primarily driven by heightened contribution from the VM muscle. This study shows, for the first time, that athletes with PFP have impaired control of muscle force output and increased MU firing variability, especially under isolated joint conditions with higher mechanical demands. These MU adaptations likely reflect neuromuscular adjustments to ongoing PFP, acting as compensatory strategies to maintain force production despite compromised motor control and potentially reducing pain during multi-joint exercises.

38. **Supraspinal Integration of Proprioception: A Crucial Factor in Homolateral Leg Coordination**

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Stable locomotion requires precise foot placement, which in bipedal humans is guided by

visual input. However, in quadrupedal mammals, posture likely limits visual step guidance, particularly for the hind limbs. Here, we investigate whether proprioceptive feedback from the homolateral forelimb, rather than vision, determines hind limb foot placement after the swing phase in quadrupedal mice. We recorded electromyographic (EMG) activity from multiple hind limb muscles during treadmill locomotion with and without perturbation. Perturbation was induced via saphenous nerve stimulation to elicit the 'stumbling corrective reaction'. Kinematic analyses measured hind limb foot placement at swing termination relative to forelimb foot placement at stance termination. We further employed Egr3-KO mice, which lack muscle spindles, as well as chemogenetic and optogenetic approaches to manipulate supraspinal centers. Our findings reveal that hind limb foot placement aligns with the forelimb foot during locomotion, but only under perturbation conditions. This mechanism is dependent on proprioceptive feedback from muscle spindles and requires supraspinal processing, as propriospinal circuits alone are insufficient. Specifically, we identify the cuneate nucleus in the medulla oblongata as a critical node in this supraspinal proprioceptive pathway. This study uncovers a proprioceptive mechanism that ensures safe hind limb placement in response to perturbations and identifies the supraspinal pathway underlying homolateral leg coordination in quadrupedal locomotion.

39. **History-dependence of force affects motor unit firing behaviour**

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Following active lengthening or shortening contractions, isometric steady-state force is higher and lower, respectively, compared to fixed-end isometric contractions at the same muscle length and level of activation. Active lengthening increases steady-state force, a phenomenon known as residual force enhancement (rFE), while active shortening decreases it (i.e., residual force depression (rFD)). While the mechanisms underlying history-dependence of force are well studied at the muscle level, less is understood about history-dependent property effects on motor unit (MU) firing patterns, and estimates of intrinsic motoneuron properties. This study aimed to investigate prolongation of MU firing following active lengthening and shortening contractions and determine if motoneuron excitability is altered during rFE and rFD. Participants performed active lengthening (85° to 115°; rFE) and active shortening (115° to 85°; rFD) contractions followed by isometric holds at 30% of their maximal voluntary contraction. Using high-density surface electromyography, we identified MU spiking activity with a convolution kernel compensation algorithm during isometric and isokinetic dorsiflexion. Isometric contractions at 85° and 115° were concatenated with rFD and rFE trials to identify common MUs across conditions. We then used paired MU analysis to quantify discharge rate hysteresis (ΔF). There was activation reduction during rFE and activation increase during rFD. MUs displayed greater prolongation of firing and lower discharge rates after active

lengthening, with the opposite observed following active shortening. This suggests that motoneuron excitability is increased during rFE, and reduced during rFD, which may have implications for the control of forces in the real-world.

40. **Sequential vs. synchronous wide pulse high frequency neuromuscular electrical stimulation: impact on force development and fatigability**

