Oral Presentation Abstracts

The following abstracts are in the order that they appear on the program.

Cooperative gating by clustered voltage-gated Cav1.3 channels enhances inward currents in neurons

Monday, June 16 (AM)
Presenter: Marc Binder

Authors:
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L-type voltage-gated Cav1.3 (α1D) channels play a critical role in the amplification of synaptic inputs and the generation of persistent activity in motoneurons. As is the case for the other classes of voltage-gated channels, it is widely assumed that individual Cav1.3 channels behave independently with respect to voltage-activation, channel open time, and inactivation. With super-resolution fluorescent imaging, optogenetic, and electrophysiology measurements we have results that -- contrary to this long-held view -- suggest that Cav1.3 channels form functional homomeres. Our data reveal that a vast majority of Cav1.3 channels associate in clusters of two or more channels that undergo coupled gating through a physical interaction of their carboxy-terminus tails. Membrane depolarization induces Cav1.3 carboxy tail-to-carboxy tail interactions, but indirectly, by increasing calcium influx. Calcium binding to calmodulin is necessary for Cav1.3 C-tail oligomerization. We propose that cooperative gating of Cav1.3 channels is a general mechanism for the amplification of calcium currents and facilitation of neuronal discharge.

The subprimary range and force generation in the cat

Monday, June 16 (AM)
Presenter: Hans Hultborn

Authors:
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It has recently been suggested that mice motoneurons exhibit somewhat different properties to cat motoneurons (Manuel et al 2009). They display high frequency subthreshold mixed mode oscillations (MMOs) creating a third range in the I-f slope, the sub-primary range (SPR), in additional to the previously described primary and secondary ranges. Surprisingly, experiments have shown that mouse motor units appear to develop nearly maximal force during the SPR (Manuel & Heckman 2011). It was suggested that MMOs and the SPR are unique to mice motoneurons, although they have since been demonstrated in rat motoneurons. Using intracellular recording we have also observed both MMOs and SPRs in cat motoneurons (decerebrate, unanaesthetised or barbiturate anesthetised). To see MMOs it was necessary to use 8 kHz (DCC mode) or bridge mode on the amplifier. It was difficult to obtain the SPR range using square pulses as spike frequency adaptation meant that firing was not usually sustained for long at this low range. That may explain why the SPR was

originally missed (or deemed not to be important) in the cat. To assess the contribution of the SPR to force generation, ramp injections of current were made into cat gastrocnemius motoneurons with simultaneous force recordings of the muscle. Very little force was generated during the SPR except under certain circumstances. We will present a possible explanation of the apparent discrepancy between the mice and our current recordings in cats and question the assumption that mice motoneurons behave in a fundamentally different manner.

The bistable lumbar motoneuron: a four-stroke engine in producing plateau potentials

Monday, June 16 (AM)
Presenter: frédéric brocard

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The development and the ionic nature of bistable behavior in lumbar motoneurons were investigated in rats. One week after birth, almost all (~80%) recorded in whole-cell configuration displayed self-sustained spiking in response to a brief depolarization and emerged when the temperature was raised above 30°C. The blockade of the persistent sodium current (INaP) abolished them. When hyperpolarized, bistable motoneurons displayed a characteristic slow afterdepolarization (sADP). The sADPs generated by repeated depolarizing pulses promote a plateau potential. The sADP was tightly associated with the emergence of Ca2+ spikes. Substitution of extracellular Na+ or chelation of intracellular Ca2+ abolished both sADP and the plateau potential without affecting Ca2+ spikes. These data suggest a key role of a Ca2+-activated nonselective cation conductance (ICaN) in generating the plateau potential. In line with this, the blockade of ICaN by flufenamate abolished both sADP and plateau potentials. Furthermore, the 2-APB, a common activator of thermo-sensitive vanilloid transient receptor potential (TRPV) cation channels, promoted the sADP. Among TRPV channels, only the selective activation of TRPV2 channels by probonecid promoted the sADP to generate a plateau potential. In sum, bistable motoneurons behave as a four-stroke engine (see figure) such as the INaP-dependent spiking activity (step 1) triggers Ca2+ entry through L-type channels (step 2) which initiates a Ca2+-induced Ca2+-released process (step 3) that in turn activates ICaN (step 4) and so forth.

Action potential threshold of motoneurons during fictive scratch and swim

Monday, June 16 (AM)
Presenter: Aidas Alaburda

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Motoneurons integrate excitatory and inhibitory synaptic inputs and when the membrane potential of the motoneuron is depolarized sufficiently to exceed the threshold potential, an action potential is generated. Threshold potential is dynamic and it provides a mechanism for modulation of the excitability of the neuron. During spinal neural network activity motoneurons receive intense synaptic input, and this could modulate the threshold for action potential generation. In the present study we investigated how the threshold potential changes during functional spinal neural network activity. We performed intracellular electrical recordings from adult turtle motoneurons in integrated spinal cord preparation. Recordings were made during stimulation of motoneurons with suprathreshold current pulses and during functional spinal neural network activity - fictive scratching and fictive swimming. Motoneurons generated bursts of action potentials during fictive scratching and fictive swimming. The threshold potential during generation of bursts was compared with the threshold of action potentials induced by current pulses. As it was previously reported, during repetitive firing induced by current pulses threshold potential gradually depolarizes until it becomes about 10 mV higher than the threshold of the first action potential. We found that synaptic inputs during spinal neural network activity did not change the threshold of the first action potential. The threshold of action potentials during generation of bursts depolarized in a similar manner as during repetitive firing induced

Memory in spinal motoneurons

Monday, June 16 (AM)
Presenter: Michael Johnson

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Intrinsic properties strongly effect neuron behavior. One intrinsic property in particular, persistent inward currents (PICs), is known to greatly influence spinal motoneuron (MN) behavior. PICs can produce prolonged outputs in response to a brief input. This is usually manifest at the cellular level as plateau potentials and self-sustained firing. Here we explore a population effect of PICs on motor unit (MU) behavior that constitutes a memory trace of sensory events. We use a multi-electrode recording technique, described in our companion presentation, to determine how MNs already in a self-sustained state respond to additional inputs. Brief (2s), repeated tendon vibration of the cat soleus muscle produced a progressive build up of force as well as long lasting after force. In this same muscle recordings of multiple (~15) MUs showed that no individual unit replicated the overall force pattern. Units responded with step increases in firing reaching a steady level for the duration of the sensory input, and then returned to a steady self-sustained level when the input ceased. The progressive increase in force with each successive input was the result of the recruitment of additional MUs. This population coding pattern constituted a memory trace robust to up to 7 events. We hypothesize that this behavior is due to the tendency of PICs in individual MNs to gradually activate when they are near their activation threshold.

Time course of human motoneuron recovery from sustained activity at a constant firing rate

Monday, June 16 (AM)
Presenter: Martin Héroux

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In humans, prolonged firing of a single motor unit (MU) at a constant rate is associated with a progressive increase in EMG activity. The need for greater excitatory input to maintain the same firing rate may reflect altered intrinsic motoneuron (MN) properties. Here we determined the time course of recovery of human MNs from sustained firing. Twenty-five healthy subjects performed low-level voluntary isometric contractions to maintain constant firing (+/- 1Hz) of a triceps MU. Over time, triceps surface EMG increased. Once it increased by ~10-20% (after 3-7 min), subjects relaxed for a predetermined period (Exp. 1: 30s, 60s, 120s, or 240s; Exp. 2: 2s, 15s, or 30s) and then resumed contraction with the target MU firing at the same rate for an additional 15s. For each trial, surface EMG was compared between the post-recovery contraction and initial 15s of sustained contraction to determine whether the MN had recovered. In Exp. 1, MUs were almost but not completely recovered after a 30s rest ($p<0.05$), and fully recovered after 60s ($p>0.05$). Results from Exp. 2 confirmed MUs do not recover from a sustained contraction after a 30s rest, and revealed that the majority of recovery occurs in the initial 15s ($p<0.05$). These findings confirm MNs require increasing voluntary drive to maintain a constant firing rate and describe the time course of recovery of the associated adaptations of MN intrinsict properties. After a low-level sustained contraction, 30-60s rest is required for MNs to recover fully. However, the majority of recovery occurs quickly, in the initial 15s.

Inhibitory Post-synaptic Potentials in Standing

Monday, June 16 (AM)
Presenter: Jayne Garland

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Motor unit discharge rates show only modest increases with external loading in standing; a finding consistent with the activation of persistent inward currents (PICs) and an increase in motoneuron excitability that renders the motor units highly sensitive to inhibitory post-synaptic potentials (IPSPs) and less so to excitatory post-synaptic potentials (EPSPs) (Heckman & Enoka, 2012). IPSPs in plantarflexor muscles, produced by non-noxious stimulation of the sural nerve (7 times perceptual threshold), are compared in standing with isometric contractions performed in supine in healthy subjects. The experiment is performed on a tilt-table so the main difference between the two positions is the postural component of standing. High-density surface EMG (grid with 64 electrodes) and fine wire electrodes are used to record motor units from the medial gastrocnemius muscles. Peristimulus frequency histogram and CUSUM are used to quantify the IPSP. We hypothesize that if

PICs are active during standing, then inhibitory cutaneous inputs should produce larger IPSPs in standing than in supine.

Double discharges and afterhyperpolarization in human motoneurons

Monday, June 16 (AM)
Presenter: Maria Piotrkiewicz

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During isometric voluntary contractions of a healthy human muscle, motoneurons (MNs) fire with low mean rates, rarely exceeding 25/s. However, there are MNs capable of firing double discharges (doublets) with interspike interval of few ms, interspersed in regular rhythmic activity. This is observed seldom in normal MNs but does so more often in neuromuscular disorders, where it is considered to be an early sign of MN dysfunction. The "true" doublets occur consistently in the same relationship to one another and are usually followed by a prolonged post-doublet interval. It is commonly accepted that the doublets are fired by MNs exhibiting delayed depolarization with a prominent hump that may spontaneously cross the firing threshold and evoke an extra spike. Other distinguishing features of these MNs are largely unknown. Kernell (1964) noted that in cat MNs a definite depolarization hump was more commonly observed in MNs with short afterhyperpolarization (AHP). We will present experimental evidence that also human MNs capable of doublet firing usually possess shorter AHP. We will also demonstrate an intriguing firing pattern of few observed triplets and some speculations on their origin.

