Banff 2022 Abstracts:

Ion channels

1 19 12/22/2021 **Marc D. Binder** mdbinder@u.washington.edu Department of Physiology & Biophysics, University of Washington School of Medicine oral

Cooperative Gating of Ion Channels: Mechanisms and Physiological Implications Ion channels play a central role in the regulation of nearly every cellular process. Dating back to the classic 1952 Hodgkin-Huxley model of the generation of the action potential, ion channels have always been thought of as independent agents. A myriad of recent experimental findings exploiting advances in electrophysiology, structural biology, and imaging techniques, however, have posed a serious challenge to this long-held axiom as several classes of ion channels appear to open and close in a coordinated, cooperative manner (Physiol Rev https://doi.org/10.1152/physrev.00022.2021) The extent of ion channel cooperativity ranges from variable-sized oligomeric cooperative gating in voltage-gated, dihydropyridine-sensitive Cav1.2 and Cav1.3 channels to obligatory dimeric assembly and gating of voltage-gated Nav1.5 channels. Potassium channels, transient receptor potential (TRP) channels, hyperpolarization cyclic nucleotide-activated (HCN) channels, ryanodine receptors (RyRs), and inositol trisphosphate receptors (IP3Rs) have also been shown to gate cooperatively. In all cases, the requisite anatomical substrate for cooperative gating is the aggregation of channels into dense, homogeneous clusters that are formed through a stochastic, self-assembly process (J Gen Physiol https://doi.org/10.1085/jgp.201912327). Cooperative gating of ion channels affects several basic physiological processes including excitation-contraction coupling in muscle cells, regulation of cardiac function and vascular tone, modulation of action potential initiation and conduction velocity in neurons and cardiac cells, generation of sustained discharge and facilitation in motoneurons, and control of pace-making activity in the heart. I will outline the different mechanisms underlying the cooperative gating of ion channels and posit how alterations in cooperative gating may induce a range of clinically significant pathologies.

2 03/25/2022 **Frederic Brocard** frederic.brocard@univ-amu.fr Institut de Neurosciences de la Timone, CNRS Ph.D None "RÃ©mi Bos1, Benoit Drouillas1, Mouloud Bouhadfane1, Emilie Pecchi1, Virginie Trouplin1, Sergiy M. Korogod2, FrÃ©dÃ©ric Brocard1.1 Institut de Neurosciences de la Timone (UMR7289), Aix-Marseille UniversitÃ© and

CNRS, Marseille, France 2 Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine; Kyiv, Ukraine." oral

TRPM5 CHANNELS ENCODE BISTABILITY OF SPINAL MOTONEURONS AND ENSURE MOTOR CONTROL OF HINDLIMBS IN MICE. "It is often assumed that the postural tone originates from supraspinal centers by providing a prolonged excitation to spinal motoneurons. On the other hand, motoneurons in vertebrates can process descending commands through nonlinear integrative functions, the most distinctive being a self-sustained spiking activity mediated by a plateau potential and evoked by a brief excitation. Even if this bistable state makes sense for maintenance of posture, its functional significance remained unclear. We recently published in Nature communications congruent evidences that bistable motoneurons are integral part of the postural control in mice (Bos et al., 2021).

There has been a consensus that the activation of the L-type Ca2+ persistent inward current mediates the plateau potential in bistable motoneurons. We here show that Ca2+ is not the main charge carrier but is required for triggering a Na+ conductance which drives the plateau potential. In addition to update the ionic nature of plateau potentials, we identify the Ca2+-activated Na+-permeable Trpm5 channels as the molecular bases for bistability. Pharmacological, genetic or computational inhibition of Trpm5 specifically occlude motoneuronal bistable behaviors. Furthermore, silencing Trpm5 in lumbar motoneurons by genetic interference techniques leads to a pronounced paresis of hindlimbs.

In sum, we bring a support to the concept that the postural tone is associated with bistable properties of motoneurons. We also provide new biological insights in bistability of motoneurons by demonstrating the significant role of Ca2+-activated Na+-permeable Trpm5 channels, and how these channels work in tandem with L-type Ca2+ channels to produce the plateau potential."

3 03/29/2022 **Benoit Drouillas** benoit.drouillas@univ-amu.frInstitut de Neurosciences de la Timone, CNRS Ph.D student Student "BenoÃ®t Drouillas, CÃ©cile Brocard, SÃ©bastien Zanella, RÃ©mi Bos, Jean-Charles Viemari, Loubna Khalki, HÃ©lÃ¨ne Bras and FrÃ©dÃ©ric Brocard.Institut de Neurosciences de la Timone (UMR7289), Aix-Marseille UniversitÃ© and CNRS, Marseille, France" oral

PERSISTENT NAV1.6 CURRENT PROMOTES BISTABILITY IN LUMBAR MOTONEURONS TO SUPPORT HINDLIMB POSTURAL TONE IN MICE. "The rhythmogenic module of the spinal central pattern generator (CPG) comprises glutamatergic interneurons endowed with inherent membrane oscillations at a frequency range similar to locomotor rhythms. Numerous experimental evidences support a fundamental role of the persistent Na+ current (INaP) in mediating membrane oscillations in premotor interneurons and thereby in generating the locomotor rhythm (Brocard et al., 2013). Beyond its rhythmogenic role, INaP also enables self-sustained spiking activity triggered in spinal motoneurons by a brief excitation (Bouhadfane et al., 2013). Despite the numerous studies on the roles of INaP within the spinal locomotor network, we still know little regarding the identity of Nav channel isoform(s) that give rise to INaP, and the manner in which they interplay have yet to be

deciphered. Our attention turned to Nav1.1 and Nav1.6 because of their strong expression within the ventral spinal cord while Nav1.2 and Nav1.3 are predominantly found in dorsal horn sensory neurons.

We here highlight the axonal Nav1.6 as the main molecular player for INaP in lumbar motoneurons. The pharmacological or genetic inhibition of Nav1.6, but not of Nav1.1, impairs INaP, bistability and postural tone in hindlimbs. In interneurons of the CPG region Nav1.6 and Nav1.1 equally mediate INaP and the inhibition of both are required to abolish oscillatory bursting activities and the locomotor rhythm. Overall, Nav1.6 plays a significant behavioral role in posture by governing INaP-dependent bistability in motoneurons, and works in tandem with Nav1.1 for producing INaP-dependent rhythmogenic properties required for locomotion.

4 04/04/2022 **Laura Schmid** laura.schmid@imsb.uni-stuttgart.de Institute for Modelling and Simulation of Biomechanical Systems, University of Stuttgart, Stuttgart, Germany PhD Student Student "Thomas Klotz: Institute for Modelling and Simulation of Biomechanical Systems, University of Stuttgart, Stuttgart, Germany Oliver RÃ¶hrle: Institute for Modelling and Simulation of Biomechanical Systems, University of Stuttgart, Stuttgart, Germany; Stuttgart Center for Simulation Sciences (SC SimTech), University of Stuttgart, Stuttgart, Germany Randall K. Powers: Department of Physiology and Biophysics, University of Washington, Seattle, WA, United States of America Francesco Negro*: Department of Clinical and Experimental Sciences, UniversiÃ degli Studi di Brescia, Brescia, Italy Utku S. Yavuz*: Biomedical Signals and Systems, Universiteit Twente, Enschede, The Netherlands *These authors contributed equally to this work." oral

Relating in vitro and in vivo post-inhibitory rebound excitation in motoneurons using a computational model Recordings of human motoneurons during reciprocal inhibition show a rebound effect - a period of excitation following the inhibition. Interestingly, in vitro studies previously demonstrated membrane potential overshoots in motoneurons after injections of hyperpolarizing current steps. This was attributed to HCN-channels. In this study, we used a motoneuron model to investigate if the characteristic HCN-channel behaviour can explain the rebound excitation seen in experimental recordings performed on human subjects. The experimental results reveal that, depending on the time lag between the last action potential and the inhibitory stimulus, the motoneuron was either inhibited or, unexpectedly, excited. Only stimuli delivered in the second part of the interspike-interval could reduce the firing rate. For stimuli applied during the first part of the interspike-interval the neurons increased their firing rates, leading to the observed rebound excitation. This behaviour was replicated by the motoneuron model when including HCN-channels. However, the amplitude of the rebound excitation was considerably smaller than in the experimental study. Notably, the rebound amplitude was influenced by the magnitude and variability of the overall injected current. The simulation results show that HCN-channels can increase the excitability of a motoneuron after it is inhibited. However, the model could not completely explain the significant rebound effect seen in human subjects.

5 04/06/2022 **Remi Bos** remi.bos@univ-amu.fr CNRS Aix-Marseille University Principal Investigator None "Harris-Warrick R.* (1), Caron G. (2), Baczyk M. (3), Pecchi E. (4), Drouillas B. (4), Zytnicki D. (2), Brocard F.* (4), Bos R.* (4) (1) Department of Neurobiology and Behavior, Cornell University, Ithaca, NY, USA (2) Centre de Neurophysique, Physiologie et Pathologie, UMR 8119, CNRS/UniversiteÂ´ Paris Descartes, 45 rue des Saints-Peres, 75270 Paris, France (3) Department of Neurobiology, PoznaÅ„ University of Physical Education, Poland (4) Institut de Neurosciences de la Timone (UMR7289), Aix-Marseille UniversiteÂ´ and Centre National de la Recherche Scientifique (CNRS), Marseille oral

A size principle for bistability in mouse lumbar motoneurons Bistability in spinal motoneurons supports tonic spike activity in the absence of excitatory drive. Based on earlier work in cats, it has been assumed that smaller motoneurons innervating slow motor units associated with antigravity musculature might be more prone to generate bistability for postural maintenance. We studied the bistable properties as a function of the size of motoneurons in neonatal and juvenile (P25) Hb9-eGFP mice. We first established a bistability score from 0 to 4 points, (0 = no bistability, 4 = full bistability). This score takes into account the following parameters: the presence of (i) a slow afterdepolarization (sADP: 1 point), (ii) a sustained discharge in response to a brief depolarizing current (1 point), (iii) negative hysteresis during ramp depolarizations (1 point) and (iv) delayed, accelerating firing during long current steps (1 point). We showed that only the largest Î±-motoneurons (GFP+/NeuN+/MMP9+), with soma diameter â‰¥ 400Âµm2, display full bistable properties. Smaller neurons showed none or only one of the bistability criteria. The proportion of bistability increases over development (from P2 to P25). Only the large motoneurons expressed a large persistent inward current, and a Kv1.2 delayed rectifying K+ current typical of bistable neurons. As repeatedly shown before, serotonin can evoke bistability, but only in large, partially bistable motoneurons. Finally, in vivo studies showed that, in adult (P50-P60) mice, bistability occurs only in motoneurons displaying a negative hysteresis and a delayed accelerating firing. In sum, it appears that, in mice, large bistable motoneurons unexpectedly display a fast phenotype.

6 04/07/2022 **Calvin Chad Smith** calvin.smith@ucl.ac.uk University College London Postdoctoral Research Associate Post-doc Robert Brownstone, University College London oral

Kv2.1 channels are not required for C-bouton amplification of motor output Neural motor systems have evolved complex circuits that afford animals a range of behaviours essential for survival. C-bouton synapses arising from cholinergic V0C interneurons increase muscle contraction force by amplifying activity of innervating motoneurons. Recent work in neonatal mouse motoneurons suggests that delayed rectifier currents carried by post-synaptically clustered Kv2.1 channels are crucial to C-bouton amplification. Here we use a motoneuron conditional Kv2.1 knockout to show that while Kv2.1 modulates maximal firing in neonates, its removal minimally affects either mature motoneuron firing, or firing rates in response to the C-bouton

agonist, muscarine. Additionally, amplification of electromyography activity during high force tasks was unchanged following Kv2.1 deletion. We next show that Kv2.2 is also expressed by spinal motoneurons and co-localises with Kv2.1 opposite C-boutons. We suggest that the primary function of Kv2 proteins Kv2.1 and Kv2.2 is non-conducting in motoneurons, and that Kv2.2 can function in the absence of Kv2.1, perhaps to ensure the integrity of the synapse.

7 P 04/06/2022 **Ricardo Goncalves Molinari** molinari@gmail.com 1. Neural Engineering Research Laboratory, Center for Biomedical Engineering, University of Campinas, Campinas, SP, Brazil. 2. Department of Electronics and Biomedical Engineering, School of Electrical and Computer Engineering, University of Campinas, Campinas, SP, Brazil.PhD Student Student "Francesco Negro3, and Leonardo Abdala Elias1,2 1. Neural Engineering Research Laboratory, Center for Biomedical Engineering, University of Campinas, Campinas, SP, Brazil. 2. Department of Electronics and Biomedical Engineering, School of Electrical and Computer Engineering, University of Campinas, Campinas, SP, Brazil. 3. Department of Clinical and Experimental Sciences, UniversitÃ degli Studi di Brescia, Brescia, Italy. " remote poster

Dendritic transformation of synaptic currents and its influence on discharge properties of alpha motor neurons Force generation during isometric voluntary contractions is hypothesized to be regulated by the common synaptic input, a signal composed of presynaptic inputs impinging on the dendrite of motoneurons (MNs). However, it is unclear how the morphology and electrophysiological properties of MN dendrite mold the bandwidth of the synaptic input signal into the neural drive to the muscle under different synaptic bombardment (SB) conditions. We conducted computer simulations with morphologically detailed S- and FF-type MN models to investigate how the dendritic transformation of synaptic inputs would influence the MN discharge. The cut-off frequency of the dendritic frequency response (DEND_CF) did not change, while the interspike interval variability (ISI_VAR) linearly increased and exponentially decayed with the increase of SB in both S- and FF-type MNs, respectively. Activation of excitatory synaptic inputs with a distal distribution reduced DEND_CF and ISI_VAR. Concomitant activation of inhibitory and excitatory synaptic inputs increased DEND_CF and ISI_VAR. Activation of dendritic Ca++ persistent current produced an increased gain of the dendritic frequency response in the low-frequency (<5Hz) band, with a reduction in both DEND_CF and ISI_VAR. The results suggest that the bandwidth of the common synaptic input to the MN is shaped in the output neural drive by a complex interplay of dendritic morphology, intrinsic MN electrophysiology, the combination of excitatory and inhibitory synaptic inputs, and SB intensity. Also, the changes in MN discharge variability shed light on the importance of considering the above-mentioned factors when evaluating the influence of the neural drive to the muscle on force control.

Development

1 20 01/12/2022 **Simon Arthur Sharples** simon.sharples87@gmail.com University of St Andrews Postdoctoral Fellow Post-doc Gareth B. Miles, University of St Andrews oral

Postnatal integration of active properties shapes motoneuron recruitment The ability to produce graded changes in muscle force is dependent on the orderly recruitment of motor units. The size principle underlies the orderly recruitment of motor units; however, motoneuron size is a poor predictor of recruitment amongst functionally defined motoneuron subtypes. Whilst intrinsic properties are key regulators of motoneuron recruitment, the underlying currents involved are not well defined. Whole-cell patch-clamp electrophysiology was deployed to study intrinsic properties, and the underlying currents, that contribute to the differential activation of delayed and immediate firing motoneuron subtypes. Motoneurons were studied during the first three postnatal weeks in mice to identify key properties that contribute to rheobase and may be important to establish orderly recruitment. We find that delayed and immediate firing motoneurons are functionally homogeneous during the first postnatal week and are activated based on size, irrespective of subtype. The rheobase of motoneuron subtypes becomes staggered during the second postnatal week, which coincides with the differential maturation of passive and active properties, particularly persistent inward currents (PICs), conducted by NaV1.6 in both subtypes, in addition to L-Type calcium channels in delayed firing motoneurons. The rheobase of delayed firing motoneurons increases further in the third postnatal week due to the development of a prominent hyperpolarization-activated inward current that is active at resting membrane potential. Our results suggest that motoneuron recruitment is multifactorial, with recruitment order established during postnatal development through the differential maturation of passive properties and sequential integration of persistent and hyperpolarization-activated inward currents.

2 04/13/2022 **Joline Brandenburg**, MD brandenburg.joline@mayo.edu Mayo Clinic Consultant, Assistant Professor None "Matthew Fogarty, PhD Mayo Clinic Gary Sieck, PhD Mayo Clinic" oral

Mouse Models of Phrenic Motor Neuron Loss during Postnatal Development During embryonic and early postnatal development, the number and size of motor neurons is established. Developmental changes in neurotransmission and/or neurotrophic factor (i.e. BDNF via TrkB receptor) signaling can impact postnatal survival of motor neurons. We employed two genetic models: 1) spaGlrb-/- mice with mutated glycine receptors and 2) TrkBF616A mice with a 1NMPP1 sensitive knockin allele that inhibits TrkB kinase activity. In TrkBF616A mice, 1NMPP1 was added to drinking water of dams at P0 and continued through P21. The impact altered neurotransmission or BDNF/TrkB signaling on the number and size of phrenic motor neurons (PhMNs) was assessed at P28. PhMNs were retrogradely labeled using rhodamine and imaged in 3D by confocal microscopy (Olympus Fluoview 40X 1.4 NA objective). Both spaGlrb-/- and TrkBF616A had fewer PhMNs (~15% and ~20% , respectively) compared to wildtype mice. In addition, the somal surface area of surviving PhMNs in both spaGlrb-/- and

TrkBF616A mice were ~10% smaller than wildtype mice at P28. In spaGlrb-/- mice, the number and diameter of primary dendrites was comparable to wildtype mice, whereas the number of primary dendrites in TrkBF616A mice was ~15% greater than wildtype but their average diameter was similar. While likely to be very different mechanistically, removal of either effective glycinergic neurotransmitter inputs to or BDNF/TrkB signaling at PhMNs during development results in fewer PhMNs and reduced PhMN somal size by P28. These developmental changes will undoubtedly affect neural control of the diaphragm muscle in adults, reducing motor unit diversity.

3 04/13/2022 **Kimberly Dougherty** kjd86@drexel.edu Drexel University Associate Professor None "Shayna Singh, D. Leonardo Garcia-Ramirez, Ngoc Ha, Erik Li, Nicholas Stachowski, Lihua Yao Drexel University" 2 Oral

Developmental and injury-induced changes in cellular and synaptic properties of spinal premotor interneurons expressing Shox2 Locomotor rhythm and pattern is generated by neuronal networks in the spinal cord. Three populations of spinal neurons have been linked to rhythm generation thus far. One of these populations is a group of excitatory interneurons identified by the transcription factor Shox2. Recent experiments have used electrophysiological techniques in spinal slice and reduced isolated spinal cord preparations to explore mutual excitatory interactions and intrinsic properties related to rhythmicity, modulation of rhythmicity by synaptic inputs from other locomotor-related neurons, and primary afferent pathways providing feedback to these neurons. Through these experiments, we have determined that gap junctional coupling within functionally related populations of Shox2 neurons supports synchronization of activity, and may amplify weak intrinsic rhythmogenic currents found in a subset of Shox2 neurons in neonates. Further, subsets of Shox2 are excited and others are inhibited by activation of sensory afferent pathways that perturb ongoing locomotion. In adult Shox2 neurons, potential rhythmogenic currents strengthen, gap junctional coupling is no longer detected, and neurons spontaneously oscillate in slice. Afferent pathways to Shox2 neurons seem to remain similar in the adult to those observed in neonate. Following spinal cord injury, Shox2 neurons maintain their intrinsic and oscillatory properties but sensory pathways to Shox2 neurons are altered. Considered together, the data demonstrate intrinsic and synaptic mechanisms influencing activation and rhythmicity in different conditions, and provide insight into network functioning, of a putative rhythm generating population.

4 P 04/14/2022 **Marie Roussel** marie.roussel.1@ulaval.ca University Laval PhD Student Student "Authors: Marie Roussel (1), Louise Thiry (2), and Frederic Bretzner (1)Institutions:(1) Centre de recherche du CHU de Quebec University Laval (2) Montreal Neurological Institute-Hospital, McGill University" "3 people: 2 PhD students and our PI. We will follow the virtual meeting. " Remote Poster

Dual role of DSCAM in the development of the spinal locomotor circuit "Locomotion results from motoneuronal activity under the control of spinal interneuronal circuits generating the appropriate rhythm and pattern of muscle contractions. Recently, we have shown that a systemic mutation of DSCAM (Down Syndrome Cell Adhesion Molecule), Dscam2J, induces

anatomical and neurophysiological changes in the lumbar spinal circuit, contributing to locomotor deficits. Here we identified the neuronal populations underlying these functional changes by conditionally impairing DSCAM expression in either excitatory or inhibitory interneurons. Vglut2-CRE or Vgat-CRE mice were crossed with Dscamflox/flox mice in order to conditionally delete DSCAM in Vglut2+ or Vgat+ neurons. Motor control was assessed by quantifying the posture, the intralimb and interlimb coordination and electromyographic (EMGs) recordings during treadmill locomotion. In comparison to their controls (Vgat-cre mice), Vgat-DS mutant mice exhibited a duck-like gait with a longer swing phase. This longer swing phase was associated with a longer burst duration in the EMG activity of the ankle flexor, while the stance phase and the extensor burst activity were normal. In contrast to their controls (Vglut2-cre mice), Vglut2-DS mutant mice presented either an extended or normal posture with a hyperflexion of their hindlimb during the swing phase. In addition, the end of the EMG activity in the ankle flexor overlapped with the beginning of the activity in the extensor. Our current results suggest that conditional mutations of DSCAM in either excitatory or inhibitory spinal interneurons impair differently the spinal locomotor circuit.

Funding: Natural Sciences and Engineering Research Council of Canada (NSERC)"

5 04/16/2022 **Sydney Dudley** sdudle@midwestern.edu Midwestern University Research Assistant None Dudley SK, Kurup A, Jeong KT, Banayat T, Koziol SM, White M, Buhlman LM, Revill AL. 4 oral

"Muscarinic acetylcholine receptor subtype contribution to inspiratory bursting at hypoglossal motoneurons in neonatal mice in vitro

Mechanisms through which muscarinic acetylcholine receptor modulation contributes to changes in hypoglossal motoneuron excitability remain to be fully elucidated. Previous data indicate a net excitatory effect of muscarinic modulation of inspiratory bursting in neonatal brainstem slices, yet a net inhibitory effect in adult rats. We hypothesize that there is a developmental shift in muscarinic modulation effects at hypoglossal motoneurons. To test this, we confirmed using immunofluorescence that hypoglossal motoneurons express M1, M2, M3, and M5 muscarinic subtypes and that there are maturational shifts in expression patterns. We then tested local application of muscarine (100ï• -M) into the hypoglossal nucleus using the in-vitro rhythmic slice preparation (CD1 mice). While inspiratory burst amplitude was potentiated from postnatal (P) 0-9, the magnitude of potentiation appears to change after P4. We next evaluated the contribution of excitatory and inhibitory muscarinic receptor subtypes on muscarinic modulation of inspiratory bursting. Blocking M1 receptorsâ€¯locally (pirenzepine, 100â€¯Î¼M) significantly decreased the muscarine-mediated potentiation of inspiratory bursting (72Â±14% of control muscarine response, n=9). Preliminary data indicate bath application of 4-DAMP (M3 antagonist) attenuated muscarinic potentiation of inspiratory bursting (range: 7%-25% of control, n=3). Modulating M5 receptors locally with a positive allosteric modulator VU 0238429 (1000 Î¼M) had little effect on burst amplitude (119Â±4% of control, n=3]). Blocking M2 receptors locally (methoctramine, 2ÂµM) had little effect on inspiratory burst potentiation

(71.14Â±14.59% of control p=0.4653, r2=0.04944, n=25. Bath application of methoctramine similarly had no effect (62.94Â±80.35% of control, n=9). Preliminary analysis suggests there may be an increasing effect of blocking M2 receptors with postnatal maturation.

