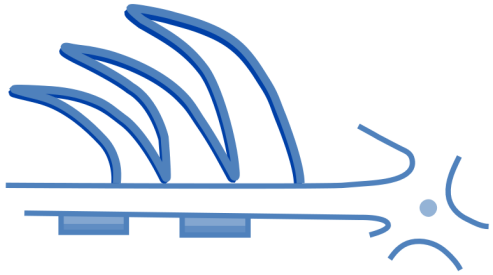


International Motoneuron Meeting
Sydney 2012

Dear Delegates

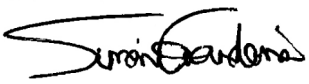
Welcome to the International Motoneuron meeting – Sydney 2012. This is the 8th Motoneuron meeting in its biennial format (Boulder 2000 and 2004; Groningen 2002, Copenhagen 2006; Seattle 2008; Paris 2010) following the inaugural meeting in Warsaw (1997).

Over the 4 days we will have 12 sessions of oral presentations and 2 periods dedicated to poster viewing and discussions. A wide range of 'motoneuron' topics will be covered from both a fundamental and clinical perspective.

To host the meeting we have been helped by sponsors (Cambridge Electronic Design; ADInstruments), and by Neuroscience Research Australia and Brain Sciences UNSW. We are also grateful to Glenguin Estate for their contribution to our Welcome drinks.

We hope that you enjoy the science, the company and the local environment.

Sincerely



Simon Gandevia on behalf of the organising group

Janet Taylor
Jane Butler
Chris McNeil
Penelope McNulty
Annie Butler
Matthew Kiernan
Richard Fitzpatrick

We are grateful for local help from Andrea Riley, Michael Cartwright and Andrew Cartwright at NeuRA, and overseas assistance from the international advisory group of CJ Heckman, Roger Enoka, Daniel Zytnicki, Jean-François Perrier and Marc Binder. In the background, Doug Stuart, Hans Hultborn and Maria Piotrkiewicz provided invaluable advice.

9th International Motoneuron Meeting: Halifax 2014 (Rob Brownstone)
10th International Motoneuron Meeting: Istanbul 2016 (Kemal Türker)

Monday, 23 July

8.00 am **Registration and coffee**

8.30 am – 10.15 am **Axons of motoneurons (Chair: Simon Gandevia)**

8.30am	Simon Gandevia	Welcome
8.45am	Hugh Bostock	In vivo assessment of motor axon and muscle membrane properties
9.10am	François Couraud	Properties of the axon initial segment
9.35am	Janet Taylor	Changes in the recurrent discharge of human motoneurons
9.50am	Claire Meehan	Compensation in mouse model of amyotrophic lateral sclerosis (ALS)

10.15 am – 10.45 am **Morning Tea**

10.45 am – 12.15 pm **Motoneuron properties (Chair: Robert Fyffe)**

10.45am	Ken Rose	Distribution of synapses, channels, and transporters: the view from the motoneuron dendritic tree
11.10am	Adam Deardorff	Expression of Ca^{2+}-activated potassium (SK) channels in normal and axotomized α-motoneurons
11.25am	Rodolfo Delgado-Lezama	Extrasynaptic $GABA_A$ receptors regulate excitability of motoneurons in the mature spinal cord
11.40am	Peter Noakes	Independent regulation of motoneuron survival during development by central synaptic activity
11.55am	Ian Johnson	Rescue of motoneurons: an ageing problem

12.15 pm – 1.30 pm **Lunch**

Monday, 23 July (cont.)

1.30 pm – 3.15 pm **Voluntary control of motoneurons (Chair: Gabrielle Todd)**

1.30pm	David Burke	Measuring the excitability of the motoneurone pool
1.55pm	Dario Farina	Origin of coherence peaks in the spike trains of populations of motor units
2.10pm	Minoru Shinohara	Corticomuscular coherence with and without EMG rectification during single and multi tasks in young and elderly adults
2.25pm	Tjeerd Boonstra	Mechanisms and dynamics of oscillatory input to motoneurons
2.40pm	Maria Piotrkiewicz	Impact of synaptic noise on the stimulus-correlated motoneuron firing (computer simulation study)
2.55pm	CJ Heckman	Reverse engineering of motor unit firing patterns to estimate the synaptic structure of motor commands

3.15 pm – 3.45 pm **Afternoon Tea**

3.45 pm – 4.45 pm **Historical session. Motoneurons: on the shoulders of giants (Chair: David Burke)**

3.45pm	Doug Stuart	Pioneers of active CNS inhibition I: Ivan Sechenov, Charles Sherrington, and John Eccles
4.05pm	Robert Callister	Pioneers of active CNS inhibition II: David Curtis and the search for inhibitory neurotransmitters in the spinal cord
4.25pm	Paul Foley	The MND/ALS concept: Alfred Campbell's pre-WWII contributions

5.00 pm – 6.30 pm **Welcome Drinks**

Tuesday, 24 July

8.30 am – 10.15 am **Rhythmic control of motoneurons (Chair: Claire Meehan)**

8.30am	Reggie Edgerton	Sources of control of the rhythmicity of stepping
8.55am	Hans Hultborn	Motoneuron properties during fictive rhythmic movements in the decerebrate cat
9.10am	Aidas Alaburda	Temporal integration during initiation of scratching
9.25am	Gareth Miles	Spinally-derived modulation of motoneuron output
9.40am	Katrina Maluf	Age-related changes in chest wall movement and respiratory modulation of motor unit behavior in the trapezius muscle
9.55am	Jack Feldman	Rhythmic drive to motoneurons

10.15 am – 10.45 am **Morning Tea**

10.45 am – 12.30 pm **Motoneuron disease (Chair: Zev Rymer)**

10.45am	Matthew Kiernan	Motoneurons: what have we learned from ALS?
11.10am	Jacques Durand	Common early alterations in lumbar motoneurons of $SOD1^{G93A}$ and $SOD1^{G85R}$ juvenile mice
11.25am	Daniel Zytniki	Degeneration of motoneurons in SOD1 mice: the size hypothesis
11.40am	Sherif Elbasiouny	G85R and G93A-h models of ALS: similar size abnormality but opposite alterations in membrane biophysical properties
11.55am	Matthew Fogarty	Motoneuron hyperexcitability in SOD^{G93Ah} mice is associated with increased dendritic arborisation and glutamatergic synapses
12.10pm	Michael Swash	Motor unit recruitment in ALS and other UMN lesions

12.30 pm – 1.30 pm **Lunch and Posters**

Tuesday, 24 July (cont.)

1.30 pm – 3.00 pm **Poster Session # 1 – odd numbers**

3.00 pm – 3.30 pm **Afternoon Tea**

3.30 pm – 5.00 pm **Motoneuron adaptation (Chair: CJ Heckman)**

3.30pm	Rob Brownstone	Spike frequency adaptation in motoneurons: is it an artefact?
3.55pm	Piotr Krutki	Effects of the muscle overload on the medial gastrocnemius motor units and motoneurons
4.10pm	Jayne Carberry	Respiratory muscle adaptation following chronic hypoxia during postnatal development
4.25pm	Curtis Manning	Fatigue of motoneurons
4:40pm	Chris McNeil	Motoneuron excitability following a locomotor vs. an isometric fatigue task

Wednesday, 25 July

9.00 am – 10.30 am **Motoneuron behaviour in people (Chair: Inge Zijdewind)**

9.00am	Andy Fuglevand	Motor unit synchrony: cortical imprint or not
9.25am	Jacques Duchateau	Initial conditions influence the discharge of single motor units during fast contractions
9.40am	Martin Héroux	Triceps surae muscle motor unit behaviour during human standing balance
9.55am	Kemal Türker	Frequency analysis of the reflex responses of human masseter motor units
10.10am	Paul Hodges	Non-uniform effects of nociceptive input to motoneurons during experimental pain

10.30 am – 11.00 am **Morning Tea**

11.00 am – 12.30 pm **Motoneurons and persistent inward currents, etc (Chair: Marc Binder)**

11.00am	Jørn Hounsgaard	Motoneurons, what makes them tick?
11.25am	Randy Powers	Contributions of sodium and potassium currents to synaptic integration in motoneurons
11.40am	Bob Lee	Interaction between Na PICs and motoneuron firing properties
11.55am	Jessica D'Amico	Role of constitutively-active 5HT2 receptors in involuntary muscle spasms after spinal cord injury in humans
12.10am	Dave Collins	Driving motoneurons with repetitive reflexive inputs after a spinal cord injury

12.30 pm – 1.30 pm **Lunch and Posters**

1.30 pm – 3.00 pm **Poster Session # 2 – even numbers**

3.00 pm – 3.30 pm **Afternoon Tea**

Wednesday, 25 July (cont.)

3.30 pm – 5.00 pm **Motoneurons after spinal cord injury (Chair: Penelope McNulty)**

3.30pm	Gary Sieck	Recovery of phrenic motoneuron activity after cervical spinal cord injury
3.55pm	Chris Thompson	Supramaximal volitional torque in humans with spinal cord injury: reflexive and perceptual consequences
4.10pm	George Hornby	Sustained amplification of volitional motor function in spinal cord injury: role of PICs in rehabilitation
4.25pm	Christine Thomas	Reduced spinal inhibition increases stretch-induced activation of muscles paralyzed by spinal cord injury
4.40pm	Krzysztof Kowalski	Respiratory motoneuron activation during high frequency spinal cord stimulation (HF-SCS)

6.00 pm **CONFERENCE DINNER**
Waterfront Restaurant, 27 Circular Quay W, The Rocks.
(BUSES LEAVE FROM CONFERENCE HOTEL AT 6.00 PM AND RETURN AT 10.30 PM)

Thursday, 26 July

9.15 am – 10.15 am **Motoneurons and other matters (Chair: Rob Herbert)**

9.15am	Jan Celichowski	The decomposition of fast and slow motor unit tetanic contractions evoked by random stimulation pattern
9.30am	Véronique Marchand-Pauvert	Evidence for direct central effect of Botulinum neurotoxin A in human spinal cord
9.45am	Marin Manuel	Properties of single adult mouse motor units recorded in vivo. Implication for their function
10.00am	Tim Carroll	A new method for online reconstruction of joint torque from EMG recordings

10.15 am – 10.45 am **Morning Tea**

10.45 am – 12.30 pm **Motoneurons after stroke (Chair: Janet Taylor)**

10.45am	Zev Rymer	The quandary of motoneuron behavior in stroke: higher excitability, but lower sustained firing rates
11.10am	Allie Hyngstrom	Motor unit behavior following fatigue in stroke survivors
11.25am	Jayne Garland	Postural responses of gastrocnemius motor units to external loading in standing in people post-stroke
11.40am	Penelope McNulty	Changes in EMG activation with post-stroke rehabilitation
11.55am	Aneesha Suresh	Examination of AHP duration changes in motoneurons innervating paretic muscles in stroke survivors
12.10pm	John Rothwell	Importance of possible corticoreticulospinal inputs to motoneurons after stroke or training

12.30 pm – 1.30 pm **Lunch**

Monday, 23 July

8.30 am – 10.15 am Axons of motoneurons (Chair: Simon Gandevia)

***In vivo* assessment of motor axon membrane properties.**

Bostock H[1]., Z'Graggen W.J.[2], Tan S.V.[3]

[1]Institute of Neurology, University College London, London, U.K.; [2]Inselspital, Bern University Hospital and University of Bern, Bern, Switzerland; [3]Guy's and St. Thomas' NHS Foundation Trust, London. UK

Nerve excitability testing, using a semi-automated sequence of different excitability measures (the TROND protocol) with the QTRAC threshold-tracking program, has proved useful in detecting membrane depolarization and ion channel dysfunction in peripheral neuropathies. Recently a similar approach has been applied to studying muscle membrane properties in myopathies. Of the different components of the TROND protocol, only the recovery cycle readily translates to muscle, by using the velocity of action potential propagation rather than electrical excitability as an indirect index of membrane potential. Muscle velocity recovery cycles (MVRCs) can be recorded reliably using direct muscle stimulation and recording, with monopolar and concentric needles respectively. Brachioradialis and tibialis anterior muscles yield similar results.

In MVRCs as in nerve recovery cycles, refractoriness is followed by a period of superexcitability (supernormality), related to the depolarizing afterpotential. Supernormality is markedly dependent on membrane potential and reduced or abolished by ischaemia. However, late subexcitability in nerve, related to slow (KCNQ) potassium currents, has no counterpart in muscle. Instead there is a broad late phase of supernormality, presumably related to t-tubule potassium accumulation, that is enhanced by trains of 5 conditioning stimuli. Further information about muscle membranes can be obtained from responses to intermittent repetitive stimulation at frequencies up to 30 Hz. Multiple excitability measures derived from these recordings have been used to investigate the pathophysiology of uraemic and critical illness myopathy and show promise as a new tool the help diagnose muscle channelopathies due to mutations in sodium, chloride and inward rectifier channels.

Properties of the axon initial segment

Fréal A., Duflocq A., Le Bras B., Davenne M. and Couraud, F.

CNRS UMR 7224, INSERM, UMRS 952, UPMC, Paris, France

The axon initial segment (AIS) constitutes a key microdomain in neurons that emerged as a structural and functional entity in the last few years. It is responsible for the initiation and modulation of action potentials since it is highly enriched in specific voltage-gated ion channels. Another crucial and more recently described function of the AIS is to contribute to the maintenance of neuronal polarity.

The AIS is composed of three types of proteins: 1) Proteins involved in the maintenance of the AIS molecular organization: the scaffold protein ankyrin G that interacts with the actin cytoskeleton via betaIV-spectrin, with microtubules through the plus-end-binding protein EB3 and with extracellular matrix through neurofascin-186 and NrCAM; 2) Ion channels, which sustain the neuron excitability: Nav1.1, Nav1.2, and Nav1.6 sodium channels, KCNQ2/3 (Kv7.2/7.3), Kv1.1, Kv1.2 and Kv1.4 potassium channels and T and R type calcium channels; 3) Regulatory proteins like protein-kinase CK2 and CAMKII.

The presence of these regulating components as well as recent data on activity-dependent AIS plasticity suggest that the AIS structure could be more dynamic than originally imagined. Furthermore in some neurons, including spinal motor neurons, the distribution of ion channels at the surface of the AIS is not homogenous and thus defines functional subdomains.

Finally the formation of the AIS during neuronal development is a long process that starts at the very beginning of axonogenesis, including the progressive restriction of ankyrin G at the initial part of axons and then the appearance of the different ion channels.

Changes in the recurrent discharge of human motoneurones after voluntary activity

Janet Taylor, Serajul Khan, Simon Gandevia

Neuroscience Research Australia, Randwick
The University of New South Wales, Kensington, Australia

In people, repetitive firing of motoneurones in voluntary contractions can reduce the response of the motoneurones to corticospinal input both during and after contractions (1,2). The reduced response may reflect presynaptic factors or it may depend on changes in motoneurone properties. In the current study we examined the effect of voluntary contraction on evoked motoneuronal discharges that do not depend on synaptic input. Recurrent discharges of motoneurones occasionally occur after an antidromic action potential reaches the cell body. The precise mechanism of these discharges is unknown but may involve re-excitation of the axon initial segment. The responses can be evoked in human muscles by stimulation of the peripheral motor axons and are known as F waves.

We examined F waves evoked in the resting abductor digiti minimi muscle (ADM) by supramaximal stimulation of the ulnar nerve before and after maximal voluntary contractions (MVCs) of ADM. Stimuli were given at 0.5 Hz in sets of 30. MVCs were of 2-s, 10-s, 1-min or 2-min duration. Prior to MVCs, F waves in ADM occurred frequently. They were seen after >95% of stimuli. After MVCs of all durations, there was an immediate decrease in the occurrence and the mean area of F waves averaged over each set. The depth and duration of the decrease became greater with longer contractions. After a 2-s MVC, mean area decreased by 27±18% for 1 min, whereas after a 2-min MVC, it was initially decreased by 48±20% and remained depressed for ~9 min. Submaximal M waves (direct muscle response to motor axon stimulation) measured after 2-s and 2-min MVCs did not show the same time course of depression. The results indicate an activity-dependent depression of the F wave in ADM. We propose that voluntary activity alters the properties of the axon initial segment or soma of the motoneurones.

1. Gandevia et al (1999) J Physiol 521: 749-759
2. McNeil et al (2011) J Physiol 589: 3533-3544

Compensation in a mouse model of Amyotrophic Lateral Sclerosis

Veronika Bonnevie, Anne Hedegaard, Lillian Grøndahl, Claire Meehan

Copenhagen University, Denmark.

We have recently obtained evidence suggestive of a hyper-excitability of adult motoneurones in a SOD-1 mouse model of the motoneurone disease Amyotrophic Lateral Sclerosis (recorded in vivo). This confirmed findings of others showing an increased excitability of motoneurones recorded in culture or in neonatal preparations. Thus, this increased excitability appears to persist into adulthood. One flaw in the hypothesis relating this excitability to possible excitotoxic death is the question, "why then are these levels not excitotoxic in the neonatal period but may become so in the adult?" Using electrophysiology and anatomy, we are exploring the hypothesis that the axon initial segment may be able to compensate for this enhanced excitability, recorded at the cell body, and investigating the possibility of changes occurring in the adult that may counteract this compensation.

Monday, 23 July

10.45 am – 12.15 pm Motoneuron properties (Chair: Robert Fyffe)

Distribution of synapses, channels, and transporters: the view from the motoneuron dendritic tree

Ken Rose, Ethan Zhao, Steven Montague, Rob Maratta, Hao Shi, Katie Lin, Monica Neuber-Hess, Keith Fenrich

Centre for Neuroscience Studies, Department of Biomedical and Molecular Sciences, Queen's University, Kingston ON Canada

Motoneuron input-output properties are dynamically regulated by serotonin (5-HT) and noradrenalin (NA). We have recently found that 5-HT and NA synapses are, respectively, 6 and 10 fold higher on small compared to large diameter dendrites. This relationship is responsible for the 2 to 4 fold higher density of 5-HT and NA synapses on distal dendrites.

5-HT and NA modulate 5 types of channels on motoneurons including a hyperpolarization-activated current called Ih. In addition 5-HT increases the strength of inhibitory synapses by increasing the expression of the chloride transporter, KCC2. We have developed quantitative immunocytochemical techniques to map the distribution of KCC2 and the HCN1 subunit of Ih channels on the membranes of proximal and distal dendrites of motoneurons. Both effectors were arranged in a patchwork of hotspots that were rarely more than 10 μm apart. These hotspots were found in all regions of the dendritic tree. Quantitatively, the density of KCC2 was 50% greater on distal (> 1500 μm from the soma) compared to proximal (< 250 μm from the soma) dendrites. The density of the HCN1 subunits followed a similar pattern in 7 of 10 motoneurons. The proximal to distal increase varied from 50% to 300%. In the remaining motoneurons, there was no detectable proximal to distal gradient. These results suggest that NA and 5-HT synapses are strategically placed to regulate the strength of Ih and inhibitory synaptic transmission.

Expression of Ca^{2+}-activated potassium (SK) channels in normal and axotomized α-motoneurons

Adam S. Deardorff, Shannon H. Romer, Robert E.W. Fyffe

Department of Neuroscience, Cell Biology, and Physiology; Wright State University Boonshoft School of Medicine; Dayton, Ohio, USA

It is well established that small conductance Ca^{2+}-activated potassium (SK) channels mediate the medium duration afterhyperpolarization (mAHP) of spinal and hypoglossal motoneurons (MNs). mAHP conductance contributes to regulation of MN firing frequency and there are well documented relationships between mAHP duration and motor unit type. In cat and rat spinal αMNs, the mAHP is of larger amplitude and longer duration in S-type cells innervating slow-twitch muscle fibers than F-type cells innervating fast twitch muscle fibers (Eccles et al., 1958; Gardiner et al., 1993). Moreover, several studies suggest type-specific changes in AHP properties following peripheral axotomy, including a shortening of AHP duration in cells innervating slow twitch muscle fibers and a lengthening of AHP duration in cells innervating fast twitch muscle fibers (Kuno et al., 1974; Gustafsson and Pinter, 1984). Here, we used immunohistochemistry and *in vivo* electrophysiology to investigate the specific distribution and expression patterns of two homologous SK channel subunits (SK2 and SK3) in rodent spinal MNs and to determine post-axotomy modification of expression patterns and AHP parameters. We hypothesize that differential SK channel expression underlies variability in AHP duration in control MNs and that specific changes in SK expression by injured motoneurons may contribute to the previously observed changes in AHP parameters following axotomy.

