International MotoNeuron Meeting Halifax 2014

Poster Abstracts

Poster Themes:
A – Firing
B – Plasticity
C – ALS
D – Development
E – Pathophysiology
F – Circuits

P1 – Poster Session 1: Monday June 16 & Tuesday June 17
P2 – Poster Session 2: Wednesday June 18 & Thursday June 19

The poster board numbers work in the following way:
Session – Theme – Board Number (E.g P1-A-1)

Poster session 1

- Set up – 7:30AM – 8:00AM, Monday June 16
- Removal – By 5:30PM, Tuesday June 17

Poster Session 2

- Set up – 7:30AM – 8:00AM, Wednesday June 18
- Removal - By 5:30PM, Thursday June 19

P1-A-1 A new approach to evaluate near synchronous inputs to motor units

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The tendency of motoneurons activated during contraction for synchronous discharge has been long observed by cross correlating the discharge times (short term synchrony) or time varying mean discharge times (common drive). Although looking through different perspectives and using different time frames, the concepts of "short-term synchronization" and "common drive", are both devised to be able to evaluate near synchronous inputs to motoneurons i.e. common EPSPs, common IPSPs, a mutual increase (or decrease) in discharge rates. A possible weakness common to both measures is that near coincident inputs that have an opposite sign cannot be quantified using either concept; furthermore, such inputs of conflicting signs would disrupt the measures used to evaluate short term synchronization and common drive. To overcome this obstacle the proposed method aims to provide a more robust measure to evaluate near synchronous inputs and possibly discriminate synchronous and anti-synchronous inputs to units by creating separate histograms selecting only the discharge times of motor units that result in shortened or lengthened ISIs compared to the previous ISI (Figure 1). Selecting such subsets of the discharge times in this way to produce histograms may provide the opportunity to discriminate the nature of the near synchronous inputs to the motoneurons.

P1-A-2 Deficits in synaptic transmission in two mouse models of Charcot-Marie-Tooth Disease Type 2D (CMT2D).

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Patients with CMT2D report slowly worsening idiopathic weakness and fatigue. The cause of these symptoms is unknown and treatment options are limited. We used two mouse models of CMT2D, which differ in disease severity, to test the possibility that defective synaptic transmission is an unrecognized feature of CMT2D that could point to new treatments. CMT2D, a length-dependent distal neuropathy, is caused by mutations in glycyl tRNA synthetase(Gars). The two strains studied carry different mutations(C201R and P278KY), and express mild and severe CMT2D, respectively. Voltage clamp studies show significant changes in synaptic transmission at the neuromuscular junctions (NMJ) in both strains. Quantal content was reduced, miniature endplate currents were unchanged, but frequency was lower, and release probability tended to increase at both C201R and P278KY synapses. Results suggest a presynaptic defect in NMJs of CMT2D mice, but ongoing work shows that these defects are not accounted for by fewer vesicles or release sites. Interestingly, data were obtained in a proximal muscle which, compared to distal muscles in both strains, otherwise shows only mild disease involvement. Also, at ~50 days of age P278KY (severe) mice have defects at >90% of NMJs, while only ~20% of C201R (mild) NMJs were clearly affected, but by 180 days the number of affected synapses in C201R mice was similar to P278KY. Together, results confirm the presence of synaptic dysfunction in CMT2D mice and support the notion that progressive synaptic dysfunction may be an unacknowledged component of CMT2D.

P1-A-3 Analysis of individual motor units in the decerebrate cat from multi-channel EMG

Francesco Negro[1], Chris Thompson[2], Michael Johnson[2], Matthew Holmes[2], Ales Holobar[3], Charles Heckman[2], Dario Farina[4]

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Reliable identification of individual motor units (MU) in the decerebrate cat has long posed a challenge for investigators. The classic approach has been to use intramuscular fine wires, however this method is limited by high selectivity that limits the stability of the MU action potential waveforms and yields a low number of recorded units per trial. In this study, we have developed a new approach for MU identification in cats, based on the decomposition of surface multi-channel EMG recordings. A matrix of 64 electrodes detects EMG activity from the gastrocnemius and soleus muscles in vivo and the convolution kernel compensation (CKC) technique is applied to extract the discharge activity of single MUs. This approach was validated by recording from gastrocnemius and soleus concurrently with both array and fine wire electrodes during stretch, TVR and sustained firing. The intramuscular EMG signals were decomposed using the EMGLAB program. This two-source validation allowed 69 units from 16 analyzed trials (4 ± 3 MUs per trial) to be identified with an average accuracy of 96 ± 8 %. A further advantage of the surface array is that we were able to identify the same MU over several trials, which

was not possible with intramuscular electrodes. In conclusion, this study proposes and validates a new technology for the study of motor unit behavior in decerebrate cat across a range of conditions.

P1-A-4 Comparison of intrinsic discharge characteristics and contractile properties of rat triceps surae motoneurons.

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We are investigating the relations between current needed to evoke discharge over the primary range, discharge frequency, and the forces produced at those frequencies in rat hindlimb motor units. Current-frequency, current-force, and force-frequency relations were assessed by measurements of force and discharge frequency during injection of triangular currents in ketamine-xylazine anesthetized rats. Force-frequency relations were also determined by injection of 0.5-second trains of current pulses. Discharge rates at the start of the primary range of the current-frequency relation are sufficient to produce most of a unit's force (>75% of maximum force) in most motor units. However, some units produce less force at the start of the primary range (as little as 30% of maximum). Corresponding current-force relations were found, with steep increases in force at near-threshold currents or more gradual increases, respectively. These results indicate that many rat hindlimb motor units resemble mouse motor units in developing most of their force at the start of the primary range (Manuel and Heckman, 2011), consistent with all-or-none usage. However, some rat motor units have properties consistent with force gradation by rate modulation.

P1-A-5 First systematic study of PICs in anesthetized adult mice using voltage clamp technique

Seoan Huh[1], Marin Manuel[2], CJ Heckman[1]

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In Amyotrophic Lateral Sclerosis (ALS) motoneurons progressively degenerate and die, however the mechanism of degeneration is still poorly understood. One potential mechanism is excitotoxic cell death, due to hyperexcitability of motoneurons. One possible source of hyperexcitability in ALS may be a change in the intrinsic properties of the cell; persistent inward currents (PICs) are essential in setting intrinsic excitability as they serve to amplify synaptic input. The SOD1 mouse was developed as a model of ALS and allows a unique window into the disease. For example, it has been shown in the SOD1 mouse that motoneurons have markedly increased PICs compared to those of wild type animals. However, it is unclear whether increased PICs in ALS are a direct influence of the disease or develop as a compensatory mechanism to the increased input conductance. In the current study the prevalence of PICs will be measured in vivo at different stages of disease progression using a single electrode voltage clamp technique. This will be the first systematic investigation of PICs in an anesthetized, un-paralyzed mouse preparation. PICs have been recorded in motoneurons of control animals during a slow voltage ramp. We predict that the PICs will be larger in SOD1 mice compared to those in control mice. As the disease progresses, however, we anticipate that the PIC increase will no longer be able to compensate

for the increase in input conductance of the diseased motoneurons, causing these motoneurons to become hypoexcitable.

P1-A-6 Torque vs frequency relationships when neuromuscular electrical stimulation is applied over the muscle belly, nerve trunk or alternated between the two.

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Introduction. When neuromuscular electrical stimulation (NMES) is applied over a muscle belly (mNMES) or nerve trunk (nNMES) contractions fatigue rapidly, due in part to high discharge rates of recruited motor units (MUs). To reduce discharge rates during NMES we developed interleaved NMES (iNMES) in which pulses are alternated between mNMES and nNMES sites. We propose that this reduces discharge rates (by half) because mNMES and nNMES recruit different MUs. Objectives. 1) describe torque-frequency relationships for mNMES, nNMES and iNMES and 2) estimate the overlap between MUs recruited by mNMES and nNMES. Methods. Dorsiflexion torque-frequency curves were generated from data collected using mNMES (tibialis anterior), nNMES (common peroneal nerve) and iNMES. Two 2 s trains of each type of NMES were delivered at 10, 20, 30, 40, 60, 80 and 100 Hz in 10 participants. NMES was set to generate 20% of a maximal voluntary isometric contraction (MVIC) at 20 Hz. The sum of torque produced by nNMES and mNMES was used to predict torque that would be produced by iNMES if there was zero MU overlap between the two sites. Results and Conclusions. At 100 Hz, nNMES and mNMES produced ~30% MVIC torque while iNMES produced 51% MVIC. Torque produced by iNMES was the sum of that produced by nNMES and mNMES, consistent with zero overlap of MUs recruited by the two sites. iNMES may reduce contraction fatigue by reducing MU discharge frequencies. Further, iNMES may produce contractions with less discomfort since more torque is produced by a given NMES intensity than during nNMES or mNMES.

