THE NEUROMUSCULAR JUNCTION
IN HEALTH AND DISEASE

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a life-time, a part:
neuromuscular junction -
mind meeting matter!

The “Final Common Path”…

…leading to the “Ultimate Synapses”
Every normal (or diseased) motor unit has a characteristic signature in extracellular electromyographic (EMG) recordings.

Sir Bernard Katz (1911-2003)

“The neuromuscular junction... [is] an experimentally favourable object whose study could throw considerable light on synaptic mechanisms elsewhere”
Fenn Lecture, IUPS Glasgow, 1993
1. Course Overview
2. EMG Recording Exercise
3. NMJ Review
1. Course Overview

2. EMG Recording Exercise

3. NMJ Review

http://www.dns.ed.ac.uk/rrrweb/NMJHDhons/NMJhonsIndex.htm
The Aims of the "NMJiHaD" course are to:

- Enhance your knowledge and understanding of the anatomy, physiology and cell biology of neuromuscular junctions; and facilitate your understanding the importance of synaptic strength and synaptic homeostasis in the healthy nervous/neuromuscular system and after injury or in disease;

- Develop your evidence-based reasoning and critical skills in appraisal and integration of findings reported in original research literature, in the context of knowledge, understanding and research on neuromuscular junctions;

- Provide generic skills training in problem solving, team working, presentation of research material, orally and in writing.
Outline

The aims of the "NAUHG72" course are to:

- Enhance your knowledge and understanding of the anatomy,physiology and cell biology of neuromuscular junctions, and facilitate your understanding of the importance of synaptic strength and synaptic homeostasis in the healthy neuromuscular system and under injury or in disease;
- Develop your evidence-based reasoning and critical skills in appraisal and integration of findings reported in original research literature in the context of knowledge, understanding and research on neuromuscular junctions;
- Provide generic skills in effective written and oral presentation of scientific material, orally and in writing.

The course this year will include student presentations and computer-based assessments. There is no in-course examination. However, an examination ("mini-symposia") will be held at the end of the course to test your understanding of the course material. This examination will be held at the end of the course.

The mini-symposia will take place in Weeks 25, 26, 27, 28 and 29 of the course. The sessions in Weeks 25, 26, 27, 28 and 29 will include student presentations and computer-based assessments. There is no in-course examination. However, an examination ("mini-symposia") will be held at the end of the course to test your understanding of the course material. This examination will be held at the end of the course.

Weeks 25 and 26 are for self-directed learning, when you will be given computer-based exercises in data analysis and interpretation based on data generated either by computer models or from real experiments.

Week 27 will comprise a discussion of final exam questions, including what examiners expect of the answers, and how they are marked. We will use examples from last year's exam. You will have an opportunity to practice writing an essay with these marking criteria in mind.

Week 28 will comprise a revision session, in which we will also go over past exam problems. There will also be an optional demonstration of neuromuscular junction physiology and pharmacology in Week 29.

You will find this website a useful resource. You should familiarise yourself with the background knowledge and discuss the content of this course with other students in the second year course. Neurophysiology with Pharmacology 2 (NPAP2) and the first year course, Neurone Function 1 (NFT1), will also be covered in this course. Further useful background reading is listed in the Additional Reading section.

Structure and Function of Motor Units and Neuromuscular Junctions

The course is examined by written examination only. Please refer to the exam paper for guidance. There are several optional assignments you may attempt in preparation for the exam. Feedback will be provided on this website. In the final stages of the course, you will also have access to feedback on your oral presentations. Finally, you will receive your feedback, which helps to improve the course for students in the next year. Forms are available for students to submit their feedback.

Check the website regularly for announcements.

Table:

<table>
<thead>
<tr>
<th>Week/Date</th>
<th>Topic</th>
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<tbody>
<tr>
<td>Wk 1-2</td>
<td>Introduction to Motor Units and Neuromuscular Junctions</td>
</tr>
<tr>
<td>Wk 23-26</td>
<td>Neuroscience and Pharmacology 2 (NPAP2)</td>
</tr>
<tr>
<td>Wk 27-28</td>
<td>Revision and Final Exam Questions</td>
</tr>
<tr>
<td>Wk 29-30</td>
<td>Final Exam and Course Feedback</td>
</tr>
</tbody>
</table>

For more information, please refer to the following resources:

1. Course Overview

2. EMG Recording Exercise

3. NMJ Review

http://www.innerbody.com/anim/arm.html
How might you quantify what you have observed?