Principles governing the subthreshold input-to-output properties of motoneurons - the view from the dendritic tree

Monday, June 16 (AM)
Presenter: Ken Rose

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The dendrite trees of motoneurons are contacted by over 30,000 excitatory, inhibitory, and neuromodulatory synapses and possess numerous voltage-dependent channels. How these components work together to meet the demands of different motor tasks is unclear. In the present study, we have used anatomically and physiologically realistic models to identify a strategic partnership between dendritic tree structure, L-type Ca++ channels, HCN channels, and serotonergic modulation. We compared the subthreshold responses of small

(dendritic surface area <415,000 um2) and large (dendritic surface area >560,000 um2) motoneurons to sinusoidal changes in the activity of 600 uniformly distributed, excitatory synapses. HCN channels were distributed in accordance with the known differences in the proximal to distal gradients of HCN channels on small and large motoneurons. After accounting for differences in electronic properties, we found that HCN channels preferentially increased (20%) the response of small motoneurons to low frequency (< 2 Hz) changes in excitatory synaptic activity. This bias was increased to 50% by L-type Ca++ channels assigned to hotspots located proximally on small motoneurons and distally in large motoneurons. Increasing the conductance assigned to HCN channels in proportion to the density of serotonergic synapses amplified the bias to 230%. These results suggest that the actions of serotonin and HCN and L-type Ca++ channels are tailored to match the subthreshold input-to-output properties of small motoneurons to the slow contractile properties of their motor units.

Homeostatic plasticity in motoneurons from mice with glycine receptor mutations

Monday, June 16 (PM)
Presenter: Bob Callister

Authors:
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Inhibitory synaptic inputs to hypoglossal motoneurons (HMs) are important for modulating their excitability. We tested whether long-term impairment in glycinergic transmission, as occurs in three murine mutants with distinct naturally occurring mutations in the glycine receptor (GlyR), leads to intrinsic and/or synaptic homeostatic plasticity. Whole cell recordings were obtained from HMs in brainstem slices from wild type (wt), spasmodic (spd), spastic (spa) and oscillator (ot) mice (~ P21). Passive and action potential (AP) properties in spd and ot HMs were similar to wt. In contrast, spa HMs had lower input resistances, more depolarized resting membrane potentials, higher rheobase currents, smaller AP amplitudes and slower AHP current decay times. The excitability of HMs, assessed by gain in current/firing-frequency plots, was similar in all strains whereas the incidence of rebound spiking was increased in spd. The difference between recruitment and de-recruitment current (ie, delta-I) for AP discharge during ramp current injection was more negative in spa and ot. GABAa mIPSC amplitude was increased in spa and ot but not spd, suggesting diminished glycinergic drive can lead to compensatory adjustments in the other major fast inhibitory synaptic transmitter system. Overall, our data suggest long-term reduction in glycinergic drive to HMs results in changes in intrinsic and synaptic properties that are consistent with homeostatic plasticity in spa and ot, but not in spd. We propose such plasticity is an attempt to stabilize HM output, which succeeds in spa but fails in ot.

The effects of passive cycling following a spinal transection on serotonin receptor mRNA expression in hindlimb flexor and extensor alpha-motoneurons

Monday, June 16 (PM)
Presenter: Jeremy Chopek

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Sacral motoneuron (mn) gene expression is altered following a spinal transection. Of interest is the regulation of the serotonin (5-HT) receptors, which mediate mn excitability and locomotor recovery post-transection. However, the examination of 5-HT receptors in lumbar mns post-transection has not been fully examined. As well the influence of neuromuscular activity on 5-HT gene expression post-transection has not been examined, which is vital as activity is used to promote locomotor recovery post-transection. The purpose of this study was to examine gene expression of various 5-HT receptors at three months following a complete thoracic spinal cord transection, with and without the inclusion of daily passive cycling. Physiological hindlimb extensor and flexor mns were differentially identified with two retrograde fluorescent tracers, allowing for the identification and harvesting of extensor and flexor mns with laser capture microdissection, and the subsequent examination of mRNA content using qRT-PCR analysis. We demonstrate that three months post transection, 5-HT1AR, 5-HT2CR, and mGluR1 expression is down-regulated, whereas the 5-HT2AR is up-regulated. Three months of passive cycling influenced gene expression in extensor but not flexor mns resulting in a further enhancement in 5-HT2AR expression as well as increase in 5-HT7R and KCC2 gene expression. Our results demonstrate that daily passive cycling influences serotonin gene expression and that extensor mns compared to flexor mns are likely more responsive to interventions aimed at locomotor recovery.

How plastic is the motoneurone axon initial segment?

Monday, June 16 (PM)
Presenter: Claire Meehan

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Much research has focused on the mechanisms underlying long term and short term potentiation as mechanisms of activity dependent learning in neurones. Homeostatic mechanisms respond to ensure stability of the system and prevent excitoxicity. Action potentials are initiated at the distal region of the axon initial segment (AIS), Thus, the AIS would appear to be an optimal hotspot for controlling the overall level of activity of the neurone. Recent elegant studies by other groups using auditory neurons and in vitro studies using hippocampal neurones have demonstrated that the AIS of neurons can respond homeostatically to changes in inputs/activity levels by changing its size and location. Spinal motoneurones have to maintain an optimum level of excitably with constantly changing levels of inputs and activity. Little is known, however, regarding the role of

the AIS in this. We will present evidence from in vivo studies showing that the AIS of spinal motoneurones can, in fact, also demonstrate both extensive and more subtle forms of plasticity both developmentally and following changes in inputs.

Time-related changes of motoneuron properties after chronic compensatory muscle overload

Monday, June 16 (PM)
Presenter: Piotr Krutki

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Our aim was to determine whether chronic muscle overload has measurable effect on electrophysiological properties of motoneurons (MNs), and whether duration of this overload influences intensity of adaptations. The compensatory overload was induced in the rat medial gastrocnemius (MG) by bilateral tenotomy of its synergists (lateral gastrocnemius, soleus, and plantaris); as a result, only the MG was able to evoke the foot plantar flexion. To assure regular activation of the MG muscle, rats were placed in wheel-equipped cages and subjected to a moderate treadmill exercise. The intracellular recordings from MG motoneurons were made after 5 or 12 weeks of the overload, and in the control group of intact rats. Some of the passive and threshold membrane properties as well as rhythmic firing properties were considerably modified in fast-type MNs, while remained unaltered in slow-type MNs. The significant changes included a shortening of the spike duration and the spike rise time, an increase of the AHP amplitude, an increase of the input resistance, a decrease of the rheobase, and a decrease of the minimum current necessary to evoke steady-state firing. The data point on higher excitability of fast-type MNs innervating the overloaded muscle, and a shift towards "slower" physiological properties. All the adaptations could be observed after 5 weeks of the compensatory overload with no further development after 12 weeks. This indicates that the response to an increased level of chronic activation of MNs is relatively quick and stable. Supported by the NCN grant 2012/04/M/NZ4/00190.

Hebbian plasticity in the spinal cord?

Monday, June 16 (PM)
Presenter: Andrew Fuglevand

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A fundamental process underlying learning and memory is a long-lasting change in the effectiveness of synaptic communication among neurons. The principle that governs such changes in synaptic 'strength' was originally formulated by Hebb in the 1940s. Hebb postulated that synaptic connections among neurons that are co-active become stronger whereas synaptic connections among neurons that are active at different times are weakened.

While a substantial body of research has largely confirmed Hebb's rule underlying plasticity in various brain structures, little is known about the capability of neurons in the spinal cord to exhibit Hebbian plasticity. The goal of this project was to determine whether the strength of synaptic connections between corticospinal neurons and spinal motor neurons (MNs) could be changed by a period of training that involved their repetitive co-activation. As such, we measured the extent of short-term synchronized spiking between MNs supplying two hand muscles before and following 4 weeks of training on a computer game that required precise matching of the forces exerted by the two muscles. Because such synchronization reflects the strength of synaptic inputs projecting across the two motor nuclei, training-related increases in synchrony were assumed to reflect Hebbian-like increases in the strength of common synaptic inputs. Despite marked improvements in game performance, no changes in synchrony were observed. It may be that the molecular machinery needed to support Hebbian plasticity is absent at MN synapses.

The recurrent discharge of human motoneurones is reduced by voluntary but not antidromic activation

Monday, June 16 (PM)
Presenter: Simon Gandevia

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The output of human motoneurone pools decreases with fatiguing exercise, but the mechanisms are uncertain. Recurrent motoneurone discharges, recorded as F waves in the EMG, reflect motoneurone excitability and are depressed following maximal voluntary contractions (MVCs; Khan et al 2012; J Physiol 590: 4957-69). Here, we investigated the link between this post-contraction depression of F-waves and non-voluntary and voluntary activation of motoneurones. Supramaximal electrical stimuli of the ulnar nerve evoked F-waves in a hand muscle, abductor digiti minimi (ADM) during relaxation. Two sets of 30 stimuli (0.5 Hz; ~ 30 s between sets) were delivered prior to interventions and up to 10 sets over 15 min afterwards. Study 1: F-waves were evoked in ADM before and after supramaximal tetanic stimulation of the ulnar nerve for 10 s at 25 and 40 Hz. This stimulation did not depress F-wave area or persistence ($P>0.5$; $n=12$) despite repetitive antidromic activation of all motoneurones supplying ADM. Study 2: F-waves were measured in ADM and in the first dorsal interosseous (FDI) before and after a sustained 3-min MVC of ADM's antagonist muscle. F waves in ADM were depressed (area by ~20%; persistence by ~13%; $P<0.02$; $n=20$) for ~15 min. However, F waves in FDI were unchanged ($P>0.05$). Our findings suggest that depression of F-waves after contractions does not solely depend on repetitive activation of the motoneurones but may require descending voluntary drive. Furthermore, sustained voluntary activation of one motoneurone pool may depress nearby, but not distant, motoneurones.

Daily use of hand muscles with and without spinal cord injury

Monday, June 16 (PM)
Presenter: Christine Thomas

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Few studies have examined the importance of daily activity in maintaining muscle contractile properties. Since most motor units in human hand muscles are recruited during weak contractions (by 30% maximum), which is typical use of these muscles, changes in activity may impact most motor units. To explore these issues, we have compared activity in thenar muscles of uninjured and spinal cord injured (SCI) individuals from 24-hour recordings of electromyographic activity (EMG) to measures of muscle EMG, strength, speed and fatigue. Uninjured muscles were active from 4 to 11 hours per day with >99% of EMG recorded during wake time. Low intensity contractions were common but usually strong enough for the EMG to form an interference pattern. After SCI, thenar muscles could be as active (3-13 hours) but were weaker, slower and more fatigable which may relate to many motor units firing at low frequencies during both wake and sleep time. Funded by National Institutes of Health grant NS30226 and The Miami Project to Cure Paralysis

En passant axonal inhibition far from the presynaptic terminal, produced by 5-HT1D receptors on sensory afferents.

