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Abstract booklet



Motoneuron excitability dysfunction in ALS

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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 our recent efforts to investigate the membrane mechanisms underlying cell death in the disease.

Title: Inhibitory synapses in ALS: dysfunction and response to chemogenetic and intrabody modulation

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Excitation/inhibition balance alterations are hypothesized to be major contributors to motoneuron vulnerability and degenerative process occurring in ALS. Structural and functional abnormalities in excitatory synapses have been described (Baczyk et al., 2020). The existence of any pathogenic or compensatory change at the level of inhibitory synapses, which may reduce or amplify the excitatory dysfunction, is matter of debate. We have employed several histological readouts and direct and indirect manipulations of inhibitory synapses in MN to investigate the extent of inhibitory synapses involvement and the MN response to their abrogation. Quantitative confocal microscopy reveals at P45 (SOD1G93A-high copy) downregulation of GlyR clusters but the upregulation of GABAR clusters at inhibitory synapses of MN, together with the disruption of the Gephyrin architecture. Both GlyR and GABAR cluster size meaningfully respond to chemogenetic alterations of MN excitability, with increased excitation driving increase in inhibitory clusters. Expression of a functionalized intrabody aimed at inducing Gephyrin degradation (Gross et al., 2016) results in the substantial decrease in GABAR and GlyR clusters without affecting the number and structure of excitatory synapses; this intrabody also results in the increase in pCREB and decrease in pPDH indicating increased net excitation. Interestingly, degradation of gephyrin results in the downregulation of misfSOD, LC3A and oxidative stress markers, whereas the expression of an untargeted ubiquitin ligase is ineffective. Finally, selective removal of GABAR by CRISPR also alters disease pathways. These data demonstrate the involvement of inhibitory synapses in the early phases of disease pathogenesis, the persistence of homeostatic loops regulating inhibitory synapses at these stages and the beneficial effect of a net excitation increase on disease pathways.

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Oral Presentation

Title

Non-canonical adrenergic neuromodulation of motoneuron intrinsic excitability through β -receptors in wild-type and ALS mice

Authors

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Abstract

Altered neuronal excitability and synaptic inputs to motoneurons are part of the pathophysiology of Amyotrophic Lateral Sclerosis. The cAMP/PKA pathway regulates both of them but therapeutic interventions at this level are limited by the lack of knowledge about suitable pharmacological entry points. Here we used transcriptomics on microdissected and *in situ* motoneurons to reveal the modulation of PKA-coupled receptorome in SOD1(G93A) ALS mice, vs WT, demonstrating the dysregulation of multiple PKA-coupled GPCR, in particular on vulnerable MN, and the relative sparing of β -adrenergic. *In vivo* MN electrophysiology showed that β 2/ β 3 agonists acutely increase excitability and input/output relationship demonstrating a non-canonical adrenergic neuromodulation mediated by β 2/ β 3 receptors both in WT and SOD1 mice. The excitability increase corresponds to the upregulation of immediate-early gene expression and dysregulation of ion channels transcriptome. However the β 2/ β 3 neuromodulation is submitted to a strong homeostasis, since a ten days delivery of β 2/ β 3 agonists results in an abolition of the excitability increase. The homeostatic response is largely caused by a substantial downregulation of PKA-coupled GPCR in MN from WT and SOD1 mice. Thus, β -adrenergic receptors are physiologically involved in the regulation of MN excitability and transcriptomics, but, intriguingly, a strong homeostatic response is triggered upon chronic pharmacologic intervention.

What about spontaneous neural activity in the embryonic spinal cord of the SOD1^{G93A} mouse model of amyotrophic lateral sclerosis?

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Amyotrophic lateral sclerosis (ALS) is typically diagnosed in adulthood, suggesting a primary impact on the mature central nervous system. However, a growing body of evidence indicates that ALS onset follows a long prodromal phase, shifting the origins of the disease to early developmental stages. Interestingly, embryonic developmental stages are characterized by the presence of spontaneous neural activity (SNA) leading to giant depolarizing potentials (GDPs). We know that SNA plays crucial role in various ontogenic processes, including axon path-finding decisions, regulation of synaptic strength and gene expression. Here, we aimed at investigating the generation of the SNA during embryonic development in the SOD1^{G93A} mouse model of ALS by using the whole-cell patch clamp recording of lumbar motoneurons (MNs). We focused on the critical embryonic days 13.5 (E13.5) and E14.5. Comparing SOD1^{G93A} mice with littermate wild-type (WT), we observed no changes in SNA at E13.5. However, notable alterations in SNA were observed at E14.5 in SOD1^{G93A} MNs, including delayed activity, decreased surface area, and amplitude of giant depolarizing potentials (GDPs). Importantly, these alterations were not attributed to the persistent sodium current (INaP), which is expressed in embryonic MNs and is known to be involved in the generation of the SNA. As SNA shapes synaptic development, we looked at synaptotagmin-2 and found a significant downregulation in SOD1^{G93A} lumbar spinal cords at E14.5, indicating a delay in the maturation of the synaptic transmission. In conclusion, our study points out a putative altered construction of the SOD1^{G93A} spinal motor networks and highlights the importance of investigating the early stages of ALS development.

Key words: SOD1^{G93A} ALS mouse model; spontaneous neural activity (SNA); embryonic development.

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Bistability in spinal motoneurons: New molecular insights and implications for motor control

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Pioneering studies from Copenhagen showed that spinal motoneurons (MNs) not only transmit central commands to muscles, but also shape motor outputs through nonlinear firing properties. One such property is bistability, whereby MNs switch between two stable states, silent and tonically active. This bistable behavior arises from a prolonged depolarization known as a “plateau potential”, usually preceded by a slow voltage transition. There has been a consensus that L-type Ca²⁺ channels mediate a large part of the plateau potential. We offer an alternative view where Ca²⁺ is not the main charge carrier for the plateau potential, but rather it triggers a Ca²⁺-sensitive Na⁺ conductance that sustains it (Bos et al., 2021). We identify the Ca²⁺-activated Na⁺-permeable *Trpm5* channels as the molecular basis for this current. We also pinpoint the persistent *Nav1.6* current, along with L-type Ca²⁺ current, as the gateway leading to self-sustained spiking (Drouillas et al. 2023). Silencing *Trpm5* or *Nav1.6* in lumbar MNs disrupts bistable behaviors and leads to hindlimb paresis. We also show that the slow voltage transition to the plateau is mainly mediated by a slow inactivation of a nifedipine-sensitive K⁺ current through *Kv1.2* channels, which plays a role in the windup of rhythmic motor outputs upon the initiation of locomotion (Bos et al., 2018). Finally, our latest research on genetically labeled α -MNs shows that larger ones, with the attributes of fast types, prominently display bistable properties (Harris-Warrick et al., 2024). Overall, these new findings have identified previously unknown molecular players involved in nonlinear properties of MNs and offer new insights into the mechanisms underlying motor behavior control.

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CHANGES OF RECTUS FEMORIS LENGTH INFLUENCE MOTOR UNIT DISCHARGE BEHAVIOUR OF VASTII MUSCLES

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The quadriceps muscle group works in a synergistic way performing knee extension. While vastus lateralis (VL), medialis (VM) and intermedius are monoarticular muscles crossing the knee joint, rectus femoris (RF) is a biarticular muscle involved in both knee and hip joint movements. Since the control of knee extension force output relies on the interaction between quadriceps mechanical properties and neural inputs to spinal motor neurons of these muscles, changes in length of one muscle within this synergistic group may directly influence the neural output of the other muscles. In this study, we investigated the effect of modifying the length of the RF muscle, by altering the hip joint position, on motor unit discharge rate of the synergistic VM and VL muscles. Four women and five men (31.6 ± 7.7 years, 70.3 ± 23.8 kg, and 1.74 ± 0.9 m) participated in this preliminary study. After participants were positioned on an isokinetic dynamometer, they were asked to perform three maximal voluntary contractions (MVC) of isometric knee extension with the hip joint positioned at 90° (seated) and at 180° (supine position). Subsequently, they performed two trapezoidal contractions for each hip joint position at 10% MVC. During submaximal contractions, high-density surface electromyography (HDsEMG) signals were recorded from VM, RF and VL muscles and decomposed into individual motor unit (MU) spike trains using a convolutive blind source separation algorithm. MUs were then tracked between hip joint positions. The mean discharge rate of matched MUs was calculated and compared using linear mixed models (LMM). Our results showed no statistical differences in peak MVC between hip joint positions (Wilcoxon-test; $p=1.00$). The number of decomposed MUs for VM were 5 ± 1 , from which 3 ± 1 were matched. For VL, 10 ± 8 were decomposed, from which 5 ± 8 were matched. Changing the hip joint angle from 90° to 180° (i.e., lengthening RF muscle) induced an increase in mean discharge rate of both VM (from 6.7 [5.28, 8.13] pps to 7.82 [6.39, 9.24] pps; LMM; $p=0.01$) and VL (from 7.69 [6.36, 9.01] pps to 8.51 [7.18, 9.83] pps; LMM; $p=0.01$). Our preliminary results suggest that to maintain the same force output between 90° and 180° hip joint angles, the neuromuscular system relies on a higher discharge rate of the synergistic VM and VL muscles, subsequently coping with the decreased force generation capacity of RF.

1 **Discharge characteristics of motor units in two hamstring** 2 **muscles during submaximal isometric contractions**

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12 Based on surface electromyographic (EMG) recordings, there are mixed findings on
13 the involvement of the hamstring muscles in knee flexion at different knee joint angles.
14 The purpose of our study was to explore the differences in motor unit (MU) discharge
15 characteristics between the Semitendinosus (ST) and Biceps femoris (BF) muscles.
16 Fifteen young healthy participants performed steady isometric contractions with the
17 knee flexors at four submaximal target forces [10, 20, 40, and 60% of the maximum
18 voluntary contraction (MVC)] at two knee joint angles (0° = full extension, and 90°) with
19 the hips fully extended. High-density EMG (HDsEMG) signals were recorded from ST
20 and the long head of BF muscles. HDsEMG signals were decomposed with a blind-
21 source separation approach to assess MU properties, including mean discharge rate
22 (MDR), coefficient of variation of interspike interval (CoV ISI) and standard deviation
23 of filtered cumulative spike train (SD fCST). To investigate whether the MUs in the two
24 muscles received significant levels of common synaptic input, we calculated the cross-
25 correlation between their smoothed discharge rates.

26 Linear mixed-effects models were performed to compare the MUs discharge
27 characteristics between the two muscles (ST and BF), knee angles (0° and 90°), and
28 target forces (10, 20, 40, and 60% MVC). In all models, muscle, knee angle, and target
29 force were specified as fixed factors with participants as a random factor. The MVC
30 force was greater at the 0° knee angle (long length) ($p = 0.001$), whereas the CoV for
31 force was greater at 90° ($p = 0.004$). The MDR ($p = 0.001$) and the variability in neural
32 drive (SD fCST) ($p = 0.003$) was greater for BF than ST. In contrast, the variability in
33 discharge times (CoV ISI) was greater for ST than BF ($p = 0.02$). The correlation
34 between the smoothed discharge rates between the MUs in ST and BF was relatively
35 low.

36 These findings indicate that BF exhibited greater MDR as well as greater variability in
37 neural drive compared with ST. Additionally, the low correlation of the smoothed
38 discharge rates suggests that these two muscles receive distinct neural drives. These
39 results are consistent with the differences in the morphological and mechanical
40 characteristics of the two muscles. Our results underscore the need to consider
41 individual muscle characteristics when addressing hamstring involvement in knee
42 flexion. This insight is crucial for designing targeted interventions and optimizing
43 clinical outcomes.

1 **Differential Responses in Rate of Force Development and Motor Unit Discharge**
2 **Characteristics During Unilateral and Bilateral Contractions of Dorsiflexors**

3
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9
10 **Abstract**

11 The bilateral limb deficit (BLD) describes the phenomenon where the maximal force
12 production during a bilateral contraction is lower compared to the sum of force
13 produced from both limbs during unilateral contractions. Previous studies suggest that
14 maximal force may differ between bilateral and unilateral conditions, but findings are
15 mixed. This study aimed to investigate the differences in rate of force development
16 (RFD) and motor unit (MU) discharge characteristics between unilateral and bilateral
17 contractions of the tibialis anterior (TA) muscle during submaximal explosive
18 contractions. Eighteen young adults performed maximal and submaximal explosive
19 isometric contractions at 80% of maximal voluntary contraction (MVC) with the
20 dorsiflexors of the right and left legs. Explosive force was calculated at different time
21 points (F50, F100, F150 & Fmax). High-density EMG (HDsEMG) signals were
22 recorded from TA and MUs discharge characteristics analyzed for both legs including
23 recruitment threshold (RT), max discharge rate (MDR), speed of recruitment (average
24 time for the recruitment of one MU) and neuromechanical delay (NMD, latency
25 between the neural drive to muscle and force). Linear mixed effects models were
26 employed to compare the MUs discharge characteristics between the two conditions
27 (unilateral & bilateral). Results revealed a significant $19.7 \pm 9.8\%$ (7 - 36%) bilateral
28 limb deficit during MVC. Additionally, a consistent reduction in explosive force and RFD
29 for bilateral contractions was also observed across all time points. In particular, the
30 BLD for the first 50, 100 and 150 ms was $21.3 \pm 10.3\%$, $21.4 \pm 13\%$ and $26.7 \pm 10\%$,
31 respectively. Further, the MDR was greater during bilateral contractions ($P < 0.05$).
32 Moreover, the NMD during bilateral contractions (-49 ± 13 ms) was longer compared
33 to the unilateral (-39.5 ± 12 ms) contractions ($P < 0.05$). Additionally, the MDR of MUs
34 was found to be correlated with force variables, underscoring the importance of motor

35 unit behavior in force production. The results suggest that a shorter NMD indicates a
36 larger spectrum of control, which enables to maximize the accuracy and control of
37 swifter movements. This study identified distinct MUs discharge characteristics
38 indicating specific neural mechanisms among bilateral and unilateral contractions
39 providing valuable insights into the neuromuscular mechanisms of BLD.

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TITLE

Directional tuning of human spinal motoneurons during standing balance

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ABSTRACT

Neural control of synergistic muscles is commonly thought to involve a shared synaptic drive to these motor pools. We demonstrate that the synergists within the human triceps surae can receive entirely opposite neural drives, enabling independent control of synergist muscles in a task-dependent manner. Ten healthy young adults participated in a series of dual and single-leg standing trials on force plates, during which high-density electromyography (EMG) was collected from the right soleus (SOL), medial gastrocnemius (MG), and lateral gastrocnemius (LG). Offline, the EMG data were decomposed into individual motor units and cleaned using a semi-automated algorithm. The center of pressure (COP) was calculated for each trial. A rotation matrix was iteratively applied to the 2-dimensional COP data, generating 360, 1-dimensional time series corresponding to COP movement about 360 degrees. Prominent peaks were identified in each COP time series. These peaks were averaged into the EMG data to calculate the amplitude of event-related EMG, thereby constructing EMG tuning curves. During two-legged standing, all active muscles of the triceps surae showed uniform EMG tuning curves, with maximal activity oriented primarily in the anteroposterior plane. However, during one-legged standing, significant deviations in the tuning curves were observed, with the LG showing a nearly orthogonal activation pattern compared to the SOL and MG. In particular, LG was maximally activated during eversion but inhibited during inversion, whereas SOL and MG were maximally activated during inversion but inhibited during eversion. These findings were confirmed with peristimulus frequencygrams generated from the decomposed motor unit data. These results demonstrate that, depending on the nature of the balance task, muscles of the triceps surae can contribute to corrective ankle torques outside of the sagittal plane. The muscles of the triceps surae act as a functional unit during bipedal standing but operate remarkably independently during unipedal standing. This independence allows muscles that normally function as synergists to act as antagonists, accommodating the biomechanical constraints of specific tasks.

Peroneii muscles are receiving shared common input during abduction of the foot

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The main function of peroneii muscles include mediolateral stability of the ankle (Louwerens et al., 1995) and prevention of sudden and involuntary ankle inversion (Konradsen et al., 1998; Sutherland, 2001). However, Peroneus Longus (PL) and Peroneus Brevis (PB) may have different roles as PL acts on plantar flexion from the first ray of the foot and ankle eversion, and PB acts on a total of 63% of the ankle eversion power (Koutsogiannis et al., 2022). Our study aimed to identify individual MUs from PL and PB to investigate their discharge characteristics and the presence of common input between the motor neuron pools of the two muscles. Sixteen active men volunteered to participate. The experimental procedure comprised isometric abduction of the foot at five target forces (5, 10, 20, 40 and 60% of MVC) during which high-density electromyographic (HD-EMG) recordings were obtained from PL and PB. Force fluctuations were quantified as Coefficient of Variation for force (CoV force) and MU discharge characteristics of PL and PB were analyzed by computing the Recruitment Threshold (RT), Mean Discharge Rate (MDR), Coefficient of Variation for InterSpike Interval (CoV ISI) and Standard Deviation of filtered Cumulative Spike Train (SD fCST). Additionally, the presence of common input between and within muscles was evaluated by performing cross correlation between smoothed discharge rates. Then, the ratio between significant to non-significant correlation coefficients were calculated for each target force. Linear mixed effects model was used to compare the two muscles at five target forces during abduction. In all models, muscles and target forces were specified as fixed factors, while participants were assigned as random factor. The RT was significantly greater for PL compared to PB and gradually increased with force ($p < 0.05$). For the MDR and the CoV ISI models, a significant main effect of target force was observed ($p < 0.05$). For the SD fCST, a significant main effect of muscle was revealed ($p < 0.05$) without any significant interaction. Correlation ratio within and between muscles were relatively high (> 0.60) across all target forces. Our results indicate that PL and PB are sharing high proportion of common input, but more drive is provided to PB relative to PL. The shared common input is probably associated with the role as main evertors and stabilizers of the foot on the sagittal plane.

Mechanisms of bistability in spinal motoneurons: a comparative analysis

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Spinal motoneurons represent the output elements of spinal circuitry providing activation of skeletal muscle to produce motor behaviors. The firing behavior of many motoneurons is characterized by bistability allowing them to maintain a self-sustained activity initiated by a brief activation and terminated by a brief inhibition. The underlying biophysical mechanisms likely involve nonlinear interactions of specific ionic channels. Experimental and modeling studies have identified the channels and corresponding ionic currents that can be involved in motoneuronal bistability, including the persistent sodium (I_{NaP}), Ca^{2+} -activated sodium (I_{CAN} in conjunction with high-voltage activated Ca^{2+} current, I_{CaL}), and potassium Kv1.2 (slowly inactivating A-type potassium, I_{KA}) currents. Interestingly, the bistability in motoneurons can be induced or amplified by serotonin, underlying its role in different motor behaviors. We have developed conductance-based mathematical models incorporating these currents to analyze conditions and characteristics of bistability. We show that each of the above currents can independently produce bistability within a special range of injected currents (or synaptic inputs), if the ratio of its maximal conductance to the leak conductance exceeds some threshold. The resulting bistable behaviors differ in their dynamics, including changes in spike amplitude, frequency, baseline membrane potential, and presence/magnitude of undershoot (post-spike hyperpolarization). Notably, existing literature supports the potential for each mechanism, either alone or in combination. We analyze these differences and compare them with the previously published recordings of motoneuron activity. Regarding the influence of serotonin, our simulations and previous experimental data suggest that serotonin can induce or amplify bistability via modulation (increasing) of I_{NaP} (e.g., through 5-HT₂ receptor activation), or I_{CAN} (e.g., via increased intracellular Ca^{2+} concentration due to an increased I_{CaL} or via 5-HT₃ receptor activation), or by reducing leak conductance, potentially involving all three mechanisms. Our analyses support each possibility, offering valuable insights into motoneuron bistability mechanisms and their potential roles in motor control.

Propriospinal V3 neuron innervation of motoneurons mediates posture and standing.

While considerable progress has been made in understanding the neuronal circuits that rhythmically drive motoneurons during locomotor behaviors like walking (CPG), less is known about the circuits that tonically drive motoneurons to provide adequate postural tone to stand and walk. Here we show that an excitatory propriospinal neuron population (V3 neuron, in Sim1⁺ mice) forms a large part of the total excitatory interneuron input to motoneurons (~20%) across all hindlimb muscles. In addition, V3 neurons make extensive connections among themselves and with other excitatory premotor neurons (such as V2a neurons). These circuits allow local activation of V3 neurons at just one segment (optogenetic activation at L2) to rapidly depolarize and amplify motoneuron output at all lumbar segments, in both the in vitro spinal cord and the awake adult mouse. Functionally, there is a strong extensor bias that enables a brief activation of V3 neurons to induce coordinated bilateral standing in awake intact or spinal cord injured mice. This can after injury overcome hindlimb paralysis to provide weight support during locomotion, whereas silencing these neurons impairs weight support. V3 neuron activation of standing occurs independently of walking; these neurons are not rhythmically active during locomotor activity (calcium imaging), can terminate ongoing walking in favor of standing, or can initiate a sit-to-stand posture that precipitates walking. In addition to their direct innervation of motoneurons, V3 neurons are ideally suited to maintaining posture because they possess large persistent inward sodium currents (Na PICs) that prolong their output, and receive key sensory and supraspinal input (vestibulospinal) needed to control posture. Thus, V3 neurons appear to act as command neurons that initiate postural activity, largely bypassing the CPG to robustly produce extensor postural tone and standing.

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Title: Neural mechanisms mediating lumbar locomotor-related V3 interneuron excitation of thoracic sympathetic preganglionic neurons and their autonomic targets

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Abstract:

Sympathetic preganglionic neurons (SPNs), located in intermediate laminae of T1-L2 spinal segments excite sympathetic tissues/organs that provide homeostatic and metabolic support during movement and exercise. We hypothesized and demonstrated that ascending lumbar V3 interneuron projections provide direct excitatory synaptic input onto thoracic SPNs throughout thoracic intermediate laminae. Optical stimulation (OS) of lumbar V3 interneurons in-vitro elicited action potentials in SPNs, demonstrating a functional connection between lumbar locomotor and thoracic sympathetic circuitry. We are now investigating neural mechanisms and pathways mediating V3 interneuron activation of thoracic SPNs and their autonomic target tissues/organs. We developed a neonatal in-vitro whole spinal cord (SC) preparation to enable simultaneous monitoring of lumbar and thoracic ventral roots, and thoracic sympathetic ganglia. Bath application of neurochemicals elicits rhythmic spinal sympathetic activity (~0.1 Hz) concomitantly with lumbar locomotor-like activity. Combinations of OS, lesioning and selective bath application of neurochemicals are ongoing to investigate neural mechanisms mediating this concomitant activation of spinal locomotor and sympathetic circuitry. In parallel, in the adult mouse in-vivo preparation, we are monitoring whole-body sympathetic responses during OS of lumbar V3 interneurons. Preliminary results demonstrate brief alterations in heart rate, depending on spinal depth and rostro-caudal site of stimulation in intact, anaesthetized mice. These preliminary findings support the need for further experiments to understand the capability and distribution of neurons within the SC integrating movement and autonomic functions. This will help develop SC stimulation strategies aimed at increasing excitatory drive for both motor and sympathetic functions, namely after spinal cord injury.

Possible role of propriospinal commissural (PSC of lamina VIII) neurons in postural control in cat?

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Little is known about the spinal circuits that control posture, even though they are necessary to initiate locomotion. In recent preliminary work, Bennett and colleagues have unexpectedly found that excitatory propriospinal commissural neurons (PSC neurons) that express the Sim1 transcription factor (V3 neurons) produce robust standing when they are optogenetically activated, including in mice that are paralyzed after spinal cord injury (SCI). These V3 neurons may be ideally suited to maintaining posture: directly innervating extensor motoneurons throughout the limb and axial muscles, and possessing large persistent inward sodium currents (Na PICs) that prolong their output. Thus, V3 neurons seem to essentially bypass the central pattern generator (CPG) to robustly produce extensor tone. The ventral V3 neurons in particular form descending commissural tracts that innervate motoneurons, much like the previously described lamina VIII neurons in cat, as detailed by Jankowska et al (PSC neurons). Thus, our goal of this work was to examine whether cat lamina VIII PSC neurons are likewise involved in postural control in the decerebrate cat preparation. In the feline preparation an intercollicular decerebration was performed. Intracellular electrodes were advanced into lamina VIII neurons and identified as descending PSC neurons by antidromic stimulation of the contralateral ventral PSC tracts one segment caudal. We found that these PSC neurons fired steadily, and a spike triggered average of the contralateral ventral root demonstrated that they monosynaptically contributed to the extensor motoneuron tone in this cat preparation. Furthermore, PSC neurons were monosynaptically excited by sensory stimulation (tibial or common peroneal) that produced a very long-lasting EPSP, with a remarkably similar long-time course to that observed in mice V3 neurons. Repeated sensory stimulation (at 20 – 100 Hz) evoked markedly increased steady PSC neuron firing and motoneuron output associated functionally with a crossed extensor reflex. Finally, PSC neurons have axons that appear to bifurcate: sending one axon locally to ipsilateral motoneurons and another across the midline, since antidromic activation of the contralateral axon evoked marked EPSPs in ipsilateral motoneurons; this provides a novel way to activate these PSC neurons that may be useful in promoting muscle tone. In summary, PSC neurons in cat fire spontaneously and provide extensive direct excitation to motoneurons, helping to support extensor tone, providing a new target to help restore posture after SCI.