What important questions arise?

How could you go about finding the answers?

What could go wrong with motor neurones/NMJ’s and what would be the consequences?

How would you fix it?

http://www.innerbody.com/anim/arm.html
The Neuronal Junction in Health and Disease

Essential Background Knowledge

The following background material describes various aspects of the neuromuscular junction, including its molecular and structural features, and its role in health and disease.

- Development of the neuromuscular junction involves the interaction of motor nerve terminals and muscle fibers.
- Synaptic transmission is mediated by the release of neurotransmitters, such as acetylcholine, from the nerve terminal and their binding to receptors on the muscle fiber.
- The synapse is composed of the presynaptic terminal, the synaptic cleft, and the postsynaptic membrane.
- The synapse is highly specialized and maintains high rates of neural transmission.
- Pathologies affecting the neuromuscular junction can lead to muscle weakness and other neurological symptoms.

![Diagram of the 'Life Cycle' of Neuromuscular Synapses](image)
Neuromuscular Junctions in Health and Disease

Recommended Reading

The background knowledge expected for students taking the course is outlined here.
Slater, CR. Reliability of neuromuscular transmission and how it is maintained. Handbook of Neurology. 2006: 91, 27-101. [PDF]

From Neuron to Brain

From Molecules to Networks
1. Course Overview
2. EMG Recording Exercise
3. NMJ Review
Mini Symposia:

1. Structure and function of motor units and NMJ
2. Neuromuscular transmission
3. Development, degeneration and regeneration of NMJ
4. Synaptic Homeostasis: the Drosophila NMJ
5. Revision of Quantal Analysis
Presenting papers:

Talks (15-20 mins) should focus on the important information in the figures but have a:

**Beginning** (Introduction, Aims of the study, summary of Methods used);

**Middle** (presentation of Results; include original figures)

**End** (strengths and weaknesses, summary of Conclusions, Suggestions for further work).

Bear in mind the mantra of good lecturing:

"Tell 'em what you're gonna tell 'em;
Tell 'em;
Tell 'em what you've told 'em."
Optional Assignments with Formative Assessment and Feedback

Hand in your practice essay(s) and/or question-analysis problems any time before the end of Week 8 if you wish to receive feedback before the examination at the end of the semester. The work may not be marked and feedback cannot be guaranteed if you submit your assignments after that date.

1. Write an essay (4000 words) on either:
   "The molecular physiology of synaptic transmission at the neuromuscular junction." This essay would include: for example, a review of the ultrastructure of the active zone, the molecular composition of synaptic vesicles, and the mechanisms of synaptic vesicle recycling, or alternatively, focus on the organization and function of acetylcholine receptors, sodium ion channels and cholinergic proteins, or the functions of molecules in the basal lamina, including scabellin in red cell membranes.

2. Or:
   "Electrophysiological studies of neuromuscular synaptic development, structure and function in the rat spinal cord." This essay should critically evaluate the application of molecular genetic techniques to determine how the size and strength of synapses is regulated during development.

In either case, discuss the relevance of the findings to the understanding of neuromuscular disease. If you wish to write both essays, both will be marked and feedback provided.

2. Choose a question from last year's Exam Paper and write an essay on that. To help you prepare for this, in Week 7 we will discuss examiners' guidelines notes, marking criteria and a couple of examples of answers that were given to one of the questions on last year's exam paper.

Some tips on writing good essays are here.

3. Measure the amplitudes of the EPSPs and calculate the mean quadrant of the EPSPs using the Direct Method, the Variance Method, and the Method of Failure in the following tissue:

Click here to download a powerpoint file containing all the tutorial powerpoint files. Click here for a pdf handout on quadrantal analysis.

A handy tool for measuring distances in images is the free software package ImageJ. To use this, save the EPSP tissue as a jpeg file from the powerpoint file then load them into ImageJ. Some further analysis problems and guidance handouts are here.

Home - Contents

RMU In Health and Disease

What makes a good essay?