Extrasynaptic $GABA_A$ receptors regulate excitability of motoneurons in the mature spinal cord

Andrés C.[1], Loeza-Alcocer M.[1], Aguilar J.[1], Felix R[2]., Delgado-Lezama R[1].

[1]Department of Physiology, Biophysics and Neuroscience, Center for Research and Advanced Studies of the National Polytechnic Institute (Cinvestav-IPN), Mexico City, Mexico; [2] Department of Cell Biology, Cinvestav-IPN, Mexico City, Mexico.

γ-Aminobutyric acid (GABA) plays many of its key roles in functioning of the central nervous system by acting on ligand gated chloride-permeable channels known as $GABA_A$ receptors ($GABA_AR$). In the last 25 years, extrasynaptic $GABA_ARs$ have been identified to be widespread in soma and dendrites in many brain structures with a subunit composition that allows them to be tonically active by low ambient GABA concentrations in the extracellular space generating a form of tonic inhibition which has a relevant role in controlling neuron excitability. We have previously demonstrated that a GABAergic tonic inhibition is present in spinal cord motoneurons. In a recent study, we found that motoneuron excitability is controlled by $\alpha_{4/6}$ and α_5 subunit-containing $GABA_A$ receptors. In motoneurons recorded intracellularly the frequency-current curve was shifted to the left, the rheobase current reduced and the input resistance increased in the presence of furosemide (200 μM) and L-655708 (20 μM), and antagonists of $\alpha_{4/6}$ and α_5GABA_A receptors respectively. Furthermore, blockade of α_5GABA_A receptors facilitated the monosynaptic reflex while dorsal root potential was depressed. In addition, we demonstrated that tonic excitability of primary afferents synapsing on motoneurons is mediated by α_5GABA_A receptors. By using RT-PCR, Western blot and immunohistochemistry we confirmed the expression of $\alpha_{4/6}$ subunits in motoneurons and α_5 subunit in primary afferents as well in cell bodies in the dorsal root ganglia. Our results suggest that excitability of motoneurons is modulated by $\alpha_{4/6}$ and α_5GABA_A receptors while primary afferent fiber excitability might be modulated by α_5GABA_A receptors.

Independent regulation of motor neuron survival during development by central synaptic activity.

Peter G. Noakes[1, 2], Matt Fogarty, John Lee, Heinrich Betz, Mark Bellingham

1 School of Biomedical Sciences. 2 Queensland Brain Institute,
University of Queensland, QLD, 4072, Australia.

The activity elicited in motor neurons (MNs) by convergent synaptic inputs somehow determines their level of excitability, and regulates their survival during development. So far, our studies have shown that central glycinergic transmission does play an essential role in these processes. However, whether this role is independent of neuromuscular activity-dependent feedback is a long unanswered question. This issue has not been addressed in the developing neuromotor system, as it has been very difficult to manipulate central synaptic activity in the absence of neuromuscular activity.

In this study we have used mutant mice lacking glycinergic (gephyrin deficient) and/or neuromuscular (muscle specific kinase [MuSK] deficient) synaptic transmission to examine if central glycinergic synaptic activity has an independent direct effect on MN survival during development and muscle innervation, during the normal motor neuron cell death period during development (embryonic days 13 to 18). We have examined MN number, MN morphology, glutamatergic synapses on MNs, and muscle innervation between E13 to E18-postnatal day 1, in mice lacking either glycinergic-based transmission (gephyrin-/- mice), neuromuscular transmission (MuSK-/- mice) or both (gephyrin-/-MuSK-/- mice). Mice missing either glycinergic or neuromuscular transmission show significant changes in MN number, MN morphology including glutamatergic synapse number, and peripheral muscle innervation. In double mutant mice missing both glycinergic and neuromuscular synaptic transmission, we have observed that changes in these parameters were greater than those observed in either single mutant strain.

This suggests that central glycinergic synaptic activity has a direct role in influencing neuromotor development independent of activity-dependent feedback from muscle, during the period when motor neurons are regulating their final adult numbers (i.e. period of naturally occurring motor neuron cell death).

Rescue of motoneurones: an ageing problem

Ian Johnson, Viythia Katharesan, Joshua Mahadevan, Shamsul Khan, Geoffrey Goldspink[1]

University of Adelaide, Australia and [1]University College London, UK

We previously found that an isoform of insulin-like growth factor -1 (IGF-1) isolated from active muscle (MGF) rescued twice as many adult rat motoneurones than the commonly-used liver-type isoform[1]. Here, we have used nerve avulsion to provoke motoneuronal death across the lifespan of rats to compare the neuroprotection afforded by MGF and two well-known motoneurone trophic factors: glial cell-derived neurotrophic factor (GDNF) and liver-type IGF-1.

Facial nerve avulsion of rats aged 8d (pups) and 3m (adults) caused marked (70-85%) motoneurone loss by 7d (pups) and 14d (adults). Facial nerve avulsion of 24m-old rats (ageing) caused no motoneurone loss by 7-14d and only moderate (41%) loss by 28d ($p<0.05$). When a Gelfoam plug soaked in 1µg/µl of neurotrophic factors was placed in the stylomastoid foramen immediately after nerve avulsion, significant (50-80%) motoneurone rescue by 28d was found in adult rats for GDNF and MGF, but not for liver-type IGF-1. MGF did not rescue avulsed motoneurones in rat pups (7d survival) or in ageing rats (28d survival).

Our results indicate that ageing affects the magnitude and time-course of motoneurone death as well as the extent of neurotrophic rescue. This may be important when neurotrophic rescue strategies based on studies of immature motoneurones are adduced to age-related neurodegenerative conditions, such as Motor Neurone Disease.

1. Aperghis M, Johnson IP, Cannon J, Yang SY, Goldspink G: Different levels of neuroprotection by two insulin-like growth factor-I splice variants. Brain Res 2004, 1009:213-218.

Monday, 23 July

1.30 pm – 3.15 pm Voluntary control of motoneurons (Chair: Gabrielle Todd)

Measuring the excitability of the motoneurone pool

David Burke

Institute of Clinical Neurosciences, Royal Prince Alfred Hospital and The University of Sydney

What limits the size of the H reflex? There are many reasons. The stimulus excites too few Ia afferents. Motoneurone responsiveness to afferent inputs may be low (*Note:* opposite responsiveness to corticospinal and reflex inputs). Efferent axons are excited and the antidromic motor discharge prevents the H wave being seen. There is too much background presynaptic inhibition of the test Ia volley. There are afferents in the test volley that impede the discharge (Ib afferents). The motor discharge itself can provide feedback that impedes the reflex discharge of higher-threshold motoneurones (recurrent inhibition).

How can we assess the excitability of the motoneurone pool?

- The H reflex recruits MNs in the same sequence as voluntary effort but is subject to factors affecting the afferent volley and postsynaptic inhibition through disynaptic inhibitory pathways ("Ib", recurrent).
- F waves provide a flawed measure of motoneurone excitability for many reasons, most importantly because voluntary and reflex inputs (and the MEP) recruit low-threshold motoneurones preferentially, but the F waves cannot appear in these motoneurons if they have discharged reflexly to the supramaximal volley.
- MEP: TES at 150 V can induce I waves. For upper-limb motor pools, the threshold response is a D wave *only* with weak TES and dependent on coil orientation and stimulus intensity. For the lower limb, TES at the vertex produces I waves at threshold. Multiple descending inputs to the MN pool create complications (largely addressed by Magistris' triple stimulation technique).
- CMEP: Comparing changes in the CMEP and the H reflex should be valid, *provided that* the synapses are located in similar sites and the sequence of recruitment of MNs is the same.

What are the effects of natural activity within circuits?

Any change in activity of a neuronal circuit will alter the excitability of the axons in that circuit, and the same stimulus will excite a smaller volley, *but* it will not depress the afferent volley produced by natural stimulation of receptors. However there could be "benefits" of activity-dependent hyperpolarisation of Ia afferent axons: the hyperpolarisation probably reaches the Ia afferent terminals, and afferent terminal hyperpolarisation should boost transmitter release, possibly offsetting both presynaptic inhibition and post-activation depression.

Origin of coherence peaks in the spike trains of populations of motor units

Dario Farina & Francesco Negro

Department of Neurorehabilitation Engineering, Bernstein Focus Neurotechnology Göttingen, Bernstein Center for Computational Neuroscience, University Medical Center Göttingen, Georg-August University, Göttingen, Germany.

Motor neurons operate a non-linear transformation on the synaptic input they receive. Therefore, the frequency content of the synaptic input to a motor neuron is modified at its output. Nevertheless, it can be theoretically proven that the common synaptic input to several motor neurons is transmitted approximately linearly to the cumulative sum of the spike trains of motor neuron populations. Therefore, the frequency spectrum of the cumulative spike train of populations of motor units or the coherence function between groups of motor units reflect the frequency distribution of the common synaptic input received by the population. This analysis is intrinsically dependent on the motor unit discharge rates and such dependency cannot be removed by normalization. To minimize the influence of discharge rate, coherence functions should be computed on rather large populations of motor units rather than pairs. This population analysis allows the investigation of the origin of coherence peaks. This lecture will provide the theoretical bases for estimating coherence functions between populations of motor units and for their interpretation.

Corticomuscular coherence with and without EMG rectification during single and multi tasks in young and elderly adults

Ashley N. Johnson[1] and Minoru Shinohara[2]

[1]School of Electrical and Computer Engineering, [2]School of Applied Physiology, Georgia Institute of Technology, Atlanta, Georgia USA

The purpose of the study was to clarify the difference in corticomuscular coherence between young and elderly adults during the performance of a unilateral fine motor task and concurrent motor and cognitive tasks. Healthy young and elderly adults performed unilateral motor, bilateral motor, concurrent motor-cognitive, and cognitive tasks. Peak corticomuscular coherence between the electroencephalogram (EEG) from the primary motor cortex and surface electromyogram (EMG) from the first dorsal interosseous muscle was compared during steady abduction of the index finger with visual feedback. Corticomuscular coherence was calculated in cases with unrectfied and full-wave rectified EMGs. In the alpha band (8-14 Hz), corticomuscular coherence was significantly greater in elderly than young adults especially during the motor-cognitive task whether unrectified or rectified EMG was used. Beta-band (15-32 Hz) corticomuscular coherence was significantly higher in elderly than young adults across tasks, with a greater difference using unrectified EMG compared with rectified EMG. There was a negative association between beta-band corticomuscular coherence and motor output error during the motor-cognitive task only in young adults. This association was statistically significant only when unrectified EMG was used. The results suggest that 1) corticomuscular coherence is increased in senior age with a greater influence of an additional cognitive task in the alpha-band and 2) individuals with greater beta-band corticomuscular coherence may exhibit more accurate motor output in young, but not elderly adults, during steady contraction with visual feedback. The results imply that full-wave rectification of EMG may attenuate the sensitivity for detecting statistical significance in assessing corticomuscular coherence.

Mechanisms and dynamics of oscillatory input to motoneurons

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The input to motoneurons shows a rich repertoire of oscillatory activities whose mechanisms and functional implications remain incompletely understood. We use an integrated experimental and computational approach to investigate the electrophysiology of corticomuscular and intermuscular coherence. Previous studies have shown that the transfer function of the motor unit pool is largely linear. Hence, deviations from a linear response can be used to characterize the underlying physiology. In particular, the recent debate on the rectification of surface EMG highlights how experimentally observed differences between rectified and non-rectified EMG inform the mechanisms of oscillatory activity in EMG recordings. In a previous study we have shown that the presence of oscillatory activity strongly depends on the distribution of motor unit action potentials. In the present study we focus on the properties of the oscillatory input to the muscles. Different types of oscillatory activity have been identified, e.g. periodic modulations of noisy spike trains or oscillations in motoneuron excitability. Using a computational model of the motor unit pool we test the effect of different types of oscillatory input on the resulting surface EMG signals and compare these findings against empirical results. The differential effect of rectification on corticospinal and intermuscular coherence suggests distinct mechanisms underlying both types of synchronization. These findings are discussed in terms of the different excitatory and inhibitory pathways that innervate the motor unit pool.

Impact of synaptic noise on the stimulus-correlated motoneuron firing (computer simulation study)

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It was hypothesized that the frequency characteristics of synaptic noise may influence the results of stimulus-correlated motoneuron firing and their interpretation. This hypothesis was tested by computer simulations based on the threshold-crossing motoneuron model developed in our laboratory. Three types of synaptic noise with different frequency spectral content were tested, and their impact on the correlation measures (peristimulus time histogram and frequencygram) was compared. The comparison yielded quantitative differences only, so we conclude that the interpretation of experimental results based on computer simulations does not depend on the time structure of synaptic noise applied in the motoneuron model.

Reverse engineering of motor unit firing patterns to estimation the synaptic structure of motor commands.

CJ Heckman, Randy Powers, Michael Johnson, Nina Suresh, Zev Rymer, Tom Sanderock

Northwestern University, Rehabilitation Institute of Chicago, University of Washington

Motor unit spikes recorded in muscles of human subjects are normally one-to-one with the action potentials of their motoneurons. Hence, motoneurons are the only cells in the CNS whose firing patterns can be readily recorded in human subjects. This remarkable window onto CNS function has yet to be exploited to its fullest extent. The goal of this collaboration is to carry out parallel experiments in animal and human subjects on motor unit firing patterns and to develop realistic computer simulations to quantitatively relate the two data sets. The primary goals of the animal experiments are to identify synaptic input weights from various ionotropic systems, to assess effects of neuromodulatory input systems on motoneuron firing patterns and to provide data on muscle behavior to allow generation of more realistic muscle models. The goals of the human experiments are to identify specific features that characterize firing patterns of both normal subjects and patients with various types of CNS dysfunction. The computer simulations then allow quantitative exploration of the sets of inputs and intrinsic properties that can produce a given form of firing pattern. An example of the potential of this reverse engineering approach is demonstrated by recent studies suggesting that the organization of excitation and inhibition is a critical determinant of normal patterns and that this organization may be significantly disturbed in patients with cerebral stroke. The possibilities for improving and extending this reverse engineering approach will be discussed.

Monday, 23 July

3.45 pm – 4.45 pm Historical session: Motoneurons: on the shoulders of giants (Chair: David Burke)

Pioneers of active CNS inhibition. 1. Ivan Sechanov, Charles Sherrington, and John Eccles

Stuart D.G.

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In the late 19th and early-mid 20th C Ivan Sechenov, Charles Sherrington, and John Eccles promulgated that inhibition is an active process within the central nervous system (CNS). From 1862 to 1868 Sechenov demonstrated central inhibition in several reports. He showed, e.g., that (1) chemical or electrical activation of a midbrain region in the frog inhibited the hind-limb flexor-withdrawal reflex, and (2) this reflex in humans was inhibited by co-activation of other reflexes. His initial results influenced his renowned 1863 essay, *Reflexes of the Brain*. Sechenov is now considered the father of Russian physiology and a key 19th C figure in physiological psychology. From 1893 to 1909, Sherrington employed electrical stimulation and kymographic measurements of muscle length changes to infer central inhibition in 14 reports on reciprocal inhibition in spinal reflexes of largely cat hindlimb muscles. In his 1932 Nobel Prize lecture he emphasized the importance of active central inhibition for coordinating muscle activity. From 1951 to 1954 Eccles, a former trainee of Sherrington, and his group introduced intracellular recording in the CNS in a series of seminal works. These included demonstration of action potentials in cat spinal motoneurons (1951), EPSPs and IPSPs in motoneurons (1952), and actions of inhibitory interneurons in spinal recurrent Renshaw (1953) and reciprocal inhibitory (1954) pathways. This work underpinned Eccles' 1963 Nobel Prize. Together, these three protagonists provided experimental results and insights that had not only immediate international impact but also strengthened a concept that is now ubiquitous in synaptic, circuit, and systems neuroscience.

Pioneers of active CNS inhibition. 2. David Curtis and the search for inhibitory neurotransmitters in the spinal cord

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After recording inhibitory post synaptic potentials in motoneurons in the early 1950s, John Eccles and colleagues concluded synaptic transmission in the central nervous system was chemically mediated and proposed *"inhibitory synaptic action is mediated by a specific transmitter substance"*. The Australian neuropharmacologist David Curtis, who trained with Eccles, took up the search for inhibitory transmitter substances by using multi-barreled electrodes that allowed chemicals to be delivered in the immediate environment of recorded neurons (ie, iontophoresis). In the late 1950s he used iontophoresis to show that GABA, which was then considered a candidate neurotransmitter in the brain, "depressed" action potential discharge in spinal neurons. Soon after, in a monumental study on the effects of over 100 compounds on the discharge of spinal neurons, Curtis and Watkins (1960) showed glycine was also a depressant in the spinal cord. Later, Curtis showed the convulsant strychnine had no effect on the action of GABA, but completely blocked the action of glycine (1968-69). Subsequent studies explored other convulsants and demonstrated that the plant alkaloid bicuculline inhibited the action of GABA, but not glycine (1970). Thus by 1970, glycine and GABA were considered potential neurotransmitters at inhibitory synapses in the spinal cord, and the action of each could be distinguished by their selective blockers strychnine and bicuculline, respectively. These important discoveries were crucial for future investigation of the mechanisms that mediate fast inhibitory synaptic transmission in the brain and spinal cord.

The MND/ALS concept: Alfred Campbell's pre- WWII contributions

Paul Foley

Neuroscience Research Australia

Alfred Campbell (1868-1938) was effectively the first specialist neurologist in Australia. During his incredibly fruitful period in England (1889-1905), he conducted research and produced a series of publications that were highly regarded internationally; his mapping of the cerebral cortex was compared favorably with that by Brodmann. One of these publications was his 1897 review for Brain "On the tracts of the spinal cord and their degenerations", in which he discussed at length the current state of knowledge regarding amyotrophic lateral sclerosis. This work, his own contributions to the investigation of the disorder, and those of other Australians prior to the Second World War will be discussed.

Tuesday, 24 July

8.30 am – 10.15 am Rhythmic control of motoneurons (Chair: Claire Meehan)

Sources of control of the rhythmicity of stepping

Edgerton, V. R.[1-4], Roy, R. R.[1,4] and Gerasimenko, Y.[5]

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The cycle period of a step is inversely related to and determined to a large degree by the speed at which an animal locomotes. There are several sources of control of these cycle period lengths within the neuromotor axes. There is volitional control that is presumably derived from cortical input, input from the mesencephalic locomotor region, and afferent input from multiple proprioception and cutaneous sources from the periphery. In addition, it has been hypothesized that the spinal locomotor related circuitry consists of neurons specifically designed to control the "rhythm" of the movement, whereas other spinal neurons are designed to control the "pattern" of activation of motor pools. While it is clear that the cycle period can be modulated volitionally perhaps via a sense of "effort" and by increasing the intensity of tonic stimulation of the mesencephalic locomotor region, the evidence for modulation by spinal neurons designed to impose a rhythm on the movement is not convincing. We now present evidence that clearly demonstrates the lack of necessity for rhythmic neurons to control the speed of locomotion. In fact, such a mechanism could actually be a disadvantage given the detailed control that can be exerted by proprioceptive and cutaneous input. More specifically, data will be presented that show that animals without any supraspinal input to the spinal neural circuitries, but with intact cutaneous and proprioceptive influences, can readily adapt to the speed and direction of locomotion on a moving treadmill belt.

Motoneurone properties during fictive rhythmic movements in the decerebrate cat.

Hans Hultborn and colleagues

Department of Neuroscience and Pharmacology, University of Copenhagen, Denmark

I will summarize findings in cat motoneurones during fictive locomotion, fictive scratching and fictive respiration in decerebrate cats.

During all those fictive movements, the excitatory inputs (drive potentials) are voltage-dependent (increase with depolarization). Whether this is due to addition of persistent inward current (PICs, plateau potentials) or to NMDA components in the drive potential is more difficult to determine. However, even despite strong labeling for Cav1.3 we have hardly found any evidence for PICs in phrenic motoneurones under *any* circumstances – still the respiratory drive potentials are strongly voltage-dependent. In intercostal motoneurones, however, it was often easy to recognize the initiation of plateau potentials as a sign a significant PIC.(see Enriques-Denton et al 2012) The same is true for lumbar motoneurones (mainly during fictive locomotion).