Temporospatial organization of V3 subpopulations attributed to their complex functions in motor control

Ying Zhang

Interneuron (IN) circuits within the spinal cord play a critical role in orchestrating patterned, rhythmic, and adaptable motor control. These circuits exhibit remarkable heterogeneity, presumed to facilitate the coordination of intricate movement patterns amidst changing environments. Our recent investigation focuses on elucidating the diverse framework of excitatory V3 INs during developmental stages. We unveil that distinct identity of V3 subtypes is based on their timing of neurogenesis and their final laminar settling positions. We delineate early-born and late-born temporal subclasses, each further diversifying into spatially and molecularly discrete identities. Furthermore, our studies unveil unique behavioral recruitment patterns among temporally distinct V3 IN subtypes, underscoring their functional diversity.

While our previous research highlighted the crucial role of V3 INs in generating robust and stable gaits, recent findings uncover additional layers of their contributions to motor control. Specifically, we reveal how V3 INs aid in synchronizing diagonal fore-hind limb movements during trotting. Moreover, our most recent study demonstrates that V3 neurons establish broad excitatory inputs to motoneurons across all hindlimb muscles and form extensive connections among themselves and with other excitatory premotor neurons. These circuits enable localized activation of V3 neurons rapidly depolarizing and amplifying locomotor-related motoneuron output in both in vitro spinal cord and awake adult mice. Furthermore, V3 neurons are found to be essential for extensor activity during locomotion, as genetic silencing results in slower and weaker mice with reduced ability to increase force with locomotor intensity. This highlights another facet of V3 function: increasing the gain of motoneuron and premotor neuron output, thus serving as global command neurons that amplify locomotion intensity. Our current data start unveiling the significance of the temporospatial organization of V3 subpopulations in their complex functions within locomotor control.

Modulation of respiratory-related motoneuron output by spinal cholinergic interneurons

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Breathing must be readily adjusted to meet changing metabolic demands. It is well established that the rhythm generating circuits that control breathing reside within the brainstem; however, mounting evidence points toward roles for cervical spinal interneurons in adjusting respiratory output.

We used a combination of mouse genetics, immunohistochemistry, and electrophysiology to study spinal cholinergic modulation of breathing and interrogate underlying neural mechanisms. We focused on C boutons; large cholinergic modulatory synapses derived from Pitx2+ interneurons previously shown to facilitate locomotor-related motoneuron output in a task-dependent manner, via M2 muscarinic receptor signalling.

Anatomical studies, utilizing mice expressing a red fluorescent protein in Pitx2+ interneurons and intrapleural injections of cholera toxin B subunit to retrogradely label motoneurons, revealed that C bouton synapses are present on phrenic motoneurons, which innervate the diaphragm. Next, functional integration of Pitx2+ interneurons within respiratory circuits was demonstrated using whole-cell patch clamp recordings of genetically labelled Pitx2+ interneurons within brainstem-spinal cord preparations in which respiratory-related activity can be recorded from C3/4 ventral roots. Integration of Pitx2+ interneurons within respiratory circuits was evidenced by volleys of synaptic inputs and bursts of action potential output, which were tightly phase-locked to respiratory output recorded from ventral roots. The roles of Pitx2+ interneurons and C boutons within respiratory circuits were then revealed using pharmacological blockade of M2 receptors within brainstem-spinal cord preparations. M2 receptor blockade led to a reduction in the amplitude and an increase in the frequency of respiratory-related activity. The reduction in amplitude was reproduced when M2 receptors were selectively blocked in the cervical spinal cord. Given that brainstem-spinal cord preparations were obtained from neonatal mice, we also assessed whether the roles of cholinergic modulation persist into adulthood by using working heart-brainstem preparations obtained from adult rats. Blockade of M2 receptors in these preparations again caused a reduction in the amplitude of respiratory-related output recorded from phrenic nerves, confirming that cholinergic modulation of phrenic motoneurons is present in adulthood.

Taken together, these data demonstrate a role for spinally-derived cholinergic pathways, likely mediated by Pitx2+ interneurons and C boutons, in modulating and maintaining the respiratory output of phrenic motoneurons.

DEVELOPMENTAL CHANGES IN THE EXPRESSION AND MODULATION OF A PERSISTENT SUBTHRESHOLD POTASSIUM CURRENT IN LARVAL ZEBRAFISH PRIMARY MOTONEURONS

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Locomotion is invaluable to vertebrates. Crucially, animals undergo refinement of spinal circuits early in life to locomote effectively. Stereotyped transitions to progressively more refined locomotor behaviours are observed in larval zebrafish in the first few days of development. During this time, new neurons are being incorporated into existing circuits, new connections are being formed, and intrinsic properties of individual neurons are maturing. How changes in intrinsic properties of individual neurons during development of spinal networks may influence circuit-wide locomotor output is currently unknown in zebrafish. We have identified, for the first time, the persistent subthreshold potassium current I_M – a current with known involvement in dampening neuronal excitability – in larval zebrafish spinal circuits for locomotion. We demonstrate that primary motoneurons involved in fast and large amplitude movements express I_M differentially at 3 days post-fertilization compared to 4 days post-fertilization and older. Our patch-clamp electrophysiology data show that the amplitude of I_M in primary motoneurons is reduced by just over 50% by 4 days and nearly 70% by 5 days post-fertilization. We also find that I_M is differentially modulated at 3 days versus 5 days post-fertilization. Our data demonstrate that serotonin (20 μ M) hyperpolarizes the activation voltage of I_M at 4-5 days but not at 3 days post-fertilization. Furthermore, our behavioural data of tactile-evoked escape responses suggest that the expression of I_M may influence primary motoneuron recruitment for execution of the movement. We propose a role for I_M in primary motoneuron recruitment during the escape response that can be modulated by serotonin by the time larval zebrafish execute their most mature form of swimming by 4 days post-fertilization. Understanding how intrinsic properties of individual neurons change with the progression of movement execution throughout development is important to piece together fundamental mechanisms of operation of spinal circuits. How these circuits are modulated sheds light onto the mechanisms by which circuits appropriately adjust their locomotor output given the context – an essential feature of locomotor circuits across species.

Wednesday 19th

09:00	MN pathophysiology - Chair: F.Roselli
	J.Brandenburg
	M.Calon
	Y.Ma
	G.Sieck
	E.Reedich
10:30	Coffee / Posters
11:00	Plenary conference
	Frédéric Bretzner
11:45	rapid talks 2
12:15	
	Lunch / Posters
14:15	Motor units / sensorimotor - Chair: V. Rozand
	S.Garland
	G.Pearcey
	C.Calkins
	B.Kopicko
	V.Malejac
15:45	Coffee / Posters
16:15	ALS models - Chair: MD. Calabria Gallego
	S.Carracedo
	M.Baczyk
	Z.Melamed
	Y.Issa
	Z.Zhao
	T.Wells
	History conference
18:15	Daniel Zytnicki
19:00	Bus to Gala dinner

Title: Postnatal Motor Neuron Survival and Growth Contributes to Neuromotor Disorders

Authors: Joline E. Brandenburg^{1,2}, Matthew J. Fogarty³, Wen-Zhi Zhan³, and Gary C. Sieck³

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Abstract:

During embryonic and early postnatal developmental the number and size of motor neurons is established, which is crucial in determining the diversity in motor unit recruitment. Previously, in a mouse model of congenital hypertonia, *spa* mice, we showed that mature mice had significantly fewer phrenic motor neurons (PhMN) with these PhMNs having a significantly smaller somal surface area. In *spa* mice, which have homozygous insertion of LINE-1 in the Gly receptor β subunit gene, resulting in a 90% reduction of Gly receptors, the onset of spasticity symptoms (abnormal locomotion, muscle rigidity, myoclonic jerks, and exaggerated startle response) occurs at the 2nd to 4th postnatal week. It is not known whether the reduced number and altered morphology of PhMNs relates to the onset of spastic symptoms. We hypothesized that the reduced number and altered morphology of PhMNs evolves concomitantly with the onset of spasticity in the postnatal development of *spa* mice.

At postnatal day 21 (P21), P28, and maturity, *spa* (n=5/age) and WT (n=8/age) mice underwent unilateral retrograde labeling of PhMNs via tetramethylrhodamine phrenic nerve dip. After 24 h, mice were euthanized, perfused with 4% paraformaldehyde and the cervical spinal cord was excised and processed for longitudinal cryosectioning at 70 μm . The number of labeled PhMNs and measurements of somal surface area were obtained from 3D confocal microscopic images rendered using ImageJ. Statistical analysis consisted of 2-way ANOVA with Bonferroni *post-hoc* tests where appropriate. Results reported as mean \pm 95% CI.

There was no significant difference in number of PhMNs between WT and *spa* mice at P21. However, at P28 (WT: 202 \pm 13; *spa*: 166 \pm 14; $p=0.01$) and maturity (WT: 208 \pm 7; *spa*: 147 \pm 4; $p<0.001$), *spa* mice had 18% and 30% fewer PhMNs, respectively. Both age and genotype had significant effects on mean somal surface area of PhMNs. Compared to WT mice, PhMN somal surface area was significantly smaller in *spa* mice at P21 (WT: 1908 \pm 148 μm^2 ; *spa* 1394 \pm 132 μm^2 ; $p=0.002$) and maturity (WT: 2338 \pm 217 μm^2 ; *spa*: 1769 \pm 292 μm^2 ; $p=0.001$) with no difference at P28 (WT: 2028 \pm 223 μm^2 ; *spa*: 1791 \pm 34 μm^2 ; $p=0.84$).

In *spa* mice, significant differences in PhMN survival and morphology are evident in the postnatal period with these differences appearing to arise in the period when spastic symptoms occur. The combination of smaller PhMNs and reduced inhibition via impaired Gly signaling likely contribute to the spastic phenotype. This work provides early evidence that PhMN abnormalities in a mouse model of congenital hypertonia evolve during postnatal development, providing a developmental window for exploring disease modifying interventions.

Distal SMA-inducing mutations cause axonal transport defects in human motor neurons

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Axonal transport defects are observed in many neurological disorders including motor neuron diseases. Analyses in animal models showed that impaired axonal transport can cause neurodegeneration or impinge on the development of neural circuits. In support of such a role in humans, mutations in *DYNC1H1* encoding dynein heavy chain and its partner BICD2, two core components of the intracellular transport machinery, cause a rare neurodevelopmental motor neuron (MN) disease termed Spinal Muscular Atrophy with Lower Extremity Dominance (SMALED) in which limb-innervating MNs are preponderantly affected. This suggests a graded dependency on intracellular transport among neuronal subtypes and that MN, and even specific MN subtypes, are the most vulnerable cells to defects in this process. To address the mechanisms underlying MN degeneration in SMALED, we have generated human induced Pluripotent Stem Cells lines (hiPSCs) from SMALED patient cells carrying mutations in BICD2 or DYNEIN and differentiated them into MNs including limb-innervating-like MNs. Using new optimized conditions for human MN culture, we showed that SMALED mutations do not impact on MN specification and early survival. However, live imaging of vesicular cargoes indicates a decrease in the speed of their axonal transport. Data in animal models show that the development of specific limb-controlling motor circuits and MN survival critically depends on the retrograde axonal transport of signals induced by target derived neurotrophic factors. In agreement with the reduced speed of axonal cargo transport, our preliminary results using MN culture in microfluidic chambers indicate that neurotrophic factor retrograde signaling might be impaired in SMALED MNs. Overall, our results suggest that mutations in core components of the transport machinery impair the axonal transport of cargoes with possible consequences on neurotrophic factor-retrograde signaling, a key pathway for limb motor circuit development and maintenance.

Single-nuclei RNA sequencing reveals novel functional network contributing to motor neuron degeneration in spinal muscular atrophy

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Motor neuron degeneration in spinal muscular atrophy (SMA) makes it the number genetic killer of toddlers, affecting one in every 6000 live birth. SMA is caused by mutations in *SMN1* (*survival motor neuron 1*) gene, leading to reduced SMN protein level and selective motor neuron degeneration. Better understanding of mechanisms underlying motor neuron degeneration in SMA is needed.

To study the comprehensive landscape of transcriptome alternations occurring at the single cell level in the SMA spinal cord, we performed single-nuclei RNA sequencing on the lumbar region spinal cords of pre-symptomatic mice of the most widely used SMA mouse model. The Delta7 SMA mice have been demonstrated to closely recapitulate every aspect of SMA pathogenesis, from the genetic mutation, to motor neuron degeneration, motor behavioral defects, and reduced life span. We choose the pre-symptomatic stage to distinguish transcriptome changes that occurs earlier than and leads to SMA pathogenesis. We prepared lumbar spinal cord samples from 3 male mice including one WT, one Het, and one KO (SMA), as well as two females including one WT and one KO mice. We sequenced ~10,000-12,500 cells per sample, with ~30,000 reads per cell. We identified and clustered nervous system cell types, primarily including neurons, microglia, astrocytes, fibroblasts, oligodendrocytes and macrophages. We determined cell type specific differentially expressed genes between SMA disease vs. non-diseased (KO vs WT) samples. We investigated critical cell-cell interactions and receptor-ligand interactions that may contribute to SMA pathogenesis using CellChat. In addition, we studied the effects of gender and SMN heterozygosity on gene expression at the single cell level in different cell types.

We identified novel functional network regulating translation, neuroinflammation and mitochondrial defects that may lead to selective motor neuron degeneration in SMA. This is different from the traditional role of SMN in snRNP biogenesis and RNA splicing. We also revealed the unexpected roles of fibroblasts and microglia in the novel functional network in SMA pathogenesis, as well as the effects of gender and SMN expression levels on transcriptome alternations occurring at the single cell level. Findings from this study may also provide insights for the understanding of ALS pathogenesis.

Age-Related Phrenic Motor Neuron Loss Due to Reduced BDNF/TrkB Signaling in Rats

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Brain derived neurotrophic factor (BDNF) signaling through its high-affinity tropomyosin receptor kinase B (TrkB.FL) is known to have potent effects on motor neuron survival during embryonic and early postnatal development, and dysfunctional signaling may play a role in neurodegenerative diseases. However, the specific effect of reduced BDNF/TrkB.FL signaling on motor neuron survival in adults is not well established. In older rats (i.e., 24-month) we found a loss of ~30% of phrenic motor neurons (PhMNs), especially larger PhMNs. We also found that the facilitating effect of BDNF/TrkB.FL signaling on neuromuscular transmission was markedly dampened in older rats. Based on these findings, we hypothesized that BDNF/TrkB.FL signaling is reduced in older animals resulting in PhMN loss. In this study, we employed a novel TrkB^{F616} rat model with a 1NMPP1 sensitive knock-in allele that in the presence of 1NMPP1 inhibits TrkB.FL kinase activity. In initial experiments using young (3-month; female and male) TrkB^{F616} rats, we showed that BDNF/TrkB.FL signaling induces CREB phosphorylation at serine 133 (pCREB^{S133}) that was blocked by 1NMPP1 added to drinking water. In a second series of studies in young TrkB^{F616} rats, PhMNs were labeled by intrapleural injection of Alexa-Fluor647 cholera toxin B (CTB). After 14 days of 1NMPP1 or vehicle treatment, labeled PhMNs were imaged in 3D using confocal microscopy. We found that 14 days of 1NMPP1-induced TrkB.FL kinase inhibition reduced the total number of PhMNs by ~20% with an ~25% reduction in mean PhMN somal surface area and an ~38% reduction in PhMN dendritic surface area. In a third series of studies, we confirmed that there is an ~30% loss of PhMNs in older (24-month) rats. We also found that in older rats, intrathecal BDNF treatment did not induce pCREB^{S133} phosphorylation. Based on these results, we conclude that 1NMPP1 induced inhibition of BDNF/TrkB.FL signaling in TrkB^{F616} rats leads to PhMN loss. We further conclude that BDNF/TrkB.FL signaling is reduced in old age due to a loss of TrkB.FL kinase activity – not BDNF availability, and that this underlies age-related PhMN loss.

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Motor unit development in a rabbit model of cerebral palsy

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Cerebral palsy (CP) is the most common motor disability in children, occurring in 1:500 live births. Symptoms of CP include hyperreflexia, hypertonia, muscle weakness, and fatigue. The mechanisms through which CP-causative injuries like hypoxia-ischemia (HI) cause motor deficits remain unresolved. However, motor unit (MU) development occurs in the perinatal period when CP-causative injuries occur, and depends on spinal motoneuron (MN) activity, which is increased in the HI rabbit model of CP partly through enhanced serotonergic neuromodulation. We are testing the hypothesis that prenatal HI injury alters serotonin receptor composition in MNs, dysregulates neuromuscular junction (NMJ) maturation, and disrupts the development of MU physiological types (S, slow; FR, fast fatigue-resistant; and FF, fast fatigable). To test our hypothesis, we are using immunofluorescence to quantify serotonin receptor expression in MNs and to track the emergence of mono-innervation at the NMJ, an anatomical hallmark of maturity. We are recording single MUs *in vivo* in anesthetized sham-operated control and HI rabbits using the split ventral root method, and characterizing MU contractile properties throughout the early postnatal period. Measuring muscle fiber type composition provides further insight into muscle force-generating capacity and fatigability. We are using immunostaining to label type I, IIa, IIx, and IIb myofibers and are evaluating differences in fiber type distributions of sham-operated control and HI rabbit muscles. We find that serotonin receptor expression is different in MNs from neonatal HI rabbits compared to age-matched sham-operated controls and our preliminary data suggests that NMJs undergo delayed maturation in HI rabbit skeletal muscle. The impact of prenatal HI injury on MU electrophysiology will be presented, and our preliminary analysis indicates that in the third postnatal week when poly-neuronal innervation is eliminated, HI skeletal muscle has a slower, weaker, and less fatigable fiber type profile than that of typically developing rabbits. A slower muscle fiber type profile in HI muscle may reflect chronic, low frequency MU activity consistent with CP. Overall, this project elucidates whether aberrant MU development and electrophysiology after prenatal HI injury contribute to motor dysfunction in the rabbit model of CP.

Genetic dissection of brainstem circuits pertaining to control and recovery of locomotion after spinal cord injury

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Spinal cord injury (SCI) disrupts the descending command from the brain and causes a range of motor and locomotor deficits. Combining detailed kinematics and electromyographic recordings in freely behaving mice with optogenetic manipulation, we investigated changes in reticulospinal efficacy during spontaneous motor recovery from SCI. We found that sites evoking stronger excitatory motor responses in intact conditions were the most impaired after injury, whereas those associated with weaker motor responses were potentiated, thus supporting plasticity. We also tested whether stimulation of these neuronal populations can boost motor recovery after chronic SCI. Whereas long trains of photostimulation delivered above glutamatergic neurons located in the most ventral nuclei of the medullary reticular formation initiated and accelerated locomotion after chronic SCI, those of glutamatergic neurons located in the more dorsal medulla stopped locomotion. Moreover, long trains of photostimulation also improved stepping ability and foot clearance of chronically impaired SCI mice during treadmill locomotion. As a therapeutical approach, we also showed that tonic activation of glutamatergic neurons of the medullary reticular formation for priming the descending drive improved skilled locomotor control after chronic SCI on a horizontal ladder. Taken together, these results highlight the resilience and capacity for reorganization of the glutamatergic reticulospinal command of the medullary reticular formation after SCI.

Maintenance of the upright standing posture is achieved by activation of populations of motor units in the ankle plantarflexor muscles. While motor units have been recorded during quiet stance and in the steady state phase after a perturbation, little is known about the behaviour of motor units during the immediate response to a balance perturbation. Advances in High Density Surface Electromyography (HD-sEMG) decomposition techniques have made it possible to extract motor units from brief synchronous activation. Participants stood in a comfortable stance with a foot on a separate force platform. Perturbations were elicited using an external load of 3% body mass in four different directions; 30°, 60°, 90°(anterior) and 120°, counter-clockwise from 0° being directly to the right, The load was attached to a belt secured around the participant's pelvis and was dropped into a basket via a cable-pulley mechanism. Two types of load drops were performed: 1) the load was dropped and remained in the basket for 10 -15s (performed twice), 2) the load was dropped and immediately removed from the basket (repeated 38 times) and spaced randomly (4 to 10 s apart) to reduce anticipation. With the participant secured in a standing frame, 5 isometric plantarflexion ramp and hold contractions with 5% MVC/s ascending ramp, 10 s holding and amplitude of 10 to 50% MVC were performed in standing. HD-sEMG signals were recorded from the right medial gastrocnemius (MG) and soleus (SOL) muscles using 64-channel grids in monopolar mode. HD-sEMG was decomposed with the blind source separation method (Holobar and Zazula, 2007) implemented in the DEMUSE tool software. The MU decomposition filters were identified from the isometric contractions and the post-perturbation holds and afterwards, applied to the EMG response following load drop perturbations. Eight healthy adults (2 female and 6 male) participated in the study. Between 10 and 30 MUs per contraction were identified in decomposition filters. When filters based on isometric contractions were applied to EMG response during load drop perturbations, not more than 10% of the MUs were found in the brief synchronous bursts. These MUs were predominantly low threshold (below 10% MVC and not exceeding 20% MVC). The filters based on the load holding were more successful, with approximately 70% of the MUs found to be active in the bursts, although not in all of them. This suggests that different sets of MUs are activated during postural tasks and isometric contractions performed in the same standing posture.

Identifying motor unit recruitment in fast burst contractions in plantarflexor muscles during dynamic standing perturbations.

S Jayne Garland, Tanya D Ivanova, Ales Holobar

Ischaemic blockade of large diameter afferents alters human motoneuron function

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Large diameter afferents facilitate homonymous motoneuron discharge and inhibit antagonist motoneurons (i.e., reciprocal inhibition). When blood flow occlusion is sustained, efferent transmission can be maintained for a short period of time after large diameter afferent transmission is abolished. We tested the hypotheses that reduced afferent transmission would 1) reduce motor unit (MU) discharge rates and impair force control; and 2) increase contribution of PICs to MU discharge. In all experiments, test contractions were performed whilst myoelectric activity (EMG) of the tibialis anterior was measured using high-density EMG arrays and decomposed into individual MU spike trains. Following the pre-test contractions, a sphygmomanometer cuff was inflated to 200 mmHg just above the knee to induce an ischaemic nerve block. Soleus H-reflexes were monitored to determine when Ia afferent transmission was diminished, after which test contractions were repeated whilst occlusion was maintained. In separate experiments, test contractions included: maximal isometric dorsiflexion (MVC); triangular isometric ramp contractions to 30% of the pre-occlusion MVC; triangular isometric ramp contractions to 30% of MVC of the corresponding timepoint; and one-minute isometric contraction to 10% of the pre-occlusion MVC. Blood flow occlusion did not alter maximal M-wave amplitudes, whereas H-reflex was abolished, indicating reduced Ia afferent transmission. Ischaemic blockade of afferent transmission 1) reduced MVC force to ~50% of pre-occlusion, 2) increased peak MU discharge rate, onset-offset hysteresis, and non-linearity of discharge rate during triangular ramp contractions performed to the same absolute force level, 3) increased onset-offset hysteresis during triangular ramp contractions performed to the same relative force level but had no effects on peak MU discharge rate and non-linearity of discharge rate increase, and 4) increased force fluctuations by 2-fold during low force sustained contractions. Taken together, the results support our hypotheses and suggest that Ia afferent feedback is important for the maintenance of maximal force output, the control of submaximal force output, and constraining persistent inward current behaviour. These findings provide insight into the importance of large diameter afferent input for normal motor behaviour and may help explain motor impairments in conditions with peripheral neuropathies.

The Effect of Visual Feedback on Motor Unit Properties in Young Adults

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Torque control improves and motor unit discharge variability increases with high compared to low visual gain in young adults. However, the influence of biological sex on torque control and motor unit firing properties during contractions with high and low visual gain are unknown. The purpose of this study was to examine if manipulating visual gain by a factor of ten would influence torque steadiness and motor unit firing properties between the sexes. It was hypothesized that: 1) high visual gain would result in greater torque steadiness, and 2) there would be increased motor unit firing rate variability and reduced oscillations in common synaptic input with high, compared to low, visual gain. 20 Young (10 female, 23.5 +/-4.37 years old) participants performed isometric ankle dorsiflexion for twenty seconds at 10% and 25% of their maximal voluntary contraction with low and high visual gain, differing by a factor of 10, and trials were completed in a randomized order. Sixteen seconds of the torque plateaus were analyzed for torque steadiness to avoid the adjustment from increasing and decreasing torque at the beginning and end, respectively of a constant submaximal contraction. There were no significant findings on the effect of sex ($p=0.29$), gain ($p=0.24$) or contraction intensity ($p=0.12$) on torque steadiness. Therefore, there is no evidence for the influence biological sex, gain or contraction intensity on torque control. However, there is ongoing analysis of motor units decomposed from high-density surface electromyograms to determine if smoothed firing rates, variability in motor unit interspike intervals and common synaptic input, and torque signal complexity that will be presented at the conference.

Motor unit discharge in response to multi-directional forcefields in the human upper extremity

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Muscles, and the motor units that comprise them, are directionally tuned, able to produce optimal force in a particular direction. It is not entirely clear if the CNS activates these motor units following this same manner. Here we examine the effect of imposed multidirectional forces on upper extremity muscles in nine healthy human participants. Using a programmable robotic endpoint system, we quantify upper extremity muscle activation while provided with either ramp or hold forcefields in one of eight directions. During these tasks, participants were provided real time feedback of their hand position and a stable target in which they were able to maintain the position. High density electromyography was collected from the long head triceps (TriLong), the long head of the biceps (Bic), and the brachioradialis (BR). Our initial findings reveal that TriLong motor units are activated only with varying degrees of elbow extension loads, whereas Bic and BR motor units are activated with both elbow extension and flexion loads. Loads orthogonal to the elbow flexion/extension produced minimal activation of TriLong, Bic, and BR. These data suggest TriLong motor units are directionally tuned to a single direction, whereas Bic and BR motor units are tuned in multiple directions: agonist activation and coactivation. These data provide new insight into the activation of upper extremity motor units outside of traditional force-controlled task. Additionally, this framework will provide new avenues of investigation of motor unit excitability with involuntary coactivation in addition to effort as applied through orthogonal forcefields.