The word "essay" is synonymous with "attempt" thus, in any essay you are attempting to explain something. At Honours level, essays are not simply regurgitated or rearranged facts that you have stumbled upon or read about. We are looking for originality, creativity, rigour and application of critical skills. Like all science, neuroscience is evidence based.

So, you must focus your essay on the evidence underpinning your attempted explanations for neuromuscular synaptic phenomena, processes, or mechanisms. You may be asked to write a detailed essay, in which case you should ensure that the evidence for any argument in scientific writing is clearly stated in terms of its quality - i.e. in terms of its verifiability, supplemented by the reference to relevant and other literature. This essay should provide the reference for the work that you cite as evidence for such a statement in a bibliography in the form of x references at the end of the essay e.g. Del Castillo, J., & Katz, B. (1949) Quantitative components of the end-plate potential. J. Physiol. 108:523.

Examiners of essays for this course will be looking for evidence that you have assimilated and understood the main findings in the relevant literature that we have discussed in class. Evidence based reasoning will be given marks as evidence of critical thinking and understanding of the subject, provided it shows knowledge and understanding relevant to the question. ALWAYS ANSWER THE QUESTION ASKED, NOT the question you would have liked to have been asked.

General features of good essays include:

- Good form and structure (introduction/hypothesis/description/conclusion/summary)
- Good use of English (style, grammar, spelling)
- Understanding of context (the 'big picture')
- Sound factual background knowledge
- Critical analysis using case appraisal and logical or statistical analysis
- Evidence-based reasoning
- Coherence and rigor of the arguments, including alternative explanations
- Clear statement of conclusions
- Specific suggestions for further work
- Conclusions/Summary
- Bibliography (not expected under closed-book/unseen exam conditions)

Samples and answers provided to examiners of essays are here.
Neuromuscular junctions in Health and Disease

Neuromuscular transmission/Quantal Analysis Problems

1. In an experiment on a partially excised frog neuromuscular junction, acetylcholine (ACh) was applied to the endplate by iontophoresis, using 1 mA, 1 ms current pulses at a frequency of 3 Hz. A train of evoked endplate potentials (EPPs) was then evoked by stimulating the muscle nerve at 60 Hz. The iontophoretic pulses were repeated within 20 min of the start of the stimulus train. The following data were obtained:

Mean ACh response before EPP train = 1.03 ± 0.12 mV (mean ± S.D., n=10)
Mean ACh response after EPP train = 1.51 ± 0.10 mV (mean ± S.D., n=7)

<table>
<thead>
<tr>
<th>EPP response (mV)</th>
<th>0.2</th>
<th>0.4</th>
<th>0.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude (mV)</td>
<td>1.2</td>
<td>2.1</td>
<td>3.0</td>
</tr>
</tbody>
</table>

a) calculate the amount of charge delivered by each of the iontophoretic current pulses.
b) sketch the characteristic responses to ACh and nerve stimulation indicating the time course of the responses;
c) how might the iontophoretic responses to ACh change, if a slow concentration of ACh (10 μM) were also continuously present in the medium?
d) is the hypothesis that short-term synaptic depression is caused by desensitisation of ACh-receptors supported or refuted by these data? Give your reasoning.

2. Intracellular recordings were made from a mouse neuromuscular junction. The nerve supply was stimulated 100 times at 5 Hz. The mean size of the EPP evoked was 1.00 mV. Five of the stimuli evoked no response (i.e. there were 5 failures).
a) What was the mean quantal content at this neuromuscular junction?
b) What do you predict for the size of the EPP (the amplitude of the quantal event MPP)
c) how many of the failures would you predict to be quantal contents of 1.25 and 4 quanta?
d) What do you predict would be the standard deviation of the EPP amplitudes?
1. Course Overview
2. EMG Recording Exercise
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Paper 1: Motor unit structure in adult muscle
Paper 2: MND: Spinal Muscular Atrophy (SMA)
Paper 3: Cytology of the mammalian NMJ
Paper 4: MND: Amyotrophic Lateral Sclerosis (ALS)
Paper 1: Motor unit structure in adult muscle

Paper 2: MND: Spinal Muscular Atrophy (SMA)