What determines the firing during such fictive rhythmic behavior? The threshold for the action potential is lowered, but besides that, it seems likely that it is still the sum of excitation, inhibition (and the post-spike AHP) at the initial segment that determines the timing and frequency of firing. It is notable that increasing the injected current during such fictive motor activity results in an increased firing rate (with an f/I-slope similar to that seen without fictive motor activity). The firing is more irregular reflecting the strong synaptic input. The input resistance is certainly decreased – during scratching by (by 20 - 60 % of the control) – somewhat more pronounced during the “antagonist” hyperpolarized phase. This agrees with positive evidence for strong active synaptic inhibition during this phase.

Reference: Enríquez Denton M, Wienecke J, Zhang M, Hultborn HR, Kirkwood PA. Voltage-dependent amplification of synaptic inputs in respiratory motoneurones.J Physiol. 2012 Apr 10.

Temporal integration during initiation of scratching

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In the turtle a gentle mechanical rub to the carapace within reach of the hindlimb induces rhythmic scratching aimed at the site of stimulation. Thus a brief train of action potentials in a few sensory afferents recruits a network of an estimated 10,000 neurons that generates the rhythm for the scratch form that targets the site of stimulation. Three different scratch forms have evolved for the scratch to reach targets in a caudal, a pocket and a rostral receptive field around the limb. Specific subsets of neurons are involved to perform particular goal directed behavior. However, how neurons are recruited to perform complex motor task is ill defined. We used an integrated preparation from adult turtle to investigate this issue. Induction of scratching was controlled by varying the frequency and intensity of the electrical cutaneous nerve stimulus. The isolated low intensity stimuli do not evoke activity in the motor nerve while repeated stimulation (≥0.2Hz) leads to temporal integration of sensory inputs and onset of a scratch episode. The mechanisms of temporal integration were investigated by intracellular recordings from motoneurons.
We were not able to find any changes of intrinsic properties of motoneurons during initiation of scratch. Our results suggest that activity of premotor interneurons contribute to gradual induction of the scratch network activity.

Spinally-derived modulation of motoneuron output

Miles G.B.

School of Biology, University of St Andrews, UK

Neuromodulatory systems are important for the control of motoneuron input-output relationships (motoneuron excitability). Regulation of motoneuron excitability in turn contributes to the flexibility of motor output generated by the CNS and ultimately the adaptability of motor behaviours. Although most research has focused on descending neuromodulatory systems arising from the brain stem, there are also a number of potential sources of modulation intrinsic to the spinal cord. We have studied a range of spinal sources of modulation in the rodent spinal cord including glutamatergic, cholinergic, nitrergic and purinergic systems. In this presentation the sources and cellular mechanisms of these intrinsic neuromodulatory systems will be discussed. In addition, the functional roles of intrinsic modulators will be considered with a particular focus on their regulation of locomotor-related motoneuron output.

Age-related changes in chest wall movement and respiratory modulation of motor unit behavior in the trapezius muscle

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Respiratory function declines with age, which may place a greater demand on accessory muscles that assist with respiration. The purpose of this study was to compare chest wall movement and respiratory modulation of motor unit behavior in the upper trapezius muscle between young and old adults. Twenty-seven young (mean (SD) age: 27.4(4.5) years) and 24 older (62.4(9.4) years) adults breathed normally while performing a computer mousing task. Intramuscular motor unit recordings were collected from the dominant upper trapezius muscle, and chest wall movement was monitored with a strain gauge placed around the upper rib cage. The amplitude of chest wall movement during quiet breathing was greater (26.3(10.8)% vs. 20.7(6.0)% maximum capacity) and more variable (CV = 52.9(11.2)% vs. 41.0(21.6)%) in old compared to young adults. Mean discharge rates did not differ across groups (10.0(1.8) pps vs. 10.2(1.4) pps), however, more motor units were recruited and discharged more variably (CV = 28(9)% vs. 25(8)%) in older adults. Covariance between instantaneous discharge rate profiles and movements of the chest wall ranged from r=0.02-0.56 in young adults, with a distinct peak at -250 to -500ms in the phase lag histogram. Covariance for older adults ranged from r=0.00-0.73, with a broad distribution of phase lags. These findings provide the first direct evidence of respiratory discharge rate modulation in the human trapezius muscle, and suggest that older adults may require greater recruitment of motor units to compensate for more variable timing of discharge rates to assist with movement of the chest wall during quiet breathing.

Rhythmic drive to motoneurons

Jack L. Feldman

David Geffen School of Medicine at UCLA

Rhythmic movements require the coordinated activation of many motoneuron pools, some in phase, some in antiphase, and others at various subphases, but all at the same frequency. Are there multiple oscillators driving the pattern for different (sets) of motoneuron pools, or is there a unitary oscillator that broadcasts timing signals that are transformed downstream into the proper patterns of input, or something in between? I will present data that for inspiration, the timing signals are broadcast from the preBötzinger Complex, and that there are separate oscillators driving inspiratory or expiratory motoneurons.

Tuesday, 24 July

10.45 am – 12.30 pm Motoneuron disease (Chair: Zev Rymer)

Motoneurons: What have we learned from ALS?

Matthew C. Kiernan

Neuroscience Research Australia and Prince of Wales Clinical School, University of New South Wales, Sydney, Australia.

The past two decades, beginning in the 1990s with the decade of the brain, have witnessed a surge of interest across the motor neurone disease landscape, both locally and internationally. Advances in our understanding of the glutamine neurotransmitter system and the discovery of causal genes linked to the development of familial amyotrophic lateral sclerosis (ALS) have re-ignited research interest. Problems related to clinical heterogeneity have been identified – not all ALS is equal. It has become clear that survival in ALS is determined by many factors, including the clinical phenotype, rate of disease progression, the early presence of respiratory failure and the patient's nutritional status. Further improvement in survival seems dependent on improving our understanding of the pathogenesis of ALS, leading to the development of early and specific diagnostic methods. The development of novel biomarkers to objectively assess disease progression will greatly refine treatment trials and significantly reduce trial costs. This presentation will cover recent findings that have established hyperexcitability with upregulation of persistent Na^+ conductances in ALS; and further research that indicates cortical hyperexcitability develops before the onset of clinical features of ALS, thereby suggesting that cortical hyperexcitability drives the process of anterior horn cell degeneration in ALS. As inevitably occurs with greater research focus, the potential for new therapies and approaches are being realised. In contrast to the previous century of limited progress in the field of motor neurone disease, these recent developments offer realistic hope that new treatments will emerge.

Common early alterations in lumbar motoneurons of $SOD1^{G93A}$ and $SOD1^{G85R}$ juvenile mice

Jacques Durand[1], Anton Filipchuk[1], Arnaud Pambo Pambo[1], Sylvie Liabeuf [1], Cécile Brocard[1], Iryna Kulagina[2], Sergey Korogod[2] and Jean-Patrick Guéritaud[1]

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In superoxide dismutase 1 (SOD1) mutant mice, the standard animal model of familial Amyotrophic Lateral Sclerosis (ALS), it has been shown that spinal motoneurons have altered electrical and morphological properties at a postnatal age. In the present study we examined the morphology and the electrical properties of lumbar motoneurons in two strains of mutant mice ($SOD1^{G93A}$ and $SOD1^{G85R}$) to determine common postnatal alterations.

In the whole isolated brainstem/spinal cord preparation, we recorded lumbar motoneurons in wild type (WT) and in SOD1 juvenile mice using intracellular recording and staining. Following histological procedures, the labelled motoneurons were reconstructed in 3D using Neurolucida^{TM} system for morphometric and topologic parameters analysis. We used computer models of 3D reconstructed WT and SOD1 motoneurons to assess the functional consequences of the morphological changes. In another set of experiments, we used specific antibodies directed against K^+/Cl^- co-transporter KCC2, and NR2A and GLUR1 subunits of NMDA and AMPA receptors, respectively.

We show that both $SOD1^{G93A}$ and $SOD1^{G85R}$ motoneurons exhibit an aberrant number of dendritic branches at postnatal days P8-P9 as compared to non transgenic WT motoneurons and develop a precocious hypoexcitability (lower F/I slopes calculated in the steady state) probably due to variation of synaptic input. Furthermore western blot analysis of NR2A subunit and KCC2 expressions in lumbar segments revealed differences between WT and SOD1 mice at postnatal days corresponding to the aberrant morphology. These results demonstrate several common abnormalities in the two SOD1 mutant strains and suggest very early imbalance of afferent inputs at the spinal level.

Degeneration of motoneurons in SOD1 mice: the size hypothesis

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*. Authors in alphabetical order

Amyotrophic Lateral Sclerosis (ALS) is an adult onset neurodegenerative disease of motoneurons (MNs) leading to muscle paralysis. In the SOD1 G93A mouse model of ALS, degeneration progresses from the periphery (neuromuscular junctions) to the cell body. Motor units (MUs) have a differential vulnerability according to their physiological type : FF (Fast-contracting, Fatigable) MUs degenerate first (P50), FR (Fast-contracting, fatigue Resistant) MUs degenerate later (P80) while S (Slow-contracting) type MUs do not seem to degenerate (Pun et al., 2006; Hegedus et al., 2008).

Our recent electrophysiological recordings showed that the excitability parameters (recruitment current, gain) of the MNs were unchanged by the disease, despite the fact that the input conductance of adult motoneurons was larger in SOD1 mice than in WT animals. These results suggest that the MNs undergo homeostatic changes to maintain their excitability. On the other hand, the increase in conductance suggests that the increase in size, observed at very early neonatal ages (Amendola & Durand, 2008; Quinlan et al., 2011), continue all the way to the age of the degeneration of the MUs. This competition between the increase in size and the increased metabolic demand to maintain the excitability could be a triggering factor for the neurodegeneration.

During my talk, I will present our recent progress in characterizing the alterations in electrophysiological properties as well as the morphological alterations observed in MNs at different stages of the disease. Our results lead us to formulate a new mechanism that explains the specific order of the degeneration of MUs: the “size hypothesis”.

The G85R & G93A-h models of ALS: similar size abnormality but opposite alteration in membrane biophysical properties

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Amyotrophic Lateral Sclerosis (ALS) is an adult-onset neurodegenerative disease characterized by the degeneration of upper & lower motoneurons (MNs). In the G85R and G93A-h (high expressor line) models, spinal MNs degenerate in a size order from large to small. In the G85R model, spinal MNs experience early alterations in their size and electrical properties, in which they have increased dendritic area and dendritic overbranching, and increased input conductance (Amendola & Durand 2008; Elbasiouny et al 2010). Furthermore, simulations of realistic models revealed increased specific membrane conductance (G_m) of SOD MNs relative to wild-type (WT) (Elbasiouny et al 2010). In the present study, we investigated the change in size and membrane biophysical properties of SOD MNs in the G93A-h model and compared them to those in the G85R model.

Our results indicate that SOD MNs (< Postnatal day 10, P10) of the G93A-h model experience early increase in their dendritic size that persisted to adulthood at P30 and P50. Realistic computer models indicated that G_m was decreased by 37% in SOD MN models relative to WT models. The latter observation is opposite to what has been found in the G85R model, in which G_m was increased in SOD MNs by 34%.

These results indicate that: 1) the increase in size of SOD MNs is a common abnormality in the G85R and G93A-h models, and 2) the change in G_m could represent a different homeostatic response of SOD MNs in the G85R and G93A models to the size increase.

Motoneuron hyperexcitability in SOD^{G93Ah} mice is associated with increased dendritic arborisation and glutamatergic synapses

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Purpose: Hypoglossal motoneurons (XII MNs) in neonatal mice carrying the human SOD1^{G93A} mutation are hyper-excitable (1). In this study, we investigated the relationship between XII MN hyper-excitability and morphology in pre-symptomatic SOD1^{G93A} mice (a mouse model for Amyotrophic Lateral Sclerosis), compared to wild-type (WT) littermates. **Methods:** XII MNs in 300μm brainstem slices were prepared from embryonic day 18 to postnatal (P) day 25 WT (*n*=68) and SOD1^{G93A} (*n*=51) mice. XII MNs were filled with Neurobiotin™ (NB) by semi-loose seal electroporation, fixed in 4% paraformaldehyde, washed in buffer and then incubated in Cy3-streptavidin. These slices were then double immuno-stained for glutamatergic terminals with anti-VGLUT2 and post-synaptic specializations with anti-PSD95. These antibodies were located using Alexa 488 and Cy5 2nd antibodies. Slices were imaged using a Zeiss confocal microscope. Neurons were traced using Neurolucida. Co-localisation of VGLUT2 and PSD95 (within 1 μm) on NB filled XII MNs were analyzed using IMARIS software. **Results:** Examination of XII MN morphology revealed that SOD1^{G93A} XII MNs had increased dendritic length and increased branch complexity by P12, and this continued throughout the pre-symptomatic period (P12 to P25). In addition, spine density was increased in SOD1^{G93A} XII MNs when compared to WT XII MNs. This was observed as early as P2 and persisted though to P25. The number of VGLUT2/PSD95 excitatory synaptic co-localisations on NB filled XII MNs was also significantly increased compared to WT by P8 (55% increase). **Conclusion:** These data show XII MNs in SOD1^{G93A} mice display increased dendritic complexity and increased density of glutamatergic synapses well before onset of amyotrophic lateral sclerosis symptoms (i.e. at P60-72). This supports the idea that MNs in SOD1^{G93A} mice are hyper-excitable and that this may be an early trigger leading to premature death of MNs in these mice.

1. van Zundert *et al* (2008) J.Neurosci, 28:10864-74

Motor unit recruitment in ALS and other UMN lesions

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Introduction: Motor unit potential (MUP) firing on volition has been neglected in ALS
Methods: We investigated MUP recruitment order and inter-MUP recruitment rate variation (coefficient of variation; CV) in normal subjects and patients with different LMN and UMN disorders. Only tibialis anterior muscles with normal strength (normal walk on heels) were investigated. We studied 42 controls, 37 patients with ALS, 14 with progressive muscle atrophy (PMA), 37 with polyneuropathy, 23 with primary lateral sclerosis (PLS), and 14 with other thoracic/cervical spinal cord lesions (UMN lesion). MUPs were analysed as recorded during a minimum of 60 seconds steady, unquantified, mild muscular contraction, which usually activated 2 to 5 motor units. The same MUP was observed in at least 10 consecutive firings before analysis. Amplitude, area and duration of each MUP, order of recruitment and firing rate were assessed.
Results: In PMA, ALS and neuropathy, MUP amplitudes, areas and durations were increased, as expected, compared with the other groups. Correlation analysis did not reveal any specific abnormality of recruitment order in the different patient groups within the limited conditions of our recordings. In the patients with spinal cord lesion (UMN) and with PLS, the variability of the coefficient of variation of the firing rate was less than in the other groups of patients. Control subjects had a non-significantly higher CV than PLS and UMN groups. In patients with ALS the CV of the firing rate was higher than PLS and UMN subjects, but the variation of the CV was much larger, suggesting a complex interplay of different physiological abnormalities within the group of lumbar motor units recruited in ALS.
Discussion: Our data confirms reduced variation in MUP firing rates with UMN lesion. The limits of this abnormality need further evaluation at different voluntary firing rates. The abnormality in ALS probably reflects not only the UMN lesion but also destruction of the neuronal organisation at spinal segmental level in the disease.

Tuesday, 24 July

3.30 pm – 5.00 pm Motoneuron adaptation (Chair: CJ Heckman)

Spike frequency adaptation in motoneurons: is it an artefact?

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Spike frequency adaptation (SFA) is the slowing of the rate of repetitive firing over time. Over the past five decades, SFA has been characterised in motoneurons largely through intracellular injection of constant current steps. These experiments have shown that there are several phases during which the firing rate in response to a constant current pulse slows down. These studies have led to important insights into the membrane currents present in motoneurons.

But is repetitive firing produced by current injected through the micropipette – whether intracellularly or extxracellularly – informative about membrane currents during behaviour? Some evidence suggests that SFA in motoneurons may not be present during behaviour, and that the nervous system is able to minimise potentially detrimental effects of SFA. Perhaps SFA is only present in minimalist conditions and not during most motor behaviours?

In this talk, I will describe SFA from an historical and phenomenological perspective, mention the ionic mechanisms underlying SFA, and discuss examples of behaviours where SFA may or may not be present. Discussion will be encouraged in an attempt to provide a consensus answer to the title of the talk!

Effects of the muscle overload on the medial gastrocnemius motor units and motoneurons

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Functionally isolated motor units (MUs) of the medial gastrocnemius muscle were investigated in rats subjected to a 3-month compensatory overload, induced by bilateral tenotomy of its synergists. To assure voluntary activation of the operated muscles, surgical procedures were followed by keeping animals in wheel-equipped cages and treadmill exercise. Electrophysiological properties of spinal motoneurons innervating the medial gastrocnemius were investigated in rats after 5 weeks of the muscle overload. Control groups of intact rats were used for comparison.
The muscle adaptation to the overload included an increase in the muscle mass and the muscle-to-body weight relation. MUs' proportion was modified towards higher percentage of the S and FF types and lower contribution of FR MUs. The overload-evoked changes in contractile properties of MUs concerned: a shortening of the half-relaxation time, a decrease of the twitch force and of the twitch-to-tetanus ratio, the higher post-tetanic potentiation (in all MU types), and an increase of the tetanus force (in FR and S types). Changes in fatigue resistance were observed only in fast MUs: the lower fatigue index for the FF, while higher for the FR type.
The electrophysiological properties of motoneurons were considerably modified by the 5-week overload, most of all in fast-type motoneurons. The changes included: a shortening of duration of the action potential, and the AHP time-to-peak, an increase of the AHP amplitude, a decrease of the rheobase, the spike generation threshold, and the minimum current necessary to evoke steady-state firing. The reverse changes were observed for the unloaded lateral gastrocnemius and soleus motoneurons.

Respiratory muscle adaptation following chronic hypoxia during postnatal development

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Chronic hypoxia (CH) is encountered in healthy individuals at high altitude and in patients with chronic obstructive pulmonary disease. It is also common in premature babies and in infants with cardio-respiratory disease. Hypoxic-induced plasticity in the respiratory control system is well described but the effects of chronic hypoxia on respiratory muscle adaptation are underexplored, particularly during postnatal development. Wistar rats were exposed to 1 week of hypobaric hypoxia (450mmHg) or normoxia at various time-points during development (postnatal day (P)1-P31). Sternohyoid and diaphragm muscle contractile and endurance properties were assessed *in vitro*. Muscle oxidative capacity, myosin heavy chain (MHC) isoform composition and fibre cross-sectional area were determined. The putative role of reactive oxygen species in hypoxia-induced muscle remodelling was assessed. CH significantly increased sternohyoid muscle force and fatigue in early but not late development – effects that persisted for several days after return to normoxia. (e.g. early hypoxia peak force 4.6 ± 1. vs. 8.2 ± 1.2 N/cm^2; fatigue index 83.2 ± 7 vs. 58.5 ± 8 %; control (n=6) vs. CH (n=8), $P<0.05$ and late hypoxia peak force, 8.2 ± 1.3 vs. 10.6 ± 1.6 N/cm^2; fatigue index, 36 ± 5 vs. 38 ± 3%; control (n=8) vs. CH (n=6)) CH-induced functional plasticity in the sternohyoid muscle was not attributable to MHC fibre type transitions (areal density of MHC slow 7 ± 1 vs. 10 ± 2%; MHC 2a 27 ± 2 vs. 32 ± 3%; MHC 2b 41 ± 2 vs. 36 ± 2%; control (n=9) vs. CH (n=6) respectively) or a decrease in oxidative capacity. Chronic supplementation with the superoxide scavenger – Tempol did not prevent CH-induced increases in sternohyoid muscle force or fatigue, suggesting that mechanisms unrelated to oxidative stress underpin CH-induced adaptation in respiratory muscle. CH increased force in the diaphragm muscle only when exposed to hypoxia in the first week of life (peak force 8.3 ± 0.8 vs. 13.5 ± 0.9 N/cm^2, control (n=6) vs. CH (7), $P<0.05$). We conclude that chronic hypoxia elicits functional adaptation in neonatal, but not juvenile respiratory muscles. Our findings are consistent with the view that there are critical windows in early life where perturbations to the developing respiratory control system are significant and may persist into later life. Airway muscle remodelling may have consequences for the control of airway patency *in vivo*.

