Development of neuromuscular innervation

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Main topics for this lecture

• The neurobiological context..
• Motor Neurone birth
• Neuromuscular synapse formation
• Motor Neurone death
• Neuromuscular synapse elimination
• Molecular changes in ACh Receptors
• Neonatal disease of motor units: Spinal Muscular Atrophy (SMA)
• Development of myelin sheaths
• Motor nerve sprouting and regeneration: recapitulation of development
The neurobiological context....
Proliferation

Migration

Induction

Progressive

Axon outgrowth

Aggregation

Synapse formation

Regressive

Cell Death

Synapse Elimination
Remodelling of connections is a feature of development.
Remodelling of connections is activity-dependent
Activity-dependent remodelling may be based on competition for neurotrophic factors

"When an axon of cell A is near enough to excite a cell B and repeatedly and persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A’s efficiency as one of the cells firing B is increased."


From
“The neuromuscular junction... [is] an experimentally favourable object whose study could throw considerable light on synaptic mechanisms elsewhere”

Sir Bernard Katz, Fenn Lecture, IUPS Glasgow, 1993
Overview of motor units and their development…
The “Final Common Path”....
Motor neurone cell bodies occupy the ventral horn of grey matter
Neuromuscular connections frequently occupy a tight band in skeletal muscle.
Mature NMJ’s are mononeuronally innervated
Confocal microscopy of the NMJ

30 µm
Four cell types at the NMJ

- KC
- NT
- tSC
- MF

1 μm
Motor units are expanded and muscle fibres hyperinnervated in neonates.
Neonatal muscle fibres are polyneuronally innervated ($\pi$)
Remodelling Neuromuscular Synapses

Development → Regeneration → Sprouting → Adult → Elimination → Consolidation
Summary of key stages in the development of rodent NMJ’s

- NMJ Expand
- NMJ Reshape
- AChR $\gamma$->$\epsilon$
- NMJ Elim
- Myelin Form
- NMJ Form
- MF Form
- MN Die
- MN Form

Progressive
Regressive
Remodel
Rodent NMJ's are stable in form but grow throughout life

Figure 5. Growth of individual neuromuscular junctions is predominantly intercalary. Top, inset is a camera lucida tracing of the 2 month

Quantal Content (variance method) at NMJ of rat HD

(Based on Kelly & Roberts, 1977 and Kelly, 1978)
Formation of motor neurone pools…
The progenitors of motor neurons and interneurons are formed within distinct regionally-restricted domains of the ventral neural tube. The p0–p3 domains give rise to various interneuron subtypes, whereas the pMN domain is the source of motor neurons. The progenitor domains are identified by segmental expression of sets of transcription factors that are activated or repressed by different threshold concentrations of sonic hedgehog (Shh). The Shh gradient is denoted by pink dots. Shh signalling is thought to regulate the initial expression of transcription factors in the ventral neural tube (for example, Nkx2.2, Olig2, Pax6 and Irx3), which subsequently engage in cross-regulatory interactions to sharpen and maintain the domain boundaries. Finally, combinatorial interactions between transcription factors expressed in each domain regulate downstream genes that determine progenitor identity. The positions of the floorplate (FP), an important source of Shh proteins, and the roofplate (RP), a source of bone morphogenetic proteins (BMPs), are shown.
Other transcription factors specify medial-lateral (intrasegmental) and rostro-caudal motor neurone identity.

Selective outgrowth of axons…
S. Ramon y Cajal, ca 1900, identifies neuronal growth cones
Growth cones both extend and retract

http://growthcones.neuroscience.umn.edu/Videos.html
Growth cones are semi-autonomous

Remove
Cell body...

...a few minutes later...
Growth cones respond to adhesive and chemical gradients

http://growthcones.neuroscience.umn.edu/Videos.html
In immature spinal cord *in vivo*, netrins attract and semaphorins repel motor neurone growth cones.
Growth cone properties may underly the specificity of connections.
Topographic projections are determined by neural identity, not location

Formation of muscle fibres and NMJ’s…
Neuromuscular connections frequently occupy a tight band in skeletal muscle
A Mouse
a Early stage

Phrenic nerve growth cone → Nucleus → Anural AChR cluster → Muscle fiber

b Late stage

Neural AChR cluster → Phrenic nerve → Muscle fiber
Two types of synapse formation: FaSyn & DeSyn

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<tr>
<th>A</th>
<th>B</th>
<th>C</th>
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<td><strong>Prepattern</strong></td>
<td><strong>Induction</strong></td>
<td><strong>Maturation</strong></td>
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<td><em>FaSyn Muscles</em></td>
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P0
Acetylcholine receptors cluster under the influence of Agrin
ACh Receptor maturation…
"Plaque"

"Pretzel"

Fig. 9. Postnatal maturation of junctional AChR distribution seen after labeling with fl-α-HgTx. (a) Newborn, (b) 1 week, (c–e) 2 weeks, (f) 3 weeks, and (g) adult. All from EDL muscles, ×1000.