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Neuromuscular electrical stimulation (NMES) is widely used for (re)training purposes, but discomfort and exaggerated fatigability limits its use in frail populations (Maffiuletti et al. 2018). The use of wide pulse (1 ms), high frequency (100 Hz) stimulation (WPHF-NMES) may overcome these limitations, as it favours reflexive motor unit recruitment that may lead to a progressive increase in force production during the stimulation ('extra force') (Popesco et al. 2024). Spatially-distributed sequential NMES has also been proposed to reduce fatigability, presumably through reduced motor unit discharge rate (Bergquist et al. 2017). However, the potential effectiveness of sequential WPHF-NMES has not been tested yet. The aim of this study was to explore the effects of sequential WPHF-NMES on maximal evoked torque and fatigability as compared to synchronous WPHF-NMES at the knee extensor level. Fifteen healthy participants (26±3 years) took part in two experimental sessions (sequential vs. synchronous WPHF-NMES) comprised of maximal electrically-induced evoked force determination, a series of 20x10s NMES trains and the evaluation of knee extensor neuromuscular function (maximal voluntary contraction (MVC) force, activation level and contractile properties). Maximal evoked force was comparable between both stimulation modalities (sequential:53±14 vs. synchronous:63±24% MVC force, $p>0.05$). When targeting 20% MVC force, extra force was higher in response to sequential stimulation (7.2±9.4 vs. 1.7±5.3% MVC force, $p<0.05$). The reduction in evoked force was attenuated in response to the 20 NMES trains (sequential:-53±24% vs. synchronous:-76±25%, $p<0.05$). In conclusion, sequential stimulation may increase the effectiveness of WPHF-NMES in intervention programs by increasing extra force and mitigating fatigability.

41. **MyoGestic: EMG Interfacing Framework for Decoding Multiple Spared Motor Dimensions in Individuals with Neural Lesions**

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Restoring motor function in individuals with spinal cord injuries (SCI), strokes, or amputations is a critical challenge, as it directly impacts their quality of life and independence. Despite assistive hardware becoming comparable to their biological counterparts, achieving intuitive and adaptive control of assistive devices remains a hurdle. Recent studies show that spared motor neurons can still be voluntarily controlled using surface electromyography (EMG), even without visible movement. Translating these findings breakthroughs into the next generation of assistive devices requires a platform that not only identifies and leverages the manifolds of spared motor activity for control algorithms but also facilitates continuous refinement — a missing cornerstone in the myocontrol community. To bridge this gap, we developed MyoGestic — an open-source framework that adapts machine learning algorithms to users' motor capabilities while ensuring ease of development for myocontrol researchers. Using MyoGestic, we successfully decoded the spared movement manifolds in real-time and mapped them into motor intent from two participants with traumatic SCI, two with spinal stroke, and three with amputations using a 32-channel EMG bracelet. Additionally, we achieved the same with two SCI participants using custom multi-wire iEMG implants, enabling multiple controllable motor dimensions within minutes. The decoded neural signals could control a digital hand, an orthosis, a prosthesis, or a 2D cursor. These experimental scenarios — spanning diverse acquisition systems, algorithms, and output devices — demonstrate MyoGestic's ability to meet the growing demands of the myocontrol field. By providing a versatile and accessible platform, MyoGestic invites collaboration across disciplines and accelerates the translation of discoveries into clinical and everyday applications.

42. **MyoGen: An Open-Source Framework for Realistic In-Silico Modeling of Spinal Motor Neurons, Motor Units, and Electromyography**

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Advancements in understanding and restoring sensorimotor functions through EMG-based interfaces require robust tools to model and interpret the input–output functions of motor unit activity across diverse physiological and technical conditions. For example, the modeling of intrinsic motor neuron properties, development of myocontrol algorithms, and validation of EMG decomposition methods have all been hindered by the lack of flexible and accessible simulation platforms that span from detailed intrinsic motor neuron activity to motor unit force and EMG generation. To address this need, we introduce MyoGen, an open-source, realistic motor neuron, motor unit, and EMG simulation framework that supports both intramuscular (iEMG) and surface (sEMG) modalities. MyoGen generates motor neuron spiking activity using a realistic human and feline motor neuron model

(Watanabe & Kohn, 2015), and EMG signals by integrating the well-established volume conductor model by Farina et al. (2004) with the advancements introduced by Konstantin et al. (2019) for multi-channel EMG simulation. Unlike other simulation tools typically tuned to cat data, MyoGen is pre-tuned with parameters aligned with experimental motor unit data from human intramuscular and surface EMG recordings. Fully developed in Python, MyoGen offers modularity and extensibility, facilitating seamless integration with existing pipelines in motor unit physiology, machine learning, control systems, and signal processing. Designed for broad applicability, it generates ground truth data for EMG decomposition algorithms, benchmarks myocontrol strategies under controlled conditions, and can model cortical and spinal inputs (Watanabe & Kohn, 2015) through interactive simulations. By offering biologically precise yet accessible simulations of neuromuscular dynamics, MyoGen lays the foundation for reproducible research and cross-disciplinary collaboration in neural engineering, rehabilitation, and motor neuroscience.