Monday, June 16 (PM)
Presenter: David Kennedy

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GABA inhibits the monosynaptic reflex (MSR) to motoneurons by activating presynaptic GABA receptors on primary sensory afferent terminals in the ventral horn, in classic presynaptic inhibition. Serotonin (5-HT) also inhibits the MSR, but we show here by a very unusual, non-presynaptic mechanism. We specifically examined the MSR in the sacral cord of adult rats by stimulating the dorsal roots and recording the ventral roots (in vitro). We show that 5-HT1D receptors inhibit the MSR, with agonists inhibiting the reflex, and selective antagonists reversing this. Also, 5-HT1D agonists directly inhibited action potential transmission in primary sensory afferents, as measured by microstimulating the afferents within the spinal cord, and recording the antidromically propagated compound action potential on the same afferents in dorsal roots (DR-CAP). Even when the afferents were stimulated in the dorsal horn, far from the motoneurons, the DR-CAP was still inhibited by 5-HT1D receptor agonists, suggesting that these receptors were located in the dorsal horn. Indeed, using immunolabelling we show that the 5-HT1D receptors are entirely confined to the superficial dorsal horn. Also, chronic dorsal root rhizotomy eliminated all labelling, indicating that the receptors are entirely on afferents, and

confined to segments of the afferents in the superficial dorsal horn. Thus, the 5-HT1D receptor must inhibit the MSR by inhibiting the transmission of action potentials (and Na channels) on the afferents as they pass through the dorsal horn (en passant), far from the afferent terminals.

Opportunities for therapeutic development in ALS

Tuesday, June 17 (AM)
Presenter: Lucie Bruijn

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The landscape for ALS research has changed significantly in light of the recent discovery of C9orf72 mutations linked to ALS. Together with research advances and a change in industry approach to research and development there has been increasing interest in the bio sector to develop therapies for rare diseases such as ALS. The ALS Association play a pivotal role in brokering and funding these opportunities. A few of these will be highlighted and scientific advances discussed.

Hypervigilant regulation as ALS-cause: evidence from ALS patients and transgenic mouse models

Tuesday, June 17 (AM)
Presenter: Cassie Mitchell

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In a large retrospective study of approximately 1,000 Emory University ALS Clinic patients, we recently found that ALS patients, prior to their ALS diagnosis, are significantly healthier than the age-matched general population. The average prevalence of 45 common cardiovascular, metabolic, autoimmune or other neurological pre-existing or antecedent conditions in these ALS patients was approximately half that of the general population. Other studies by leaders in the field have shown a higher prevalence of ALS among previous athletes and those that lead active lifestyles. We propose a novel hypothesis, hypervigilant regulation, defined as over-zealously maintaining homeostasis by aggressively over-reacting to physiological variable perturbations, which could explain how patients with apparent "good health" later succumb to ALS. Given the physical length of motoneurons, they are already nominally susceptible to regulatory delays and neurochemical instabilities like hyperexcitability/excitotoxicity, protein aggregation, and bioenergetics and axonal transport deficiencies that could be either initiated or greatly compounded by hypervigilant regulation. We present evidence for the hypervigilant regulation hypothesis as ALS-cause by performing large-scale meta-analyses on extracted clinical data from over 1,000 patients at the Emory University ALS Clinic and experimental data from over 1,600 published G93A SOD1 transgenic mouse studies.

Mechanisms of ALS mutant FUS mediated motor neuron degeneration: evidence for a toxic gain of function in a novel mouse model of disease

Tuesday, June 17 (AM)
Presenter: Neil Shneider

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder in which selective loss of motor neurons (MNs) results in paralysis and death. Mutations in the gene FUS cause familial ALS, and FUS pathology has also been found in sporadic cases. Whether mutant FUS causes MN degeneration both by cell autonomous and non-autonomous mechanisms - as in the case of mutant SOD1 - is not known. To model the effects of ALS mutant FUS on MNs in vivo we targeted the mouse MAPT (tau (τ) locus to generate a conditional τ allele with which to control the pattern and timing of mutant or wild type human FUS expression in the nervous system. Selective expression of wild type FUS in MNs (τMN) had no effect on MN survival. In contrast, expression of ALS-associated R521C and P525L mutant FUS resulted cytoplasmic mislocalization of mutant FUS, nuclear aggregation and nuclear exclusion similar to what is seen in patients with FUS-ALS. This pathology was associated with progressive MN loss and denervation of hindlimb muscles, demonstrating the cell autonomous and mutation-dependent toxic effects of mutant FUS on MNs. Germline activation of the hFUSWT and hFUSP525L alleles in a series of τON mice revealed more pronounced MN degeneration as a consequence of the widespread expression of ALS mutant FUS in the nervous system. These studies demonstrate cell non-autonomous mechanisms of mutant FUS-dependent MN loss in this model, and suggest a pathogenic role for τ-expressing oligodendroglia. Analysis of these and related knockout mutants demonstrates a toxic gain of function.

Mechanical, electrical and molecular properties of mouse motor unit subtypes

Tuesday, June 17 (AM)
Presenter: Marin Manuel

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by the degeneration of motor units in the specific order FF>FR>S. However, until now, it was impossible to distinguish the physiological type of a motoneuron in mouse spinal cords. We have used our new in vivo mouse preparation, which allows the simultaneous recording of a motoneuron electrophysiological properties and the contractile properties of its motor unit, to identify the type of a recorded motoneuron. For the first time, we were thereby able to compare the electrophysiological and contractile properties of motoneurons type by type in mSOD1 mice, a model of

familial ALS. By injecting a dye into a type-identified motoneuron, we were furthermore able to study the expression of proteins that could constitute specific markers of type.

Is motoneuron hyperexcitability harmful in ALS ?

Tuesday, June 17 (AM)
Presenter: Daniel Zytnicki

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It has long been suggested that hyperexcitability of motoneurons induces excitotoxicity in ALS. Several works, made at different time points (embryos, neonates, adults), have led to somehow contradictory results. We would like to report new data both in neonate and in adult SOD1 G93A mice that suggest that intrinsic hyperexcitability is unlikely to cause degeneration of motoneurons. However, there is still a possibility, which remains to be fully investigated, that excitotoxicity might arise from synaptic inputs to motoneurons.

Sex specific contributions to altered motoneuron size in SOD1G93A ALS mouse model

Tuesday, June 17 (AM)
Presenter: Katharina Quinlan

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Amyotrophic lateral sclerosis (ALS) is a devastating, adult-onset neurodegenerative disease in which spinal and cortical motoneurons are lost. In most patients the disease occurs spontaneously, however ~10% of cases have a genetic cause. Among the spontaneous cases, there is an increased incidence and prevalence of spontaneous ALS in men, and in US military veterans. In a standard mouse model of the disease (SOD1G93A B6/SJL), female mice survive significantly longer than males, and male SOD1G93A B6 mice have larger C-terminals, while females show no changes. In our lab, electrophysiology has suggested that spinal motoneurons have increased in size (larger input conductance) in both juvenile and adult SOD1G93A B6/SJL mice. Since the motoneurons that are most vulnerable to degeneration are also the larger, FF motoneurons, we have directly investigated anatomical differences in spinal motoneurons in the male and female SOD1G93A B6/SJL at two presymptomatic time points, postnatal day (P)30 and 50 (overt symptom onset at P90). We focused on three parameters: soma surface area, number of stem (primary) dendrites, and total dendritic length. We found that SOD1G93A B6/SJL motoneurons are larger than SOD1WT (overexpressor control line B6/SJL) motoneurons in adulthood, confirming that mutant SOD1 motoneurons are not just increased in size during a transitory period in postnatal

maturation. In addition, larger motoneuron sizes appear more pronounced in male mice. The larger size of male motoneurons may contribute to their significantly shorter lifespans compared to female mice.

Chemogenetics and high-sensitivity performance test reveal new excitation-dependent and intrinsic vulnerability mechanisms in ALS mouse model

Tuesday, June 17 (AM)
Presenter: Francesco Roselli

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Amyotrophic lateral sclerosis (ALS) is characterized by progressive dysfunction and loss of motoneurons (MN). In the mutant SOD1 mouse model, MN subpopulations display differential vulnerability related to their activation properties, with fast-fatigable (FF) MN affected first. Vulnerable MN display biochemical abnormalities including ER stress, unfolded protein response and ubiquitin-proteasome dysfunction. The investigation of activity-related factors and the impact of biochemical dysfunctions requires the independent manipulation of each element and the development of sensitive read-out assays for FF-MN. We exploited chemogenetic tools to explore how excitation shapes MN vulnerability in vivo. The engineered ion channels were expressed in MN by intraspinal injection of AAV9 and activated by systemic administration of the agonist. Excitation delivers a critical neuroprotective signal to vulnerable MN in the early stages of disease progression, while increased inhibition is detrimental. To investigate cell-autonomous pathogenic pathways, we devised a motor test to reveal the early dysfunction of vulnerable MN, and we used it to screen small-molecule modifiers of mouse performance. Several small molecules, affecting autophagy and heat-shock protein response, showed beneficial effect on MN performance. MN excitability emerges as a key regulator of several cell-autonomous pathogenic pathways. Early changes in excitability and dysfunctional autophagic flux contribute to the pathogenic cascade that shapes MN vulnerability, impairs MN function and leads to MN degeneration.

Vibration-induced H-reflex inhibition is suppressed in ALS ? A biomarker for upper motor neuron dysfunction?