Slater (1982)
Agrin clusters ACh receptors via Muscle-Specific Kinase
Neuregulin modulates AChR synthesis via ErbB receptors
The nicotinic ACh Receptor at NMJ

8 nm

Exterior
Membrane
Cytosol

α-Helices forming gate

Pore ~0.7 nm diameter
Neonate: AChR - γ

Adult AChR - ε
Fetal: AChR - $\gamma$

Adult AChR - $\epsilon$

Fig. 3 Properties of ACh-activated single-channel currents in fetal and adult bovine muscle.
AChR-ε knockout mice continue to make AChR-γ and initially NMJ develop normally.

Witzemann et al. (1996) PNAS 93, 13286
Missias et al. (1997) Development 124, 5075
But AChR-ε are required for long-term maintenance of NMJ and survival.

Witzemann et al. (1996) PNAS 93, 13286

Missias et al. (1997) Development 124, 5075
Natural Motor Neurone Death...
MN death is a normal part of PRENATAL development
Target size regulates the number of motor neurones
Growth Factors possibly implicated in activity-dependent, negative-feedback control of motor neurone survival:

BDNF
CNTF
GDNF
TGFβ
CT-1
HGF
VEGF
Reg-2
Fas
Muscle paralysis inhibits embryonic motor neurone death
The mitochondrial Bcl-2/Bax System regulates motor neurone apoptosis
Natural Synapse Elimination...
Muscle fibres are initially “polyneuronally” innervated
Physiological methods of measuring PI

\[ PI = \frac{((A+B)-AB)}{A} \]

\[ PI = \frac{AB}{A} \]
POLYNEURONAL INNERVATION OF SKELETAL MUSCLE IN NEW-BORN RATS AND ITS ELIMINATION DURING MATURATION

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(Received 27 February 1976)

Text-fig. 1. The compound e.p.p. Intracellular recording (a.c.) from a soleus muscle fibre of a 5-day-old rat. The soleus nerve was stimulated twice during each of six superimposed sweeps. The first shock was graded in strength, while the second was kept supramaximal. Each of the three different e.p.p. components was recruited at sharply defined threshold levels. The muscle was paralysed with d-tubucurarine, 1 μg/ml.
Text-fig. 4. Percentage of soleus muscle fibres innervated by more than one axon at different ages, determined from intracellular recordings in curarized muscles. At least twenty fibres were examined in each muscle. Three or more e.p.p. components were seen in many fibres, especially at early ages, suggesting that the loss of extra synapses may be well under way before day 10. Continuous line drawn by eye to fit the observations.
Synapse elimination during postnatal development establishes mononeuronal innervation of motor endplates

*Walsh & Lichtman (2003). Neuron*
Time lapse imaging of synapse elimination

Animation courtesy of Jean Livet & Mark Terasaki
Loss of motor neurones?
Or elimination of connections?
Motor unit size can be estimated from their isometric forces

Text-fig. 5. Measurement of motor unit size in an immature muscle. A, shows the tension generated in the soleus muscle of a 3-day-old rat by stimulation of a single ventral root filament and, for comparison, the tension produced by maximal stimulation of the whole nerve. B, shows, at a fast sweep speed, the unitary action potential recorded en passage from the soleus nerve (see Methods) after stimulation of the ventral root filament. Note the very slow time course of the contraction, which is characteristic of immature muscle.
Motor unit size decreases postnatally

Text-fig. 6. Size and number of soleus motor units at different ages. Each vertical line represents observations on one animal. The ordinate gives the size of motor units expressed as a percentage of the maximal twitch to direct stimulation. Filled circles (●) give the mean size of motor units in each muscle; horizontal lines indicate the individual measurements for the lowest-threshold motor unit in each ventral root filament. Open circles (○) show the average motor unit size one would expect in the absence of polyneuronal innervation [100 x (total number of units⁻¹)]. In three muscles (2, 5 and 17 days) this value is not given because a partial nerve block in the region of the ventral roots prevented completion of the motor unit count.
Synapse elimination during postnatal development establishes mononeuronal innervation of motor endplates


Neurones retract some of their synapses while stabilising others

Is Synapse Elimination globally “programmed” or due to local “competition”…
Neuronal/synaptic competition:

“The negative effects that one neurone has on others by consuming, or controlling access to, resources at synapses that are limited in availability”

“Intrinsic” withdrawal?
Or “competitive” take-over?
The rate of synapse elimination is activity-dependent

- Stimulation
- Paralysis
- Normal
“When an axon of cell A is near enough to excite a cell B and repeatedly and persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A’s efficiency as one of the cells firing B is increased.”