Fatigue of motoneurons

Curtis D Manning, Chantelle D Murnaghan, Parveen Bawa

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When step or ramp currents are injected into mammalian motoneurons, firing rates of motoneurons exhibit adaptation to constant currents (Kernell, Acta Physiol Scand 1965). In humans, prolonged firing of motoneurons leads to silencing of active motoneurons and recruitment of additional motoneurons. When the subject tries to maintain constant discharge rates in motoneurons, switching of activity between low threshold motoneurons occurs; this phenomenon has been called rotation (Bawa & Murnaghan, J Neurophysiol 2009). We show that when a motoneuron silences after a prolonged discharge, it does so because it is unable to maintain its tonic firing given the same excitatory input. Its probability of spontaneous firing or response to phasic excitatory inputs is highly diminished. After some rest, the probability of spontaneous spikes and its response to phasic inputs increases until tonic discharge resumes. A constant discharge followed by inability to discharge given the same input, which, after rest, is followed by slow recovery to resumption of tonic firing is reported for mammalian limb motoneurons. Such behaviour is akin to the definition of fatigue used for other cells or systems rather than to adaptation defined for step injected currents.

Acknowledgements: Work was supported by a grant from NSERC of Canada.

Motoneuron excitability following a locomotor vs. an isometric fatigue task

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In isometric tasks of the upper limb, fatigue reduces motoneuron excitability in the silent period which follows a cortical stimulus (McNeil et al. J Physiol, 2009; 2011). The mechanism may represent an intrinsic property of the motoneurons and be related to their late adaptation. In the decerebrate cat, late adaptation of motoneurons is reversed by fictive locomotion (Brownstone et al. J Neurophysiol, 2011). The present study aimed to compare motoneuron responsiveness in the silent period in humans after locomotor and isometric fatigue tasks. Eight healthy males completed 10-min fatigue tasks matched for electrical activity (EMG) of the quadriceps in a random order. The locomotor fatigue task involved subjects walking uphill (12° incline) on a treadmill at 4 km/h with one step of the right leg every 1.2 s. To maximize quadriceps EMG, an elastic band was attached to the right ankle and set to allow 'normal' gait. For the isometric task, subjects sat with their right ankle strapped into a strain gauge. At the same 1.2s cadence, subjects made brief contractions to the force which reproduced the level of integrated EMG during treadmill walking. Prior to each fatigue task and in the recovery period afterward, motoneuron excitability was assessed in brief (~4 s) isometric knee extension contractions sustained at the integrated EMG produced during a 40% maximal effort. To test motoneuron excitability in the silent period, a thoracic motor evoked potential (TMEP) was elicited by electrical stimulation over the thoracic spine 100 ms after conditioning transcranial magnetic stimulation of the motor cortex. In the 5 min after the isometric task, the TMEP declined to 69±38% (mean±SD) of its pre-fatigue area with 7 of the 8 subjects showing a decrease of >20% and the other an increase of 56%. In recovery after the locomotor task, the TMEP was 105±64% of its pre-fatigue area with 3 subjects showing an increase and 5 showing a decrease. Data from the isometric task indicate the documented fatigue-related reduction in motoneuron excitability occurs in the lower limb and with an intermittent task. Although the mean data loosely support the abolition of late adaptation seen in the decerebrate cat, the majority of subjects still showed a reduction in motoneuron excitability after locomotion. This result was confirmed in a separate experiment performed on a level treadmill without resistance.

Wednesday, 25 July

9.00 am – 10.30 am Motoneuron behaviour in people (Chair: Inge Zijdewind)

Motor unit synchrony: cortical imprint or not

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Motor units within human muscles usually exhibit a significant degree of short-term synchronization. Such coincident spiking typically has been attributed to last-order projections that provide common synaptic input across motor neurons. The extent and strength of branched input arising directly from cortical neurons has often been suggested as a critical factor determining the magnitude of short-term synchrony. The relationship between motor unit synchrony and strength of cortical input, however, has not been thoroughly investigated. Here I will describe a set of studies that quantified the magnitude of motor unit synchrony in several human muscles differing in the presumed extent of cortical input to their respective motor nuclei. While our results confirm the existence of striking variations in the degree of short-term synchrony across muscles - with synchrony tending to be higher in the more distally located muscles - the greatest synchrony was found in the intrinsic muscles of the foot rather than in the hand. Furthermore, the strength of corticospinal inputs to motor neurons supplying foot muscles, as assessed through transcranial magnetic stimulation, was relatively weak. Therefore, factors other than the potency of cortical inputs to motor neurons, such as the number of motor neurons innervating a muscle, may significantly affect motor unit synchrony.
Supported by NIH grant NS070897.

Initial conditions influence the discharge of single motor units during fast contractions

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This study investigated the effects of a submaximal sustained contraction (20-25% of maximal force) on the characteristics of a subsequent contraction performed as fast as possible (ballistic action) with the ankle dorsiflexor muscles. The ballistic actions were performed under two different conditions: (a) during the sustained pre-activation (STABAL) and (b) following a brief agonist relaxation inserted between the pre-activation and ballistic action (ARBAL). The performances of these two ballistic actions were compared with ballistic contractions initiated from a resting state (BAL). Isometric force of the dorsiflexors, and surface and intramuscular electromyograms (EMG) of the tibialis anterior were simultaneously recorded.

The results indicated that when ballistic contractions were performed without pre-activation (BAL), the rate of torque development was greater (9.4 ± 1.7 vs 8.7 ± 1.8 $Nm.s^{-1}$) than in STABAL but lower than in ARBAL condition (16.3 ± 4.7 $Nm.s^{-1}$). Consistent with these results, motor unit discharge rate (mean of the first three interspike intervals) at the beginning of the ballistic action was greater in ARBAL (138.6 ± 9.0 Hz) than in BAL (115 ± 5.8 Hz) and STABAL (89.8 ± 3.8 Hz) conditions. Furthermore, motor unit discharge rate increased progressively during the first three interspike intervals in STABAL whereas it decreased during the successive discharges of ballistic actions performed in BAL and ARBAL conditions.

Together, these results indicate that the conditions from which ballistic contractions are initiated have a substantial influence on motor unit activation and rate of torque development during ballistic actions.

Triceps surae muscle motor unit behavior during human standing balance

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The human center of mass projects anterior to the ankle, resulting in an ongoing need for plantar flexion torque. Despite the agonistic role of the gastrocnemius (Gas) and soleus (Sol), important differences exist between these muscles that may reflect separate roles in standing balance. We recorded motor unit (MU) activity from medial and lateral Gas and Sol to investigate MU behavior during standing balance (two 2-min trials) and braced isometric contractions (three 1-min trials) in 6 subjects. Fifty MU (30 medial Gas, 20 Sol) were analyzed during standing balance. Sol MU were continuously active for the majority of standing trials (99% [75%-100%], median [range]); medial Gas MU activity was more intermittent (74% [2-100%]). Mean MU firing rates were not different between muscles, but interspike interval variability was significantly greater in medial Gas MU (coefficient of variation: 0.35±0.04 versus 0.19±0.035, $p<0.05$). While 45 lateral Gas MU showed sustained activity during isometric trials (mean or 90% maximum standing torque), only 1 was consistently active while standing, with 7 others firing sporadically (< 100 times). This lack of lateral Gas activity was related to higher recruitment thresholds in these MU. The continual and regular activity of Sol MU provides support to this muscle playing a key tonic role in generating plantar flexor torque during standing balance, while the medial Gas seems to provide phasic increases in torque that would summate with torque produced by the Sol. The lateral Gas plays a negligible role in torque production during standing balance.

Frequency analysis of the reflex responses of human masseter motor units

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Introduction: Previous studies indicate the importance of periodontal receptors in feedback control of mastication. However, earlier studies have been performed either on animals or on human subjects using probability-based analyses.

Aim: Our aim was to investigate the jaw reflexes using both the probability- and the discharge rate based analysis methods.

Methods: Twelve consenting volunteer subjects participated in this study. Subjects bit gently so that one selected single masseter motor unit discharged at about 10 Hz. While the subject fired the motor unit 4N stimuli were delivered to the upper right central incisor.

Results: While preceding local anesthesia the stimuli induced an inhibitory reflex. However, during local anesthetic block, stimuli induced excitatory reflex responses in the probability-based methods but not in the discharge rate based method. Since the classical methods rely upon the number of action potentials, they generate significant count and synchronization related errors as the action potentials are phase advanced or phase delayed by the stimulus-induced synaptic potentials. Discharge-rate method is however free from such errors as it is not affected by the number and density of action potentials at any particular time after the stimulus.

Conclusions: Usage of discharge rate based analysis for bringing out the genuine synaptic activity is essential for building the accurate wiring diagram for the human central nervous system. These pathways are used for determining stability, damage and recovery from damage of the central nervous system.

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Non-uniform effects of nociceptive input to motoneurons during experimental pain

Hodges P.W.[1], Tucker K.[1], Garland S.J.[2], Butler J.[3], MacDonell C.[2], Poortvliet P.C.[1], Schabrun, S.[1]

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Theories of motor adaptation to pain predict uniform inhibition of motoneuron pools of painful muscles and muscles producing painful movements. Although reduced motoneuron discharge rate during pain provides some evidence, recent data show this is accompanied by recruitment of motoneurons that were inactive before pain, and increased discharge rate of others (Tucker et al. 2009 JNeurosci). Although this suggests non-uniform effects of nociception, more direct measures of excitability are required. We used two methods to quantify excitability changes during experimental pain. First, time course of motoneuron after-hyperpolarisation (AHP) was estimated using analysis of interval death rate calculated from single motor unit discharge at three rates (Matthews 1996 JPhysiol). Second, excitability of corticospinal inputs to motoneurons was estimated using transcranial magnetic stimulation (TMS) of the motor cortex. Motor unit activity was recorded from tibialis anterior during dorsiflexion. Motoneuron excitability was maintained by matching discharge rate before and during pain. Reduced probability of motoneuron discharge in response to TMS during pain was interpreted as reduced excitability of corticomotor inputs, and visa versa. Results for 17 units in Study 1 show both shortened (n=10) and lengthened (n=6) AHP time course. Results for 11 units in Study 2 show increased (n=4) and decreased (n=5) probability of firing. These observations show non-uniform effects of nociceptive input on motoneuron pools during pain and lend support to recent theories that argue for redistribution of activity in painful muscles, rather than uniform inhibition (Hodges & Tucker 2011 Pain), potentially to modify load distribution within the muscle.

Wednesday, 25 July

11.00 am – 12.30 pm Motoneurons and persistent inward currents, etc (Chair: Marc Binder)

Motoneurons, what makes them tick?

Jørn Hounsgaard

INF, University of Copenhagen, Denmark

Motoneurons are not spontaneously active but what then cause them to tick? I will discuss the role of intrinsic response properties, modulation and synaptic input. With fictive scratch as an example I will also discuss the number and distribution of pre-motor neurons involved in functional network activity.

Contributions of sodium and potassium currents to synaptic integration in motoneurons.

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Experimental and simulation studies of synaptic integration in motoneurons have concentrated on the role of calcium-mediated persistent inward currents in amplifying and prolonging the response to synaptic inputs. However, the slow kinetics of calcium channels suggests that they can only contribute to amplification of low frequency inputs (< 5Hz). To characterize frequency-dependent amplification of excitatory synaptic post-synaptic potentials (EPSPs), we measured the averaged stretch-evoked EPSPs in cat medial gastrocnemius motoneurons in decerebrate cats at different subthreshold levels of membrane potential. EPSPs were produced by muscle spindle afferents activated by stretching the homonymous and synergist muscles at frequencies of 5 to 50 Hz. Voltage-dependent amplification was observed at all stretch frequencies but generally decreased with increasing stretch frequency. However, in many cells the amount of amplification was greater at 10 Hz than at 5 Hz. Fast amplification was generally reduced or absent when the lidocaine derivative QX-314 was included in the electrode solution, supporting a strong contribution from sodium channels. In the presence of QX-314, depolarization often reduced EPSP amplitude and area more than would be expected based only on a reduction in driving force. This and previous experimental work suggests that dendritic potassium channels also influence synaptic integration. Computer simulations using a compartmental model of a fully-reconstructed medial gastrocnemius motoneuron suggest that dendritic sodium channels and at least two different dendritic potassium channels contribute to the observed frequency-dependent amplification of stretch-evoked EPSPs. These experimental and simulation results support the view that a variety of voltage-dependent dendritic channels shape and enhance motoneuron responses to modulations in synaptic drive over a physiologically significant range of frequencies.

Interaction between Na PIC's and motoneuron firing properties

Robert Lee and Cassie Mitchell

Emory University and Georgia Institute of Technology

We have previously shown that Na PIC's form the spike threshold in motoneurons. We now present experimental and theoretical evidence that these same currents are integral to the internal regulation of F-I gain as well as some viable options for modeling the important elements of this process in computer models of motoneurons. This work has particular significance in relation to the role of persistent sodium in pathologies such as Stroke, Spinal Cord Injury, and ALS.

Role of constitutively-active 5HT2 receptors in involuntary muscle spasms after spinal cord injury (SCI) in humans

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The recovery of motoneuron excitability and the development of involuntary muscle spasms after SCI are mediated by the re-emergence of sodium and calcium persistent inward currents (PICs), despite the loss or reduction of serotonin (5HT) that facilitates the PIC. Here we examined if, like in animal models, PICs in humans are facilitated by constitutively-activated 5HT2 receptors, i.e., receptors that are activated without the ligand 5HT.

In 8 incompletely injured subjects an 8mg oral dose of cyproheptadine, which blocks both constitutive and ligand activation of the 5HT2 receptor (inverse agonist), significantly decreased both the PIC-mediated long-lasting reflex (LLR) and ΔF (measured by paired motor unit analysis) by 60% ($n=8$, $p<0.001$) and 29% ($n=6$, $p<0.05$), respectively. However, in these subjects, the selective serotonin reuptake inhibitor citalopram, significantly facilitated the LLR by 94% ($p=0.04$), indicating that there was likely residual sources of 5HT (and ligand activation of the 5HT receptor) in these motor incomplete subjects. Thus, it was unclear if cyproheptadine decreased the PIC mediated responses by solely reducing constitutive 5HT2 receptor activity.

We then tested cyproheptadine in 9 motor or motor/sensory complete SCI subjects who likely had far less to no residual sources of 5HT. Cyproheptadine again reduced the PIC-mediated LLR by 60% ($p<0.05$). In addition, chlorpromazine, which only blocks the ligand activation of the 5HT2 receptor (neutral antagonist), had no effect on the LLR at doses that were effective in reducing the ΔF in non-injured control subjects (12.5 mg). Taken together, these results suggest that following SCI, recovery of motoneuron excitability is facilitated by the development of constitutive 5HT2 receptor activation after injury.

Driving motoneurons with repetitive reflexive inputs after a spinal cord injury

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INTRODUCTION: During movement, motoneurons receive trains of impulses along reflex pathways from muscle afferents. Experimentally, when prolonged trains of electrical stimulation are delivered to the tibial nerve, soleus H-reflexes are initially depressed (post-activation depression) but can recover and this recovery is augmented after a brief train of stimulation at a higher frequency. The present experiments were designed to characterise how motoneurons respond to trains of afferent impulses after a spinal cord injury (SCI). The results provide insight into changes in spinal circuits after a SCI and have implications for rehabilitation.

METHODS: Nine individuals with a SCI participated. Soleus M-waves and H-reflexes were evoked by electrical stimulation of the tibial nerve delivered in two patterns: "constant-frequency" (15 or 20Hz for 12s) and "burst-like" (15 or 20Hz–100Hz–15 or 20Hz; 4s each phase).

RESULTS: During constant-frequency stimulation, after the initial depression from the first to the second H-reflex (from 57% to 25% of the maximal M-wave (Mmax)), H-reflexes did not recover significantly and were 37% Mmax at the end of the stimulus train. During the burst-like pattern, after the initial depression (from 62% to 30% Mmax), reflexes recovered completely by the end of the stimulation (to 55% Mmax) as they were not significantly different from the first H-reflex. Preliminary experiments show that contractions driven predominantly through H-reflexes fatigue less than those produced via M-waves.

CONCLUSIONS: These results are consistent with a general increase in reflex excitability after a SCI and show that a brief burst of relatively high frequency stimulation effectively reverses the effect of post-activation depression. Driving contractions through reflex pathways may be beneficial for rehabilitation by producing contractions that are more fatigue resistant.

Wednesday, 25 July

3.30 pm – 5.00 pm Motoneurons after spinal cord injury (Chair: Jane Butler)

Recovery of phrenic motoneuron activity after cervical spinal cord injury

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Upper cervical spinal cord injury often results in diaphragm muscle (DIAm) paralysis requiring long-term maintenance of patients on mechanical ventilation, with associated higher morbidity and mortality rates. Clearly, it is important to understand how rhythmic DIAm activity can be restored in these spinal cord injury patients. It is well established that rhythmic excitatory premotor drive to phrenic motoneurons emanates predominantly from the ipsilateral medulla. In our studies, we employed a well-established rat model of partial spinal cord injury where the C_2 spinal cord is hemisected (spinal hemisection - SH) thereby removing ipsilateral excitatory input to phrenic motoneurons causing activity to disappear on the affected side. However, there is a latent contralateral input to phrenic motoneurons that can be strengthened with time after SH (neuroplasticity) leading to functional recovery of rhythmic phrenic activity (crossed phrenic phenomenon). Converging evidence from our lab indicates that brain-derived neurotrophic factor (BDNF) acting through its high affinity receptor (TrkB.FL) plays an important role in neuroplasticity and promoting functional recovery. For example, intrathecal BDNF treatment enhances functional recovery of rhythmic phrenic activity after SH. In contrast, intrathecal treatment with TrkB-Fc, a fusion protein that quenches extracellular BDNF delays functional recovery. Unfortunately, exogenous intrathecal BDNF treatment is associated with significant negative adverse effects that preclude its therapeutic use. As an alternative, we developed a novel targeted approach to promote functional recovery after SH. We found that intrapleural injection of an adeno-associated virus (AAV-7) designed to increase TrkB.FL expression in phrenic motoneurons markedly enhanced functional recovery of rhythmic phrenic activity after SH.

Supramaximal volitional torque in humans with spinal cord injury: Reflexive and perceptual consequences

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Individuals whom suffer from chronic motor incomplete spinal cord injury (SCI) are able to produce volitional torques 20-30% above their one repetition maximum when a maximum voluntary effort (MVE) contraction is preceded by a history of high intensity efforts. One of our current goals is to better understand the neural mechanisms which may underlie this supramaximal volitional torque generation in individuals with chronic motor incomplete SCI. Here we present 3 lines of evidence to suggest high intensity volitional efforts result in supramaximal volitional motor output through alterations in spinal neuron excitability. First, intramuscular and subcutaneous motor unit recordings collected during supramaximal volitional torque generation reveal short term increases in peak firing rate and recruitment of additional motor units; at times prolonged motor unit activity is observed following cessation of high intensity volitional efforts. Second, a time- and intensity-dependent potentiation of both the tendon reflex and the stretch reflex following a range of volition efforts is observed. Third, subjects with SCI consistently overestimate the generation of a learned submaximal volitional torque generation immediately following, but not 1 minute following, a bout of MVE contractions. These alterations in motor unit firing patterns, reflex potentiation and kinesthetic sensibility are consistent with the idea that alterations in spinal neuron excitability underlie this supramaximal volitional torque generation. This work better allows us to understand how individuals with incomplete SCI generate volitional force and helps to guide current pharmacological investigations and strength training interventions.

Sustained amplification of volitional motor function in spinal cord injury: role of PICs in rehabilitation

T. George Hornby

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Individuals with motor incomplete spinal cord injury (SCI) can demonstrate substantial increases in peak volitional torques during standard 'fatiguing' protocols assessed in individuals without neurological injury. While this behavior is observed on the same or different days with substantial rest between "fatiguing" bouts, the parameters which optimize torque generation, their mechanisms, and the influence of exogenous perturbations and their contributions to functional performance are not well established. In the present study, we investigated the contribution of various parameters of repeated maximal volitional effort (MVE) contractions, the effects of serotonergic agents, and the functional consequences on individuals with SCI. Increases in volitional torque generation were only observed during higher intensity contractions and shorter durations, which were mirrored by intensity- and time- dependent increases in the short latency quadriceps tendon reflex responses following volitional efforts. The effects of serotonergic agents strongly suggest that antagonists can markedly depress the observed behaviors and impair functional mobility, whereas serotonergic reuptake inhibitors only slightly augment strength and do not improve motor activity immediately following administration. These findings suggest a functional role for increased spinal neuron excitability following volitional contractions in individuals with SCI and may provide the framework for novel rehabilitation interventions.