Paper 3: Cytology of the mammalian NMJ

Paper 4: MND: Amyotrophic Lateral Sclerosis (ALS)
Motor neurone cell bodies occupy the ventral horn of grey matter

http://www.tmin.ac.jp/english/dept07/neurology2.jpg
http://www.shef.ac.uk/content/1/6/02/25/50/jw2.jpg
Neuromuscular connections frequently occupy a tight band in skeletal muscle

The (mouse) motor neurone in perspective

Robert Hartley/Adrianna Terakidis
The 4DL Connectome

MU sizes
55
44
16
53
19
11
Total: 198 muscle fibres
Typical Human Motor Unit (TA)

<table>
<thead>
<tr>
<th></th>
<th>Diameter (µm)</th>
<th>Length (µm)</th>
<th>Number</th>
<th>Volume (µm³)</th>
<th>Re Soma Volume</th>
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<tbody>
<tr>
<td><strong>Soma</strong></td>
<td>40</td>
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<td>1</td>
<td>33510</td>
<td>1</td>
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<tr>
<td><strong>Dendrites</strong></td>
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<td>1,000</td>
<td>200</td>
<td>157079</td>
<td>4</td>
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<tr>
<td><strong>Axon</strong></td>
<td>10</td>
<td>1,000,000</td>
<td>1</td>
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<td>2345</td>
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<tr>
<td><strong>Collaterals</strong></td>
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<td>2,000</td>
<td>1500</td>
<td>37699112</td>
<td>1125</td>
</tr>
<tr>
<td><strong>Terminals</strong></td>
<td>10</td>
<td>20</td>
<td>1500</td>
<td>300000</td>
<td>9</td>
</tr>
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Summary

Each motor neurone supplies one specific anatomical muscle. Intramuscular branches innervate a set of muscle fibres. The motor neurone and the muscle fibres it innervates is called a motor unit. The set of motor units innervating a muscle is called its “connectome”.
The interosseus muscle connectome.

Lu J, Taylor JC, White DL, Lichtman JW.
Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts, USA.

Erratum in

Abstract
The complete connectonal map (connectome) of a neural circuit is essential for understanding its structure and function. Such maps have only been obtained in Caenorhabditis elegans. As an attempt at solving mammalian circuits, we reconstructed the connectomes of six interosseus muscles from adult transgenic mice expressing fluorescent probes in all motor axons. The reconstruction revealed several organizational principles of the neuromuscular circuit. First, the connectomes demonstrate the anatomical basis of the graded tensions in the size principle. Second, they reveal a robust quantitative relationship between axonal caliber, length, and synapse number. Third, they permit a direct comparison of the same neuron on the left and right sides of the same vertebrate animal, and reveal significant structural variations among such neurons, which contrast with the stereotypy of identified neurons in invertebrates. Finally, the wiring length of axons is often longer than necessary, contrary to the widely held view that neural wiring length should be minimized. These results show that mammalian muscle function is implemented with a variety of wiring diagrams that share certain global features but differ substantially in anatomical form. This variability may arise from the dominant role of synaptic competition in establishing the final circuit.

Paper 1: Motor unit structure in adult muscle

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Paper 4: MND: Amyotrophic Lateral Sclerosis (ALS)
The ‘Life Cycle’ of Neuromuscular Synapses

Adult muscle fibres are mononeuronally innervated (µ)
Neonatal muscle fibres are polyneuronally innervated (π)

Synapse elimination occurs during postnatal development, establishing the mononeuronal innervation of motor endplates

Rodent NMJ’s are stable in form but grow throughout life

Keller-Peck et al. (2001). Neuron 31, 381-394

Balice-Gordon & Lichtman (1990) J Neurosci 10, 834
Summary of key stages in the development of rodent NMJ’s

- NMJ Expand
- NMJ Reshape
- AChR \( \gamma \rightarrow \varepsilon \)
- NMJ Elim
- Myelin Form
- NMJ Form
- MF Form
- MN Die
- MN Form

-20 days Birth +30 days

Progressive
Regressive
Remodel
Agrin clusters ACh receptors via Muscle-Specific Kinase Neuregulin modulates AChR synthesis via ErbB receptors