P1-B-7 Sensitization of lumbar motoneurons by the ""pain mediator"" bradykinin

Frédéric Brocard[1], Mouloud Bouhadfane[2], Attila Kaszas[3], Laurent Vinay[2]

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Bradykinin (Bk) is a potent pain mediator. Much has been reported on the action of Bk on the sensory system, but its effects on the motor circuit remain unknown. The present work aims at determining whether Bk may sensitize lumbar motoneurons of neonatal rats. In the presence of Bk, most motoneurons (~77%) displayed a concentration-dependent (EC50=680nM) depolarization of the membrane potential and an increase in excitability as evidenced by a lower rheobase and a greater ability to generate self-sustained spiking. Calcium imaging and voltage clamp recordings showed that the depolarization was associated with both a rise of [Ca2+]i and the emergence of an inward current insensitive to TTX and TEA, indicative of a direct postsynaptic action of Bk. Overall the Bk action was prevented or mimicked by the selective B_2 receptor antagonist or agonist, respectively. Substitution of extracellular Na+ or chelation of [Ca2+]i prevented responses suggesting a role of a Ca2+-activated nonselective cation current (ICaN). In line with this, the blockade of ICaN by rhutenium red inhibited the

depolarization. The activation of ICaN involves a G-protein-dependent process as GDPβS abolished Bk responses. Examination of the downstream signaling pathway reveals that inhibition of phospholipase C, IP3 receptor or calmodulin abolished the Bk-induced depolarization. In sum, Bk may contribute, at least in part, to hyperalgesia (withdrawal flexor reflex used as a surrogate pain model) by sensitizing final motor outputs through a direct postsynaptic action of the pain mediator onto motoneurons.

P1-B-8 Evaluation of motor unit recruitment and discharge rates influence on sex-related differences in force steadiness

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The ability to maintain a steady force is less in women than men and decreases with age. This decline in force steadiness ability is associated with maximal strength which is a consequence of motor unit (MU) activity. However, when strength is equal between young and old adults force steadiness does not differ even though MU activity is dissimilar. No study has determined the effect of MU activity on force steadiness in men and women matched for strength. Six young men and 6 women matched for strength (194 ± 25N and 189 ± 16N, respectively) participated. Voluntary activation, was similar between men (98 ± 2%) and women (99 ± 2%). MU activity was recorded via intramuscular fine wire electrodes in the biceps brachii. Force steadiness, was evaluated over a 5sec ramp and 7.5sec steady state isometric elbow flexion contraction for target forces of 2.5, 5, 10, 15 and 25% of MVC. Steadiness improved with increases in force (2.5%MVC, CV=3.0±1.0%; 25%MVC, CV=0.6±0.2%); and did not differ between sexes. Approximately 200 MU trains were recorded. Average recruitment thresholds were higher in women compared with men. Higher recruitment thresholds were paralleled by higher discharge rates during the ramp phase. At 2.5%, discharge rates were ~0.5Hz higher in women, while at 25% they were ~2.5Hz higher. During steady state, MU discharge rates remained higher in women. Preliminary findings suggest the recruitment thresholds of MU are higher in women and that these units discharge at higher rates during the ramp and steady state contractions.

P1-C-9 The Involvement of Motoneuron Size in Amyotrophic Lateral Sclerosis

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In ALS, spinal motoneurons with various sizes show distinctive degenerating rates in which large motoneurons are particularly vulnerable. Data from the G93A-SOD1 mouse model suggests that ALS spinal motoneuron size is increased since neonatal stage accompanied with abnormal electrical properties. Motoneuron size enlargement may be the consequence of initial responses to morbific prodromal factors such as altered expression level of proteins related to survival and translation. From cell physiology standpoint, larger soma size indicates intensive demanding of cytoskeleton proteins and vigorous metabolism requirement which may exacerbate the aggregation-prone protein homeostasis system in ALS and accelerate degeneration. We hypothesize that the large soma size might account for the selective susceptibility of motoneurons to ALS-causal toxicities. S6K is selected for motoneuron size manipulation due to its roles in protein translation control and involvement in mTOR pathway. Our

results showed that inhibiting S6K can maintain the motoneuron size of G93A mice from abnormal enlargement at early stage. We will apply the strategy to adult G93A mice and examine the electrical properties of the maintained size motoneurons, as well as assess the effect of S6K suppression to disease progression and lifespan.

P1-C-10 Excitatory and inhibitory synaptic boutons and dendritic vacuolization in adult motoneurons of SOD1-G93A mice.

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A striking pathological feature in SOD1 mice is the presence of vacuoles in the soma, the axon and also the dendrites of their motoneurons (Dal Canto & Gurney 1994). We show that dendritic vacuolization of spinal motoneurons has already started at P40 in SOD1-G93A mice and that it precedes the denervation of muscle fibers. Afterward, dendritic vacuoles dramatically grow throughout the disease to reach diameters as large as 10 micrometers at P90. The density of excitatory (VGLUT1 and VGLUT2) and inhibitory (VGAT) boutons that contact the dendritic tree of motoneurons is unchanged at P40-50 in mSOD1 mice compared to WT mice. However VGLUT2 and VGAT boutons tend to cluster at loci where dendritic vacuolization take place. Our data suggest that there might be a causal link between synaptic activity and dendritic vacuolization in spinal motoneurons of mSOD1 mice.

P1-C-11 Dynamic profile of calcium signaling in hypoglossal motoneurons from adult wild-type and tgSOD1 mice

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Amyotrophic lateral sclerosis is a progressive neurodegenerative disease that affects specific motoneuron populations like hypoglossal and spinal motoneurons, while others e.g. those of the oculomotor system are spared. The pathophysiological basis of this selective vulnerability, which is preserved in a transgenic mouse model of amyotrophic lateral sclerosis (SOD1G93A), and the mechanism of neurodegeneration in general is unknown. Hyperexcitability and calcium dysregulation have been proposed by others on the basis of data out of juvenile mice. No studies have been done with symptomatic mice following disease progression to the disease endstage. Here, we present data from a new brainstem slice preparation for whole cell patch-clamp recordings and single cell fura-2 calcium imaging to study motoneurons in adult wildtype and SOD1G93A mice up to disease endstage. We analysed disease-stage-dependent electrophysiological properties and intracellular Ca2 handling of vulnerable hypoglossal motoneurons in comparison to resistant oculomotor neurons. Most important, we revealed a remodelling of intracellular Ca2 clearance within vulnerable but not resistant motoneurons at disease endstage. Taken together, our study indicates a clear deficit in Ca2 clearance in endstage motoneurons and suggests that a stabilisation of motoneuron Ca2 handling might be an attractive option for future therapeutic strategies.

P1-D-12 Activity-dependent synaptic plasticity at VLF-motoneuron synapses depends on the flexor-extensor nature of motoneurons and on mGluR expression during development

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In neuronal networks, synaptic strength is not constant but depends on the past activity of the synapse. This change in synaptic efficacy is called activity-dependent synaptic plasticity (ADSP). In this study, we investigated the plasticity occurring at the synapses made by reticulospinal and propriospinal neurons that project to the spinal cord through the ventrolateral funiculus (VLF) on lumbar motoneurons. Spinal cord slices from mice were studied at two developmental stages, 1-4 and 8-12 postnatal days. EPSC plasticity was examined by stimulating VLF axons at 50Hz during 2s (high frequency stimulation, HFS). Three different responses were observed following HFS: short-term depression (STD), long-term depression (LTD) or no significant effect. Interestingly, the occurrence of these different forms of ADSP is dependent on the developmental stage of the mice and is function of the functional type of motoneurons (flexor or extensor). We also found that ADSP modulation by metabotropic glutamate receptor agonists is function of the developmental stages of motoneurons. The impact of the ADSP in VLF-motoneuron synapses was tested on the synaptic transmission between commissural neurons and motoneurons. Finally, we performed experiments in the in vitro spinal cord preparation. Overall, our results reveal for the first type that the extensor or flexor nature of motoneurons determine the synaptic plasticity expressed in VLF synapses and that this plasticity is function of the developmental stage of the motoneurons.

P1-D-13 Emergence of motor circuit activity

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In the developing nervous system, ordered neuronal activity patterns can occur even in the absence of sensory input and to investigate how these arise, we have used the model system of the embryonic chicken spinal motor circuit, focusing on motor neurons of the lateral motor column (LMC). At the earliest stages of their molecular differentiation, we can detect differences between medial and lateral LMC neurons in terms of expression of neurotransmitter receptor subunits, which are reflected in subtly different activity patterns recorded by single-cell patch clamp of medial and lateral LMC neurons. Using a combination of patch-clamp recordings in single neurons and calcium-imaging of motor neuron populations, we demonstrate that inhibition of nicotinic, muscarinic or GABA-ergic activity has profound effects on motor circuit activity during the initial stages of neuromuscular junction formation. Finally, by analysing the activity of large populations of motor neurons at different developmental stages, we show that the asynchronous, disordered neuronal activity that occurs at early stages of circuit formation develops into organised, synchronous activity evident at the stage of LMC neuron muscle innervation. In light of the considerable diversity of neurotransmitter receptor expression, activity patterns in the LMC are surprisingly similar between neuronal types, however the emergence of patterned activity, in conjunction with the differential expression of transmitter systems likely leads to the development of near-mature patterns of locomotor activity by perinatal ages.

P1-E-14 Reorganization of VGLUT1 Synapses on Spinal Motoneurons Following Peripheral Nerve Injury

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Motor deficits that remain after nerve injury and successful regeneration are the result of permanent alterations that occur in central spinal cord circuits. For example, many proprioceptive IA afferents (VGLUT1-IR boutons) retract from motoneurons (MNs) after injury resulting in loss of stretch reflexes. However, electrically evoked EPSPs are still elicited in regenerated MNs by stimulation of remaining IA afferents. How these residual inputs are capable of inducing electrical EPSPs but fail to transmit stretch signals to MNs remains unknown. To better understand these differences we analyzed the normal distribution of IA afferent inputs onto single motoneurons and mapped their reorganization after injury. Adult rats underwent complete tibial nerve transection followed by microsurgical reattachment. After muscle reinnervation, one year later, regenerated MNs were intracellularly recorded and filled with neurobiotin to map VGLUT1 contacts along the entire dendritic arbor. Injured MNs showed 62% depletion in overall VGLUT1 terminals when compared to uninjured MNs. Uninjured cells had a proximal distribution of VGLUT1 contacts with approximately 50% of these terminals tightly clustered in the first 300µm of dendrite, which was lost after injury resulting in a more uniform synaptic distribution along the dendritic arbor and less clustering of synapses. We conclude that proximal clustering of IA afferent synapses is essential for transmitting natural stretch information to MNs.