Tuesday, June 17 (AM)
Presenter: Michael Lee

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UMN signs in the limbs of ALS patients are difficult to detect clinically particularly if there is prominent neurogenic weakness. In an attempt to identify a sensitive neurophysiological biomarker for UMN dysfunction, we investigated the inhibitory effects of focal vibration on the H-reflex using a threshold tracking paradigm in 25 ALS patients and 20 healthy controls and correlated the results with clinical UMN signs and ALSFRS. The marker of motoneuronal excitability was the threshold current (H-Th) needed to generate an H-reflex of 10% Mmax. This was tracked using a semi-automated program (Q-Trac). Focal vibration (50Hz) was applied over Achilles' tendon (Ach), lateral gastrocnemius (LG) and tibialis anterior (TA). Soleus H-reflex was absent in 5 ALS patients (i.e., 20%) (present in all controls). H-Th was higher in ALS patients (14 mA) compared to controls (8.5 mA; p=0.0031). In all controls, vibration of Ach, LG and TA immediately abolished the H-reflex and H-Th was elevated (by 34.1%, 29.7% and 24.3% respectively) to compensate for the vibration-induced inhibition. ALS patients (n=20) showed much smaller threshold changes during Ach, LG and TA vibration (4.2%, 3.8% and 6.6% respectively; p<0.0004). H-Th change during Ach vibration and ALSFRS were positively correlated (r=0.45, p=0.014, R^2=0.28), with the association stronger for bulbar onset ALS (n=8, r=0.67, p=0.035, R^2=0.45). Vibration-induced inhibition of H-reflex is suppressed in ALS patients with and without clinically detectable UMN signs and may assist in identifying UMN pathology in patients with possible ALS

Sensorimotor impairment in Amyotrophic Lateral Sclerosis (ALS)

Tuesday, June 17 (AM)
Presenter: Sina SANGARI

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Recent study using spinal multiparametric MRI has revealed denervation and demyelination of sensory ascending tracts in patients with ALS without any sensory clinical sign. This unexpected result raised the question whether abnormal sensory inputs could participate in the pathophysiology of ALS. Because MRI does not inform on possible functional impairment, the aim of the project was to investigate electrophysiologically sensory integration at spinal levels. Median and ulnar nerves were stimulated at wrist level to activate group Ia afferents from hand muscles (clinically impaired). Transcranial magnetic stimulation was applied over the primary motor area to elicit motor evoked potential (MEP) in triceps brachii (clinically non-affected). MEP recruitment curve and median and ulnar-induced modulations of MEP size were compared in ALS patients and sex/age matched healthy subjects. While the MEP threshold was significantly increased in the patient group, the MEP recruitment curve exhibited the same shape in both groups. This indicates that the corticospinal control was similar between groups, suggesting that synaptic activity between pyramidal cells and motoneurons was normal. While the sensory afferent input was less in patient group, the median and ulnar-nerve induced MEP facilitation was larger than in controls. This suggests that group Ia monosynaptic facilitation in motoneurons was increased in patients. Alteration of this synapse may contribute to a compensatory change of motoneuron homeostasis, leading finally to its hyper-excitability and degradation.

Human iPSC-derived motoneurons harbouring TDP-43 or C9orf72 ALS mutations are dysfunctional despite maintaining viability

Tuesday, June 17 (AM)
Presenter: Anna Claire Devlin

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterised by progressive paralysis due to the loss of motoneurons (MNs). ALS remains untreatable and incurable, largely due to our incomplete understanding of underlying disease mechanisms. With the advent of induced pluripotent stem cell (iPSC) technology, it is now possible to study human neurons from individuals with degenerative diseases at a range of time points, including those prior to overt pathology. In the present study, we have utilised whole-cell patch-clamp recording techniques to study ALS pathophysiology in human iPSC-derived MNs affected by the disease. We have demonstrated that MNs derived from human iPSCs obtained from healthy individuals or patients harbouring TDP-43 or C9orf72 ALS mutations develop appropriate physiological properties. However, patient iPSC-derived MNs display an initial hyperexcitability, followed by a progressive loss of action potential output and synaptic activity. This loss of functional output appears to result from a progressive decrease in voltage-activated Na and K currents which occurs in the absence of any changes in cell viability. These novel data from ALS-affected human MNs indicate that early dysfunction or loss of ion channels contributes to the initiation of downstream degenerative pathways that ultimately lead to MN loss in ALS. Through the use of this model system we hope to reveal new disease mechanisms and ultimately novel, highly relevant targets for much needed therapies for this devastating disease.

A shared requirement for POU3F1 in distally projecting motor neurons enables innervation of muscles critical to respiration and grasping

Tuesday, June 17 (PM)
Presenter: Kevin Kanning

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To establish synapses onto specific muscles, distinct motor pools are burdened with differential axon growth requirements as a function of target distance. We hypothesized distally projecting motor neurons may share a transcriptional program enabling distal innervation. Focusing on distinct motor nerves at cervical levels in mouse, we identified the phrenic, median and ulnar nerves as the longest axon projections at E12.5 and found

unique expression of POU3F1, a member of the POU transcription factor family renowned for roles in axon growth, common to these diverse MN populations. To test the requirement for POU3F1 in distal motor innervation, we analyzed POU3F1 null mice and found that although at birth the phrenic, median and ulnar nerves had contacted their muscle targets, they showed a striking failure to innervate more distal compartments within each muscle. A temporal analysis reveals a failure in initial outgrowth as early as E13.5, without compensation over time. Deficient innervation was not a consequence of faulty specification or early MN survival, as we found normal numbers of POU3F1-dependent MNs at E12.5. However, coincident with defects in target innervation, a significant number of MNs died by E14.5. Preventing MN loss using a compound Bax;POU3F1 mutant failed to rescue distal muscle innervation despite a surplus of MN number, clearly demonstrating a POU3F1 requirement for distal innervation independent of survival. Therefore POU3F1 regulates an intrinsic program critical for motor innervation of the muscles vital to breathing and grasping.

Fine tuning the final common path: molecular pathways driving motor neuron functional diversification and plasticity

Tuesday, June 17 (PM)
Presenter: Till Marquardt

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Motor neurons encompass types ranging from 'slow' to 'fast' whose biophysical properties govern gradation and amplitude of muscle force during movements. Such functional specialization of neurons belonging to the same morphological class is a recurrent feature of nervous system organization thought to be critical for neural network integration and output. In the talk, I will provide an update on our screening efforts for identifying determinants of motor neuron functional diversification and plasticity. For instance, we found a non-canonical Notch receptor ligand, expressed by ~30% of motor neurons, that is necessary and sufficient to promote a fast biophysical signature in motor neurons. This pathway involves global implementation of motor neuron type-specific gene programs, including genes encoding neural activity modulators. Its inactivation comprehensively shifts motor neurons towards slow biophysical and transcriptome signatures, while abolishing peak force outputs. This new work may shed light onto the development of motor neuron functional diversity and its contribution to movements. I will further highlight conceptual challenges to our current understanding of motor neuron functional organization and its significance to movement control, in addition to providing an update on our search for mechanisms promoting motor neuron adaptive plasticity.

Development of electrical and morphological properties of lumbar motoneurons in the mouse

Tuesday, June 17 (PM)
Presenter: Jacques Durand

Authors:

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Electrical and morphological properties of lumbar motoneurons (Mns) change dramatically during the first postnatal days in the mouse. Antidromically identified lumbar Mns (n=104) were intracellularly recorded in an isolated brainstem/spinal cord preparation from mice aged between P3 and P10 and some of them (n=38) were stained with Neurobiotin and reconstructed using NeurolucidaTM. The input conductance of Mns was positively correlated with the total dendrite surface area. As in other species, motoneuron rheobase and input conductance are positively correlated and both increase with age. However, both parameters can vary three to five times at the same age. Three main types of lumbar Mns were characterized by their firing profile during prolonged depolarizations (transient, early and sustained firing, delayed onset). Mns with late spiking (delayed-onset discharge) represent 30% of the recorded Mns and have a high rheobase. The proportion of Mns with delayed onset vary depending on the "in vitro" preparations. In the isolated brainstem/spinal cord preparation 70% of Mns fired with early action potentials and 30% with late spiking, whereas in the slice preparation the reversed proportions where found (Pambo-Pambo et al., 2009). This difference might be related either to different experimental recording conditions or to the spinal control by descending pathways in the entire preparation. Our data indicate that the acquisition of adult-like functional properties of lumbar motoneurons occurs near P8 in the mouse when delayed firing disappears.

Functional organization of spinal motor neurons revealed by ensemble imaging

Tuesday, June 17 (PM)
Presenter: Timothy Machado

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The isolated neonatal mouse spinal cord is capable of generating sustained rhythmic network activity, termed fictive locomotion. However, the spatiotemporal patterning of motor neuron activity in this network state has not been described at single-cell resolution. We have measured the activity of hundreds of motor neurons using large-scale, cellular resolution calcium imaging and spike inference methods to estimate the phasic tuning of each motor neuron during fictive locomotion. This approach was validated using antidromic stimulation of ventral roots to induce specific firing patterns during imaging. We found that neurons that innervate synergist muscles (which are clustered into spatial groups called columels) fire synchronously and that phase synchronization does not fall off over long distances within columnels. In contrast, neurons innervating flexor muscles from different joints displayed offset burst times. Flexor and extensor motor neurons obeyed an antiphase relationship relative to one another and were also distinguishable on the basis of their burst duration. These spatiotemporal patterns were highly reproducible between preparations. Our results demonstrate that locomotor-like network activity is intrinsically organized by muscle target identity, revealing complexity and specificity in the patterns of motor neuron recruitment. These measurements of fictive locomotion in wild type mice have provided a quantitative baseline for ongoing work in which we are analyzing the role of motor neuron identity in the formation of locomotor circuits.

Number, size and distribution of motoneurons in motor nucleus of male and female rat medial gastrocnemius

Tuesday, June 17 (PM)
Presenter: Jan Celichowski

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The cell bodies of motoneurons of male and female rats (6 months old) were labeled following a bath of the proximal stump of the transected medial gastrocnemius (MG) nerve in the horseradish peroxidase solution. The central nervous system mass was by 20% higher in males. The total number of motoneurons was higher in males than in females by 13% and amounted to 94 and 83 motoneurons, respectively. The size of stained cells was measured and motoneurons were divided into alpha and gamma types at a border value of a cell diameter of 27.5 µm. The mean of 66 alpha motoneurons was obtained in the motor nucleus of male rats, contrary to 56 alpha motoneurons in females. Similar number of gamma motoneurons was found in both sexes (28 vs 27, in males and females, respectively). The soma diameters of alpha and gamma motoneurons were bigger in males, by 6% and 9%, respectively. Moreover, the MG motor nucleus was longer for males than for females (3.7 vs 2.8 mm, respectively), but the density of stained motoneurons was lower in males.