Acetylcholine receptors cluster under the influence of Agrin
Neonate: AChR - γ

Adult AChR - ε
Motor Neurone Disease
(Spinal Muscular Atrophy; SMA)
Spinal Muscular Atrophy

- Neurodegenerative disorder with autosomal recessive genetic heredity in 95% of cases.
- Degeneration of α-motor neurons of the spinal cord, resulting in muscle weakness and progressive paralysis.
- Incidence about 5-7 per 100,000 live births. The prevalence of individuals with the carrier state is 1 in 80.
- The most common degenerative disease of the nervous system in children and the leading heritable cause of infant mortality.
- Caused by a homozygous deletion of the survival motor neuron (SMN1) gene on chromosome 5.
- SMN2 has reduced stability due to C-to-T transition in exon 7 (→ SMNΔ7 protein).
- Onset/severity of SMA varies depending on number of SMN2 gene copies (up to 8) Type I (Werdnig-Hoffman Disease) terminal in neonates; Type IV - adult onset.
- Normal function of SMN protein is unknown. It is expressed in many cell types, and has been implicated in a range of cellular functions, including small nuclear ribonucleoprotein (snRNP) assembly.
Mouse Models of SMA

- Mice possess a single \textit{Smn} gene, which has 82% amino acid identity with its human homolog and a similar expression pattern.

- Homozygous \textit{Smn} deletion results in massive embryonic cell death and lethality at birth.

- Expression of a human SMN2 transgene on the \textit{Smn}-null background rescues lethality and transgene copy number modifies severity.

- Introduction of a second transgene, containing human SMNΔ7 extends the lifespan from 6 to 13 days.
Reduced SMN protein impairs maturation of the neuromuscular junctions in mouse models of spinal muscular atrophy

Shingo Kashi way 1, Gyoo Huwon Park 1, 2, 4, Yuka Mineo-Hitachi 2, Olea Leykekhman 1, Cari Ann Leitz 1, Marc B. Aronson 1, Lynn T. Landmesser 1 and Umran R. Mosa 1, 2, 4, a

Selective vulnerability of motor neurons and dissociation of pre- and post-synaptic pathology at the neuromuscular junction in mouse models of spinal muscular atrophy

Lyndsay M. Murray 1, 2, Laura H. Comley 1, 2, Derek Thomson 1, 2, Nick Parkinson 1, Kevin Talbot 1 and Thomas H. Gillingwater 2, 4

1Centre for Integrative Physiology and 2Centre for Neuroscience Research, University of Edinburgh Medical School, Edinburgh EH10 9XD, UK and 3MRC Functional Genetics Unit, Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford OX1 3QX, UK
Morphological characteristics of motor neurons do not determine their relative susceptibility to degeneration in a mouse model of severe spinal muscular atrophy.

Euan Macdonald Centre for Motor Neuron Disease Research, University of Edinburgh, Edinburgh, United Kingdom.

Abstract

Spinal muscular atrophy (SMA) is a leading genetic cause of infant mortality, resulting primarily from the degeneration and loss of lower motor neurons. Studies using mouse models of SMA have revealed widespread heterogeneity in the susceptibility of individual motor neurons to neurodegeneration, but the underlying reasons remain unclear. Data from related motor neuron diseases, such as amyotrophic lateral sclerosis (ALS), suggest that morphological properties of motor neurons may regulate susceptibility. In ALS, larger motor units innervating fast-twitch muscles degenerate first. We therefore set out to determine whether intrinsic morphological characteristics of motor neurons influence their relative vulnerability to SMA. Motor neuron vulnerability was assessed across 10 muscle groups in SMA mice. Neither the position of the muscle in the body, nor the fibre type of the muscle innervated, influenced susceptibility. Morphological properties of vulnerable and disease-resistant motor neurons were then determined from single motor units reconstructed in Thy1-YFP-KI mice. None of the parameters we investigated in healthy young adult mice, including motor unit size, motor unit fiber length, branching pattern, motor endplate size, developmental pruning and numbers of terminal Schwann cells at neuromuscular junctions - correlated with vulnerability. We conclude that morphological characteristics of motor neurons are not a major determinant of disease susceptibility in SMA, in stark contrast to related forms of motor neuron disease such as ALS. This suggests that subtle molecular differences between motor neurons, or extrinsic factors arising from other cell types, are more likely to determine relative susceptibility in SMA.
The ‘Life Cycle’ of Neuromuscular Synapses