Effect of motor imagery training on motor unit recruitment

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Motor imagery (MI) is the mental simulation of an action without concomitant production of movement (Jeannerod, 1994). Similarly to physical practice, MI training can improve muscle strength (e.g., Yue & Cole, 1992 ; Zijdwind et al., 2003) through neural adaptations. Common hypotheses propose that MI could increase the discharge rate of the motor units and decrease the recruitment threshold, as after physical training (Del Vecchio et al., 2019). The aim of the present study was to evaluate strength gains and associated potential changes in motor units discharge rate (DR), recruitment threshold (RT) and derecruitment threshold (DT) induced by MI training of ankle dorsiflexors.

Eleven young subjects performed a 4-week MI training including 5 sessions per week. Maximal voluntary contraction (MVC) of the dorsiflexor muscles was assessed before and after the training intervention. Participants performed trapezoidal contractions (rate of increase: 5% MVC.s⁻¹; plateau: 10 s) at 35%, 50% and 70% MVC to assess motor units DR, RT and DT using high-density EMG on the tibialis anterior muscle. DR was assessed at recruitment (first four action potentials), on the plateau (first 10 action potentials) and at derecruitment (last four action potentials). RT and DT were considered as the absolute force at which the motor units were recruited and derecruited, respectively.

Following MI training, MVC increased significantly by $5.7 \pm 9.7\%$ ($p = 0.025$). A total of 720 motor units were identified across all subjects and testing sessions (pre- and post-intervention). Mean DR on the plateau remained unchanged after training ($p=0.16$), as well as mean DR during the recruitment phase ($p=0.81$) and derecruitment phase ($p=0.44$). RT and DT were not modified by the training intervention ($p=0.76$ and $p=0.78$, respectively).

A 4-week MI training intervention increased maximal strength production capacity of the ankle dorsiflexors. However, this improvement in maximal strength following MI training was not due to changes in DR, RT or DT, in contrast to what has been recently demonstrated after physical training (Del Vecchio et al., 2019). These results suggest that the motor command during MI

may be inhibited before attaining the motoneurons or may be insufficient to induce changes at the motor units level. Further investigations are needed to test potential changes in motor units behavior during maximal contractions.

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TITLE

Dual roles of neuronal and microglial P2X4 receptor in ALS

AUTHORS

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Abstract

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by a selective loss of motor neurons (MN). P2X4 receptor, which is a non-selective cationic channel activated by ATP has been recently involved in ALS. Previous studies found that both the absence of P2X4 or the expression of non-internalized P2X4KI have a beneficial outcome in the SOD1 mice. This paradoxical output suggests a complex cell-role of P2X4 in ALS, so far unexplored.

In order to address the neuroglial role of P2X4 in ALS, we have developed novel SOD1 mice, expressing either an increase on surface P2X4 or a blocking of P2X4 gene selectively in microglia/macrophages or neurons. We have found a dual role of this receptor. While the neuronal increase of P2X4 has detrimental effects on motor performance and MNs survival, a beneficial role of surface P2X4 is observed in myeloid cells. We are currently studying morphological and functional changes in microglia from these lines which may support neuronal viability through ALS progression.

Altogether, dual roles of P2X4 may be involved on the neuroimmune crosstalk occurring during ALS. Further studies need to be carried in order to study the contribution of P2X4 in the interplay between MN death and microglia reactivity over the disease progression, providing valuable insights into the cellular role of P2X4 to fight ALS.

TITLE

Functional and structural recovery of proprioceptive Ia afferent inputs to spinal MNs by chronic anodal tsDCS does not change disease dynamics in SOD1 G93A mouse model of Amyotrophic Lateral Sclerosis

AUTHORS

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ABSTRACT

We have previously identified trans-spinal direct current stimulation (tsDCS) as a potent tool to modify spinal MN intrinsic excitability and synaptic excitation levels in SOD1 G93A (SOD1) mouse model of ALS. Here we report structural and functional alterations to proprioceptive Ia afferent inputs to spinal MNs in SOD1 animals, as a result of the 2-week tsDCS protocol. Polarization was applied to p35-p40 animals for 2 weeks under isoflurane anaesthesia. During each polarization session, SOD1 mice were subjected to 100 uA anodal, cathodal or sham-control polarization for 15 min. The electrodes were arranged ventro-dorsally, with an active electrode (silver plate 5 × 10 mm) placed on the skin over Th12-Th13 vertebra, and a reference electrode (crocodile clip) located on the skin flap ventral to the active electrode. One day after the last polarization session an *in vivo* electrophysiological experiment was performed to assess the impact of chronic tsDCS on monosynaptic Ia EPSPs and intrinsic membrane properties of spinal MNs. In a subsequent group of animals subjected to the same tsDCS regime, the spinal cords were harvested for immunohistochemical analysis. We have found a significant increase (by 43%) in Ia EPSP amplitudes following anodal tsDCS, while no significant effect was found after cathodal polarization. The increase in Ia EPSPs was accompanied by a strong 10% increase in the Vglut1 fluorescence signal in the anodal group and an opposite decrease of the signal in the cathodal polarization group. Moreover, the GLuR4 subunit of the AMPA receptor, which was previously found to be decreased in SOD1 animals, was significantly recovered following anodal tsDCS. No significant changes were seen after cathodal tsDCS. Finally, the misfolded SOD1 levels were significantly decreased by anodal tsDCS, indicating a potential therapeutic effect of our intervention. Encouraged by these results, we applied life-long tsDCS to p35 SOD1 animals, and we investigated mouse functional parameters and survival during disease development. Unfortunately, tsDCS did not significantly alter disease dynamics. This study was funded by an NCN grant 2017/26/D/NZ7/00728

International MotoNeuron Meeting

Mechanism of Stathmin-2 loss and its correction to restore motor neuron function in ALS

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Abstract

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by premature loss of upper and lower motor neurons, leading to progressive, fatal paralysis and respiratory failure. ALS is associated with cytoplasmic aggregation and nuclear clearance of the RNA-binding protein TDP-43. We recently identified a critical role for TDP-43 in regulating the mRNA encoding stathmin-2 (STMN2), a neuronal microtubule-associated protein essential for axon stability and regeneration. Reduction of nuclear TDP-43 leads to suppression of stathmin-2 levels by uncovering cryptic splice and polyadenylation sites in stathmin-2 pre-mRNA, producing a truncated non-functional RNA. Stathmin-2 emerges as one of the most abundantly expressed genes in motor neurons (MNs), whereas its expression in ALS patients' motor neurons is critically suppressed. Unraveling stathmin-2 function and developing strategies to rescue its levels in affected motor neurons hold significant promise.

Here, we identify the mechanisms through which TDP-43 sustains normal stathmin-2 pre-mRNA processing and used those insights to develop methods to restore stathmin-2 synthesis in motor neurons affected by TDP-43 dysfunction. We screened and identified antisense oligonucleotides (ASOs) that block cryptic splicing and restore normal stathmin-2 pre-mRNA processing, despite reduced TDP-43 level. In iPSC-derived motor neurons, utilizing ASOs restored stathmin-2 level and rescued impaired axonal regeneration capacity after injury when TDP-43 was depleted, evidence supporting stathmin-2 as a potential therapeutic target. We then established that late-onset suppression of stathmin-2 in adult mice is sufficient to produce progressive ALS-like motor phenotypes, including: *i*) muscle denervation, *ii*) axonal collapse sufficient to rip the myelin layers, *iii*) shrinkage in the neurofilament spacing that defines axonal caliber and *iv*) a corresponding collection of dysfunctional motor behavior. Notably, in mice gene-edited to contain the human-specific stathmin-2 cryptic exon, we showed that ASO injection into cerebral spinal fluid is a viable approach to rescue stathmin-2 levels in TDP-43 proteinopathies including ALS.

IDENTIFICATION OF SPECIFIC MOLECULAR MARKERS OF ALS VULNERABLE MOTONEURONS

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Abstract:

Modification of electrical activity of motoneurons is a key factor in amyotrophic lateral sclerosis (ALS) disease progression. Experimental evidence revealed a motoneuron-type vulnerability in ALS, beginning with the low excitability fast fatigable (FF) motoneurons, while the high excitability slow (S) motoneurons are preserved. These observations have led to the hypothesis that the high task demand of the FF motoneurons is responsible for their highest vulnerability.

To broaden our understanding of the role of excitability in the selective degeneration and to improve the functional characterization of motoneurons types, we used the patch-seq method on motoneurons subtypes FF and S identified by patch-clamp electrophysiology [1] [2].

The expression of voltage gated channels was analyzed in six FF motoneurons RNA banks and six S motoneurons RNA banks. The results led us to identify several potential markers of these populations (six out of forty genes involved in action potential). Among the differentially expressed genes, *Cacna2d3*, a gene coding for $Ca_v\alpha 2\delta 3$, a regulatory subunit of high voltage activated calcium channels was selected. This subunit was significantly increased in the FF motoneurons. Our preliminary data of immunofluorescence confirmed its expression in the soma and proximal dendrites of adult spinal motoneurons, as well as at the neuromuscular junction. The functional significance of *Cacna2d3* in neurotransmission and firing properties of motoneurons will be addressed using Knock-Out mice *Cacna2d3*^{-/-} as well as its impact on ALS progression.

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Keywords:

Motoneuron vulnerability, Calcium channel, Electrical activity, Amyotrophic Lateral Sclerosis

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Aging enhances spinal motoneurone excitability in C9orf72 (C9-500) BAC mice

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Repeat expansions in the C9orf72 gene (C9orf72 RE) are the most common mutations found in both familial and sporadic Amyotrophic Lateral Sclerosis (ALS). *In vitro* recordings from iPSC cells derived from C9orf72 ALS patients have demonstrated an early increase followed by a later decrease in motoneurone excitability suggesting that neurones “crash and burn” in this disease. However, these iPSC cells represent embryonic-stage neurones so it is difficult to extrapolate to any kind of disease stage from this. Additionally, the mutant cells may simply be more vulnerable to *in vitro* conditions. Our previous work investigating motoneurone excitability *in vivo* in mouse models with C9orf72 repeat expansion found relatively normal excitability at around 250 days when mild motor symptoms first appear.

As aging is the biggest risk factor for ALS, including in C9orf72 RE carriers, we hypothesized that excitability might be impaired with later aging. We therefore performed a pilot study to investigate the effects of C9orf72 REs in aged female mice. *In vivo* intracellular recordings from spinal motoneurons were performed in 600+ day old FVB/NJ-Tg(C9orf72)^{500Lpwr}/J mice and results compared to age matched- wild type littermates. We selected mice for this study that had not shown a strong ALS (or FTD) phenotype at around 250 days and thus could be aged until 600+ days, although tail suspension tests revealed that most C9orf72 RE mice exhibited a clasping phenotype at this age. Surprisingly, at this age, rather than decreased excitability, we found a significant increase in the I-f gain and a trend towards a reduction in rheobase in the aged C9orf72 mice compared to age matched- wild type littermates. This is similar to the excitability profile that we have observed in other ALS mouse models suggesting that advanced aging is necessary to evoke hyperexcitability in this model. Our results thus confirm that hypoexcitability is not observed in motoneurons carrying C9orf72 REs when tested under *in vivo* conditions, even in very old mice.

TITLE

Interactions Between the Cholinergic and Serotonergic Motor Modulatory Systems in Health and in Amyotrophic Lateral Sclerosis

AUTHORS

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ABSTRACT

Motor neuron modulation is achieved by regulating motor neuron output and their sensitivity to neural inputs. This allows for the precise and adaptive control of movement. Understanding the interactions and contributions of diverse motor-modulatory systems is crucial for understanding motor control. Two major questions are still unanswered as to how motor modulation is achieved: Why are multiple modulatory systems necessary for regulating movement, and how do these different systems interact with one another? Here, we sought to answer these questions by investigating the interactions of two systems: the local-spinal V0c interneurons, which regulate motor neuron firing, and the descending-brainstem serotonergic system, which regulates the intrinsic excitability of motor neurons. Using genetic manipulations and measurements of neuronal activity in mice, we demonstrate the task-dependent recruitment of these populations. Notably, the collaboration of both populations is necessary for driving high motor neuron output, such as during high-intensity locomotor tasks, or in instances of significant motor neuron loss, such as in mSOD1^{G93A} model Amyotrophic Lateral Sclerosis mice. Our results identify the collaborative function of two anatomically and functionally distinct systems that modulate motor neuron activity to control motor behavior in health and in motor neuron-related disorders.

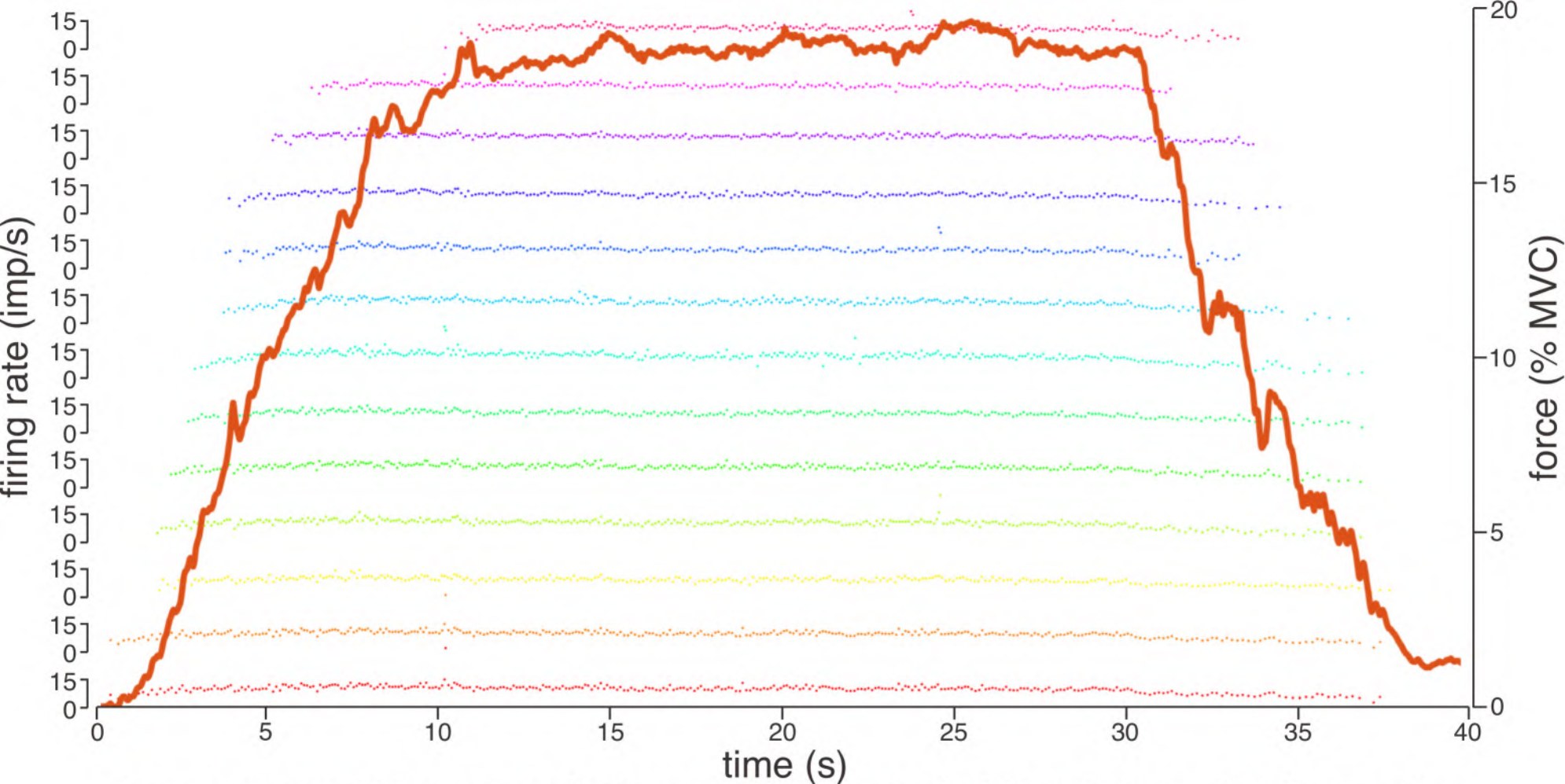
Thursday 20th

08:30	Motor units / MNs - Chair: I. Rybak
	A.Fuglevand
	A.Pascual-Valdunciel
	R.Mesquita
	N.Place
	V.Goreau
	A.Benedetto
10:15	Coffee / Posters
10:30	Plenary conference
	Véronique Marchand-Pauvert
11:15	Spinal cord injury - Chair: K.Quinlan
	M.Zaback
	A.Adewuyi
	O.Gajewska-Wozniak
12:15	Lunch box distribution
12:45	Bus to St-Emilion

Inexpensive, Easy-To-Fabricate, Reusable Intramuscular Multielectrode Arrays for Recording Motor Unit Activity

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The development of high-density surface electrode arrays in the early 2000s significantly advanced the study of motor unit (MU) physiology. Those advances were paralleled by the creation of sophisticated computational algorithms needed to extract spiking times of individual MUs from large numbers of surface-detected signals. The multiple 'viewpoints' of MU potentials offered by these arrays enabled reliable identification of large numbers of MUs during strong contractions. Yet, there remain some limitations with this approach. As with any surface electrode system, signal-to-noise ratio is smaller compared to that of intramuscular electrodes. Also, such arrays are susceptible to crosstalk – making it difficult in some situations to ascribe identified MUs to particular muscles. Furthermore, surface arrays cannot be readily used to record MU activity from deep muscles. And finally, such arrays are not easily deployed to record MUs in diminutive muscles like that found in small experimental animals. To address these limitations, recent efforts have been directed toward developing high-density, intramuscular microelectrode arrays (IMAs). These efforts have been successful. For example, Muceli et al. (*J Physiol* 2015) was able to track the activities of up to 50 MUs from human tibialis anterior using a thin-film IMA with 16 contacts. The drawbacks of these IMAs are that they require sophisticated microfabrication techniques, they are relatively expensive to build (or buy), and are somewhat delicate. To overcome those drawbacks, we have developed easy to fabricate, robust, reusable, inexpensive IMAs with up to 16 electrodes. In brief, we fabricate them using long lengths of 25, 50, or 75- μ m diameter wire that is repeatedly folded over a suspension bar so that there are 8 or 16 equal length wire segments hanging vertically from the bar. A small metal clip is attached to the base of the wires and the wires twisted into a bundle using a magnetic stirrer. Once twisted, a heat gun is used to gently fuse the insulation of the wires together to prevent unraveling. The bundle is then fed through the cannula of a 21 or 23-gauge needle with the electrode tips folded over to form a hook. Each wire in the hook is recut to slightly different lengths (\sim 1 - 2 mm interelectrode spacing). The other ends are denuded of insulation using emery paper. Once the sterilized IMA is inserted into muscle, the needle is removed, and the ends connected to amplifiers by small springs attached to a head stage. Signal-noise ratio is high and multiple MUs can be recorded with good fidelity. Following an experiment, the IMAs are straightened, cleaned, sterilized, and can be reused with no detectable loss in signal quality.



Instantaneous firing rates of 13 motor units recorded from human tibialis anterior using two 8-channel intramuscular electrode arrays.

TITLE

Characterization of spinal circuits with high density surface electromyography (HDsEMG)

AUTHORS

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ABSTRACT

Spinal circuits are fundamental in motor control, with spinal interneurons defining motoneuron excitability and shaping the patterns of motor output. Local spinal networks are affected in many neuromuscular disorders. Standard tools used to assess spinal circuits in clinics are based on the use of surface EMG which has a poor temporal resolution to identify circuit features or needle electrodes which are invasive and allow identification of only a few motor units (MUs). HDsEMG allows non-invasive identification of a pool of MUs at a time and more accurate study of motor pool properties. Only two studies have previously proposed the use of HDsEMG to study reciprocal inhibition through the decomposition of individual MUs. Latest advances on HDsEMG methods have allowed sampling larger proportion of the motor pool even at low force levels; while new protocols have been proposed to study recurrent inhibition based on stimulation of motor axons and recording MUs using intramuscular electrodes. In this study, we propose to use the state-of-the-art HDsEMG technique to characterize spinal circuits in large population of MUs.

HDsEMG grids of electrodes were placed on the Tibialis Anterior (TA) and Soleus (SOL) muscles of healthy participants whose feet were strapped to a dynamometer to produce isometric contractions at low force levels (5% and 10%) of MVC. During the steady contractions, inhibition was evoked by nerve stimulation with single pulses at an interstimulus interval (ISI) of 2s. Essentially, recurrent or reciprocal inhibitory circuits to inhibit SOL activity were evoked by nerve stimulation of the tibial nerve to stimulate SOL motor axons to antidromically activate recurrent inhibition (SOL-to-SOL); or the antagonist common peroneal to stimulate TA Ia fibres to orthodromically activate reciprocal inhibition (TA-to-SOL). A similar approach was followed for TA muscle activity: antagonist SOL Ia fibres for reciprocal, and TA motor axons for recurrent inhibition, were stimulated. Individual MUs were decomposed with a blind source separation algorithm and stimulus-triggered responses were used to determine the inhibition latency and duration using peristimulus histograms. For the first time, we non-invasively estimated the latency and duration of recurrent inhibition in the TA and SOL muscles in a large population of MUs. At the same time, we characterized optimal experimental conditions to measure both spinal circuits.

The technique and results derived from this study will allow to develop neurophysiological biomarkers which might contribute to early diagnosis or monitoring the progress of neuromuscular diseases in clinics.

TITLE: Do persistent inward currents contribute to inspiratory motoneuron firing in humans?

AUTHORS

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Background: Voltage-sensitive persistent inward currents (PICs) are known to prolong firing in limb-innervating motoneurons. While there is immunohistochemical evidence of PIC channels in respiratory motoneurons, it is unknown whether PICs contribute to their firing prolongation. Potential neuromodulatory effects may also depend on whether skeletal respiratory muscles are activated via bulbospinal (automatic breathing) or corticospinal (voluntary) pathways.

Methods: Intramuscular electromyographic signals were recorded from human inspiratory muscles to identify motor unit (MU) activity. Diaphragm MUs were identified during quiet breathing (7 participants; 1 female) and from the 1st, 3rd, and 5th parasternal intercostal muscles during quiet (no visual feedback of lung volume) and voluntary (triangular-shaped feedback) breathing (5 males). Analysis of these previously collected data [1,2] included estimation of PIC effects on firing prolongation using a paired MU analysis (firing hysteresis; ΔF). Firing prolongation of individual MUs was also quantified via a measure of firing symmetry in relation to peak volume $[(\text{Duration}_{\text{Inspiration}} - \text{Duration}_{\text{Expiration}}) / \text{Duration}_{\text{Total}}]$.

Results: In quiet breathing, a total of 169 MUs were identified (range across muscles: 28-47), with ΔF computed in 70 MUs (range: 9-26 MUs). ΔF scores were significantly greater than 1 Hz in diaphragm MUs (2.29 Hz, 95% CI: [1.08, 3.50], $p=0.001$) but not in the other muscles (1st: 1.78 Hz [0.43, 3.12], 3rd: 1.08 Hz [-0.39, 2.55], 5th: 1.55 Hz [-0.03, 3.13], $p = 0.17-0.49$). The proportion of MUs that continued to fire during the expiratory phase (duration ratio < 1) was higher in diaphragm MUs (85.1%) than in the 1st (48.9%, $p<0.001$) and 3rd (63.8%, $p=0.02$) intercostal muscles, but not different to the 5th (71.4%, $p = 0.15$). Compared to quiet breaths, duration ratios were higher in voluntary breaths (85% MUs tracked between conditions) in the 3rd ($p = 0.003$) and 5th ($p = 0.003$) intercostal muscles ($p < 0.01$), suggesting a left-shift in MU activity (less firing prolongation). However, no changes in ΔF between quiet and voluntary breaths were observed in parasternal intercostal MUs ($p > 0.05$).

Conclusion: These findings suggest that PICs may contribute to firing prolongation in diaphragm MUs during quiet breathing. However, PIC-like behaviours were not evident in parasternal intercostal MU firing during either quiet or voluntary breathing. These novel findings motivate future efforts to validate, estimate or modulate possible PIC contributions to respiratory motoneuron firing in humans and animal models.

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2. Hudson et al, Respir Physiol Neurobiol, 2011

Caffeine increases the centrally-mediated responses to wide-pulse high-frequency neuromuscular electrical stimulation

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Introduction. While neuromuscular electrical stimulation (NMES) is widely used to preserve, restore or improve neuromuscular function in healthy and clinical populations, its effectiveness depends on training intensity, i.e., the torque developed during the stimulation. Wide-pulse (1 ms) high-frequency (100 Hz) NMES (WPHF NMES) may enhance torque production through activation of persistent inward currents (PICs) leading to a progressive increase in torque production during the stimulation ('extra torque'). Caffeine is an antagonist of adenosine receptors and has been shown to increase self-sustained firing occurrence and estimates of PIC magnitude of human motor units. Therefore, the aim of the present investigation was to test the hypothesis that caffeine would increase the torque induced by WPHF NMES and to identify the potential contributing mechanisms.