ALS/MND

1 02/18/2022 **Vivian Ko** vko@health.ucsd.edu University of California, San Diego Neuroscience Graduate Student Student "Sandra Diaz-Garcia(1), Haiyang Yu(2), Melissa McAlonis-Downes(2), Alisar Shanklin(1), Brian Giang(1), Kailee Ong(1), Don W. Cleveland(2), John Ravits(1)(1) Department of Neurosciences, University of California San Diego (UCSD), La Jolla, CA, USA, 92093(2) Ludwig Institute for Cancer Research, University of California San Diego (UCSD), La Jolla, CA, USA, 92093" 1-2 people might be in attendance from the Ravits Lab oral

CK1Îµ-dependent TDP-43 phosphorylation in ALS ALS is the most common incurable adult-onset motor neuron disease. Despite heterogeneity in familial versus sporadic ALS, over 90% of all patients exhibit the key pathological hallmark of TDP-43 (TAR DNA-binding protein 43) mislocalization from the nucleus to the cytoplasm where it forms hyperphosphorylated TDP-43 aggregates which are suggested to be toxic in neurons. The role of phosphorylation in this pathological mechanism is unknown, such as whether phosphorylation is a driver or consequence of aggregation, and whether phosphorylation is protective or toxic in neurons. In our published study (Krach, et al. 2018), we used laser capture microdissection technique to isolate motor neurons in lumbar spinal cords of sporadic ALS patients for RNA-seq and eCLIP analysis. By comparing the list of genes whose mRNA levels correlated with pTDP43 burden and a list of genes whose mRNA is bound by TDP43 in the human brain, we identified the upregulation of casein kinase 1 epsilon gene (CSNK1e gene encoding CK1Îµ protein) as being tightly correlated with pTDP-43 pathology. CSNK1E is a serine/threonine specific phosphorylation kinase found in the cytoplasm of cells. Using cellular models that generate cytoplasmic phosphorylated TDP-43 inclusions, we found that inhibiting CK1Îµ kinase activity results in significant reduction of phosphorylated TDP-43 and increased cellular survival. Using an ALS mouse model with shortened survival, motor deficits, and TDP-43 abnormalities, we are testing the efficacy of CK1Îµ inhibitors, which are highly selective and CNS-penetrant, as a potential therapeutic. If proof-of-principle is established, CK1Îµ inhibitors could rapidly translate into clinical use for ALS.

2 03/03/2022 **Sherif Elbasiouny** sherif.elbasiouny@wright.edu Wright State University Professor None none Four people from my lab (including myself) will be attending. Oral

Motoneuron excitability dysfunction in ALS Motoneuron excitability dysfunction has been implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS); however, the mechanisms underlying excitability dysfunction are still unknown. In this talk, we show how

computer simulations and electrophysiological recordings were employed to examine the dynamic interaction between disease and compensatory excitability mechanisms allowing the motoneuron to maintain a pseudo-normal net excitability that masks disease progression.

3 27 03/12/2022 **Daniel Zytnicki** daniel.zytnicki@parisdescartes.fr UniversitÃ© Paris CitÃ© CNRS Research Director None "Marcin BÄ…czyk (1,3), Najwa Ouali Alami (2), Kamil Grycz (3), Nicolas DelestrÃ©e (1), ClÃ©mence Martinot (1), Linyun Tang (2), Barbara Commisso (2), Nicolas Doisne (1!, Marin Manuel (1), Francesco Roselli (2,4), Daniel Zytnicki (1)1 UniversitÃ© Paris CitÃ©, Saints-PÃ¨res Paris Institute for the Neurosciences (SPPIN), Centre National de la Recherche Scientifique (CNRS), Paris, France.2 Department of Neurology, Ulm University, Ulm, Germany.3 PoznaÅ„ University of Physical Education, PoznaÅ„, Poland 4 German Center for Neurodegenerative Diseases, Ulm, Germany." "Two people from the Paris Lab: Guillaume Caron and Daniel Zytnicki+ Marcin Baczyk from the Poznan Lab" Oral

Early reversible structural and functional impairments of excitatory synapses on ALS motoneurons "Excessive excitation is hypothesized to cause motoneuron (MN) degeneration in amyotrophic lateral sclerosis (ALS), but actual proof of hyperexcitation in vivo is missing: how are synaptic inputs to MN affected by the disease, and are they increased or decreased? We demonstrate, by in vivo intracellular MN electrophysiology, that, contrary to expectations, excitatory post-synaptic potentials evoked by electrical or mechanical stimulation of Ia sensory fibers are reduced in MNs of adult presymptomatic mutSOD1 mice. This synaptic impairment correlates with disrupted postsynaptic clustering of Homer1b, Shank, and GluR4 subunits. Moreover, this impairment has a deep impact on the whole MN biology since mechanically-induced Ia inputs translate in a reduced phosphorylation of the CREB transcription factor in MNs. Interestingly, a similar functional impairment is observed in synapses on MN originating from the brainstem descending medial longitudinal fasciculus, indicating a widespread phenomenon. Restoration of excitatory synapses can be achieved by activation of the cAMP/PKA pathway, by either intracellular injection of cAMP or DREADD-Gs stimulation. Furthermore, we reveal, through independent control of signaling and excitability in MN allowed by multiplexed DREADD/PSAM chemogenetics, that PKA-induced restoration of synapses triggers an excitation-dependent decrease in misfolded SOD1 burden and autophagy overload. In turn, increased MN excitability contributes to restoring synaptic structures. Thus, the decrease of excitation to MN is an early but reversible event in ALS. Failure of the postsynaptic site, rather than hyperexcitation, drives disease pathobiochemistry at this stage of the disease evolution.

Fundings: NIH-NINDS R01NS077863, Target ALS, Fondation Thierry Latran, ARSLA, Radala Foundation, AFM, NCN 2019/35/B/NZ4/02058"

4 28 03/14/2022 **Francesco Roselli** francesco.roselli@uni-ulm.de ulm university principal investigator None Stefano Antonucci (1), Guillaume Caron (2), Marcin BÄ…czyk (3), Daniel Zytnicki (2), Francesco Roselli (1,4). Remote Oral

The motoneuronal receptorome in ALS reveals adrenergic entry points to modulate MN excitability and firing "Modulation (up- or downregulation) of motoneuron (MN) excitability and synaptic excitation constitutes an important entry point to affect MN degeneration in several MN diseases. We have previously demonstrated that chemogenetic interventions at the level of excitability and of PKA signaling exert profound beneficial effects on synaptic integrity and disease burden in ALS MN. In order to achieve a similar upregulation of PKA signaling and MN firing through natural receptor, we explored the PKA-coupled motoneuronal receptorome in ALS. Among the receptors prioritized by screening available databases (Allen Spinal Cord Atlans, GPCR database) in situ hybridization reveals that adenosinergic, histaminergic, cholinergic and several peptidergic receptors are downregulated, whereas beta-1 adrenergic receptor is distinctively upregulated and the expression of dopaminergic D5 and beta-2 and beta-3 adrenergic receptor are preserved. Importantly, activation of Dopaminergic and beta2/3 adrenergic receptors by selective agonists results in the increase in neuronal excitability andâ€¦. , suggesting a physiological role for dopaminergic and adrenergic inputs in the regulation of MN excitation.

The ALS MN receptorome is nevertheless highly dynamic and all studied receptors are downregulated in advanced stages of disease. Notably, PKA stimulation and suppression of excitability are characterized by distinct receptoromes, with adrenergic beta-1 receptor systematically downregulated. Finally, the MN receptorome in ALS is substantially modified by pharmacological agents in clinical use. Our data show that MN display extensive entry points for modulation of their electrophysiological properties, which can be accessed with small molecules with translational potential for ALS treatment. "

5 32 03/22/2022 **Calum Bonthron** cb281@st-andrews.ac.uk School of Psychology and Neuroscience, University of St Andrews PhD Student Student "Matthew J. Broadhead: Maite F. Lopez: Jessica Valli: Fei Zhu: Noboru H. Komiyama: Colin Smith: Seth G.N. Grant: Gareth B. Miles: School of Psychology and Neuroscience, University of St Andrews" oral

Selective Vulnerability of Tripartite Synapses in Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder. Separate lines of evidence have implicated both synaptic and astrocytic pathological mechanisms underlying ALS. Given that astrocytes make specialised contacts with some synapses, called tripartite synapses, we hypothesise that tripartite synapses could act as a fulcrum of disease in ALS. To test this hypothesis, we have performed an extensive microscopy-based investigation of synapses and tripartite synapses in the spinal cord of ALS model mice and post-mortem human tissue from ALS cases. Use of the progeny of such mouse models bred with an animal expressing a GFP tag on the postsynaptic density protein PSD95 allows for characterisation of excitatory

synapses at sub- and supra-diffraction-limited resolutions. We reveal widescale synaptic changes at the early symptomatic stages of the SOD1G93a mouse model. gSTED super-resolution microscopy reveals that large complex postsynaptic structures are lost in ALS mice. Interestingly, tripartite synapses are selectively lost, while non-tripartite synapses remain in equal number to healthy controls. Such loss is seen throughout the grey matter of the lumbar cord, and is not simply limited to motor regions. Finally, we also observe a similar selective loss of tripartite synapses in human post-mortem ALS spinal cords. From these data we conclude that tripartite synaptopathy is a key hallmark of ALS.

6P 33 03/22/2022 **Tyler Laurier Wells** Tyler.Wells@dal.ca Dalhousie University Ph.D. Candidate Student "Jacob R Myles; Dalhousie University Turgay Akay; Dalhousie University" 2 poster

C-boutons and Their Relationship to Amyotrophic Lateral Sclerosis Disease Progression

Amyotrophic Lateral Sclerosis (ALS) is an adult-onset neurodegenerative disease with progressive motor neuron death, where patients usually die within five years of diagnosis. Previously we showed that the C-boutons, which are large cholinergic synapses to motor neurons that modulate motor neuron activity, are necessary for behavioural compensation in mSOD1G93A mice, a mouse model for ALS. Since the C-boutons likely increase the excitability of surviving motor neurons to compensate for motor neuron loss during ALS disease progression, we reasoned that frequency modulation through the C-boutons likely increases motor neuron stress and worsens disease progression. By comparing mSOD1G93A mice to mSOD1G93A mice with genetically inactivated (silenced) C-boutons (mSOD1G93A; Dbx1::cre; ChATfl/fl), we show that the C-boutons do not influence the humane endpoint of mSOD1G93A mice; however, histological assessment of fast-twitch leg muscle neuromuscular junctions shows that C-bouton silencing significantly improves fast-twitch leg muscle innervation over time. Using behavioural analysis, we provide evidence that C-bouton silencing in combination with swimming (mSOD1G93A/Coff swim) significantly improves the speed, balance, and grip strength of symptomatic mSOD1G93A mice. Lastly, we provide evidence that C-bouton silencing in combination with swimming in mSOD1G93A mice significantly prolongs the humane endpoint relative to C-bouton silencing alone, but not relative to mSOD1G93A mice with intact C-boutons. Our observations suggest that manipulating the C-boutons in combination with a modulatory-targeted training program may therefore be beneficial for ALS patients.

7 04/01/2022 **Marcin BÄczyk** baczyk@awf.poznan.pl Department of Neurobiology, PoznaÅ„ University of Physical Education, PoznaÅ„, PolandAdiunkt None "Tomasz Jankowiak, Department of Neurobiology, PoznaÅ„ University of Physical Education, PoznaÅ„, Poland Marcin CholewiÅ„ski, Department of Neurobiology, PoznaÅ„, University of Physical Education, PoznaÅ„, Poland" Oral

Facilitation of proprioceptive Ia excitation and intrinsic excitability of spinal motoneurons by anodal trans-spinal direct current stimulation in SOD1 G93A mouse model of Amyotrophic Lateral Sclerosis "Alterations of intrinsic excitability and synaptic excitation

levels form the hallmark of spinal motoneurons degeneration in Amyotrophic Lateral Sclerosis (ALS). We have recently shown that activation of the cAMP/PKA pathway partially restores the reduced Ia EPSPs amplitudes of SOD1 G93A mice motoneurons (MNs). Importantly, this effect was crucially dependent on MN activation. Here we provide direct evidence, based on in vivo intracellular recordings of spinal MNs, that a similar effect can be achieved by an acute application of a single session of anodal trans-spinal direct current stimulation (tsDCS). 15 minutes of 30µA anodal tsDCS facilitated submaximal monosynaptic Ia EPSPs amplitudes up to 290±320% of pre-polarization control values during the current application. Importantly, this increase of excitation was still visible 15 minutes after tsDCS was switched off (an increase up to 278±210 control). The facilitation of Ia EPSPs was accompanied by a decrease in MN activation threshold and an increase in firing frequency. Interestingly, the maximal Ia EPSPs were still facilitated up to 1h after the current application, pointing to a significant long-lasting effect of this neuromodulation. This study validates tsDCS as a powerful method to modify MN excitability in the SOD1 G93A mouse model of ALS. Funding: NCN 2017/26/D/NZ7/00728

8 04/08/2022 **Filipe Nascimento** f.nascimento@ucl.ac.uk UCL Queen Square Institute of Neurology Wellcome research fellow Post-doc "Filipe Nascimento UCL Queen Square Institute of Neurology M. GÃ¶rkem Ã–zyurt UCL Queen Square Institute of Neurology Rob Brownstone UCL Queen Square Institute of Neurology Marco Beato UCL Department of Neuroscience, Physiology and Pharmacology" Oral

Spinal circuits early dysfunction in the SOD1G93A mouse model of Amyotrophic Lateral Sclerosis There is increasing evidence that motoneuron death in Amyotrophic Lateral Sclerosis is preceded by a series of events that are not restricted to motoneurons but may involve alterations at the pre-motor circuit level. In particular, alteration in the density and strength of both excitatory and inhibitory synapses onto motoneurons have been reported in the early stages of the disease. Here we compared the strength of identified sensory afferent and motor efferent synaptic pathways in SOD1G93A mice and Wild Type littermates in the P15-P25 age range. While no changes were observed in di-synaptic Ia/Ib inhibition, we found that Ia mono-synaptic excitation was stronger in SOD1G93A mutants. The excitatory component of the recurrent circuitry was not altered, however, Renshaw cell mediated inhibition exhibited a 2-fold reduction in mutant mice, with no concomitant change in the strength of motoneurons to Renshaw cells synapses. A reduction in quantal size, demonstrated by quantal analysis and by asynchronous release experiments, is responsible for the alteration in recurrent inhibition strength. Interestingly, the impairment of recurrent inhibition is preferentially restricted to late firing motoneurons (putative fast), while early firing motoneurons (putative slow) are not affected. In vivo electromyography recordings confirmed the impairment in Renshaw inhibition in juvenile animals, but also showed that at later stages (around P60 and P90) the strength of recurrent inhibition is restored, suggesting the possibility of a compensation effect.

9P 04/08/2022 **Filipe Nascimento** f.nascimento@ucl.ac.uk UCL Queen Square Institute of Neurology Wellcome Research fellow Post-doc "M. GÃ¶rkem Ã–zyurt UCL Queen Square Institute of Neurology Filipe Nascimento UCL Queen Square Institute

of Neurology Rob Brownstone UCL Queen Square Institute of Neurology Marco Beato UCL Department of Neuroscience, Physiology and Pharmacology " 3 Poster

Electrophysiological properties of motoneurons in the FUSÎ”14 model of ALS

A proportion of familial cases of Amyotrophic Lateral Sclerosis (ALS) are caused by mutations in the Fused in Sarcoma enzyme (FUS). FUS mutations can give rise to an aggressive form of the disease, with early onset and rapid time course. In this study we used a homozygous transgenic mouse, that expresses a humanized truncated FUS protein (FUSÎ”14), and compared the cellular and synaptic properties of motoneurons at an early (P1-P5) and later (P15-P25) time points in the initial stages of disease progression. Homozygous FUSÎ”14 mice show early signs of motor unit loss, detected in juvenile animals (P25-30), measured through in vivo electromyography (EMG). These signs became more pronounced at 2 months of age in EMG along with a decline in grip strength force. In vitro experiments showed that, in homozygous FUSÎ”14 mutants, motoneurons had a larger capacitance and an increased proportion of putative fast motoneurons. Repetitive firing characteristics were not affected, but during development we observed a marked decrease in spike size, not accompanied by a change in the threshold for firing, nor by alterations in the Ih or in the persistent inward currents. Recurrent circuits were also altered, with an increase in the strength of recurrent inhibition in juveniles. The response to afferent inputs was affected in P15-25 animals, with both mono-synaptic Ia excitation and di-synaptic Ia/Ib inhibition reduced in mutant mice. Our data show that the homozygous FUSÎ”14 mouse model of ALS displays early abnormalities in both cellular properties of motoneurons and in local spinal circuits.

10P 04/13/2022 **Arauthy Gina Gnanasampanthan** agg4@st-andrews.ac.uk University of St Andrews PhD Student Student Professor Gareth B. Miles, University of St Andrews 5 Poster

Progressive changes in synaptic inputs to motoneurons and hyperpolarisation-activated inward currents in a SOD1G93A mouse model of Amyotrophic Lateral Sclerosis.

Amyotrophic lateral sclerosis (ALS) is progressive neurodegenerative disease characterised by the deterioration of upper and lower motoneurons (MN) that lead to paralysis and death. Alterations in MN output have been shown at early postnatal stages ALS model mice; however, it is unclear whether these changes are due to synaptic inputs or intrinsic properties. Here, we investigated the mechanisms contributing to altered MN output by using whole-cell patch clamp electrophysiology to study MN properties and synaptic inputs, during the first three postnatal weeks in SOD1G93A mice. Our study targeted delayed firing, fast-type lumbar MNs that are vulnerable and degenerate in ALS. Our results show that the frequency of mixed postsynaptic currents (PSCs) received by MNs is significantly increased in SOD1G93A mice at the second post-natal week. Voltage-clamp protocols were undertaken to distinguish the inhibitory PSCs which showed no significant changes in frequency or amplitude in SOD1G93A mice across all ages. These findings suggest that there is increased excitatory PSCs. The origin of this increased in excitatory drive is currently being investigated by focusing on excitatory miniature PSCs recorded in the presence of TTX. Whilst no significant changes were seen in

intrinsic properties such as capacitance, rheobase, persistent inward currents or post-discharge activity, a significant increase in the hyperpolarisation-activated inward current (Ih), an important factor driving recruitment, was observed in two-week-old SOD1G93A mice. Overall, early postnatal changes observed in synaptic inputs and Ih may both contribute to improper MN output and progressive degeneration in spinal MN in SOD1G93A ALS.

11P 04/14/2022 **Zoe Piccus** piccusze@nih.gov National Institutes of Health/Brown University Graduate Student Student Ken Gable (USUHS), Payam Mohassel (NINDS), Carsten Bonnemann (NINDS), Teresa Dunn (USUHS), Claire Le Pichon (NICHD) poster

Precision mouse models of childhood ALS caused by excessive sphingolipid synthesis

"Amyotrophic lateral sclerosis (ALS) is a fatal disease affecting motor neurons. Recently, patients were identified with de novo mutations in the gene SPTLC1, leading to an ALS onset as early as 3 years of age. SPTLC1 is a subunit of serine palmitoyltransferase (SPT), the rate-limiting enzyme of sphingolipid (SL) synthesis. SLs are an essential lipid class which are particularly abundant in myelin and enriched in the nervous system. The ALS-linked mutations occur within a transmembrane domain (TMD) of SPT required for its negative regulation; structural data predict these mutations cause unrestrained SL synthesis. These are the first metabolic mutations linked to ALS; no animal models of this syndrome exist. We hypothesized that mice expressing ALS-mutant Sptlc1 would produce excessive SLs and exhibit neurodegeneration.

Mouse and human protein sequences are highly conserved in the affected TMD, making precision mouse modeling ideal to investigate consequences of these mutations. We generated mice with the A20S disease-linked mutation in the endogenous locus to examine neurodegeneration and SL production. SLs are elevated in mutant tissues, especially in homozygotes. A20S animals exhibit neurodegeneration revealed by thin myelination and increased levels of neurofilament light chain, a protein that accumulates in serum following axonal breakdown. Mutants exhibit progressive age-related neurodegeneration. By 1 year, A20S animals show TDP-43 denuclearization, a defining pathological feature of human ALS rarely observed in mouse models. These mice provide a preclinical model to test therapies for patients with Sptlc1 ALS-linked mutations and can be leveraged to study how high SL levels lead to neurodegeneration.

12P 04/14/2022 **Anna Bak** an.bak@sund.ku.dk University of Copenhagen Medical Student Student "Svetlana Djukic Marion Kadlekova, Thomas Braustein, Dennis Bo Jensen, Claire Francesca Meehan University of Copenhagen Poster

Cytoplasmic TDP-43 accumulation drives changes in C- bouton number and size in a mouse model of sporadic ALS "An altered neuronal excitability of spinal motoneurones has consistently been implicated in Amyotrophic Lateral Sclerosis (ALS) leading to several investigations of synaptic input to these motoneurones. One such input that has repeatedly been shown to be affected are a population of large cholinergic synapses referred to as C-boutons.

Most research on these synapses during disease progression has used transgenic Superoxide Dismutase mouse models of the disease which have not only produced conflicting findings but also fail to recapitulate the key pathological feature seen in ALS; cytoplasmic accumulations of TDP-43. Additionally, they fail to distinguish between slow and fast motoneurones, the latter of which have more C-boutons but are lost earlier in the disease.

To circumvent these issues, we quantified the frequency and volume of C-boutons on traced soleus and gastrocnemius motoneurones, respectively representing predominantly slow and fast motor pools. Experiments were performed using the TDP-43NLS mouse model carrying a transgenic construct of TDP-43 devoid of its nuclear localization signal preventing its nuclear import. This results in pathological TDP-43 cytoplasmic inclusions, modelling the main pathology seen in ALS.

Our results confirmed changes in both the number and volume of C-boutons with a decrease in number on the more vulnerable, predominantly fast gastrocnemius motoneurones and an increase in number on the less vulnerable, predominantly slow soleus motoneurones. Importantly, these changes were only found in male mice. However, both sexes and motor pools showed a decrease in C-bouton volume. Our experiments confirm that cytoplasmic TDP-43 accumulation is sufficient to drive C-bouton changes."

13 04/15/2022 **Kelly A Marshall** kellymarshall2017@u.northwestern.edu Northwestern University Graduate student Student "Evangelos Kiskinis “ Northwestern University Brian Joseph â€“ Harvard University Kevin Eggan - Harvard University " 02-Jan Oral

Nuclear loss of TDP-43 causes a determinantal exon-skipping event in the RNA of the ion-channel KCNQ2 in ALS-patient neurons Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that affects neurons in the brain and spinal cord. Nuclear depletion and cytoplasmic aggregation of TAR DNA Binding Protein 43â€‰kDa (TDP-43) in patient post-mortem tissue is a neuropathological a hallmark of ALS. TDP-43 is an essential DNA/RNA binding protein predominantly localized to the nucleus where it is responsible for crucial RNA-processing and transport. Mislocalization of TDP-43 to the cytoplasm has recently been linked to the mis-splicing of several genes that appear to be human and neuron-specific targets such as stathmin-2 and UNC13A. However, the functional repercussion of these events for most of the genes, as well as their relevance to ALS pathophysiology remain unclear. We describe here that the voltage-gated potassium channel, KCNQ2 (Kv7.2) becomes significantly mis-spliced as a result of TDP-43 loss-of-function. Kv7.2 is a key regulator of neuronal excitability previously associated with severe developmental epilepsy. With TDP-43 in the cytoplasm, a significant proportion of Kv7.2 transcripts are mis-spliced leading to a removal of exon 5. Using several model system including heterologous expression of KV7 plasmids as well as stem cell derived-neurons we are finding that the deletion of exon 5 results in Kv7 protein aggregation and altered neuronal excitability.