P1-E-15 Stroke-related changes in motor unit firing behavior during fatiguing contractions

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Motor unit rate modulation is altered after stroke and may contribute to stroke-related fatigability of limb muscles. This study determined the effects of a single dose of a serotonin norepinephrine re-uptake inhibitor (duloxetine), a serotonin antagonist (cyproheptadine), or a placebo on motor unit firing behavior before and after fatiguing contractions in chronic stroke. Five stroke survivors (56±10yrs) and 5 controls (59±10 yrs) performed 5 ramp-and-hold knee extension contractions (5% to 20% of maximal voluntary contraction, MVC) for 16 s before and after a fatiguing protocol (intermittent isometric contraction at 30% MVC until task failure) for three conditions: after a single dose of duloxetine, cyproheptadine, or the placebo. Motor unit discharge rates were extracted from surface motor unit potentials recorded from the vastus lateralis using a multi-channel grid of 64 EMG electrodes. Motor unit numbers identified during the ramp and hold pre and post the fatigue for stroke subjects were 36 and 33 respectively, and 40 and 33 for controls. For the stroke subjects after fatigue, the mean motor unit discharge rates increased with duloxetine (9.6 vs 10.9 pps), decreased with cyproheptadine (9.9 vs 8.8 pps)and decreased during the placebo session (9.3 vs 9.1 pps). Discharge rates in controls increased for all 3 conditions (duloxetine 9.5 to 10 pps; cyproheptadine 9.9 to 11.4 pps; and placebo 8.6 to 10.5

pps). These results suggest that paretic motor unit firing behavior following fatiguing contractions is adaptable and sensitive to neuromodulatory input.

P1-E-16 Evidence of systemic depolarization & prolonged Ia EPSP in α motoneurons of hemispheric stroke survivors

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Muscle spasticity is one of the major impairments that limit functional recovery in stroke survivors. Clinically the muscle reacts quicker to a stretch input as if primed. Two hypothesized neural mechanism for spasticity are that α motoneurons are systemically depolarized and/or have larger prolonged Ia EPSPs since both mechanisms would increase the likelihood of α motoneurons discharge. To test the first hypothesis we estimated the response latency of motor units (MU) triggered by position controlled transient taps (Amp: 0.5-1-2 mm) delivered to the distal tendon of the passive biceps brachii muscle of both sides of stroke survivors. To test the second hypothesis we constructed the CUSUM of the PSTH of MUs recorded from both sides of 5 stroke survivors. Specifically, we asked our subjects to generate a steady MU discharge during very low isometric force tasks of the biceps brachii while a computer controlled tendon tapper precisely stretched the distal tendon of the biceps brachii. Our first results show that the latency of the motor unit discharge with respect to the tendon tap input was significantly shorter in the passive spastic muscle as compared with the contralateral muscle and this effect was consistent across multiple tap amplitudes. Our second results show that Ia EPSPs showed a prolonged time course on the spastic side on 4/5 subjects suggesting the influence of PICs. The findings provide evidence for the contribution of hyper excitable α motoneurons to muscle spasticity and also provide a reliable tool to assess spasticity post-stroke.

P1-E-17 A revisitation of motor unit firing properties in Parkinson's disease

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It has recently been recognized that a hallmark of Parkinson's disease (PD) is the presence of an abnormal pattern of muscle activity when performing voluntary movements. While the limbs of healthy adults produce fused muscle contractions of a stereotypical duration and amplitude, patients with PD exhibit a fractionated EMG consisting of multiple bursts of highly variable duration. This fractionated EMG has been shown to manifest in the earliest stages of the disease, and yet this abnormal pattern is not fully restored to normal by conventional dopaminergic therapy. While there is a considerable body of literature describing individual motor unit firing behavior in PD, recent technological developments and advances in our understanding of PD pathophysiology demand a renewed investigation of this field. Thus, the purpose of this study is to examine motor unit recruitment, rate modulation and firing properties in mild-moderate PD patients compared to healthy age and handedness-matched controls. The current study builds upon previous literature by comparing motor unit behavior in both flexors and extensors, using both conventional intramuscular EMG as well as recently-developed high-density surface EMG arrays using the convolution kernel compensation (CKC) algorithm for surface EMG

decomposition. Preliminary data suggest that motor units in PD tend to be recruited later than healthy controls and exhibit highly variable discharge rates as well as an inability to scale discharge rate with an increase in torque.

P1-E-18 Electrophysiological properties of medial motoneurons in a mouse model of mild SMA

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Spinal muscular atrophy (SMA) is a pediatric autosomal recessive neuromuscular condition caused by low levels of the survival motor neuron (SMN) protein. Patients range in disease severity from type I (most severe, neonatal mortality) to type III (mild, affects motor control in older children and adults). Newly created mouse models for mild SMA recapitulate symptomology of human patients, including motor axon loss and motor control deficits. The disease arises from deficient levels of the ubiquitously-expressed SMN protein, yet it results in the specific loss of motoneurons. This loss is preceded by dramatic changes in motoneuron excitability and loss of afferent inputs. In our current work, we compare the electrophysiological properties of mild SMA mice at just over one week of age (P8-9), long before any overt symptoms appear (lifespan ~ 2 years). Whole cell patch clamp was performed on large neurons in the medial motoneuron pools in 350 um thick transverse spinal cord slices from lower thoracic to upper lumbar regions (T10 - L2). Properties were analyzed in voltage clamp (for persistent inward currents) and in current clamp (for action potential / after hyperpolarization characteristics, frequency-current relationships, etc). In the mild SMA mouse, the etiology of motoneuron loss and time course of electrophysiological changes vs. functional losses can be much more clearly delineated, revealing new insights into disease progression and potential windows for treatment before motoneurons and /or motor axons are lost.

P1-E-19 Frog Spinal Motoneuron Synaptic Activity and its Modulation by Group II Metabotropic Glutamate Receptors.

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Several potent and subtype-selective ligands of group I, II, and III mGlu receptors are under clinical development and are among promising drugs in the treatment of neurological disorders. Group II mGluRs (mGluR2/3) are found in many stations of the pain neuraxis. We have carried out experiments on the isolated spinal cord of the frog Rana ridibunda. Previously we studied the influence of a mGluR2/3 antagonist, EGLU, on miniature synaptic activity (mPSPs) of frog lumbar motoneurons. It has been shown that group II mGluR modulation of mPSPs is presynaptic (Karamian et al., 2008). In the present study, bath application of 1 µM of DCG-IV, a potent agonist of mGluR2/3, produced a decrease of frog motoneuron mPSP frequency by 18-40% without significant changes in their average amplitude. This suggests a presynaptic mechanism of transmission release modulation. DCG-IV, 2-4 µM, resulted in a considerable increase in frequency and amplitude of sPSPs or production of motoneuron action potentials when spontaneous synaptic activity was recorded. This may reflect DCG-IV effects on presynaptic and postsynaptic NMDA receptors (Ishida et al., 1993). Using the double fluorescence

immunohistochemistry and 3D reconstruction data of frog motoneuron synaptic inputs we analyzed the localization of mGluR2/3 immunoreactivity in the frog spinal cord and showed possible synaptic structures which may possess group II mGluRs. Supported by the BR Program of the RAS Presidium No. 7.

P1-E-20 Post-activation depression changes following intramuscular injection of Botulinum toxin in post-stroke spastic patients

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Local injection of Botulinum toxin (BTx) is the referent treatment of focal spasticity. BTx acts by blocking the release of acethylcholine from motoneuron terminals at the neuromuscular junction and so, transiently relieves the excessive muscle contraction. BTx is assumed to have also a central action. In particular, by blocking also the intrafusal muscles fibers endplates, BTx might reduce the discharge from muscle spindles, which might in turn affect the functioning of the central motor networks. The aim of the present study is to see whether BTx treatment affect the synaptic transmission at the Ia/motoneuron synapses. For this purpose, Post-AD of H-reflex, assessed as frequency related depression of soleus H-reflex, was investigated before and 3, 6, 12 weeks after BTx-injections in soleus in 8 chronic hemiplegic patients presenting lower limb spasticity and requiring BTx injection in the calf muscles. H-reflex amplitude was analyzed in response to electrical stimulation of the tibial nerve at 0.1Hz and 0.5Hz. Post-AD was quantified as the ratio H0.5Hz/H0.1Hz. The Post-AD was significantly reduced in the affected side compared to the non-affected side before BTx injection. Three weeks after injection, the Post-AD was reinforced in the paretic leg and significantly higher than in pre-injection. BTx-treatment restores the Post-AD of soleus H-reflex in post-stoke paretic patients. As Post-AD amount is correlated to the severity of spasticity, it can be assumed that BTx's effectiveness in post-stroke rehabilitation is also due to induced-changes in spinal motor networks.

P1-E-21 Asymmetries in vestibular drive to ocular motoneuron pools following hemispheric stroke.