Methods. Twenty-four healthy participants (26±6 years) were recruited in this double-blind, randomized, crossover, placebo-controlled study. WPHF NMES was applied to the *triceps surae* for 10 s at an intensity evoking 10% of the maximal voluntary contraction torque before and 1h after caffeine (6 mg/kg) or placebo ingestion over two separate sessions. The torque produced during stimulation was quantified using the torque time integral (TTI, i.e. area under the torque curve). Bipolar surface electromyography (EMG) was used on the *soleus* (SOL) muscle to measure EMG activity at the end of the stimulation (sustained EMG activity, used as an index of PIC magnitude). High-density surface EMG was also recorded during voluntary triangular contractions to investigate the impact of caffeine on estimates of PIC magnitude using the paired motor unit analysis technique.

Results. The change in TTI (placebo: -1 ± 21% vs. caffeine: +21 ± 32 %, P = 0.012) and SOL sustained EMG activity (placebo: -2 ± 6% maximal EMG activity vs. caffeine: +3 ± 4% maximal EMG activity, P = 0.031) was greater after caffeine ingestion than after placebo. In the caffeine condition, there was a significant positive correlation between the change in TTI and the change in SOL sustained EMG activity (r=0.6, P=0.010). The analysis of high-density EMG data is ongoing and will be presented at the conference.

Conclusion. Our findings shows that acute caffeine supplementation increases the torque evoked by WPHF NMES, with a potential involvement of PICs.

TITLE

Estimates of persistent inward currents in α -motoneurons of the gastrocnemius medialis and soleus muscles decrease with muscle lengthening positions

AUTHORS (full name & institutional affiliation)

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ABSTRACT

Persistent inward currents (PICs) regulate the intrinsic excitability of α -motoneurons. They have been shown to increase with muscle lengthening in decerebrate cats (Hyngstrom et al., 2007). The aim of this study was to determine whether PICs are similarly modulated by muscle lengthening in humans.

We obtained non-invasive estimates of PIC magnitude from 19 male participants (aged 24.4 ± 3.9 years). High-density surface electromyography signals were recorded from the gastrocnemius medialis and soleus muscles. These signals were then decomposed using convolutive blind source separation to identify motor unit spike trains. A paired motor-unit analysis was employed to calculate ΔF , which is assumed to be proportional to the magnitude of PICs (Gorassini et al., 2002). To isolate the length-dependent effects of PICs and to account for the change in muscle force capacity with position, participants performed triangular isometric contractions in neutral ankle position (0°), plantar flexion (20°), and dorsal flexion (-20°) under two conditions: (1) reaching a peak of 40% of their maximum voluntary contraction (similar relative torque between positions), and (2) reaching the same absolute torque corresponding to each participant's 40% maximum voluntary contraction at 20° (similar absolute torque between positions).

For both relative and absolute torque conditions, ΔF of the gastrocnemius medialis was significantly (all p-values < 0.0361) lower in dorsal flexion (3.03 Hz and 3.02 Hz) than plantar flexion (3.67 Hz and 3.74 Hz) and neutral ankle position (3.83 Hz and 3.58). For both relative and absolute torque conditions, ΔF of the soleus was significantly (all p-values < 0.0253) lower in dorsal flexion (2.45 Hz and 2.92 Hz) than plantar flexion (3.61 Hz and 3.75 Hz) and neutral ankle position (3.33 Hz and 3.48).

Our results suggest that PICs are attenuated with muscle lengthening. This result aligns with the known decrease in spinal excitability as a muscle is lengthened (Forman et al., 2019) but contradicts the reported increase in PICs with muscle lengthening in decerebrate cats (Hyngstrom et al., 2007). The discrepancy with our findings in the decerebrate cat study may be attributed to the lack of descending control of inhibitory afferents at the spinal level. This may suggest a potential contribution of PICs to length-dependent muscle hyperactivities such as the spasticity occurring after the loss of descending regulation of afferent inhibition resulting from central nervous system lesions.

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Motor commands are comprised of excitatory, inhibitory, and neuromodulatory components, and disruptions in descending tracts caused by spinal cord injury (SCI) affects all three of these components. The aim of this study was to determine if these disruptions alter motor unit discharge patterns in the upper limb muscles following cervical SCI, and whether motor unit properties were associated with upper limb function. Experiments were performed on eighteen people with a chronic, incomplete SCI at the cervical level, and eighteen non-injured age-matched control participants. High-density surface electromyographic arrays were placed over the biceps and triceps brachii and participants were seated with their arm secured to a force transducer. Elbow flexion and extension maximal voluntary isometric contractions (MViC) were used to normalize subsequent contractions, after which participants performed submaximal isometric triangular and trapezoidal ramps between 20 – 60% of MViC. Blind source separation was used to identify spike times of biceps and triceps motor units and persistent inward currents were estimated using the paired-MU analysis technique, which quantifies discharge rate hysteresis (ΔF). Due to heterogeneous strength deficits, the participants with SCI were categorized into high-functioning and low-functioning sub-groups for each muscle based on their MViC values. Preliminary analysis revealed a positive association between ΔF scores and MViC magnitude for both biceps and triceps in individuals with SCI, but not in the non-injured control group. A linear mixed effects model revealed that estimates of PICs were lower ($p < 0.05$) in both of the low-functioning SCI groups (biceps: $\Delta F = 1.08 \pm 1.21$ pps, $n=7$; triceps: $\Delta F = 2.33 \pm 0.83$ pps, $n=11$) compared to the high-functioning SCI groups (biceps $\Delta F = 4.39 \pm 0.59$ pps, $n=11$; triceps $\Delta F = 5.11 \pm 0.74$ pps, $n=7$) and non-injured controls (biceps $\Delta F = 4.70 \pm 0.57$ pps, $n = 18$; triceps $\Delta F = 4.63 \pm 0.47$ pps, $n = 18$). These results suggest that PICs are impaired in low-functioning muscles after SCI, and that the preservation or restoration of motoneuron excitability after SCI may enhance functional recovery. Further analysis of motor unit behavior in these groups is ongoing and will be presented in person.

Motoneuronal persistent inward currents may contribute to preserved function in humans with spinal cord injury.

Alex Benedetto, Sophie T. Jenz, Bradley S. Heit, Matthew T. Farley, James A. Beauchamp, Sina Sangari, Charles J. Heckman, Monica A. Perez, Gregory E. Pearcey

Resting-state brain-spinal cord networks in humans assessed using innovative fMRI approach

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The spontaneous fluctuations in blood-oxygen–level dependent (BOLD) signals detected by functional magnetic resonance imaging (fMRI) in the absence of explicit tasks or stimuli have been extensively characterized in the human brain. These slow variations divide the resting brain into temporally synchronized networks comprising spatially distinct areas, known as resting-state networks (RSNs). These RSNs mirror clusters of brain regions co-activated during various sensorimotor and cognitive tasks. Identified RSNs have also been reported within the human spinal cord, including bilateral and unilateral dorsal and ventral networks, likely representing sensory and motor spinal processing. Until then, it remained uncertain whether and how the reported RSNs in both the brain and spinal cord are interconnected. To address this question, we employed a scanning protocol to simultaneously capture functional images of the brain and cervical spinal cord during resting-state periods. The protocol utilized an echo-planar imaging (EPI) sequence, enabling optimization of acquisition and shimming parameters for the brain and cervical spinal cord volumes separately. Additionally, we devised a novel processing pipeline for a joint analysis of fMRI signals in these two structures using independent component analysis (ICA) and the region of interest (ROI)–based functional connectivity method. Our results demonstrated a strong correlation between brain and cervical spinal cord activities during rest periods, revealing specific functional links between spinal cord regions and consistently reported brain sensorimotor RSNs. The functional organization of these networks adheres to well-established anatomical principles, including contralateral correspondence between spinal hemicords and brain hemispheres, as well as the segregation of sensory and motor pathways along the brain–spinal cord axis. Consequently, our findings unveil a unified functional organization of sensorimotor networks across the entire central nervous system during rest. While the impact on basic neurophysiological knowledge may seem modest, it is crucial to acknowledge the methodological significance of this novel approach in evaluating functional connectivity along the brain–spinal cord axis as a whole in humans, opening new avenues for studying its alterations in both physiological (such as motor learning; see A. Khatibi abstract) and pathological conditions.

Vahdat S et al. PLoS Biol. 2020;18(7):e3000789. doi: 10.1371/journal.pbio.3000789.

Abstract: Influence of transient afferent drive on the activation of spinal motoneurons and interneurons following chronic spinal cord injury

Authors: **Martin Zaback**, Jose Paz Amaya, Christopher K Thompson, Michel A Lemay

Spinal reflex activity is mediated by extensive interneuronal networks spanning multiple segments of the spinal cord, making it challenging to understand how these networks ultimately sculpt coordinated motor output. Here, we record from a large number of hindlimb motor pools in combination with lumbar spinal interneurons in response to peripheral nerve stimulation. In five female chronic spinal cats (T10 complete transection; 6 weeks post-injury, untrained), two 64-channel microelectrode arrays were placed into lamina VII of the spinal cord on the right side at multiple segments spanning L3-S1 at depths between 1500-3000 μm . Bifilar EMG was recorded bilaterally from multiple ankle (soleus, medial gastrocnemius, tibialis anterior), knee (vastus lateralis, biceps femoris posterior), and hip (biceps femoris anterior, sartorius) flexors and extensor muscles. The right tibial nerve was isolated and stimulated at 2 Hz from a cuff electrode at 2, 5, and 10x sciatic nerve threshold. Interneuron and muscle activity were quantified as short- (8-40 ms; SLR) and long- (50-450 ms; LLR) latency responses from the spinal multi-unit and EMG recordings, respectively. At all intensities, the EMG SLR following tibial nerve stimulation demonstrated a focal ipsilateral response in hip, knee, and ankle flexors. A robust EMG LLR became evident only at higher intensities and was more distributed across all muscles, both ipsilateral and contralateral. Spinal interneurons demonstrated a robust SLR, which was larger at more caudal recording sites. The averaged interneuronal LLR was very small and of similar amplitude across recording sites. This suggests only a small number of spinal interneurons contribute to the EMG LLR and/or the EMG LLR is largely maintained by currents intrinsic to the spinal motoneuron. The decomposition of these spinal interneuron data into the spike times of single units will allow us to assess for subpopulations of spinal intraneuronal networks that contribute to sensorimotor function following spinal lesions. Understanding how these circuits contribute to motor output will allow for targeted biomarkers and therapies to improve motor impairments following spinal lesions.

Motor Unit Number Estimation of Intralesional Forearm Muscles after Cervical Spinal Cord Injury

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ABSTRACT

Introduction: The health of infralesional lower motor neurons (LMNs) after a cervical spinal cord injury is frequently overlooked, despite its critical role in mediating effective clinical interventions for improving arm and hand function. Prior studies suggest high frequencies of infralesional lower motor abnormalities using non-quantitative measures in muscles that are potential targets for nerve transfer surgery, a procedure that has the potential to restore upper limb function.

Methods: In this prospective, two-center cohort study, we used multipoint stimulation motor unit number estimation (MPS-MUNE) to evaluate the number of motor units in clinically relevant, but rarely studied, infralesional muscles, including the predominantly C7-innervated anconeus and the predominantly C8-innervated extensor indicis (EI) in 15 individuals with cervical spinal cord injury (26 limbs) and 17 neurologically intact controls.

Results: The test-retest reliability as measured by intraclass correlation coefficient and confidence interval (CI) for the EI and anconeus were 0.84 (CI: 0.45-0.95) and 0.78 (CI: 0.36-0.93), respectively. Both compound muscle action potential (CMAP) and MUNE values were significantly lower ($p < 0.05$) for those with cervical spinal cord injury (EI CMAP: 2.0 mV \pm 1.57, EI MUNE: 33 \pm 30.5, Anconeus CMAP:2.7 mV \pm 1.9, Anconeus MUNE: 39 \pm 50.6) versus controls (EI CMAP: 6.6mV \pm 1.0, EI MUNE:137 \pm 33.9, Anconeus CMAP:6.6 mV \pm 1.3, Anconeus MUNE: 146 \pm 42.3).

Discussion: Here, we demonstrate the potential utility of MPS-MUNE for evaluating the health of LMNs. This study showed significant loss of infralesional motor units after cervical spinal cord injury. The LMN abnormalities observed underscore the significance of this approach to evaluating potential targets for nerve transfer surgery for the restoration of upper limb function.

AAV-mediated TrkB receptor enrichment of soleus motoneurons promotes motor function recovery after spinal cord transection.

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The brain-derived neurotrophic factor/tropomyosin-related kinase B receptor (BDNF/TrkB) signaling is one of the most implicated in the maintenance of synapses and neuronal survival in the CNS. It is also involved in the stability and functionality of the neuromuscular junctions (Głowacka et al., 2022). In animal models BDNF prevents lesion-induced degeneration of spinal motoneurons. Our recent studies showed that complete spinal cord transection (CST) at the thoracic (Th10) level in the rat leads to a profound (approx. 80%) decrease in TrkB expression in L3-6 motoneurons (MNs) innervating muscles in the ankle joint (Głowacka et al., 2023 FENS).

This study investigates whether enhancing the expression of the gene encoding the TrkB receptor in Soleus (Sol) MNs, which innervate Sol muscle characterized by high susceptibility to BDNF, and whose circuits are more impaired by SCT than circuits of the GL and TA MNs (Skup et al., 2012; Gajewska et al., 2023) can lead to locomotor function recovery in the adult rat.

To increase responsiveness of Sol MNs to BDNF, we used intramuscular gene transfer. TrkB with C-myc tag was produced from ssAAV6/2-hsyn-TrkB_Cmyc (8.2x10E12 v.c.) construct. Vectors were injected into the Sol muscle during the surgery of complete SCT at Th10. Activation of receptors was supported by long-term locomotor training (5 weeks), which up-regulates BDNF level in the spinal network. The control (n=5) and operated groups (n =7 each) were used: SCT, SCT-Loc, SCT-AAV-TrkB, and SCT-AAV-TrkB-Loc.. Numerous traced MNs responded to AAV-TrkB treatment by increasing the mRNA trkB level above control levels, showing Cmyc tag staining and increasing pan-TrkB protein labeling. Functional assessment was performed based on the modified BBB locomotor scale. We observed an improvement in functional capacity: the increased number of "step-like movements" and alternations at the 6th week, compared to animals receiving saline. We conclude that enriching the TrkB receptor pool, and stimulating neurotrophin signaling with endogenous ligand may be a therapeutic approach with promising potential.

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Friday 21st

08:30	ALS humans - Chair: M. El Mendili
	MD.Calabria Gallego
	O.Aousji
	C. Mitchell
	M.Piotrkiewicz
	M.Gorassini
10:00	Coffee / Posters
10:30	Plenary conference
	Cédric Raoul
11:15	Spinal cord injury - Chair: N.Suresh
	F.Alvarez
	F.Krust
	K.Godlewska
	H.Bras
12:15	Lunch / Posters
14:15	Sensory-motor - Chairs: T. Bui / M. Henry
	B.Nasserolelami
	J.Colard
	G.Niyo/F.Valero-Cuevas
	S.Gandevia
	P.Krutki
	A.Revill
	A.Czarnecki
16:15	Meeting conclusion

TITLE: Remarkable neurological improvement after two years of treatment with intrathecal Tofersen in two selected patients with SOD1-ALS

AUTHORS (full name & institutional affiliation):

María Dolores Calabria Gallego

Neurology Department. ALS Unit. University Hospital of Salamanca (Spain)

ABSTRACT

5-10% of ALS cases are familial, and of these 20% have a mutation of SOD1; 2% of sporadic also have SOD1 mutations. Neuronal degeneration in these cases are considered to be caused by toxic gain of function of the mutant protein. Tofersen is an intrathecally administered antisense oligonucleotide designed to reduce synthesis of SOD1 protein.

In this report, we communicate data collected on the outcome of treatment with tofersen in the Early Access Program of 2 selected patients.

A. 50-year-old woman, with a p.Asn140Lys variant, with onset of disease in 2019. The examination at the beginning of treatment (August 2022) showed areflexia in the lower limbs and hyperreflexia with a right (R) bicipital clonus, R Hoffman sign. Motor balance: Left (L) ankle flex-extension 2/5, R 3/5; bilateral knee extension 3/5; R knee flexion 2+/5, L 1+/5; bilaterally hip flexion 0/5; R intrinsic hand musculature 3/5, L 2/5; bilateral elbow flex-extension 4/5; R shoulder abduction 3+/5, L 2+/5. Very important amyotrophy in both feet and intrinsic musculature of hands. With successive administrations of drug, the patient began to report subjective improvement in stopping progression of disease, being able to sign again, elevation of R leg, handling knife and fork, and movement of legs in the pool.

B. A 50-year-old male, with a rs80265967 variant, who began with the first symptoms of disease in 2019. At the time of treatment initiation (March 2022), his examination showed hyporeflexia in lower limbs and hyperreflexia in upper limbs. Bilateral Babinski. Motor balance: flex-extension of R arm: 4/5, flex-extension of both wrists: 3+/5, intrinsic muscles of the fingers: 2/5, bilateral legs flexion: 3+/5, impossible walking on toes and heels. With successive administrations of the drug, the patient began to report subjective improvement on electric bicycle, recovery of ability to stand from a sitting position from a low seat, ability to whistle and movement of fingers of R hand, ability to squatting, no longer requires antequinus in R ankle, he has gone from depending on 2 canes to just 1, and a notable decrease in fasciculations throughout his body surface.

During almost 2 years of treatment, we performed determinations of neurofilaments, dynamometry and ALSFRS-R scale (complementary images).

In conclusion, with the experience of these two cases, effectiveness of the treatment seems good, although given lack of success of the VALOR study, and due to the not so good experience of other patients, it is reasonable to think that patients with that remarkable efficacy would be those with characteristics similar to those of this report (who were relatively young and with a not very advanced course of the disease).

Synaptic loss in human amyotrophic lateral sclerosis spinal cord: a clinicopathological study

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Amyotrophic Lateral Sclerosis (ALS) arises from the combined degeneration of motor neurons (MN) and corticospinal neurons; displaying important phosphorylated 43-kDa transactive response DNA-binding protein (p-TDP43) inclusions. Previous studies point to the early involvement of synapses in the disease course and their crucial role in the pathogenic cascade. However, pathology studies, with large post-mortem cohorts, mapping the pattern of synaptic disturbances over clinical and neuropathological hallmarks of disease progression, are currently not available. Thus, the appearance and progression of synaptic degeneration in human ALS patients are currently not known, preventing a full validation of the murine and in vitro models. In this study, we investigated the loss of synaptophysin-positive terminals in cervical, thoracic, and lumbar spinal cord samples from a cohort of 33 sporadic ALS patients and 9 healthy controls, and we correlated the loss of synapses against clinicodemographic features and neuropathological ALS stage. We found that, although dorsal and intermediate spinal cord laminae do not lose synapses, ALS patients displayed a substantial but variable loss of synapses in the ventral horn of lumbar and cervical spinal cord. The amount of synaptic loss highly correlates with disease duration, clinical onset of disease, and the loss of α -motoneurons, although not with the fraction of pTDP-43-immunopositive α -motoneurons. Taken together, our data validate the synaptic pathology observed in other models and suggest that pathogenic pathways unfolding in the spinal microenvironment are critical to the progressive disassembly of local synaptic connectivity.

TITLE

Elucidating the comparative dynamics of ALS using artificial intelligence

AUTHORS

[Cassie S. Mitchell](#), Raghav Tandon, Zihan Wei, David Kartchner

Laboratory for Pathology Dynamics, Department of Biomedical Engineering, Georgia Institute of Technology and Emory University School of Medicine, Atlanta, GA, USA

ABSTRACT

Artificial intelligence (AI) has provided several innovative methodologies for better exploring the dynamics of Amyotrophic Lateral Sclerosis (ALS) and other similar neuropathological disorders that share some of the same biomarkers. The goal of this study was to employ cutting-edge AI to integrate multifactorial and multi-scalar data sets that can identify new dynamic themes not otherwise obvious to the human eye. First, literature-base discovery was employed using SemNet 2.0 to compare and rank the amino acids, peptides, proteins, co-morbid disease or syndromes, and biological functions perturbed in ALS. The underlying large knowledge graph incorporated 33+ million PubMed articles to identify and rank nodes (e.g. concepts) that are most important or relevant to ALS. The initial analysis showed that, compared to other multifactorial neurological disease, ALS had stronger ties to pathways pivotal to wound healing, large-scale immune system regulatory changes that induce neuroinflammation, perturbed ATP-based energy generation and utilization, and strong associations with changes in liver function. Next, we used a novel scaled event-based modeling algorithm to examine specific biomarker dynamic changes and covariance with clinical ALS disease progression. Results illustrate distinct subtypes of ALS that have different temporal disease dynamics that may explain disease heterogeneity. Collectively, this study provides further credence to a systemic homeostatic network instability in ALS, possibly initiated by hypervigilant regulation. Future work will examine the use of large language models to extract and aggregate quantifiable clinical data across the scientific publication and clinical trial domain that can be leveraged for larger, integrative cohort generation needed for model validation.

The course of changes in motor neuron discharge characteristics in amyotrophic lateral sclerosis

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Abstract

Recent studies of the discharge characteristics of motoneurons (MNs) in ALS patients have shed light on their excitability and its changes over the time course of the disease. The paper by Trajano (2023) showed that PICs' amplitude increased in early stages of the disease and decreased as the disease progressed. This was consistent with our data on increased firing rates and decreased AHP duration in patients with the strongest muscles and the opposite results in those with the weakest muscles (Piotrkiewicz and Hausmanowa-Petrusewicz 2011).

We showed that the mean estimate of AHP duration in patients with strong muscles was shorter than in age-matched controls and began to increase rapidly after the patient's force deficit exceeded 30%. This result, reinforced by several recent studies, suggests that there are at least 2 different symptomatic stages of ALS. What happens in the pre-symptomatic phase of ALS is not measurable, but we know that at this stage the MUs are denervated and re-innervated, so that by the time the first symptoms appear, about 50% of the MNs are already dead. In my talk, I will present a hypothesis regarding the possible evolution of the duration of MN AHP in ALS from its onset.

It is known that the most vulnerable MNs innervating fast fatigable motor units (MUs) die first, which may result in short initial increase in AHP duration averaged over a MN pool. At the same time, reinnervation begins and the influence of orphaned fast muscle fibers gradually shifts the initially "slower" MNs towards faster phenotypes. This results in a shortening of average AHP duration, which continues in the early symptomatic stage until the MN pool exhausts its reinnervation potential and MN excitability begins to rapidly decline. This gradual transformation of muscle phenotype has been documented in animal models of ALS.

Title: ALS motoneurons: dysfunction and death by salt?

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Evidence from ALS rodent models indicate that the sodium channel distribution at the initial segment of motoneurons is increased, leading to enhanced persistent inward sodium currents (NaPICs). Here we show in humans evidence of increased NaPICs in ALS motoneurons. In participants with sporadic or SOD1 ALS, motor units often fired spontaneously at rest at low rates (5-7 Hz) with little variability and when voluntarily recruited during a triangular contraction, they fired with poor rate modulation. Together, the low firing rates, low variability and lack of firing rate modulation are hallmarks of a strong regenerative activation of NaPICs that slowly depolarize the membrane potential after each AHP to produce long interspike intervals (i.e., low firing rates) of consistent duration (i.e., low variability). Paradoxically, ALS motoneurons have previously been shown to have reduced AHPs and small conductance potassium (SK) currents, which should alone increase firing rates and rate modulation contrary to our observations. Thus, the NaPIC, and not the AHP, likely dominates the firing rate behaviour of ALS motoneurons. However, *consistent* with reduced AHPs, we observed many instances of doublets (~200 Hz) that we propose arise from enhanced afterdepolarizations that are unmasked from the reduced AHP and associated SK currents. Decreased SK currents would also be consistent with a more strongly activated PIC also observed in ALS. These and other indications of increased intracellular Na⁺ and chloride (Cl⁻) raise the question of whether excessive NaCl may be cytotoxic, explaining the observations of somatic swelling and dendritic blebbing of ALS motoneurons as will be discussed.

Diverse mechanisms of spinal motoneuron death during development and pathology

Cédric Raoul

GABA/Glycine synaptic activity on axotomized motoneuron cell bodies promotes motor axon regeneration.

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Motor axon regeneration in peripheral nerves after injury is possible, but very slow. Delayed muscle reinnervation impairs functional recovery because the known decreases in regenerative capacity with time after injury and the detrimental effects of long-term denervation in muscle. Electrical nerve stimulation and exercise are two techniques that enhance axonal regeneration and have showed promise in the clinic. However, the synapses driving this activity are unknown and likely limited because many excitatory synapses are lost over the cell bodies of motoneurons after axotomy. We propose that following removal of the potassium chloride cotransporter 2 from the membrane of axotomized motoneurons the excitatory drive is dominated by GABA/glycine synapses retained after injury that are likely depolarizing. To test the hypothesis that GABA/glycine synapses contribute to regeneration we injected tetanus toxin (TeTx) in the left tibialis anterior (TA) muscle to block the release of these neurotransmitters specifically on TA motoneurons. Thereafter, we axotomized all sciatic motoneurons by crushing the left sciatic nerve and analyzed the time course of muscle reinnervation by recording the recovery of compound muscle action potentials and estimating neuromuscular junction (NMJ) re-innervation quantifying motor endplate coverage. We confirm that TeTx injected in TA muscles precisely block inhibitory synapses on TA motoneurons and that muscle reinnervation of the tetanized TA muscle was significantly delayed compared to the non-injected lateral gastrocnemius muscle (LG) in the same leg or to vehicle-injected TA muscles. We conclude that GABA/glycine neurotransmission on regenerating motoneurons accelerates muscle reinnervation. This finding opens many new avenues to explore future interventions that might accelerate regeneration of motor axons in peripheral nerves.