14P 04/15/2022 **Sarah Burley** sb372@st-andrews.ac.uk University of St Andrews Postdoctoral Research FellowPost-doc "Simon Sharples -University of St Andrews Calum Bonthron -University of St Andrews Matthew Broadhead -University of St Andrews Arauthy Gina Gnanasampanthan -University of St Andrews Kimberley Morrison -University of St Andrews Gareth Miles -University of St AndrewsJudith Sleeman -University of St Andrews" PosterEither

Protein methylation as a mechanism for cellular damage in motor neuron disease

"Amyotrophic Lateral Sclerosis (ALS) is the most common form of adult-onset motor neuron disease. One potential shared mechanism across ALS is dysregulation of post-translational protein methylation. The methylation of proteins on arginine residues occurs in three forms: monomethylation (MMA) and symmetrical or asymmetrical dimethylation (sDMA, aDMA).

We have observed major defects in the sub-cellular distribution of proteins that usually contain sDMA modification in cells expressing FLAG-tagged FUS-R495X, a causative mutation for fALS. The antibody SYM10, which recognises sDMA residues, usually detects endogenous proteins resident in sub-nuclear structures: Cajal bodies and splicing speckles. In cells expressing FLAG-tagged FUS-R495X, sDMA (SYM10)-containing proteins co-mislocalise with mutant FUS in cytoplasmic aggregates. The sDMA-containing, splicing snRNP protein, SmB also shows altered localisation with a notable loss from splicing speckles, while non-methylated members of the Sm protein family are unaltered.

Mammalian cells have a single enzyme, protein arginine methyl transferase 5 (PRMT5), to convert MMA to sDMA. Reduction of PRMT5 expression by ~70% using shRNA expression redistributes sDMA-containing proteins in a similar, but less extreme, manner as does expressing FLAG-FUS-R495X, raising the possibility that alterations in sDMA protein distribution resulting from FUS-R495X expression may be mediated through competition for PRMT5 activity.

We have now moved these findings into iPSC-derived motor neurons containing the C9ORF72 gene mutation to establish whether they could reflect a shared mechanism. We additionally observed an unexpected astrocytic disruption when manipulating PRMTs. We hope this study will provide further clarity in the field of protein arginine methylation and associated cellular damage in ALS."

15 04/15/2022 **Nicolas Delestree** nd2488@cumc.columbia.edu Center for Motor Neuron Biology and Disease, Columbia University Post-doc Post-doc Evangelia Semizoglou, John G. Pagiazitis, Aleksandra Vukojicic, Estelle Drobac, Vasilissa Paushkin, George Z. Mentis Oral

Dysfunction in serotonergic neuromodulation impairs locomotor coordination in spinal muscular atrophy "Spinal Muscular Atrophy (SMA) is a neurodegenerative disease due to deficiency of SMN protein. Gait, posture and locomotion, which are largely influenced by proprioception and neuromodulation, are altered in SMA. We previously reported the

dysfunction and elimination of proprioceptive synapses. However, whether the neuromodulatory serotonergic system is affected in SMA, is unknown.

To address this, we utilized the SMN-Î”7 mouse model of SMA and investigated the characteristics of the serotonergic (5-HT) synapses on motor circuits. Functionally, 5-HT is known to modulate the monosynaptic sensory-motor reflex mediated by proprioceptive synapses on motor neurons. Using an ex vivo brainstem-spinal cord preparation, we confirmed that brainstem stimulation reduces the amplitude of this reflex by 50% in P10 WT animals. However, while this modulatory effect was preserved in SMA animals in the disease-resistant L5 spinal segment, it was almost abolished in the L1 segment. Morphologically, 5-HT synaptic coverage is reduced by 25% in vulnerable (L1 and L5-MMC) but not resistant (L5-LMC) motor neurons in SMA at P4 and this reduction progressed to ~55% in vulnerable motor pools of P10 SMA animals. Behaviorally, the dysfunction of the 5-HT synapses in SMA resulted in uncoordinated locomotor behavior, revealed by EMG recordings of the TA muscles in vivo. Selective genetic restoration of SMN in 5-HT neurons in SMN-Î”7 mice improved synaptic coverage and 5-HT neuromodulation, and more importantly, restored left-right hindlimb alternation during locomotor activity. Together, our study uncovers a severe serotonergic dysfunction in SMA mice and proposes the serotonergic pathway as a new therapeutic target."

16 04/16/2022 **Yongchao C. Ma** ma@northwestern.edu Northwestern University Feinberg School of Medicine, Stanley Manne Children's Research Institute Children's Research Fund Associate Professor in Neurobiology None "Zhaofa Xu, Saravanan Arumugam, Nimrod Miller Northwestern University Feinberg School of Medicine, Stanley Manne Children's Research Institute" Oral

Regulation of mitochondria-induced neuroinflammation and motor neuron degeneration in Spinal Muscular Atrophy (SMA) by RNA methylation "Motor neuron degeneration in SMA makes it the number genetic killer of toddlers, affecting one in every 6000 live births. SMA is caused by mutations in SMN1 (survival motor neuron 1) gene, leading to reduced SMN protein level and selective motor neuron degeneration. Although treatments based on increasing SMN protein levels have been developed, there are significant limitations of therapeutic effects. Better understanding of mechanisms underlying motor neuron degeneration and SMN function is critically needed.

RNA methylation on 6-methyladenosine was recently identified as the most prevalent and dynamic modification of mRNA and lncRNA that regulates every aspect of RNA metabolism, including biogenesis, splicing, nuclear-cytoplasmic transport, translation, and degradation. However, as a RNA-binding protein, SMN has not been linked with RNA methylation.

Through studying SMN-interacting proteins and RNA, we identified SMN as a novel methylated RNA binding protein that regulates mitochondrial function through regulating a specific program of methylated RNA. Reduced SMN protein level in SMA leads to dysfunction of methylated RNA, mitochondrial defects, mitochondrial DNA release, neuroinflammation, and motor neuron

degeneration. Therefore, our study not only identifies a novel function for SMN, a new pathogenic mechanism for SMA, but also opens up avenues for novel therapeutic strategy. "

17P 04/16/2022 **Jennifer Deng** jenniferdeng@gatech.edu Georgia Institute of Technology undergraduate research assistant Student"Sarah Bi, Dennis Tsui, Albert Lee, Irfan Al-Hussaini, Cassie S. Mitchell Georgia Institute of Technology" 2-3 (virtual) Remote Poster

Homeostatic re-stabilization of SOD1-G93A ALS via combination therapy with repurposed drugs Simultaneous multi-factorial in vivo experimental assessment of the SOD1-G93A ALS mouse etiology is inherently limited. Mitchell and colleagues have previously published the homeostatic instability hypothesis to describe multi-factorial ALS progression due to failed homeostatic regulation, which causes multi-scalar oscillatory system instability. The study objective was to model targets for ALS combination therapy in silico using system stability as the criterion for a successful treatment strategy. Computational models of wild type (WT) and SOD1-G93A (ALS) mouse physiological stability were developed using dynamic meta-analysis (DMA), mathematical optimization, and unsupervised machine learning, based on prominent regulatory mechanisms in ALS pathophysiology. Combination treatments mathematically modulated regulatory feedback. The identified treatments and stability patterns provide critical insight for experimental development and testing of translational ALS treatments and etiological hypotheses. Combination treatment targets identified from the SOD1-G93A computational mouse model are used as starting points for literature based discovery (LBD). LBD was performed using a custom built cutting-edge software, SemNet 2.0, which contains text relationships extracted from 30+ million PubMed articles. LBD recommended repurposed drugs for ALS that matched the mechanistic intent of the identified highly ranked dynamics-based combination therapies. Specific repurposed treatment candidates are proposed to mitigate oxidative stress, apoptosis, and inflammation.

18 Presenting Author **Zhenxiang Zhao** (University of Copenhagen)

Other authors Svetlana Djukic (University of Copenhagen)Lasse Mathias Holmsted Jørgensen (University of Copenhagen)Anne Bak (University of Copenhagen)Dennis Bo Jensen (University of Copenhagen)Claire Francesca Meehan (University of Copenhagen) oral

Cytoplasmic TDP-43 drives reversible hyperexcitability of spinal motoneurones.

TMS, reflex and nerve excitability studies all indicate an increased motoneurone excitability in patients with Amyotrophic Lateral Sclerosis (ALS). Our previous in vivo intracellular recordings have confirmed an increase in the intrinsic excitability of spinal motoneurones in SOD1 mice models of the disease. However, SOD1 mutations account for only ~2% of all ALS patients. What is driving the hyperexcitability in the vast majority of sporadic cases is therefore unclear. The most common key pathological feature found in ~95% of all ALS patients are cytoplasmic

aggregates of the protein TDP-43. The TDP-43(ΔNLS) mouse model successfully recapitulates this pathology and is sufficient to drive a severe ALS phenotype. We used in vivo intracellular recordings in this model to investigate whether TDP-43 pathology is sufficient to induce hyper-excitability.

Induction of the transgene in adult mice resulted in a severe hyper-excitability of spinal motoneurones after 4 weeks including a decreased rheobase, increased gain, increased input resistance and decreased AHP amplitude. Anatomical experiments showed a significant reduction in soma size, which could explain the reduced input resistance. Axon initial segments were also significantly longer and thinner in the induced mice.

Re-suppression of the transgene after 4 weeks, resulted in significant functional recovery by 6 weeks post re-suppression. This resulted in a return to normal excitability parameters with no significant difference now between suppressed and the induced-resuppressed mice with respect to both rheobase and gain. We therefore conclude that TDP-43 pathology itself is sufficient to drive a severe but reversible hyperexcitability of spinal motoneurones.

19 Hemali Phatnani, Ph.D. New York Genome Center and Columbia University Irving Medical Center oral

Spatiotemporal dynamics of molecular pathology in ALS

Paralysis occurring in amyotrophic lateral sclerosis (ALS) results from denervation of skeletal muscle as a consequence of motor neuron degeneration. Interactions between motor neurons and glia contribute to motor neuron loss, but the spatiotemporal ordering of molecular events that drive these processes in intact spinal tissue remains poorly understood. We used spatial transcriptomics to obtain gene expression measurements of mouse spinal cords over the course of disease, as well as of *postmortem* tissue from ALS patients, to characterize the underlying molecular mechanisms in ALS. We identified novel pathway dynamics, regional differences between microglia and astrocyte populations at early time-points, and found perturbations in several transcriptional pathways shared between murine models of ALS and human *postmortem* spinal cords. Here, we will describe these findings and outline how we plan to expand these studies more broadly.

MN RNA-sequencing

1P 04/14/2022 **Mor R Alkaslasi** mor.alkaslasi@nih.gov National Institutes of Health/Brown UniversityGraduate Student Student "Zoe E. Piccus, Sangeetha Hareendran, Hanna Silberberg, Li Chen, Yajun Zhang, Timothy J. Petros & Claire E. Le Pichon Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA" Poster

Single nucleus RNA-sequencing defines unexpected diversity of cholinergic neuron types in the adult mouse spinal cord In vertebrates, motor control relies on cholinergic neurons in the spinal cord that have been extensively studied over the past hundred years, yet the

full heterogeneity of these neurons and their different functional roles in the adult remain to be defined. Here, we develop a targeted single nuclear RNA sequencing approach and use it to identify an array of cholinergic interneurons, visceral and skeletal motor neurons. Our data expose markers for distinguishing these classes of cholinergic neurons and their rich diversity. Specifically, visceral motor neurons, which provide autonomic control, can be divided into more than a dozen transcriptomic classes with anatomically restricted localization along the spinal cord. The complexity of the skeletal motor neurons is also reflected in our analysis with alpha, gamma, and a third subtype, possibly corresponding to the elusive beta motor neurons, clearly distinguished. In combination, our data provide a comprehensive transcriptomic description of this important population of neurons that control many aspects of physiology and movement and encompass the cellular substrates for debilitating degenerative disorders.

2P 04/16/2022 **Mikaela Myers** mikaela.myers@midwestern.edu Midwestern University Student Fellow "Ann Revill, PhD. - Midwestern University Johana Vallejo, PhD. - Midwestern University Kimberly Bussey, PhD. - Midwestern University Cole Leiker- Midwestern University Simran Gill- Midwestern University" poster

Evaluation of the Impacts of Biological Sex and Diet-Induced Obesity on Ion Channel and mRNA Expression at the Hypoglossal Motoneurons in Mice Abstract will be submitted later per email with Dr. Monica Gorassini

3. Ariel Levine **Title: What Human Motoneurons Are Made Of** oral
Abstract: In neurodegenerative diseases of the human spinal cord, such as amyotrophic lateral sclerosis (ALS), motoneurons are particularly vulnerable to degeneration. It is hypothesized that their large size contributes to disease susceptibility, but the link between genetic variants associated with disease and cell-type specific degeneration is not clear. We characterized human spinal cord cells using single-nucleus RNA-sequencing and protein profiling. We found that human motoneurons displayed a unique expression profile characterized by factors involved in cytoskeletal structure, cell size, and degenerative disease (including ALS-associated genes SOD1, NEFH, OPTN, TUBA4A, PRPH, and STMN2) and that protein expression of these genes correlated with larger cell size in tissue. This work suggests a motoneuron-specific signature underlies their selective vulnerability to neurodegeneration.

4. Jacob Blum oral
Title: Motor neuron heterogeneity and vulnerability in amyotrophic lateral sclerosis
Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal, progressive neurodegenerative disease caused by the dysfunction and death of spinal motor neurons. Despite their outsized role in neuromuscular control and disease, these cells constitute a minute fraction of the total cells in the mammalian spinal cord. We developed a novel approach to enrich for motor neuron nuclei using fluorescence-activated nuclei sorting (FANS). Using this technique, we transcriptionally profiled ~150,000 single nuclei from the spinal cords of healthy and ALS disease mice. Here, we present a detailed characterization of spinal motor neuron populations at unprecedented resolution and depth. Analysis of these data allow us to cluster cell transcriptomes and map them onto functionally defined cell types. For the first time, we provide insight into the transcriptional differences among these physiologically important cell types. In ALS, certain populations of

motor neurons degenerate preferentially despite ubiquitous expression of mutated genes responsible for the disease. By applying our technique to a disease model of ALS, we have identified widespread transcriptional alterations in motor neuron transcriptomes—as well as the other neuronal and glial cells that support their function. By analyzing the motor neurons that remain unaffected in diseased mice, we have discovered novel markers of susceptible and resistant motor neuron populations, and generated novel hypotheses regarding the molecular underpinnings of ALS.

5. **Sam Pfaff Title: MicroRNA regulation of motor neuron survival.** oral
Abstract: The gene regulatory and phenotypic effects of altering a miRNA's in vivo abundance within motor neurons, rather than its binary gain or loss of function, is not well understood. We generated an allelic series of mice expressing varying levels of miR-218, a motor neuron-selective gene regulator associated with motor neuron degeneration and disease. Titration of miR-218 unexpectedly revealed complex, non-ratiometric target mRNA dose-responses and distinct gene network outputs. A non-linearly responsive regulon exhibited a steep miR-218 dose-dependent threshold in repression, that when crossed, resulted in severe motor neuron synaptic failure and death. This work demonstrates that a miRNA can govern distinct gene network outputs at different dose ranges. It was striking that miRNA-dependent phenotypes emerge at particular dose ranges due to hidden regulatory inflection points of the underlying gene networks. (see Amin ND, Senturk G, Costaguta G, Driscoll S, O'Leary B, Bonanomi D, Pfaff SL. (2012). A hidden threshold in motor neuron gene networks revealed by modulation of miR-218 dose. Neuron 109:3252-3267. PMID: 34450025

6. **Claire LePichon Single nucleus RNA sequencing reveals unexpected diversity of spinal cholinergic neurons in the adult mouse**

In vertebrates, motor control relies on cholinergic neurons in the spinal cord that have been extensively studied over the past hundred years, yet the full heterogeneity of these neurons and their different functional roles in the adult remain to be defined. In this study, we apply a targeted single nuclear RNA sequencing approach and use it to identify an array of cholinergic interneurons, visceral and skeletal motor neurons. Our data expose markers for distinguishing these classes of cholinergic neurons and their rich diversity, together with information about their anatomical localization. Our study provides a comprehensive transcriptomic description of this important population of neurons that control many aspects of physiology and movement and encompass the cellular substrates for debilitating degenerative disorders

Spinal cord injury, stroke, neurological injury_____

1 23 02/12/2022 **Gregory Pearcey** gpearcey@northwestern.edu Northwestern University & Shirley Ryan Ability Lab Postdoc Post-doc "Babak Afsharipour(1), Ales Holobar (2), W Zev Rymer (3), and Milap Sandhu (3) 1) University of Alberta 2) University of Maribor 3) Northwestern University & Shirley Ryan Ability Lab" Oral

Acute intermittent hypoxia effects on strength and motor unit discharge rates Acute intermittent hypoxia (AIH) is a therapeutic intervention that utilizes brief reductions of inspired oxygen to stimulate the serotonergic system, a potent neuromodulator of alpha motoneurons. AIH enhances volitional strength in spinal cord injured individuals but underlying mechanisms are unclear. Here, we decomposed motor units (MUs) from high-density surface EMG during maximal elbow flexion and extension, recorded before and 60-minutes after AIH. We hypothesized that MU discharge rates would increase after AIH, which would help explain changes in strength. Seven individuals with chronic iSCI at the cervical level completed AIH and Sham AIH interventions in a randomized order. AIH consisted of 15 ~60s periods of low oxygen (O2 = 9%) interspersed with 60s periods of normoxia, whereas Sham AIH consisted of repeated exposures to normoxia. Elbow flexion and extension torque increased by 54% (p = 0.0078; g = 0.58) and 59% (p = 0.04, g = 0.63) from baseline, respectively, whereas there was no change after SHAM. MU discharge rates increased by 3.96 pulses per second (~38%; p = 0.0015; G = 0.86), from pre to post AIH. Using generalized linear mixed models, we found that changes in discharge rates explained 54% (p = 0.0033) of the variance of changes in volitional strength. In a follow-up study, we administered AIH to neurologically intact participants and observed greater estimates of persistent inward currents after AIH. This suggests that intrinsic motoneuron excitability and/or monoaminergic drive are augmented by AIH, providing an underlying mechanism for observed AIH-induced improvements in volitional strength.

2 24 02/15/2022 **Alexandra Lackmy-Vallee** alexandra.lackmy@upmc.fr Sorbonne UniversitÃ©, CNRS, INSERM, Laboratoire dâ€™Imagerie BiomÃ©dicale, LIB, F-75005, Paris, France Engineer None "Wanalee KLOMJAI : Faculty of Physical Therapy, Mahidol University, 73170 Nakonpathom, Thailand, Mohamed Mounir EL MENDILI: Aix Marseille Univ, CRMBM, CNRS, Marseille, France, Alain GIRON: Sorbonne UniversitÃ©, CNRS, INSERM, Laboratoire dâ€™Imagerie BiomÃ©dicale, LIB, F-75005, Paris, France, APHP, HÃ´pital de la PitiÃ© SalpÃªtriÃ¨re, F-75013, Paris, France oral

UNDAMAGED HEMISPHERE ACTIVATION ENHANCES CONTROL ON SPINAL NETWORKS OF THE AFFECTED ARM POST STROKE "Objective: The functional role of ipsilateral motor cortex efferent pathways in the transmission of voluntary command to spinal motor nuclei reminds controversial in humans. However, recent imaging studies suggest that they may contribute to functional recovery after unilateral brain damage. This randomized-sham control study aims to explore to what extent ipsilateral tracts from the undamaged hemisphere may strengthen corticospinal control onto spinal motor networks following stroke.

Method: Anodal transcranial direct current stimulation (tDCS) was combined with monosynaptic H-reflex method to evaluate the variations of reciprocal inhibition (RI) in wrist flexors in 21 stroke participants. Moreover, five participants underwent imaging experiments in spinal cord to evaluate alterations in descending tracts.

Results: Anodal tDCS decreased RI in wrist flexors in stroke participants. In the affected side, anodal tDCS unmasks an ipsilateral control from the undamaged hemisphere onto spinal motor networks. In the unaffected (contralateral) side, effects were opposite to those induced in healthy subjects. Imaging experiments stressed an atrophy in spinal cord in stroke participants.

Conclusion: Stimulation of the ipsilateral undamaged cortex in stroke participants induces modulation of motor networks controlling the hemiparetic side.

Significance: Rehabilitation could leverage stimulation of the undamaged hemisphere to enhance motor recovery post stroke

3 36 03/24/2022 **Colin K. Franz** cfranz@sralab.org Shirley Ryan AbilityLab Physician-Scientist "Suning He, Northwestern University Alyssa Weston, Shirley Ryan AbilityLab Maria Jose Quezada, Northwestern University Ian Jones, University of Illinois Chicago John D. Finan, University of Illinois Chicago" oral

A human stem cell based-assay to define how a highly prevalent genetic variation increases motor neuron vulnerability to mechanical injury Lower motor neuron (MN) loss after spinal cord injury (SCI) occurs several segments above and below the level of injury, but varies a lot between patients. Genetic variation may contribute to this variation, but has proved difficult to isolate. To begin to define how genetic variation may affect SCI outcomes, we employed a stem cell-based assay to isolate the effects of the Val66Met single nucleotide polymorphism in the brain-derived neurotrophic factor (BDNF) gene. BDNF Val66Met carriers have impaired activity-dependent release of BDNF protein and this is implicated in altered clinical neurotrauma outcomes. It is also highly prevalent, carried by about 1/3rd of the US population. To test the hypothesis that carriers of the Met allele are more susceptible to motor neuron degeneration after injury, we use an in vitro stretch injury model designed for human induced pluripotent stem cell (iPSC)-derived motor neurons. We apply different strain levels to isogenic iPSC-motor neurons (gene edited to differ by only the presence or absence of the Met allele), then measured the cell viability and neurite length after the injury. The results show that motor neurons with the Met allele are more susceptible to neurodegeneration. This genotype difference could be ameliorated by adding recombinant BDNF to injured motor neurons before injury, which implies a BDNF-TrkB signaling mechanism. Transcriptomic analysis of isogenic iPSC-motor neurons by RNA sequencing reveals differential expression of 1491 genes (613 upregulated, 878 downregulated) with gene ontology enrichment analysis identifying both TrkB and other pathways altered by this genetic variation.

