Involvement of NMJ in mouse models of Motor Neurone Disease

Outline
1. Early signs of degeneration at NMJ’s: “Compartmental Degeneration”
2. Motor Neurone Disease (SMA/ALS)
3. SMA and SMN mouse model
4. ALS and SOD1 mouse model
5. Does synaptic degeneration imply synaptopathy?

Typical Human Motor Unit (TA)

|  | Diameter (µm) | Length (µm) | Number | Volume (µm³) | Re Soma Volume
|---|---|---|---|---|---|
| Axon | 10 | 1,000,000 | 1 | 700,000,000 | 2248
| Collaterals | 5 | 2,000 | 1000 | 709,941,22 | 7225
| Terminals | 10 | 20 | 1000 | 100,000 | 9

The ‘Life Cycle’ of Neuromuscular Synapses

“Compartmental Neurodegeneration” Hypothesis

Apoptosis Wallerian Degeneration “Synaptosis”

Neonatal SE Axotomised WldS MND

π, µ, δ

Poly (π), mono (µ), and denervated (δ) endplates occur together in adult EN1-mutant mouse muscles

Gillingwater, Blanco & Ribchester 2003 J Neurocytol. 32:863-81
Gillingwater & Ribchester 2001 J Physiol. 534:627-39
Synapse elimination occurs by reciprocal retraction and takeover of motor endplates.

Neurones retract some of their synapses while stabilising others.

Synapse elimination is completed over the first three postnatal weeks.

Synaptic degeneration precedes axonal degeneration after axotomy in WldS mice.

Synaptic protection by WldS is lost with age.

The time course of synaptic degeneration in young WldS mice is similar to that of developmental synapse elimination.
**Compartamental Neurodegeneration** Hypothesis

<table>
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<tr>
<th>Apoptosis</th>
<th>Wallerian Degeneration</th>
<th>Synaptosis</th>
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-/+ neonate Axotomised Wld\(^S\) MND

Gillingwater & Ribchester 2003, J Neurocytol. 32:863-81

*“Defeating Motor Neurone Diseases through Innovative Fundamental Research”*

http://www.euanmacdonaldcentre.com/

**FIVE QUESTIONS**

- Are motor neurones selectively vulnerable to disease and if so why?
- What biological processes are responsible for the induction, progression and spread of MND in contiguous regions?
- What overlap is there between MND and other neurodegenerative diseases?
- What are the molecular signalling pathways in different forms of MND and at what point(s) do they converge?
- Why is age a risk factor for MND?

**Motor Neurone Disease**

Primary lateral sclerosis (PLS)
Progressive/Spiunal Muscular Atrophy (SMA/PMA)
Amyotrophic lateral sclerosis (ALS)

**Spinal Muscular Atrophy (SMA)**

- 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 85.
- 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 Smn gene, which has 92% amino acid identity with its human homolog and a similar expression pattern
• Homozygous Smn deletion results in massive embryonic cell death and lethality at birth
• Expression of a human SMN2 transgene on the 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
Amyotrophic Lateral Sclerosis (ALS)

"La Maladie de Charcot" (1862)
Sclérose Latérale Amyotrophique (SLA)

Jean-Martin Charcot
(1825-1893)

Une leçon clinique à la Salpêtrière, André Brouillet (1887).
Signs and Symptoms of ALS

- Adult onset, mostly middle-late age
- Initial symptoms vary but may include muscle twitching (fasciculation) or cramping; difficulty swallowing
- Progressive weakness, paralysis and muscle wasting
- Intellectual capacity unaffected in most cases but some forms associated with fronto-temporal dementia

Summary of facts about MND/ALS

- Incidence 2/100,000
- Prevalence 5/100,000
- Life expectancy from diagnosis: 2-5 years
- Ca. 50-95% of cases are “sporadic”
- Ca. 5-10% of cases are “familial”
- Causes: unknown for sporadic ALS; mechanism is unknown for familial ALS
- Age is the only known risk factor
- Disease frequently has a specific initiating focus then spreads to contiguous regions
- Disease is initiated in MN but progression is dependent on defects in glia and/or other non-neuronal cells
- Motor nerve terminals degenerate before axons and motor neurone cell bodies
- Glutamate transporters are deficient in spinal cord
- Reactive astrocytes invade ventral horn grey matter
- Motor neurones show dispersal of nuclear TDP43 protein
- The only drug licenced for treatment is riluzole (suppressor of glutamate release); prolongs life by ca. 3 months with no effect on quality of life