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A neonatal mouse model for demystifying spasticity after spinal cord injury

Nejada Dingu, Florent Krust, Tony Barbay, Cécile Brocard, Jacques Durand, Rémi Bos, Frédéric Brocard.

Spasticity is a prevalent pathological condition found in several neurological disorders, including spinal cord injury (SCI). After SCI, individuals face ~80% risk of developing spasticity within the 1st year, a scenario that reduces quality of life and imposes a significant economic burden on society. While our prior research characterized spasticity using neonatal rat preparations (Brocard et al., 2016; Plantier et al., 2019), we have transitioned to a mouse model, enabling more comprehensive transgenic research.

Similar to neonatal rats, we have shown that neonatal mice, few days after SCI at birth, exhibit behavioral signs of spasticity. This is evident through the emergence of hyperreflexia and abnormal involuntary muscle contractions in their hindlimbs. Concurrently, *in vitro* isolated spinal cords became hyperreflexive and displayed numerous spontaneous motor outputs. We further characterized features of lumbar motoneurons (MNs) that were markedly hyperexcitable after SCI. This was exemplified by a lower rheobase and a resting membrane potential (RMP) more depolarized than in intact animals. In parallel, we noted an increase in sublesional MNs displaying plateau potentials that trigger self-sustained firing after a brief stimulation. Lastly, it's noteworthy that the activation of calpain-I has been positively correlated with the occurrence of spasticity (Brocard et al., 2016; Plantier et al., 2019; Kerzoncuff et al., 2024). Inhibiting calpain-I post-transcriptionally in lumbar MNs restores the normal excitability, particularly with respect to RMP, the persistent sodium current and bistability thereby alleviating the symptoms of spasticity. In conclusion, the mouse model faithfully replicates critical aspects of spasticity, providing a solid groundwork for future advancements in the comprehensive characterization of its pathophysiology through transgenic approaches.

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BROCARD C, PLANTIER V, BOULENGUEZ P, LIABEU F, BOUHADFANE M, VIALLAT-LIEUTAUD A, VINAY L, BROCARD F. Cleavage of Na(+) channels by calpain increases persistent Na(+) current and promotes spasticity after spinal cord injury. *Nature Medicine* Apr;22(4):404-11.

Does locomotor training differentially affect proprioceptive input to flexor and extensor motor neurons after spinal cord transection?

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Glutamatergic proprioceptive neurons are essential for the proper execution of movements by providing muscle sensory feedback to the central motor network. After spinal cord transection (SCT) a decreased motoneuron (MN) receptivity to glutamate (Glu), manifested by reduced expression of AMPA and NMDA Glu receptors early postlesion (Grycz et al., 2019, Ji et al., 2022), may affect signaling in the proprioceptive circuit. At that time there is a comparable downregulation of Glu receptors in tibialis anterior (TA) and gastrocnemius lateralis (GL) MNs. However, proprioceptive afferents which signal via Glu receptors are more impaired on TA than GL MNs (Grycz et al., 2019), suggesting their diversity. Proprioceptive activation by electrical stimulation of peripheral nerve causes enrichment of MN inputs (Gajewska-Woźniak et al., 2016), revealing their ability to plasticity. To examine further these phenomena long term, we asked whether proprioceptive input to MNs is amenable to stimulation by locomotor training shown to counteract synaptic loss on MNs (Macias et al., 2009).

The study was performed on intact (C, n=8), spinal (SpNt, n=14), and spinal, subjected to 5 week training (SpLoc, n=14) male adult rats. Assisted hindlimb stepping on the running treadmill was video recorded and analyzed off-line. Input-specific VGluT1 signal intensity and distribution on transverse L5 sections were measured with ImagePro Plus 7.0 software. Quantification of VGluT1 (+) terminals apposing soleus (Sol), GL and TA MNs was done on confocal images with Imaris software.

Six weeks after SCT, SpLoc rats showed over 4-fold increase in step-like movements, plantar stepping and alternations compared to SpNt rats. Number of step-like movements correlated with the number of alternations in both groups ($r=0.7$) but not with the number of plantar steps performed by SpLoc rats. No changes in VGluT1 signal intensity and laminar distribution were found in the SpNt rats, suggesting that spinalization does not impair overall primary afferent inputs. Trained animals exhibited a 35% decrease in VGluT1 signal in the ventral horn, implicating development of spinal adaptive mechanisms.

SCT led to a decrease in the number of VGluT1 (+) proprioceptive inputs to Sol but not to GL and TA MNs. Training led to a further decrease of VGluT1 inputs to Sol and opposite effect on TA MNs. This result confirmed the differential vulnerability of MNs to SCT and stimulation. In the SpLoc group we observed a positive correlation ($r=0.55$) between the number of VGluT1 inputs on Sol MNs and the number of performed plantar steps. This suggests that proprioceptive input is indispensable for proper performance of the steps.

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Key words: spinal cord, injury, VGluT1, motoneurons

Knockdown of calpain1 in lumbar motoneurons reduces spasticity after spinal cord injury in adult rats.

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Spasticity affects ~75% of patients with spinal cord injury (SCI), causing hyperreflexia, muscle spasms and co-contractions of antagonist muscles, greatly impacting life quality. The pathophysiology of spasticity is partly driven by motoneuron (MN) disinhibition due to reduced chloride extruder KCC2 and alterations in glycine receptors (Boulenguez et al., 2010; Sadlaoud et al. 2020), worsened by rising persistent inward currents (Brocard et al. 2016). This excitatory/inhibitory imbalance promotes sustained MN firing below SCI, leading to spasticity. Calpain, a Ca²⁺-activated protease, plays a crucial role in this process. It disrupts chloride homeostasis and upregulates persistent sodium currents by cleaving KCC2 and Nav1.6, marking calpains as promising targets for spasticity treatment (Plantier et al., 2019). Therefore, our study was focused on mitigating spasticity by specifically targeting calpain1 in spinal MNs. We successfully transduced lumbar MNs in adult rats with SCI, using intrathecal administration of adeno-associated virus AAV6, carrying a ShRNA sequence against calpain1. This approach significantly reduced calpain-1 expression in transduced MNs, leading to a noticeable decrease in spasticity symptoms including hyperreflexia, muscle spasms and co-contractions in hindlimb muscles, particularly evident in the second month post-SCI. Additionally, this decreases that prevented the escalation of spasticity to a severe grade, paralleled with the restoration of KCC2 levels in transduced MNs, suggesting a reduced proteolytic activity of calpain1. These findings demonstrate that inhibiting calpain1 in MNs is a promising strategy for alleviating spasticity in SCI patients. This work has been recently published (Kerzonkuf et al. 2024).

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Neurophysiological signatures of sensorimotor (dys)function in spinal cord: from cortico-muscular coherence to evoked responses

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The assessment of sensorimotor function in the spinal cord remains challenging due to its unique alignment in the spinal column. Functional Magnetic Resonance Imaging (fMRI) provides insights into the engagement of the spinal cord in sensorimotor tasks, but the limited temporal resolution of fMRI does not facilitate the interrogation of fast neural oscillations and communication across the brain, spinal cord and effector muscles. This limitation can be addressed, in part, using neurophysiological measurements such as electroencephalography (EEG), electromyography (EMG), and evoked spinal responses.

We will review findings from studies in our group that demonstrate how different novel neurophysiological methods can be used to assess sensorimotor function or dysfunction in the spinal cord.

In the first example, we will show how focal impairment in the spinal cord influences neurophysiological measurements taken from the brain and muscle rather than directly from the spinal cord. I will review the findings from our study on participants with post-polio syndrome (PPS), a condition known to involve structural damage to Lower Motor Neurons (LMN) in the spinal cord. The findings highlight the abnormal features of cortico-muscular communication as quantified by spectral coherence between EEG and EMG, which appears as a widespread gamma band coherence in primary and non-primary motor scalp locations. We will also discuss the similarity of these findings in another LMN condition, Spinal Muscular Atrophy (SMA).

In the second example, we will show how advanced experimental methods and neuroelectric signal analysis provides neurophysiological measures comparable to invasive recordings from the spinal cord. We will review the findings from our study on healthy individuals using spectral analysis of evoked responses in the spinal cord recorded from surface electrodes. Event-related spectral perturbations show that neural oscillations are present and perturbed in frequencies as high as 1200Hz. Importantly, the difference in the power of the neural oscillations measured from electrodes placed over the anterior versus posterior sides of the cervical ring electrode montage around the neck might pertain to different sources within the spinal cord that contribute to the responses.

We will discuss the limitations and advantages of different neurophysiological methods, and how they might be used in different fundamental and clinical studies. We will briefly refer to the potential applications of such neurophysiological measures as biomarker candidates for diagnosis, phenotyping and tracking the progression of neurological and neurodegenerative diseases.

Effect of muscle length on post-activation depression and recurrent inhibition during eccentric contractions of plantar flexors.

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INTRODUCTION:

It is widely accepted that post-activation depression by primary afferent depolarization (PAD) and recurrent inhibition (RI) mechanisms are specifically modulated during eccentric contractions. However, a notable gap remains regarding whether these modulatory processes can be influenced by variations in muscle length. The aim of this study was to explore how variations in muscle length change the modulation of PAD and RI activities during eccentric contractions.

METHOD:

Fifteen healthy individuals participated in four experimental sessions. Percutaneous stimulation of the posterior tibial nerve was used to evoke Hoffmann (H) reflex and M wave on the soleus muscle. PAD was evaluated with the D1 method and heteronymous Ia facilitation (HF), including recordings of submaximal H reflexes with conditioning fibular and femoral nerves stimulations (H_{cond}), respectively, and without conditioned stimulations (H_{test}). PAD and HF activities were estimated with H_{cond}/H_{test} . RI was evaluated with paired H reflex method including recordings of conditioning H reflexes without M wave (H_1) and test H reflex (H'), i.e., RI activity was estimated with H'/H_1 . H_{cond}/H_{test} , and H'/H_1 were measured at long, intermediate and short muscle lengths during all contraction types.

RESULTS:

Analyses of H_{cond}/H_{test} showed that during eccentric contraction the increase in PAD activity is significantly enhanced at long muscle length ($P < 0.001$). Analyses of H'/H_1 showed that RI activity is enhanced during eccentric compared to concentric and isometric contractions at intermediate and short muscle length, but it was not different between the three contraction types ($P = 1.000$) at long muscle length.

CONCLUSION:

This study demonstrates that both PAD and RI activities are greater when muscle length increases, regardless of contraction type. Particularly during eccentric contractions, PAD is enhanced at longer muscle lengths. In contrast, RI, which is typically greater during eccentric contraction compared to concentric and isometric contractions, was identical among the three contraction types at long muscle length. These results suggest that the cumulative inhibition observed during eccentric contractions at long muscle lengths could be adjusted by RI to ensure efficient motoneuronal output.

**Disruptions of voluntary movement by velocity-dependent stretch reflexes
can vary greatly within and across movements: Implications to sensorimotor control**

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The primary motor cortex does not uniquely or directly produce alpha motoneurone (α -MN) drive to muscles during voluntary movement. Rather, α -MN drive emerges from the synthesis and competition among excitatory and inhibitory inputs from multiple descending tracts, spinal interneurons, sensory inputs, and proprioceptive afferents. One such fundamental input is velocity-dependent stretch reflexes in lengthening (antagonist) muscles, which are thought to be inhibited by the shortening (agonist) muscles. It remains an open question, however, the extent to which velocity-dependent stretch reflexes disrupt voluntary movement, and whether and how they are inhibited in limbs with numerous multi-articular muscles. We used a computational model of a Rhesus Macaque arm to simulate movements with feedforward α -MN commands only, and with added velocity-dependent stretch reflex feedback. We found that velocity-dependent stretch reflex caused movement-specific, typically large and variable disruptions to arm movements. These disruptions were greatly reduced when modulating fusimotor feedback as idealized α - γ co-activation or an α -MN collateral to homologous γ -MNs (which scaled the velocity-dependent stretch reflexes to its α -MN output). We conclude that such α -MN collaterals are a tenable, but previously unrecognized, propriospinal circuit in the mammalian fusimotor system. These collaterals could collaborate with the posited (but yet to be clarified) α - γ co-activation, and the few β -MNs in mammals, to create a flexible fusimotor ecosystem to enable voluntary movement. By locally and automatically regulating the highly nonlinear neuro-musculo-skeletal mechanics of the limb, this fusimotor ecosystem could be a critical low-level enabler of learning, adaptation, and performance via brainstem, cerebellar and cortical mechanisms.

TITLE: Possible effects of acute intermittent hypoxia on human motoneurone output.

AUTHORS:

Simon Gandevia [1, 2], Harrison Finn [1, 2], Anandit Mathew [1, 2], Jane Butler [1, 2]

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ABSTRACT:

Acute intermittent hypoxia has shown early promise as an intervention to improve human limb motoneurone output, particularly following a spinal cord injury. Attempts to quantify the effects on the motoneurone output in non-injured individuals have resulted in conflicting results questioning the underpinning mechanisms of how AIH impacts limb motoneurone outputs. Here we summarise the acute studies in non-injured participants.

The included studies delivered one session of AIH with similar methodologies and tested the motoneurone output through stimulation, stretch and voluntary tasks. AIH consisted of alternating periods of 1-5 minutes of hypoxia (9-10% O₂) and 1-2 minute of normoxia (~21% O₂) repeated 5-15 times.

The strongest support for the ability of AIH to increase motoneurone output was the research by Christiansen et al. (2018, PMID: 29688171) who showed a strong facilitation of hand muscle motor evoked potentials (MEP) measured 15-75 minutes after AIH. In addition, evoked potentials increased following electrical stimulation of the cortex or subcortically at the cervicomedullary level suggesting the facilitation occurs below the cortex. Research by our group, Finn et al. (2022, PMID: 35338753) did not reproduce the large MEP facilitation after AIH, showing only a small change at a single time point. We additionally found no changes in the MEP input-output curve. In supporting our findings, Radia et al. (2022, PMID: 35752660) showed no change in the MEP or MEP input-output curve.

Additional tests have been performed on the effects of AIH on the motoneurone responsiveness to afferent input through H-reflex and stretch-reflex testing. Finn et al. (2022) showed some decrease in the recruitment threshold of the soleus H reflex, but no change to the maximal H reflex. Recently Tan et al. (2024, PMID: 3835624) found that the soleus stretch reflex was unchanged after AIH.

In exploring voluntary output of the motoneurons after AIH, Tan et al. (2024) showed no change in the maximal EMG activity of soleus during a maximal contraction. Furthermore, our unpublished work (Mathew et al. 2023) shows no effect of AIH on the voluntary activation of the adductor pollicis muscle and no change in maximal force.

Conclusion: Studies using AIH in a single session have provided inconsistent and irreproducible results.

Mathew AJ, Finn HT, Prajnadewie C, Gandevia SC and Butler JE, (2023). Effect of acute intermittent poikilocapnic hypoxia (AIH) and acute intermittent normoxic hypercapnia (AIC) on voluntary activation of adductor pollicis in humans. Third International Motor Impairment Conference, Sydney, Australia, Abstract #P03.

Adaptations of proprioceptive input from muscle spindles to motoneurons in response to various forms of physical training

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The adaptive plasticity to an altered level of motor activity manifests in adjustment of the properties of neurons and/or muscle fibers. The aim of this study in a rat model was to investigate whether repeated and long-lasting physical exercises, which provide powerful excitatory input from receptors in active muscles to spinal motoneurons, evoke adaptive changes in afferent synaptic transmission. Three groups of male Wistar rats were exposed to three forms of physical training: a low-volume whole-body vibration on a vibration platform, a voluntary progressive weight-lifting or an endurance running on a treadmill. Each training program was conducted for 5 weeks, and a day after the last training session an acute electrophysiological experiment was performed on each rat under general anesthesia. The respective control groups were assigned for each training protocol. Lumbar spinal motoneurons innervating the medial gastrocnemius (MG) or lateral gastrocnemius and soleus (LG-S) muscles were investigated intracellularly. The passive membrane properties were measured and monosynaptic EPSPs were evoked by electrical stimulation of group I afferent fibers from muscle spindles of synergistic LG-S or MG muscles. The central latencies, the amplitudes and the time parameters of EPSPs were analyzed. The potentiation of synaptic excitation of motoneurons, expressed by higher EPSP amplitudes and shorter rise times, was observed in fast-type motoneurons after whole-body vibration training in comparison to respective control. Similar observations concerned the weight-lifting group, in which an increase in input resistance was also noted. However, no considerable changes in EPSP parameters were found in motoneurons following endurance training, in comparison to sedentary rats. We suggest that different modes of muscle activation during particular types of training explain differences in the adaptive changes of the synaptic input evoked in motoneurons.

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Muscarinic acetylcholine receptors (mAChRs) modulate hypoglossal motoneuron (XII MN) function. In neonates, mAChR modulation potentiates inspiratory bursting whereas in adults, mAChR modulation has an overall inhibitory effect on XII MN excitability. The timing during postnatal maturation and mechanisms by which this shift occurs remain to be elucidated. The overall effect of mAChR modulation at XII MNs will in part be determined by the suite of ion channels present that mAChRs modulate. The hyperpolarization activated cyclic nucleotide gated channel (HCN1-4) is modulated by mAChRs and produces the h-current (I_h), a mixed cation current more strongly activated at hyperpolarized membrane potentials. At XII MNs, I_h amplitude increases with postnatal maturation. Increased I_h amplitude could influence muscarinic modulation at XII MNs. We used the rhythmic medullary slice preparation in neonatal (postnatal day 0 – 5) CD1 mice to test the functional effects of muscarinic modulation of I_h on inspiratory bursting in combination with pharmacological block of I_h with ZD7288. Preliminary data (n=4) indicate that I_h may contribute to the muscarinic potentiation of inspiratory burst amplitude at XII MNs in neonatal mice [muscarinic potentiation (100 μ M, 30s) % baseline: control – 112 \pm 65 %, vehicle - 131 \pm 38%; ZD7288 100 μ M, 150s - 148 \pm 44%; ZD7288 100 μ M, 330s - 107 \pm 72%]. We then used neuroanatomical techniques to evaluate changes in expression patterns of HCN1-4 at XII MNs across postnatal maturation. Previous data indicate that HCN1 and HCN2 are most prominent in adult XII MNs, whereas the expression of HCN1-4 in neonates is unknown. We performed double-labeled immunofluorescence experiments against HCN1 or HCN2 and choline acetyltransferase (ChAT) using 20 μ m transverse brainstem slices across six age groups under identical conditions. Images of XII nuclei were collected using confocal microscopy. ImageJ was used to determine the average XII MN HCN subtype intensity. Preliminary data suggest that HCN1 (n=2) labeling intensity undergoes modest decrease with maturation (P0-1= 97-100%, P4-6= 92-100%, P9-10= 85-95%, P12-14= 77-97%, P17-19= 61-67%, adult= 71-82%). By contrast, preliminary data suggest that HCN2 (n=2) expression intensity remained relatively consistent with maturation (P0-1= 97-100%, P4-6=62-84%, P9-10= 45-95%, P12-14= 67-90%, P17-19= 50-93%, adult= 75-100%). These modest changes in labeling intensity of HCN channel subtypes may not explain the increase in I_h with postnatal maturation. Future research will elucidate whether the I_h contribution to muscarinic modulation of inspiratory bursting increases with postnatal maturation.

Testing the contribution of the hyperpolarization activated cyclic nucleotide gated channel on muscarinic modulation of inspiratory bursting at hypoglossal motoneurons.

Ann Revill, Alexis Osbourne, Aleanna Melliza, Lori Buhlman, Johana Vallejo

Nicotine replacement strategies in the form of patches or electronic cigarettes are considered a good alternative to smoking. In pregnant women, it is known that nicotine can cross the placental barrier. Prenatal nicotine exposure (PNE) can cause sensory and motor deficits in the child. In rodents, studies have shown that PNE is responsible for neurodevelopmental disruptions, locomotor disturbances and hyperactivity. These findings suggest that some of the adverse effects of nicotine on development could result from chronic disruption of endogenous cholinergic signaling. Interestingly, nicotinic cholinergic signaling plays an essential role in the embryonic development of spinal motor networks. It is required for the generation of Spontaneous Network Activity (SNA), which is characterized by the activation of giant depolarizing potentials (GDPs). SNA is generated at a specific time-window between the 11th and the 14th embryonic day (E11.5 - E14.5) in the mouse spinal cord when motoneurons (MNs) group in pools and start to project to specific individual muscles. The SNA is instructive for synaptogenesis, axonal growth, guidance of motor axons to their target muscles and for the formation of intraspinal motor networks.

Our project is based on the hypothesis that the chronic activation of nicotinic receptors in the embryo may affect the SNA and the development of the SC.

In the current study, we performed whole-cell patch-clamp recordings of lumbar MNs in *ex vivo* spinal cord at E13.5 to assess the impact of the PNE on the generation of the SNA and on MNs electrophysiological properties. Our results indicate that the PNE increases the amplitude and the duration of the GDPs and the firing of MNs during the SNA. This effect correlates with an increase of the amplitude of the persistent sodium current (INaP) in MNs and the percentage of MNs exhibiting a sustained repetitive firing.

Our findings suggest that the locomotor disturbances observed in the offspring after the PNE could stem from an early alteration of the development of spinal motor networks.

Effects of prenatal nicotine exposure on the development of spinal motor circuits

Thomas Baty, Sarah Boulet, William Cazenave, Grégory Barrière, Pascal Branchereau, and **Antonny Czarnecki**

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The decoding of extensive samples of motor units in human muscles reveals the rate coding of entire motoneuron pools.

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Human muscles are versatile effectors producing forces that span several orders of magnitude. They allow humans to perform extremely diverse motor tasks with the same limbs, like a surgeon closing an incision, and a climber who grasps supports on a cliff. This versatility relies on a unique structure that converts neural inputs into muscle force; that is, the motor unit. The control signals converging to the motor unit are not linearly related to the net synaptic input, but instead emerges from interactions between ionotropic and neuromodulatory inputs to motoneurons.

While we have a clear understanding of the general mechanisms of force control, we still lack a full picture of the detailed organisation of the firing activities of motor units. This is challenged by our inability, so far, to decode the concurrent firing activity of many motor units spanning the entire range of recruitment thresholds in human muscles. Here, we took advantages of dense grids of surface electrodes and updated source-separation algorithms to decode the firing activity of extensive samples of motor units in the Tibialis Anterior (129 ± 44 per participant; $n=8$) and the Vastus Lateralis (130 ± 63 per participant; $n=8$) during isometric contractions of up to 80% of maximal force.

From this unique dataset, we characterised the rate coding of each motor unit as the relation between its instantaneous firing rate and the muscle force, with the assumption that the linear increase in isometric force reflects a proportional increase in the net synaptic excitatory inputs received by the motoneuron. This relation was characterised with a natural logarithm function that comprised two phases. The initial phase was marked by a steep acceleration of firing rate, which was greater for low- than medium- and high-threshold motor units. The second phase comprised a linear increase in firing rate, which was greater for high- than medium- and low-threshold motor units. Changes in firing rate were largely non-linear during the ramp-up and ramp-down phases of the task, but with significant prolonged firing activity only evident for medium-threshold motor units.

Our results demonstrate that the firing rate of each motor unit can follow a large variety of trends with force across the pool. From a neural control perspective, these findings indicate how motor unit pools use gain control to transform inputs with limited bandwidths into an intended muscle force. We will also show how these results can help to design linear or non-linear decoders that aim to predict muscle activation or muscle force from descending inputs recorded in supraspinal centres, with the will to generalise their performance to movements.

CHANGES IN COMMON SYNAPTIC OSCILLATIONS OF SYNERGISTIC MUSCLES DURING THE ACQUISITION OF A NEW MOTOR SKILL TASK

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The acquisition of a new motor skill involves the activation and integration of different mechanisms at the supraspinal and spinal levels. Although these supraspinal adaptations have been well characterized in the literature, the effects of motor skill learning on spinal synaptic connectivity remains relatively less explored. This study investigated whether short-term learning of a new motor skill modulates the common synaptic oscillations within and between synergistic muscles. Twelve participants performed maximal isometric voluntary (MVC) knee extension contractions followed by 15 trials of a challenging isometric force-matching task at 10% MVC. During this learning task, high-density surface electromyograms (HDsEMG) were recorded from the vastus medialis (VM) and lateralis (VL) muscles. Two trials were selected, one with the highest (pre-learning) and one with the lowest (post-learning) error between the force output and target trace. HDsEMGs were decomposed into motor unit spike trains using a convolutive blind source separation algorithm. The motor units were then matched between trials and subsequently, mean discharge rate (MDR) and coefficient of variation of the inter-spike interval (COV_{ISI}) were calculated. Common synaptic oscillations, within and between muscles, were estimated using coherence analysis for delta (1-5 Hz), alpha (5-15 Hz) and beta (15-35 Hz) bands. The results showed significant improvements in force-matching between pre- and post-learning, however no significant changes in MDR were observed in either muscle ($p > 0.16$ for both). For COV_{ISI} , a significant reduction between trials was observed for VL ($p < 0.01$) but not for VM ($p = 0.12$). Regarding estimated common synaptic oscillations within muscle, significant reductions between pre- and post-learning were observed for VL, specifically within the alpha band ($p < 0.002$). In addition, shared synaptic oscillations between VM and VL significantly decreased between trials for both delta ($p < 0.008$) and alpha ($p < 0.03$) bands. Our results indicate that the acquisition of a force-matching skill entails reductions in alpha (VL and between muscles) and delta (between muscles) band oscillations. These preliminary findings indicate that specific changes in shared synaptic oscillations within and between synergistic muscles occur between pre- and post-learning, aimed at enhancing force output precision.