4 04/04/2022 **Amanda Pocratsky** a.pocratsky@ucl.ac.uk University College London Postdoctoral fellow Post-doc "Filipe Nascimento (2), M. GÃ¶rkem Ã–zyurt (2) , Ian White (3), Roisin Sullivan (4), Calvin C. Smith (1), Sunaina Surana (1,5), Marco Beato (2), Robert M. Brownstone (1)(1) Department of Neuromuscular Diseases, Institute of Neurology, University College London, London, WC1N 3BG, UK.(2) Department of Neuroscience, Physiology, and Pharmacology, University College London, London, WC1E 6BT, UK. (3) Electron Microscopy Laboratory, MRC Laboratory for Molecular Cell Biology, University College London, London, WC1E 6BT, UK.(4) Department of Molecular Neuroscience, Institute of Neurology, University College London, London, WC1N 3BG, UK. (5) UK Dementia Research Institute, University College London, London, WC1E 6BT, UK." oral

Pathophysiology of Dyt1 dystonia is mediated by spinal cord dysfunction Dystonia, a neurological disorder defined by abnormal postures and disorganised movements, is thought to be a neural circuit disorder with dysfunction arising within and between multiple brain regions. Given that spinal circuits are the de facto final common pathway for motor control, we sought to determine their contribution to the movement disorder. To do so, we confined a dystonia-related mutation to the spinal cord, which behaviourally and physiologically recapitulated a severe form of inherited, early-onset, generalised dystonia. These data reveal that spinal circuitopathy is a key factor in dystonia pathophysiology, thus challenging our current understanding of dystonia and opening the door to new therapeutic targets.

5P 04/12/2022 **Timothy S. Pulverenti** Timothy.Pulverenti@csi.cuny.edu City University of New York Post doctoral Research Fellow Post-doc "Timothy S. Pulverenti1, Morad Zaaya1, and Maria Knikou1, 2 1 Klab4Recovery Research Laboratory, Department of Physical Therapy, College of Staten Island, The City University of New York, Staten Island, NY USA 2 PhD Program in Biology and Collaborative Neuroscience Program, Graduate Center of The City University of New York and College of Staten Island, New York, NY USA " Timothy S. Pulverenti and Maria Knikou poster

Brain and Spinal Cord Paired Stimulation Coupled with Locomotor Training Affects Polysynaptic Flexion Reflex Circuits in Human Spinal Cord Injury Neurorecovery from locomotor training is well established in human spinal cord injury (SCI). However, neurorecovery resulting from combined interventions has not been widely studied. In this randomized clinical trial, we established the tibialis anterior (TA) flexion reflex modulation pattern when transcranial magnetic stimulation (TMS) of the primary motor cortex was paired with transcutaneous spinal cord (transspinal) stimulation over the thoracolumbar region during assisted step training. Single pulses of TMS were delivered either before (TMS-transspinal) or after (transspinal-TMS) transspinal stimulation during the stance phase of the less impaired leg. Eight individuals with chronic incomplete or complete SCI received at least 20 sessions of paired stimulation during assisted step training. Each session consisted of 240 paired stimuli delivered over 10-min blocks for 1 hour during robotic assisted step training with the Lokomat6 ProÂ®. Body weight support, leg guidance force and treadmill speed were adjusted based on each participantâ€™s ability to step without knee buckling or toe dragging. Both the early and late TA

flexion reflex remained unaltered after TMS-transspinal and locomotor training. In contrast, the early and late TA flexion reflexes were significantly depressed during stepping after transspinal-TMS and locomotor training. Reflex changes occurred at similar slopes and intercepts before and after training. Our findings support that targeted brain and spinal cord stimulation coupled with locomotor training reorganizes the function of flexion reflex pathways, which are a part of locomotor networks, in humans with varying levels of sensorimotor function after SCI.

6P 04/12/2022 **Timothy S. Pulverenti** Timothy.Pulverenti@csi.cuny.edu City University of New York Post doctoral research fellow Post-doc "Timothy S. Pulverenti 1, Morad Zaaya 1, Ewelina Grabowski 1, Monika Grabowski 1, and Maria Knikou 1, 21 Klab4Recovery Research Laboratory, Department of Physical Therapy, College of Staten Island, The City University of New York, Staten Island, NY USA2 PhD Program in Biology and Collaborative Neuroscience Program, Graduate Center of The City University of New York and College of Staten Island, New York, NY USA" poster

Activity-Based Brain and Spinal Cord Paired Stimulation Facilitates Motor Output in Human Spinal Cord Injury Combined interventions for neuromodulation leading to neurorecovery has gained great attention by researchers to resemble real clinical rehabilitation approaches. In this randomized clinical trial, we established changes in motor neuron output when transcranial magnetic stimulation (TMS) of the primary motor cortex was paired with transcutaneous spinal (transspinal) stimulation over the thoracolumbar region during assisted step training. TMS was delivered before (TMS-transspinal) or after (transspinal-TMS) transspinal stimulation during the stance phase of the less impaired leg. Eight individuals with chronic incomplete or complete SCI received at least 20 sessions. Each session consisted of 240 paired stimuli delivered over 10-min blocks for 1 hour during robotic assisted step training with the Lokomat6 ProÂ®. Body weight support, leg guidance force and treadmill speed were adjusted based on each subjectâ€™s ability to step without knee buckling or toe dragging. Most transspinal evoked potentials (TEPs) recorded before and after each intervention from ankle and knee muscles during assisted stepping were modulated in a phase-dependent pattern. Transspinal-TMS and locomotor training affected motor neuron output of proximal (knee, hip) and distal (ankle) muscles that were modulated in a more physiological pattern. TMS-transspinal and locomotor training increased motor neuron output for proximal but not for distal muscles. Our results support that targeted brain and spinal cord stimulation alters responsiveness of neurons over multiple spinal segments in people with chronic SCI. Supported by the NYSDOH, SCIRP (C32095GG), and in part by the NICHD/NIH (RO1 HD100544).

7 04/13/2022 **Frederic Bretzner** Frederic.Bretzner.1@ulaval.ca UniversitÃ© Laval Professor None "Marie Roussel, Nicolas Josset, David Lafrance-Zoubga, Maxime Lemieux UniversitÃ© Laval" oral

Plasticity and contribution of mesencephalic locomotor region nuclei to functional motor recovery after chronic spinal cord injury in the mouse "Spinal cord injury (SCI) results in a

disruption of information between the brain and the spinal locomotor circuit. Although the spinal cord contains all the neural circuits to generate locomotion, people with SCI are unable to walk due to the absence of descending commands from the brain. Electrical stimulation of supraspinal locomotor centers, such as the Mesencephalic Locomotor Region (MLR), can promote locomotor recovery in acute and chronic SCI rodent models. Although clinical trials are currently underway in SCI patients, there is still debate about the organization of this supraspinal locomotor center and which anatomical correlate of the MLR should be targeted to promote functional recovery. Combining kinematics, electromyographic recordings, anatomical analyses, and mouse genetics, our study reveals that glutamatergic neurons of the cuneiform nucleus contribute to locomotor recovery by enhancing its motor efficacy in flexor and extensor hindlimb muscles and by increasing locomotor rhythm and speed during treadmill and overground locomotion as well as during swimming in chronic SCI. In contrast, glutamatergic neurons of the pedunculopontine nucleus slow down locomotion. Therefore, our study identifies the cuneiform nucleus and its glutamatergic neurons as a therapeutical target to improve locomotor recovery in patients living with SCI. Funding: Wings for Life and Canadian Institutes of Health Research"

8P 04/14/2022 **Matthew Bryson** matthew.bryson@emory.edu Emory University PhD Candidate Student Shawn Hochman, Emory University; Sandra Garraway, Emory University; Heidi Kloefkorn-Adams, Oregon State University 4 Poster

SCI-induced spinal hyperexcitability shares common circuitry with 4-AP induced synchronous bursting "Spinal cord injury (SCI) commonly leads to increased hyperexcitability in spinal sensory and motor circuits with associated dysfunction (e.g. neuropathic pain and spasticity, respectively). Here, we compared rostrocaudal changes in dorsal horn circuitry excitability several months after sham or T12 contusion SCI using an isolated ex vivo intact spinal cord preparation. Population activity was recorded from the dorsal root (DR) entry zone of multiple roots bilaterally above and below the lesion as well from Lissauerâ€™s tract as intracellular field potentials. A prominent observation was the presence of spontaneous antidromic bursts in various dorsal roots (dorsal root reflexes DRRs) with greatly increased incidence of synchronous bursting across roots observed in the SCI population. Bursting interacted with electrically evoked afferent responses in a phase-dependent manner and was strongly depressed with several GABAA receptor antagonists. Rhythmic antidromic bursts appear similar to those reported previously in cat following administration of 4-aminopyridine (4-AP), there shown to correspond to activity in medium and slower conducting cutaneous and muscle afferents that could also synchronize with activity in ventral roots. Here, 4-AP similarly led to the emergence of rhythmic antidromic DRRs in naÃ¯ve/sham mice but did not alter spontaneous bursting properties already expressed in SCI mice, supporting actions via common spinal circuits. Studies are underway to identify afferents involved in DRRs and whether their activity leads to modifications in the excitability of peripheral afferents controlling sensory and motor behavior (funded by 5R01NS102850-03)."

9 04/14/2022 **Alex M Laliberte** alalibe4@uottawa.ca University of Ottawa Postdoctoral Fellow Post-doc"Riham Khodr [1], Tuan V Bui [1,2] [1] University of Ottawa, Department of Biology [2] UOttawa Brain and Mind Research Institute" oral

Pre-motor dI3 interneurons regulate hindlimb motor tone in spinalized mice The dI3 population of spinal cord interneurons (INs) is a group of excitatory INs that receive varied sensory afferent input and project ipsilaterally to motoneurons. Prior experiments have discovered that the permanent silencing of dI3 INs has a minimal impact on locomotor function in intact animals, but substantially interferes with locomotor rehabilitation/recovery after spinal cord injury. To gain insight into how dI3 INs are involved with locomotor function, the inhibitory or excitatory DREADD receptor (hM4Di or hM3Dq) was expressed in dI3 INs using a hybrid transgenic mouse line (Isl1-Cre:Vglut2-Flp) combined with an hM4Di reporter line, or an intraspinally-injected adeno-associated virus expressing hM3Dq. Consistent with prior experiments, transient inhibition of hM4Di-expressing dI3 neurons with the DREADD agonist JHU37160 (0.5mg/kg) did not significantly impact locomotor function in intact animals. However, following T9-T10 transection, transient silencing of dI3 INs resulted in a significant loss of flexor motor tone, demonstrated by an increase in the resting ankle joint angle (+43.9Â° Â± 11.6Â°, n=7, t-test, p=0.0065). This coincided with hindlimb flaccidity during a treadmill locomotor task. Conversely, activation of dI3 interneurons in dI3hM3Dq mice resulted in a tonic increase in tibialis anterior EMG activity that was partially modulated by passive stretch or relaxation. Viral anterograde tracing of dI3 projections (GFP+) to motoneurons associated with fluorogold-injected gastrocnemius or tibialis anterior muscles identified substantial innervation of gamma motoneurons (ERR3+) in addition to alpha motoneurons. Based on these results, dI3 INs appear to regulate hindlimb motor tone, possibly through the modulation of fusimotor drive and stretch reflexes.

10P 04/15/2022 **Narges Karimi** narges.karimi.1@ulaval.ca Centre de recherche du CHU de QuÃ©bec, UniversitÃ© Laval, QuÃ©bec, Canada PhD student Student "Maxime Lemieux, Centre de recherche du CHU de QuÃ©bec, UniversitÃ© Laval, QuÃ©bec, Canada FrÃ©dÃ©ric Bretzner, Centre de recherche du CHU de QuÃ©bec, UniversitÃ© Laval, QuÃ©bec, Canada " poster

Contribution and plasticity of glutamatergic neurons of the gigantocellular reticular nucleus to locomotor recovery after spinal cord injury "Although anatomical studies have shown plasticity of the reticulospinal axons of the gigantocellular reticular nucleus (Gi) after spinal cord injury (SCI), little is known about the functional contribution of the Gi to locomotor recovery. Using kinematic and electromyographic measurements in VGluT2-cre mice, we investigated changes in the motor efficacy of glutamatergic neurons of the Gi in relation to locomotor recovery following a lateral thoracic hemisection. Before SCI, short photostimulation delivered in the Gi evoked excitatory motor responses in the flexor muscle of the ipsilesional hindlimb. Although these motor responses decreased in more than half of mice 1 week after SCI, they recovered or increased 7 weeks after SCI. Interestingly, these changes in motor responses correlated with changes in the locomotor performance. Furthermore, long trains of photostimulations delivered in the Gi reduced the variability in the stepping ability and improved

the position of the ankle prior to the swing phase. Finally, conditioning photostimulations of the Gi also improved voluntary locomotion 7 weeks after SCI. In summary, our findings show that glutamatergic neurons of the Gi contribute to locomotor recovery after SCI and can improve motor functions in chronic SCI. Funding: Wings for Life Foundation and Craig H. Neilsen Foundation"

11 04/15/2022 **Han Zhang** hzhang20@ualberta.ca Faculty of Rehabilitation Medicine, University of Alberta Post-doc Post-doc "Aysan Khatmi Faculty of Rehabilitation Medicine, University of Alberta Keith Fenrich Faculty of Rehabilitation Medicine, University of Alberta David J. Bennett Faculty of Rehabilitation Medicine, University of Alberta" oral

Spinal lumber V3 INs are crucial in the recovery of locomotion after SCI Many rehabilitation practices, such as locomotor training to activate spinal motor circuits, have been combined with electrical spinal cord stimulation (epidural stimulation) to activate sensory afferents, with the aim to reorganize spinal motor circuitry and improve recovery after spinal cord injury (SCI). Currently, however, the major limitation to advancing these potential rehabilitation strategies is that the underlying mechanisms and neuroplastic changes caused by locomotor training and epidural stimulation remain poorly understood. Genetically identified spinal interneuron (IN) populations have been shown their distinctive roles in sensory-motor functions, yet very few of them have been investigated in SCI models. Among them, V3 INs, one of the major groups of excitatory spinal neurons that play crucial roles in generating robust and stable locomotor activities, have shown great potential as a therapeutic target to treat SCI. Firstly, we found that after spinal cord transection at the thoracic level 10 (T10), V3OFF mice (with V3 silencing) still had severely impaired hindlimb locomotion six weeks after surgery, while V3ON mice already started walking well with weight support. Furthermore, with epidural stimulation training every other day for six weeks after T10 spinal cord transection, V3OFF mice still could not recover the locomotion. Optogenetic stimulation applied to lumbar V3 INs that express ChR2 leads to substantial recovery of locomotion after SCI. In addition, I also found that V3 INs receive strong sensory input. Therefore, I hypothesize that V3 INs are crucial for locomotor recovery after SCI by mediating cutaneous or/and proprioceptive sensory feedback that assists walking.

12 04/18/2022 **David Leonardo Garcia Ramirez** dg679@drexel.edu Drexel University Postdoc Post-doc "Nicholas J. Stachowski SebastiÃ¡n J. Atoche, Lihua Yao, Simon F. Giszter Kimberly J. Dougherty Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA 19129, USA" oral

Spinal cord injury-induced plasticity of sensory afferent input pathways and serotonergic modulation of Shox2 interneurons following epidural stimulation in mouse Epidural stimulation (ES) is a strategy used after spinal cord injury (SCI) to counteract the deficits in descending control of the spinal circuitry and restore locomotor function. In mice, interneurons (INs) expressing the transcription factor Shox2 have been linked to the generation of the locomotor rhythm and pattern. Studying the effects of SCI and ES on Shox2 INs is an opportunity to understand the therapeutic mechanisms and further improve current strategies to

recover locomotor function after SCI. Here, we analyzed the impact of SCI and ES on the serotonin (5-HT) modulation of and sensory afferent pathways to Shox2 INs. Shox2::Cre;Rosa26-lsl-tdTomato mice were uninjured, received a complete spinal transect at thoracic T8/T9 (SCI), or received sub-motor-threshold ES at L2 after SCI (SCI+ES). Lumbar spinal slices were prepared from mice 6-10 weeks after surgery. We performed whole cell patch clamp recordings from Shox2 neurons while either electrically stimulating dorsal roots or administering 5-HT. In slices from uninjured mice, 5-HT inhibits and excites Shox2 INs in a dose dependent manner and Shox2 INs received afferent inputs mediated by both excitatory and inhibitory sensory pathways. However, SCI enhances the excitatory response of Shox2 INs to 5-HT and Shox2 INs received almost exclusively excitatory input from sensory pathways. Interestingly, 5-HT mainly inhibits Shox2 INs from SCI+ES mice, while afferent-evoked inhibitory inputs to Shox2 neurons were observed more often than excitatory inputs. This suggests that ES is able to prevent or restore the plastic changes observed at the level of Shox2 INs after SCI.

13P **Mingchen Jiang1**, Derin V Birch[2], Charles J Heckman[1,2,3], Vicki M Tysseling[1,2] [1]Department of Physiology, Feinberg School of Medicine, Northwestern University, Chicago, IL, United States; [2]Physical Therapy and Human Movement Sciences, Feinberg School of Medicine, Northwestern University, Chicago, IL, United States; [3]Physical Medicine and Rehabilitation, Feinberg School of Medicine, Northwestern University, Chicago, IL, United States m-jiang@northwestern.edu poster

Involvement of NMDA receptors in spontaneous muscle spasms in a mouse model of chronic SCI Clinical surveys indicate that more than 30% of people with chronic spinal cord injure (SCI) suffer spontaneous muscle spasms. However, the tendency for these spasms to occur spontaneously has not been previously investigated. Using a mouse model of chronic SCI with complete cord transaction at T10, we investigated the occurrence of spontaneous spasms by recording muscle electromyography (EMG) in the hind legs and ventral root activities at sacral segments 1-3 (S1-S3). The EMG recordings showed spontaneous bursting firings from hind leg muscles that were correlated with muscle spasms and could be similarly induced by electrical stimulation. The ventral root recordings showed that similar bursts were found in 63% SCI mice (24 out of 38 mice; 3.62 ± 2.65 seconds, mean and SD). The bursts were synchronized in the ventral roots contra- and ipsi-laterally in the 10 second time window, blocked by Ketamine and Zolmitriptan and sensitive to the concentrations of Magnesium. The bursts could also be induced by electrical stimulation on dorsal root (>1.5 times of threshold) inconsistently when the stimulation was delivered every 1 minute. The bursts were enhanced by the addition of NMDA. However, higher concentration of NMDA could only induce tonic firing after blocking AMPA receptors with DNQX. Taken together, these data suggest that a role of NMDA receptors in premotor network relating to central pattern generation in the spontaneous muscle spasms and a new therapeutic target.

14 **Jeremy Weinberger (PhD student),** Guillaume Caron, Marie-Pascale Côté jeremy@drexel.edu oral

Multisite Electrode Array to Optimize Epidural Stimulation for Spasticity Following Spinal Cord Injury Spasticity manifests in approximately 80% of spinal cord injury (SCI) patients.[1] Spastic individuals can display involuntary muscle movements, co-contraction of muscles, and hyperreflexia, making daily activities challenging. Current pharmacological treatments for spasticity have severe side effects such as seizures and dizziness, and depress overall spinal reflex excitability and muscle activity, further impeding motor recovery.[2] Here, we examine epidural stimulation (ES) as an alternative form of treatment for spasticity following SCI. ES studies have historically focused on locomotor recovery, while spasticity has

been seldomly explored.[3] First, we investigated the therapeutic potential of individually tailored ES after chronic SCI in rats featuring a severe thoracic contusion injury. A 15-channel epidural electrode array, with close resemblance to electrodes used in SCI people, was positioned over hindlimb motoneuronal pools to allow for individually optimized ES with the control of both anode and cathode positions. During a terminal experiment performed nine weeks after a severe (250kdyn) contusion injury, animals received a single individually optimized ES treatment session (40Hz, 15 min). Optimized anode and cathode positions were identified for each animal. The rate-dependent depression of the H-reflex was recorded from the plantar muscle in response to stimulation of the tibial nerve, and improvements were seen following optimized ES treatment. Our results suggest that tailored ES has the potential to decrease spasticity in SCI individuals without the use of pharmacological intervention. 1) Elbasiouny, S. M., Moroz, D., Bakr, M. M., & Mushahwar, V. K. (2010).Management of spasticity after spinal cord injury: current techniques and futuredirections. Neurorehabilitation and neural repair, 24(1), 23–33. 2) Nair, K.P.S. and J. Marsden. The Management of Spasticity in Adults. British Medical Journal. 349, g4737. (2014).3) Barolat G, Singh-Sahni K, Staas WE Jr, Shatin D, Ketcik B, Allen K. Epidural spinal cord stimulation in the management of spasms in spinal cord injury: a prospective study. Stereotact Funct Neurosurg. 1995;64(3):153-64.

15P. Guillaume Caron*, Bilchak J., Côté M-P. poster

Bumetanide increases postsynaptic inhibition after spinal cord injury The decrease of pre- and post-synaptic inhibition is often associated with spasticity in individuals with spinal cord injury (SCI). Because a shift in chloride homeostasis contributes to decrease spinal inhibition after SCI, we investigated whether restoring chloride homeostasis with bumetanide, a FDA approved NKCC1 (chloride intruder) antagonist, improves preand/or post-synaptic inhibition. In decerebrated rats, we recorded H-reflexes evoked by stimulating the tibial nerve and recorded dorsal root potentials (DRP) evoked by stimulating the posterior biceps and semitendinosus (PBSt) nerve. H-reflexes conditioned by PBSt nerve stimulations (4 pulses, 1.5T, 250Hz) at different conditioning-test intervals determine the strength and duration of the inhibition evoked by PBSt group I afferents. A change in DRP amplitude and in long-lasting H-reflex inhibition evoked by PBSt group I afferents were used to estimate the presynaptic inhibition, whereas a change in the short-lasting H-reflex inhibition was used to estimate the postsynaptic inhibition. Our results show that a chronic bumetanide treatment increases short-, but not long-lasting inhibition of the H-reflex, nor the DRP evoked by PBSt group I afferents. We further performed in vivo intracellular recordings of motoneurons from triceps surae or common peroneal nerves and recorded inhibitory postsynaptic potentials (IPSP) evoked by antagonists group I afferents. Our preliminary data indicate that bumetanide hyperpolarizes the reversal potential for IPSP after SCI. Together, these results suggest that a chronic bumetanide treatment increases postsynaptic inhibition by acting on chloride homeostasis. Such a treatment might be considered to improve postsynaptic inhibition after SCI and thereby to reduce spasticity.

Spinal/sensory /descending inputs to MNs_____

1 21 01/27/2022 **Maria Piotrkiewicz** mpiotrkiewicz@ibib.waw.pl Nalecz Institute of Biocybernetics & Biomedical Engineering, PASHanna GoszczyÅ„ska, Nalecz Institute of Biocybernetics & Biomedical Engineering, PAS oral

Evaluation of new methods for estimating the characteristics of inhibitory postsynaptic potentials in human motoneurons (computer simulation study) "Human spinal circuitry can be studied by nerve stimulation and analysis of induced changes in MN firing patterns. The aim of this study was to verify the existing methods and establish those that will allow the estimation of the IPSP duration with the maximum possible precision. The study was performed by means of computer simulation based on a simple threshold-crossing model of a rhythmically firing MN.

The output measures applied were: peristimulus time histogram (PSTH), peristimulus frequencygram (PSF) and the modified raster plot that presented the MN responses to each stimulus (test). The discharge times were represented by different symbols according to their timing relative to the target interval, TI, and all tests were sorted in order of increasing time lag between the MN discharge initiating TI and the IPSP arrival time. The results obtained for 3 IPSP durations are presented as functions of MN firing rate.

The most reliable estimate was the maximum elongation of interspike interval, determined from the raster plot. However, this measure corresponded to the half- time of simulated IPSP, while the values obtained from the other two estimates were closer to the IPSP duration. The simulations confirmed that the estimates decreased as the MN firing rate increased. IPSP durations been shown to be overestimated at the lower rates and underestimated at the higher rates. The results closest to the real values were obtained for firing rates from 11 to 14/s. The rationale for the selected estimation methods will be described in the accompanying poster."