Electrical stimulation of nerves causes muscles to contract
The Cell Vizio fluorescence Confocal MicroEndoscope (f-CoME)

Neuromuscular junctions in living thy1.2-YFP mice viewed with fibre-optic confocal microendoscopy (CME)
Combining EMG with CME

Desaki & Uehara, 1981

Rosalind Brown

Hamster

Desaki & Uehara, 1981
Inactivation

\[
\text{CH}_3\text{C}---\text{OCH}_2\text{CH}_2\text{N(CH}_3\text{)}_3 \xrightarrow{\text{Acetylcholinesterase}} \text{CH}_3\text{C}---\text{OH} \xrightarrow{0} \text{Acetic acid} + \text{HOCH}_2\text{CH}_2\text{N(CH}_3\text{)}_3
\]
Hamster

Desaki & Uehara, 1981

Desaki & Uehara, 1981

Wood & Slater (1997)
The 'Life Cycle' of Neuromuscular Synapses

DEVELOPMENT

REGENERATION

SPROUTING

ADULT

ELIMINATION

CONSOLIDATION

Sprouts

Axon

μ

π

10 μm
Axonal sprouting is preceded by Schwann cell sprouting

Son et al (1996) TINS 19,280
Summary
Confocal microscopy, electron microscopy, immunocytochemistry, electrophysiology and transgenic tools have established the cellular composition and sub-cellular organization of key components of the NMJ critical for synaptic transmission.

Neuromuscular junctions comprise four types of cells
Identity, developmental restriction and reactivity of extralaminar cells capping mammalian neuromuscular junctions.

Court FA, Gillieswater TH, Melrose S, Sherman DL, Greenshields KJ, Morton AI, Harris JE, Wilson HJ, Ritchie RR.

Euan MacDonald Centre for Motor Neurone Disease Research, The University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ, UK.

Abstract

Neuromuscular junctions (NMJs) are normally thought to comprise three major cell types: skeletal muscle fibres, motor neuron terminals and perisynaptic terminal Schwann cells. Here we studied a fourth population of junctional cells in mice and rats, revealed using a novel cytoskeletal antibody (2166). These cells lie outside the synaptic basal lamina but form caps over NMJs during postnatal development. NMJ-capping cells also bound rH5, iNM-HLM, CD94 and cholera toxin B subunit. Bromodeoxyuridine incorporation indicated activation, proliferation and spread of NMJ-capping cells following denervation in adults, in advance of terminal Schwann cell sprouting. The NMJ-capping cell reaction coincided with expression of tenascin-C but was independent of this molecule because capping cells also dispersed after denervation in tenascin-C-null mutant mice. NMJ-capping cells also dispersed after local paralysis with botulinum toxin and in atrophied muscles of transgenic R6/2 mice. We conclude that NMJ-capping cells (proposed name: "synaptic cytes") represent a neglected, synovial cellular constituent of neuromuscular junctions where they could play a permissive role in synaptic regeneration.

PMID: 19021504 (PubMed - indexed for MEDLINE) Free full text

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Motor Neurone Disease
(Amyotrophic Lateral Sclerosis; ALS)
Motor Neurone Disease

Primary lateral sclerosis (PLS)

Amyotrophic lateral sclerosis (ALS)

Progressive Muscular Atrophy (PMA)

Transverse section of partially-denervated/neuropathic muscle

http://www.neuropathologyweb.org/chapter13/images13/13-7n.jpg
Some cruel facts about MND/ALS

- incidence 2/100,000
- prevalence 5/100,000
- life expectancy from diagnosis: 2-5 years
- ca. 90-95% of cases are “sporadic”
- ca. 5-10% of cases are familial
- ca. 2% of cases are attributed to mutations in SOD1
- cause is unknown for sporadic ALS; mechanism is unknown for familial ALS
- age and gender are risk factors (20% higher incidence in men)
- disease frequently has a specific initiating focus then spreads to contiguous regions
- disease is initiated in MN but progression is more likely due to defects in glia and/or other non-neuronal cells
- glutamate transporters are deficient in spinal cord
- motor neurones contain inclusions of TDP-43 protein
- the only drug licenced for treatment is riluzole (supressor of glutamate release); prolongs life by ca. 3 months with no effect on quality of life
ALS/MND Duration