From
Transgenic expression of a growth factor, GDNF, delays elimination

Evidence for competition: no change in size of surviving motor units after partial denervation at birth

Betz et al. (1980)

Evidence against competition: surviving motor unit size continues to decline after partial denervation at birth

Fladby & Jansen, 1988
We can determine motor unit size in thy1.2-YFP mice by counting the number of muscle fibres that are innervated.
Neonate

~ 1 month after partial denervation

Adrianna Teriakidis
Motor unit sizes 2 days after partial denervation are larger than motor unit sizes 4-6 weeks later.
Is every one of a dominant motor neurone’s synapses a winner?

Motor units may compete in a “dominance hierarchy”

<table>
<thead>
<tr>
<th>MU</th>
<th>Label</th>
<th>MU size (number of muscle fibres)</th>
<th>Average synaptic occupancy (entire MU)</th>
<th>Number of co-innervated junctions</th>
<th>Outcome</th>
<th>Average synaptic occupancy (co-innervated junctions)</th>
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<td>1</td>
<td>CFP</td>
<td>102</td>
<td>64 ± 8%</td>
<td>12</td>
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<td>76 ± 3%</td>
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<td>15 ± 4% (yellow)</td>
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<td>brYFP</td>
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<td>dYFP</td>
<td>12†</td>
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<td>3 (5 versus 7)</td>
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<td>13 ± 2%</td>
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<td>2 (6 versus 7)</td>
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<td>85 ± 4% (yellow)</td>
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<td>89 ± 2% (yellow)</td>
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Disease correlate: Spinal Muscular Atrophy

- SMA Type I (Werdnig Hoffman disease)
- Neonatal, “floppy baby”
- Fatal, no cure
- Incidence 1:15,000 births
- 95% cases have mutations in SMN1 gene
- Compensation by SMN2 gene in other SMA types
- Mouse KO/transgenic models show NMJ defects
Impaired NMJ form and function in neonatal NMJ’s in smo knockout mouse models of SMA
Summary of key stages in the development of rodent NMJ’s

NMJ Expand
NMJ Reshape
AChR $\gamma \rightarrow \epsilon$
NMJ Elim
Myelin Form
NMJ Form
MF Form
MN Die
MN Form

-20 days  Birth  +30 days

Progressive
Regressive
Remodel
Myelin formation...
Schwann cells arise from the neural crest

Myelin forms from compacted Schwann cell membranes.
Myelin sheaths form postnatally

Fig. 3. Number of myelinated axons in extramuscular SOL nerve at different ages. Each point refers to one nerve.

Regeneration...
Partial denervation triggers axonal sprouting
Axonal sprouting is preceded by Schwann cell sprouting

Son et al (1996) TINS 19,280
Regeneration reverses axonal sprouting

**Neuromuscular Synapse Elimination in Reinnervated Adult Skeletal Muscle**

A 15 days

B 30 days

C 45 days

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Fig. 3. Examples of isometric twitch (left) and tetanic (right) tension measurements in partially denervated lumbrical muscles after crushing the lateral plantar nerve, showing regression of sprouted motor units and recovery of reinnervated motor units with time. In each case, the lowest trace is the response to stimulating the regenerating LPN axons; the middle trace is the response to stimulating intact SN motor axons; and the top trace is due to combined nerve stimulation. The recovery of the LPN responses was accompanied by a decrease in the latency of the isometric twitch, as conduction velocity in the regenerating axons also recovered. A, 15 days after LPN crush; muscle containing four SN motor units. B, 30 days post crush; three SN motor units. C, 45 days post crush; three SN motor units.
Polyneuronal innervation ($\pi$) in reinnervated adult muscles resembles development.
Polyneuronal innervation in reinnervated muscle is also activity-dependent …
...but some polyinnervation persists at some NMJ after activity resumes
SUMMARY

• Neuromuscular Junctions (NMJ’s) are an excellent model system for studying synaptic development

• Motor neurone (MN) birth, muscle fibre growth, myelin formation and endplate expansion are progressive features of motor unit development

• MN death and synapse elimination at NMJ’s are regressive features of motor unit development

• Endplates additionally undergo structural remodelling and adjustments in AChR expression

• Some aspects of MN/NMJ development are competitive, establishing hierarchies of motor units

• Motor unit development goes awry in diseases such as Spinal Muscular Atrophy, due to mutations in SMN genes

• Axonal and synaptic regeneration after nerve injury partially recapitulates neuromuscular development