43. **Human Motor Unit Firing Patterns During Slow Lengthening and Shortening Contractions**

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While motor unit (MU) firing patterns are well-studied in the tibialis anterior (TA) during isometric conditions, behaviour in dynamic conditions is not well-characterised. Due to increased sensory feedback during dynamic output, we hypothesized alterations in MU firing patterns compared to isometric contractions. Isometric dorsiflexion MVCs were performed at 80°, 90°, and 100° ankle angles to generate a predictive algorithm to normalise torque feedback, help with MU identification, and maintain relative effort across joint angles. Participants then performed triangular dorsiflexion contractions to 30% and 50% MVC, both isometric and while an isokinetic dynamometer moved the ankle joint from 80° to 100° (eccentric) or 100° to 80° (concentric). High-density surface electromyograms from the TA were decomposed into MU spike trains, and common MUs were tracked by concatenating and transferring MU filters across isometric joint angles and dynamic tasks. Peak firing rates were higher during concentric (50%: 22.6 [21.9, 23.2] pps; 30%: 18.1 [17.5, 18.7] pps) compared to the eccentric (50%: 20.9 [20.3, 21.6] pps; 30%: 16.4 [15.8, 17.0] pps). Input-output hysteresis (i.e., ΔF) was condition- and intensity-dependent. ΔF was higher during 50% (4.89 [4.47,5.3] pps) compared to 30% (4.30 [3.94,4.65] pps), but only differed significantly between contraction types at 50% (eccentric: (5.02 [4.45,5.58] pps; concentric: 4.25 [3.73,4.77] pps). Brace height (BH) was intensity-dependent (50%: 28.9 [26.9,30.9] %rTri; 30%: (33.8 [32.3,35.3] %rTri), but there were no significant differences across contraction types. These findings suggest that differences in firing patterns during dynamic actions may result from altered patterns of inhibition, rather than neuromodulatory input.

44. **Perisynaptic Schwann cells cannabinoid type-1 (CB1) receptors promote neuromuscular junction repair following peripheral nerve injury**

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Perisynaptic Schwann cells (PSCs), glial cells at the neuromuscular junctions (NMJs), are essential for NMJ maintenance and repair. They extend processes from denervated endplates towards innervated ones to promote the reinnervation process. Cannabinoids are frequently used in the treatment of neuropathic pain related to nerve injury. However, despite evidence for their roles in the axonal guidance and synapse formation during development and management of pain in the peripheral sensory system, their possible contribution in response to peripheral nerve injury remains unclear. Here we present a novel role of glial CB1Rs in motor recovery following nerve injury. CB1R absence, through treatment with the antagonist AM251 or in CB1R-KO, accelerated NMJ denervation with an increased expression of the phagocytic marker MAC-2. Also, CB1R absence delayed the NMJ reinnervation and denervated NMJs were observed up to 21 days post-injury. Importantly, these results were completely replicated glial GFAP-CB1R-KO mice. Reflecting the repair mode of PSCs, a reduced muscarinic activation of PSCs is normally observed at reinnervating NMJs. However, at reinnervating NMJs of CB1-KO and GFAP-CB1-KO animals, PSC Ca2+ responses induced by local application of muscarine were altered compared with their WT littermates. Lastly, CB1R activation enhances reinnervation after nerve injury, as revealed by a faster and more complete reinnervation, suggesting a positive role of this receptor in nerve repair processes. Our results highlight a novel role of endocannabinoids in NMJ repair, opening a possible therapeutic strategy for facilitating nerve repair following injury or to address inadequate NMJ maintenance observed in motor neuron-related neurodegenerative diseases.