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Deficits in eye movements are common post-stroke, however the role that vestibular pathways play in mediating these deficits in ambiguous. We recently demonstrated that there are asymmetries in vestibular drive to neck motoneurons (MN) following hemispheric stroke. Given the influence vestibular pathways have on ocular MN activity, we hypothesized that there would be asymmetries in vestibular drive to the ocular MN following hemispheric stroke. In this study we assessed ocular vestibular evoked myogenic potentials (OVEMPs), a vestibular-dependent biphasic surface potential representing the modulation of ocular MN activity, in 10 hemispheric stroke survivors to quantify the relative levels of ascending vestibular drive to the clinically-affected (CA) and clinically-spared (CS) ocular MN pools. Side-to-side differences in normalized OVEMP amplitude were expressed as an asymmetry ratio (AR), a proxy

for the relative amount of vestibular drive to the CA and CS ocular motor nuclei. CA OVEMPs were larger than CS VEMPs in 9/10 subjects, AR (%): 20.4 ± 19.6. The mean OVEMP amplitude for the CA and CS sides were 3.6 ± 2.3 and 2.1 ± 0.7, respectively. The population CA OVEMP was larger than the population CS OVEMP, however the difference was not significant (n=11, paired t-test, p=0.07). Vestibular drive to ocular MN pools is asymmetric in hemispheric stroke survivors, supporting our hypothesis that there is an imbalance in ascending vestibular drive. We speculate this imbalance is a consequence of the disruption of corticobulbar fibers that project to the vestibular nuclei.

P1-E-22 Increased central activation during eccentric contractions is associated with decreased spinal inhibition during muscle lengthening in human spinal cord injury

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Recent data demonstrate individuals with incomplete spinal cord injury (SCI) centrally activate the knee extensors more during eccentric maximal voluntary contractions (MVCs) than during isometric or concentric MVCs (submitted manuscript). This pattern of activation is markedly different than that of healthy adults, who often demonstrate central activation deficits only during eccentric MVCs. The normal inhibition in healthy adults of Ia-α motoneuron excitability during muscle lengthening may partially explain such deficits. In light of these findings, the current study aims to assess central activation during isometric and anisometric MVCs of the plantarflexors, and to compare modulation of soleus H-reflexes during isometric, shortening, and lengthening conditions in SCI and healthy subjects. Preliminary results (n=5 SCI, 3 controls) demonstrate that in SCI subjects, similar to the knee extensors, central activation ratios (peak voluntary T/peak superimposed T) are higher during eccentric MVCs (0.78±0.13) than isometric (0.64±0.17) or concentric (0.55±0.29). Consistent with previous data, controls demonstrate an inhibition of H-reflexes during muscle lengthening (see attached figure). In contrast, SCI subjects demonstrate similar maximal H-reflex amplitudes across all conditions (figure), indicating decreased spinal inhibition during muscle lengthening compared to controls. These data suggest contrasting patterns of muscle activation between SCI and healthy subjects may be related to differences in modulation of Ia-α motoneuron excitability across contraction types.

P1-F-23 Synaptic mechanisms underlying C bouton-mediated modulation of motoneurons

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C-bouton synapses are a prominent class of neuromodulatory input to motoneurons (MNs) that contribute to task-dependent regulation of MN excitability. Although it is known that C-boutons arise from Pitx2 spinal interneurons (INs), the synaptic mechanisms underlying their modulatory effects remain unclear. We have therefore utilised conditional expression of channelrhodopsin in Pitx2 INs to directly examine the effects of C-bouton activation on individual MNs. We injected a Cre-dependent adeno-associated virus expressing channelrhodopsin (AAV.lsl.hChR2-YFP) directly into the lumbar spinal cord of neonatal Pitx2::Cre;ROSA.lsl.tdTom mice. After 6-10 days, spinal cords were isolated and a longitudinal, diagonal slice performed, removing the dorsal horn on one side of the cord such that both

the central canal and motor pools are near the surface of the tissue. In this preparation Pitx2 INs were stimulated using blue light (480nm), while whole-cell patch-clamp recordings were obtained from either Pitx2 INs or MNs. Depolarising currents were observed in Pitx2 INs in response to light (74.1±15.8pA, n=36). Light also increased the firing rate of tonically active Pitx2 INs, while stimulating spiking in INs that were not active at rest. Preliminary data suggests that activation of Pitx2 INs leads to a reduction in the after-hyperpolarisation and a decrease in the current threshold for action potential generation in MNs. These data support that activation of C-boutons leads to an increase in the excitability of MNs via multiple cellular mechanisms.

P1-F-24 Control of Motoneuron Excitability Via C-boutons

Adam Deardorff[1], Shannon Romer[1], Robert Fyffe[1]

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Regulation of motoneuron excitability can be mediated by various neuromodulatory systems that regulate both inward and outward currents to generate firing rates appropriate for specific motor commands. One important cholinergic modulatory locus, the C-bouton, may contribute both to state-dependent and pathologic alterations in motoneuron firing rate. Recent work to identify the source and functional significance of C-boutons has prompted new questions regarding the molecular actions that control motoneuron excitability via these enigmatic synapses. Data from our laboratory and others define a unique complement of proteins and structures that exist at the C-bouton synapse. The molecular/cellular ensemble includes presynaptic active zone proteins, postsynaptic m2 acetylcholine receptors, and various ion channels including SK2/3, Kv2.1 and CaV2.1/2.2 as well as a postsynaptic subsurface cistern. Here, using our current understanding of both anatomical and physiological properties, we propose a cholinergic mechanism of state dependent modification of motoneuron excitability through an effect on outward potassium currents at the C-Bouton. Our desire in presenting this hypothesis is to aid in the development of new experimental strategies to answer questions regarding molecular events that occur at the C-bouton, as well as to understand their impact on human health and disease.

P1-F-25 Stromatoxin-Sensitive Currents Maintain Firing Rate and Increase Excitability in Postnatal Rat Lumbar Motoneurons

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Despite their relative abundance on the motoneuron soma and proximal dendrites, the physiological role of specific delayed rectifier potassium ion channels (e.g. Kv2.1) in mammalian motoneurons is unknown. The tarantula toxin stromatoxin (ScTx1) has been identified as a potent gating modifier with a high affinity for Kv2.1, shifting the activation to more positive potentials thus functionally blocking the channels (Escoubas et al, 2002). Here, we show, through whole cell patch clamping of motoneurons in rat postnatal lumbar spinal cord slices, that ScTx1-sensitive currents maintain motoneuron repetitive firing properties. Specifically, 100nM ScTx application causes a decrease in motoneuron firing rate, rheobase and F-I gain. These results are consistent with observations in other cell types where Kv2

channels relieve interspike Na channel inactivation to maintain repetitive firing (Guan et al 2013, Johnston et al 2008).

P1-F-26 Decoding static hand position from human brain activity patterns

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The brain uses a range of afferent proprioceptive signals to produce accurate movements. However, it is unknown how these signals are integrated and applied. In this study, we investigated how the brain monitors the current position of the hand after passive movements. Participants (n=13) were lying in a magnetic resonance imaging (MRI) camera (3T). They were asked to stay relaxed and attend to the position of their left hand, while it was moved passively into different positions (~20° flexion, ~20° extension, and neutral). The functional MRI data were pre-processed using SPM8. We then applied a multi-voxel pattern analysis (MVPA; LIBSVM implementation) to decode the position of the hand (flexion vs. extension) for the whole brain ($p<0.05$ corrected for multiple comparisons using family-wise error rate, FWE). We also performed small-volume corrections (SVC; $p<0.05$ FWE) in regions of interest derived from the literature. Hand position could be decoded from multiple brain regions. In the contralateral (right) sensorimotor cortex, we found three significant clusters. Furthermore, we found significant peaks in the right parietal operculum (corresponding to the secondary somatosensory cortex), the supplementary motor area, and the right inferior frontal cluster (Area 44). Passive changes in hand position modulate the patterns of cortical activation in several areas, such that the position of the hand can be decoded from activity in these regions.

P1-F-27 Expiratory motoneurons: bi-functional respiratory and locomotor spinal neurons?

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The central nervous system contains neural networks that can generate rhythmic motor drive in absence of sensory feedback. These neural networks are commonly called central pattern generators (CPGs) and are involved in many vital functions, such as locomotion or respiration. In certain circumstances, these neural networks must interact to produce a motor behavior adapted to environmental constraints and to satisfy the basic needs of an organism. In this context, our work aimed to explore the neurogenic mechanisms engaged in the coordination between the medullary respiratory networks and the spinal locomotor CPGs. To address this question, we used an isolated in vitro brainstem-spinal cord preparation from neonatal rat in which the respiratory and the locomotor networks were kept intact. Using electrophysiological, pharmacological and neuroanatomical approaches, we studied the mechanisms involved in the coordination between locomotor and respiratory motor rhythms. In a recent previous paper, we reported the existence of an ascending excitatory influence from spinal locomotor CPGs to the respiratory networks, acting particularly on the parafacial respiratory group during locomotion (Le Gal et al., 2014, PLoS ONE). Here, we show that this locomotor ascending influence also modulates the activity of thoracic and lumbar expiratory

motoneurons (Mn, see illustration). These data provide the first evidence for the existence of bi-functional respiratory-locomotor neurons in the newborn rat spinal cord.

P1-F-28 The roles of central synaptic activity in shaping the morphology and activity of hypoglossal motor neurons during development.

Peter Noakes[1] M.J. Fogarty*, R. Kanjhan*, M.C. Bellingham and P.G. Noakes.