2P 04/01/2022 **Grace Niyo** niyo@usc.edu University of Southern California, LA Ph.D. student Student Lama Almofeez and Francisco J Valero-Cuevas from University of Southern Califronia, LA

Current Approaches to the Neural Control of Movements Must Account for the Fusimotor System. Current conceptual and computational models of neuromuscular control emphasize the cortico-spinal drive as the main command signal to a muscle. However, intraneuronal, sensory, and proprioceptive signals combine with the cortico-spinal drive at the spinal cord to generate the actual alpha motoneuron (a-MNs) drive to a muscle. Skeletal muscles have muscle spindles that provide muscle velocity and length signals to ongoing motor commands at the spinal cord via primary (Ia) and secondary (II) sensory neurons through a spinal stretch reflex loop. These fusimotor projections, modulated by gamma motoneurons (Î³-MNs) include the homologous monosynaptic excitatory connection to the a-MNs that is the foundation of muscle tone and the stretch reflexes. The extent to which this spindle afferent signal disrupts limb kinematics, however, is not well understood. Here we quantify the functional consequences of excitatory spindle afferent signals on limb kinematics to determine the extent to which cortico-spinal drive

needs to be modulated or adapted to counteract them and generate appropriate alpha-MN command signals to muscles. We used a rhesus macaque arm model and systematically added homologous excitatory monosynaptic drive to each muscle during free arm movements. We then recorded the departure of the original endpoint kinematics when using 5 different gains of homologous excitatory drive proportional to the change of length and velocity of each muscle with respect to the initial posture. As expected, adding the spindle afferent signals disrupted limb kinematics. For some movements, muscle afferentation did not cause significant disruption even when the gain levels were increased.

3 04/07/2022 **Olivier D. Laflamme** olivierdlaflamme@dal.ca Dalhousie University PhD Student Student "Olivier D. Laflamme1, Rachel Banks1, Sergei Markin2, Simon Danner2 and Turgay Akay11: Dalhousie University, Department of Medical Neuroscience, Halifax, Canada2: Drexel University, Department of Neurobiology and Anatomy, Philadelphia, PA, USA" oral

The Involvement of V0 and V3 Commissural Interneurons in Crossed Reflexes

Sensory information is used to generate various behavior including protective and corrective reflexes. Following perturbation in one leg, motor activity can be elicited in the contralateral side of the body known as crossed reflex. V0 and V3 interneurons are commissural interneurons (CINs) important for coordinating left and right leg movements during locomotion: the V0 interneurons are responsible for left-right alternation, and the V3 interneurons are important for producing a robust locomotor pattern. However, if and how these two groups of CINs transmit sensory information to the contralateral side is unknown. To investigate this, we used mutant mouse models to kill (V0kill) or silence (V3off) interneurons, combined with in vivo electrophysiological recording. We stimulated either the tibial or sural nerve to evoke proprioceptive or cutaneous afferents and recorded multiple contralateral flexors and extensor muscles using electromyographic electrodes in awake resting and walking mice. Our results show that in WT mice, the crossed reflexes include excitatory and inhibitory components which are modulated during locomotion depending on the activity of the muscle prior to the stimulation. We were not able to identify any differences in crossed reflexes between V0kill and WT mice. In V3off mice, we observed a decrease in the excitatory response, and significant disruption in the inhibitory crossed reflex responses. Our data suggest that V0 CINs are not involved in crossed reflexes, but the V3 CINs are involved in both the inhibitory and the excitatory crossed reflex pathways.

4P 04/08/2022 **Marco Beato** m.beato@ucl.ac.uk University College London Professor None "M. Gorkem Ã–zyurt UCL Queen Square Institute of Neurology Julia Alonso-Ojeda UCL Department of Neuroscience, Physiology and Pharmacology Marco Beato UCL Department of Neuroscience, Physiology and Pharmacology Filipe Nascimento UCL Queen Square Institute of Neurology" poster

In vitro longitudinal lumbar spinal cord preparations to study sensory and recurrent motor microcircuits of juvenile mice "Studies using in vitro intact spinal cord preparations from rats and mice are limited to early postnatal days, because the spinal cord cannot be maintained in vitro at mature ages. While transverse slices can be viable in adult age, the cutting procedure severs many synaptic pathways, especially in a non-layered structure like the spinal cord.

We describe here that by performing a coronal cut along the length for the lumbar cord, the tissue from animals up to 1 month old can be maintained in vitro for up to 4-5 hours. Two preparations are described, both giving optical and electrophysiological access to the L4-L5 dorsal motor nuclei, that innervate ankle flexor and extensor muscles.

1) The dorsal horn-ablated preparation is obtained by performing a coronal cut at the level of the central canal. The entire ventral horn and its circuits are preserved and ventral roots can be stimulated to elicit antidromic spikes in motoneurons. This preparation is useful for measuring the synaptic strength of recurrent inhibition as well as recurrent excitation between motoneurons;

2) The ventral horn-ablated preparation is obtained by performing a coronal cut between the central canal and the ventral commissure. All dorsal horn circuits are preserved and it is possible to stimulate the dorsal roots to elicit mono-synaptic excitatory inputs from Ia afferent and di-synaptic inhibitory inputs from Ia/Ib interneurons

The in vitro coronal preparations are useful to study spinal motor circuits in juvenile mice (>P14) that have reached a mature stage of motor development.

5 04/08/2022 **M. Gorkem Ozyurt** g.ozyurt@ucl.ac.uk UCL Queen Square Institute of Neurology Royal Society Newton Research Fellow Post-doc "M. GÃ¶rkem Ã–zyurt UCL Queen Square Institute of Neurology Filipe Nascimento UCL Queen Square Institute of Neurology Marco Beato UCL Department of Neuroscience, Physiology and Pharmacology" oral

The connectivity pattern of recurrent excitatory connections between motoneurons "Anatomical and electrophysiological evidence shows that motoneurons collaterals can form synaptic contacts with other motoneurons in the lumbar spinal cord. We have recently shown that late firing motoneurons (putative fast) receive 10-fold stronger recurrent excitation than their early firing (putative slow) counterpart. This finding raises the question of whether connections between motoneurons are dominantly between fast motoneurons, or whether it is slow motoneurons that preferentially connect to fast ones.

We performed paired recordings from motoneurons labelled from either lateral gastrocnemius (ankle extensor) or tibialis anterior (ankle flexor) muscles. Once a pair of connected motoneurons was identified, we characterized their properties and determined the frequency and strength of connections between the two types of motoneurons. First, for synapses within the same motor nuclei, there was a large dominance of connections between fast motoneurons and only rarely we identified a slow motoneuron as the pre- or post-synaptic partner. In preparations

in which both nuclei were labelled, we tested whether it was possible to find connections between motoneurons belonging to antagonist nuclei. Surprisingly, the connectivity across antagonist nuclei was similar to that observed within nuclei, and the synaptic strength of individual connections did not differ. The connectivity been antagonist is similarly biased towards fast to fast connections.

The function of these recurrent circuits is unclear, but given the preferential fast to fast connectivity, it is possible that recurrent excitatory circuits are recruited during motor tasks that require the activation of a large number of fast motor units, for instance during explosive movements.

6P 04/12/2022 **Hojeong Kim** hojeong.kim03@gmail.com DGIST Senior Researcher

Estimation of intracellular inputs to the motoneuron for linear force production of its motor unit The input-output properties of spinal motoneurons and muscle fibers comprising motor units are highly non-linear. Thus, it has been challenging to infer the stimulation patterns over motoneurons that may generate desired force production by motor units. A physiological model of the motor unit was constructed that included intracellular and synaptic stimulation of the motoneuron under neuromodulatory inputs from the brainstem and variation in muscle length. Both continuous and discrete current stimulation was considered for the motoneuron. Waveforms of continuous current intensity and impulse current frequency were inversely estimated for the motor unit to develop and relax the muscle force in a linear form within a broad range of contraction speeds and levels during isometric contraction at various muscle lengths. The exponential shape of the stimulation waveform was sufficient to induce linear force production at all levels of force production and muscle length under discrete stimulation. However, the sinusoidal shape of the stimulation waveform was needed for high force levels at shorter-than-optimal muscles under continuous stimulation. Only discrete stimulation could produce maximum force and control force relaxation at all muscle lengths. In contrast, continuous stimulation could not reach maximum force level and control force relaxation at high contraction levels in shorter-than-optimal muscles due to persistent inward current saturation on the motoneuron dendrites. These results may provide insights into synaptic control of motoneurons for proper force generation at the motor unit level and a basis for designing stimulation patterns for precise movement control via neural interface under pathological conditions.

7 04/12/2022 **Amr Mahrous** amr.mahrous@northwestern.edu Northwestern University Postdoctoral Fellow Post-doc "'- Matthieu Chardon - Northwestern University- Michael Johnson - Northwestern University- Jack Miller - Northwestern University- CJ Heckman - Northwestern University" oral

Rebound extensor response following electrical stimulation of the lumbar spinal cord Spinal cord stimulation (SCS) is a very promising therapeutic approach for spinal cord injury (SCI). However, it does not generate enough muscle activation in many patients to enable weight-bearing. Here, we study SCS in the decerebrate cat using a wide range of stimulation

parameters in an attempt to diversify and enhance the elicited motor output. Our study has uncovered a new type of response to SCS, a potent post-stimulation rebound excitation (PSRE), directed solely to extensor muscles and controlled by stimulation parameters. Therefore, PSRE has great clinical potential to assist postural movements, such as sit-to-stand transitions. We have tested multiple parameters and found that PSRE is best evoked by stimulation of the L5-L7 segments at 10 to 40 Hz for several seconds. The amplitude and duration of PSRE are mainly dependent on stimulation intensity. To investigate the origin of PSRE, we performed intracellular recordings of single motoneurons where PSRE was recorded at different membrane potentials. The depolarization during PSRE increased at hyperpolarized potentials indicating a synaptic origin. Furthermore, interneurons exhibited increased firing during PSRE, confirming the circuit origin of this behavior. Importantly, PSRE disappeared after acute and chronic (1 mo.) transection of the spinal cord. The initial pharmacology experiments show that serotonergic modulators can fully restore PSRE following acute injury. Our study has thus unveiled a novel motor response to spinal stimulation, investigated its mechanism, and provided pharmacological interventions to restore it after injury. These results have the potential to enhance the benefits of electrical stimulation in patients with SCI.

8P 04/14/2022 **Giulia Benedetta Calabrese** gbc1@st-andrews.ac.uk University of St Andrews, UK PhD student Student "Simon A. Sharples, University of St Andrews, UKGareth B. Miles, University of St Andrews, UK" poster

Cholinergic modulation of respiratory-related motor output. "Breathing is arguably the most fundamental biological function and must be readily adjusted to meet changing metabolic demands. While it is well established that the respiratory rhythm is generated in the brainstem, there is mounting evidence that cervical spinal interneurons may play a role in adjusting respiratory output. However, we still do not know how this spinal modulation is achieved. Here we aim to investigate cholinergic spinal modulation of respiratory-related activity with a focus on the C-bouton system, a large cholinergic modulatory synapse previously shown to facilitate the output of lumbar motoneurons, via M2 muscarinic receptor signaling, in a task-dependent manner. We used mouse genetics and in-vitro electrophysiology to study cholinergic modulation of breathing and interrogate the underlying neural mechanisms. Respiratory-related activity was recorded with extracellular suction electrodes from C3 and C4 ventral roots in isolated brainstem-spinal cord preparations obtained from mice at postnatal days 3 and 4.

We found that blocking the M2 receptor signaling reduced the amplitude and increased the frequency of respiratory-related activity, suggesting a role for M2 receptors in both rhythm generation and motor output. Refining this approach with the use of a split bath suggested that the amplitude effect is mostly driven through modulation of brainstem rhythm generating circuits. Moreover, chemogenetic inhibition of V0c interneurons, the source of C-bouton synapses, did not affect respiratory-related activity, suggesting that these neurons might not be active during eupneic breathing in-vitro.

Together, these data suggest that C-boutons may not control respiratory-related activity under eupneic states in neonatal mice in-vitro."

9P 04/16/2022 **Carly L. O'Sullivan** cosulliv@ualberta.ca University of Alberta Graduate student Student "Neil Tyreman - University of Alberta,Vivian K. Mushahwar- University of Alberta" poster

Comparing the effectiveness of epidural stimulation and intraspinal microstimulation

Spinal cord injury (SCI) has devastating effects to an individualâ€™s function and quality of life. It also comes with many complications and if SCI could be reversed, many of these could be reduced or even avoided. With this goal in mind, spinal cord neuromodulation has been explored as a method of restoring standing and walking following SCI. Two promising methods of achieving this are epidural stimulation (ES) and intraspinal microstimulation (ISMS). The overall goal of this research is to determine the type and distribution of neurons that are activated during ES and ISMS to better understand the mechanisms and functional outcomes of these spinal cord stimulation modalities. To do this, 24 intact female domestic pigs are divided into 6 acute treatment groups including stimulation, sham, surgical and naive control. At the end of each treatment/control paradigm, the pigs are euthanized, perfused, and the spinal cords removed for immunohistochemical analysis. Preliminary findings showed a very different pattern of neuronal activation between the ES and ISMS, with ES primarily activating neurons in lamina I-III with low scattered activation throughout the gray matter. ISMS primarily showed strong activation of neurons in laminae V-IX. This suggested that different pathways are engaged during ES and ISMS, with neuronal activation with ISMS focused primarily in the region where the locomotor networks reside. Additional immunohistochemistry is expected to identify the types of neurons activated with each stimulation modality. This research will help with our understanding of the mechanisms of action of ES and ISMS.

10 04/16/2022 **Martin Zaback** martin.zaback@temple.edu Temple University, Philadelphia, PA, USA Postdoctoral fellow "FrancescoNegro, Universita degli Studi di Brescia, Brescia, ItalyMichel A. Lemay, Temple University, Philadelphia, PA, USA Christopher K. Thompson, Temple University, Philadelphia, PA, USA" oral

Assessing the functional connectivity of spinal neurons "Background: Spinal interneurons play a critical role in motor output. A given interneuron may receive convergent input from multiple ascending and descending sources and relay this information to multiple targets. This convergence of inputs and divergence of projections has the potential for substantial functional connectivity. Here, we quantify the functional connectivity of spinal neurons through the concurrent recording of populations of lumbar interneurons and hindlimb motor units in the decerebrate cat during activation of distinct afferent pathways.

Methods: In three female cats, two 64-channel microelectrode arrays were placed into lamina VII of the spinal cord at L3 and L6/7 segments, while a 64-channel electrode array was placed on the surface of the exposed soleus. Contralateral tibial and ipsilateral sural nerves were stimulated at multiple frequencies, and time- and frequency-domain correlations were examined between stimulus pulse times and interneuron and motoneuron spike times.

Results: Both modes of afferent drive elicited similar changes in motoneuron and interneuron discharge rate. However, the prevalence and magnitude of correlated activity underlying these two forms of afferent drive differed substantially. Activation of the ipsilateral sural nerve

resulted in highly correlated activity, particularly for interneurons recorded at the L6/7 segment. In contrast, the contralateral tibial nerve resulted in less, but more widespread correlated activity.

Conclusions: These data suggest that the ipsilateral sural nerve has dense projections onto caudal lumbar spinal neurons, while contralateral tibial nerve has sparse projections. This approach will allow us to quantify changes in functional connectivity of spinal neurons following spinal cord injury.

11 Discharge rate method to study human Renshaw inhibition

Kemal Sitki Türker, Betilay Topkara Arslan, and Mustafa Görkem Özyurt

Istanbul Gelisim University, Turkey; University College London, U.K. oral

The precise role of the interneuronal networks such as the Renshaw circuitry in motor control is not fully understood. To improve our understanding of this circuitry's function, we electrically stimulated the lowest threshold motor axons in the tibial nerve. We analysed the stimulus-correlated changes in the discharge rate of voluntarily recruited single motor units from the soleus muscle. The orthodromic component of the motor axons stimulation generated direct motor response on surface electromyography and antidromic impulse, on the other hand, evoked recurrent inhibition (RI) that subsequently reduced the discharge rate of the soleus motor units (Özyurt et al., 2019). The current study used the peristimulus frequencygram (PSF) method and some ideas from our previous brain slice experiments where inhibitory postsynaptic potentials (IPSP) of known profiles were injected into regularly discharging motoneurons in rat brain slices (Türker and Powers 2005 Using IPSPs of known profiles, the brain slice experiment indicated that the actual duration of an IPSP could be achieved when the discharge rate of a motoneuron was firing at around 2.88 Hz (Topkara-Arslan et al., unpublished). Since it is challenging to evoke motor unit firings at this low frequency voluntarily, we employed the extrapolation method that wields the relationship between the discharge rate and duration of the IPSP. The actual duration of RI on active soleus motor units that corresponds to extrapolated discharge rate at 2.88 Hz was found to be around 50 ms, almost twice as long as the previous reports. In summary, the present study employed the extrapolation method to estimate the duration of RI with precision in the human subject and hence improved our understanding of the temporal properties of RI that regulate human motor control.

Özyurt MG, et al. The Journal of Physiology (2019) Apr;597(8):2185-2199
Türker KS and Powers RK. Trends in Neurosciences (2005) Jul;28(7):379-386

12 30 03/20/2022 **Yasin Yousef Dhaher** yasin.dhaher@utsouthwestern.edu UT Southwestern Medical Center, Dallas, USA Professor None "1. Yu-Chen Chung, UT Southwestern Medical Center, Dallas, USA Jonathan Shemmell, University of Wollongong, Wollongong , Australia" oral

Dissecting the separate effects of descending tracts on spinal motor circuits

Neuromodulation of human spinal motor circuits could be accomplished using invasive

(e.g., epidural stimulation) and noninvasive (e.g., operant conditioning) means, yet understanding the mechanisms underlying their effects remain elusive. In this study we explored the effect of descending tracts trans-spinal magnetic stimulation (TSMS) on spinal networks. Specifically, the magnetic stimulus was applied at the T12 spinal segment to condition S1 spinal circuits. Three sub-motor TSMS intensities were tested (including sham) for muscles innervated by the cord S1 segment, with no evoked motor potentials observed. To pair peripheral nerve stimulation on the posterior tibial nerve with the T-12 TSMS, a paired-pulse stimulus paradigm was employed. Testing this paired-pulse paradigm using varying inter-stimulus intervals (ISI, 2-20 ms) in 12 young males revealed consistent and intriguing responses. While sham stimulation yielded no changes in soleus H-reflex across ISIs compared to the unconditioned state, the signature response exhibited three unique components: suppressed H-reflex at the shorter ISIs, muted effects at the intermediate ISIs, and a second phase of suppression at the higher ISIs. Based on mammalian descending tracts conduction velocities, it is likely that the observed expressions may be a manifestation of antidromic and orthodromic activation of spinal tracts to the soleus muscle spinal motor circuits. Given the non-selective nature of TSMS, further work is needed to delineate the relative contribution of the different spinal structures to the two phases of suppression.

13 35 03/23/2022 **Evangelos A. Christou** eachristou@ufl.edu University of Florida Professor None "Evangelos A. Christou, University of Florida Stefan Delmas, University of Florida YoonJin Choi, University of Florida Basma Yacoubi, University of Florida" oral

Signal-dependent noise of the neural drive does not explain the exacerbated motor variability of low forces The signal-dependent noise hypothesis assumes that motor variability results from random neural variations (noise) whose amplitude increases proportionally with effort. So far, however, no study has examined if this hypothesis occurs at the neural drive to the muscle. Here, we tested if greater neural activity (signal amplitude) will result in proportionally greater neuronal and motor variability (â€œnoiseâ€•). Fifteen young adults exerted pre-planned contractions with the index finger and aimed to match a force target either at 10% or 40% MVC at 160 ms (Experiment 1) or to match a 40% MVC force target at either 160 or 280 ms (Experiment 2). The magnitude of the neural drive was quantified as the summated motor unit FDI activity (mean discharge rate â€“ MDR; coefficient of variation of discharge rate â€“ CVDR). Neuronal variability across the 100 trials was quantified with the coefficient of variation (CV). Motor and neuronal variability was significantly greater at 10% MVC, despite that the magnitude of the motor output and neural drive was significantly greater at 40% MVC. The exacerbated motor variability at 10% associated with greater inconsistency across trials in the CV of DR (R2 = 0.31). In experiment 2, motor and neuronal variability was not significantly different for the two temporal conditions (160 vs. 280 ms), despite the greater magnitude of the neural drive (area of EMG burst) for the 280 ms. These findings refute the signal-dependent noise hypothesis as an explanation of neuronal and motor variability, at least for low force levels.

14P 37 03/25/2022 **Jacob Thorstensen** jacob.thorstensen@griffithuni.edu.au Griffith University PhD student Student "Janet Taylor:Edith Cowan University and Neuroscience Research Australia Justin Kavanagh: Griffith University" poster

5-HT2 receptor antagonism reduces motoneuron output to antidromic activation but not to brief synaptic input from corticospinal stimulation

In vitro recordings of motoneurons isolated from animals indicate that serotonin (5-HT) increases motoneuron excitability by activating 5-HT_2 receptors on the somato-dendritic compartment. However, few experiments have examined if/how this receptor affects *in vivo* human motoneuron excitability. In the current study we examined the effects of 5-HT_2 receptor antagonism on human motoneuron excitability. The 5-HT_2 receptor antagonist cyproheptadine (8 mg) was administered to ten healthy participants in a double-blind, placebo-controlled, crossover trial. Electrical cervicomedullary stimulation evoked cervicomedullary motor evoked potentials (CMEPs) in the biceps brachii. In addition, we used supramaximal peripheral nerve stimulation to evoke F-waves in the abductor digiti minimi. Compared to placebo, we found that 5-HT_2 antagonism reduced the amplitude (placebo: 96.4 ± 13% of pre-pill amplitude, cyproheptadine: 66.3 ± 23.6%, $P = 0.002$) and persistence (placebo: -1.1 ± 4.7% difference in persistence from pre-pill, cyproheptadine: -9.4 ± 14.9%, $P = 0.013$) of F-waves. However, 5-HT_2 antagonism did not affect CMEPs. We also assessed drug effects on CMEPs after contraction of the opposite-limb elbow flexors, and on maximal elbow flexion performance. We found that 5-HT_2 antagonism did not affect CMEPs after remote contraction but reduced maximal force (placebo: 303.6 ± 88.5 N, cyproheptadine: 284.6 ± 74.5 N, $P = 0.016$). Overall, reductions in F-waves with 5-HT_2 antagonism suggest that the drug intervention affected the initial segment or soma to reduce excitability. Conversely, we suggest that the 5-HT mediated amplification of synaptic input is strongest when motoneurons discharge repetitively and 5-HT sensitive persistent inward currents are activated.