45. **Transcutaneous Spinal Cord Stimulation Inhibits Tibialis Anterior Motor Neurons**

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Transcutaneous spinal cord stimulation (tSCS) is a non-invasive method of stimulating the spinal cord. tSCS was designed to target dorsal root afferents and improve voluntary movements in individuals with a spinal cord injury. tSCS intensity is often set relative to threshold for evoking a short-latency excitatory response (SER) in relaxed muscles (SERthresh). SERthresh is thought to reflect dorsal root afferent activation sufficient for α-

motoneurons in the motor pool to reach their activation threshold. However, tSCS is often applied below SERthresh, making it difficult to determine whether dorsal root afferents are still activated. The purpose of this study was to determine the intensity that tSCS begins to recruit dorsal root afferents and influence the tibialis anterior (TA) motor pool. Nine participants held a small TA contraction while tSCS was applied at 0.3 Hz at 50%-100% of SERthresh over the lumbar enlargement. Stimulation was also delivered 10 cm lateral at 100% of SERthresh. Unexpectedly, tSCS inhibited TA at a latency of ~40-80 ms when delivered at intensities as low as 50% of SERthresh. Despite holding a contraction, tSCS evoked an SER in only 3/9 participants. During lateral stimulation, the SER dissipated, whereas inhibition remained. The absence of a SER during contractions suggests that in most participants, the SER in TA may be due to stimulating ventral, not dorsal roots. We propose that the inhibition is due to stimulating cutaneous afferents in the skin of the back and that this input may play an important role in how tSCS exerts its beneficial effects.

46. **UNMASKING MOTONEURON DIVERSITY: HYBRID A-MNS BRIDGING SLOW AND FAST PHENOTYPES**

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Alpha-motoneurons (α-MNs) have traditionally been classified into three types—slow (S), fast fatigue-resistant (FR), and fast-fatigable (FF). However, these categories represent a spectrum of properties that underlie graded force production and smooth movement. By applying a novel combination of markers, we developed advanced immunohistochemistry protocols capable of co-labeling six distinct α-MN types—along with intermediary subtypes—throughout the mouse lumbar spinal cord in unprecedented detail. Intriguingly, these protocols revealed greater heterogeneity and diversity in molecular profiles than previously recognized. Some α-MNs exhibited a blend of molecular, electrical, and morphological traits characteristic of both slow and fast phenotypes. Electrophysiological recordings confirmed the presence of these mixed-feature α-MNs, highlighting that motoneuron identity is more varied than current classifications suggest. These findings underscore the complexity within the α-MN population.

47. **Real-time Control of Ankle Spared Motor Dimensions after Spinal Cord Injury using High-Density EMG and Functional Electrical Stimulation**

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Neural lesions in the brain and spinal cord affect millions of people worldwide, causing among others a condition known as "drop foot", where individuals are unable to voluntarily recruit pools of spinal motor neurons that innervate the ankle dorsiflexor muscles. Lower limb neuro-orthoses based on functional electrical stimulation (FES) can assist in the movement by delivering electrical pulses to the dorsi-/ plantarflexor muscles to increase the user range of motion and correct their gait patterns. However, current commercial FES systems estimate the user foot position with inertial measurement units to trigger stimulation, limiting the intent detection to the voluntary pathological range of movement. Building on our previous work in humans with paralyzed hands (Oliveira et al., Brain, 2024), we show that five participants with spinal cord injury (SCI) can control multiple spared motor dimensions in real-time using a wearable 32-channel high-density surface electromyography (EMG) bracelet placed over the tibialis anterior and triceps surae muscles and a machine learning model. All users exhibited multiple distinct muscle activation patterns for dorsiflexion, plantarflexion, inversion and eversion while controlling a 2D cursor. Additionally, participants achieved two levels of EMG activation for dorsi-/ plantarflexion, irrespective of their pathological range of motion. Finally, one participant with a chronic SCI (45 years) successfully used his spared EMG to trigger and stop electrical stimulation from two FES channels on demand, significantly increasing the range of motion of the affected foot. These results highlight the potential of high-density EMG and FES for precise motor control with significant implications for clinical rehabilitation.