Poster Title: Estimating Synaptic Inputs in Human Tibialis Anterior Through Supercomputer-driven Modeling

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Abstract:

There is a lack of understanding in how synaptic inputs shape motor output as well as intrinsic differences in the motor unit (MU) firing behavior across multiple human muscle groups. During a slow submaximal triangular contraction, human tibialis anterior (TA) MUs have been shown to have faster firing frequencies and a slightly reduced MU recruitment range compared to its antagonistic pair – soleus (SOL) (Beauchamp et al., 2022). One reason behind this observation may lie in how these muscles receive or respond to synaptic inputs. Unfortunately, there is a lack of techniques that can study motoneurons (MNs) at the pre-synaptic level – especially in humans. Recently, Chardon et al. (2023) proposed a reverse-engineering (RE) approach to estimate synaptic inputs to an MN pool using computer MN models and supercomputers. In summary, this reverse-engineering approach has a high potential to address the question of how motor output is shaped by a complex set of input combinations. The work presented here is an attempt to apply RE approaches proposed by Chardon et al. to search for appropriate model parameters (exact or a set) that closely resemble human TA muscle during a submaximal slow triangular contraction (30% max voluntary torque (MVT)). This pool of model MNs was then subjected to 12,285 combinations of excitation, inhibition, and neuromodulation to find suitable model parameters that most resemble human TA output as seen in experimental data collected via high-density surface electromyography (HD-sEMG). In addition to synaptic inputs, intrinsic MN model parameter values setting afterhyperpolarization (AHP) and voltage-threshold for persistent inward current (PIC) activation were added to the input combinations. The hypothesis here is that TA MU firing patterns can be explained with a shorter AHP as well as a reduced voltage threshold for PIC activation. Preliminary results from the simulations show that a shorter AHP can provide a good match to TA experimental data. However, only a narrow range of PIC voltage thresholds provide a good match. While the work presented here focuses on TA, this technique can be expanded to also find a model parameter set to match human SOL muscles from experimental data. In this study, nearly five million simulations have been carried out by supercomputers at the Laboratory Computing Research Center at Argonne National Laboratory and at the National Energy Research Scientific Computing Center at Lawrence Berkeley National Laboratory to study MUs firing patterns. Our preliminary results are related to a larger

ongoing project to optimize existing human computer models for multiple muscles in both the lower and upper limbs.

MOTOR UNIT DISCHARGE BEHAVIOUR IN HUMAN MUSCLES: A SYSTEMATIC-REVIEW AND META-ANALYSIS.

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Since the seminal work of Sherrington, the motoneuron has drawn much interest as the final common pathway of the neuromuscular system. The study of motoneurons in humans has focused on the activity of the motor unit (MU) during various tasks. Increased interest and technological advancements have led researchers to attempt to characterize MU behaviour throughout force gradation. This review aimed to summarize the current wealth of knowledge in the study of MU behaviour between 1950 and 2023 to answer critical questions and address current knowledge gaps in the literature as we strive to better understand human motor control. The primary research question addressed discharge behaviours of MUs in various human muscles during isometric voluntary contractions. The secondary questions addressed the influence of force output on MU behaviour in various muscles and differences in MU behaviour between the upper, trunk and lower limbs, proximal to distal and small to large. Included studies were primary surface or intramuscular electromyography (EMG) MU behaviour investigations using voluntary isometric contractions on baseline or control human subjects ≥ 18 years and who were free of neuromuscular impairment. PubMed, Medline, and Web of Science searches resulted in 14,759 papers identified, 8,931 remained after removing duplicates. 561 screened papers were identified for full text retrieval (34 not retrieved). Of the 527 remaining papers, after screening, 432 were included for data extraction. A data table was constructed from retrieved data and analysed to address the above research questions. From a subgroup of the first 100 papers (alphabetically), meta-means of MU discharge rate (MUDR) were calculated using a random-effect meta-analysis with an inverse-variance method. Subgroup analyses splitting the force outputs into HIGH ($>60\%MVC$) and LOW ($\leq 30\%MVC$) were performed for the tibialis anterior (TA), vastus lateralis (VL), biceps brachii (BB), and first dorsal interosseous (FDI). There were significant increases in MUDR from the LOW to HIGH force output for all muscles (All $p < 0.001$; TA: 63%; VL: 41%; BB: 58%; FDI: 54%). Although all muscles MUDR increased from LOW to HIGH force output, the greatest differences were seen in the TA, BB and FDI compared to the VL which may be related to differences in central and/or peripheral mechanisms. Overall, in the preliminary data, there is a significant increase in MUDR from LOW to HIGH force output suggesting the reliance on rate coding in the modulation of force, regardless of muscle size, function, or location.

TITLE: Motor unit discharge characteristics in post-menopausal females and the effects of hormone replacement therapy

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ABSTRACT:

Oestrogen and progesterone are the predominant female sex hormones which potentially influence neuronal activity via excitatory and inhibitory effects [1] [2]. The menopausal transition marks the cessation of the female reproductive cycle and is characterised by a rapid decline in these sex hormones. This significant hormonal shift is not observed in males and may contribute to sex-based disparities in the development of frailty [3]. Hormone Replacement Therapy (HRT) has been shown to have beneficial effects on physical function in post-menopausal females [4], however, the effects of HRT on individual motor unit (MU) characteristics have not yet been fully investigated.

Eight post-menopausal females aged 59.5 yrs (± 7.4) were recruited according to NICE guidelines, and all participants reported ≥ 12 consecutive months without menstruation. HRT users (n=4) were taking combined hormone therapy for a minimum of 6 months, and control females (n=4) were not using any form of hormone therapy. Maximum isometric dorsiflexor force was recorded from the right leg. High-density surface electromyography (HD-sEMG) signals were recorded from the tibialis anterior (TA) during trapezoid contractions (3s ramp up, 12s hold, 3s ramp down) normalised to 25% of maximum force, and decomposed into individual MU spike trains.

The mean number of TA MUs recorded from these older individuals was 15 ± 5 . Non-HRT users had a maximum dorsiflexion strength of 94.8N, compared to 117.2N in the HRT group. Mean MU discharge rate (MUDR) at the point of recruitment was 9.23Hz in the non-HRT group, and 9.01Hz in the HRT. At de-recruitment, mean MUDR was 7.58Hz in the non-HRT, and 7.84Hz in the HRT. During the sustained phase held at 25% of maximum force, mean MUDR was 12.94Hz in the non-HRT and 13.41Hz in the HRT group.

Although the current pilot data preclude definitive outcomes, there are indications muscle strength may differ across these hormonally distinct groups, with little difference between MU characteristics at this single contraction intensity. The current data and the accompanying ongoing study highlight the plausibility of exploring the effects of exogenous hormone administration in older humans and help alleviate the minimal MU data available in older females. This has the potential to identify clinically relevant interventional targets to help address sex-based differences in sarcopenia and frailty.

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Remote muscle contraction influences persistent inward current strength and the response to wide-pulse, high-frequency neuromuscular electrical stimulation

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Abstract

Introduction: Neuromuscular electrical stimulation (NMES) is a method classically used in athletes or patients to improve neuromuscular function. Yet, its use is commonly accompanied by exacerbated fatigue and discomfort, which limits its efficiency. The use of wide pulses (WP, 1 ms) and high stimulation frequencies (HF, >80 Hz) delivered at low stimulation intensity can promote motor unit (MU) central recruitment through the depolarization of Ia sensory axons. When applying WPHF NMES, a progressive increase in torque, called 'extra torque', and the persistence of surface electromyographic (EMG) activity after the end of the stimulation, called 'sustained EMG activity', can be observed in some individuals and are interpreted as a consequence of persistent inward current (PIC) generation due to MU recruitment through central pathways. Thus, interventions aiming at maximizing PICs, such as the contraction of a remote muscle, could improve the effectiveness of WPHF NMES. The aim of this study was to assess the effect of a remote handgrip contraction during WPHF NMES on the magnitude of extra torque and estimates of the PIC contribution to motoneuron firing in the plantar flexors.

Methods: Ten participants performed triangular-shaped contractions to 20% of maximal plantar flexion torque before and after three different interventions: 20 s of rest (Control), a 20-s train of WPHF NMES (1 ms, 100 Hz) at the intensity required to elicit 10% of their maximal voluntary torque (WPHF) and the same 20-s train of stimulation with a handgrip contraction at ~40% of estimated maximal handgrip torque (WPHF+Remote). Extra torque – the relative difference between the initial and final torque during stimulation – as well as *soleus* sustained EMG activity were assessed. High-density EMG signals were recorded during the triangular-shaped contractions to calculate an estimate of PIC contribution to motoneuron firing (ΔF).

Results: While extra torque was not significantly increased with remote contraction (WPHF+Remote) as compared with WPHF alone ($+37 \pm 63\%$, $p = 0.112$), *soleus* sustained EMG activity was higher in this condition than WPHF ($+3.9 \pm 4.3\%$ maximal EMG activity, $p = 0.017$). The increase in ΔF was greater in WPHF+Remote than Control (respectively $+0.35 \pm 0.30$ pps vs. $+0.03 \pm 0.1$, $p = 0.028$). A positive correlation was found between the change in ΔF and extra torque in WPHF+Remote ($r = 0.862$, $p = 0.006$).

Conclusion: The findings suggest that the addition of a remote contraction to WPHF NMES enhances the central contribution to torque production, which may be related to an increased PIC contribution to motoneuron firing.

INCREASED RATE OF FORCE DEVELOPMENT AFTER TMS-INDUCED SILENT PERIOD IN THE KNEE EXTENSORS.

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Explosive strength is the capacity of the neuromuscular system to maximise the rate of force development (RFD) of a muscle. The higher the recruitment speed and the firing rate of the motor units, the higher the RFD of a ballistic (explosive) contraction. It has been shown, that when a pre-contraction silent period (absence of muscle activity and motor units firing before contraction onset) is present, higher firing rate of the recruited motor units and RFD is achieved. In the present study we artificially silenced the motor units of the knee extensor muscles before maximal ballistic contractions onset by transcranial magnetic stimulation (TMS). We hypothesized to find increased RFD and electromyographic (EMG) activity during maximal ballistic contractions with TMS-induced silent period in comparison to control contractions. Six participants performed 10 sets (2 min rest in-between) of 2 isometric ballistic contractions of the knee extensors, separated by 20 s. Participants were instructed to contract as fast as possible as soon as they heard the TMS discharge after a countdown. In the first contraction (w/SP), TMS was used (at rest) to induce a corticospinal silent period overlapping with ballistic contraction onset. The TMS output was set up to induce a silent period of ~180 ms in the vastus medialis EMG activity during isometric contractions at 20% maximal voluntary contraction (MVC) force. In the second condition (w/oSP), the TMS discharge sound was triggered, but no silent period was elicited. Between sets, the order of w/SP and w/oSP were alternated. Reaction time (from TMS trigger to EMG onset) was longer w/SP (187 ± 62 ms) than w/oSP (143 ± 30 ms; $P = 0.04$). EMG amplitude in the windows 0-50, 0-100, and 0-200 ms from onset (root mean square divided by maximal compound muscle action potential amplitude; from vastus medialis) was higher w/SP (73.3 ± 48.8 , 44.3 ± 36.2 , and 18.6 ± 15.4 %, respectively; $P < 0.04$). Peak RFD (in a 30 ms-window, divided by MVC force) was 32.5 ± 24.9 % higher w/SP than w/oSP (7.0 ± 2.0 vs. 5.2 ± 0.6 MVC force/s, respectively; $P = 0.03$). A silent period from TMS superimposed on ballistic contractions onset increased EMG activity in the first 200 ms. By silencing corticospinal excitatory neurons in the primary motor cortex, descending neural drive may be delayed but more synchronous, yielding higher rate of recruitment and firing rate of motor units, and higher peak RFD.

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Reliability of estimated persistent inward currents in tibialis anterior motoneurons within the same session

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ABSTRACT

The modulation of muscle force is primarily achieved by the voluntary command from the motor cortex to the alpha-motoneurons, but also depends on excitatory and inhibitory afferent inputs projecting onto the motoneurons. Typically, the response of spinal motoneurons to synaptic input greatly depends on the activation of persistent inward currents (PICs), which amplify and prolong the effects of the ionotropic system. The contribution of PICs to motoneuron firing cannot be directly measured in humans but can be estimated through the paired motor unit technique using high-density electromyography (HD-EMG). Yet, the intra-session test-retest reliability of this measurement remains to be fully established.

Twenty males performed isometric triangular dorsiflexion contractions to 20 and 50% of maximal voluntary contraction (MVC) torque at baseline and after a 15-min resting period. Ascending and descending phases of the triangular contractions lasted 10 s. HD-EMG signals of the tibialis anterior were recorded with a 64-electrode matrix. HD-EMG signals were decomposed, and motor units tracked across time points to estimate the contribution of

PICs to motoneuron firing through quantification of motor unit recruitment-derecruitment hysteresis (ΔF).

A total of 260 (16.2 ± 9.4 per participant) and 256 (15.8 ± 8.6 per participant) motor units were tracked during the 20% and 50% MVC ramps, respectively. A good intraclass correlation coefficient (ICC = 0.75 [0.63, 0.83]) and a large repeated measures correlation coefficient ($R_{(rm)} = 0.65$ [0.49, 0.77]; $p < 0.001$) were found between ΔF values obtained at both time points for 20% MVC ramps. For 50% MVC ramps, a good ICC (0.77 [0.65, 0.85]) and a very large repeated measures correlation coefficient ($R_{(rm)} = 0.73$ [0.63, 0.80]; $p < 0.001$) were observed. Additionally, ΔF was moderately associated with the recruitment threshold of test units in 20% MVC ramps, whereas this correlation was either small or absent for 50% MVC ramps.

Our data suggest that ΔF scores can be reliably investigated in tibialis anterior motor units during both low- and moderate-intensity contractions within a single experimental session, and future studies should investigate inter-session reliability. Moreover, our data suggest that motoneurons recruited at low levels of force may exhibit less recruitment-derecruitment hysteresis in triangular contractions, in comparison with motoneurons recruited at higher levels of force.

TITLE

Motor unit tracking across two testing visits using independent HDsEMG signals decomposition

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ABSTRACT

Background. High-density surface electromyography (HDsEMG) systems have been used to identify the activities of motor units (MUs) during voluntary contraction. Adaptation of the neuromuscular system to interventions, injuries, or diseases may affect the behavior and properties of MU. To evaluate changes in individual MU, tracking of MUs is required. The aim of this study was to propose an algorithm to track motor units across testing visits.

Methods. Fourteen healthy females (26 ± 4 yr., BMI 24.1 ± 3.3 kg/m², mean \pm SD) were tested on a number of visits within a span of 30 days. They were placed in a pronate position, their knees were fully extended, and their ankle joint was secured in a boot. A 64-channel HDsEMG grid (8 mm inter electrode distance) was placed on the tibialis anterior muscle with columns parallel to the proximal-distal muscle axis. The monopolar HDsEMG signals and torque were recorded at a sampling rate of 2 kHz while the subjects performed isometric dorsiflexion following a trapezoidal torque trajectory: 10 s sustained torque at 10% of the maximal voluntary contraction (MVC) and 2% MVC/s increment and decrement rate.

HDsEMG signals from two visits separated by 12–16 days were randomly selected. After independent MU decomposition in monopolar configuration using the Convolution Kernel Compensation algorithm, single differential MU action potential (MUAP) shapes were calculated using spike-triggered averaging. Tracking of the MUs was then performed using the MUAP image registration method on MUs with a pulse-to-noise ratio (PNR) >28 dB. The proposed algorithm was evaluated by comparing the PNR, precision, and sensitivity of the MU firing patterns obtained using the MU filter with reinforcement before and after application of the algorithm.

Results. The Wilcoxon paired test showed no significant difference in MVC (31.7 ± 8.0 Nm and 32.4 ± 8.1 Nm, $p = .71$), number of identified MUs (16 ± 7 and 14 ± 5 , $p = .11$), and average PNRs (34.2 ± 3.5 dB and 35.2 ± 4.9 dB, $p = .85$) between the two visits. On average, the proposed algorithm could track 64% of the identified MUs in 12 participants and failed in 2 participants. There was a significant improvement in the average PNR from 26 dB to 32 dB ($p < .05$), an increase in the average sensitivity from 61% to 85% ($p < .05$), and an increase in the average precision from 67% to 85% ($p < .05$).

Conclusion. We proposed an algorithm to identify the expression of mutual MUs in two testing visits. This approach will prove useful in expanding our understanding on the characteristics of MUs across testing visits.

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Association Between Brain and Upper Cervical Spinal Cord Atrophy Assessed by MRI and Disease Aggressiveness in Amyotrophic Lateral Sclerosis

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BACKGROUND AND PURPOSE

Significant brain and spinal cord atrophy was evidenced in patients with ALS, predicting progression and survival. However, the contribution of upper (UMN) and lower motor neuron (LMN) degeneration to disease aggressiveness as well as their relationship are far from being clarified.

The objective of this study was to characterize the relative contributions of brain and upper cervical spinal cord compartmental atrophy to disease aggressiveness in ALS, using a conventional brain MRI scan.

MATERIALS AND METHODS

Twenty-nine ALS patients and 24 age- and gender-matched healthy controls (HC) were recruited. Disease duration and the ALSFRS-R at baseline, 3- and 6-months follow-up were assessed. Patients were clinically differentiated into fast (n=13) and slow (n=16) progressors according to their ALSFRS-R progression rate.

Brain grey (GM) and white matter, brainstem sub-structures volumes and spinal cord cross-sectional area (SC-CSA) at C1-C2 vertebral levels were measured from a 3D-T1-weighted MRI sequence at 3T.

RESULTS

Fast progressors showed significant GM, medulla oblongata and SC atrophy compared to HC ($p=5.10^{-4}$, $p=0.013$ and $p=0.008$) and significant GM atrophy compared to slow progressors ($p=0.008$).

GM volume correlated with the ALSFRS-R progression rate ($Rho/p=-0.487/0.007$), the ALSFRS-R at 3-months ($Rho/p=0.622/0.002$), and ALSFRS-R at 6-months ($Rho/p=0.407/0.039$). Medulla oblongata volume and SC-CSA correlated with the ALSFRS-R at 3-months ($Rho/p=0.510/0.015$ and $R/p=0.479/0.024$). MRI measures showed high performance to discriminate between fast and slow progressors (receiver operator characteristic analysis: area under curve/specificity/sensitivity/accuracy=0.764/0.813/0.692/0.759).

CONCLUSION

Our study suggests an association between compartmental atrophy and disease aggressiveness. This result is consistent with the combination of upper and lower motor neuron degeneration as the main driver of disease worsening and severity in ALS. Our study highlights the potential of brain and spinal cord atrophy measured by MRI as biomarker of disease aggressiveness signature.

Ia glutamatergic synapses on spinal motoneurons (MNs) are functionally and structurally impaired in the SOD1 G93A mouse model of amyotrophic lateral sclerosis at a presymptomatic stage (1). However, we do not know the consequence of such a synaptic impairment on intracellular activity-dependent signaling. Indeed, activity-dependent CREB phosphorylation is crucial to induce the transcription of immediate early genes as well as genes involved in cellular adaptations and synaptic plasticity (2). CaMKIV is an important mediator of nuclear calcium signaling (3) whereas S6 is related to neuronal-activity-induced protein synthesis (4).

To investigate this issue, we developed the following protocol. Unilateral small vibrations (0.6mm peak to peak) at high frequencies (400Hz during 0.2s, repeated every 1.8s, for 20 minutes) were applied to the Achilles tendon in order to activate the spindle primary endings in triceps surae (TS), eliciting Ia inputs to homonymous motoneurons. In vivo intracellular recordings of TS MNs showed that the vibration-induced EPSPs mostly remained below the threshold for spiking. 5 days before applying vibration, ipsi- and contralateral TS MNs were labeled by intramuscular injections of CTb-555. Vibration-induced phosphorylation of CREB/CaMKIV and S6 were probed with quantitative immunofluorescence in CTb+ TS MNs from vibrated vs. non-vibrated side.

The vibration-induced Ia EPSPs induced a substantial phosphorylation of CREB, S6 and CaMKIV in MN from WT mice but not in the SOD1 mice, indicating that in the latter, Ia inputs are uncoupled from activity-dependent signaling pathways. This impairment does not seem to be caused by the reduced EPSPs size in SOD1 mice since treatment with Ampakine (40mg/kg), known to prolong the opening of AMPA receptors, increased the EPSP size but does not restore phosphorylation of CREB, S6 and CaMKIV after vibrations. However, treatment with selective phosphodiesterase-4 inhibitor (Rolipram, 1mg/kg), which prevents the degradation of cAMP, restores CREB and S6 phosphorylation in response to the Ia vibration-induced inputs. This occurs despite Rolipram does not increase the size of Ia EPSP.

We are further investigating the mechanisms of the uncoupling between synaptic activity and activity-dependent signaling pathways in SOD1 animals.

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Ia inputs are uncoupled from activity-dependent intracellular signaling in motoneurons from SOD1 mice.

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Glutamate excitotoxicity is canonically viewed as an important mediator in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS). In both human and animal studies, neural networks exhibit elevated extracellular glutamate concentrations coupled with hyperexcitability which ultimately beget denervation. Not surprisingly, the contribution of synaptic glutamate has been heavily scrutinized in ALS etiology; the role of ambient (tonic), extrasynaptic glutamate, however, has yet to be examined in this regard. In the central nervous system (CNS) ambient glutamate is regulated by the cystine/glutamate antiporter, system x_c^- , which imports cystine from the extracellular compartment and exports glutamate in exchange. Importantly, system x_c^- , with specific protein subunit xCT, is markedly upregulated in both animal ALS models and human patients. Remarkably, transgenic mice lacking a functional system x_c^- (xCT^{-/-} mice) exhibit extended lifespan as well as 60-80% lower ambient glutamate concentrations when compared to wild-type (WT) controls. In contrast, SOD1^{G93A} mice, a transgenic model for ALS pathology, display truncated lifespan, enhanced system x_c^- activity, and elevated ambient glutamate concentrations. In the current study, we explored the role of ambient, extrasynaptic glutamate in ALS by assessing the antiporter's contribution to the deleterious hyperexcitability of motoneurons. Thus, we employed a novel *in vitro* spinal cord preparation to electrophysiologically measure motoneuronal excitability in xCT^{-/-}, WT, and SOD1^{G93A} mice. Additionally, cerebral spinal fluid (CSF) was sampled from each genotype, and glutamate concentrations were analyzed using mass spectrometry. Our results revealed decreased motoneuronal excitability in xCT^{-/-} mice, as evidenced by enhanced short-term depression (STD), when compared to WT and SOD1^{G93A} counterparts. Contrarily, SOD1^{G93A} mice exhibited attenuated STD as compared to xCT^{-/-} and WT mice, thus revealing increased motoneuronal excitability. Furthermore, the decreased excitability in xCT^{-/-} mice was concomitant to reduced CSF glutamate levels, whereas the increased excitability in SOD1^{G93A} mice was attendant to elevated glutamate levels. These preliminary findings suggest that ALS-induced system x_c^- upregulation, and the obligate release of ambient glutamate, drive the motoneuronal hyperexcitability which leads to degeneration. Collectively, our data portend a novel therapeutic intervention which may ameliorate the noxious effects of glutamate excitotoxicity in ALS thereby attenuating or precluding motoneuronal dysfunction.

Ambient glutamate and motoneuronal excitability: the role of system x_c^- in ALS pathogenesis

Bradley Stavros Heit, Mingchen Jiang, C.J. Heckman

3D vs 2D motoneuron anatomical reconstructions: implications on cell characterization and ion channels' expression analysis in amyotrophic lateral sclerosis Morgan Highlander, Sherif Elbasiouny

Relying on 2D representations of somas and considering only small portions of protein expression on the somatic membrane, existing methods for 60x immunohistochemistry morphological analysis are subject to sampling error and lack of repeatability caused by analyzer subjectivity. However, characterizing soma size and somatic protein expression precisely and reliably is of great importance for linking structural and functional changes of ion channels in amyotrophic lateral sclerosis. Here, we present our novel analytical approach using 3D soma reconstructions and validate our somatic measurements and complete protein expression analysis by comparison to 2D analysis.

Quantitative Brainstem and Spinal MRI in Amyotrophic Lateral Sclerosis: Implications for Predicting Noninvasive Ventilation Needs

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Background.

Respiratory complications resulting from motor neurons degeneration are the primary cause of death in amyotrophic lateral sclerosis (ALS). Predicting the need for non-invasive ventilation (NIV) in ALS is important for advance care planning and clinical trial design. The aim of this study was to assess the potential of quantitative MRI at the brainstem and spinal cord levels to predict the need for NIV during the first six months after diagnosis.