Reduced spinal inhibition increases stretch-induced activation of muscles paralyzed by spinal cord injury

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Involuntary contractions (spasms) are common in muscles paralyzed by spinal cord injury (SCI). The importance of reduced inhibition versus increased motoneuron excitability in generating these contractions remains unclear. Our aim was to examine how paralyzed muscles (under no voluntary control) respond to repeated, sustained passive stretch, an approach often used by people with SCI to manage muscle spasms. Surface electromyographic (EMG) signals (vastus lateralis, hamstrings, tibialis anterior, medial gastrocnemius, soleus) and joint angles (knee, ankle) were recorded during passive stretch of each muscle group (10 static stretches held for 30 seconds each, with 15 seconds of rest in between) in SCI and uninjured (control) subjects. The control experiment for each subject was without stretch. Most muscles responded to the changes in muscle length (lengthening or shortening). The EMG responses were stronger in SCI subjects, persisted from one stretch to the next without habituation, and spread to arm and hand muscles. Stretch-induced contraction of paralyzed muscles was greater when there was less depression of the soleus H-reflexes with 4 Hz stimulation. H-reflex depression at 1 Hz was minimal after SCI. Contraction intensity was not related to the maximal soleus H-reflex to M-wave ratio or the magnitude of F waves. These results suggest reduced spinal inhibition facilitates involuntary contraction of muscles paralyzed by SCI.

Funded by National Institutes of Health grant NS30226 and The Miami Project to Cure Paralysis.

Respiratory motoneuron activation during High Frequency Spinal Cord Stimulation (HF-SCS)

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Objective: HF-SCS is a novel technique of inspiratory muscle activation involving spinal cord pathways. The purpose of the present study was to compare the distribution of motor drive to the intercostal muscles during spontaneous breathing with that occurring during HF-SCS.

Methods: In 7 anesthetized dogs, stimulation was applied via a disc electrode located on the ventral surface of the spinal cord at the T2 level following C2 section. Fine wire recording electrodes were used to assess single motor unit activation of the external intercostal muscles in the dorsal portion of the 3rd ($EI3_D$) and 5th ($EI5_D$) interspaces and ventral portion of the 3rd ($EI3_V$).

Results: Rib cage contribution to inspired volume was significantly higher during HF-SCS compared to spontaneous breathing (200±4 ml vs. 98±4 ml). Mean maximum discharge frequency of the $EI3_D$ (18.8±0.3 Hz) was significantly greater than that of the $EI3_V$ (12.2±0.2 Hz) and the $EI5_D$ (15.3±0.3 Hz) ($p<0.05$) during HF-SCS. While these values were significantly greater than those occurring in the same animals during spontaneous breathing, the pattern of responses was similar.

Conclusion: HF-SCS results in the same differential pattern of activation of the various intercostal muscles as that which occurs during spontaneous breathing. The neural circuitry involved in the physiologic distribution of drive to the external intercostal muscles exists within the spinal cord and does not require input from medullary respiratory centers.

Support: NIH-NINDS (R01 NS064157).

Disclosure: Dr. DiMarco has a significant financial interest in Synapse BioMedical, Inc, a manufacturer of diaphragm pacing systems.

Thursday, 26 July

9.15 am – 10.15 am Motoneurons and other matters (Chair: Rob Herbert)

The decomposition of fast and slow motor unit tetanic contractions evoked by random stimulation pattern

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The unfused tetanic contractions evoked by stimulation at variable interpulse intervals (IPIs) were recorded for 10 fast fatigable (FF), 10 fast resistant (FR) and 10 slow (S) motor units (MUs), and decomposed using a mathematical algorithm into trains of twitch-shape responses to successive stimuli. The mean stimulation frequencies were matched for each MU to evoke tetani of similar fusion degrees, whereas the variability range of IPIs was 50-150% of the mean IPI. Force and time parameters of decomposed twitches were related to the first response. Considerable variability of twitch parameters was observed in each MU, although the largest range of variability concerned slow MUs. In general, the decomposed twitch responses had longer duration and higher force than single twitches, although for 9 FF and 6 FR MUs some of decomposed responses were slightly weaker (but not faster) than the first twitches of these MUs. Comparison of the strongest decomposed twitch to the first decomposed twitch revealed ratios of forces amounting up to 2.35, 3.33 and 6.89 for FF, FR and S MUs, ratios of force-time areas up to 3.54, 4.67 and 14.26 for FF, FR and S MUs, whereas for the contraction times the ratios of the longest decomposed twitch to the first twitch amounted to 2.46, 2.07 and 3.52 for FF, FR and S MUs, respectively. The contractile responses to successive action potentials are considerably variable, especially for slow MUs, and these conclusions are likely true for voluntary movements, when especially low-threshold motoneurons produce discharges with variable interpulse intervals.

Evidence for direct central effect of Botulinum neurotoxin A in human spinal cord

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In animal models, the botulinum neurotoxin type A (BoNT-A) is retrogradely transported by motoneurones and central neurones, transcytosed to afferent synapses, which enables it to block the neurotransmission not only in peripheral cholinergic synapses but also in central synapses. An analogous direct central effect in humans is still debated. However, when injected at muscular level, a retrograde transport of the toxin along the motor axons could primarily affect the cholinergic transmission between intraspinal recurrent collaterals and Renshaw cells mediating recurrent inhibition to spinal motoneurones. The study was designed to address the question whether BoNT-A modifies recurrent inhibition in humans. To avoid methodological bias, the recurrent inhibition from an injected muscle (soleus) was investigated on an untreated muscle (quadriceps), and stimulation parameters (producing recurrent inhibition) were monitored on a third non injected muscle but innervated by the same nerve as soleus (flexor digitorum brevis, FDB). The experiments were performed on 14 stroke patients exhibiting spasticity in ankle plantarflexors, candidates for BoNT-A. One month after BoNT-A, the level of recurrent inhibition was found depressed, especially in patients who received the toxin for the first time, and the loss of inhibition was related to the number of injection sites and the change in muscle tone. Indirect possible effects of BoNT-A will be discussed but the conclusion will be that the toxin-induced depression of recurrent inhibition was probably due to retrograde transport of the toxin and the blockade of the cholinergic synapse between motor axon recurrent collaterals and Renshaw cells. Functional repercussions on motor synergy during locomotion will be addressed.

Properties of single adult mouse motor units recorded in vivo. Implication for their function.

Marin Manuel* & CJ Heckman

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by the specific loss of cortico-spinal neurons and spinal motoneurons. In the latter population, the neurodegeneration appear to follow a precise pattern based on the physiological types of the motor units (Pun, 2006; Hegedus, 2008). However, the study of this particular aspect of the disease has been precluded by our inability to readily identify the type of the motor units in the mouse, which is of critical importance since it is our model to study ALS.

This is the reason why we set off some years ago to develop a new preparation allowing the simultaneous recording of the electrical activity of the motoneurons and the force developed by their muscle targets. In my talk, I will present our recent progress in this endeavor. I will show how one can identify the type of a mouse motor unit, the correlations between the electrical properties of a motoneuron and its physiological type, and attempt to provide some insight on the differential sensitivity of the various motor units to ALS.

A new method for online reconstruction of joint torque from EMG recordings.

Aymar de Rugy and Timothy J Carroll

Centre for Sensorimotor Neuroscience, School of Human Movement Studies,
The University of Queensland, Brisbane, Australia

Current methods to reconstruct muscle contributions to joint torque usually combine electromyograms (EMG) with cadaver-based estimates of biomechanics, but both are imperfect representations of reality. Here we describe a new method that enables *online* reconstruction of joint torque, in which we optimize a "virtual" representation of muscle biomechanics. We first obtain tuning curves for the five major wrist muscles from the mean, rectified EMG during the hold-phase of an isometric aiming task, in which a cursor is driven by actual torque recordings. We then apply a custom, gradient-descent algorithm to determine the set of "virtual pulling vectors" that best reach the target torques when combined with the observed muscle activity. When these pulling vectors are multiplied *online* by the rectified and low-pass filtered (1.3 Hz) EMG of the five muscles, the reconstructed "torque" provides a close spatiotemporal match to the true torque exerted at the wrist. The technique works equally well for surface and fine wire recordings, and is sensitive to postural modifications. The method provides new avenues to study the relationship between neural control and limb biomechanics, since the "virtual biomechanics" can be systematically altered at will. For example, we recently studied muscle activity changes during a torque aiming task when a muscle was "virtually cut" such that its activation had no effect on the cursor, or when the signal-dependent noise associated with its activation was increased. Interestingly, the results observed were contrary to predictions from optimal control theory[1].

[1] De Rugy, Loeb, Carroll. *J Neurosci.* (in press).

Thursday, 26 July

10.45 am – 12.30 pm Motoneurons after stroke (Chair: Janet Taylor)

The quandary of motoneuron behavior in stroke: higher excitability, but lower sustained firing rates

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Motoneurons in stroke survivors display an array of properties that are not readily reconciled with any single physiological disturbance.

In paretic limb muscles, motoneuron discharge rates are often inappropriately low, and recruitment profiles are compressed, presumably reflecting ineffectual force generation by newly recruited motor units. The origins of this rate reduction are unclear but it may result from prolonged afterhyperpolarization potentials.

In spastic limb muscles, the threshold for stretch reflex activation is routinely lower than that in contralateral muscles, yet the origins of this threshold reduction also remain uncertain.

There are at least three potential mechanisms worth considering.

1. Larger muscle afferent responses to stretch: There have been no extensive descriptions of spindle afferent responses in stroke, but existing data suggest this is not a factor
2. Changes in intrinsic motoneuron properties including voltage-gated conductances. We have no evidence supporting systematic change in these membrane properties, but our methods here are imprecise.
3. Depolarizing synaptic drive: Here, we seek a strongly lateralized source of excitation from the brainstem or above- this likely excludes reticulospinal, raphe, and locus ceruleus, which each exerts bilateral actions. One possibility is that vestibulospinal projections are involved. Vestibular nuclei have been shown to be important in mediating the rigidity recorded in decerebrate animal preparations.

   We have used vestibulospinal responses to high intensity sound to test the role of vestibular projections in spasticity. Our findings here are that vestibulospinal actions on neck muscles are highly asymmetric in stroke survivors, strongly favoring the spastic side.

Motor unit behavior following fatigue in stroke survivors

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Abnormal fatigue-related firing behavior of single motor units in paretic leg musculature, such as the knee extensors, could contribute to impairments in force generation and limit walking function. Using novel and non-invasive technology for the investigation of motor units from the decomposition of high-density 64 channel surface electrode EMG, we investigated the influence of muscle fatigue on knee extensor single motor unit firing behavior in 5 stroke survivors and 5 controls. Motor unit discharge rates of the vastus lateralis were extracted during five 16 s ramp-and-hold knee extension contractions (5% to 20% of maximal voluntary contraction, MVC) and a 2-min sustained contraction (10% MVC), which were performed before and after a sub-maximal isometric fatiguing protocol. The discharge rates at recruitment, peak, and derecruitment for stroke survivors did not differ between the pre (7.1 ± 1.6, 8.1 ± 1.2, 4.5 ± 0.9 pps) and post measurements (6.7 ± 2.3, 8.5 ± 4.9, 4.9 ± 1.5 pps, $P>0.05$). Conversely, the three discharge rates increased for controls between pre (7.4 ± 1.5, 8.9 ± 1.9, 5.6 ± 1.3 pps) and post measures (8.6 ± 1.6, 13.9 ± 2.9, 6.7 ± 1.7 pps; $P<0.05$). Additionally, all post-fatigue discharge rate measurements in the stroke subjects were lower than control rates ($P<0.05$). We provide evidence that fatigue-related paretic motor unit behavior is blunted, potentially limiting the ability to generate sufficient force during fatigued knee extensor contractions that are important for daily function.

ACKNOWLEDGEMENTS: NIH KL2RR031972; Bernstein Focus Neurotechnology No. 1GQ0810; European Research Council Advanced Grant DEMOVE No. 267888.

Postural responses of gastrocnemius motor units to external loading in standing in people post-stroke

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Maintaining upright stance during external loading involves the activation of motor units (MUs) in the medial gastrocnemius (MG) muscles in an inverted pendulum model of postural control. Previously, we applied a series of five external loads at the hips in healthy subjects by adding weight in 0.45 kg increments every 30 seconds to a maximum of 2.25 kg. The MUs were classified as low-load or high-load threshold, when recruited with the first two loads or the last three loads, respectively. In healthy subjects, low-load threshold MUs demonstrated limited firing rate modulation and reliance on MU recruitment to respond to increasing external loads, whereas high-load threshold MUs increased their firing rate. The purpose of this study was to examine how the motor units in MG would respond to the aforementioned external loading paradigm in people after stroke. We also employed motion capture and force platform technology to quantify the whole body response to the external perturbation. Participants post-stroke had lower motor unit firing rates and more sporadic MU firing than healthy controls and limited firing rate modulation or MU recruitment with increasing loads. Rather than simply overusing the non-paretic side, the kinematics and kinetics revealed a compensatory strategy at the hip and knee when external loading moved the center of pressure towards the limits of stability. These data are among the first to combine kinematics and kinetics with motor unit recordings to demonstrate the effects of distal paresis on postural reactions after stroke.

Discharge behaviour of single motor units after stroke

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The properties of single motor units change after stroke. Firing rate, discharge variability and dynamic range are reduced on the more-affected side. The activity of 24 spontaneously-active and 5 task-driven biceps brachii single motor units were discriminated from surface EMG recordings in 6 patients during sustained elbow flexor voluntary contractions 10% of maximal (task-driven) or in otherwise relaxed muscles (spontaneously-active). The incidence of 4.8 units per subject was lower than previous studies in the lower leg (6.7 units) or wrist (7.7 units), or for controls (11.1 and 7.2 units, respectively). The variable discharge behaviour of task-driven units precluded further analysis. Unlike previous studies, no antagonist single motor units were discriminated. Mean firing rates were lower on the more-affected compared to the less-affected side, 9.5±0.5 and 12.2±0.9 Hz, respectively ($p=0.008$). The coefficient of variation 12.0±0.8% vs 14.0±1.2% ($p>0.05$) and dynamic range 7.5-14.7 vs 8.7 vs 16.0 Hz were also lower on the more-affected side. For all parameters biceps motor-unit behaviour fell between that of wrist and lower-leg motor units. The mean firing rate differed between sites ($p<0.001$) but biceps units were not different to either wrist or lower-leg units (which were different, $p<0.05$). Despite different functional roles, the pattern of post-stroke changes in motor-unit behaviour was consistent. Differences between wrist and biceps motor units presumably reflect the greater reliance of biceps brachii on recruitment- than rate-coding. Decreased motor-unit firing rates and discharge variability on the more-affected side after stroke reflect reduced descending drive from the lesioned cortex, particularly for the upper limb.

Examination of AHP duration changes in motoneurons innervating paretic muscles in stroke survivors

Aneesha K. Suresh, Xiaogang Hu, Randall K. Powers, William Zev Rymer

Rehabilitation Institute of Chicago, Northwestern University, University of Washington

The after hyperpolarization (AHP) of a motoneuron is a primary determinant of motoneuron firing rate. Any increase in its duration or amplitude could alter normal motor unit (MU) firing rate properties in stroke, and potentially impact muscle force generation. The objective of this preliminary study was to examine potential differences in afterhyperpolarization duration of motoneurons innervating paretic and contralateral limb muscles of stroke survivors. A novel surface EMG (sEMG) electrode was used to record from the FDI muscle of two hemiparetic stroke survivors. sEMG data was decomposed to derive single motor unit events, which were utilized to produce interval histograms of the motor unit discharge. Interval Death Rate (IDR) analysis was then used to transform ISI histograms into death rate plots. [1] The prescribed IDR analysis method involves a final transformation of death rate plots into an estimated AHP time course. The present study uses a modified method of interpreting death rate plots to determine AHP duration. AHP durations from this analysis are similar to durations obtained from ISI variability analysis. [2] Results from two subjects indicate that on average, motor units on the paretic side have a longer AHP duration than the contralateral side, potentially contributing to lower firing rates, and to less efficient force production in paretic muscles.

1. Matthews, P.B., *Relationship of firing intervals of human motor units to the trajectory of post-spike AHP and synaptic noise*. J Physiol, 1996.
2. Piotrkiewicz, M., et al., *Age-related change in duration of AHP of human motoneurones*. J Physiol, 2007.

Importance of possible corticoreticulospinal inputs to motoneurons after stroke or training

Damon Hoad and John Rothwell

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TMS can often evoke ipsilateral MEPs in proximal and trunk muscles, but their strength relative to the contralateral projection varies between individuals. Since ipsilateral MEPs occur several ms later than contralateral MEPs and are more complex, it has been suggested that they represent activity in corticoreticulospinal pathways. The presence of ipsilateral projections is increased after stroke and the size correlates with functional measures of trunk function or of shoulder movement on the paretic side. Such inputs could form a substantial fraction of the supraspinal drive to motorneurones and could potentially contribute to any changes in motoneurones properties that are observed.

We also investigated whether intensive (>10,000 hours) training could increase the amplitude of the ipsilateral control in healthy subject. Ten elite canoe polo players who train shoulder and upper body musculature to extremes were examined. The excitability of the ipsilateral responses evoked by TMS on both sides of the body in pectoralis major, trapezius and external oblique muscles, expressed as a ratio with the contralateral response was increased by a factor of 2-3 relative to non-canoeists. The effect was less prominent in a small sample of novice canoe polo players. We suggest that corticoreticulospinal excitability can be changed by training, although this may require many years of practice at least in healthy individuals.

Posters (on display throughout meeting)

Presenters to attend posters on:

Tuesday, 24 July, 1.30 pm – 3.00 pm (odd numbers) or
Wednesday, 25 July, 1.30 pm – 3.00 pm (even numbers)

P01.Short-interval cortical inhibition increases during exposure to psychosocial stress in healthy adults

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Previous studies have documented altered activation of inhibitory networks within the motor cortex among a variety of patient populations with impaired muscle relaxation. These impairments are often exacerbated by psychosocial stress, yet the effects of stress on intracortical inhibition are currently unknown. The purpose of this study was to investigate changes in intracortical inhibition during exposure to an acute psychosocial stressor in healthy adults. Transcranial magnetic stimulation was used to assess short-interval intracortical inhibition (SICI), previously shown to be mediated by $GABA_A$ neurotransmission involving inhibitory interneurons within the primary motor cortex. Participants were instructed to relax their neck and shoulder muscles as background muscle activity and motor evoked potentials were recorded with surface electrodes on the upper trapezius muscle at baseline and during periods of low and high psychosocial stress. The high stress condition comprised difficult mental math performed with time and accuracy constraints in the presence of an authoritative investigator. Perceived anxiety and cardiovascular responses increased significantly between the low and high stress conditions, demonstrating the efficacy of the stress protocol. Intracortical inhibition also increased in the high stress compared to the low stress condition ($p = 0.018$), whereas motor thresholds and background muscle activity remained unchanged across conditions. These findings suggest that healthy adults increase the activation of intracortical inhibitory pathways to help maintain a relaxed muscle during exposure to acute psychosocial stressors. Future research is necessary to determine whether impairments of intracortical inhibition contribute to the inability of some patient populations to relax their muscles under stress.

P02. A moving motor homunculus? Dynamic representations of intrinsic hand muscles in two arm postures

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Background: The human primary motor cortex is somatotopically organized; different body parts are represented in different territories, and single muscles also have their representations. This study examined the assumption that such maps are stable across limb postures. By measuring corticospinal excitability using transcranial magnetic stimulation (TMS) over a grid of sites (5x5 cm) centered on the left primary motor cortex (n=13), we mapped the motor representation of two intrinsic hand muscles (first dorsal interosseus, FDI, and abductor digiti minimi, ADM) as the right relaxed arm was placed in two different postures.