ALS onset occurs mostly in middle to late age

Courtesy of Michael Strong, UWO
SOD1<sup>G93A</sup> mice develop progressive hindlimb paralysis

“Symptomatic”

Endstage
Loss of motor neurones and glutamate transporter in SOD1 mouse spinal cord

Synapses degenerate before axons in SOD1^{G93A} mice

12 week old - asymptomatic

50 µm

thy1.2:YFP16/SOD1^{G93A}

Robert Hartley
SOD1<sup>G93A</sup> NMJs: synaptic “autotomy”?  

Confocal Z-series projection

Quantitative morphometry of axonal atrophy

Axon Thinning

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SOD1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axon Thickness µm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
</tbody>
</table>
Synapses degenerate before axons in SOD1\textsuperscript{G93A} mice

Symptomatic 8 month-old mouse

thy1.2:YFP/SOD1\textsuperscript{G93A}

30 µm

Synaptic degeneration in SOD1\textsuperscript{G93A} mice detected by CME

Symptomatic thy1.2:YFP16/SOD1\textsuperscript{G93A}
In vivo CME showing degeneration of distal axons and NMJ in SOD1<sup>G93A</sup> mice

A

0d

B

+4d

C

D

E

In-vivo Imaging with Confocal Microendoscopy

11-week (Presymptomatic) SOD1<sup>G93A</sup><sub>low</sub>
Degenerating neuromuscular junctions in the SOD1<sup>G93A</sup> mouse model of MND/ALS - Symptomatic

Other motor units in SOD1<sup>G93A</sup> mice compensate by sprouting
Motor neurone disease (eg ALS)

Early vulnerability to ischemia/reperfusion injury in motor terminals innervating fast muscles of SOD1-G93A mice.

David G. Nguyen & Brent EF
Department of Physiology and Biophysics, University of Miami Miller School of Medicine, USA. gnvnd@med.miami.edu

Abstract
In mouse models of familial amyotrophic lateral sclerosis (ALS), motor neurons are especially vulnerable to oxidative stresses in vitro. To determine whether this increased vulnerability also extends to motor nerve terminals in vivo, we assessed the effect of tourniquet-induced ischemia/reperfusion (IRI) injury on motor terminals innervating fast and slow hindlimb muscles in male G93A-SOD1 mice and their wild-type littermates. These mice also expressed yellow fluorescent protein (YFP) in motor neurons. We report that in SOD1-G93A/YFP mice the motor terminals innervating two predominantly fast muscles, extensor digitorum longus (EDL) and plantaris, were more vulnerable to IRI injury than motor terminals innervating the predominantly slow soleus muscle. The mean duration of EDL ischemia required to produce a 50% reduction in endplate innervation in SOD1-G93A/YFP mice was 26 min, compared to 45 min in YFP-only mice. The post-IR destruction of EDL terminals in SOD1-G93A mice was rapid (>24 h) and was not duplicated by cutting the sciatic nerve at the tourniquet site. The increased sensitivity to IRI injury was evident in EDL muscles of SOD1-G93A/YFP mice as young as 11 days, well before the onset of motor neuron death at approximately 90 days. This early vulnerability to IRI injury may correlate with the finding (confirmed here) that in ALS mice motor nerve terminals innervating fast hindlimb muscles degenerate before those innervating slow muscles, at ages that precede motor neuron death. Early vulnerability of fast motor terminals to IRI injury thus may signal, and possibly contribute to, early events involved in motor neuron death.
Summary

1. Each motor neurone supplies one specific anatomical muscle. Intramuscular branches innervate a set of muscle fibres. The motor neurone and the muscle fibres it innervates is called a motor unit. The set of motor units innervating a muscle is called its “connectome”.

2. Confocal microscopy, electron microscopy, immunocytochemistry, electrophysiology and transgenic tools have established the cellular composition and sub-cellular organization of key components of the NMJ critical for synaptic transmission.

3. NMJ are the first components of the motor neurone to undergo degeneration in SMA and ALS.