[1]The University of Queensland

We have investigated the roles of glycinergic and GABAergic synapses onto motor neurons (MNs) and how MNs respond when such connections are perturbed. To achieve this, we have used mice lacking glycinergic, GABAergic and mice lacking both glycinergic and GABAergic transmission to study the development of hypoglossal (XII) MNs. Brainstem slices from wild type and mutant mice were used to record spontaneous synaptic currents from XII MNs that post recording were filled with Neurobiotin. These slices were then immuno-stained for the distribution of excitatory glutamergic and inhibitory GABAergic synapses. In mice lacking glycinergic or GABAergic synapses, XII MNs responded by increasing the complexity of their dendritic branches, and the number of dendritic spines. We also observed that excitatory glutamergic synapses were increased, but not inhibitory synapses. Physiological analyses showed increased excitatory post-synaptic potentials and frequency of action potential generation in XII MNs from mice lacking glycinergic or GABAergic transmission. However, we saw no compensatory increases in inhibitory transmission in either mutant. Analyses of XII MNs in mice lacking both glycinergic and GABAerigic transmission showed similar changes. In all three mutant mice, XII MNs had become more active. This lead to few numbers of XII MNs, post the period of naturally occurring neuronal death. Our results suggest that glycinergic and GABAergic synaptic regulate XII MN morphology, network properties and their final number.

Poster Session 2:

P2-A-29 Development of method to record motor unit activity during voluntary contractions in the rat

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The main experimental approach used to study motor neuron activity during natural behavior involves motor-unit recording in human subjects. While access to neural activity in an awake, intact, and unanesthetized preparation has provided important insights into motor neuron function, application of powerful pharmacological and electrophysiological tools is clearly limited in human subjects. Therefore, we are developing a system that enables reliable recordings of motor unit activity in awake rats while they make controlled, voluntary muscle contractions like that typically performed by human subjects in motor unit studies. Rats were comfortably secured in an apparatus with their hind-paw attached to an isometric force transducer. Then, using an automated operant-conditioning paradigm, rats were readily trained to perform ramp and hold contractions of the tibialis anterior. Liquid reward was provided to the

rat when the rate of rise of force and the holding force were within specified limits. Following training, activity was recorded from pairs of motor units with tungsten microelectrodes during repetitive performances of the ramp and hold task. Ultimately, we plan to record such activity before and following pharmacological blockade of specified ion channels via chronically implanted intrathecal catheters. These methods should provide critical insight into the role played by specific ion channels in shaping motor neuron activity in response to natural synaptic drive.

P2-A-30 The amplitude of reflex inhibition follows the discharge rate rather than the size principle

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It has been shown that the excitatory muscle spindle afferent input is distributed on motor neurons according to the size principle. On the other hand, the discharge rate of a motor neuron (MN) is one of the factors that can change the probability of occurrence of a reflex response to excitatory or inhibitory post synaptic potentials. In this study, it is hypothesized that the dependence of reflex response on the discharge rate of the tested MN is the mechanism that determines the observation of a relation between MN size and inhibitory post synaptic potentials. The tibial nerve of 4 healthy subjects was electrically stimulated and motor unit (MU) activities were recorded at 10% and 20% of the maximum voluntary contraction force using high-density surface and intramuscular EMG electrodes. The correlation between reflex amplitude and pre-stimulus mean discharge rate of the MUs were calculated. The hypothesis was also tested simulating a model of 100 motor units as their membrane resistance changed gradually while the afferent input received was kept constant. There was a significant positive correlation between discharge rate and reflex amplitude for all subjects (R^2=0.46±0.30, for 10% MVC; R^2=0.33±0.19 for 20% MVC) and simulations. Moreover, reflex amplitudes of MUs were greater when the same MUs discharged at higher rate. In conclusion, the results suggest that motor units firing at higher discharge rate have a higher probability to be inhibited at low or moderate contraction level. This study was supported by European Research Council (ERC, DEMOVE, No. 267888).

P2-A-31 Characterization of motor units in behaving adult mice shows a wide primary range

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The mouse is essential for genetic studies of motor function in both normal and pathological states. Thus it is important to consider whether the structure of motor output from the mouse is in fact analogous to that in larger animals and in humans. There is a striking difference in the basic electrical properties of mouse motoneurons compared to those in rats, cats and humans. The firing evoked by injected currents produces a unique frequency-current (F-I) function that emphasizes recruitment of motor units at their maximum force. These F-I functions, however, were measured in anesthetized preparations that lacked two key components of normal synaptic input: high levels of synaptic noise and neuromodulatory inputs. Recent studies suggest that the alterations in the F-I function due to these two components are essential for recreating firing behavior of motor units in human subjects. In this study

we provide the first data on firing patterns of motor units in the awake mouse, focusing on steady output in quiet stance. The resulting firing patterns did not match the predictions from the mouse F-I behaviors, but instead revealed rate modulation across a remarkably wide range (10 to 60 Hz). The low end of the firing range may be due to changes in the F-I relation induced by synaptic noise and neuromodulatory inputs. The high end of the range may indicate that, unlike larger species, quiet standing in the mouse involves recruitment of relatively fast twitch motor units.

P2-B-32 Plasticity-dependent modulation of mitochondrial biogenesis determining motor neuron function and vulnerability

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Behavior programs generated in the central nervous system are converted into body movements by a specialized class of neurons in the spinal cord: the motor neurons (MNs). MNs control skeletal muscle contractions through direct neuromuscular synapses. Thus, MNs are particularly active cells with a high energetic demand at locations distant from the cell body. Paradoxically, although the particularly high-energy demand of MNs could make them more prone to energetic stress, endurance exercise appears to be beneficial in specific neurodegenerative conditions. Since tight regulation of mitochondrial biogenesis is generally important for meeting elevated energy demands in neurons, mitochondrial plasticity may adapt MN metabolic properties to increased energetic stress. This study investigates the response of mouse MNs to exercise in terms of differential mitochondrial-regulating gene expression levels. For this, isolated spinal MNs from both sedentary and long-term voluntary trained animals are submitted to a transcriptomic analysis. In parallel, the study addresses the modifications of the mitochondrial web organization following chronic neuromuscular activity. Using nanomicroscopy, live-imaging and 3D-reconstruction, exercise-induced changes in mitochondrial network morphology, size and function are measured from genetically tagged mitochondria in MNs and neuromuscular junctions. The study is designed to ultimately provide insights into mechanisms modulating mitochondrial biogenesis and stress pathways and their contribution to MN function, movement control and vulnerability.

P2-B-33 Spike-Timing-Dependent Plasticity in the Human Spinal Cord is Enhanced by a Greater Number of Stimulus Pairs

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In humans, both motor responses to corticospinal stimulation and voluntary motor output can be altered by repeated paired stimuli designed to facilitate the efficacy of corticospinal-motoneural synapses (1, 2). We examined whether more conditioning pairs induced more facilitation of evoked responses. Transcranial magnetic stimulation (TMS) over motor cortex elicited volleys in corticospinal axons and electrical stimulation at brachial plexus antidromically activated the motor axons innervating biceps brachii. Paired stimuli were timed so that responses interacted at a spinal level. Subjects (n=10) completed 4 conditioning protocols on 4 days: 50 paired stimuli (0.1 Hz), 100 pairs, two blocks of 50 pairs (15 min apart), and 50 TMS alone. Biceps EMG responses to cervicomedullary stimulation

(cervicomedullary motor evoked potentials, CMEPs) were recorded before and for one hour after conditioning. Sizes of CMEPs differed significantly between conditioning protocols (ANOVA, $F(3,27)=3.52$, $p=0.028$) and across time ($F(1.5,13.35)=5.47$, $p=0.025$) with a significant interaction ($F(12,108)=2.45$, $p=0.007$). CMEPs were larger after conditioning with 100 pairs in one block (46±55% increase; $F(1,9)=6.94$, $p=0.027$) or two blocks (71±99% increase; $F(1,9)=5.51$, $p=0.044$) than after TMS alone (6±28% decrease). Our findings showed considerable variability but suggest that increasing the number of paired conditioning stimuli increases facilitation induced at corticospinal-motoneuronal synapses. 1) Taylor & Martin J.Neurosci. 29:11708-11716, 2009 2) Bunday & Perez Curr.Biol. 22:2355-2367, 2012

P2-C-34 One subpopulation of spinal motoneurons, likely S-type, is hyperexcitable in neonatal SOD1-G93A mice.

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In neonatal spinal alpha-motoneurons, firing is either delayed in response to a liminal pulse of current (delayed firing) or it starts at the onset of the pulse (immediate firing). We provided electrical, morphological and molecular evidence that strongly suggest that the immediate firing motoneurons are S-type motoneurons whereas the delayed firing ones are F-type. Remarkably, each type is not equally affected in mSOD1 mice, a model of familial Amyotrophic Lateral Sclerosis. Immediate firing motoneurons are more excitable in mSOD1 mice than in WT animals. In sharp contrast, the excitability of delayed firing motoneurons is unaffected. Therefore, only the subpopulation of motoneurons that are likely S-type is hyperexcitable in mSOD1 mice.