Preliminary results: Overall corticospinal excitability was higher for FDI than for ADM ($p=0.023$). It differed between the two arm postures differently for the two muscles ($p=0.026$), FDI being higher with the hand on the left and ADM on the right, without any change in peripheral muscle output. Further we observed dynamic changes of maps with the peak response spatially shifting depending on posture. Furthermore, corticospinal excitability in the site of best response for one arm posture was higher than the response at the same site with the arm in the other posture (FDI: $p<0.037$; ADM: $p<0.014$).

Conclusions: Changes in arm posture induced changes in the general corticospinal excitability recorded from intrinsic hand muscles. Furthermore, changes in arm posture cause changes in the location of the site of best response in the motor cortex. These findings indicate that the proprioceptive information related to arm posture dynamically modulates the representation of intrinsic hand muscles in the motor cortex.

*equal contribution

P03. Muscle fatigue affects dual task performance, but not more in patients with Multiple Sclerosis

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1) Dept. of Neuroscience, 2) Dept. of Neurology, University Medical Center Groningen, Groningen, The Netherlands

Multiple sclerosis patients suffer from symptoms of fatigue and reduced information processing speed. Several experiments investigated these symptoms; however, studies that investigated the relation between these symptoms are scarce.
MS patients (relapsing remitting; EDSS range 0-3; mean age 40) and controls participated in experiments performed on two occasions separated by a week. Subjects performed a cognitive task concurrently with either a fatiguing or a less-fatiguing motor task. The motor task consisted of a contraction of a hand muscle (FDI, abduction of the index finger). The cognitive task consisted of an auditory choice-reaction-time task (CRT). Each session consisted of: 1) practice of the cognitive task. 2) Assessment of the maximal voluntary force (MVC). 3) Single cognitive task (auditory CRT task). 4) A fatiguing (30% MVC) or less-fatiguing (10% MVC) dual task. 5) Single cognitive task. Task performance was measured as percentage of correct responses, reaction times, and force variability.
Preliminary analysis (9 MS patients; 9 controls) showed that the dual task was more difficult than a single task (number of errors, $p=0.001$, reaction time, $p=0.012$). Performance during the fatiguing dual task was more affected than during the less-fatiguing dual task (number of errors, $p=0.003$, reaction time, $p=0.006$, force variability, $p=0.009$). No interaction effect with MS was found for any condition.
The data showed that muscle fatigue has a detrimental effect on concurrent cognitive task performance. The relatively large variability in response times during the dual task performance in MS patients masks potential effects of MS in this relatively small sample size.

P04. Fatigue-sensitive group III/IV muscle afferents reduce voluntary activation and force in the hand

David Kennedy [1,2], Chris McNeil [1], Simon Gandevia [1,2], Janet Taylor [1,2]

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INTRODUCTION: The firing of group III/IV muscle afferents in large muscles of the arm during fatigue can decrease voluntary activation of the fatigued muscles (1). However, it is unknown if fatigue-sensitive group III/IV muscle afferents in a small hand muscle have a similar effect on voluntary activation. AIM: The purpose of the study was to determine if the firing of fatigue-sensitive afferents reduces voluntary activation of the adductor pollicis muscle. METHODS: Paired electrical stimuli (doublet) were delivered to the median nerve at the elbow to evoke increments in force (superimposed twitch) from the adductor pollicis muscle of the right hand and provide a measure of voluntary activation. Inflation of a blood pressure cuff about the forearm was used to block blood flow to the fatigued muscle and maintain firing of group III/IV muscle afferents. On two days, subjects (n=10) performed a 2-min maximal voluntary contraction (MVC) of the adductor pollicis with or without a subsequent 2-min period of ischaemia. In the 2 min after the sustained MVC, subjects performed 5 brief MVCs. Doublets were delivered during each brief MVC. A resting doublet was delivered following each brief contraction. Voluntary activation was quantified using the equation: voluntary activation (%) = [1-(superimposed twitch/resting twitch)] X 100. Data are reported as the mean of the 5 brief MVCs. RESULTS: Following a 2-min fatiguing adductor pollicis contraction, maximal voluntary force was reduced with ongoing ischaemia compared to without (39.2±10.3% versus 64.7±11.6% of initial maximal force; $P=0.001$). Voluntary activation was also significantly less with ongoing ischaemia than without (55.5±17.8% versus 76.5±14.6%; $P=0.02$). CONCLUSION: Maintained ischaemia following a fatiguing contraction of a small hand muscle prevents recovery of maximal force production. The findings suggest that voluntary activation is impaired because of continued discharge of fatigue-sensitive group III/IV muscle afferents and contributes to the reduction in force.

REFERENCES: 1. Gandevia et al. J Physiol., 1996 490:529-36.

P05. Corticospinal control from ventral premotor cortex in humans.

Giboin L, Sangari S, Marchand-Pauvert V.

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It is known that the ventral premotor cortex contains "mirror" neurons implicated in the learning of observed grasping actions. In humans, during the observation of such movements, the spinal cord excitability is modulated as to prevent the limb from replicating seen actions (Baldissera et al., 2001). It is also known that in the monkey, the ventral premotor cortex projects onto C3-C4 cervical segments which correspond to the location of short propriospinal neurons (He et al., 1993).

The present study aims to demonstrate that human C3-C4 propriospinal neurons are used by the ventral premotor cortex to control the spinal cord excitability. Thereby, we studied the effect of ventral premotor cortex transcranial magnetic stimulation on C3-C4 propriospinal inhibition evoked in wrist extensor motoneurons by median nerve stimulation. The premotor stimulation facilitates the propriospinal inhibition. The existence of a direct pathway from the ventral premotor cortex to propriospinal neurons was also investigated using a paired continuous theta burst over M1. The premotor-induced reinforcement of propriospinal inhibition was maintained after virtual lesion of M1. This suggests a direct corticospinal control from the ventral premotor cortex onto propriospinal neurons.

This study shows that C3-C4 propriospinal neurons can serve as a relay for ventral premotor cortex signal transmission onto motoneurons in human.

P06. Changes in the corticospinal control of human wrist extensor motor units during an attention-demanding visuomotor task

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A specific tuning of the peripheral and central synaptic inputs to motoneurones is liable to contribute to the fine adjustment of motoneuron activity to the behavioural constraints involved in the motor task performance. We previously showed that proprioceptive inputs contributed more greatly to controlling the motoneuron firing pattern during an attention-demanding task. The aim of the present study was to examine the cortical motor control. For this purpose, the responsiveness of single motor units (SMUs) to transcranial magnetic stimulation (TMS) was tested in a wrist extensor muscle while the subjects were maintaining a steady wrist extension force using visual force feedback set either at low or high gain, which requires different levels of attentiveness to perform the task.

The effects of TMS on tonic SMU discharge were displayed in PSTH and trial by trial rasters and analyzed in terms of inter-spike interval (ISI) duration, which makes it possible to assess the inhibitory effects of the stimulation-elicited volley on the spike trains of SMU. We indeed observed that the duration of the ISI in which the corticospinal afferent volley occurred was significantly increased in lack of excitatory response, which highlights that TMS activates inhibitory mechanisms.

Results show a reinforcement of both excitatory and inhibitory effects of TMS on motoneurons during the more demanding task, which indicates an increased overall corticospinal excitability. An attention-related increase in excitability of inhibitory intracortical networks might explain the enhanced inhibition whereas the increased excitatory effects might be induced by the increase in proprioceptive inputs to motoneurons.

P07. The direction of the associated contralateral force during unilateral contractions of hand muscles in stroke survivors

Inge Zijdewind[1], Nina Suresh[2] and Zev Rymer[2]

[1]Dep. Neuroscience, UMCG, Groningen, Netherlands; [2]Rehabilitation Institute Chicago, USA.

During unilateral hand muscle contractions, in addition to activation of target muscles, contralateral muscles also become active. In stroke survivors, this 'associated contralateral activation' is often more evident. Furthermore, after a unilateral stroke, the precision to produce forces in various directions in the affected hand is largely reduced. It was our aim to study the direction of the target force and contralateral associated force in the affected and less-affected side of stroke survivors.

Subjects were seated with both hands immobilized in the set up. Forces in horizontal and vertical planes of both sides were measured while subjects tried to move their index finger in four directions (up, down, left and right). The target finger changed between affected and less-affected side, and the position of the target or contralateral hand was changed from pronated to neutral position (halfway between pronation and supination) or vise versa. In the second experiment, we changed the position of the other hand.

Preliminary analysis showed that precision of the target contraction differed between sides (affected versus less-affected side) and movement direction (interaction: $P<0.001$); precision was better for the less-affected side during abduction, adduction and extension. The associated-contralateral force showed large variability between subjects. Repeated-measures ANOVA showed that the direction of the associated movements differed with movement, side and hand position (interaction: $P<0.005$).

The observation that associated-contralateral index finger movements are often observed and that the direction of associated force differs between affected and non-affected sides promises an interesting tool to study motor control in stroke survivors.

P08. Effects of Deep Brain Stimulation on M-wave and motor unit cross-correlation in Parkinson's disease

Aldewereld Z.T., Huang H., Montgomery Jr. E.B.

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Evidence whether Deep Brain Stimulation (DBS) increases or decreases synchronization of neuronal activity is contradictory. As DBS improves Parkinson's disease (PD), the mechanisms of DBS have been thought to be the converse of the presumed pathophysiological mechanisms, which many hold to be increased neuronal synchronization. Consequently, the effects of DBS on motor unit (MU) synchronization were studied. Four subjects with PD and DBS and four normal controls performed an isometric wrist flexion force at 30% of maximum voluntary force during which a suprathreshold electrical stimulation was applied to the ulnar nerve behind the medical epicondyle and in the axilla. Stimulation-induced surface and intra-muscular EMG were recorded from the flexor carpi ulnaris. The different latencies of the initial EMG changes with stimulation at different points allowed amplitude measurement of the M-wave. The operational hypothesis is that smaller M-waves suggest that more axons were in their refractory periods at the time of nerve stimulation and hence, were synchronized within the duration of the axonal refractory period. Compared to normal subjects, PD subjects without DBS tended to have more low-amplitude M-waves suggesting possible increased synchronization of axonal action potentials. With DBS, the M-wave of PD subjects increased the number of low-amplitudes suggesting increased synchronization with DBS. Cross-correlograms were constructed between simultaneously recorded MUs. Of the 103 pairs studied, 64 had a peak in the cross-correlograms. Of those, 36 (56%) pairs demonstrated no difference with DBS, 10 (16%); showed decreased correlation with DBS; 3 (5%), increased with DBS; and 15 (23%) mixed.

P09. Reversal of size principle in Parkinson's Disease and normalization with Deep Brain Stimulation

Huang H.[1], Watts R.L.[1], Guthrie B.L.[2], Walker H.C.[1], and Montgomery Jr. E.B.[1]

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Background: Orderly recruitment of motor units, progressing from small to large as force demands increase, is critical to normal motor control. The effects of Parkinson's disease (PD) and subthalamic nucleus Deep Brain Stimulation on motor unit recruitment order were studied.

Methods: Normal subjects and Parkinson subjects with and without stimulation performed isometric progressive wrist flexion forces. Intramuscular electrodes identified motor units and estimated size. Slopes of linear regressions of force at motor unit onset and motor unit size were calculated. PD subjects were tested following an overnight fast from medications. Stimulated subjects were tested at 0 pulses per second and clinically effective high frequency stimulation. Non-stimulated subjects were tested again one hour after administration of their usual first morning doses of anti-Parkinson medications.

Results: Five subjects were in each PD group and five were normal. In the PD stimulated group the slopes under high frequency were statistically different from the 0 pulses per second condition (two-tailed paired t-test, $p < 0.05$). Within the medication group a trend did emerge ($p < 0.15$) between their "off" or "on" medication conditions. No normal or high frequency stimulation subject had negative slopes, while 6 of 10 Parkinson subjects in the untreated condition did, and the incidence of negative slopes were significantly different (Fischer Exact test $p < 0.03$).

Conclusions: Subjects with untreated PD have a higher incidence of reversed motor unit recruitment, which normalizes with high frequency stimulation, suggesting a role for the basal ganglia in determining motor unit recruitment order.

P10. Is mediodorsal thalamic nucleus a higher-order or first-order thalamic nucleus?

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Objectives: Thalamus is concerned with the transmission of information to cortex. Thalamic nuclei are classified as first-order and higher-order. The first-order relay messages to the cortex from subcortical structures whereas the higher-order relay messages from cells in layer 5 of one cortical area to another cortical area. The aim of this study is to identify the connections of mediodorsal thalamic nucleus (MDn) and to define whether it is a higher or first-order thalamic nucleus.

Materials & Methods: Retrograde tracer (fluorescent Fluoro-Gold) is injected to the mediodorsal nucleus of Wistar albino rats. After 7-10 survival days animals were decapitated with transcardiac perfusion. 40 μm thick serial sections were taken and the connections of the MDn were identify using a fluorescent microscope.

Results: Cortical connections of the MDn were to layer V and/or IV of the cingulate, somatosensory, prelimbic, midline and insular regions of the prefrontal cortex (Pfc), including the anterior limbic, and most ventral part of the precentral agranular Pfc, as well as the agranular insular cortex.

Conclusion: Mediodorsal nucleus is regarded as higher-order thalamic nucleus. However, MDn receives driver afferents both from subcortical structures and conveys them to layer 3 and 4 of cortex and also receives driver afferents from layer 5 of cortex. Therefore, MDn can be classified as a mixed nucleus having both the properties of first-order and higher-order. The MDn has been shown to play a role in the mechanism of epilepsy, schizophrenia and Parkinson's disease. The detailed knowledge of MDn will aid to reveal the former mechanisms.

P11. Spinal pathways mediating phrenic activation during High Frequency Spinal Cord Stimulation (HF-SCS)

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Objective: HF-SCS is a novel and more physiologic method of inspiratory muscle activation which involves activation of spinal cord pathways. The specific pathways by which inspiratory motoneurons are activated by this method however are unknown. The aim of the present study was to evaluate the potential role of upper cervical inspiratory neurons on the observed responses.
Methods: In 7 anesthetized and spinalized (C1 level) dogs, HF-SCS was applied at the T2 level. Airway pressure generation (Paw) was monitored as an index of the degree of inspiratory muscle activation before and following sequential spinal sections at the C4 level (to eliminate upper cervical propriospinal neurons) and subsequently at the C8 level.
Results: During HF-SCS (2mA, 300Hz, 0.2ms), Paw at FRC was 64±4 cmH_2O. Sequential bilateral dorsal columns (DC) section, lateral funiculi (LF) section and complete section (CS) at C4 resulted in no significant changes in Paw (66±5, 66±3 and 63±4 cmH_2O respectively, NS). While DC section at C8 resulted in no changes in Paw (60±6 cmH_2O), LF section resulted in significant reduction in Paw to 16±3 cmH_2O ($p<0.05$). Subsequent CS resulted in no further reduction in Paw (13±2 cmH_2O, NS).
Conclusion: During upper thoracic HF-SCS, activation of the phrenic motoneuron pools does not involve upper cervical propriospinal inputs. The phrenic motoneuron pools are activated via spinal pathways located bilaterally in the lateral funiculus.

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P12. Effects of aging on motor units in the genioglossus muscle of humans

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The effects of aging on motor units in the genioglossus are unknown. We recently reported that motor unit potentials (MUPs) in genioglossus do not demonstrate remodeling up to the age of 50 years (1). To assess possible changes associated with aging we compared quantitative parameters related to motor unit potential morphology derived from EMG signals in a sample of older (n=12; >50 years; *new data*) versus younger (n=27; <50 years; *historic data set: see 1*) individuals. Diagnostic sleep studies (Apnoea Hypopnoea Index; AHI) to confirm the presence or absence of obstructive sleep apnoea (OSA) were performed in all subjects. Quantitative EMG was obtained using DQEMG to extract concentric needle-detected MUPs. Muscle activity was recorded with concentric needles with a recording area of $0.07mm^2$. The needle was positioned at >10 sites/subject, after ultrasound measurements. 2040 MUPs from 39 subjects (mean AHI 28.9 events/h) were decomposed from genioglossus muscles. MUPs in the older individuals were 26% longer in duration (13.6ms *versus* 10.8ms; $R^2=0.4$; $p=0.05$) but had similar amplitudes (314.8mV *versus* 320.8mV) compared to younger controls. The complexity of the MUP waveforms {as assessed by the relative irregularity coefficient (normalized length of MUP)} was decreased by 19% in the older individuals (17.2 *versus* 14.0; $p<0.05$). These results confirm that remodeling of genioglossus is present in older individuals above 50 years. The role of these findings in OSA pathogenesis and aging related upper airway changes remains unresolved.

1. Saboisky et al. 2012 *AJRCCM* 185: 322-329.

P13. Human trapezius motor units exhibit tonic and phasic activity associated with respiration

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Primary respiratory muscles such as the diaphragm are known to exhibit characteristic patterns of tonic and phasic motor unit (MU) activity that correspond to distinct phases of the respiratory cycle. Indirect evidence suggests that the trapezius muscle may function as an accessory muscle to respiration, however, a direct association between trapezius MU activity and respiration has not yet been established. The purpose of this study was to characterize respiratory modulation of MU discharge behaviors in the human trapezius muscle. Chest wall movement was recorded with a strain gauge and intramuscular single MU recordings were collected from the dominant upper trapezius muscle while subjects performed a postural task at a computer workstation. Correlations between the respiratory signal and instantaneous discharge profiles of discriminated MUs were determined. MUs were classified as phasic when they were recruited and derecruited intermittently, with peak discharge rates corresponding to either the inspiratory (11.8%) or expiratory (17.7%) phases of the respiratory cycle. MUs were classified as tonic when they fired continuously through both phases of the respiratory cycle, with peak discharge rates corresponding to either inspiration (0%) or expiration (17.7%). Motor units exhibiting both tonic and phasic respiratory discharge behaviors were classified as mixed (35.3%), and motor units displaying discharge behaviors that were not significantly associated with chest wall movement were classified as unmodulated (17.7%). These findings indicate that that trapezius motor unit activity is modulated by and adaptable to respiratory inputs, suggesting that this muscle plays an accessory role in respiration, particularly during expiration.

P14. Scratch reflex generation beyond the lumbar enlargement in spinal cord of adult turtle

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Scratch reflex is a motor response to remove an irritant from body surface. The central pattern generator (CPG) for scratching is located in spinal cord. Pocket scratch reflex is one of three scratch reflex forms generated by spinal cord of turtle. Afferent inputs for this scratch reflex enter spinal cord close to lumbar segments (D6-D8) and the motor response recruits motoneurons in lumbar part (D8-S2) of spinal cord. Previous studies showed that pocket scratch reflex can be evoked within a few (D8-D10) or a single (D7 or D8) segment of spinal cord of turtle. It was suggested that key elements of CPG of all scratch forms reside within the lumbar spinal cord segments (D7-D10). These studies identified the minimal network for scratch generation. However, how functional network activity is distributed in the intact spinal cord is not known.
In this study we recorded neurons from thoracic (D4) segment of spinal cord from adult turtle during pocket scratch. Two-thirds of neurons receive synaptic inputs during pocket scratch reflex. About 40% of the cells were phasically active during pocket scratch reflex, mostly in phase with HF nerve activity. Substantial increase in conductance suggests intense synaptic inputs during scratch reflex.
These results show that the real distribution of functional neural network is wider than was thought before.

P15. Central contribution to "extra torque" during neuromuscular electrical stimulation

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Introduction: Neuromuscular electrical stimulation (NMES) generates contractions through peripheral and central mechanisms. The central contribution can be augmented by 100Hz bursts of NMES which can generate "extra" torque. We have shown that extra torque was abolished during a nerve block, providing strong evidence for a central origin.[1] In contrast, Frigon et al. (2011) used a slightly different protocol and showed that the additional torque generated by bursts of 100Hz NMES was not abolished during a nerve block, providing strong evidence that it was not of central origin.[2]
Aim: Compare torque generated using Frigon's protocol[2] (#1, below) and our own[1] (#2&3).
Methods: Plantarflexion torque was measured using a Biodex dynamometer (hip and ankle at ~90°; n=2 participants). Protocol 1) Knee extended (170°-180°) NMES over the gastrocnemius. 2) Knee flexed (90°), NMES over gastrocnemius and soleus. Protocol 3) Knee as above (#2), NMES over the tibial nerve. Three trains of NMES (20–100–20Hz for 3–2–3s, respectively) were delivered 60s apart. Torque was calculated at Time1 (2–3s into the train) and Time2 (7–8s into the train) and was normalized to maximal voluntary isometric torque.
Results: Torque increased 29, 61 and 95% from Time1 to Time2, for protocols 1, 2 and 3, respectively.
Conclusion: The protocol used by Frigon et al. generated less extra torque than protocols used in our lab. To generate extra torque from a central origin in the plantarflexors, the stimulation may need to be applied to activate soleus and not only gastrocnemius.