15P 04/14/2022 **Justine R. Magnuson** justine.magnuson@ubc.ca University of British Columbia Okanagan PhD Candidate "Christina D. Bruce, Brian H. Dalton, Chris J. McNeil University of British Columbia Okanagan " poster

Corticomuscular coherence and neuromuscular function with two submaximal fatigue tasks Isometric submaximal fatiguing tasks typically involve a matched-torque design, which leads to increased motoneuron pool output to compensate for contractile decrements. To limit the influence of increased descending drive on indices of neural excitability, an alternative design is a matched-electromyography (EMG) contraction that maintains motoneuron pool output, but leads to reduced torque production. It is unknown how these different fatiguing tasks influence connections between the motor cortex and contracting muscles (corticomuscular coherence, CMC), ratings of perceived effort (RPE), or neuromuscular capacity. To address this knowledge gap, on separate days, 16 participants sustained a 10-min isometric elbow flexion at 20% maximal voluntary contraction (MVC) torque or the integrated biceps brachii EMG recorded at 20% MVC. Electroencephalography and EMG signals were used to assess CMC for biceps and

triceps brachii during the first and last minute of the fatiguing tasks. Pre-task and at task termination, participants performed brief 100, 75, and 50% MVCs, with transcranial magnetic stimulation to calculate voluntary activation (VA). Biceps brachii CMC increased with matched-torque (37.9 Â± 55.9%) but not matched-EMG, with no fatigue-related changes for triceps brachii. End-task RPE was higher for the matched-torque (8.0 Â± 1.8) than matched-EMG contraction (6.3 Â± 2.0). Fatigue-related reductions in MVC torque and VA were greater with matched-torque (32.9 Â± 17.0% and 9.5 Â± 9.9%) than matched-EMG (20.6 Â± 18.6% and 3.0 Â± 5.7%). These results suggest increased common oscillatory input to agonist muscles during a matched-torque, but not matched-EMG contraction, presumably to compensate for greater reduction in torque-generating capacity.

16P 04/15/2022 **Matthieu Chardon** m-chardon@northwestern.edu Northwestern University Research Assistant Professor New Investigator 5 years "Matthieu Chardon, Northwestern University, USA Curtis Wang, CalState LA, USA Marta Garcia, Argonne National Laborarty, USA Randy Powers, Washington University, USA CJ Heckman, Northwestern University, USA" poster

Supercomputers, Neural Network Simulations and Reverse Engineering of Neuron Firing Patterns Our recent studies combine 3 advances: 1) novel array electrodes to measure firing patterns of populations of motoneurons in humans, 2) highly realistic computer simulations of these motoneurons and 3) implementation of these models using the High Performance Computing facility at Argonne National Labs. The huge advantage of the supercomputer approach is that their massive parallelism allows thousands (soon millions) of simulations to be carried out simultaneously. Here we use this computational power as the basis of a brute force approach to reverse engineer motoneuron firing patterns to identify the organization of their synaptic inputs. More specifically our goal is to identify the patterns of excitatory, inhibitory and neuromodulatory inputs. Our initial simulations show that, although a given motor output pattern could potentially be generated by a huge number of combinations of these three types of input, neuromodulatory input makes motoneuron input-output properties so nonlinear that the effective â€œsolution spaceâ€• is restricted. These high levels of neuromodulatory inputs are coupled to strong inhibitory inputs, with interaction between these two input types providing the â€œamplifierâ€• upon which excitation acts to execute the pattern of the movement. This is a novel insight into the synaptic organization of motor commands in humans. Overall, we show that this reverse engineering approach achieves deep insights about the organization of synaptic inputs that drive a set of neuronal firing patterns. We feel that this novel technology has the power to answer many theoretical motor-command question, we are thus aiming at making it available to the community.

17 04/15/2022 **Robyn L. Mildren** rmildre1@jhmi.edu Johns Hopkins University Postdoctoral fellow Kathleen E. Cullen oral

Vestibular contributions to neck postural muscle activity during natural motion experienced in everyday life The ability to maintain posture during our everyday activities requires the integration of multiple sensory and motor signals. Notably, the vestibular system provides vital information about motion of the head in space, which can elicit compensatory

muscle responses to stabilize head and body posture. We aimed to examine the mechanisms by which the vestibular system, independently and in combination with other sensory cues, contributes to the sensorimotor control of posture. First, we applied sinusoidal and broadband yaw angular motion at frequencies from 0-20Hz in alert rhesus monkeys. Single motor units in splenius capitis (SPL) increased their activity during contralateral motion (e.g., leftward rotation activated right SPL motor units) to stabilize the head in space, and gain increased with frequency up to 16Hz. Responses were attenuated at low frequencies in the presence of higher frequencies, a phenomenon previously observed in central vestibular neurons. Next, to distinguish contributions from different sensory systems and examine the integration of multisensory and autonomic inputs, we changed visual feedback about self-motion in healthy and bilateral vestibular loss (BVL) monkeys and manipulated autonomic arousal. Results showed that SPL motor unit responses were driven by vestibular input, and accurate visual self-motion information did not enhance responses in healthy monkeys nor substitute for absent vestibular feedback following BVL. However, increasing arousal using a positively-valanced social paradigm did enhance responses. Altogether, our results demonstrate the vestibular system has an important contribution to postural neck muscle activity across the range of dynamic motion experienced during everyday life in primates.

18P___ 04/15/2022 **Basma Yacoubi Keyhani** basma@ufl.edu University of Florida Research assistant professor New Investigator 5 years "Agostina Casamento-Moran: Department of Applied Physiology and Kinesiology, University of Florida, USA

Roxana G. Burciu: Department of Applied Physiology and Kinesiology, University of Florida, USAS. H. Subramony: Department of Neurology, University of Florida, USADavid E. Vaillancourt: Department of Applied Physiology and Kinesiology, University of Florida, USA, Department of Neurology, University of Florida, USA & Department of Biomedical Engineering, University of Florida, USAEvangelos A. Christou: Department of Applied Physiology and Kinesiology, University of Florida, USA & Department of Neurology, University of Florida, USADepartment of Neurology, University of Florida, USA"2 Poster

Pre-planned Ballistic and Closed Loop Feedback Dependent Control of Force in cerebellar patients Discrete ballistic and sustained contractions are generally considered to rely on different neural processes (pre-planned vs. closed-loop control). However, there is a growing body of evidence in cerebellar patients that link the cerebellum to both control processes. Here, to investigate this hypothesis, we compared accuracy and variability of ankle dorsiflexion during a visually guided force tracing task (15% MVC for 20 s; 15 trials) and a ballistic goal-directed task (15 % MVC, 180 ms; 50 trials) of cerebellar patients (Spinocerebellar ataxia 6, SCA6; N=13) with that of age-matched healthy controls (N=13). We found that impairments in accuracy and steadiness of SCA6 during the force matching task were predicted from the force trial-to-trial variability during the ballistic goal-directed task (R2=0.27 and R2=0.55, respectively). This supports the hypothesis that adequate sensorimotor integration is dependent upon adequate serial pre-planning of ballistic actions. We also found that during the force tracing task accuracy was predicted from Cerebellar Lobule 8a, Vermis crus1, and Cerebellar Lobule 7b

grey matter (GM) volume (R2=0.82), and steadiness from Vermis Crus1 GM volume (R2=0.38). Interestingly, the trial-to-trial variability during the fast goal-directed task was predicted by fractional anisotropy of the Superior cerebellar peduncle (R2=0.72) which suggest that impaired intermittent control during visually guided movements may be related to a deficit in relaying information from the cerebellum to the cerebral cortex via the red nucleus and the thalamic nuclei. These findings are particularly relevant to understanding the link between the degradation of pre-planned and closed-loop control systems in cerebellar patients.

Sympathetic Neurons

1 04/13/2022 **Yaqing Li** yaqing.li@emory.edu Emory University Instructor New Investigator 5 years "Krishna Pusuluri (Emory University)Michael Sawchuk (Emory University)Astrid Prinz (Emory University)Shawn Hochman (Emory University)" oral

Plasticity of vasomotor sympathetic paravertebral postganglionic neurons after spinal cord injury High thoracic spinal cord injury (SCI) significantly severs descending autonomic control systems resulting in several dysautonomias. Loss of descending excitatory drive onto spinal sympathetic preganglionic neurons controlling vasculature results in hypotension. One compensatory plastic response to reduced sympathetic drive is enhanced vascular smooth muscle adreno-sensitivity. Sympathetic postganglionic neurons (SPNs) represent the final neural step for sympathetic output. Whether vasomotor SPNs also undergo compensatory changes in function after SCI remains unknown. The adrenergic SPNs innervating vasculature co-express neuropeptide Y (NPY). Therefore, to study vasomotor SPNs intrinsic and synaptic plasticity after SCI, we performed T2 spinal transection in adult NPY::TdTomato mice, and undertook whole-cell patch recordings from mid-thoracic paravertebral chain ganglia SPNs in an ex vivo preparation. SPNs from chronic SCI mice were significantly more excitable (rheobase value 44% of sham controls). Interestingly, voltage-dependent Na+ and IA current amplitudes were significantly depressed after SCI. To assess possible consequence on SPN output, maximum and sustained firing frequency were plotted in relation to injected current steps to generate f-I curves. We observed an overall leftward shift and increased maximum and sustained f-I curve slope after SCI supporting an amplified output gain. As spontaneous synaptic input frequency was also observed to double after SCI, we hypothesize that, in response to significantly reduced preganglionic drive, vasomotor SPNs undergo compensatory homeostatic changes to increase vasomotor tone. Such changes would also be expected to contribute to exaggerated vasoconstrictor responses to nociceptive stimuli seen in autonomic dysreflexia

2P 04/15/2022 **Shawn Hochman + Mallika Halder**mhalder@emory.edu Emory University Graduate Student Student "Yaqing Li, Alan Sokoloff, Michael Sawchuk, Yuxuan Wu, Astrid A Prinz, Shawn HochmanEmory University" oral + poster

Spike conduction in preganglionic axons is modifiable and has the capacity to shape sympathetic output divergence across paravertebral ganglia "Spinal sympathetic preganglionic neurons (SPNs) issue divergent branching axonal projections onto ganglionic

neurons across multiple paravertebral chain ganglia. We explored whether spike conduction failures that occur across ganglia are a physiologically modifiable feature of divergence control. Adult mouse thoracic chain ganglia were dissected to remain in continuity in situ. SPN axons stimulated from ventral roots and evoked population responses were recorded across branching axons. Evidence of spontaneous conduction block or unblock in individual SPNs was always seen across â‰¥ 3 ganglia at all temperatures tested (22 - 36°C). Magnitude of population block increased with Tâ• °. Conditioning stimulus spike trains of 5 & 10 Hz (10 pulses) facilitated overall population conduction across â‰¥ 3 ganglia for several seconds. As SPNs express GABAA receptors including Î±5-GABAA receptors, we undertook pharmacological studies to explore a possible modulatory role in axonal spike conduction. Bicuculline and L655,708 (negative allosteric modulator for a5-GABAA) reduced population response amplitude, supporting a role for constitutively-active GABAA in spike conduction. A computational model assessed the effect of axon branch point geometry between mother and daughter branches, TÂ°, GABAA Cl- conductance (gGABA) and ECl on branch point failure. Branch point failure was associated with increases in TÂ°. gGABA near axonal branch points could influence signal propagation with failures more likely for larger gGABA and more hyperpolarized ECl. In sum, we show SPN capacity for axonal conduction block that is modifiable in a distance-, temperature-, activity- and neuromodulation-dependent manner. Supported by NS102871, NS116724, NS121850."

3 04/15/2022 **Nicholas Au Yong** nauyong@emory.edu Emory University School of Medicine Assistant Professor New Investigator 5 years"Dario I. Carrasco, Emory UniversityMatthew Bryson, Emory University Shawn Hochman, Emory University" oral

The Influence of Phrenic Sympathetic Activity on Diaphragm Contractile Properties

The execution of motor behaviors relies on high-fidelity neurotransmission at the nerve-muscle interface. Sympathetic fibers innervating skeletal muscles are generally associated with vasomotor control of intramuscular microcirculation. However, recent studies employing modern immunohistochemical and neurophysiological techniques have revitalized a historically sidelined concept; skeletal muscles neuromuscular junctions (NMJs) are dually innervated by both motor and sympathetic fibers. Skeletal muscle sympathetic innervations have been suggested to play a critical role in 1) maintaining healthy NMJ anatomy and 2) modulation of neuromuscular transmission. Of all muscles examined, the diaphragm is particularly rich with sympathetic terminals colocalizing with the NMJs constituting the phrenic-diaphragm neural interface. Using optogenetics mice that allow for selective light-activation of motor or sympathetic fibers, we utilized an ex-vivo phrenic-diaphragm preparation to systematically examine the direct effects of 1) exogenous application of adrenergic agonists/antagonists and 2) selective phrenic sympathetic recruitment on diaphragm contractile properties. Our preliminary findings demonstrate that exogenously applied adrenergic agonists increase diaphragm force production whereas application of an antagonist greatly impairs phrenic-diaphragm neuromuscular transmission. Electrically-elicited submaximal diaphragm twitch and tetanic force (ranging from threshold to 50% of maximum force) can be significantly enhanced with concurrent recruitment of phrenic sympathetic fibers. Together our results suggest that neuromuscular transmission at the phrenic-diaphragm interface is sensitive to phrenic

sympathetic activity and exogenously applied adrenergic agonist/antagonist. Accordingly, these findings may have bearing on the prescribing of adrenergic agonist and antagonist medications to patients with a weakened phrenic-motor drive.

4. Jeremy Chopek: University of Manitoba oral
Locomotor related spinal V3 interneurons innervate sympathetic preganglionic neurons in the mouse. Spinal electrical stimulation is a promising strategy to promote locomotor recovery after spinal cord injury. When stimulation is applied over the lower thoracic and lumbar regions, not only are improvements seen in walking, but also in autonomic function(s) mediated by neurons many segments away from the site of stimulation. We believe these autonomic improvements are mediated through long ascending propriospinal neurons originating in the lumbar spinal cord. Here we demonstrate that genetically defined lumbar spinal interneurons (INs), termed V3s, previously shown to produce stable and robust locomotion, also synapse on thoracic sympathetic preganglionic neurons (SPNs). Our results demonstrate that synaptic input arising from V3 neurons accounts for 20% of VGluT2 contacts apposing SPNs. Using whole cell patch clamp recording, we further demonstrate that optogenetic stimulation of lumbar V3 cell bodies or axons elicits action potentials in thoracic SPNs. These preliminary results demonstrate an ascending anatomical and functional connection between lumbar spinal V3 neurons involved in locomotion and thoracic spinal neurons involved in autonomic function.

5 04/13/2022 **Benjamin I Goodlich** benjamin.goodlich@griffithuni.edu.au Griffith University PhD Candidate Student "Alessandro Del Vecchio, Friedrich-Alexander-University Sean Horan, Griffith University Justin Kavanagh, Griffith University" 03-Feb oral
5-HT2 receptors play a critical role in motor unit discharge rate in humans Animal preparations have revealed that serotonin (5-HT) is a neuromodulator which can regulate the gain of spinal motoneurons by binding to somato-dendritic 5-HT2 receptors on motoneurons. However, the role of 5-HT2 receptor activity in modulating human motor unit (MU) activity during contractions of different intensity has yet to be assessed. Ten healthy participants (25.1â€‰Â±â€‰1.9â€‰yr) ingested 8mg of cyproheptadine, a competitive 5-HT2 antagonist, in a repeated-measures, double-blinded, placebo-controlled experiment. MU activity of tibialis anterior (TA) was assessed with high-density surface electromyographic decomposition during steady-state contractions of 10%, 30%, 50% and 70% of maximal voluntary contraction (MVC). TA activity was assessed at baseline and post-pill ingestion on both the drug day and placebo day. MUs were tracked within and between days. Baseline measures for MVC force ($p = 0.30$) and discharge rate for the tracked MUs ($p = 0.25$) did not differ between days. Main effects of drug were identified, where MU discharge rate was significantly lower post-pill ingestion on the cyproheptadine testing day compared to pre-pill ingestion ($p < 0.01$). Similarly, MU discharge rate was significantly lower post-pill ingestion on the cyproheptadine day compared to post-pill ingestion on the placebo day ($p < 0.01$). Overall, these findings reinforce the critical role that 5-HT has on regulating discharge rate of motoneurons, where 5-HT2 receptor blockade reduced human motoneuron discharge rate. These effects were not specific to an individual contraction intensity, which supports the notion that 5-HT regulates the gain of spinal motoneurons across a full range of forces.

Peripheral Nerve Injury

1 03/22/2022 **Travis Rotterman** travis.rotterman@biosci.gatech.edu Georgia Institute of Technology Post-doc Post-doc "Travis M. Rotterman - School of Biological Sciences, Georgia Institute of Technology Violet Garcia - School of Biological Sciences, Georgia Institute of Technology Paul Nardelli - School of Biological Sciences, Georgia Institute of Technology Oreoluwa Amosu - School of Biological Sciences, Georgia Institute of Technology Sebinne Lee - School of Biological Sciences, Georgia Institute of Technology Timothy C. Cope - School of Biological Sciences, Georgia Institute of Technology" oral

Ia afferent synapses are temporarily restored but not retained on motoneurons by minocycline treatment following peripheral nerve injury Peripheral nerve injury (PNI) results in the permanent reorganization of spinal motor circuitry. One example is the degradation of the proprioceptor Ia afferent synapses on axotomized motoneurons which ultimately results in the absence of the stretch reflex. Our previous work demonstrated that this loss is due to a central microglia-macrophage mediated neuroinflammatory response. Therefore, we hypothesized that suppressing inflammation would result in the preservation of Ia connections and ultimately the restoration of the stretch reflex. To investigate this, we transected and repaired the medial gastrocnemius nerve in adult rats. Rodents were then treated with an anti-inflammatory drug (minocycline) or vehicle for two weeks post-injury. Anatomical preservation and functional connectivity were assessed at 3 and 6+ months using retrograde tracing techniques and in vivo intracellular motoneuron recordings. At 3 months post-axotomy motor axons had regenerated and reformed neuromuscular junctions in both treated and untreated animals alike, but sensory axon reinnervation was limited. However, retrograde labeling revealed that motoneurons retained Ia afferent synapses in minocycline treated rats compared to the vehicle treated cohort. Curiously, however, Ia afferent synapses were not retained, but instead retracted at 6 months post-injury when peripheral regeneration and muscle reinnervation were substantial for both motor and sensory axons in minocycline treated rats. This reduction in Ia synapses at 6 months corresponded with weakened stretch-evoked synaptic potentials and loss of the stretch reflex. In sum, successful muscle reinnervation by severed peripheral nerves is not sufficient to sustain the temporary restoration of central Ia-motoneuron synapses achieved by minocycline.

2P 03/30/2022 **Conor O'Croinin** ocroinin@ualberta.ca University of Alberta Undergraduate student Student "Indiresh Akil Mangra-Bala, University of Alberta Siyu Du, University of Alberta, Bethany Jantz, University of Alberta, Kelvin Jones, University of Alberta"Two Poster

Are correlations between axonal excitability outcomes, motor unit number estimates and age consistent with the selective loss of fast axons with age? Background: Healthy aging in the human neuromuscular system is characterized by a progressive loss of muscle mass, axonal degeneration and an increased proportion of slow-type motor units. Motor axons innervating slow and fast fibers have different electrophysiological properties and the changes observed with aging may reflect selective degeneration of axons that previously innervated fast fibers. Aim: To examine the relationship between motor unit number and motor axon excitability

to determine if changes are consistent with selective loss of fast motor axons during aging. Methods: A cross-sectional sample (N = 54, 52% female, ages 18-81) reported to the lab either twice (N = 17) or a single occasion for electrophysiological measures of motor unit number (MUNE) and nerve excitability testing (NET). Testing was done on the right side in both the upper (APB) and lower limb (TA). Results: Test-retest reliability was 'moderate' for MUNE (ICC(2,1) = 0.642). As expected, age was negatively correlated with MUNE (Pearson r = -0.35 & -0.41) and peak compound muscle action potential (CMAP) (Pearson r = -0.33 & -0.41) in the upper and lower limb. The NET measure of strength-duration time constant was correlated with age only in the upper limb (r = 0.35) and the hyperpolarizing I/V slope was correlated with age in both the upper and lower limbs (r = 0.35 & 0.50). Conclusion: Age-related declines in MUNE and peak CMAP were demonstrated. Data from both the upper and lower limb are consistent with a selective loss of fast motor axons with age.

3P 04/14/2022 **Hongkai Wang** h.wang@u.northwestern.edu Northwestern University and Shirley Ryan Abilitylab Student Student "Hongkai Wang1,2, Grace Wickerson3,4, Jordan Walters1, Dom Dâ€™Andrea1, Yeon Sik Choi3,4, Yasmine Bouricha1, Hak-Young Ahn3, Sumanas W. Jordan5, John A. Rogers 3,4,6,7,8,9, Colin K. Franz1,10 1Laboratory of Regenerative Rehabilitation, Shirley Ryan AbilityLab, Department of Physical Medicine and Rehabilitation, 2 Northwestern University Interdepartmental Neuroscience Program, 3 Center for Bio-integrated Electronics, Querrey Simpson Institute for Bioelectronics, 4 Department of Materials Science and Engineering, 5 Division of Plastic and Reconstructive Surgery, Biologics, Shirley Ryan AbilityLab, 6 Department of Biomedical Engineering, 7 Department of Neurological Surgery, 8 Department of Chemistry, Department of Electrical and Computer Engineering, 10 The Ken and Ruth Davee Department of Neurology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA" poster

Comprehensive electrical stimulation therapy to improve phrenic nerve regeneration and recovery of diaphragm muscle function on rats Phrenic nerve injury leads to diaphragmatic paralysis, causing shortness of breath, recurrent pneumonia, anxiety, insomnia, morning headache, excessive daytime somnolence, orthopnea, and fatigue. There is lack of epidemiological studies to show the prevalence of phrenic nerve injury due to underrecognizing the injury, but small observation studies showed idiopathic neuritis to be the most common single cause of phrenic nerve injury. Natural recovery not only takes a year but still leaves two thirds of patients with unsatisfactory diaphragmatic function. Here, we propose an integrated therapeutic electrical stimulation to both the phrenic nerve and affected diaphragm with novel, wirelessly powered battery free devices to achieve a better functional recovery. These devices reduce the risk of infection inherent with transcutaneous temporary leads, avoid the use of externally powered hardware that can be dislodged when caring for a patient, and do not require a secondary removal surgery. The phrenic nerve stimulator uses advanced materials that bioresorb harmlessly to benign products and the elastomeric polymer cuff design interfaces seamlessly with the proximal nerve stump. With the right stimulation parameters, there is an improvement on the speed and efficiency of axon regeneration and reinnervation. The diaphragm

stimulator utilizes highly stretchable, bioresorbable wire electrodes that can be attached directly to the muscle surface and ensure an ideal electrical interface. With direct, paced, stimulation, we can decrease the muscle degeneration during the denervated period and maintain respiratory function. Together, we demonstrated a comprehensive electrical stimulation therapy to enable better recovery from phrenic nerve injury on a rat model.