Signalling through MuSK and the acetylcholine receptor mediate the retention of neuromuscular connections

Tuesday, June 17 (PM)
Presenter: William (Bill) Phillips

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Muscle Specific Kinase (MuSK) is a transmembrane tyrosine kinase in the postsynaptic membrane at the neuromuscular junction (NMJ). MuSK is vital for NMJ formation during embryonic development. A subset of myasthenia gravis patients display autoimmune antibodies against MuSK. Daily injections of IgG from anti-MuSK patients into mice caused a reduction in postsynaptic staining for MuSK. Subsequent slow declines in postsynaptic acetylcholine receptor (AChR) density and endplate potential amplitude culminated in neuromuscular transmission failure and whole-body weakness after 12-14 days. The postsynaptic AChR clusters became fragmented with a slow wastage of AChRs. Motor nerve terminals displayed reduced alignment with postsynaptic AChRs. In the diaphragm muscle the adaptive increase in quantal release (which normally follows a reduced quantal amplitude) failed. There was also evidence for the complete disappearance of many neuromuscular connections. During embryonic development activation of postsynaptic AChRs and associated and calcium influx drives a negative feedback loop that can disperse AChRs. This mechanism seems to remain

active at adult NMJs. In mice injected with anti-MuSK IgG, the cholinesterase inhibitor drug, pyridostigmine (which prolongs the activation of AChRs) exacerbated the dispersal of postsynaptic AChR clusters. Our findings suggest that signalling through MuSK and AChR converges to regulate the size and effectiveness of the adult NMJ. This has implications for diseases where the retention of neuromuscular connections comes under challenge.

Loss of β2-Laminin Alters Calcium Sensitivity and Voltage Gated Calcium Channel Maturation of Neurotransmission at the Neuromuscular Junction

Tuesday, June 17 (PM)
Presenter: Peter Noakes

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Beta 2-laminin is a key mediator in the differentiation and formation of the skeletal neuromuscular junction. Loss of beta 2-laminin results in significant structural and functional aberrations such as decreased number of active zones and reduced spontaneous release of transmitter. In-vitro β2-laminin has been shown to bind directly to the pore forming subunit of P/Q-type voltage gated calcium channels (VGCCs). Neurotransmission is initially mediated by N-type VGCCs, but by postnatal day 18 switches to P/Q-type VGCC dominance. We have investigated the changes in neurotransmission during the switch from N- to P/Q-type VGCC mediated transmitter release in mice lacking beta 2-laminin. Analysis of the relationship between quantal content and extracellular calcium concentrations demonstrated a decrease in the calcium sensitivity, but no change in calcium dependence at beta 2-laminin deficient junctions. Electrophysiological studies on VGCC sub-types involved in transmitter release indicate N-type VGCCs remain the primary mediator of transmitter release at matured beta 2-laminin deficient junctions. β2-laminin deficient junctions also maintained presence of N-type VGCC clustering within the pre-synaptic membrane, which supported our functional findings. We conclude that β2-laminin is a key regulator in development of neuromuscular junction function, with loss resulting in decreased calcium sensitivity stemming from a failure to switch from N to P/Q type VGCCs, as well as alterations in the pre-synaptic density of existing VGCCs.

Spinal neuron identity and survival in the absence of neurosecretion

Tuesday, June 17 (PM)
Presenter: Chris Law

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Spinal cord development is critically dependent upon intercellular communication via the regulated secretion of morphogens, neurotrophins and neurotransmitters. Electrical activity influences neural circuit development by affecting gene transcription, neural tube patterning and axon guidance, though understanding of these effects has been hampered by the imprecise nature of pharmacologically-induced electrical activity manipulations. The Munc18-1 protein is critical to neurosecretion and regulates docking of vesicles containing neurotrophins or neurotransmitters. We examined development of the spinal cord of mice lacking Munc18-1 to probe the role of patterned activity and neurally-derived neurotrophin secretion in spinal neuron patterning and survival. Mice lacking Munc18-1 lack patterned activity, though numbers of molecularly-defined motor- and interneurons are normal, demonstrating that patterned electrical activity is not critical for neuronal specification. However, in Munc18-1 mutants there is an increase in cell death both in vivo and in vitro. As we also observe a dysregulation of neurotrophin receptor localisation, we hypothesise that the observed apoptosis is due to inhibited neurotrophin signalling. When Munc18 is ablated only in motor neurons, they are spared from apoptosis at E13.5, suggesting that it is a non-cell autonomous phenotype. Given that Munc18 mutants have a normal response to peripheral limb-derived neurotrophins, our hypothesis is that there is a remarkable dependence of motor neurons and interneurons on central neurotrophic signals.

Mechanisms of cholinergic synapse formation in mouse spinal cord

Tuesday, June 17 (PM)
Presenter: Kuo-Fen Lee

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Neuronal networks in the vertebrate spinal cord control many facets of locomotor behavior. Effective locomotion requires coordinated and adaptable firing of motoneurons (MNs) during specific motor tasks. Several morphologically and physiologically distinct types of synapses on MNs have been identified. The excitability of MNs is heavily regulated by modulatory synapses, including the cholinergic C-boutons for which the V0c interneurons provide the presynaptic terminals. The rhythmic activity of V0c interneurons during locomotion is phase locked with the activity of their target MNs. C-boutons on MN surface are closely aligned with clusters of m2 muscarinic receptors (M2Rs) and Kv2.1 voltage gated potassium channels. Activation of M2Rs increases the excitability of MNs by reducing the amplitude of the afterhyperpolarization. But the mechanisms of cholinergic synapses formation are not understood. We found that neuregulin 1 (NRG1) protein is clustered on MN membrane opposed by the C-boutons. NRG1 and their erbB receptors play multiple roles in neural development and function via both forward and reverse signaling pathways. We determine if NRG1 is required for the formation of cholinergic synapses in mice lacking NRG1 expression in MNs. M2Rs are no longer clustered on postsynaptic membrane, and the muscarinic-evoked increase in excitability of motoneurons is reduced. However, presynaptic specialization is not affected. Surprisingly, M2R clustering is normal in mice that

lack erbB receptors either in pre-motor interneurons or in MNs. These results demonstrate that NRG1, independent of erbB receptors, is required for cholinergic synapse formation.

Non-cell autonomous mechanisms induce dysfunction of motor neurons in a mouse model of Spinal Muscular Atrophy

Wednesday, June 18 (AM)
Presenter: George Mentis

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Spinal muscular atrophy (SMA) is a neurodegenerative disease caused by reduced levels of SMN protein. The hallmarks of SMA are loss of motor neurons (MNs), muscle atrophy and abnormal postural reflexes. The mechanisms leading to selective motor deficits are poorly understood. Using SMA mice, we have shown that sensory-motor circuit dysfunction precedes motor neuron loss. Vulnerable MNs innervating axial muscles are abnormally hyperexcitable and exhibit reduced reflexes compared with resistant MNs. To address whether this hyperexcitability is due to premotor synaptic dysfunction or due to SMN deficiency per se in MNs, we first measured the intrinsic membrane properties from vulnerable SMA MNs in presymptomatic stages. Our results reveal that ~50% of SMA MNs, at P2, were significantly hyperexcitable compared with their WT counterparts. At the same time, the monosynaptically-evoked EPSPs after proprioceptive fibers stimulation revealed a significant reduction. The rest 50% of SMA MNs exhibited normal intrinsic and evoked EPSPs. In addition, we investigated the intrinsic properties of MNs in animals, in which SMN has been selectively restored in proprioceptive neurons only, using novel Cre/Lox technology. Our results reveal that the hyperexcitability observed in vulnerable SMA motor neurons is significantly corrected. These findings provide evidence that SMN deficiency in proprioceptive neurons alters the intrinsic excitability of SMA motor neurons through non-cell autonomous mechanisms, consistent with SMA being a disease of motor circuits.

Mutations in glycyl-tRNA synthetase that cause peripheral neuropathy CMT2D create a neomorphic protein that antagonizes VEGF-Nrp1 signaling

Wednesday, June 18 (AM)
Presenter: Sam Pfaff

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Charcot-Marie-Tooth (CMT) diseases are the most common hereditary peripheral neuropathies. Nevertheless, our understanding of the pathological mechanisms that underlie CMT are limited, which has hindered the identification of effective therapies. A subtype of the diseases--CMT2D--is caused by dominant mutations in

GARS, encoding the ubiquitously-expressed enzyme glycyl-tRNA synthetase (GlyRS). Despite the broad requirement of GlyRS in all cells, mutations in this gene cause a selective degeneration of peripheral axons leading to deficits in distal motor function. Here we report that wildtype and mutant GlyRS are both cosecreted with exosome vesicles, but mutant GlyRS selectively antagonizes the interaction between Neuropilin 1 receptor (Nrp1) and vascular endothelial growth factor (VEGF). Lentivirus-mediated expression of VEGF in mice with CMT2D-mutations improves their motor function, synaptic connectivity, and spares motor axon degeneration. These findings suggest that VEGF-Nrp1 signaling contributes to motor neuron survival, and this pathway may be a useful target to exploit for CMT2D treatment.

'Roid 'Rage - The mechanism of neurosteroid-mediated excitation of motor neurons

Wednesday, June 18 (AM)
Presenter: Mark Bellingham

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Neurosteroids, such as alfaxalone, are effective anaesthetic agents with desirable properties including a high margin of safety, minimal cardiorespiratory depression, multiple routes of administration, and ability to give multiple top-up doses. However, in many species, alfaxalone anaesthesia can complicated by prominent signs of pyschomotor excitation. Intraperitoneal alfaxalone administration (20 mg/kg body weight) in adult Wistar rats (n=20) produced alfaxalone plasma levels equivalent to those of 2 and 5 mg/kg intravenous (IV) injections (n=5 each). Muscle twitching during alfaxalone anaesthesia induction or recovery was a consistent effect in rats (n=20). We measured glycinergic responses of single hypoglossal motor neurons (HMs), which control tongue muscles, to alfaxalone using whole cell patch clamp electrophysiological recordings from in vitro slices of juvenile (10-18 day old) rat brain tissue. Alfaxalone produced a dose-dependent reduction in spontaneous and evoked glycinergic IPSCs, consistent with a presynaptic reduction in glycine release. Our data show that alfaxalone causes 1) psychomotor excitation in rats in vivo and 1) reduction in glycine receptor-dependent inhibition of rat motor neurons in vitro.

Mechanisms Regulating Axonal Transport and Motor Neuron Degeneration

Wednesday, June 18 (AM)
Presenter: Yong-Chao Ma

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Motor neurons are characterized by long axons connecting cell bodies with targets. Coordinated transport of organelles and molecules along axons is essential for neuronal functions, which are modulated by

neurotransmitters. However, little is known about how axonal transport is regulated by signal transduction pathways. Here we report a novel signaling mechanism regulating axonal transport. Dysregulation of this mechanism leads to defective axonal transport and selective motor neuron degeneration in spinal muscular atrophy (SMA). Using SMA mouse and zebrafish models, we found that the phosphorylation of histone deacetylase 5 (HDAC5) on Serine 279 (S279) within the nuclear localization sequence (NLS) is increased in motor neurons affected by SMA. HDAC5 S279 is phosphorylated by cyclin-dependent kinase 5 (Cdk5), and dephosphorylated by protein phosphatase 2A (PP2A) activated by the cAMP-PKA signaling pathway. In addition, we identified HDAC5 as a novel tubulin deacetylase, regulating microtubule-based axonal transport. Using in vivo confocal live imaging, we have found that increased phosphorylation of S279 on HDAC5 leads to cytoplasmic localization of HDAC5, enhanced deacetylation of tubulin, and defective motor axon transport in motor neurons affected by SMA. Altogether, our findings reveal a new mechanism by which signal transduction pathways regulate axonal transport. Dysregulation of this mechanism leads to defective axonal transport, which may be particularly exacerbated in motor neurons with long axons, contributing to the specific motor neuron degeneration in SMA.