1. Bergquist et al. (2011) Eur J Appl Physiol. 111(10):2409-26.
2. Frigon et al. (2011) J Neurosci. 13;31(15):5579-88.

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P16. The spatial distribution of recruited motor units differs during electrical stimulation over a muscle versus a nerve

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Introduction: Neuromuscular electrical stimulation is used to generate contractions for rehabilitation. Presently, we compared the spatial distribution of motor units recruited in tibialis anterior (TA) by the depolarization of motor axons when stimulation was delivered over the TA muscle belly versus the common peroneal nerve trunk.
Methods: Muscle activity was recorded using fine wires inserted into superficial and deep portions of TA. M-wave recruitment curves were constructed from responses to single pulses delivered at each stimulation site.
Results: During stimulation over the muscle belly, current required to reach M-wave threshold was not different between recording sites; however, more current was required to reach both the maximum M-wave (M_{max}) and 50% of M_{max} at the deep than superficial recording site. During stimulation over the nerve trunk, there were no significant differences in the current required to reach threshold, 50%M_{max} or M_{max} between recording sites. M_{max} recorded at the superficial site did not differ between stimulation sites; however, M_{max} was smaller with stimulation over the muscle belly than over the nerve trunk at the deep recording site.
Conclusions: Stimulation over the muscle belly recruited motor units from superficial to deep with increasing stimulus amplitude. Stimulation over the nerve trunk recruited superficial and deep motor units equally, regardless of stimulus amplitude. The deep portion of TA was not activated in some participants with stimulation over the muscle belly, even in this relatively small muscle. These results provide further support for the idea that *where* stimulation is applied markedly affects *how* contractions are produced.

P17. Comparing quadriceps contractions between nerve trunk and muscle belly stimulation

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Introduction: Neuromuscular electrical stimulation can be delivered over a nerve trunk or muscle belly and both can generate contractions through *peripheral* (by depolarising motor axons) and *central* (by depolarising sensory axons) pathways. Generating contractions through peripheral pathways is associated with a non-physiological motor unit recruitment order which may limit the efficacy of stimulation for rehabilitation. In contrast, recruitment through *central* pathways follows Henneman's size principle.

Aim: Compare recruitment through *peripheral* and *central* pathways for contractions of similar amplitude evoked by stimulation applied over the femoral nerve versus the quadriceps muscle.

Methods: Stimulation was delivered to evoke 10 and 20% of a maximum voluntary contraction 2-3s into the stimulation ($Time_1$). Two patterns of stimulation were delivered; 1) Constant-frequency: 15Hz for 8s and 2) Step-frequency: 15-100-15Hz and/or 25-100-25Hz for 3-2-3s, respectively. Torque and electromyographic activity (M-waves: *peripheral* pathway; H-reflexes: *central* pathway) recorded from vastus-lateralis were quantified at the beginning ($Time_1$) and end ($Time_2$; 6-7s into the stimulation) of each train.

Results: Torque generated by stimulation over the nerve and muscle did not differ in mean amplitude for either contraction amplitude. In contrast, M-waves were ~7-10 times smaller and H-reflexes ~8-9 times larger during stimulation over the nerve compared to over the muscle.

Conclusion: Stimulation over the muscle produced contractions primarily through *peripheral* pathways. Stimulation over the nerve produced contractions with greater recruitment through *central* pathways, which may help reduce muscle atrophy and fatigue for NMES rehabilitation.

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P18. A comparison of two electrical stimulation protocols for increasing cortical excitability for a hand muscle

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Introduction: Neuromuscular electrical stimulation (NMES) generates a large sensory volley that increases the excitability of corticospinal (CS) pathways. Overtime, NMES strengthens these pathways, leading to enduring improvements in motor function. These effects have been studied using many NMES protocols, although the influence of different NMES protocols on increasing CS excitability is not well-defined. The purpose of this study was to compare changes in CS excitability following a single session of either functional electrical stimulation (FES) or somatosensory stimulation (SS). We hypothesized that because FES generates a larger sensory volley, FES would increase CS excitability more than the SS.

Methods: Both NMES protocols were delivered over the median nerve at the wrist. FES was delivered at 100Hz (20s on–20s off) for 40min to evoke an M-wave that was 15% of maximal. SS was delivered at 10Hz (0.5s on–0.5s off) at motor threshold for 2h. CS excitability was quantified as the amplitude of 10 motor evoked potentials (MEPs) evoked using transcranial magnetic stimulation delivered immediately before and after each NMES session.

Results: MEPs recorded from abductor pollicis brevis increased significantly following both FES (66 ± 7% increase) and SS (49 ± 6% increase), but the magnitude of these increases was not significantly different.

Conclusion: These results suggest that that 40 min of FES can increase CS excitability, and provide rehabilitative benefits, to the same extent as 2 h of SS. We are currently conducting experiments to quantify the time-course of changes in CS excitability induced by FES and SS.

P19. Influence of tendon vibration on sex-related differences in motor unit activity and force steadiness

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The short (SBB) and long (LBB) head of the biceps brachii are often studied with the assumption that muscle activity between the two heads is homogenous. Yet, surface and intramuscular electromyography demonstrate compartmentalization but the cause is unknown. The purpose of this study is to examine Ia afferent contribution to compartmentalized activation of the biceps brachii in males and females. Sixteen young, healthy males (n=8;21 years) and females (n=8;22 years) participated. Subjects performed 4 ramp isometric tracking tasks at 15% MVC. Tendon vibration (100Hz) was applied for 5 seconds in the middle of the 35 second contraction to increase activation of Ia afferents. Motor unit activity was recorded with intramuscular fine wires in the SBB and LBB. Data were analyzed pre and post vibration in Spike 2. Males had a higher discharge rate than females ($p<0.001$); however, the decrease in DR with vibration was similar (-12.9%Δ). The LBB discharge rate was higher than the SBB pre vibration ($p=0.04$) but DR following vibration did not differ between heads. Males and females were 43% less steady after vibration ($p<0.001$). Vibration of the biceps tendon resulted in preferential depression of motor unit DR in the LBB compared to the SBB. This provides evidence that common Ia input modulates firing rates independently between the two heads contributing to compartmentalization. The absence of sex differences due to vibration indicates spinal connections from Ia afferents are similar between males and females. The functional consequence of these alterations in motor unit DR is reduced force steadiness.

P20. Ia afferent feedback generates low-frequency coherence between motor neuron spike trains

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Coherence analysis between motor neuron spike trains in voluntary contractions has been used to characterize the correlation between the discharge times of different motor neurons. For example, low-frequency coherence (<2 Hz) has been related to the common drive while coherence at higher frequencies (>15 Hz) has been associated to motor unit short term synchronization.

A novel computational model of neural activity including Ia and Ib afferent neurons of an antagonist pair of intrinsic hand muscles during voluntary dynamic contractions was used to simulate realistic patterns of motor neuron spike trains. The simulated agonist contraction level was 10% MVC. Furthermore, motor neuron spike trains were obtained experimentally from the Abductor Digiti Minimi from 120 s voluntary contractions at a contraction level of 10% MVC in four subjects. For both datasets, the coherence between composite spike trains consisting of pairs of motor neurons were computed in 10 s windows.

Simulated as well as experimental data consistently showed a peak in the coherence at 6-8 Hz. When the simulations were repeated without monosynaptic Ia afferent input to the motor neuron population this peak was absent.

The results shows that coherence between pairs of motor neuron spike trains of a muscle at 6-8 Hz is related to Ia afferent feedback, and that the magnitude of this peak may be used as a measure of the synaptic strength of the Ia afferent feedback.

P21. Age-related effect of vision on the modulation of Ia presynaptic inhibition during upright stance

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This study investigated the influence of vision on the modulation of soleus Ia afferent input at a presynaptic level in young and elderly adults during quiet upright stance in rigid and foam surface. Changes in Ia presynaptic inhibition was assessed by a single motor unit method based on the stimulation of homonymous Ia afferents of the soleus that evokes a peak in the motor unit discharge probability measured with a peri-stimulus time histogram. Changes in homonymous Ia afferent input onto motor neurones was investigated during the first 0.5 ms of the peak of discharge probability. Twenty four motor units of soleus muscle (12 motor units for each age group) are successfully conditioned by electrical stimulation applied to the tibial nerve for only one surface condition (rigid or foam) or both. Motor unit discharge probability decreased when vision was absent when standing on the rigid or foam surface. This particular modulation within the first 0.5 ms of the peak was observed for the 18 motor units recorded during upright stance on rigid surface ($P < 0.001$) and the 13 motor units recorded when standing on foam surface ($P = 0.001$). Moreover, the decrease in discharge probability in the absence of vision was greater for elderly than for young adults ($P = 0.026$). The main new finding of the study was an increase in Ia presynaptic inhibition during upright stance when vision was suppressed, with a greater modulation for elderly adults.

P22. Properties of hypoglossal motoneurons in mice with impaired inhibitory synaptic transmission

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Inhibitory synaptic input to hypoglossal motoneurons (HMs) plays a crucial role in circuits that generate the respiratory rhythm. We have shown previously that inhibitory drive to HMs in mice with naturally occurring mutations in the glycine receptor (GlyR) is markedly reduced (Graham et al., 2006, J Neurosci 26: 4880). Here we ask whether the intrinsic properties of HMs in spasmodic, spastic and oscillator mice are altered under conditions of reduced inhibitory drive. Mice were anaesthetized (Ketamine 100 mg/kg i.p.) and transverse brainstem slices (300 μm thick) containing the hypoglossal nucleus were prepared. Whole-cell recordings were obtained from IR-DIC visualized HMs (23^{o}C, KCH_3SO_4 internal) in the mutant and wild type (C57/Bl6) mice. The intrinsic and AP properties of spasmodic and oscillator HMs (n = 48 and 57, respectively) were similar to wild type (n = 64). In contrast, spastic HMs (n = 61) exhibited lower input resistances, more depolarized resting membrane potentials and smaller AP amplitudes. Subtle differences were also observed in AP discharge elicited in GlyR mutant HMs during injection of both depolarizing and hyperpolarizing square step currents (50 pA increments, 1 s duration), and triangular ramp currents (1 nA peak, 7 s duration). Together, these data suggest the reduced glycinergic drive to HMs in spasmodic, spastic and oscillator mice is accompanied by subtle changes in intrinsic and AP discharge properties that when combined, reduce excitability in mutant HMs. We propose these changes contribute to the stabilization of this important respiratory circuit in the face of reduced inhibitory drive.

P23. Persistent inward currents in the human tibialis anterior muscle investigated from populations of motor units

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During a ramp contraction, the difference between the discharge rates of a low-threshold motor unit (reporter) in the time instants corresponding to the recruitment and derecruitment of a high-threshold motor unit (test) is believed to be related with the activation of persistent inward currents (PICs) of the motor neurons [1]. Current techniques for recording active motor units (MUs) *in vivo* are limited to a small number of identified units, therefore the evaluation of PICs on relatively large populations of MUs have been performed only on simulation studies [2]. In this study, we estimate the amount of PICs developed during ramp contractions of the TA muscle using two 16 channel thin-film iEMG electrodes [3]. One subject participated in the experiment. The subject performed three ramps contractions at 7.5 and 10 % MVC with a slope of 2.5 MVC/s. iEMG data were decomposed using a multichannel decomposition algorithm [4]. Recruitment and derecruitment thresholds relative to force (ΔForce) and discharge rate (ΔF) of the first recruited MU were calculated. A total of 65 MUs were identified in all contractions. The average ΔForce was 0.48 ± 0.92 % MVC and 1.07 ± 0.51 % MVC for the 7.5 and 10 % contractions, respectively. The average ΔF was 5.7 ± 1.3 and 2.3 ± 0.4 pps for the two cases. In each contraction there was a significant relation between the two variables ($R^2 = 0.83$, $P<0.05$ for 7.5 % and $R^2 = 0.37$, $P<0.05$ for 10 %). In conclusion, according to previous reports [5], the investigation of PICs in human MUs needs further evaluation.

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P24. Segmental arrangement of the motor neurons supplying the mouse forelimb

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Introduction: Our current focus is exploring strategies to deliver therapeutic genes to specific populations of motor neurons. This can be achieved via targeted intramuscular injections of viral vectors and the ensuing retrograde transport of the genes of interest into corresponding motor neurons. We have previously described the organisation of the motor columns supplying the rat forelimb. However, with the increasing prevalence of mouse models of central nervous system disease and injury, there is a rising need to define the organisation of the motor neurons supplying the mouse forelimb.

Aim: To define the precise relationship between muscles of the mouse forelimb and the motor neurons that innervate them.

Method: The forelimb motor end plates (MEP) were mapped using acetylcholinesterase histochemistry. This map was then used as a guide to perform intramuscular injections of retrograde tracer(s) along the entire MEP region of targeted muscles. 1-2 weeks later, the animals were perfused and the spinal cords were dissected, sectioned and analysed under epifluorescence. For each muscle, labelled motor neurons were plotted on a spinal cord schematic representation and stacked thereafter to create a motor neuron map.

Results: This study revealed that motor neurons supplying the mouse forelimb are arranged in columns spanning multiple spinal cord segments. These motor columns substantially overlap in all axes.

Conclusion: Both the MEP and the motor column maps constitute a valuable guide for the selection of appropriate muscle(s) for the delivery of therapeutic genes into specific motor neurons within the cervical spinal cord.

P25. Intrinsic properties of human ventral horn neurons during early foetal development

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Electrophysiological investigations on the development of rodent ventral horn neurons (VHNs) have documented dramatic changes in the intrinsic properties and excitability of VHNs during late foetal and early postnatal development. Similar data are not available for VHNs in the human. Here, we examine the intrinsic properties of human VHNs during early foetal development. Transverse slices (400 μm thick) were obtained from spinal cords of terminated human foetuses (10-18 weeks gestation; WG). Whole cell patch clamp recordings were made from visualized VHNs (32^{o}C, KCH_3SO_4 internal). We recorded from 61 VHNs at three gestational ages (10-12, 13-15, ≥ 16WG). Resting membrane potential became more hyperpolarized (-54.7 ± 2.6 vs. -64.6 ± 2.7 mV) and input resistance decreased (875 ± 120 vs. 331 ± 88 MOhm) over the time points examined. Action potentials (APs) were elicited, during current injection, in 14/21 VHNs at 10WG and 22/23 at ≥16WG. AP afterhyperpolarization amplitude also changed significantly during the same period (-8.3 ± 2.2 vs -18.8 ± 2.0 mV). Step current injection (50 pA steps, 1s duration) resulted in tonic AP discharge in most VHNs (12/19 at 10WG and 15/23 at ≥16WG). These data suggest human VHNs, some of which will be motoneurons, are electrically excitable by 10 WG and are consistent with reports of muscle activity in the very young foetus.

P26. Spontaneous recovery vs exercise training: influences on select spinal neuron properties after spinal cord injury

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After spinal cord injury (SCI) a degree of spontaneous functional recovery occurs and exercise training after injury is known to promote this recovery. The mechanisms mediating improvements, however, are unknown. Here we studied the effect of exercise training after SCI on the intrinsic properties of spinal neurons in the vicinity of a lesion. Male mice (C57Bl/6; ~P42) received a spinal hemisection (T8-10) and following one week of recovery, were divided into trained (treadmill exercise for 3 or 6 weeks) and untrained (no exercise) groups. After 3 or 6 weeks, mice were sacrificed and horizontal spinal cord slices (T5-T12, 250 μm thick) were prepared for whole cell patch clamp recording. Intrinsic neural properties such as resting membrane potential, input resistance, rheobase current, action potential (AP) threshold and AHP amplitude were similar in trained and untrained groups at both 3 (trained=20 neurons; untrained=32) and 6 weeks (trained=50; untrained=45). Neurons were grouped into four AP discharge categories according to their response to current injection. The proportions of tonic firing, initial bursting, single spiking and delayed firing neurons were similar between trained and untrained groups at both 3 and 6 weeks, but differed significantly across time points (ie. 3 vs 6 weeks). These data show treadmill exercise has no effect on the intrinsic properties of spinal neurons after SCI, however, the discharge properties of spinal neurons appear to change during recovery.

P27. Reduced sodium conductances of median motor axons in chronic stroke survivors

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The study of axon properties in-vivo may provide important insights into motoneuron adaptation following central nervous system lesions. We compared motor axon properties of the paretic and non-paretic limbs in 18 persons (61±9 y) who had unilateral hemiparesis due to chronic stroke (12±7 y). The median nerve was stimulated at the wrist while monitoring target compound muscle action potentials of the abductor pollicis brevis, using threshold tracking techniques. The TROND protocol was applied to record stimulus-response, strength duration time constant, threshold electrotonus, current-threshold relation, and the recovery cycle.

Compared to the non-paretic side, the strength duration time constant in paretic muscle was significantly reduced, but rheobase and maximal compound muscle action potentials were unchanged. The paretic side threshold electrotonus exhibited distinctive changes with less threshold decline during depolarization and, similar to a previous study (Jankelowitz et al., 2007), greater threshold increase during hyperpolarization. In the recovery cycle, refractoriness and relative refractory period of paretic muscles were increased, and superexcitability and subexcitability were decreased. Most of these non-paretic axon excitability data were similar to published values of healthy controls of similar age.

These paretic limb axon properties could be reproduced in a mathematical model (MEMFIT, H. Bostock, 2006) by reducing the percent persistent sodium channels, nodal sodium permeability, and hyperpolarization-activated conductance by 19%, 12% and 33%, respectively. The novel finding of reductions in sodium conductance may reflect compensatory down-regulation of sodium channel expression resulting from heightened motoneuron reflex activity following stroke.

P28. Matching motoneuron and muscle fiber phenotype: the chicken or the egg?

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Alpha motoneurons can be classified as fast or slow based on their electrophysiological properties. Motoneurons and their muscle unit phenotypes match. However, it is unclear if motor unit phenotype is determined by the motoneuron or the muscle fiber. Developmental studies on avian and rodent embryos suggest that muscle fiber phenotype is innervation-independent. On the other hand, cross-reinnervation studies indicate that motoneurons direct muscle fiber phenotype. In order to investigate this further, mouse immature motoneurons were co-cultured with either fast or slow myotube populations. We hypothesized that electrophysiological properties of recorded motoneurons would match the muscle fiber types with which they were cultured.

Embryonic stem cell-derived motoneurons were plated on either slow myotubes obtained from the external adductor muscle or fast myotubes harvested from the pectoral muscle of E10 chick embryos. After one month, muscle contractions were chemically blocked to study motoneuron properties using patch electrodes.

The two sets of motoneurons had similar whole cell capacitances, input resistances, and rheobases. Medium after-hyperpolarization amplitude, half-width, and half-decay time were also similar in both conditions. Firing frequencies were nearly identical with first interval maximal frequencies of 150 Hz and steady state firing frequencies of 30 Hz.

We conclude that one month old embryonic stem cell derived motoneurons demonstrate similar electrophysiological properties whether grown on fast or slow myotubes. Such findings indicate that either motoneuron phenotype is independent of the phenotype of the innervated muscle fibers or that ES cell derived motoneurons are unable to change their phenotype in response to different muscles types.