4. **Stephen N. Housley**[1,3], Paul Nardelli[1], Travis M. Rotterman[1], and Timothy C. Cope[1,2,3]
[1]School of Biological Sciences, Georgia Institute of Technology, Atlanta, Georgia 30332.[2]W.H. Coulter Department of Biomedical Engineering, Emory University and Georgia Institute of Technology, Georgia Institute of Technology, Atlanta, Georgia 30332. [3]Integrated Cancer Research Center, Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, 315 Ferst Drive, Atlanta, GA, 30309, USA. oral

Sensorimotor Circuit Collapse After Cancer Treatment Cancer survivors rank sensorimotor disability among the most distressing long-term consequences of chemotherapy. The underlying mechanisms are poorly understood, and there are no approved treatments or preventative strategies. Disorders in gait, balance, and precision movements are commonly assigned to damage of peripheral sensory neurons without consideration of the deterministic role played by neural circuits in translating sensory information into movement. Recently, we discovered the first direct evidence that chemotherapy chronically reduced intrinsic motoneuron excitability resulting in erratic firing in response to current injection, reduced spiking probability, suppressed firing frequencies, and uncoupled rate-modulation. While intrinsic motoneuron defects contribute to sensorimotor impairments, it is unclear whether extrinsic factors such as synaptic inputs and neuromuscular transmission play a role. Such information is prerequisite for reversing sensorimotor disability following cancer treatment. We rectified this omission by studying the operation of a spinal sensorimotor circuit *in vivo* in a rat model of chronic cancer treatment. Major sequential events were studied in the circuit translation of mechanosensory information into synaptic potentials that modulate motoneuron and muscle activity. Defective firing expressed by all mechanosensory neurons reduced accurate sensory representation of muscle biomechanics to 55% of normal. Accuracy in synaptic translation of sensory information through spinal premotor pathways fell an additional 43% of normal, resulting at least partially from synaptic impairment. Further, sporadic failure of the one-to-one relationship between motoneuron spiking and muscle force generation suggests dysfunction extends to the neuromuscular junction. Collectively, these sequential peripheral and central inaccuracies compound to drive the sensorimotor circuit to functional collapse.

Sensory afferent conduction (PAD)

1P 04/14/2022 **Jonathan Jair Milla Cruz** jonathan.millacruz@ucalgary.ca University of Calgary Postdoctoral Associate None "Jorge Ramon Calvo Martinez (Physiology, Biophysics and Neuroscience, Cinvestav-IPN, Mexico City, Mexico), Carlos Miguel VillalÃ³n Herrera (Pharmacobiology, Cinvestav-IPN (Unidad Sur), Mexico City, Mexico), Shawn Hochman (Dept. of Physiology, Faculty of Medicine, Emory Univ., Atlanta, GA, USA), Jorge Noel quevedo DurÃ¡n (Physiology, Biophysics and Neuroscience, Cinvestav-IPN, Mexico City, Mexico)"

The activation of D2 and D3 receptor subtypes inhibits pathways mediating primary afferent depolarization (PAD) in the mouse spinal cord "Somatosensory information can be modulated at the spinal cord level by primary afferent depolarization (PAD), known to produce presynaptic inhibition (PSI) by decreasing neurotransmitter release through the activation of presynaptic ionotropic receptors. Descending monoaminergic systems also modulate somatosensory processing. In the present work, we investigated the role of D1-like and D2-like receptors on pathways mediating PAD in the hemisected spinal cord of neonatal mice.

Experiments were performed in the sagittally-hemisected spinal cord of P6 mice with dorsal roots and peripheral nerves attached for afferent stimulation. Stimulus strength was based on multiples of threshold (xT) of the most excitable fibers recorded from the incoming afferent volley, with strengths 2xT recruiting only myelinated afferents. PAD was inferred from dorsal root potential (DRPs) recorded at spinal L3-L4 dorsal roots and monosynaptic transmission of afferent fibers from the extracellular field potentials (EFPs).

We found that DA (10Î¼M) depressed low-threshold evoked DRPs by 43±2% (n=47) of control, with no effect on EFPs. These effects on DRPs were mimicked by the D2-like receptor agonist quinpirole (35±2% of control, n=7), but not by the D1-like receptor agonist SKF38393. In addition, the selective D2 (L-741,626) and D3 (SB277011-A) receptor antagonists effectively prevented DRPs depression by quinpirole (17±1% and 19±5%; n=4 and n=5, respectively).

These results suggest that DA modulates somatosensory transmission at the spinal cord level through the activation of D2/D3 receptors located in the neuronal pathways mediating PAD. The activation of these receptors produces a relative facilitation of synaptic transmission associated with a decrease in PAD-related PSI."

2 04/15/2022 **Lucy Liang** lul49@pitt.edu University of Pittsburgh Graduate Student Student "Josep M. Balaguer, University of Pittsburgh, Dept. of BioEAmr A. Mahrous, Northwestern University, Feinberg School of Med.Jonathan C. Ho, University of Pittsburgh, School of MedicineErinn M. Grigsby, University of Pittsburgh, Dept. of PM&RVahagn Karapetyan, University of Pittsburgh, Dept. of BioE

Peter C. Gerszten, University of Pittsburgh, Dept. of Neurological Surgery Jorge A. Gonzalez-Martinez, University of Pittsburgh, Dept. of Neurological Surgery Charles J. Heckman, Northwestern University, Feinberg School of Med.Marco Capogrosso*, University of Pittsburgh,

Dept. of Neurological Surgery Elvira Pirondini*, University of Pittsburgh, Dept. of PM&R" oral

Corticospinal Tract Modulation of Sensory Information Through GABAergic Interneurons

The corticospinal tract (CST) is the major efferent pathway for motor control. Evidence suggest damage to this pathway causes sensory deficits in addition to motor deficits, yet the mechanisms are largely unknown, limiting effectiveness of therapies for central motor diseases. Most CST axons terminate in the intermediate zone of spinal gray matter where GABAergic interneurons reside. Recent studies showed GABAergic axo-axonic synapses generate primary afferent depolarization (PAD) to facilitate primary afferent spike transmission. Here we explored the role of CST in modulating upper-limb proprioceptive information by observing changes in PAD, motor evoked EMG, and intra-spinal activity in response to paired afferent-CST stimulation. We implanted an electrode in the internal capsule to stimulate CST of the upper limb in 3 monkeys. The proprioceptive afferents were stimulated through nerve cuffs on the radial and median nerves. We recorded PAD from a cut dorsal rootlet with a hook electrode, EMGs from arm and hand muscles, as well as intra-spinal neuronal activity with a multichannel linear array placed in the C5/C6 spinal segment. We then conditioned the spinal cord with a burst stimulation of the CST, followed by single pulse stimulation of a peripheral nerve at 2Hz. We observed a clear increase in PAD with CST conditioning, as well as modulation in EMG and intra-spinal activity. These results directly demonstrate that CST modulates sensory inputs into the spinal cord, and one mechanism is through modulation of the GABAergic interneurons. This understanding will help us develop better targeted therapies for central motor syndromes.

3. 04/14/2022 **Krishnapriya Hari** krishnap@ualberta.ca University of Alberta, Edmonton, Canada PhD student Student "Krishnapriya Hari1, Ana M. Lucas-Osma1,2, Krista Metz1, Shihao Lin1, Monica A. Gorassini1,3, Keith K. Fenrich1,2, Yaqing Li1,4, David J. Bennett1,21Neuroscience and Mental Health Institute, University of Alberta, Edmonton, AB, T6G 2R3, Canada2Faculty of Rehabilitation Medicine, University of Alberta, Edmonton, AB, T6G 2G4, Canada3Department of Biomedical Engineering, Faculty of Medicine and Dentistry, T6G 2V2, University of Alberta, Edmonton, AB, Canada4Department of Physiology, Emory University, Atlanta, GA, 30322, USA" oral

Constitutive GABAA receptor activity contributes to exaggerated sensory transmission to motoneurons and muscle spasms after spinal cord injury

While GABA is considered to be an inhibitory transmitter in the adult CNS, we have recently shown that it has a marked excitatory action on sensory axons, which increases proprioceptive spike transmission to motoneurons. Specifically, $GABA_A$ receptors near nodes of Ranvier in myelinated central branches of Ia afferents cause a depolarization (primary afferent depolarization, PAD) that helps facilitate spike initiation and propagation through the many nodes and complex branch points in these afferents (termed nodal facilitation), without which spike propagation failure is common. Neuronal circuits that activate $GAD2^+$ GABAergic neurons, which directly innervate afferent nodes, cause a phasic PAD and associated nodal

facilitation, because optogenetically activating or inhibiting GAD2 neurons respectively increases or decreases PAD and afferent spike conduction to motoneurons. Additionally, more general neuronal circuit activity leads to a spillover of GABA onto extrasynaptic α5 GABAA receptors at nodes, causing a tonic PAD and further nodal facilitation, because inhibiting these α5 receptors with L655708 decreases tonic PAD (hyperpolarizes afferents) and afferent conduction to motoneurons. Considering that sensory transmission to motoneurons is increased with spinal cord injury (SCI) and this leads to muscle spasms, we wondered whether a part of this increased transmission was due to exaggerated GAD2 neuron and α5 GABAA receptor activity, leading to an exaggerated tonic PAD and reduced afferent spike propagation failure at branch points. We explored this in the sacrocaudal spinal cord, maintained in vitro, from adult mice following a chronic S2 spinal transection (1 – 2 months prior). As expected, we found that there was more tonic PAD after SCI, since L655708 caused a larger hyperpolarization of afferents than in uninjured mice. This PAD led to a markedly greater conduction of afferents with SCI (both antidromic and orthodromic conduction), to the point where afferents rarely failed to conduct after SCI, with no evidence of branch point failure. This left no headroom for further nodal facilitation, since optogenetic activation of GAD2 neurons produced little if any increase in spike conduction or transmission to motoneurons, unlike in uninjured mice. Furthermore, this increased afferent conduction with SCI was reduced by blocking extrasynaptic GABAA receptors with L655708, which ultimately decreased monosynaptic reflexes and spasms, both in vitro and in vivo. However, we unexpectedly found that afferent spike conduction was not reduced by blocking circuit activity with glutamate receptor antagonists, unlike in uninjured mice where conduction was substantially reduced. This implies that the tonic PAD that enables spike conduction after SCI is not supported by neuronal circuit activity from GAD2 neurons, unlike in uninjured animals. Indeed, blocking all circuit activity with TTX after SCI left a substantial tonic PAD that was blocked by L655708, but not gabazine. Interestingly, L655708 acts as an inverse agonist that blocks constitutive activity in GABAA receptors, whereas gabazine is a neutral antagonist that does not, implying that the tonic GABAA receptor activity in SCI is largely from constitutive activity, in the absence of GABA. In contrast, L655708 had weaker effects after TTX in uninjured mice, implying that GABAA receptor activity was largely driven by GABA arising from circuit activity. Overall, these results imply that, unlike in conventional clinical practice, blocking GABA receptors, rather than activating them, might be useful in reducing spasms and restoring function after SCI

Human motor unit/ H-reflex studies_____

1P 29 03/18/2022 **Matheus Machado Gomes** mmgomes@usp.br School of Physical Education and Sport of RibeirÃ£o Preto, University of SÃ£o Paulo, SP, Brazil; Department of Physical Therapy and Human Movement Sciences, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; Department of Neuroscience, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; Assistant Professor None " Sophia T. Jenz (1,2); James A. Beauchamp (1,3); C.J. Heckman(1,2,4,5); Gregory E.P. Pearcey (1,2,4,5) Affiliations:1 - Department of Physical Therapy and Human Movement Sciences, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; 2 - Department of Neuroscience, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; 3 - Department of Biomedical Engineering, McCormick School of Engineering, Northwestern University, Chicago, IL, USA; 4 - Department of Physical Medicine and Rehabilitation, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; 5 - Shirley Ryan AbilityLab, Chicago, IL, USA. " poster

Voluntary co-contraction of human ankle muscles reduces the discharge rate hysteresis of motoneurons Contrary to most motor behaviors, where contraction of an agonist muscle causes reciprocal inhibition of its antagonistic pair, an isometric co-contraction task demands simultaneous contraction of antagonistic muscles and likely alters the composition of excitatory, inhibitory, and neuromodulatory commands to motoneurons. In this study, we compared estimates of persistent inward current (PIC) magnitude, a proxy for neuromodulatory drive, during both isometric dorsiflexion and co-contraction tasks about the human ankle. Discharge patterns of the tibialis anterior (TA) motor units (MUs) were discriminated using high-density surface electromyography and a convolutive blind source separation algorithm. To estimate PIC magnitude, we quantified discharge rate hysteresis (Î”F) by comparing the onset and offset of a higher-threshold MU in relation to the discharge rate of lower-threshold MUs. Participants randomly performed four triangular ramps of both co-contraction (simultaneous dorsiflexion and plantarflexion) and isometric dorsiflexion. In both cases, participants received visual feedback of the smoothed TA electromyogram, with triangular contractions consisting of 10 s ascending and descending phases to a peak of 30% of that achieved during maximal voluntary co-contraction. Î”F was significantly lower ($F(1, 318) = 70.3$; p <0.001) during co-contractions (4.32 Â± 2.06 pps) than isometric contractions (6.09 Â± 1.90 pps). This suggests that co-contraction imparts reciprocal inhibition from the antagonist onto the agonist and, since PICs are highly sensitive to inhibition, reduces discharge rate hysteresis of the agonist motoneurons. This diversity in the structure of motor commands during co-contraction may have major implications for the control of stabilizing tasks, and certainly for voluntary co-contraction training.

2P 03/21/2022 **Sophia T. Jenz** sophia-jenz@northwestern.edu Department of Physical Therapy and Human Movement Sciences, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; Department of Neuroscience, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA Graduate Student"James A. Beauchamp; Matheus M. Gomes,. C.J. Heckman;Gregory E.P. Pearcey; poster

Persistent inward currents may underlie sex differences in motoneuron discharge behavior.

Non-invasive recordings of motor unit (MU) spike trains help understand how neural drive is modulated by various physiological conditions. Most study participants in human and non-human animal studies are male, and it is assumed that these findings apply similarly to females. Paradoxically, there are known sex differences in neurological impairment and performance that warrant the study of sex differences broadly. To address this topic, we examined persistent inward currents, a proxy for the level of neuromodulatory drive, in both sexes by quantifying discharge rate hysteresis (Î”F). We decomposed MU spike trains from the tibialis anterior (TA), medial gastrocnemius (MG), and soleus (SOL) using high-density surface electromyography and blind source separation algorithms. Ten participants of each sex (26.4Â±5.2 years) performed triangular contractions to 30% MVC. We used linear mixed effects models to determine if peak discharge rate and Î”F were predicted by the fixed effects of sex, muscle, and their interaction. Across muscles, Î”F was larger (Ï‡2(1) = 5.79, p=0.0161) in females (F: 4.7Â±0.18pps; M: 3.7Â±0.22pps). An interaction between sex and muscle (Ï‡2(2) = 31.47, p<0.001) revealed that peak discharge rate was higher in female plantarflexors (F: MG = 13.2Â±0.0.5pps and SOL = 11.4Â±0.51pps; M: MG = 11.76Â±0.6pps and SOL = 9.37Â±0.6pps), but not dorsiflexors (15.78Â±0.5pps vs. 15.51Â±0.6pps). These findings suggest that neuromodulatory drive and/or motoneuron excitability differs between the sexes and may contribute to differences in MU discharge patterns. This calls for future studies to include equal numbers of female and male participants and analyze sex as a biological variable."

3P 03/30/2022 **Melanie HENRY** melanie.henry@ulb.be Laboratory of Applied Biology, Research Unit in Applied Neurophysiology (LABNeuro), ULB-Neurosciences Institute (UNI), UniversitÃ© libre de Bruxelles (ULB), 808 route de Lennik, 1070 Brussels, Belgium PhD Candidate Student "Jacques DUCHATEAU and StÃ©phane BAUDRY Laboratory of Applied Biology, Research Unit in Applied Neurophysiology (LABNeuro), ULB-Neurosciences Institute (UNI), UniversitÃ© libre de Bruxelles (ULB), 808 route de Lennik, 1070 Brussels, Belgium" poster

Age-related changes of the Hoffmann-reflex pathway in the flexor carpi radialis "Wide-pulse (>0.2ms) stimulus preferentially recruits muscle spindle afferents than motor axons (1). However, the age-related alterations in sensory afferents (2) may impact these electrophysiological properties. We investigated the influence of age and stimulus pulse width on several Hoffmann-(H)-reflex parameters.

H-reflex and M-wave recruitment curves were recorded at rest in the flexor carpi radialis of 12 young (YA, 21-36 yrs) and 12 older adults (OA, 62-80 yrs) by stimulating the median nerve with 1-ms, 0.2-ms and 0.05-ms pulse widths. We measured the ratio between the maximal H-reflex and M-wave amplitude (Hmax/Mmax ratio), the H-reflex amplitude for a stimulus intensity evoking an M-wave of 5%Mmax (H-M5%), the M-wave amplitude at Hmax (M-Hmax) and the ratio between the intensities eliciting half Hmax and Mmax amplitude (iH50/iM50 ratio).

OA had smaller Hmax/Mmax ratio but greater M-Hmax and iH50/iM50 ratio than YA, regardless of pulse width (p-valuesâ‰¤0.025). H-M5% was smaller in OA than YA for pulse width â‰¥0.2ms (p<0.001), with a lesser effect of pulse width on H-M5% in OA (p=0.005).

The age-related changes in H-reflex parameters could reflect changes at axonal or synaptic levels. However, the lesser influence of pulse width on H-M5% in OA suggests a change in strength-duration time constant (1) or a loss of larger sensory afferents (2,3). Overall, these results may reflect changes in the excitability of muscle spindle afferents with ageing.

1. Panizza et al. 1989. Muscle Nerve.12,576-579.2. Kim et al. 2007. J Physiol.582,525â€“538. 3. Veale et al. 1973. J. Neurol. Neurosurg. Psychiatry.36,75â€“86."

4 03/31/2022 **James (Drew) Beauchamp** james.beauchamp@northwestern.edu Northwestern University PhD Candidate Student "James A. Beauchamp1,2, Gregory E.P. Pearcey2,3,4,5, Obaid U Khurram6, Julius P.A. Dewald1,2,4, CJ Heckman2,3,4,5 1 Department of Biomedical Engineering, McCormick School of Engineering, Northwestern University, Chicago, IL, USA" oral

Potential deficits in isometric ankle torque control introduced by persistent inward currents in humans Voltage-sensitive persistent inwards currents (PICs) are monoaminergic-dependent depolarizing currents that augment excitatory synaptic input to motoneurons, often prolonging discharge and sculpting the behavior of motoneurons to meet task demands. Here, we highlight this relation and show that prolongation of motoneuron firing depends on task demands in the human. In the first experiment, we used paired motor unit (MU) analysis (DeltaF) to estimate PICs during triangular isometric plantarflexion/dorsiflexion torque contractions at various ankle joint angles, where both mechanical properties and afferent input (i.e. muscle length and reciprocal inhibition) may influence PICs. Across sampled muscles, DeltaF was 0.385 pps higher (95%CI: [0.174 0.595]) when the agonist muscles were at relatively shorter lengths. In a second experiment, we investigated the influence of torque profile on persistent MU discharge using a novel sombrero paradigm, comprised of a triangular contraction superimposed upon a steady low-level contraction. In this paradigm, ~50% of MUs recruited in the triangular region sustained discharge into the low-level contraction and created difficulties in task performance as evidenced by a 1.5 fold increase in torque variability. Combining parameters from both experiments, we found that a greater proportion of MUs exhibited sustained discharge during sombrero contractions at shorter lengths in the tibialis anterior (Short:278/538, Long:114/361) and medial gastrocnemius (Short:378/623, Long:41/237) but not soleus (Short:24/68, Long:24/43). These collective findings suggest motoneurons at the ankle exhibit a greater propensity for sustained discharge at shorter muscle lengths during voluntary efforts and that PICs, once activated, may introduce potential limitations in isometric ankle torque control.

5P 04/01/2022 **Ghazaleh Mohammadalinejad** ghazale1@ualberta.ca University of Alberta student Student Monica Gorassini, Babak Afsharipour, Jennifer Duchcherer University of Alberta poster

Development of Intrinsic Motoneuron Properties in Humans Motoneuron properties and their firing patterns change by age and in response to CNS injury. We investigated intrinsic motoneuron properties in 51 typically developing (TD) participants from the ages of 7 to 60 years and in 3 participants with cerebral palsy (CP) between the ages of 26 - 39. One of the main objectives of this ongoing study is to understand how activation of persistent inward calcium currents (CaPICs) contribute to the excessive activation of motoneurons during spasticity in CP. To do this, we measured multiple single motor units from the ankle dorsiflexor muscle tibialis anterior (TA) from a 64-electrode surface EMG grid. CaPIC amplitude was estimated from the amount of synaptic current (measured from the motor unit firing rate profiles) that is needed to de-recruit the motor unit compared to the amount needed to recruit the motor unit (or DeltaF) given that the CaPIC provides sustained depolarization of the motoneuron at sub-threshold levels. Interestingly, DeltaF is large in young children and adults (up to 24 years of age) and then plateaus to a steady amplitude during later adulthood. So far, DeltaF measured in participants with CP is within TD ranges. In summary, it appears that CaPICs decrease with age and are not affected by insult to the developing brain in CP. However, other properties of motoneuron firing such as the sensitivity or gain of the motoneuron to synaptic inputs does seem to be affected in CP and we are investigating this further.

6P 04/12/2022 **Maria Knikou** Maria.Knikou@csi.cuny.edu City University of New York Professor "Andreas Skiadopoulos1, Rachel Burau1, Stephan Heddon1, Nicole Saulnier1, Timothy S. Pulverenti1, Maria Knikou1,21 Klab4Recovery Research Laboratory, Department of Physical Therapy, College of Staten Island, The City University of New York, Staten Island, NY USA 2 PhD Program in Biology and Collaborative Neuroscience Program, Graduate Center of The City University of New York and College of Staten Island, New York, NY USA " Timothy S. Pulverenti and Maria Knikou poster

Physiological effects of different thoracolumbar transspinal stimulation electrode configurations in humans Spinal cord stimulation has gained prominent attention, with research investigations spanning from animals to humans, and from mathematical modeling to intraspinal, epidural, and transcutaneous (i.e. transspinal) delivery of electrical current to affect locomotor, respiratory, and bladder function. In this study we established the neurophysiological properties of transspinal evoked potentials (TEPs) in response to different configurations of the cathode electrode. The cathode was a rectangular electrode (Protocol P-Klab4Recovery), a square electrode (P-2), two square electrodes with 1 cm distance placed vertically on spinal process (P-3), or a square electrode placed on each paravertebral side (P-4). TEPs were recorded bilaterally from ankle and knee muscles, and we determined the latency, and duration, as well as recruitment curves and strength of pre-motoneuronal control (low-frequency and post-activation depression) for each protocol. We found that TEPs onset latency was significant different across muscles and protocols, with latency being different P-Klab vs. P4, P-Klab vs. P2, P3 vs. P4, and P3 vs. P2. The strength of low-frequency depression was not significant different across protocols, but was stronger for ankle TEPs compared to TEPs recorded from knee muscles

suggesting that a different proportion of afferents are involved in manifestation of TEPs across muscles. A shift in the slope and threshold intensity to the right was found for P-4, suggesting that more stimulation intensity is needed to evoke TEPs of similar amplitude. Our findings suggest the use of the most optimal stimulation configuration based on physiological evidence. Supported by the NIH/NICHD (RO1 HD100544) and the NYSDOH/ SCIRP (C35594GG).

8P 04/13/2022 **Ellen Pereira Zambalde** ellenzamb@gmail.com University of Campinas PhD. Student Student "Carina Marconi Germer, University of Pernambuco Leonardo Abdala Elias, University of Campinas"None. A colleague from my lab (Ricardo) will be attending the meeting in the virtual environment.