Spinal plasticity in stroke patients after botulinum neurotoxin A injection in ankle plantarflexors

Wednesday, June 18 (AM)
Presenter: Veronique Marchand-Pauvert

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The effect of botulinum toxin (BT) after stroke has been attributed to its peripheral action only. However, it depressed recurrent inhibition of lumbar motoneurons, likely due to its retrograde transportation along motor axons affecting synapses to between antagonists, we tested whether this inhibition, particularly affected after stroke, could recover after BT. The effect of posterior tibial nerve (PTN) stimulation on tibialis anterior (TA) EMG was investigated in stroke patients during walking before and 1 month after BT injection in ankle plantarflexors. Before BT, PTN stimuli enhanced TA EMG during the swing phase. After, the PTN-induced reciprocal facilitation in TA motoneurons was depressed at the beginning of swing and reversed into inhibition in midswing, but at the end of swing, the reciprocal facilitation was enhanced. This suggests that BT induced spinal plasticity leading to the recovery of reciprocal inhibition likely due to the withdrawal of inhibitory control from Renshaw cells directly blocked by BT. At the end of swing, the enhanced reciprocal facilitation might be due to BT-induced modification of peripheral afferent inputs. Therefore, both BT central and peripheral actions can modify muscle synergies during walking: i) limiting ankle muscle co-contraction in the transition phase from stance to swing, to assist dorsiflexion, and ii) favoring it from swing to stance, which blocks the ankle joint and thus assists the balance during the single support phase on the paretic limb.

Motor unit number estimation and functional tasks

Wednesday, June 18 (AM)
Presenter: Stephane Baudry

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Introduction The number of functioning motor units (MU) has been reported to decrease with ageing (McNeil et al. 2005), but the influence of motor unit number on motor control remains uncertain (Enoka et al. 2003). The present work re-investigates the relation between motor unit number estimation (MUNE) and performance in motor tasks in young and elderly adults. Methods Motor unit number estimation (MUNE) in tibialis anterior is computed with the spike-triggered average technique from intramuscular MU recordings during dorsiflexion isometric contractions. For each subject, at least 10 MU and a minimum of 3 trains of 50 action potentials for each MU was used to compute the mean surface motor unit action potential (sMUAP). MUNE is calculated by dividing sMUAP by the compound muscle action potential. The motor tasks investigated in relation to MUNE are submaximal contractions and postural tasks. Results Up to now, MUNE has been calculated for 4 young (23-39 yrs) and 4 elderly adults (69-76 yrs), and tends to be greater in young (range: 122-155) than in elderly adults (range: 84-124). The range of MU recruitment threshold was 5-30% and 15-50% of maximal torque for young and elderly adults, respectively. No relation exists between sMUAP amplitude and MU recruitment threshold. Conclusion The current data, although incomplete, are in agreement with previous work reporting a reduced number of MU with ageing in the tibialis anterior (McNeil et al. 2005). Data collection is still under progress and relations between MUNE and motor task performance will be presented at the congres

Anomalous EMG-force relations during stretch reflex responses in stroke survivors

Wednesday, June 18 (AM)
Presenter: Nina Suresh

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EMG force relations derived from human data have been described extensively. The slope of the EMG-force curve has been reported to be significantly greater in paretic muscle as compared to non-paretic muscle during voluntary isometric force contractions. It is assumed that the larger EMG increment for a given force increment in stroke is a result of increased motor unit(MU) recruitment needed to compensate for reduced MU discharge. Multiple factors confound interpretation of the observed slope increases during voluntary tasks. To address this we assessed the EMG-force relation of reflexive responses to brisk tendon taps in stroke survivors. Experiments were performed on the biceps brachii of both sides of three hemiparetic stroke individuals. Bipolar surface EMGs were recorded from both the biceps and triceps brachii muscles. Reflexes were elicited at varying depths of tendon indentation while the subjects were asked to remain at rest. Surface EMG response magnitude was

measured by calculating the RIEMG (rectified and integrated EMG). A least squares regression line was fit to the RIEMG of the reflex response versus the maximum reflex force and the slope of this regression line was compared for the involved and contralateral sides of each subject for both conditions. All three subjects exhibited a substantially greater slope on the impaired side as compared to the unimpaired side under resting conditions. These results suggest the possibility that EMG force slopes are a function of increased excitability of motoneurons innervating paretic/spastic muscle.

Mechanisms of Spasticity in Cerebral Palsy

Wednesday, June 18 (AM)
Presenter: Monica Gorassini

Authors:
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Individuals with cerebral palsy (CP) often have spasticity as their primary motor impairment, potentially mediated by an altered activation of spinal motoneurons by sensory pathways. We have measured how cutaneomuscular afferents, which recruit both inhibitory and excitatory spinal interneurons, activate the motoneuron. To estimate the profile of sensory-evoked post-synaptic potentials (PSPs) in the human, we used the technique of Peristimulus Frequencygrams where the firing rate of a tonically active motor unit is plotted time-locked to a sensory stimulation. Motor unit activity from the soleus was recorded in response to a stimulus train (5 pulses, 300 Hz, just below pain threshold) applied to the foot medial arch during a weak contraction. In non-injured controls (n = 13), medial arch stimulation produced an early-latency pause in motor unit firing followed by a cluster of motor unit firing rates below background. Based on intracellular recordings, this profile is indicative of a strong, spinally-mediated inhibitory PSP. Afterwards, there was a second period of elevated firing above background lasting for ~300 ms, indicating a subsequent excitatory PSP. In the CP participants with varying levels of dysfunction (n = 17), some displayed responses similar to controls with evidence of IPSP and EPSP activation while others showed only evidence of pure EPSP activation. CP participants having a lack of IPSP activation typically were not functional walkers. We propose that in CP, walking helps to maintain the integrity of spinal inhibitory networks.

Evidence of systemic depolarization & prolonged Ia EPSP in α motoneurons of hemispheric stroke survivors

Wednesday, June 18 (AM)
Presenter: Matthieu Chardon

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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.

Contradictions in motoneurone discharge behaviour after stroke: comparing activity from the more-affected side, less-affected side and healthy subjects

Wednesday, June 18 (AM)
Presenter: Penelope McNulty

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Motoneurone activity, discharge variability, and dynamic range are reduced on the more-affected side after stroke. Data from three experiments demonstrate a more complex pattern of impaired motoneurone control. The more-affected biceps brachii stimulus-response curves to transcranial magnetic stimulation were reduced but parallel to control data. Higher stimulus intensities were required on the less-affected side for a given proportion of muscle output until the maximum response was reached which was equal to control data. On the more-affected side torque error and regularity were higher during ankle dorsiflexion. Although the EMG-torque relationship was not statistically disrupted the ratio was lowest on the less-affected side and the pattern of spontaneous activity was different. Spontaneous motoneurone discharge rates were equivalent to task-driven activation, and discharge rates were lower on the more-affected side as expected after stroke. However the pathological activation was demonstrated on the less-affected side with discharge rates higher than either the more-affected side or control data which were not different. The complex bilateral impairments revealed here may reflect the influence of diaschisis, compensation for learned non-use, and asymmetric interhemispheric inhibition. They emphasise the difficulty of both hemispheres to drive descending connections; that cortical control of motoneurone discharge becomes more important; and that the less-affected side cannot be used as a control for more-affected side motoneurone behaviour after stroke.

Optogenetic control of muscle contraction attenuates denervation atrophy ? who needs motor neurons?

Wednesday, June 18 (AM)
Presenter: Victor Rafuse

Authors:
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Exogenously induces the contraction of non-functioning muscles using electrical currents has been studied for over 100 hundred years. While functional electrical stimulation has proven to be useful in a limited number of applications, its broader use is plagued by damaging side effects caused by electroporation, thermal damage and the production of toxic products. To circumvent these issues, we generated transgenic mice expressing channelrhodopsin 2 in skeletal muscles (Sim1::ChR2 mice). We then showed that light activated twitch and tetanic contractions were as efficient as nerve stimulation. Furthermore, contractile force could be graded by varying light intensity or through repetitive light stimulation. Finally, to ascertain whether this approach could attenuate a clinical relevant condition known as denervation atrophy, we denervated the hindlimb of Sim1::ChR2 mice and activated thigh muscles transcutaneously with light for one hour a day. Hindlimbs were denervated in a second group of mice, but were not light activated. Ten days later, medial gastrocnemius muscle force, wet weight, whole muscle cross-sectional area, and mean muscle fiber cross-sectional areas were quantified. All four measures were significantly greater in the light activated muscle group compared to denervation alone. These results indicate that optogenetics can be used to efficiently control muscle contraction acutely and attenuate denervation atrophy chronically. Supported by CIHR.

Sensorimotor control circuits in dragonfly prey capture

Thursday, June 19 (AM)
Presenter: Anthony Leonardo

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Dragonflies are aerial predators that intercept small flying insects. These interception flight paths are hypothesized to arise from a simple rule based on stabilizing the visual angles to the moving prey. Within the nervous system, a target-selective class of descending neurons (TSDNs), sensitive to prey angular drift, is hypothesized to influence the firing rhythm of wing motorneurons by unknown circuitry. We will discuss two topics related to these points. First, using a novel camera array system, we will show that the structure of the dragonfly's interception flight path is driven largely by motor constraints related to head and body orientation, rather than primarily by prey drift. Second, we present new anatomical data, based on double backfills of descending TSDNs and flight motorneurons. This data suggests a compelling circuit architecture in which TSDNs influence steering by synapsing directly onto the apical trunks of wing motorneurons dendrites. This allows

TSDN input to bypass the microcircuitry of the flight motor and directly contribute to the activation of individual wing muscles. Additional anatomical data reveal that neck proprioceptors, carrying body state information, make GABAergic synapses in the same location, creating a specialized circuit point where target information, body information, and flight motor oscillation patterns all converge concurrently onto wing motorneurons. Based on this data, we propose a simple computational logic for interception steering.