P29. Distinct timecourse of forelimb and hindlimb neuromuscular junction development in the Western Grey Kangaroo (*Macropus fuliginosus*)

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The principles of synapse formation have been largely unraveled by investigations at the somatic neuromuscular junction (NMJ). Such experiments are commonly performed on placental mammals (e.g. mouse), where all four limbs develop on a similar timescale. By contrast, some Australian marsupials have very segmented limb development, where forelimbs are precociously developed to assist the journey to the pouch, while hindlimbs are comparatively unformed. We explored the implications of this novel developmental pattern for the morphological development of marsupial NMJs. Western Grey Kangaroo neonates (n = 8) aged postnatal day (P) 0-100 were ethically sourced from licensed shooters. Multiple (5-9) muscles from each specimen were embedded, cryosectioned, and stained immunohistochemically for NMJ compartment proteins. Confocal micrographs of adult kangaroo NMJs revealed small oval endplates with a central internal perforation. Standard indices of synapse maturation (e.g. ACh receptor clustering) confirmed significant NMJ development from P0-P100. Notably, forelimb NMJs were considerably more developed than hindlimb junctions at P0. From P0-100, kangaroo forelimb NMJs developed at a slower rate than hindlimb NMJs, so that all limbs were similarly developed by P100. Interestingly, within the hindlimb, a detailed comparison of 5 muscles suggested a single, homogenous developmental profile, in contrast with recent studies in mouse suggesting both fast and delayed synapsing muscle populations (Pun *et al.,* 2002). The segmented nature of NMJ development in Western Grey Kangaroos combined with the relative accessibility provided by a protracted maturation in the pouch, suggest that marsupials may present novel opportunities for studying synapse maturation compared with placental mammals.

P30. Mice lacking β2-laminin present immature neuromuscular synapses: functional investigation of calcium channel subtypes involved in transmitter release.

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Background: β2-laminin has been shown to be involved in the organisation of the presynaptic region in the developing neuromuscular junction (NMJ). Mice lacking β2-laminin present disrupted presynaptic differentiation with scattered active zones and invasion of Schwann cell processes into the synaptic cleft. At the NMJ transmitter release is initially dependent upon N-type voltage gated calcium channels (VGCCs) but later switches to P/Q-type. *In vitro* β2-laminin is able to interact directly with P/Q -channels suggesting a role in organizing VGCCs in close proximity to transmitter release sites. We examined the role of β2-laminin in organising the arrangement of N- and P/Q-type VGCCs at the active zones of developing end-plates using specific VGCC toxins and by examining neurotransmission.

Methods: The VGCC toxins, ω-agatoxin-IVA and ω-conotoxin-GVIA were used to specifically target P/Q- and N-type channels respectively to assess the relative contribution of each sub-type to neuromuscular transmission in β2-laminin mutant and wild-type mice. Diaphragm and innervating nerves were dissected from P18 mutant and wild-type mice. Intracellular recordings of end -plate potentials were taken prior to and after the application of ω-agatoxin-IVA [5 x 10^{-9} M] followed by ω-conotoxin-GVIA [1 x 10^{-8} M] for both wild-type (n=5) and β2-laminin mutant mice (n=6).

Results: Application of ω-agatoxin-IVA resulted in approximately 70% decrease in end-plate potential amplitude in wild-type mice ($p<0.001$) compared to a 20% decrease in mutants ($p<0.001$). Subsequent addition of ω-conotoxin-GVIA resulted in a further 20% decrease in wild-type mice ($p<0.05$) while a 60% decrease was observed in mutants ($p<0.001$).

Conclusion: Our present findings indicate the neuromuscular synapse of β2-laminin mutants remain immature with N-type VGCCs the primary mediator of neurotransmission.

P31. Gender differences in the number of motor units in rat medial gastrocnemius muscle

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The gender differences in the number of motor units (MUs) in the rat medial gastrocnemius (MG) and the number of alpha and gamma motoneurons in motor nucleus of the muscle were studied. Experiments were performed on Wistar rats under general anesthesia. Functional isolation of MU was achieved by electrical stimulation of single axons from the ventral roots of L4 – L5 spinal nerves whereas whole muscle force was measured during stimulation of the sciatic nerve. The number of MUs in the MG was estimated by comparison of the whole muscle tetanic force to the mean tetanic force of its MUs. The number of MUs was calculated as 57 in males and 52 in females. In the second series of experiments the cell bodies of motoneurons of the same age male and female rats were labeled following a bath of the proximal stump of the transected MG nerve in horseradish peroxidase solution. The number of male and female MG motoneurons were determined from serial microscopic images of stained sections using the Sony CCD-Iris Camera and MultiScanBase System. The mean number of alpha motoneurons was 13% higher in males than in females and amounted to 66 and 56 motoneurons, respectively whereas the number of gamma motoneurones was similar for both genders (28 motoneurons).

P32. Motor unit properties from the three heads of the triceps surae

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The soleus and two heads of the gastrocnemeii comprise the triceps surae and together contribute >80% of plantar flexion torque. Despite these muscles sharing a common innervation and function, the monoarticular soleus is composed of >80% slow twitch muscle fibers; whereas the gastrocnemeii span the ankle and knee joints and have greater fast twitch muscle fiber composition (50%) than the soleus. The active postural soleus is known to have lower motor unit (MU) discharge rates at 100% maximal isometric voluntary contraction (MVC) than other limb muscles tested (Enoka & Fuglevand 2001). However, MVC MU discharge rates have not been reported for the gastrocnemeii. Thus, the purpose was to record MU discharge rates from the three heads of the triceps surae concomitantly to compare the rate coding capacity during plantar flexion MVC. Participants performed brief isometric plantar flexion contractions from 25%-100% MVC in a seated upright position. Tests were conducted on the dominant (right) leg with the hip, knee, and ankle angles at 90°. Intramuscular motor unit properties were sampled from the medial and lateral gastrocnemeii and soleus with tungsten microelectrodes and fine-wires. Steady-state MU discharge rates in the soleus were ~16 Hz during MVC, while rates exceeded 25 Hz for both heads of the gastrocnemius. Despite the compromised torque-production position of the gastrocnemeii with the knee flexed, mean rates were ~25% lower in the soleus than the gastrocnemeii. The current findings are likely explained by the greater slow twitch muscle fiber composition and greater level of habitual activity of the human soleus than the gastrocnemeii.

P33. Localization of motor units in the human medial gastrocnemius muscle

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It has been suggested that the control of movement may occur via regional control of sub-volumes (or "compartments") within muscles. In the human medial gastrocnemius (mGas), muscle fibers comprising single motor units have been suggested to extend no longer than 4 cm along the longitudinal axis of the muscle based on surface electromyography (EMG). In this study we used indwelling EMG to examine the spatial localization of single motor units found in the human mGas. Seven subjects had 7 fine-wire indwelling electrodes inserted equidistantly along the longitudinal axis of their right mGas. Three tungsten microneurography electrodes were inserted into the proximal, middle, and distal electrode sites. Subjects were required to isolate single motor units on all three needle electrodes, and to perform ten 1-2 minute ramp-and-hold isometric contractions (up to 35% MVC). Preliminary results (n=43 motor units) indicate that motor units can span up to 12.5 cm (or 75% of mGas). While 8 motor units were collected only at one electrode site, motor units extending to 2 or more sites spanned an average of 6.9 ± 3.2 cm (39% ± 18%) of the longitudinal length of the mGas. It was also found that single motor units extending to 2 or more sites from the middle (n=11, 8.4 ± 3.6 cm, 49% ± 21%) and distal (n=15, 7.3 ± 2.4 cm, 41% ± 13%) sites spanned a greater length than those found at the proximal site (n=8, 4.4 ± 2.3 cm, 26% ± 15%), possibly due to changes in the angle of pennation of muscle fibers. Results from this study support the hypothesis of compartmentalization of the human mGas, however the spatial localization of single motor units may be much larger than previous reported.

P34. Using surface electromyography to assess impaired motor unit control in paretic muscle post stroke

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The objective of this preliminary study was to examine the possible contribution of disordered control of motor unit recruitment and firing patterns in muscle weakness post-stroke. A novel surface EMG (sEMG) recording and decomposition system was used to record sEMG signals and extract single motor unit activities from the first dorsal interosseous muscle of two hemiparetic stroke survivors. To characterize motor unit reorganization, an estimate of the motor unit action potential amplitude was derived using spike triggered averaging of the sEMG signal. The motor units suitable for further analysis were selected using a set of statistical tests that assessed the variability of the morphological characteristics of the motor unit action potentials. Our preliminary results suggest a disrupted orderly recruitment based on motor unit action potential size, a compressed recruitment range, and reduced firing rates evident in the paretic muscle compared with the contralateral muscle of one subject with moderate impairment. In contrast, the motor unit organization was largely similar bilaterally for the subject with minor impairment. The preliminary results suggest that motor unit organizational changes with respect to recruitment and rate modulation can contribute to muscle weakness post-stroke. The contrasting results of the two subjects indicate that the degree of motor unit reorganization may be associated with the degree of the functional impairment, which reveals the possible differential diagnostic capability of the sEMG decomposition system.

P35. Low-frequency component of rectified surface EMG reflects the variability of motor unit discharge rate

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Common drive to a pool of motor units manifests as low-frequency oscillations in motor unit discharge rate, producing force fluctuations. The low-frequency component of rectified surface EMG (< 5 Hz) may represent the variability in motor unit discharge rate due to such oscillations. The aim of the study was to examine the temporal correlation between instantaneous motor unit discharge rate and rectified EMG in low frequency. Eight young subjects contracted the first dorsal interosseus muscle. Subjects matched the ipsilateral force with the target as a unilateral or bilateral force matching task (5-20% of maximal force). Surface EMG and motor unit potentials by fine wire electrodes were recorded from the ipsilateral muscle. Surface EMG was band-pass filtered (5-1000 Hz), full-wave rectified, and then low-pass filtered <5 Hz. Cross correlation function (CCF) between low-frequency rectified EMG and instantaneous motor unit discharge rate were determined. In each identified motor unit (n = 11), instantaneous discharge rate was significantly and positively correlated with rectified EMG as confirmed by the distinct peak in CCF. Peak in CCF was greater (P < 0.05) in bilateral (0.35 ± 0.11) than unilateral contraction (0.22 ± 0.08). Coefficient of variation was greater (P < 0.05) for both motor unit discharge rate and force in bilateral (15.8 ±1.6% and 2.3 ± 0.2%, respectively) than unilateral contraction (12.2 ± 1.0% and 1.9 ± 0.2%, respectively). The results indicated that low-frequency component of rectified EMG (< 5 Hz) reflects the temporal characteristics and magnitude of the variability of motor unit discharge rate.

P36. Comparison of the 3D reconstruction of the dendritic tree of adult SOD1 and WT spinal motoneurons

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In the SOD1 mouse model of Amyotrophic Lateral Sclerosis (ALS), alterations of the electrophysiological properties have been observed at very early neonate ages (Bories et al., 2007; Quinlan et al., 2011). These changes were accompanied by an increase in the complexity of the motoneuron dendritic tree (Amendola et al., 2008), which contributes in part to the observed reduction of the input conductance of these neurons (Elbasiouny et al., 2010).

In adult SOD1 mice, at the age that precedes the first denervations of motor units, the input resistance remains much lower in SOD1 mice. This lead us to hypothesize that an excessive increase of the dendritic arbor might be one cause of the degenerative process. This might account for the order of degeneration according to the motor unit physiological type (FF first, followed by FR and S last) since motoneurons innervating the FF motor units are the largest whereas those that innervate the S motor units are the smallest

The goal of this work is to check this hypothesis by making intracellular labeling of triceps surae motoneurons whose intrinsic properties have also been recorded. Quantitative analysis of the full 3-D reconstruction of motoneurons will reveal if the geometry is different in SOD1 compared to WT mice. We will present here our first results.

P37. Influence of microglia on motor neuron viability in an *in vitro* model of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by loss of motor neurons (MN) rapidly leading to death. Studies indicate an important role for reactive microglia in that process and their activation seems to be linked to the secretion of both neuroprotective and cytotoxic molecules.

In order to investigate the role of microglia in different phases of ALS disease, highly purified (90%) microglial cell cultures (MCC) were obtained from spinal cord of post natal day 1 (P1) or 60 (P60) $SOD1^{G93A}$ transgenic (TG) and wild-type (WT) mice. MN cell culture was performed from P1 pups of both genotypes.

TG and WT MN were treated with conditioned medium (CM) of activated TG or WT MCC, from both ages, or with neuronal medium (n=3). Alternatively, MCC was co-cultured with MN. MN extensions were accessed by stereology at culture days one and five. Neuronal death was also quantified by Fluoro Jade C at the culture day five.

CM from P1 MCC did not lead to neuronal alterations. Longer MN extensions were observed after treatment with WT P60 microglial CM comparing to TG P60 microglial CM or neuronal medium. Furthermore, neuronal number was not altered between groups at both periods of culture. Higher neuronal death was performed when MN were co-cultured with TG microglia.

Data suggest that mature $SOD1^{G93A}$ transgenic microglia shift from neuroprotective towards neurotoxic actions, leading to motor neuron death in ALS.

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P38. Metallothionein and its derivatives may extend survival in the SOD1-G93A ALS mouse model

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Background Metallothionein-I/II (MT-I/II) is an antioxidant protein which also displays neuroprotective properties in models of traumatic brain injury. Emtin peptides, synthetic analogues of the MT-I/II protein, display similar neuroprotective properties and can cross the blood-brain barrier. Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterised by loss of both upper and lower motor neurons. Disease progression is hastened in the transgenic SOD1-G93A ALS mouse model when endogenous MT-I/II is genetically ablated. We postulated that administration of exogenous MT-I/II or emtin peptides might prolong survival of SOD1-G93A mice. **Methods** Female symptomatic SOD1-G93A mice received either emtin peptide (EmtinA or EmtinB, 30mg/kg/week) or vehicle control, from 14 weeks of age until endpoint. Female pre-symptomatic mice received MT-IIA protein (20mg/kg/week) from 6-16 weeks of age. Survival time, body weight and stride length were examined as outcome measures. **Results** Survival time for mice receiving EmtinA or EmtinB was greater than control mice; but this difference was not statistically significant (average±standard error: EmtinA 169.7± 2.2days, EmtinB 169.3±2.7days, vehicle 162.8±3.8days, p=0.105 from Kaplan-Meier analysis). Survival time for mice receiving MT-IIA was also longer than control, but not significantly so. The maintenance of stride length with disease progression seemed to be subtly enhanced with either emtin or MT-IIA treatment; however body weight and stride length were not significantly affected by either treatment when compared weekly by ANOVA. We are currently undertaking histological analysis, and statistical modelling to account for different responses of individual mice. We will report any further results to date at this conference.

P39. The complement factor C5a contributes to the pathology in hSOD1^{G93A} mouse model of amyotrophic lateral sclerosis

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There is increasing evidence that the complement system is implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS). Our previous studies in the hSOD1^{G93A} rat model of ALS demonstrated that excessive complement activation in the lumbar spinal cord leading to C5a generation contributed to motoneuron death. The hSOD1^{G93A} rats treated with the selective C5a receptor (CD88) antagonist PMX205 reduced gliosis and improved behavioral deficits, consistent with reduced neuropathology. This study aimed to determine the expression and localization of CD88 at both the mRNA and protein levels in the hSOD1^{G93A} mice, and also to elucidate the role of CD88 in the disease progression of hSOD1^{G93A} mouse. Lumbar spinal cord from high-copy number hSOD1^{G93A} mice and their wild-type littermates were obtained at 4 different ages where expression and localization of CD88 was examined using qPCR, *in-situ* hybridization, western blotting and immuno-histochemistry. Circulating levels of C5a were also examined using ELISA. The survival of hSOD1^{G93A} mice was also compared between PMX205 and vehicle treated mice. We found consistent upregulation of plasma C5a levels and CD88 mRNA and protein levels in the lumbar spinal cord during disease progression. Immuno-localization showed that CD88 is expressed on motoneurons and predominantly on the microglia surrounding the regions of motoneuron death. There was also an increase in survival of hSOD1^{G93A} mice treated with PMX205, and also hSOD1^{G93A} mice lacking CD88 (hSOD1^{G93A}-CD88-/-). These results indicate that C5a-CD88 signaling may play an important role in the pathogenesis of ALS. Hence reducing complement-induced inflammation could be an important therapeutic strategy to treat ALS.

P40. The SOD1$^{G93A\text{-}high}$ mutation only affects a subpopulation of lumbar motoneurons in neonate mice.

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We have analyzed the electrophysiological properties of motoneurons (MNs) from wild-type (WT) and transgenic (SOD1$^{G93A\text{-}high}$) neonate mice (P6-P11). Whole-cell patch-clamp recordings of MNs were conducted on lumbar slices keeping a ventral rootlet in continuity for antidromical stimulation.

Two firing patterns were observed in WT MNs in response to the injection of long lasting (5s) liminal current pulses. 30% of the cells (*immediate* MNs) started to discharge at the pulse onset and exhibited spike frequency adaptation. In the remaining 70% MNs (*delayed* MNs), the discharge was delayed up to several seconds and then displayed an increasing firing frequency.
We demonstrated that the complex delayed pattern is due to two K^+ currents acting at two different time scales (a fast I_A–like current and an ultra-slow I_{KSlow} current). In WT MNs the passive electrical properties (R_{in}, C_m, L) and the firing properties (direction of the hysteresis, gains of the F-I curve, recruitment current) proved to be highly different between *immediate* and *delayed* MNs. For instance input resistance is much higher (70.8±70.6MΩ n=79) in *immediate* MNs than in *delayed* MNs (32.9±30MΩ n=137). Also 80% of *delayed* MNs showed counter-clockwise hysteresis of the I-F curve whereas 82% of *immediate* MNs had a clockwise hysteresis.

In transgenic (SOD1$^{G93A\text{-}high}$) neonate mice we also observed the two firing patterns in the same proportions. Strikingly only *immediate* MNs were affected by the mutation. Their input resistance is much lower (40.4±47.1MΩ n=24) in mutant than in WT mice and the proportion of clockwise hysteresis dropped from 80% to 35%. In sharp contrast *delayed* MNs in SOD1 mice retained features of the WT (input resistance 30.6±16.5MΩ n=59 and proportion of counter-clockwise hysteresis 77%).

It remains to be seen whether these two populations, differentially affected by the disease, match with any MNs functional subgroups (alpha or gamma, innervating slow or fast muscle fibers….).

P41. Measuring the progression of MND with S50 – a new method to enhance the clinical utility of the CMAP scan.

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The CMAP scan is a practical tool extending standard nerve conduction studies. Using surface stimulation and recordings it assesses all of the CMAP and can exploit the fact that large motor units (MU) (as found on EMG) are present due to collateral reinnervation. Bayesian analysis uses the CMAP scan to determine MU number and other properties but is not practical for common use. Integrated software has now been developed to calculate other potential electrophysiological markers to track the progression of motor unit loss in MND. One promising marker, the S50, calculates the number of steps required to obtain 50% of the CMAP in any nerve. This value can be obtained automatically once the CMAP scan has been performed, and has the advantage of ease of collection and interpretation. 8 MND patients with 3-8 serial studies were assessed using both the S50 and Bayesian analysis and a similar decline in MU number was observed. Patients with large and small MU counts as well as unstable MUs were assessed. The method appears reproducible using paired CMAP scans but is limited when large MU numbers are present. Further work with studies from other centres is needed.

P42. Serotonin induces central fatigue by inhibiting action potential generation at the AIS of motoneurons

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Motor fatigue induced by physical activity is a recurring everyday experience characterised by decreased ability to produce muscle force. Factors in both muscles and the central nervous system are involved. The central component of fatigue is revealed by the inability of motoneurons to activate muscle. It secures rotations of motor units. Indirect evidence indicate that central fatigue is caused by serotonin (5-HT), but the cellular mechanisms are unknown. We show that increased release of serotonin, as during motor activity, acts directly on motoneurons by an extrasynaptic action at the axon initial segment (AIS) to downregulate motoneuron responsiveness. In a slice preparation from the spinal cord of the adult turtle, we found that prolonged stimulation of the raphe-spinal pathway activated 5-HT1A receptors (5-HT1AR), which in turn decreased the excitability of motoneurons. The suppression of firing was caused by the inhibition of the Na+ channels responsible for action potentials. We showed that the somatodendritic membrane of motoneurons was densely innervated by serotonergic synaptic boutons. The AIS by contrast, was devoid of 5-HT terminals suggesting that extrasynaptic 5-HT1AR in this compartment are activated by spillover of 5-HT. Prolonged stimulations of the raphe-spinal pathway increased extracellular 5-HT in the vicinity of the AIS to a concentration sufficient to activate 5-HT1AR.
In conclusion prolonged 5-HT release during motor activity spills over from its release sites to the AIS of motoneurons. Here activated 5-HT1AR inhibit firing and thereby muscle contraction. To the best of our knowledge, this is the first cellular mechanism for central fatigue.