Methods.

Forty-one ALS patients underwent MRI and spirometry shortly after diagnosis. The need for NIV was monitored according to French health guidelines for 6 months. The performance of four regression models based on: clinical variables, brainstem structures volumes, cervical spinal measurements, and combined variables were compared to predict the need for NIV within this period.

Results.

Both the clinical model ($R^2 = 0.28$, AUC = 0.85, AICc = 42.67, BIC = 49.8) and the brainstem structures' volumes model ($R^2 = 0.30$, AUC = 0.85, AICc = 40.13, BIC = 46.99) demonstrated good predictive performance. In addition, cervical spinal cord measurements model similar performance ($R^2 = 0.338$, AUC = 0.87, AICc = 37.99, BIC = 44.49). Notably, the combined model incorporating predictors from all three models yielded the best performance ($R^2 = 0.60$, AUC = 0.959, AICc = 36.38, BIC = 44.8). These findings are supported by observed positive correlations between brainstem volumes, cervical (C4/C7) cross-sectional area, and spirometry-measured lung volumes.

Conclusions.

Our study shows that brainstem volumes and spinal cord area are promising measures to predict respiratory intervention needs in ALS.

Keywords: Amyotrophic lateral sclerosis, motor neuron disorder, biomarkers, MRI, spinal cord, brainstem, Non-invasive ventilation, spirometry

DYSFUNCTION OF CORTICAL INHIBITORY INTERNEURONS IN AMYOTROPHIC LATERAL SCLEROSIS

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Background

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder affecting human motor system. ALS disease is characterised by a progressive loss of lower motor neurons (LMN, bulbar and spinal motoneurons) and upper motor neurons (UMN, pyramidal cells in the motor cortex). It was shown that imbalance of excitation / inhibition, leading to hyperexcitability of UMN is a key mechanism in ALS. Previous studies using transcranial magnetic stimulation (TMS), have reported a depressed short-Intracortical Inhibition (sICI) and an enhanced Intracortical Facilitation (ICF) in ALS patients. (Vucic & Kiernan *Handb Clin Neurol* 2013; Singari *et al. Clin Neurophys* 2016). To shed light on mechanisms causing neuron excitability disorders, it is crucial to consider afferent interneurons that project onto UMN.

Objective:

The present study aims to investigate inhibitory interneurons afferent to UMN in ALS. Paired pulse TMS (ppTMS) was used to evaluate sICI in 20 ALS patients and in 16 age- and -gender matched healthy subjects (HS) (Kujirai *et al. J Physiol* 1993).

Methods

Electromyogram recordings and ppTMS of the motor cortex were used to evoke sICI in abductor digit minimi (ADM). To stress changes in inhibitory interneuron excitability, sICI was evaluated in different conditions: 1) Test TMS intensity was set at 1.2 of the resting motor threshold (RMT). Two intensities of conditioning TMS were tested (0.7 X RMT and 0.7 of the active MT, AMT) 2) sICI was compared at rest and during 10% of the maximal voluntary contraction.

Results

Consistent with previous studies, The RMT was higher in ALS patients compared to HS. Given that test TMS intensity was stronger in ALS patients, the amplitude of the test motor evoked potential (MEP) was about 10% of the maximal motor action potential (Mmax) while it was about 5% Mmax in HS. Thus, the test MEP in ALS patients was optimal to follow the modulations of sICI across conditions (Lackmy & Marchand-Pauvert *Clin Neurophysiol* 2010; Lackmy-Vallée *et al. Eur J Neuros* 2012). At rest sICI was weaker in ALS patients than in HS. Interestingly, we found that sICI was not modulated by the tonic contraction in ALS patients while in HS the contraction depressed the sICI.

Conclusion

These findings suggest a dysfunction of inhibitory interneurons afferent to UMN, at early stage of ALS. This opens avenues to new approaches to counteract the imbalance of E/I observed in the motor cortex in attempt to slow down UMN loss in ALS.

Acknowledgements:

We would like to thank all subjects involved in the study.

Title

The synaptic connectivity between Renshaw cells and lumbar motoneurons is altered in the early stages of disease progression in the SOD1G93A mouse model of Amyotrophic Lateral Sclerosis (ALS)

Authors and affiliations

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Abstract (2131/2300 characters max)

In neurological conditions that affect the mammalian motor system, such as Amyotrophic Lateral Sclerosis (ALS), alterations in spinal circuits precede motoneuron loss and may represent homeostatic responses to help preserve force output. We tested this possibility in the widely studied SOD1G93A mouse model of ALS, through a combination of *in vivo* and *in vitro* electrophysiology, and super-resolution microscopy. We found that, in early juvenile SOD1G93A mice, recurrent inhibition mediated by Renshaw cells is reduced by 50% , preferentially in motoneurons exhibiting delayed firing profile (putative fast-type). *In vitro* recordings from Renshaw cells revealed no alterations in the strength of their synaptic excitation received following antidromic stimulation of lumbar motoneurons, suggesting that these early changes in recurrent circuitry might be occurring at Renshaw - motoneuron contacts. We used Bayesian Quantal Analysis to assess quantal parameters of Renshaw-motoneuron synapses, and we found that quantal size was reduced by ~30% in mutant animals. Additional electrophysiological testing was done by recording from affected motoneurons and measuring asynchronous synaptic release following Renshaw cell activation in the presence of high concentrations of Sr^{2+} , which further corroborated a quantal size impairment. Since quantal size impairments are usually associated with postsynaptic alterations, we utilized super-resolution stimulated emission depletion (STED) microscopy to measure glycine receptor (GlyR) clustering at Renshaw-motoneuron

contacts. Through STED we found that the density of surface postsynaptic GlyRs distribution was reduced in early juvenile mutants, thus possibly contributing to the early impairment in recurrent inhibition that we observed. Additionally, *in vivo* motoneuron and electromyographic recordings revealed that the reduction in recurrent inhibition is compensated at later time-points, thus indicating that the inhibition mediated by Renshaw cells may go through multiphasic homeostatic compensations as disease progresses in the SOD1G93A model of ALS.

Spinal circuits comprising pelvic motoneurons: A novel approach to study neuroplasticity in ALS

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Previous reports suggested that in mouse models of ALS, spinal circuits undergo a series of early homeostatic changes at premotor synapses to preserve appropriate motor output. Defining changes to these synapses throughout the course of the disease could provide valuable information about the aetiology of disease progression. However, ALS-vulnerable and resistant MNs innervating the limbs (where the first symptoms occur) are not amenable to in vitro recordings in adult tissues. MNs of the pelvic system are known to be viable in adult in vitro preparations, and importantly, ALS-vulnerable ischiocavernosus (IC) and ALS-resistant external urethral sphincter (EUS) muscles are adjacent, potentially allowing comparisons of how these two groups of MNs change as disease progresses.

Anatomical and electrophysiological experiments targeted at labelled MNs were conducted 3-5 days after selectively injecting these muscles in adult mice with retrograde tracers (CTB-Alexa). We first aimed to confirm the selective vulnerability of MNs innervating IC. At the early symptomatic stage (~P90), the number of IC MNs in SOD1G93A mice was reduced compared to age-matched controls, whereas the number of EUS MNs was unchanged (N=10 slice/genotype/MN group, from 2 WT and 2 ALS mice). At a similar time point, anatomical analysis revealed a lower number of C-boutons on ALS IC MNs but not on EUS MNs compared to controls (number of MNs analysed: 23 WT-EUS, 23 ALS-EUS, 40 WT-IC, 10-ALS IC). Next, we performed patch clamp recordings in slices and confirmed that these labelled MNs are amenable to electrophysiological recordings in vitro at ~P90. We then confirmed that these MNs receive recurrent inhibitory inputs which are impaired at the early stages in lumbar MNs (tested in 2 WT mice). We next asked whether changes to vulnerable MNs might affect functionality of neighbouring resistant-EUS MNs. Through EUS EMG combined with cystometry, we observed that ALS mice at early symptomatic stage (~P90) had longer EUS bursts and shorter burst intervals, suggesting an increase in excitability of ALS-resistant EUS MNs (N=2 ALS mice and 2 WT mice).

These preliminary results show a spectrum of changes on vulnerable and resistant MNs in ALS. As a future goal, the collective understanding of circuit-level modifications at different time points would show how early microcircuit changes affecting vulnerable MNs contribute to neighbouring resistant MN re-wiring and how these changes are reflected in behaviour.

Student name: Reynaldo Popoli – Ph.D. Candidate

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Pharmacological cholinergic modulation as a therapeutic approach to ALS

In amyotrophic lateral sclerosis, symptom onset does not occur until a significant number of motor neurons have died, suggesting the existence of a compensatory mechanism. Our previous research has shown that genetically silencing C-boutons in ALS mutant mSOD1^{G93A} mice leads to earlier symptom onset and worsening of behavioural performance, indicating that these synapses are involved in delayed symptom onset relative to motor neuron death. We have shown that C-boutons are upregulated during intensive exercise routines, such as swimming. The literature suggests that this type of exercise is detrimental to disease progression in mSOD1^{G93A} mice. However, when the C-boutons are genetically silenced and mice are exercised three times a week in the form of swimming, an activity where these synapses would typically be upregulated, behavioural performance is dramatically improved. Genetic manipulations, however, are not used in clinical settings, presenting a challenge to improving neurological care in patients with ALS. In our most recent study, we investigated a clinically relevant approach through the use of two cholinergic antagonists in two conditions: (i) frequent exercise under the influence of these pharmacological agents and (ii) resting conditions. These groups were compared among themselves, along with saline controls. Statistical analyses were performed with sample sizes sufficiently large to provide a statistical power of at least 80%. Our results show that both pharmacological agents improve behavioural performance and increase lifespan by approximately 8%, suggesting it may improve the quality of life in patients with ALS. Histological analyses also show that one of the cholinergic antagonists significantly improves muscle innervation at end-stage. Based on these results, however, it is unclear whether these positive effects are C-bouton dependent or not since these drugs act on several cholinergic systems in the body. In order to reveal whether the mechanism behind these positive results is C-bouton dependent, we repeated the previous experiments in mice with genetically silenced C-boutons (mSOD1^{G93A}; Dbx1::cre; ChAT^{fl/fl}; mSOD1^{G93A}/C^{off}). Our preliminary results suggest that behavioural performance does not seem to be affected by the cholinergic antagonist in the absence of functional C-boutons. However, weight, lifespan, and muscle innervation at end-stage seem to improve in the treatment group compared to controls. These results suggest that the beneficial effects of the cholinergic antagonists may be due to a combination of both central and peripheral effects.

Viktória Reháková*, Sandra Dræby*, Arauthy Gnanasampanthan, Michael Guldbrandsen, Karin Sørig Hougaard, Claire Francesca Meehan

Gene Environment interactions influencing neuronal excitability in C9orf72

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative condition affecting motoneurons. One of the biggest challenges in developing better treatments for this disease is that, for the vast majority of cases we still do not know what is actually causing the disease. The most common mutations found in both sporadic and familial ALS are non-coding repeat expansions in the C9orf72 gene (C9orf72REs). However, the penetrance of ALS in patients carrying C9orf72REs is not 100%, meaning that not every carrier of the mutation develops the disease. This indicates that some external trigger may be necessary to induce disease penetrance in C9orf72RE carriers. Carriers with higher exposure to diesel exhaust particles have a higher risk for this disease, implicating diesel exhaust particles as a potential trigger.

An increased excitability of the motor system has been consistently observed in Amyotrophic Lateral Sclerosis (ALS) patients at both the cortical and lower motoneuron levels, including in C9orf72RE carriers. These changes appear to depend on whether C9orf72RE carriers are symptomatic or not. Therefore, we have begun to explore the impact of environmental factors on neuronal excitability in C9orf72 (C9-500) BAC mice.

We initially focused our experiments on C9orf72RE mice that showed a slowly progressing motor phenotype raised in a clean environment. *In vivo* intracellular recordings from spinal motoneurons were performed at around 250 days of age in the C9orf72 and WT littermates. Most basic excitability parameters such as rheobase, voltage threshold and I-f gain were unchanged, except motoneurons from C9orf72RE mice showed signs of increased persistent inward currents. The changes seen in this model are, therefore, less extreme than those seen in other ALS mouse models at more advanced disease stages. Next, we tested the impact of diesel exhaust particle exposure, modelling this by applying 3 x intratracheal instillations of carbon black particles, (a model of diesel exhaust particles) at around 100 days of age. We then measured neuronal excitability 150 days later. Our preliminary results showed that motoneurons in exposed C9orf72 mice show increased spontaneous action potential firing compared to both non-exposed C9orf72 and exposed WT mice. Our results suggest that gene-environment interactions are necessary to induce neuronal hyperexcitability in 250 day-old C9orf72RE mice.

*Authors contributed equally

TITLE: The impact of trans spinal direct current stimulation on motoneuron firing properties in SOD-1 G93A mouse model of ALS.

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ABSTRACT: Trans spinal direct current stimulation (tsDCS) is a non-pharmacological neuromodulatory technique that allows treatment of several neurodegenerative conditions. We have recently shown, in SOD1 G93A mouse model of Amyotrophic Lateral Sclerosis, that anodal tsDCS evokes an acute and long-lasting increase in the amplitudes of monosynaptic EPSPs evoked by Ia afferent stimulation in spinal motoneurons (MNs). Here we show that in addition to the synaptic activity, anodal tsDCS also significantly alters MN intrinsic excitability and firing pattern. We have performed 2 sets of experiments to test the impact of both acute and chronic tsDCS application. In the first set of experiments, tsDCS was applied with a silver ball electrode located one spinal segment rostral to MN recording site. The reference electrode (crocodile clip) was positioned on the skin flap ventral to the dorsal electrode. 30 μ A anodal tsDCS significantly increased firing frequency and reduced recruitment current in the same MNs recorded before and during tsDCS. However, when groups of MNs recorded before tsDCS were compared to MNs recorded within 1h after tsDCS, a marked depolarization of voltage threshold, and an increase of the recruitment current were seen. This indicates that increased intrinsic excitability during tsDCS transfers into reduced intrinsic excitation after tsDCS application. In the next series of experiments, we applied chronic 10-day, 60 μ A anodal tsDSC to SOD1 mice in 15-minute daily sessions. This time tsDCS was delivered in a rostrocaudal arrangement, with the rostral plate electrode positioned on the skin over the Th12-Th13 vertebra, and the caudal plate electrode placed over the sacral vertebra. This polarization protocol provoked significant alterations in passive membrane properties of spinal MN expressed in a decreased peak and plateau input resistance and increased sag ratio. This was further linked to a decrease of MN firing gain in response to intracellular injection of triangular ramp of depolarizing current. Interestingly, despite the apparent reduction in MN excitability, a larger proportion of anodally polarized SOD1 MNs were able to produce repetitive firing. This study was founded by NCN grant 2017/26/D/NZ7/00728 and JPND 2022 grant 2022/04/Y/NZ4/00117

Pharmacological activation of air-stepping behavior in spinal-cord injured newborn rats.

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Following spinal cord injury, communication between the brain and spinal cord is disrupted, resulting in partial or total paralysis. Despite the complexity introduced by the location, extent, and timing of injuries, many individuals retain potential for walking recovery due to incomplete lesions. Because locomotor function relies on specialized spinal networks that are regulated by descending neuromodulatory pathways, one avenue to restore function below a SCI is to compensate for the loss of descending neuromodulatory inputs using pharmacological strategies. Here, we investigate the efficacy of L-DOPA, a precursor of catecholamines, in reactivating locomotor functions below an experimental SCI in newborn rats. In addition, we explore the benefit of co-administrating L-DOPA inhibitors that decrease catecholamine/monoamine degradation. The efficacy of these treatments was evaluated by analyzing their capacity to trigger air-stepping activities. In addition, we used a biochemical approach to decipher their actions at the spinal cord level and establish how spinal monoamines correlate with lesion severity, locomotor parameters. To mimic clinical scenarios, we induce spinal cord lesions of varying severity in newborn rats, ranging from moderate to highly severe. Our findings reveal that the effectiveness of L-DOPA largely depends on the severity of the lesion. However, when combined with a monoamine oxidase inhibitor, the potency of L-DOPA is significantly enhanced, irrespective of lesion severity. Biochemical data indicate that increasing dopamine levels below the injury site plays a key determinant to activate the locomotor program.

Hindlimb motor units shift towards slow type in a mouse model of DYT-TOR1A dystonia

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DYT-TOR1A is the most common genetic form of dystonia, a neurological disorder characterised by disorganised movements and abnormal posturing. We recently revealed that confining a biallelic conditional knockout (cko) of *Tor1a* to the developing spinal cord (spinal *Tor1a* d-cko) leads to a mouse model that recapitulates a severe form of the human movement disorder. In this study, we investigated underlying pathological changes in hindlimb motor units in spinal *Tor1a* d-cko mice, from pre-symptomatic postnatal day 1 (P1) to P18, when the movement disorder is fully penetrant.

We previously showed that in P1-P13 spinal *Tor1a* d-cko mice, L4-L5 motoneurons (MNs) show reduced whole-cell capacitance and increased input resistance, suggesting that *Tor1a* depleted MNs are smaller (PMID: 37134150). When investigating intrinsic firing properties, we discovered that the vast majority of L4-L5 MNs had a putative slow-type firing profile (~75% vs ~25% in control). Ongoing targeted patch-clamp experiments (P16-20) of labelled tibialis anterior (TA) and gastrocnemius motor pools (primarily fast-twitch muscle groups) corroborate the shift from putative fast-to-slow type firing properties.

Given the shift in MN firing properties, we next asked whether the peripheral targets of the MNs – muscle fibres – also undergo a fast-to-slow shift. P18 whole distal hindlimbs (11 muscle groups) were embedded, cryo-sectioned, stained for mature muscle fibre type isoforms (Types 1, 2A, 2X, 2B), imaged, and analysed via a semi-automated unsupervised segmentation and fibre type classification protocol. We found that in spinal *Tor1a* d-cko mice, there was an overt reduction in fast-twitch Type 2B fibres, and an overall shift in the spectrum of muscle fibre composition towards a slower type. Preliminary findings at the pre-symptomatic (P1) and early-symptomatic (P7) timepoints suggests that there may be a progressive decrease in mature fast type fibres across the development of the disease.

In summary, these data suggest that in a phenotypically penetrant mouse model of early onset dystonia, there is a functional shift from fast-to-slow type motor units in the distal hindlimb. These data may provide further insight into the relationships between motoneurons and muscle fibres in health and disease, as well as whether changes to muscle fibre type composition contribute to the symptoms and signs of DYT-TOR1A dystonia.

Title: The Curious Case of Motor Neuron Dendritic Spines

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Abstract:

Dendritic spines were first observed by Cajal in his seminal late-19th century efforts meticulously classifying many canonical neuron types. In the late 1950s, dendritic spines were confirmed as the location of synapses by Grey. Since the advent of molecular methods, dendritic spines have been demonstrated to be incredibly dynamic cytoskeletal organelles, replete with multiple glutamatergic receptor subtypes and possessing different active and passive electrical properties. Changed in dendritic spine morphology and/or abundance occurs in development, ageing and during neurodegenerative or psychiatric conditions. In all “dendritic spine” returns ~13,000 publications in PUBMED. Curiously, reports of dendritic spines on motor neurons are conspicuous in their absence, with a couple of dozen in the literature, mostly in mice.

Here, we evaluate dendritic spine abundance and morphology in brainstem and lumbar motor neurons in a size-dependent manner in adult Fisher 344 rats. Layer V pyramidal cells in the motor cortex were used as a methodological control (i.e., ensuring dendritic spines could be adequately identified). 2 male and 2 female rats at 6 months old and 2 male and 2 female rats of 24-months old were heavily anaesthetized and exsanguinated, with fresh cortex, brainstem, and spinal cords dissected and processed for golgi-cox impregnation. Cortical (coronal), brainstem (transverse) and spinal cord (longitudinal) sections were cut at 180 μm and imaged in mosaics using a 40 objective on an Olympus confocal. Neurons were traced using Neurolucida software (MBF), with motor neurons identified by having a long axis diameter of $> 30 \mu\text{m}$. Motor neurons exhibited dendritic spines (although to a lesser density than cortical neurons of the same rat) in brainstem and lumbar regions, with younger rats illustrating a positive effect of motor neuron size on the abundance of dendritic spines. Lower spine abundance and a blunting of the size-dependent relationship was evident in ageing rats.

In conclusion, given the appropriate preparation and imaging, dendritic spines may be a robust measure of pathology in ageing, particularly in motor pools with age-associated motor neuron death. We provide context to our study with timeline for some early qualitative identification of motor neuron dendritic spines.

Title:

Primary afferent depolarization and hyperreflexia in cerebral palsy

Authors:

Elvia Mena Avila, Dave Bennett, Monica Gorassini, [Katharina Quinlan](#)

Perinatal injuries can result in lifelong motor impairments collectively known as cerebral palsy (CP). Clinical hallmarks of CP include spasticity, muscle stiffness / hypertonia, and hyperreflexia (both increased amplitude of reflexes and farther spread of reflex activity to unrelated muscle groups). Few treatments are available to reduce the impact of developmental injuries once they occur, and rodent models of CP do not display prominent motor deficits after developmental injuries, making the disorder difficult to study. In contrast to rodents, New Zealand White rabbits have a developmental pattern more closely aligned to that of humans and display prominent hypertonia and hyperreflexia after exposure to prenatal hypoxia-ischemia (HI). Altered sensorimotor processing in reflex circuits is present in humans and in the rabbit model and could provide an avenue for prevention / treatment of motor dysfunction. In this context we investigated primary afferent depolarization (PAD) in the rabbit HI model of CP. Briefly, we performed HI or sham surgery (where the pregnant dam was anesthetized but fetuses did not experience HI) at 70-80% gestation. At birth, we performed a battery of behavioral tests to assess motor disfunction including modified Ashworth, righting reflex, and joint torque. Roughly half of the HI kits have motor deficits (HI affected), and the other half were classified as HI unaffected. At postnatal days 1-5, we isolated spinal cords from kits and recorded from dorsal and ventral roots from several adjacent segments of lumbar hemicords while perfusing drugs and stimulating dorsal roots. In all rabbits tested, evoked, short duration phasic PAD was dependent on GABA and glutamate transmission, and the time course of PAD was unchanged. Comparing sham to HI motor-affected and HI motor-unaffected, we found increased reflex irradiation (the intersegmental spread of dorsal root potentials) specifically in HI motor-affected spinal cords compared to both sham and HI unaffected cords only when high threshold afferents were stimulated using 5 and 10 x threshold stimulation parameters. Serotonergic 5HT_{1D} receptor activation (zolmitriptan 600nM) resulted in similar hyperpolarization in sham and HI groups. These results suggest there is a similar pharmacology of PAD in sham and HI rabbits. Future studies will examine the mechanisms of increased reflex irradiation particularly the role of nociceptor-driven activation of GAD2 interneurons.

TITLE: spinal plasticity induced by movement-patterned focal muscle-tendon vibration in subacute stroke

AUTHORS : **Arnaud PREUILH**, Alexandra Lackmy-Vallée, Florian Machi, Bertrand Pichon, Eléonore Bayen, Véronique Marchand-Pauvert

ABSTRACT

Corticospinal plasticity after stroke results in spastic hemiparesis, which is characterized by exacerbation of myotatic reflexes and reduced presynaptic inhibition of group Ia terminals and reciprocal inhibition between antagonists. After undergoing rehabilitation, patients often face challenges in returning to work due to their walking difficulties. Among various factors, walking speed stands out as the most significant predictor of successful return to work. Interestingly, focal muscle-tendon vibration (FMV) therapy conducted over several weeks has demonstrated functional improvements, yet its specific impact on spinal excitability and its correlation with walking speed have not been thoroughly investigated in stroke patients. FMV induces stretch sensation and alleviates spasticity. Notably, studies indicate that a 30-minute session of FMV decreases the H-reflex in both agonist and antagonist muscles. Therefore, this study aims to investigate whether multiple synchronized FMV mimicking lower limb movement induce changes in spinal excitability correlated with walking speed.

Twelve hemiparetic patients in the subacute phase of moderate and severe stroke, unable to ambulate independently, were randomized into a FMV group (n = 6) or a placebo group (n = 6). The protocol spanned 5 weeks with three 30-minute sessions of FMV or placebo, on lower limb muscles per week. Electrophysiological examinations were performed to assess the H-reflex and M-wave in soleus muscles on both sides after popliteal tibial nerve stimulations. Reciprocal and presynaptic inhibition were evaluated by studying common peroneal nerve stimulation-induced H-reflex modulation. Walking speed was measured using both the 10-meter test and treadmill, assessing both comfort and maximum speed. The walking speed was then correlated with electrophysiological metrics. Criteria were assessed before, during, and after the protocol.

The Hmax/Mmax ratio is anticipated to decrease more significantly in the FMV group compared to the placebo group. Restoration of reciprocal and presynaptic inhibition is expected to occur more rapidly in the intervention group. Walking speed is expected to exhibit correlations with electrophysiological metrics.

TITLE: Similarity in reflex responses with and without background activation in chronic stroke survivors with spasticity.