P2-C-35 Long-term measurement of muscle denervation and motor impairment in vivo in ALS model mice

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The increasing availability of mouse models of human degenerative and injury related diseases affecting motor behavior raises the importance of in vivo methodologies that allow measurement of physiological and behavioral changes over an extended period of time in individual animals. I will present a method that provides long-term measurements of muscle denervation and its behavioral consequences in individual mice over a period of several months. I applied this method to mSod1G93A mice, which model human amyotrophic lateral sclerosis (ALS), an adult-onset paralytic disorder, characterized by the selective death of motor neurons (MNs), and is uniformly fatal. The denervation process of two ankle extensor muscles (gastrocnemius and soleus) in mSod1G93A mice is demonstrated for up to three months. The data suggest that as muscle denervation progresses, massive behavioral compensation occurs within the spinal cord that allows animals to walk almost normally until late ages. The first sign of abnormal movement during walking occurs around 84 days of age, as an abnormal ankle flexor muscle (tibialis anterior) activity profile, manifested in subtle but abnormal swing movement during walking. Finally, I show in later ages (~100 days), the task dependent amplitude modulation in walking versus

swimming of the extensor muscle, gastrocnemius, is significantly decreased in the mSod1G93A mice. The methods provide sensitive and objective measures of motor impairment and could be used to test new potential therapeutic interventions.

P2-D-36 Genetic regulation of motor neuron birth

Jessica Clark[1], Angelo Iulianella[1]

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Spinal motor neurons (MNs) diversify into a variety of subtypes during development with each group dedicated to different a motor function. Each MN subtype develops from the same precursor pool in the early embryonic spinal cord. It is not currently clear what controls the generation and specification MN subtypes. Here we describe a role for the two homologous Cut-like homeobox factors Cux1 and Cux2 in the developemnt of motor neuron pools in the mammlian spinal cord. Our preliminary findings shows reveal a role for Cux1 in the early formation of lateral motor neurons (LMC), while Cux2 promotes medial motor neurons (MMC) formation. These distinct functions of the Cux factors reflect the complimentary expression patterns for Cux1 in LMCs and Cux2 in MMCs. Lastly, we suggest that the Cux factors can affect the ratio of MMC vs LMC MNs in part by regulating cell cycle exit through Cip/Kip cell cycle inhibitory protein expression in MN neuroblasts in the ventral spinal cord.

P2-D-37 Contribution of DSCAM in the development of the spinal locomotor circuit

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Several molecular pathways contribute to the normal development of the spinal locomotor circuit. Although the role of Netrin-1 and DCC is well documented during development, much less is known about the role of DSCAM: a Cell Adhesion Molecule associated to Down Syndrome. However, given its role in topographical organization, axonal guidance, and dendritic arborisation in the central nervous system, DSCAM might contribute to the formation of the spinal locomotor circuit. Our studies of locomotor activity in the neonatal isolated spinal cord from DSCAM mutant mice revealed several anomalies including a higher variability in the locomotor pattern and rhythm, as well as in the left-right coordination, with episodes of synchronization. Kinematic analysis in adult DSCAM mutant mice confirmed a higher variability in the coordination of their left-right hindlimbs during treadmill locomotion, suggesting a rabbit-like hopping gait. This hopping-like activity was independent of the locomotor frequency during both neonatal and adult locomotion. Taken together, our results suggest that DSCAM play an important role in the normal development of the spinal locomotor circuit.

P2-E-38 Plasticity of the serotonergic system after spinal cord injury: activity-dependent neurotransmitter re-specification?

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The spinal cord of turtles regenerates spontaneously after complete transection, leading to a substantial recovery of locomotion. The plasticity of the Because 5HT modulates pre-motor circuits and motoneurons the plasticity of the serotonergic system may be involved in functional recovery. Our aim was to determine changes in the number of serotonergic neurons in the lumbar spinal cord after a complete transection at mid-thoracic level. Sham and injured turtles (Trachemys dorbignyi and T. scripta elegans) were injected with BrdU (200 mg/kg, i.p.) during 4 consecutive days (from the 1st or 7th day after injury). Control, sham and injured (35-40 days) turtles were anaesthetized (pentobarbital 60 mg/kg) and fixed by perfusion with 10% paraformaldehyde. Sections (60-80 µm) of the lumbar enlargement were obtained with a vibratome, processed to detect BrdU and 5HT by immunohistochemistry and observed with a confocal microscope. We found a reduction in 5HT fibers and terminals in injured compared to uninjured animals. However, we found an increase in the number of serotonergic cells in the peri-ependymal region and neighboring ventral horn of injured animals (n= 157) compared to the control group (n=63; n=4 pairs of injured-control animals; $P< 0.05$). 5-HT+ cells did not incorporate BrdU suggesting that new 5HT+ cells are not newborn neurons but nerve cells that changed their neurotransmitter phenotype. It is reasonable to speculate that this phenotypic change is a compensatory mechanism for the loss of serotonergic transmission from higher centers.

P2-E-39 Rescuing motor unit numbers and muscle properties after sciatic nerve crush in neonatal rats: Lamotrigine vs. Riluzole

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A large proportion of motoneurons die after injury to their axons shortly after birth, and some of these motoneurons can be rescued by blocking glutaminergic receptors, but systemic application of some glutamate antagonists increases mortality. In the present investigation we reduced the excitability of motoneurons using the antiepileptic and neuroprotective drugs Lamotrigine and Riluzole. Sciatic nerve crush was carried out at P0 in rat pups from three experimental groups treated with daily intraperitoneal injections of saline, Lamotrigine (5mg/kg for 7 days) or Riluzole (16mg/kg for 14 days). Three months later the numbers of motoneurons innervating extensor digitorum longus (EDL) and soleus muscles were assessed by counting the motor units Moreover, muscle contractile properties were established by recording isometric twitch and tetanic contractions. Both Lamotrigine and Riluzole prevented the reduction of motor unit numbers induced by nerve injury. However, Lamotrigine treatment was superior to Riluzole for promoting improvement of muscle performance. In the Lamotrigine treated animals the force produced by soleus was 84% and by EDL 88% of control, significantly higher than that obtained after Riluzole, where the force produced by soleus was 32% and by EDL 30% of that in control muscles. In rats treated with saline the soleus muscles produced only 16% and EDL 10% of force of control muscles. We show that both drugs enhance motoneuron survival after neonatal nerve injury, but the muscle recovery after Lamotrigine is superior to that after Riluzole.

P2-E-40 Bite-force tremor during force tracking is altered in patients with Bruxism

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The excessive grinding/clenching of the teeth that defines Bruxism is of unclear neurophysiological origin. Abnormal peripheral feedback from the teeth and jaw may be involved. If Bruxism is marked by a mishandling of afferent information, we would expect differences in bite-force control between patient populations and healthy subjects. We therefore investigated bite-force control in subjects with and without Bruxism, during a force tracking task. Subjects used visual feedback of their own bite-force to follow a triangular target trajectory. Force tremor and associated masseter EMG activity were compared between patients and controls, as these measures provide a window into the neural mechanisms of sensorimotor integration. We found that the tremor profile of the patient group differed significantly from that of the control group, with the patient group having more force tremor and masseter EMG activity at ~8 Hz. This suggests that the ongoing closed-loop control of jaw force is altered in patients with Bruxism. Since isometric bite-force tremor at ~8 Hz is associated with the activity of periodontal mechanoreceptors, our results imply that Bruxism is marked by dysfunction in this feedback pathway. Whether such dysfunction is a cause or consequence of the condition remains an open question, however, it does appear that afferent feedback pathways may be an important target for future investigation and/or clinical intervention.

P2-E-41 The Effects of Motor Neuron Pre-Conditioning with Botulinum Toxin

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Peripheral nerve regeneration is improved when the nerve lesion under consideration has followed a prior nerve injury. This is known as the conditioning lesion effect (CLE). It increases the rate of axon re-growth, extent of collateral sprouting, and the regenerating axons ability to overcome inhibitory cues. Previously, we described a new paradigm for the CLE in pre-symptomatic SOD1g93a rats when a pre-conditioning nerve crush resulted in protection of motor function, motor neurons and neuromuscular junctions [Franz et al. 2009, PLoS ONE 4(10):e7367]. Since a nerve crush treatment is not clinically translatable, an alternative may be pre-conditioning with transient denervation from Botox ® (onabotulinumtoxinA). For proof of principle we studied the pre-conditioning effect of Botox ® on axon regeneration. We injected the triceps surae muscle of mice with 0.25U of Botox® or Sham. One week later we performed a tibial nerve crush surgery. Then, another week later we performed a second surgery for retrograde labeling of motor neurons as well as to harvest a tibial nerve biopsy. Excitingly, we found that Botox® pre-treatment significantly increased the distal nerve reinnervation 1 week after tibial nerve crush (myelinated axon counts: 366±24 vs 175±40, p=0.004; motor neuron counts: 313±19 vs 205±39, p=0.040). Thus, transient chemo-denervation with Botox® replicated the CLE and ultimately represents a potential neuroprotection strategy for motor neuron disease.

P2-E-42 Effects of acute spinal cord injury on the sensory input of mouse deep dorsal horn neurons

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Spinal cord injury (SCI) causes loss of tonic serotonin (5HT) inhibition of the excitatory deep dorsal horn neurons (DHN), likely contributing to the exaggerated excitatory drive to the spinal motoneurons (MN). The enhancement of excitatory input over inhibitory one to MNs results in hyperreflexia and muscle spasms commonly seen in chronic SCI patients. We hypothesize that DHNs become hyperexcitable after SCI by showing prolonged plateau potentials and strong persistent inward currents. Moreover, if the 5HT deficiency is the cause of the hyperexcitable DHNs, the selective 5HT1B/D receptor agonist zolmitriptan should reduce the neuron hyperexcitability. We have been testing these hypotheses by using the in vitro sacral cord preparation from the adult mice for the model of acute SCI. We analyzed the effect of zolmitriptan and NMDA on the extracellularly recorded responses of DHNs located in spinal lamina III-V and ventral root activity. The synaptic activation of DHNs and ventral root activity was achieved via dorsal root stimulation at four stimulus strengths. Compared with controls, bath application of zolmitriptan (1 µM) increased spike threshold of DHNs, thus generating fewer or no spikes at a given stimulus strength. However, not on all DHNs did we observe the inhibitory effect of zolmitriptan. Surprisingly, NMDA had dual effects on the firing activity of DHNs. While NMDA (100 µM) facilitated bursting in one DHN population, it suppressed the evoked responses in the other. These preliminary results suggest the differential sensory input processing of DHNs in acute SCI.