Correlation between the approximate entropy of the isometric muscle force and the neural drive to a hand muscle Approximate entropy (ApEn) is a statistical measure that has been used to quantify the regularity of isometric force signals. A recent computer simulation study predicted that several properties of the neuromuscular system would influence force ApEn, including the input bandwidth, the number of recruited motor units (MUs), and the contractile properties of muscle fibers. Also, the simulations predicted that the correlation between ApEn of common input to the motor neuron (MN) pool and force was low. Here we analyzed human experimental data to validate modelâ€™s predictions. Eleven participants performed an isometric force task (index finger abduction) at two contraction intensities (2.5% and 5%MVC). Force signals and MU spike trains (decomposed from high-density EMG) were used to perform a correlation analysis using both ApEn and CoV (coefficient of variation) of muscle force and the smoothed cumulative spike trains (sCST; filtered at 5Hz). Results indicated that both CoV and ApEn of force and sCST signals reduced with contraction intensity. sCST CoV presented a moderate correlation with force CoV ($ï•^2 = 0.55$), while the correlation between sCST and force ApEn was low. The latter, however, depended on the number of recruited MUs used to estimate the sCST, so that the increased number of MUs used in the sCST resulted in a higher correlation coefficient ($ï•^2 = 0.31$ for a group of 9 MUs per CST). Therefore, our results support that the regularity of low-frequency components of the common input to the motor neuron pool is linearly transmitted to muscle force regularity.

9P 04/13/2022 **Christina D. Bruce** nique1965@gmail.com University of British Columbia - Okanagan PhD Student Student "Justine R. Magnuson & Chris J. McNeilSchool of Health and Exercise Sciences and Centre for Heart, Lung and Vascular Health, The University of British Columbia, Kelowna, BC"

Assessing voluntary activation using two different methods to set intensity of transcranial magnetic stimulation According to current guidelines, when measuring voluntary activation (VA) using transcranial magnetic stimulation (TMS), stimulator output (SO) should not exceed the intensity that, during a maximal voluntary contraction (MVC), elicits a motor evoked potential (MEP) from the antagonist muscle >15% of its maximal M-wave amplitude. However, VA is based on agonist evoked-torque responses (i.e., superimposed twitch; SIT and estimating resting twitch; ERT), which means limiting SO based on electromyographic (EMG)

responses will often lead to a submaximal SIT and ERT, possibly underestimating VA. Therefore, the purpose of this study was to compare elbow flexor VA calculated using the original method (i.e., intensity based on MEP size; SOmep) and a method based solely on eliciting the largest SIT at 50% MVC torque (SOsit), regardless of triceps brachii MEP size. Fifteen healthy, young participants performed 10 sequences of brief contractions at 100, 75, and 50% MVC torque, with TMS delivered at SOmep (73.0Â±13.5%) or SOsit (92.0Â±10.8%) for five sequences each. Although the mean ERT torque was greater using SOsit (15.2Â±4.8Nm) compared to SOmep (12.9Â±3.7Nm; P=0.03), the SIT amplitude at 100% MVC torque was not different (SOmep: 0.69Â±0.49Nm vs. SOsit: 0.74Â±0.52Nm; P=0.60). Despite the ERT disparity, VA scores were not different between SOmep (94.6Â±3.5%) and SOsit (95.0Â±3.3%; P=0.57). Although SOsit did not lead to a higher VA score than the SOsit method, it has the benefit of yielding the same result without the need to record antagonist EMG or perform MVCs when determining SO, which can induce fatigue prior to measuring VA.

10 04/14/2022 **Francesco Negro** francesco.negro@unibs.it Universita' degli Studi di Brescia Associate Professor None "Tea Lulic-Kuryllo1, Marco Benedini1, Marta Cogliati1, Simone Piva2, Claudio Orizio1, Nicola Latronico21Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy2Department of Anethesia, Critical Care and Emergency, ASST Spedali Civili University Hospital, Brescia, Italy" oral

A longitudinal study on motor unit adaptations in COVID-19 post-intensive care syndrome patients Introduction: COVID-19 post-intensive care syndrome (PICS) patients present with persistent fatigue and weakness. The longitudinal effects of COVID-19 on motor unit (MU) properties have not been investigated. The aim of the study was to assess if COVID-19 survivors have altered MU properties at 3- and 6-months and 6- and 12-months post-ICU discharge. Methods: Two groups of critically ill patients were recruited from the ICU of Spedali Civili Hospital in Brescia with a confirmed COVID-19 infection. The two patient groups consisting of 20 and 16 patients were followed at 3 to 6 or 6 to 12 months, respectively. Patients performed submaximal isometric contractions scaled to 30%, 50%, and 70% MVC. One 64 channels matrix was placed over the belly of the tibialis anterior. HD-sEMG signals were decomposed, following which mean discharge rate, discharge rate at recruitment and de-recruitment, recruitment and de-recruitment threshold, and discharge variability were quantified. Results: Recruitment, and de-recruitment discharge rates of low-threshold MUs increased by 14% and 9%, while de-recruitment threshold increased by 16% at 6 months compared to 3 months. For high-threshold MUs, recruitment threshold decreased by 6% at 6 months compared to 3 months. There were no significant differences between 6 and 12 months in low- or high-threshold MU properties. There was an increase in MVC across the follow-up. Discussion: MU properties adapt and are altered in the first 6 months but recover by 12 months following ICU discharge. These adaptations may be due to the disease-causing autoinflammatory process.

11P 04/14/2022 **Ricardo N. O. Mesquita** r.mesquita@ecu.edu.au Edith Cowan University, Australia PhD candidate / Senior research assistant Student Janet L. Taylor (Edith Cowan University, Australia), Gabriel S. Trajano (Queensland University of Technology, Australia), AleÅ¡ Holobar (University of Maribor, Slovenia), BasÃ-lio A. M. GonÃ§alves (Griffith University, Australia), Anthony J. Blazevich (Edith Cowan University, Australia) poster

Does mental stress modulate the activity of persistent inward currents in motoneurons? It (probably) depends! Background: Persistent inward currents (PICs) in motoneurons greatly influence their responsiveness to synaptic input. As PICs are enhanced by monoamines, we hypothesised that increased noradrenaline release induced by mental stress would increase PICs in humans. Methods: Surface electromyograms (EMG) collected using a 32-electrode matrix on gastrocnemius medialis during voluntary, isometric, ramp plantarflexions were decomposed to estimate PICs using the paired motor unit technique (delta frequency, Î”F). Additionally, another technique (VibStim), which combines Achilles tendon vibration and triceps surae neuromuscular electrical stimulation (NMES) to evoke involuntary self-sustained contractions was used. VibStim-induced muscle activity may reflect PIC activation. Trials of each technique were performed with and without mental stress, which was induced by a mental arithmetic task. Results: Reported stress and heart rate increased during mental stress. Î”F was not altered by mental stress (p=0.147, n=14, 5 females). In VibStim (n=19, 10 females), mental stress induced greater responses in torque (p=0.010) and soleus EMG (p=0.005) during vibration at the end of NMES, in the torque increase throughout the trial (i.e., warm-up) (p=0.005), and in sustained torque 0.5 s post-vibration (p=0.017), but not in post-vibration EMG (p=0.201). Conclusion: Mental stress increased the magnitude of involuntary contractions (VibStim) but not Î”F. Thus, effects of increased noradrenaline release on PIC contribution to motoneuron firing might depend on the background level of neuromodulation and ionotropic input. Alternatively, as only gastrocnemius medialis Î”Fs were calculated, mental stress might have differentially influenced plantarflexor synergists. Finally, mechanism(s) other than increased PICs could have contributed to the greater VibStim responses.

12P 04/14/2022 **Popesco Timothee** timothee.popesco@unil.ch University of Lausanne PhD Student Student"Gilles Andreotti, Laurenne Dulac, Chris Donnelly, Nicolas Place Institute of Sport Sciences, Faculty of Biology and Medicine, University of Lausanne, Switzerland" poster

Amplifying the torque induced by wide-pulse, high-frequency neuromuscular electrical stimulation "Wide-pulse high-frequency neuromuscular electrical stimulation (WPHF NMES) may induce a progressive increase in torque during the stimulation (â€˜extra torqueâ€™ (ET)) at low stimulation intensity (Wegrzyk et al. 2015). The mechanisms responsible for ET production remain largely unknown but persistent inward currents (PICs) are thought to play an important role (Collins 2007). The aim of this study was to test the hypotheses that a remote contraction (i.e. contraction of an unrelated muscle) would (i) increase ET during WPHF NMES of the plantar flexors and (ii) lead to higher PIC estimates.

Twenty-five healthy volunteers (26Â±6 years, 7 women and 18 men) took part to the protocol. The experiment was performed in two conditions: (i) Control and (ii) Remote, in which the participants sustained an isometric elbow flexion at ~20% of their maximal voluntary contraction (MVC) torque. WPHF NMES 20-s long trains were delivered at the intensity necessary to evoke 5% MVC torque. High-density electromyographic recordings from the soleus (SOL) and gastrocnemius medialis (GM) muscles were used to estimate PIC strength during triangular voluntary contractions using the deltaF method (Gorassini et al. 2002).

ET, quantified as the relative change in torque during the 20-s evoked contraction, was higher in Remote compared to Control (+55Â±180% vs. +15Â±113%, p=0.003). PIC estimates did not differ between Control and Remote for SOL and GM (p>0.05). Our findings do not support a direct link between PIC and ET in response to WPHF NMES. Nevertheless, the effect of the remote contraction on ET production may offer new therapeutic perspectives. "

13P 04/14/2022 **Antonio Gogeascoechea** antonio.gohz@gmail.com University of Twente PhD candidate Student "Rafael Ornelas Kobayashi, University of Twente Utku S. Yavuz, University of Twente Massimo Sartori, University of Twente" poster

Identification of Motor Unit Twitch Responses in the Intact Human In Vivo: Shifting the Paradigm in Electromyography-driven Modeling"Current electromyography (EMG)-driven modeling frameworks aim at representing complex neuromusculoskeletal processes. However, they often fail to capture the interaction between neural and mechanical mechanisms of movement. The ability to decode motor units (MUs) from high-density EMGs enables extending current neuromusculoskeletal models into MU-specific formulations where the neural information is preserved. Herein, we propose a high-resolution framework to generate MU-specific neuromusculoskeletal models based on the identification of MU-twitch properties.

For this purpose, we recorded torque and high-density EMGs from the lower leg during isometric dorsi-plantarflexion contractions across multiple activation levels and ankle positions. We decomposed the EMGs into MU spike trains and calculated their recruitment thresholds and discharge rates. We computed a linear combination of these neural features and mapped them into contractile properties found in humans. We employed the resulting properties to design twitch models as impulse responses of a second-order system. The MU-specific activation dynamics were defined as the convolution between the MU-twitch responses and their corresponding spike trains. The resulting activation profiles were used to drive a subject-specific musculoskeletal model which allowed computing joint moments. Moreover, we compared our methodology with the conventional EMG-driven framework.

For the MU-driven models, the normalized RMSE values between the reference and predicted torques were below 0.5 across all conditions. Contrastingly, the EMG-driven models were unable to adapt to all conditions, providing greater errors in the plantar-flexed and low activation conditions.

Our proposed methodology showed robustness in predicting torque across multiple conditions and provides a deeper insight into force-generation processes of human movement.

14P 04/14/2022 **Trevor S Barss** tbarss@ualberta.ca University of Alberta Postdoctoral Fellow Post-doc "Behdad Parhizi - University of Alberta Vivian K Mushahwar - University of Alberta" poster

Neuromodulation across multiple segments of the spinal cord with transcutaneous spinal cord stimulation Transcutaneous spinal cord stimulation (tSCS) has the potential to modulate circuitry of the spinal cord non-invasively. Currently, little is known as to how tSCS influences spinal and corticospinal excitability across multiple remote segments of the spinal cord. Two studies aimed to determine the effect of cervical, lumbar, or combined tSCS on cervico-lumbar connectivity and corticospinal excitability during static and rhythmic arm and leg tasks. During study 1 (n=13), Hoffman (H)-reflexes were elicited in the soleus muscle (SOL), while in study 2 (n=14), the amplitude of H-reflexes and motor evoked potentials (MEPs) were evaluated in the flexor carpi radialis muscle (FCR). These were assessed during 2 tasks: 1) Static; 2) Rhythmic cycling of the arms (study 1) or legs (study 2). In both studies, conditions included 1) No tSCS; 2) cervical tSCS; 3) lumbar tSCS. Study 2 also included combined cervical and lumbar tSCS. As expected, SOL H-reflex amplitude was suppressed by 19% during arm cycling relative to arms static (without tSCS) and cervical tSCS with arms static induced a similar 23% reduction in SOL H-reflex amplitude. Leg cycling suppressed the FCR H-reflex by 14%, while lumbar tSCS facilitated the FCR H-reflex amplitude by 11% when the legs were static. Interestingly, combined cervical and lumbar tSCS produced a convergence in the upper limbs (FCR muscle) that increased H-reflex and MEP amplitude by 20% compared to static No-tSCS. Collectively, these results indicate that tSCS alters excitability across multiple segments of the spinal cord and converge to modulate both spinal and corticospinal transmission.

15P 04/15/2022 **Melissa Elena Fajardo** melissa.fajardo@northwestern.edu Northwestern University PhD Student Student "Laura Miller-McPherson (Washington University in St. Louis) CJ Heckman (Northwestern University) James A. Beauchamp (Northwestern University) Sophia Jenz (Northwestern University) Gregory EP Pearcey (Northwestern University)" poster

Comparing Estimates of Persistent Inward Currents & Motor Unit Discharge Patterns in the Human Upper Arm Muscles within a limb play various roles in the generation of movement. For example, proximal muscles in the upper limb tend to stabilize and produce gross movements with the arm while distal muscles are mainly responsible for fine movements. All motor pools are innervated by corticospinal- and brainstem-mediated projections in the mammalian upper limb, but proximal muscles are biased towards brainstem-mediated innervation and distal muscles are biased towards corticospinal innervation. This may be significant because brainstem pathways provide neuromodulatory input to motoneurons (MN) which can alter excitability and alter the input-output patterns of MN discharge in response to incoming excitatory/inhibitory motor commands. Monoamines originating from the brainstem facilitate persistent inward currents (PICs), which amplify and prolong excitatory inputs, thus facilitating

MN discharge. The purpose of this ongoing study is to compare estimates for PIC magnitude as well as motor unit discharge patterns decomposed from high density surface EMG arrays over various muscles of the upper limb in healthy young adults. The anterior and intermediate deltoid, biceps brachii, lateral head of the triceps brachii, flexor digitorum superficialis, extensor digitorum, and the first dorsal interossei were studied during triangular-shaped ramp contractions in a single session. Preliminary data suggest that motor unit discharge characteristics differ as one moves from proximal to distal muscles while estimates of PIC magnitude are higher in proximal muscles when compared to distal muscles. This may have functional implications for the normal control and rehabilitation of movement after neurological impairment.

16P 04/15/2022 **Christopher Taylor** taylor.chris@temple.edu Temple University PhD Student Student "Francesco Negro (UniversitÃ degli Studi di Brescia) Christopher Thompson (Temple University)"

The Effects of Caffeine on Human Spinal Motoneuron Excitability Human spinal motoneurons are partially governed by the activation of persistent inward currents that contribute to changes in excitability. These changes in motoneuron excitability are regulated by monoamines, such as serotonin and norepinephrine. Caffeine, one of the worldâ€™s most popular performance-enhancing supplements, elicits its ergogenic benefits through stimulating the volumetric release of monoamines. However, little is known on how caffeine may affect motoneuron excitability and discharge characteristics. We utilized a double-blind, inactive placebo-controlled, crossover study design (clinical trial: NCT04891393) to examine and quantify the effects of caffeine (3 mg/kg) on motoneuron excitability and discharge characteristics. Utilizing high-density electromyography from the right tibialis anterior and soleus muscles of 20 adults with no history of neurological injury, we decomposed dozens of concurrently active motor units during a series of sub-maximal (20%) isometric contractions of the ankle, while a registered nurse documented vital signs, at 30 minute intervals before and after the ingestion of either caffeine or placebo. In the caffeine group, we observed significant changes in cardiovascular physiology (mean arterial pressure +8.8% (+7.7mmHg); heart rate -15.3% (-11.7 bpm)) 30 minutes after caffeine consumption, however, no changes in estimated motoneuron excitability (DeltaF), motor unit yield, maximum discharge rate, recruitment threshold, and derecruitment threshold were observed. In the placebo group, no significant changes were observed in either cardiovascular physiology, motoneuron discharge characteristics, or excitability. These findings suggest that caffeineâ€™s ergogenic benefits may be unrelated to changes in motoneuron excitability or discharge properties.

17P 04/15/2022 **Jessica Leverett** leverett@ualberta.ca University of Alberta Student "Bronder, Porozni, & Collins, University of Alberta" poster

Functional electrical stimulation and transcutaneous spinal cord stimulation: Better together? Transcutaneous spinal cord stimulation (tSCS) holds great promise for restoring lost motor function after a spinal cord injury. tSCS is thought to work by increasing the

excitability of neural circuits that control movement, thereby â€œboostingâ€• residual voluntary descending commands and enhancing movement. Functional electrical stimulation (FES), on the other hand, was developed in the 1970s to generate contractions, restore movement, and maintain muscle mass and cardiovascular health. FES generates contractions predominantly by depolarsing motor axons, however, sensory axons also depolarise and contractions can also arise from signals traveling along circuits through the CNS. We propose that if tSCS increases the excitability of such circuits, then when tSCS is on, contractions produced by FES will be larger than when tSCS is off, and contraction amplitude will increase with tSCS intensity. FES will be delivered over the tibial nerve to produce sets of 5 (7s long) plantarflexion contractions (10s between) that generate ~5% of a maximum voluntary contraction under control (FES only) conditions. In separate trials, FES will be delivered while tSCS (4kHz alternating current pulses at 30Hz) is delivered for 10 min at each of 4 intensities (0.6, 0.8, 1.0, and 1.2 times dorsal root reflex threshold). Preliminary results suggest contractions can be 50% larger when tSCS is on compared to off and the effect scales with tSCS intensity. If these results hold true, we propose that FES and tSCS will be better together for producing contractions that are larger, more fatigue-resistant and accompanied by less spasticity than FES alone.

18P 04/16/2022 **Matt Topley** topley@temple.edu Temple University Doctoral Student Student "Christopher K. Thompson, PT, DPT, PhD, Martin Zaback, PhD" poster

Estimating motoneuron excitability during isometric contractions with and without antagonist co-contraction "Introduction: The nervous system is suggested to utilize distinct descending pathways to mediate flexion, extension, and cocontraction. Other evidence suggests both motor pools receive concomitant excitatory and inhibitory inputs during cocontraction. Though fundamental to motor control, neither of these theories consider the role of motoneuron excitability in cocontraction. In this study, we hypothesize that motoneuron excitability will be reduced during co-contraction of antagonist muscles.

Methods: High-density surface electromyography (HD-sEMG) of the tibialis anterior (TA) was recorded in three healthy individuals (male age 30-35). First, subjects performed 10% maximal voluntary isometric dorsiflexion (DF) ramps with visual feedback of TA EMG amplitude. Ramps with a target 10% TA EMG amplitude were repeated, but while producing zero dorsiflexion torque, thus requiring co-contraction of plantarflexors (PFs; Coco). The HD-sEMG signals were decomposed into single motor unit action potentials and motoneuron excitability was examined using a paired motor unit analyses (Delta-F).

Results: 339 and 329 motor unit spike trains were extracted during the DF and Coco ramps, respectively. Unitwise Delta-F values were calculated for every test unit that met established criteria and had a minimum of four valid comparisons. The average TA motoneuron excitability was similar between DF (3.74 Â± 0.99pps) and Coco (3.62 Â± 1.29pps) ramps.

Conclusion: Our preliminary results demonstrate similar motoneuron excitability during dorsiflexion and antagonist co-contraction in TA motoneurons. These data are consistent with

the hypothesis that the magnitude of inhibition to TA spinal motoneurons is not different between isolated TA contractions and contraction."

19 04/16/2022 **Gabriel Siqueira Trajano** g.trajano@qut.edu.au Queensland University of Technology Senior Lecturer None "Patrick Rodrigues (Queensland University of Technology, Australia) Karen Mackay (Queensland University of Technology, Australia) Anthony Blazevich (Edith Cowan University, Australia) David Borg (Queensland University of Technology, Australia) Tiago Souza (Queensland University of Technology, Australia) Raphael Sakugawa (Federal University of Santa Catarina, Brazil) Anthony Shield (Queensland University of Technology, Australia) Lucas Orssatto (Queensland University of Technology, Australia)" oral

Intrinsic motor neurone excitability is increased after resistance training in older adults

This study investigated the effects of high-intensity resistance training on estimates of the motor neurone persistent inward current (PIC) in older adults. Seventeen participants (68.5±2.8 years) completed a 2-week non-exercise control period followed by 6 weeks of resistance training. Surface electromyographic signals were collected using two 32-channel electrodes placed over soleus to investigate motor unit discharge rates. Paired-motor unit analysis was used to calculate delta frequency (DF) as an estimate of PIC amplitudes during a) triangular-shaped contractions to 20% of maximum torque capacity, and b) trapezoidal- and triangular-shaped contractions to 20% and 40% of maximum torque capacity, respectively, to understand their ability to modulate PICs as contraction intensity increases. Maximal strength and functional capacity tests were also assessed. For the 20% triangular-shaped contractions, DF (0.58-0.87 pps; $p<0.015$) and peak discharge rates (0.78-0.99 pps; < 0.005) increased after training, indicating increased PIC amplitude. PIC modulation also improved after training. During control period, mean DF differences between 20% trapezoidal-shaped and 40% triangular-shaped contractions were 0.09-0.18 pps ($p=0.448$ and 0.109, respectively), which increased to 0.44 pps ($p< 0.001$) after training. Also, moderate-to-very large correlations ($r=0.39$-0.82) were observed between changes in 20% triangular-shaped contractions DF and changes in peak discharge rates and measures of motor function. Our findings indicate, for the first time, that increased motor neurone excitability is a potential mechanism underpinning training-induced improvements in motor neurone discharge rate output, strength, and motor function in older adults. This increased excitability is likely mediated by enhanced PIC amplitudes, which are larger at higher contraction intensities.

20P 04/14/2022 **Joshua Cohen** jcohen66@uwo.ca Western University PhD Candidate Student "Taian Vieira - Politecnico di Torino Tanya D. Ivanova - Western University Jayne Garland - Western University" poster

Differential control of distinct motoneuron pools in the ankle plantarflexors It has been shown that when humans lean in various directions, the central nervous system (CNS) recruits different motoneuron pools for task completion; common units that are active during different

leaning directions, and unique units that are active in only one leaning direction. We used high-density surface electromyography (HD-sEMG) to examine if motor unit (MU) firing behaviour was dependent on leaning direction, muscle (medial and lateral gastrocnemius; soleus), limits of stability, or whether a MU is considered common or unique. Fourteen healthy participants stood on a force platform and maintained their center of pressure in five different leaning directions. HD-sEMG recordings were decomposed into MU action potentials and the average firing rate (AFR), coefficient of variation (CoVISI) and firing intermittency were calculated on the MU spike trains. During the leaning directions that demanded larger force production, both unique and common units had higher firing rates ($F = 31.31$, $p < 0.0001$). However, the unique units achieved higher firing rates compared to the common units (mean estimate difference = 3.48 Hz, $p < 0.0001$). The CoVISI increased across directions for the unique units but not for the common units ($F = 23.65$. $p < 0.0001$). Finally, intermittent activation of MUs was dependent on the leaning direction ($F = 22.21$, $p < 0.0001$), with less intermittent activity occurring during diagonal and forward-leaning directions. These results provide evidence that the CNS can preferentially control separate motoneuron pools within the ankle plantarflexors during voluntary leaning tasks for the maintenance of standing balance.