48. **Electrically Evoked Sensory Reflexes in Motor Units: Does Recruitment Threshold Shape Excitability?**

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Neuromuscular reflexes elicited by sensory nerve stimulation provide valuable insights into neural motor control pathways. To accurately analyse reflex responses to electrical nerve stimulation, decomposition algorithms extract motor unit (MU) firings from electromyographic (EMG) signals, enabling investigation at the motor pool and individual MU levels. Little is known about how individual MUs respond to sensory stimulation, particularly whether they exhibit distinct reflex characteristics and what factors influence them. This study addresses this issue by investigating reflex behaviour in a large number of MUs. While neuromuscular reflexes to cutaneous electrical stimulation have been widely studied, most analyses remain at EMG level or solely focus on single tasks and muscles. As a secondary aim, this study establishes a baseline of expected reflex responses in healthy humans across several stimulation sites, muscles, and movements. We show individual tibialis anterior MUs (n=303) exhibit notable differences in reflex excitability to fibular nerve stimulation (2.5x perceptual threshold) at the foot instep. A

moderate to strong correlation (mean r=0.62) between MU recruitment threshold and individual excitation probability was found in all subjects (n=4, one female) at all recorded force levels (10%, 20%, 30% of maximum force). We also show across the pool of MUs for each force level, the response magnitude increases with increasing force level. Minor latency differences were observed, but showed no correlation to recruitment threshold. These preliminary findings suggest reflex excitability in individual MUs is closely related to their recruitment threshold and thus their size, offering insight into individual MUs' contribution to sensory reflexes.

49. **Demonstration of Axonal Excitability Techniques Using the TROND Protocol**

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I'll give a quick demonstration of how easy it is to perform a nerve excitability test, by stimulating the median nerve at the wrist, and tracking the threshold required to elicit a target CMAP response from the Abductor Pollicis Brevis. The same technique can be used to study motor and sensory axons in humans in vivo, in preclinical animals (under anaesthesia), in cell preparations, and is well supported by a simple but effective mathematical model.

50. **Transcutaneous Spinal Cord Stimulation after Chronic Spinal Cord Injury May Decrease Spastic Sensory Evoked-Responses and Restore Presynaptic Inhibition**

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Spasticity arises in ~75% of individuals with spinal cord injury (SCI) within one year of injury. However, therapeutic treatment is often limited to pharmacological options with undesirable side effects. Spasticity after SCI results from a loss of synaptic inhibition and an increase in motoneuronal excitability driven in part by larger persistent inward currents and disrupted chloride homeostasis. This contributes to aberrant co-contraction of antagonist muscles and hyperreflexia. We previously showed that transcutaneous spinal cord stimulation (tSCS) prevents the development of hyperreflexia when initiated early after injury. Here, we evaluate the effectiveness of tSCS to treat spasticity after chronic SCI. Sprague Dawley rats received a severe T9 contusion. 4-weeks post-injury, rats received tSCS or sham

stimulation (18 min/day, 5x/week) for 6 weeks. Spasticity was assessed by recording electromyographic activity of an ankle flexor and extensor in response to toe pinch and passive ankle stretch. In terminal experiments, hyperreflexia was assessed using the plantar H-reflex to determine 1) H/M_{max} ratio, 2) frequency-dependent depression, and 3) presynaptic inhibition (PSI) evoked by PBSt. $VGluT1^+$ afferents sprouting and expression of the chloride extruder, KCC2, were quantified in the lumbar enlargement. Our data suggests that tSCS initiated after spasticity develops decreases co-contraction in response to passive stretch, reduces hyperreflexia and $VGluT1^+$ afferents sprouting, and restores PSI and motoneuronal KCC2 expression close to intact levels. Overall, this indicates that tSCS not only prevents the development of hyperreflexia and spasticity but also contributes to its decrease, once established, potentially by normalizing $VGluT1^+$ afferents innervation and motoneuronal KCC2 expression.

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