Types of ALS

- 90% “Sporadic”
- 10% “Familial”

ALS onset occurs mostly in middle to late age

Normal ALS

Muscle fibre-type grouping in ALS

Normal ALS

(Source: unknown - slide courtesy Brian Dickie, MNDA)

Few, large motor unit potentials in ALS

Normal EMG

Motor neurone disease (eg ALS)

Types of ALS

SOD1-dependent ALS

- 1993: ~2% of total cases have mutations in SOD1, the most common familial form of the disease
- 1994: Transgenic mice overexpressing mutant SOD1 develop ALS-like signs of MND. Onset and progression of disease in these mutants are proportional to transgene copy number
- 1994: Riluzole benefits MND/ALS patients
- 1996: Riluzole extends life in SOD1 transgenic mice
- 1996+: SOD1 mice become the predominant animal model of ALS.

90% “Sporadic”
10% “Familial”
2% SOD1 mutations
**SOD1**
- One of three forms: (Cu/Zn) superoxide dismutase
- Homodimer of molecular weight 32,500 kD
- Catalyses reaction: \( \text{O}_2^- + 2\text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2 \)
- Ubiquitously expressed
- 100 different mutations found throughout protein
- Many have normal enzyme activity

\[ \rightarrow \text{"Toxic Gain-of-Function"?} \]

Possible mechanisms of SOD1-induced motor neurone toxicity:
- Excitotoxicity
- ER stress
- Inhibition of proteasome
- Mitochondrial damage
- Abnormal secretion of mutant SOD1
- Generation of extracellular superoxide
- Disrupted axonal transport
- Microhaemorrhages of spinal capillaries


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SOD1<sup>G93A</sup> mice develop progressive hindlimb paralysis

"Symptomatic" Endstage

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

Summary of disease onset and progression in SOD1 mice

Kaplan-Meier Plots: Onset

Low copy # (7-15)  High copy # (20-25)

Kaplan-Meier Plots: Survival

Low copy # (7-15)  High copy # (20-25)


Gurney et al. (1994) Science 264,1772-1774


Synapses degenerate before axons in SOD1G93A mice

12 week old - asymptomatic

Conventional

Confocal Z-series projection

SOD1G93A muscle NMJs: synaptic "autotomy"?
Degenerating neuromuscular junctions in the SOD1\textsuperscript{G93A} mouse model of MND/ALS -

Symptomatic

Synapses degenerate before axons in SOD1\textsuperscript{G93A} mice

Skeletal muscle injection

Other motor units in SOD1\textsuperscript{G93A} mice compensate by sprouting

Symptomatic 8 month-old mouse thy1.2:YFP/SOD1\textsuperscript{G93A}

Caveats

- Both onset and progression of disease in hSOD1\textsuperscript{G93A} mouse mutants are proportional to transgene copy number
- No human cases are due to overexpression of SOD1
- Neither knockout nor overexpression of normal mSOD1 in mice produces ALS
- No convincing treatments that ‘rescue’ hSOD1 mice or substantially delay onset or progression of disease
- Reported benefits treatments in hSOD1 mice usually ineffective or harmful in human clinical trials
The minocycline debacle...
(Minocycline is a broad spectrum tetracycline antibiotic)


The minocycline debacle...

Additive Neuroprotective Effects of Minocycline with Creatine in a Mouse Model of ALS

The presence of ... uncontrolled confounding variables in the screening system, and the failure of ... several drugs to demonstrate efficacy in adequately designed and powered repeat studies, leads us to conclude that the majority of published effects are most likely measurements of noise in the distribution of survival means as opposed to actual drug effect.
Infamy! Infamy!!.. They've all got it in for me!!!
And finally…

... “Caveat emptor”

Synaptic failure precedes axon degeneration

Wallerian Degeneration

Synaptic vesicles are degraded within 6-15 h

6h axotomy 15h axotomy


SUMMARY

1. Selective degeneration of synapses in development, after nerve injury, or in disease is consistent with a “compartmental” model of neuronal maintenance and degeneration.

2. NMJ abnormalities are early pathophysiological signs in several forms of motor neurone disease.

3. SMN mice appear to be a good model of SMA; SOD1 mice may not be such a good model of ALS.

4. Synaptic degeneration does not necessarily mean synaptopathy: presynaptic terminals react badly to malfunctions elsewhere in the motor neurone.

5. Protecting synapses may nevertheless mitigate progression of disease and, perhaps, ameliorate quality of life.