P2-E-43 Development of miniaturized, wirelessly powered neuromuscular recording devices for use in mice.

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We are developing implantable recording/stimulating devices that are powered by magnetic resonance coupling, and operate wirelessly to provide simultaneous recordings of nerve and muscle activity in awake, freely moving mice. The miniaturized, biocompatible design should allow for monitoring of neuromuscular activity for the lifespan of the animal. This works takes devices under development for human prosthetic control and refines/adapts them for use as a research tool in rodents. We will outline key solutions for transceiver design, power management, signal conditioning, and biocompatible packaging.

P2-E-44 Motor unit firing behavior during spasms of paralyzed thenar muscles

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Involuntary contractions (spasms) of paralyzed muscles are common after human spinal cord injury (SCI). Many of these contractions are strong, which may reflect recruitment of many motor units and/or, high motor unit firing rates including doublets. To examine these possibilities, we have recorded the firing rates of motor units during spasms of thenar muscles that have been paralyzed by cervical SCI. The spasms involved submaximal and maximal contractions, when the force was compared to that evoked by median nerve stimulation at 50 Hz. There were strong positive relationships between surface EMG

and force during spasms. Firing rate modulation varied widely across units, even in the same spasm. Motor units also reached higher firing rates when the spasms were stronger, ranging from 2-12 Hz during weak spasms (<20% maximum) and from 8-25 Hz during strong contractions. When the firing behavior of pairs of units (n=18) were compared during the same spasm, 13 units (72%) were derecruited in the order expected from their recruitment order. Of these, 9 units had a lower derecruitment than recruitment frequency (range: 1.0-7.9 Hz) while the derecruitment frequency was higher in 4 units (31 %; 1.3-4.8 Hz). The threshold difference for the 5 units (18%) that showed reversals at derecruitment varied from 1-9% maximum force. Other units showed sustained motor unit firing after spasms, but always at low rates (~5-6 Hz). These results suggest that afferent inputs play an important role in driving motor units to high firing rates, resulting in strong muscle spasms.

P2-E-45 Developmental nicotine exposure enhances motor neuron responsiveness to serotonin

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Nicotine, an agonist of nAChRs, is a neuroteratogen associated with cellular and clinical respiratory abnormalities. Hypoglossal motor neurons (HMNs) control tongue muscles and have a key role in numerous oromotor behaviors including breathing. Chronic nicotine exposure during development is associated with enhanced excitability in HMNs. Here we tested the possibility that this augmented responsiveness is due to increased sensitivity of HMNs to neuromodulators such as serotonin (5HT). In the presence of bath-applied 5HT, HMNs from neonatal rats exposed in utero to nicotine exhibited reduced threshold current, increased input resistance, hyperpolarized threshold potential, increased frequency-current gain, and reduced maximum firing rate compared to control cells. In addition, triangular ramp current injection during 5HT application evoked firing patterns indicative of persistent inward current activation exclusively in nicotine-exposed cells. Occasionally, 5HT application evoked spontaneous 'spikelets' thought to arise from unclamped spikes in electrically coupled HMNs. Such 'spikelets' were only present in control cells suggesting less coupling in nicotine-exposed HMNs. Overall, these results suggest that HMNs exposed to nicotine during development have greater sensitivity to 5HT, which could underlie their enhanced excitability.

P2-E-46 Reinnervation following delayed transplantation of embryonic stem cell-derived motoneurons

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We have shown that embryonic stem cell-derived motoneurons (ESCMNs) transplanted at the time of injury into a peripheral nerve reinnervate muscle. For clinical relevance, we directed our efforts towards delaying the transplantation by various intervals after denervation. Successful reinnervation progressively decreased as the denervation period lengthened: 50%, 33%, 17% and 0% of transplanted animals demonstrated reinnervation at 3 months after immediate, 1-week delayed, 4-week delayed and 8-week delayed transplantation respectively. Teratocarcinomas developed in 47% of transplants.

Tumor-derived cells were maintained as colonies on mouse embryonic feeders up to one month, expressed pluripotent markers and could be repetitively passaged while maintaining the ability to differentiate into motoneurons. ESCMNs were found to harbored a residual pluripotent population (8%) expressing the marker SSEA-1. Exposure to mitomycin (MTOC) rendered 62% of the SSEA-1 population apoptotic by 12 hours and eliminated the SSEA-1 population by 3 days in vitro while permitting motoneuron survival. Transplantation of MTOC-treated ESCMNs prevented tumor formation and restored twitch and tetanic force after chronic denervation similar to animals transplanted acutely after denervation. We conclude that despite differentiation, cells derived from stem cells carry a risk of tumorigenicity, which can be controlled by the anti-mitotic agent mitomycin. Transplanted ESCMNs are able to survive in chronically denervated nerve, reinnervate muscle, and restore muscle force production.

P2-F-47 Maturation of C-bouton regulation of motoneuron excitability

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Regulation of motoneuron (MN) excitability is essential to the production of appropriate motor output. It has been suggested that C-bouton synapses are involved in regulating MN input-output gain. Early postnatal maturation of these synapses involves clustering of delayed rectifier KV2.1 channels and type 2 muscarinic receptors (m2R) at the postsynaptic site. Here, we study postnatal development of small-conductance Ca2+-gated K+-channels (SK2 & SK3) and m2R, which contribute to MN excitability. Using spinal cord slice preparations, we show that SK channels contribute to MN excitability in immature (P3) and mature (P20+) mice; but the SK-blocker apamin (100nM) is less effective in neonatal MNs (f-I slope increase of 238 ± 101% vs. 544 ± 464% in adult MNs; ANOVA interaction P=0.0005). This may be due to the lack of SK clustering (demonstrated with immunohistochemistry), differences in SK density, or quantitative differences in SK2 vs. SK3 in neonatal and adult MNs. Channel clustering may also be needed for functional cooperation between other C-bouton elements. Since SK cluster with m2R, and m2R activation reduces SK-mediated post-spike afterhyperpolarisation, the effect of muscarine on neonatal MN output was tested. Muscarine (50µM) was significantly more effective in increasing excitability in adult MNs (f-I slope increase, ANOVA interaction P=0.0088), buttressing the thesis that clustering and colocalization of SK, m2R and perhaps other channels with C-boutons is essential for tuning MN output. Furthermore, immature C-boutons regulate a narrower range of f-I gain changes.

P2-F-48 Are acetylcholine and glutamate released by the same bouton at the motoneuron-Renshaw cell synapse ?

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In newborn mice, excitation of Renshaw cells (RCs) by motoneurons (MNs) involves the co-release of glutamate and acetylcholine (ACh) and the activation of AMPA and NMDA receptors as well as of homomeric α7 and heteromeric α*β* nicotinic receptors (nAChRs). The kinetics of the post-synaptic receptors is spanning a large range of time constants, from a few ms (the nicotinic α7 receptors) to tens

of ms (the NMDA receptors). The synaptic currents mediated by these four receptors differ in both their rise times (RTs) and their decay times (DTs) (Lamotte d'Incamps and Ascher, 2008; Lamotte d'Incamps et al., 2012). We have used these differences to examine if co-release leads to "mixed " miniature synaptic currents (mEPSCs) activating both glutamatergic and nicotinic receptors. We could not find any mixed miniature currents, suggesting that the two neurotransmitters are released by independent synaptic vesicles. The simplest explanation of our results is that ACh and glutamate are stored in and released by distinct vesicles. However, one cannot exclude that the two transmitters be stored in the same vesicles and that the separation of cholinergic and glutamatergic mEPSCs be due to a postsynaptic segregation of receptors (see Dugué et al. 2005). Supported by CNRS, Université Paris Descartes and AFM (grant 16026).