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ABSTRACT The neural mechanisms that contribute to hyperreflexia in individuals with spasticity post-stroke remain largely unknown. One possibility is that the observed reduction in reflex threshold may be a manifestation of a motoneuron pool that is easily excitable. To address this, we tested the stretch reflex in the biceps brachii using brisk tendon taps during a passive state and with voluntary activation. **METHODS** Experiments were performed on both the affected and contralateral sides of four hemispheric stroke individuals. The forearm was cast and immobilized. Bipolar surface EMGs were recorded from both the biceps and triceps brachii muscles. Tendon reflexes were elicited in both arms of each subject at approximately five second intervals using a tendon hammer. This device contained a load cell attached at the point of contact with the forearm to measure the force with which the biceps tendon was struck. For the stretch reflex trials, the subjects were first asked to remain at rest and were then asked to maintain a low level of force so as to evoke a minimal amount of EMG (background) activity. The magnitude of the surface EMG reflex responses were measured by calculating the root mean square (RMS) of the response. A least squares regression line was fit to the RMS EMG of the reflex response versus the (force)magnitude of the tendon tap (TT) response, and the slope of this regression line was compared for the involved and contralateral sides of each subject for both conditions. **RESULTS** Three of the four subjects exhibited a substantially greater slope on the affected side as compared to the contralateral side under resting conditions. In these three subjects, biceps activation did not significantly alter the slope derived from the spastic side. However, while exerting similar levels of voluntary activity, there was a significant increase in the slope of the regression line when compared to the resting condition, in the contralateral biceps of our tested subjects. **DISCUSSION** The main finding of our study is that background activation did not significantly increase the reflex response as a function of tap force in the affected side of tested stroke subjects. This data would suggest that the motoneuron (MN) pool on the spastic side is in a hyperexcitable state during rest, thus slight voluntary activation may not result in increased activation of the MN pool, as is observed on the non-spastic side.

The ablation of supraspinal structures modifies membrane properties of neonatal lumbar motoneurons.

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The spinal cord from neonatal rodents, isolated from the brainstem or cervical-thoracic levels to cauda equina, is a widely used experimental model for tracing the functional organization of spinal networks during development. In addition, intracellular recordings from single motoneurons using micro-nano electrodes defined membrane properties of post-natal spinal motoneurons, which preserve most of their pre-synaptic input. However, in isolated spinal cord preparations, the functional impact of descending modulatory input from suprapontine structures over lumbar motoneurons is underestimated. To explore this contribution, recently, a novel preparation of the whole CNS isolated from neonatal rats has been designed (Mohammadshirazi et al., 2023). Fast surgical procedures conducted on young newborns in the first two days of life allow the entire preparation to remain alive and functional for over 4 hours, with optimal histological preservation and tissue oxygenation. Selective surgical ablations at the level of superior colliculi changed numerous properties of the rhythmic and reflex motor output derived from spinal ventral roots, demonstrating the functional presence of subtle descending input from suprapontine structures already at birth. The novel preparation of the whole CNS kept at 27° C allowed, for the first time, to intracellularly define the membrane properties of single lumbar motoneurons that maintained descending input intact. Recordings were performed before and after a complete cervical transection that fully disconnected the spinal cord from the brain, revealing small changes in input resistance, without though affecting membrane resting potential, spike threshold and features of antidromic action potentials. Pooled data from numerous lumbar motoneurons recorded from both, the whole CNS in vitro and the isolated spinal cord, showed a significant increase of input resistance in motoneurons disconnected from supraspinal structures, likely reflecting the removal of a descending modulatory input over lumbar motor pools after ablation of supraspinal structures. Future studies will characterize the neurotransmitter pathways involved and anatomically trace the descending fibers impinging on lumbar motoneurons at birth. The current study provides a proof of concept about stable and consistent intracellular recordings from single motoneurons derived from a more intact isolated preparation of the whole CNS in vitro kept at 27° C. The presence of a functional albeit weak modulatory tone over lumbar motoneurons spurs the adoption of the whole CNS preparation to identify the evolution of supraspinal control over the motor output.

“I’m so excited” – why the neurosteroid anesthetic alfaxalone causes motor excitation and how to prevent it

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Neurosteroids increase open probability of GABA A channels, producing sedative and anesthetic properties. Neurosteroid anesthesia possesses many ideal features – rapid onset, short duration, wide safety index and low cardiovascular or respiratory depression. Once widely used, neurosteroid anesthetics have virtually vanished from human use, due to anaphylactoid reactions to the solvents used for these highly lipophilic compounds. In contrast, veterinary anesthesia uses Alfaxane, the neurosteroid alfaxalone solubilized in hydroxylpropyl-beta-cyclodextrin which is not suitable for human use for induction, for injectable anesthesia and sedation in a broad range of species. One shortcoming of Alfaxan when used without sedative premedication is that animals can show agitation – paddling, vocalization, twitching, reaction to external stimuli – on induction or recovery. Rats given alfaxalone consistently show this reaction.

We investigated the effects of alfaxalone on hypoglossal (XII) motor neurons in rat brainstem slices. Whole cell patch clamp recordings found that therapeutic levels of alfaxalone (100nM-25 uM) significantly depressed spontaneous glycinergic IPSC frequency without effect on amplitude, rise time or half width. In contrast, alfaxalone had no significant effect on spontaneous glutamatergic EPSCs. Evoked glycinergic IPSCs were also significantly altered at these doses, with significant reduction of amplitude and prolongation of decay time, but not paired pulse ratio, suggesting that alfaxalone selectively reduced glycinergic inhibition by postsynaptic modulation of glycine receptor activity.

We then investigated the effects of cannabinoid receptor (CBR) modulation of these alfaxalone effects. AM251 (1uM), a CB1R inverse agonist, or NESS0327, a CB1R competitive antagonist, blocked effects of alfaxalone on spontaneous IPSC frequency, evoked IPSC amplitude and decay time. WIN55,212-2 (300 nM), a CB1R agonist, mimicked evoked IPSC amplitude depression, and partially blocked effects of alfaxalone on evoked IPSCs.

These data are consistent with alfaxalone-induced endocannabinoid signalling indirectly activating CB1Rs, to reduce glycinergic receptor activity and presynaptic release of glycine onto XII motor neurons, suggesting that CB1R receptor modulation may be a way to reduce motor excitation during neurosteroid anesthesia.

Title: Neural mechanisms mediating lumbar locomotor-related V3 interneuron excitation of thoracic sympathetic preganglionic neurons and their autonomic targets

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Abstract:

Sympathetic preganglionic neurons (SPNs), located in intermediate laminae of T1-L2 spinal segments excite sympathetic tissues/organs that provide homeostatic and metabolic support during movement and exercise. We hypothesized and demonstrated that ascending lumbar V3 interneuron projections provide direct excitatory synaptic input onto thoracic SPNs throughout thoracic intermediate laminae. Optical stimulation (OS) of lumbar V3 interneurons in-vitro elicited action potentials in SPNs, demonstrating a functional connection between lumbar locomotor and thoracic sympathetic circuitry. We are now investigating neural mechanisms and pathways mediating V3 interneuron activation of thoracic SPNs and their autonomic target tissues/organs. We developed a neonatal in-vitro whole spinal cord (SC) preparation to enable simultaneous monitoring of lumbar and thoracic ventral roots, and thoracic sympathetic ganglia. Bath application of neurochemicals elicits rhythmic spinal sympathetic activity (~0.1 Hz) concomitantly with lumbar locomotor-like activity. Combinations of OS, lesioning and selective bath application of neurochemicals are ongoing to investigate neural mechanisms mediating this concomitant activation of spinal locomotor and sympathetic circuitry. In parallel, in the adult mouse in-vivo preparation, we are monitoring whole-body sympathetic responses during OS of lumbar V3 interneurons. Preliminary results demonstrate brief alterations in heart rate, depending on spinal depth and rostro-caudal site of stimulation in intact, anaesthetized mice. These preliminary findings support the need for further experiments to understand the capability and distribution of neurons within the SC integrating movement and autonomic functions. This will help develop SC stimulation strategies aimed at increasing excitatory drive for both motor and sympathetic functions, namely after spinal cord injury.

INVOLVEMENT OF 5-HT_{5A} RECEPTORS IN THE MODULATION OF MINIATURE GLYCINERGIC ACTIVITY OF LUMBAR MOTONEURONS.

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It is known that impaired inhibition is a feature of circuit deficits in the brain and spinal cord. One of potential factors of a shift in balance between excitation/inhibition is reduced neuromodulation. Using intracellular recording we have been studied the involvement of 5-HT_{5A} receptors in the modulation of miniature glycinergic activity (glymIPSP) of motoneurons on isolated superfused lumbar segments of the frog spinal cord. For pharmacological testing, 5-CT, a serotonin receptor agonist with high affinity for 5-HT_{5A} R, and SB69955, a highly specific 5-HT_{5A} receptor antagonist, were used. In a medium containing TTX, CNQX, D-AP5, bicuculline, application 5-CT (10 μM) led to a suppression of frequency by 86% and the disappearance of high-amplitude glymIPSPs (200–500 μV) at preservation of rare potentials with an amplitude of about 100 μV. . The addition of methysergide, a blocker of 5-HT_{1,2} receptors, to the medium reduced the average frequency of glymIPSPs by 67%, the frequency of high-amplitude events by 5 times and their average amplitude by 20%. These effects may indicate the participation of 5-HT_{5A} receptors in pre- and postsynaptic modulation in glymIPSPs of motoneurons. Application of 1 μM 5-CT led to a decrease in the frequency of glymIPSPs by 49% without a noticeable change in the amplitude of glymIPSPs, and the subsequent introduction of SB-699551, a selective antagonist of 5-HT_{5A} receptors, into the solution increased the frequency of events by 41%, which confirms the involvement of 5-HT_{5A} receptors in presynaptic modulation of glymIPSPs.

Immunofluorescence study showed the possibility of postsynaptic modulation of motoneuron activity through 5-HT_{5A} receptors. It is confirmed by the point-like fluorescence of the 5-HT_{5A}Rlike+ signal on the dendrites and bodies of labeled motoneurons. Double labeling with antibodies to the 5-HT_{5A} receptor and the Ca²⁺-binding protein, parvalbumin, revealed 5-HT_{5A}Rlike+ localization in the myelin sheath of dorsal and ventromedial funiculi fibers. Also in preparations after long-term stimulation of the ventral roots through suction electrodes when labeling motor neurons with biocytin, a bright 5-HT_{5A}Rlike+ signal was detected in the myelin of motor axons, dorsal root fibers entering the brain and individual fibers of the ventromedial funiculus. The involvement of extrasynaptic 5-HT_{5A} receptors in the functioning of feedback circuits of lumbar motoneuron activity, with the possible participation of glial elements in these circuits, is discussed.

A biophysical model of non-rigid motoneuron control

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Supraspinal regions control motoneuron activity by modulating their firing patterns to produce the desired muscle force. A substantial portion of these descending synaptic inputs projects to multiple motoneurons within a pool, imposing a rigid constraint on their behaviour. Recent studies, however, have challenged this longstanding view of motoneuron control, suggesting that it may be more flexible. For example, Marshall et al. (Nature Neurosci, 2022) reported that the responses of motoneuron pairs evoked by intracortical microstimulation varied in latency and magnitude depending on the cortical stimulation site. Additionally, Bräcklein et al. (eLife, 2022) observed that participants could learn to invert the derecruitment of motoneuron pairs, consistent with activity-dependent changes in motoneuron excitability. These observations, which conflict with the most rigid view of motoneuron control, can be potentially explained by neuromodulatory processes.

We present a computational model to understand how neuromodulation via persistent inward currents (PICs) could lead to a more flexible control of motoneurons. Our biophysical model builds on the Hodgkin-Huxley model and implements components that seek to capture neuromodulatory influences on motoneuron pool control. Our first objective is to characterise the mechanisms that facilitate the inversion of motoneuron derecruitment by dissecting the distribution and interplay of PICs and inhibitory mechanisms with synaptic input. All motoneurons receive common synaptic inputs as well as neuromodulatory and inhibitory signals, which drive the spiking activity of single motoneurons. Model parameters are optimised using recordings from large populations of motoneurons (n~130 per participant) from two leg muscles during isometric contractions. Our second objective is to model feedback control to identify the biophysical mechanisms underlying derecruitment inversion during volitional control. We will model the experiments by Bräcklein et al., in which participants had to control a cursor in two dimensions by modulating the activity of two motoneurons from the same muscle.

Therefore, we expect our model to clarify the unique contributions of neuromodulatory inputs to motoneuron control, in enabling a degree of flexibility that was unsuspected until very recently.

Greater force-reproduction accuracy when using auditory rather than visual feedback is not associated with differences in force steadiness or motor unit activity

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The proprioceptive sense of force can be assessed with a force-reproduction task that involves matching a target force with feedback of the applied force (target phase) and reproducing it without feedback (reproduction phase). In young adults, the sensory modality used to provide the feedback influences the force-reproduction performance, with greater accuracy when using auditory feedback relative to visual feedback (1). Our project aimed to determine if differences in force steadiness and motor unit activity in the two feedback conditions were associated with the accuracy of the applied force during the reproduction phase. We hypothesized that force steadiness and variability in motor unit discharge characteristics would be associated with more accurate force-reproduction performance.

Thirteen middle-aged (47 ± 4 yrs) and 13 older (72 ± 6 yrs) adults performed force-reproduction tasks at 5% and 20% of maximal index finger abduction force (MVC). Participants received either auditory or visual feedback of the applied force during the target phase. Accuracy during the reproduction phase was quantified by normalizing the applied force relative to that produced during the target phase. The outcome measures included the applied force, force steadiness (coefficient of variation [CV] for force), and motor unit discharge characteristics (CV for interspike interval [ISIs] and SD of the normalized cumulative spike train [CST]).

Preliminary results indicate that auditory feedback when compared with visual feedback was associated with:

- More accurate force reproduction at 5% MVC ($p < 0.001$)
- Greater CV for force at 5 and 20% MVC ($p < 0.001$)
- Similar CV for ISIs at 5% ($p = 0.095$) or 20% MVC ($p = 0.066$)
- Similar SD of the normalized CST at 5% ($p = 0.88$) or 20% MVC ($p = 0.080$).

None of the variables differed between age groups ($p > 0.09$). The force-reproduction accuracy was not associated with either force steadiness or motor unit activity ($r^2 < 0.01$; $p > 0.20$).

The absence of associations between force-reproduction accuracy and both force steadiness and fluctuations in the normalized CST indicates that performance was not related to common low-frequency oscillations in the discharge rate of the activated motor units (2). Instead, force-reproduction accuracy was related to feedback modality, being better when using auditory feedback than with visual feedback at the lower target force and likely reflecting differences in availability and calibration of sensory inputs with target force (1) and feedback modality (3).

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Spinal cord's mediatory role in the cortico-muscular connectivity during motor learning in humans

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While it is established that the excitability of the spinal cord network is altered during learning, discerning between plasticity arising from changes in descending drive and intrinsic spinal cord plasticity poses a considerable challenge in human model studies. Furthermore, our understanding of how the brain and spinal cord interact during motor learning and the resulting changes in their connectivity still needs to be completed. We recently showed that a 6-day motor sequence learning regimen leads to the creation of spinal networks and changes in the brain and spinal cord connectivity. In the current study, we investigated changes in the mediatory role of the spinal cord networks in the connectivity between motor cortex activity and executive muscular activity (ECR/FCR) in the periphery from the beginning to the end of the learning day. The brain's and spinal cord's activity was extracted using a mask created from the area that demonstrated learning on the first training day. Mediation analysis results showed that the activity of the spinal cord at the beginning of the training mediated the relationship between the brain and muscular activity. Still, in the last five training blocks, at the end of the first day of training, the mediatory role of the spinal cord disappears and the correlation between spinal activity and muscular activity increases. These findings further support the active involvement of the spinal cord and its neuroplasticity in motor learning.

Influence of dendritic PIC location on firing rate saturation in a heterogeneous motoneuron pool model

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Spinal motoneurons (MNs) can show the acceleration and saturation of firing output in response to ramp excitatory synaptic input. This nonlinear behavior of the MN has been known to be mediated by the activation of persistent inward currents (PICs) mainly generated over the MN dendrites. In this study, we investigated whether and how the dendritic location of PICs influences the degree of firing saturation following the firing acceleration during the ramp synaptic excitation to the MN. The computational approach was used to systematically evaluate this issue, varying the distribution of dendritic PIC locations over the heterogeneous MN pool comprising different MN types. A conductance-based two-compartment modeling framework was employed for a single MN that reflected type-specific electrophysiological properties such as the system input resistance, membrane time constant, signal attenuation between the soma and the dendrites, rheobase, afterhyperpolarization, and the effective PIC at the soma. 10 MN models were constructed to mimic the MN pool innervating the cat's gastrocnemius muscle. The degree of firing saturation was more significant in low-threshold MNs than in high-threshold MNs when the PICs were located closer to the somata in low-threshold MNs than in high-threshold MNs. The opposite result was also observed when the PICs were located more distal to the somata in low-threshold MNs than in high-threshold MNs. These results suggest that the spatial distribution of PIC channels across the MN pool may shape the systematic variation of firing rate saturation in heterogeneous pools of MNs.

Characterization of post-stimulation rebound excitation following spinal cord stimulation in cats

Amr Mahrous, Matthieu Chardon, Michael Johnson, Jack Miller, CJ Heckman

Spinal cord stimulation (SCS) has emerged as a promising therapeutic tool for spinal cord injury (SCI). While many studies focused on using SCS to evoke locomotion, little attention was paid to enabling standing which is a prerequisite of walking. In this study, we fully characterized a new type of response to SCS, a potent post-stimulation rebound excitation (PSRE) evoked immediately following a few-seconds stimulation train. PSRE is directed solely to extensor muscles and is controllable via changing stimulation parameters. Therefore, it has great clinical potential to assist postural movements.

We used the decerebrate cat model to avoid the suppressive effects of anesthesia, and combined EMG and force measurement in the hindlimb with intracellular and arrays recordings in the lumbar spinal cord. Stimulation trains were delivered via bipolar electrodes placed on the cord surface. We have tested different combinations of stimulation locations, amplitudes, and frequencies to find the optimum parameters for evoking PSRE. The best stimulation location to evoke PSRE varied slightly among animals but was generally restricted to lower lumbar segments (L5-L7). Stimulation frequencies of 10-40 Hz were tested with some preparations responding better to the lower or higher end of the range. Importantly, the amplitude and duration of PSRE were controlled by the stimulation intensity.

To investigate the mechanism of PSRE, we performed intracellular recordings of single motoneurons and induced PSRE while the cell membrane potential was varied. In 70% of recorded motoneurons, the peak depolarization during PSRE increased at hyperpolarized potentials indicating a synaptic origin, while the other 30% of cells had a more prominent PICs component. This indicates that PSRE is mediated by an excitatory synaptic current that recruits, and thus gets augmented by, motoneuron PICs. Furthermore, we recorded the activity of spinal interneurons using intracellular sharp microelectrodes as well as linear intraspinal multi-electrode arrays that showed changes in interneuron firing during PSRE, confirming the circuit origin of this behavior. To identify the type of afferents involved in eliciting PSRE, we used shorter stimulation pulses for SCS to activate proprioceptive afferents more selectively, as well as stimulation of cutaneous nerves in the hindlimb to activate cutaneous afferents. Smaller PSRE was successfully evoked in each condition, indicating that both types of afferents are probably involved.

Our study has thus characterized this novel postural motor response to SCS which has the potential to expand the applications of SCS in patients with SCI.

Maturation of abducens motoneurons involved in the angular vestibulo-ocular reflex during larval development.

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Keywords : extraocular, abducens, motoneurons, ontogenesis, vestibulo ocular reflexes, xenopus

During Gaze stabilization extraocular muscles produce reflexive compensatory eye movements to fix the image on the retina. Among such a complex sensorimotor transformation the vestibulo-ocular reflexes (VOR), generating compensatory eye movements in response to head motion, require vestibular inputs which are integrated by extraocular motoneurons.

In *Xenopus* larvae, the onset of the angular VOR (aVOR) is delayed compare to other visuo-vestibular reflexes. This delay could imply the existence of distinct parallel pathways in vestibulo-ocular networks, involving distinct motoneuron functional subpopulations.

Although relevant for understanding the establishment of these parallel pathways, the maturation processes of abducens motoneurons have not been elucidated. This led us to investigate how their properties differ during the establishment of the aVOR during pre-metamorphosis life.

Firstly, we performed electrophysiological recordings of unitary nerve discharges on *in vitro* isolated head preparations during passive head rotations. The abducens nerve discharge analysis, based on their spike amplitude, revealed different motor unit groups with distinct maturation patterns from the aVOR onset (stage 49-50) to a more mature stage (stage55-56). These results will be complemented with immunohistochemistry (Kv1.1, Kv3.1b, Cav3.3) and electron microscopy experiments, investigating the molecular phenotypes the axon myelination and neuronal size between these two stages.

In the future, we intend to perform the same approach on other extraocular motor nuclei, involved in ocular reflexes mature earlier, and in which we suspect different developmental patterns. These findings enhance our understanding of the development in neural networks controlling gaze-stabilizing ocular behavior in vertebrates.

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Poster Title: From bench to model: Exploring a MultiModal Markov Chain Monte Carlo (MM-MCMC) applied to Renshaw cell data from mice.

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Ionic-based models are essential to understanding neuron activity measured in laboratories. However, coupling multiple ion channels into a single model forms a system of ordinary differential equations with underdetermined coefficients. We have recently shown that a new parameter search technique, MM-MCMC, can quickly find solutions to these systems (Wang et al., 2022). Specifically, we have demonstrated that MM-MCMC can replicate the spiking output of a model of the stomatogastric ganglion neuron provided by Alonso-Marder (Alonso and Marder, 2019). MM-MCMC varied the conductance of the model's eight ion channels to reproduce its spiking and bursting behavior. This previous work informed our hypothesis: MM-MCMC can find appropriate conductances for real laboratory data. This work aims to generate models that accurately mirror all key characteristics of Renshaw cells from mice. To validate our hypotheses, we applied our MM-MCMC technique concerning Renshaw cell data provided by the Alvarez laboratory at Emory University. We developed two in-silico experiments using this data to determine if varying conductances alone can replicate experimental data. Each model sought to mimic the critical characteristics contained within the data over an array of applied currents. Experiment 1: We asked the community to give us the most likely channels that work. The first iteration of the model attempted to fit the data by varying conductance for a few selected channels: general sodium, potassium delayed rectifier, potassium-based afterhyperpolarization (K-AHP) channel, and leak. Experiment 2: We implemented all channels from Channelpedia (repository of channels), expanding our parameter space to sixteen channels with Hodgkin-Huxley representations. Furthermore, we varied the bounds of the search space to allow for better channel selection. Experiment 1 proved inadequate in describing the data. We presumed the addition of more channels could resolve these issues. With an improved cost function, greater conductance bounds, and a more extensive ion channel selection, experiment 2 failed to describe the data accurately. The results of the experiments indicate that a parameter space solely limited to conductances is inadequate. The next step in the work is to establish a model utilizing generic channels, allowing for variation in activation rate, half activation, activation slope, deactivation rate, half deactivation rate, and deactivation slope for each channel.

Transcutaneous spinal cord stimulation and primary afferent depolarization mechanism

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Introduction. Transcutaneous spinal cord stimulation (tSCS) is an emerging stimulation technique which consists of electrically stimulating the spinal cord via paravertebral and abdominal surface electrodes. This stimulation has the great advantage of evoking concomitant responses in all muscles of the lower limb, called posterior root muscle (PRM) reflexes, when the electrode is placed over the thoracic or lumbar level. These responses share some neurophysiological similarities with the H-reflex, evoked by peripheral nerve stimulation. It is known that the H-reflex is sensitive to inhibitory projections of the antagonist's afferences inducing primary afferent depolarization (PAD), mediated by interneurons that inhibit the release of neurotransmitters at the synapse between the Ia afferents and the motoneuron. The purpose of this study was to assess PAD phenomenon on H-reflex and PRM reflex in order to compare these two types of stimulation and obtain a better comprehension of the nature of PRM reflex.

Methods. Twelve subjects participated in 2 experimental sessions. During each session, EMG activity of the soleus muscle was recorded. Stimulations were delivered at the lumbar level and at the tibial nerve to elicit PRM and H-reflex, respectively. These soleus responses were conditioned by stimulation of the common peroneal nerve (inhibition) and of the femoral nerve (heteronymous facilitation) at different interstimulus intervals (ISIs): 0, 5, 10, 15, 20, 25, 50, 100 and 200ms before the test stimulation (H- or PRM reflex) for D1 and D2 inhibition, while for heteronymous facilitation, stimulation was applied at 0, 2, 4, 6, 8, 10ms after the stimulation (for tSCS, a delay of 10ms was applied for all ISIs). The degree of inhibition or facilitation was accessed as the amplitude of the conditioning response compared to the amplitude of the test response (i.e., H_{PSI}/H_{TEST} , H_{FAC}/H_{TEST} , PRM_{PSI}/PRM_{TEST} , PRM_{FAC}/PRM_{TEST}).

Results. Results did not show any statistical difference between H_{PSI}/H_{TEST} and PRM_{PSI}/PRM_{TEST} ($P=0.62$) not between H_{FAC}/H_{TEST} and PRM_{FAC}/PRM_{TEST} ($P=0.85$). An effect of the different tested ISIs was observed for the two types of stimulation ($P<0.001$ both for inhibition and heteronymous facilitation). For both stimulation types, the ISI that induced the greatest inhibition was the 5-ms ISI and the greatest facilitation the 6-ms ISI.

Conclusion. The responses evoked by tSCS are sensitive in the same way as the H-reflex regarding the PAD mechanism. This suggests that under the present stimulation conditions, both peripheral nerve stimulation and tSCS activate the same neural pathways.