How rhythmic inputs to motoneurons produced: We were wrong!

Thursday, June 19 (AM)
Presenter: Jack Feldman

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During rhythmic movements including breathing and locomotion, coordinated bursts of motoneuronal activity drive coordinated muscle contraction. These bursts are downstream manifestations of rhythmic bursting in neural circuits driving the rhythm. We have considered this bursting to be essential to the rhythmo¬genic process. WRONG1! We have considered inhibition within the rhythmo¬genic circuits to be essential for rhythm. WRONG2! We have considered pacemaker neurons as a key element for rhythmogenesis. WRONG! What mechanisms are left? An essential one that has been ignored largely because it has been experimentally refractory is the neural microcircuit, and we now are discovering some basic properties that are indicative of a critical role in rhythm generation3. We will propose a thesis for rhythmogenesis that reflects what we now know about the kernel for breathing.

Motoneurons, orchestrated by modular clocks or global networks?

Thursday, June 19 (AM)
Presenter: Jorn Hounsgaard

Authors:
Jorn Hounsgaard[1]
[1]University of Copenhagen

The coherent activity of hundreds of Motoneurons is what makes limbs move. But what brings them to it ..... And what keeps them at it? Simple hindlimb movements like scratching are thought to be driven by flexor and extensor half-center networks in the lumbar spinal cord. However, in an ex vivo preparation from the adult turtle we find that the scratch network extends into non-motor segments of the thorasic spinal cord. As in the lumbar cord most thorasic ventral horn neurons are recruited during scratching, each with a cell specific phase relation to the scratch rhythm. The active periods of the population interneurons are evenly distributed over the scratch cycle with no signs of clustering in the extensor and flexor phases. During scratch episodes these neurons receive intense concurrent rather than phasic synaptic inhibition and excitation. Taken together our

results suggest that even simple limb movements involve a widely distributed network of neurons that seems to be organized as a global neural network rather than local clockworks of interacting segmental modules. So what is the physical origin of modules and rhythms?

Motoneurones keep calm and carry on: Pain during fatigue does not change the excitability of motoneurones of the leg

Thursday, June 19 (AM)
Presenter: David Bennett

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Group III/IV muscle afferents affect motoneurones of agonist/antagonist muscles of the arm during fatigue (Martin et al. J Neurosci 2006). We assessed their effect on the corticomotoneuronal path of knee extensors. In 2 studies, subjects (n=10) sat with the right leg strapped to a transducer to measure knee extensor force. In study 2 we also measured knee flexor force. Surface EMG was recorded from vastus lateralis (VL) and biceps femoris (BF). In study 1, on 2 days, subjects performed brief maximal voluntary contractions (MVCs) of knee extensors before and after a 2-min MVC of the extensors. Study 2 was similar to study 1 except subjects performed a 2-min MVC of the knee flexors prior to brief extensor MVCs. On 1 day a pressure cuff occluded circulation of the fatigued muscles, and so maintained firing of group III/IV muscle afferents after the sustained 2-min MVC. EMG responses [motor evoked potentials (MEP) and thoracic motor evoked potentials (TMEP)] were evoked by transcranial magnetic stimulation and thoracic electrical stimulation delivered during MVCs. After the 2-min extensor MVC, maintained firing of group III/IV muscle afferents had no effect on the size of VL MEPs or TMEPs (P=0.18 and P=0.50, respectively). Likewise, after the 2-min knee flexor MVC, maintained firing of these muscle afferents showed no effect on VL MEPs or TMEPs (P=0.69 and P=0.34, respectively). Fatigue-sensitive group III/IV muscle afferents do not alter the excitability of the motor cortical output cells or motoneurone pool of the knee extensors during maximal efforts during fatigue.

Group Ia reciprocal excitation between ankle antagonists in a plantigrade animal

Thursday, June 19 (AM)
Presenter: Adam Deardorff

Authors:
Adam Deardorff[1], Robert Fyffe[1], Timothy Cope[1]
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We expect the obvious differences in hindlimb movements of adult rats and cats to manifest species difference(s) in segmental circuit function. Digitigrade operation of the ankle joint in cats requires plantar and

dorsiflexors adopt a role in ankle stabilization distinct from plantigrade posturing of the rat or human. Nichols & Koffler-Smulevitz (1991) assign an important task in stabilizing the ankle joint in cat to reciprocal Ia inhibition, and Hongo et al (1984) propose species differences in this segmental circuit may underlie plantigrade vs digitigrade limb use. We tested these notions by studying the IA reciprocal pathways, which are so well documented in cat, for the first time in the adult rat. Intracellular records of synaptic potentials were obtained in vivo from motoneurons of acutely decerebrated or ketamine/xylazine anesthetized adult rats. Following selective physiological activation of tibialis anterior Ia afferents, we observed few instances of reciprocal inhibition in rat medial gastrocnemius motoneurons. Rather, we frequently observed marked reciprocal excitation at latencies consistent with a tri-synaptic pathway. Notably, excitatory potentials occurred without preceding di-synaptic inhibition. Our results demonstrate a pathway for Ia excitation of antagonist muscles which may represent a useful adaptation for species (eg: human) exhibiting plantigrade gait.

Modulation of Motoneuron Firing by Recurrent Inhibition in Adult Rat in vivo

Thursday, June 19 (AM)
Presenter: Timothy Cope

Authors:
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Recent reports emphasize the role of synaptic inhibition in sculpting the temporal pattern of firing by postsynaptic neurons, e.g. synchronized inhibitory input from cerebellar Purkinje cells is shown to affect instantaneous but not average firing rate of nuclear neurons (Person and Raman, Nature 2012). This concept motivated us to return to unsettled questions about the role of recurrent inhibition (RI) in modulating the firing of spinal motoneurons. In in vivo electrophysiological studies of anesthetized adult rats, we isolated the effects of RI (produced by antidromic electrical stimulation of peripheral nerves in rats with severed dorsal roots) on repetitive firing induced by intracellular current injection into lumbosacral motoneurons. The results provide definitive evidence in living animals that RI can dramatically influence the temporal pattern and/or instantaneous rate of motoneuron firing without altering average rate.

A new method to determine reflex latency induced by high rate stimulation of the nervous system

Thursday, June 19 (AM)
Presenter: KEMAL TURKER

Authors:
Kemal Turker[1], Ilhan Karacan[2], Halil Cakar[3], Oguz Sebik[1], Gizem Yilmaz[1], Muharrem Cidem[2], Sadik Kara[3]
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High rate stimulations of the neuromuscular system are used in the physiological research with an increasing interest. The exact neuronal circuitries underlying these reflex responses remain unclear due to the problem of determining the exact reflex latencies during high rate stimulations. We present a new method to determine the reflex latency during high rate stimulation of the nervous system. Our method relies on the fact that each stimulus, no matter what the interstimulus interval is, generates a reflex response with the same latency. In other words, when the stimulus pulses delivered at varying rates are superimposed, the reflex responses that correspond to each stimulus overlap at a time point that indicates the response latency. Reflex latency calculated using this "cumulated average method" for the whole body vibration experiments was significantly shorter than the value obtained using the classical cumulant density calculation. Simulation results illustrated that the cumulated average method is a reliable and more accurate method compared with the classical method. We suggest that the cumulated average method is able to determine the high rate stimulation induced reflex latencies more accurately than the classical method.

Effects induced by motor cortex anodal transcranial direct current stimulation on wrist muscles in stroke patients

Thursday, June 19 (AM)
Presenter: Alexandra Lackmy-Vallee

Authors:
Alexandra Lackmy-Vallee[1], Wanalee Klomjai[1], Mounir EL Mendili[1], Pascale Pradat-Diehl[1], Bernard Bussel[2], Nicolas Roche[3], Rose Katz[4]
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Transcranial direct current stimulation is used to modify non invasively cortical brain excitability in humans. It consists in delivering a weak current trough two electrodes: one applied over the motor cortex and a second in supra-orbital position. Effects induced by tDCS are polarity dependent: anodal tDCS lowers the resting membrane potential of neurons which increases cortical excitability whereas cathodal tDCS decreases cortical excitability. Recently we showed in healthy subjects that anodal tDCS applied over the motor cortex can be used to modify descending inputs relayed by corticospinal tract on reciprocal inhibition pathways innervating wrist muscles. We found that effects induced by anodal tDCS in wrist extensors and in wrist flexors are opposite: tDCS increases reciprocal inhibition in extensors but decreases reciprocal inhibition in flexors. It appears that modulations induced by anodal tDCS on reciprocal inhibition pathways favor the contraction in wrist extensors. Motor deficit in wrist extensors is usual in hemiplegic patients. Preliminary results in hemiplegic patients suggest

an increase of reciprocal inhibition in affected arm when anodal tDCS is applied over the non lesional hemisphere (ipsilateral motor cortex) contrary to the results found in healthy subjects. Then, electrophysiological data will be confronted to anatomical data from magnetic resonance imagery of the cervical spinal cord.

Motor unit firing patterns during abnormal multi-joint coupling in chronic hemiparetic stroke

Thursday, June 19 (AM)
Presenter: Laura Miller

Authors:
Laura Miller[1], Francesco Negro[2], CJ Heckman[1], Dario Farina[2], Jules Dewald[1]
[1]Northwestern University, [2]University Medical Center Göttingen

After chronic hemiparetic stroke, abnormal co-activation patterns often exist in the affected arm whereby movements of the shoulder, elbow, and hand are coupled together. We examined motor unit (MU) discharge of 3 arm muscles within the context of the flexion synergy (FS), one such pattern in which activation of shoulder abductors is coupled with that of elbow and finger flexors. This coupling may reflect an underlying neural coupling, possibly due to increased influence of brainstem motor pathways. Three severely impaired post-stroke subjects and 5 controls generated shoulder abduction (SABD), elbow flexion, and finger flexion (FF) torques at 10, 20, 30 and 40% of max. MU discharges were extracted from high-density surface EMG placed over deltoid, biceps, and wrist/finger flexors (WFF). In total, 478 MUs were isolated. On average, mean firing rates were significantly lower in the stroke group for each muscle (deltoid: 10.7 vs. 13.3 pps; biceps: 10.4 vs. 13.2; WFF: 8.5 vs. 13.0). Coherence among WFF MU during volitional activation (FF trials) was compared to that during FS activation (SABD trials). During volitional trials, WFF coherence seen in controls in alpha/beta frequency bands was absent in the stroke group, but low frequency common drive, possibly reflective of activity of non-cortical sources, was preserved. During FS trials, in addition to common drive, WFF coherence emerged between 10-15 Hz, a band suggested to reflect activity of brainstem pathways. Results suggest different neural circuits may contribute to MU discharge of volitional vs. FS-driven contractions.