P2-F-49 Motor axon synaptic boutons are enriched in aspartate

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Motoneuron synapses on Renshaw cells activate nicotinic, AMPA and NMDA receptors consistent with co-release of acetylcholine and excitatory amino acids (EAA). However, whether these synapses express vesicular glutamate transporters (VGLUTs) capable of accumulating glutamate into synaptic vesicles is controversial. An alternative possibility is that these synapses release other EAAs, like aspartate, not dependent on VGLUTs. To clarify the EAA concentrated at motor axon synapses we performed a quantitative postembedding colloidal gold immunoelectron analysis for aspartate and glutamate on motor axon synapses (identified by immunoreactivity to the vesicular acetylcholine transporter; VAChT) contacting calbindin-immunoreactive (-IR) Renshaw cell dendrites. The results show that 71% to 80% of motor axon synaptic boutons on Renshaw cells contained aspartate immunolabeling two standard deviations above average neuropil labeling. Moreover, VAChT-IR synapses on Renshaw cells contained, on average, aspartate immunolabeling at 2.5 to 2.8 times above the average neuropil level. In contrast, glutamate enrichment was lower; 21% to 44% of VAChT-IR synapses showed glutamate-IR two standard deviations above average neuropil labeling and average glutamate immunogold density was 1.7 to 2.0 times the neuropil level. We conclude that motor axon synapses co-express aspartate and glutamate, but aspartate is concentrated at higher levels than glutamate

P2-F-50 Effect of Direct Triceps Brachii Tendon Vibration on Motor Unit Activity in the Contralateral Homonymous Muscle

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Using biceps brachii (BB) tendon vibration we have previously shown a ~40% decline in elbow flexor steadiness and a ~13% decline in motor unit (MU) discharge rates and disproportionate increase in recruitment between the short (SH) and long head (LH) of the BB. Combined with the observed vibration-induced shift to lower surface intermuscular coherence, results infer a differential influence of afferent inputs upon the motor neuron pool which may account for the compartmentalized muscle activity and reduced steadiness. To further explore differential activation of the SH and LH the influence

of polysynaptic connections should be considered. The purpose of this study was to evaluate the effect of contralateral triceps brachii tendon vibration on MU activity and force steadiness of the elbow flexors. With intramuscular electrodes MU activity was recorded in the SH and LH. Force steadiness was evaluated over a 9-s steady state 5% isometric elbow flexion contraction. Contralateral triceps tendon vibration decreased MU recruitment thresholds and increased discharge rates ~3Hz of the SH and LH. There was a slight decrease in force steadiness with vibration (~1%) coincident with an increase in discharge rate variability (~10%). These preliminary data indicate that tendon vibration of the contralateral antagonist limb does not mediate MU activity in the SH and LH independently as is observed via the stretch-related monosynaptic reflex pathway. Thus, it is unlikely that polysynaptic connections from the contralateral limb contribute to a decline in isometric force steadiness.

P2-F-51 Motor unit activity in biceps brachii in response to contralateral neuromuscular electrical stimulation

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Introduction The purpose of the study was to determine the influence of NMES pulse width on the modulation of motor unit (MU) activity in a contralateral limb. Methods MU activity was recorded in left biceps brachii of young adults. Subjects performed submaximal isometric contractions (≤30 s in duration) to maintain the discharge of an isolated MU. In the middle 5 s of each contraction, NMES was applied to the right biceps brachii with either of two pulse durations (0.2 or 1 ms) or three currents (below motor threshold, 10%, and 20% of MVC force). The primary outcome was the influence of NMES on the discharge characteristics of the isolated MUs. Results (1) Both NMES pulse widths elicited a variable number of longer interspike intervals, even when the NMES current was less than motor threshold; and (2) Wide pulses increased the coefficient of variation (CoV) for interspike interval (ISI) for some MUs, but not others. An NMES-evoked contraction at 20% MVC force, for example, did not change the CoV for ISI (29.2%) of one MU during narrow-pulse NMES, whereas the CoV with wide-pulse NMES was 15.3% before, 28.8% during, and 18.1% after. Discussion The differential influence of NMES pulse width on the discharge characteristics of a MU may depend on its recruitment threshold. The capacity of NMES to modulate MU activity in the contralateral arm may depend on pulse width. References Bergquist AI, Clair JM, Lagerquist O, Mang CS, Okuma Y, Collins DF. (2011). Eur J Appl Physiol, 111, 2409-2426. Garnett R, Stephens JA. (1981). J Physiol, 311, 463-473.

P2-F-52 The function of spinal V3 interneurons in locomotion

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V3 interneurons (INs) are the major excitatory commissural interneurons in the mouse spinal cords. Our previous work has shown that genetic deletion of V3 INs caused the animal to exhibit an unstable and incoherent gait. In order to further understand the functional role of V3 INs in the locomotor circuits, we selectively blocked the synaptic transmission of V3 INs by deleting the expression of vesicular glutamate transporter 2 (VGLUT2). The mutant mice could survive and didn't show obvious behavior defects in the

cage. When they were put on a treadmill with various speeds, however, these mutant mice couldn't run faster than 15 cm/s, while wild-type mice could run at a rate of above 60 cm/s. On the other hand, during swimming, the mutant mice could reach the same high speed as wild types. Furthermore, electromyography (EMG) recordings in different hind limb muscles have shown that some extensor muscles, such as gastrocnemius-soleus and vastus lateralis muscles, showed little change of activity in mutant mice when they altered from running to climbing or to swimming, while there was a significant increase in the activity of these muscles in control animals under the same condition. These results indicate that V3 INs may be involved in regulating the activity of extensor muscles of the hind limbs in controlling animal's locomotor activity, instead of directly setting the rhythms of limb movement.

P2-F-53 Sim1 is required for the proper formation of subpopulations of V3 interneurons in the mouse spinal cord

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V3 interneurons in the spinal cord are a group of excitatory commissural interneurons that play an important role in producing balanced and stable gaits in animals. In the developing mouse spinal cord, V3 interneurons arise from the ventral-most progenitor domain, p3, and express the transcription factor single-minded 1 (Sim1) at embryonic day (E) 9.5. Although Sim1 is used as a molecular marker to identify V3 neurons in the spinal cord, its function during V3 development is still unknown. To reveal its function, we have used transgenic mouse models to knock out Sim1 expression and trace the fate of mutant V3 neurons during development. After leaving the progenitor domain, V3 neurons migrate dorsally and laterally, settling at birth as clusters in laminas VIII (ventral), VII (intermediate), and IV-V (dorsal). Analysis of axon projections at different embryonic stages indicates a unique progression. V3 neurons initially have equally strong ipsilateral and contralateral projections. Through development, this profile changes to become almost entirely contralateral-projecting. V3 interneurons lacking Sim1 show a defective migration and axon projection pattern. In Sim1 mutants, V3 neurons cluster with a less organized pattern. At P0, dorsal cells are mostly absent, while intermediate cells are significantly increased. Furthermore, we found a drastic decrease in the number of V3 neurons able to form proper axon projections. At both E14.5 and P0, ventral neurons show decreased rostral and caudal projections, while non-ventral neurons show decreased rostral projections.

P2-F-54 The characterization of calretinin expressing V3 interneurons in the mouse spinal cord

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V3 interneurons (INs) in the spinal cord are a major group of excitatory commissural INs that innervate contralateral motoneurons as well as other interneuron types. V3 are essential in establishing a robust and balanced locomotor rhythm during walking. During development V3, as marked by the expression of the transcription factor Sim1, exit from the most ventral progenitor domain, p3, at embryonic day (E) 9.5 in the mouse spinal cord. In order to better understand the embryonic development of V3, the expression of calretinin (CR) in V3 has been investigated. CR is a calcium binding protein that has been

implicated as a neuronal sub-population marker in specific areas of the developing nervous system. V3 were identified by their expression of tdTomato fluorescent protein in Sim1Cre/+; Rosafoxstop26TdTom mice. Approximately 20-30% of V3 begin to express CR at E14.5 in the lumbar and sacral spinal cord. Of these cells two subpopulations of CR expressing V3 are identified. The first is a group of ventral and intermediate V3 that transiently express CR from E14.5 to P0. The second is a cluster of V3 forming a subpopulation at the intermediate region of the L6-S2 spinal cord that lasts into adulthood. The effect of sim1 on CR expressing V3 during development was further studied. In Sim1 null mice, CR positive V3 significantly decreased in the transient-expressing group, although the total number of V3 didn't change. Sim1 did not affect the V3 subgroup that persistently expressed CR. Further investigation of these cells function in spinal neural networks is needed.

P2-F-55 Spinal sensorimotor circuits involved in locomotor circuit plasticity

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Following spinal cord injury, motor training can lead to improvement in spinal motor output in animals and humans. However, the underlying sensory-motor circuits responsible for this improvement in function remain to be fully understood. Given the role that dI3 interneurons play in sensorimotor integration for hand function (Bui et al., 2013), that dI3 interneurons are active in locomotion, and that they are involved in low threshold cutaneous-evoked locomotor activity, we sought to determine whether they may also be involved in motor circuit plasticity following spinal cord transection. After complete spinal transections at thoracic levels in mice in which glutamatergic output from dI3 interneurons was eliminated, we assessed hind limb locomotor performance over a 4-month period in comparison to control-spinalized animals. We will report our preliminary data here, and will discuss implications for locomotor recovery after spinal cord injury.

P2-F-56 Are Hb9 interneurons involved in walking?

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Central pattern generators (CPGs) are neuronal circuits that are responsible for the production of rhythmic behaviours such as locomotion. It has been suggested that a population of spinal interneurons (INs), known as Hb9 INs, are a possible component of the spinal locomotor CPGs. Whether these glutamatergic INs (expressing vesicular glutamate transporter vGluT2) are involved in generating the rhythm of locomotion remains unknown. To address this question, we used genetic strategies to alter the output of Hb9 INs. Hb9::CreERT2 animals were crossed for two generations with VGluT2$^{lox/lox}$ animals. Injection of tamoxifen generated animals that are Hb9-VGluT2^{OFF}, i.e. animals in which glutamate would not be released by Hb9 INs. There were no significant differences observed in treadmill locomotion of Hb9-VGluT2^{OFF} animals compared to hemizygotes or wild-type controls. To further assess their possible role, the distribution of glutamatergic boutons of Hb9 INs were analyzed in Hb9-CreERT2;Rosa26-lox-stop-lox-synaptophysin-tdTom animals. The boutons of Hb9 INs were distributed in

the rostrocaudal axis of the spinal cord in intermediate laminae. While their role appears to be limited in locomotor activity, the locations of Hb9 IN boutons may provide insight into the role of these interneurons.