Serotonergic control of motoneuron activity during locomotion in adult rats

Thursday, June 19 (PM)
Presenter: Larry Jordan

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We investigated the serotonin receptors involved in controlling motoneuron output during locomotion. We used spinalized animals to eliminate descending pathways; rats were subjected to spinal cord transection at the Th9/10 level. In some animals grafting of embryonic serotonergic cells was performed one month after the

transection: embryonic tissue (E14) containing serotonergic neurons was injected through a glass micropipette into the spinal cord at the T10/11 level. Two months after grafting, stepping was induced by tail stimulation. Locomotor trials with 5-HT agonists commenced 2-3 months after the transection in non-grafted rats. Intrathecal cannulae were implanted over the lumbar enlargement for application of 5-HT antagonists in spinal and in otherwise intact animals. Locomotor activity was monitored using EMG (soleus and tibialis anterior muscles) and video recordings during treadmill trials and during trials on a 2m runway. Stretch reflexes were induced by dorsi- and plantar flexion at the ankle. 5-HT agonists or grafting of 5-HT neurons induced prolonged extensor bursts that occupied the entire stance phase of locomotion, and increased amplitude of EMG activity. Motoneuron output was diminished by 5-HT2 receptor antagonists, shown by reduced EMG peak amplitude, reduced duration of extensor EMG bursts, and blockage of stretch reflexes. Selective 5-HT7 or 5-HT2C antagonists had no apparent action on motoneuron output. Our results suggest that 5-HT2A receptors and not 5-HT2C or 5-HT7 receptors are implicated in the control of limb motoneuron output during locomotion.

Beyond the size principle - Motoneuron pool organization and recruitment during locomotion

Thursday, June 19 (PM)
Presenter: Abdel ElManira

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A salient feature of motor behavior is the orderly recruitment of motor pools and the coordination of their activity to produce movement with precise force, speed and timing. Traditionally spinal locomotor circuits have been believed to be composed of a unit CPG that with increased activity produces faster movements. In this view, all motoneurons receive uniform inputs from premotor interneurons and their recruitment follows the size principle. Our recent results from adult zebrafish challenge this view. We show that slow, intermediate and fast motoneuron pools are connected to different excitatory V2a interneurons and their order of recruitment is not dictated by their size or input resistance. The order of recruitment of the different motoneuron pools emerges from their selective wiring with the premotor circuit. Indeed, the spinal circuits can be deconstructed into three microcircuit modules. Each microcircuit encompasses a subset of interconnected excitatory V2a interneurons and a motoneuron pool that are recruited sequentially from slow, to intermediate and fast during swimming with increased speed. The connectomics, cellular properties, and function of each microcircuit are attuned to the properties of the muscle fibers they activate. Thus our findings reveal a circuit principle governing the recruitment of motor pools that is unrelated to the size principle. In addition we show that the spinal locomotor network display a specific connectivity map with ensemble microcircuit organization that acts as an intrinsic gearshift to change the speed of locomotion.

Application of concurrent motor unit recordings in the unparalyzed, unanesthetized, decerebrate cat

Thursday, June 19 (PM)
Presenter: Christopher Thompson

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Christopher Thompson[1], Michael Johnson[1], Francesco Negro[2], Matthew Holmes[1], Dario Farina[2], Charles Heckman[1]
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Understanding the actions of both synaptic drive and intrinsic excitability across the motor pool can provide us with novel insights into the motor system in both healthy and disease conditions. Recent methods for decomposing multi-channel EMG recordings provide us with the means for this analysis. Our current strategy allows us to investigate the concurrent discharge of 10-20 motor units in vivo in the soleus of the cat using a grid of 64 electrodes. With this method, we first focused on quantifying the contributions of both rate coding and recruitment of additional motor units underlying the progressive windup of force generation during tendon vibration. Second, we have applied coherence analyses to assess common synaptic input to the motor pool. This approach can accurately identify the high-frequency Ia synaptic input to the motor pool produced during tendon vibration, though the discharge of any single unit remains relatively low (7-9 pps). Lastly, we estimated the post synaptic potential using PSTHs and PSFs for multiple concurrently active units, providing us a novel means to assess the variability of synaptic input across the motor pool. By examining the relative contributions of rate coding and recruitment, coherence, and post synaptic potentials across the motor pool, we can develop a novel understanding of the synaptic input to motoneurons that drives motor output. Importantly these methods can be directly translated to humans and foster parallel animal and human investigations.

Microstimulation of single human motor axons: A comparison of the contractile responses to irregular and regular trains of stimuli

Thursday, June 19 (PM)
Presenter: Michael Leitch

Authors:
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During voluntary contractions, human motoneurones discharge with a physiological variability of ~20%. However, studies that have measured the contractile responses to microstimulation of single motor axons have used regular trains of stimuli with no variability. We tested the hypothesis that irregular (physiological) trains of stimuli produce greater contractile responses than regular (non-physiological) trains of identical mean frequency but zero variability. High-impedance tungsten microelectrodes were inserted into the common peroneal nerve and guided into fascicles supplying a toe extensor muscle. Selective microstimulation was achieved for 14 single motor axons. Contractile responses were measured via an angular displacement transducer over the relevant toe. After recording the responses to regular trains of 10 stimuli extending from 2-100 Hz, irregular trains of 10

stimuli - based on the interspike intervals recorded from single motor units during voluntary contractions - were delivered. Finally, the stimulation sequences were repeated following a 2 min period of continuous stimulation at 10 Hz to induce muscle fatigue. Regular trains of stimuli generated a sinusoidal increase in displacement with frequency, whereas irregular trains - emulating the firing of volitionally-driven motoneurones - displayed significantly greater responses over the same frequency range (8-24 Hz). This was maintained even in the presence of fatigue. We conclude that physiological discharge variability, incorporating irregularity, offers an advantage to the neuromuscular system.

Excitotoxic vulnerability of motoneurons and AHP regulation

Thursday, June 19 (PM)
Presenter: Robert Lee

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The relationship between action-potential size and AHP size results in a serious excitotoxic vulnerability for motoneurons wherein attempts by a motoneuron to "gracefully" reduce its excitability by inactivation of sodium currents paradoxically increase excitability (f-I gain). We present experimental and theoretical evidence that this vulnerability is neutralized in healthy cells by low levels of just-su-threshold activation of AHP-associated potassium channels (aka SK) driven by equally low levels of calcium current activation. In short, SK activation has to be tonically active in the just subtreshold voltage region to protect the cells ability to protect itself. We also suggest (from other's published experimental data) that G93A has reduced this activation, potentially making the cells both more excitable and more vulnerable to excitability.

Reverse engineering motor unit discharge patterns to estimate the structure of motor commands

Thursday, June 19 (PM)
Presenter: Randy Powers

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The firing rate of motoneurons could simply reflect their net excitatory input. However, in the presence of neuromodulatory drive, persistent inward currents (PICs) are enhanced, and their activation changes the time course of firing rate. PIC activation is very sensitive to inhibitory input, so that motor unit firing rate depends upon 3 components of the motor command: excitation, inhibition and neuromodulation. To estimate the contribution of these 3 components to a given motor output, we record the discharge of multiple motor units from ankle extensor muscles in the decerebrate cat, and compare this to the output of a set of computer models representing a range of motoneurons of different excitability. A subset of the motor unit discharge records are

used as targets, and we vary the levels of neuromodulation, inhibition and excitation applied to a matching subset of models to find which combinations provide the best matches to the targets. The solution space for good matches to individual targets is relatively large. However, additional constraints based on what is known about the distribution of synaptic inputs across the motoneuron pool, together with additional measures of population behavior, greatly restricts our estimates of the 3 input components. To validate this approach, we will compare these estimates to voltage-clamp measurements of the synaptic currents evoked by the same stimuli. Our ultimate goal is to apply this analysis to the discharge patterns of human motor units to estimate motor command structure in different tasks and following CNS damage.

Motoneurons: choosing the right computer model

Thursday, June 19 (PM)
Presenter: Sherif Elbasiouny

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Computer motoneuron models represent the complex anatomy of the cell as a set of electrically coupled isopotential compartments (in which the membrane potential does not change along the length of each compartment). Based on the extent of dendritic morphology representation, computer motoneuron models range from reduced (where the soma is connected to a single unbranched cable, SUC model) to full (where the soma is connected to a realistic 3D dendrite; R3D model) models (Fig. 1). Due to computing speed requirement in large network and single 3D neuron simulations, SUC models are usually employed for their simplicity and fast simulation runs; however, these models are unwarranted under active conditions. Using simulations of R3D models and their SUC counterparts, here we illustrate that SUC models fail to simulate a number of key active behaviors, especially those originating from the dendrites. Specifically, oversimplification of the dendritic morphology and lack of spatial segregation of dendritic active conductances in the SUC model were shown to cause significant underestimation of those behaviors. To address this limitation, SUC models were modified by adding key branching features in steps. Addition of primary dendritic branching restored partially some active behaviors while addition of secondary dendritic branching restored most of the active behaviors. Importantly, the proposed modified models successfully replicated the active properties without sacrificing model simplicity, making them attractive candidates for running R3D single neuron and network simulations.

Comparison of the firing patterns of human postganglionic sympathetic neurones and α motoneurones

Thursday, June 19 (PM)
Presenter: Vaughan Macefield

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Microelectrode recordings from individual sympathetic postganglionic motoneurones in awake human subjects have revealed common firing properties. Motoneurones supplying blood vessels in muscle tend to fire only once per sympathetic burst, even in states of physiologically or pathophysiologically elevated central drive, and it may be that the short duration of the burst limits the number of times a sympathetic motoneurone can fire. Indeed, when human alpha motoneurones are volitionally activated to fire in brief bursts that emulate muscle sympathetic bursts, their spike distribution is identical to that of sympathetic postganglionic neurones. Given the probabilistic nature in which these motoneurones fire, and that most can intermittently generate spike doublets or triplets with very high instantaneous frequencies (>100 Hz), I have argued that this reflects the near-coincident firing of at least two preganglionic neurones possessing strong synaptic coupling with an individual postganglionic neurone; modelling studies support this interpretation. This differs from the way in which alpha motoneurones fire, but there are still similarities: both types of motoneurone possess essentially fixed recruitment orders, and rely on recruitment and rate coding to grade output, but recruitment is a more important strategy for increasing sympathetic outflow. And that both types of motoneurone can intermittently generate high instantaneous frequencies means that effector organ responses can be augmented by discharge irregularity and short interspike intervals.