Involvement of NMJ in the SOD1 mouse model of Motor Neurone Disease

Outline
1. Motor Units and Amyotrophic Lateral Sclerosis (ALS/MND)
2. hSOD1<sup>G93A</sup> transgenic mouse model
3. Neuroprotective challenges - the Wld<sup>S</sup> mouse revisited

**The 'Life Cycle' of Neuromuscular Synapses**

**Typical Human Motor Unit (TA)**

<table>
<thead>
<tr>
<th>Description</th>
<th>Diameter</th>
<th>Length</th>
<th>Number</th>
<th>Volume</th>
<th>Re Soma Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soma</td>
<td>1 µm</td>
<td>40 µm</td>
<td>1</td>
<td>335 µm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td>Axon</td>
<td>10 µm</td>
<td>1,000 µm</td>
<td>1</td>
<td>785 µm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2345</td>
</tr>
<tr>
<td>Terminals</td>
<td>4</td>
<td>2,000 µm</td>
<td>1500 µm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>9</td>
<td>37699112</td>
</tr>
</tbody>
</table>

**Drosophila 3rd Instar Larva: Motor Unit**

Soma diameter ~ 10 µm ; Volume ~ 500 µm<sup>3</sup>
Axon length ~0.3-3 mm ; Volume ~ 750 - 3000 µm<sup>3</sup>
NMJ length ~ 100 µm x 3 = 100 boutons: Volume ~ 500µm<sup>3</sup>

Segment A1: fibre 6/7 NMJ
Segment A2: fibre 8/7 NMJ
"Compartmental Neurodegeneration" Hypothesis

<table>
<thead>
<tr>
<th>Apoptosis</th>
<th>Wallerian Degeneration</th>
<th>Synaptosis</th>
</tr>
</thead>
</table>

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

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

Jean-Martin Charcot (1825-1893)

"Defeating Motor Neurone Diseases through Innovative Fundamental Research"

http://www.euanmacdonaldcentre.com/

ALS onset occurs mostly in middle to late age

ALS/MND Duration

Signs and Symptoms:
- Usually late-onset (Mean 45-56 years)
- Initial symptoms vary (eg muscle twitching or cramping; difficulty swallowing)
- Progressive paralysis and muscle wasting
- Life expectancy 2-5 years from diagnosis
- Intellectual capacity unaffected

ALS/MND Duration

Courtesy of Michael Strong, UWO
"The finding of a cure for ALS lies in a better understanding of the biological mechanisms that lie behind the loss of motor neurones. It can only be won through basic research conducted over the long term."

Ulla-Carin Lindquist, Rowing without Oars, published by John Murray, London

### ALS Genetics

<table>
<thead>
<tr>
<th>Gene</th>
<th>Inheritance</th>
<th>Age of onset</th>
<th>Locus</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD1</td>
<td>AD</td>
<td>Adult</td>
<td>21q22.1</td>
<td>Normal lifespan</td>
</tr>
<tr>
<td>FIG4</td>
<td>AD</td>
<td>Adult</td>
<td>1p36.2</td>
<td>Familial + sporadic</td>
</tr>
<tr>
<td>VAPB</td>
<td>AD</td>
<td>Adult</td>
<td>20q13</td>
<td>Normal lifespan</td>
</tr>
<tr>
<td>TARDBP</td>
<td>AD</td>
<td>Adult</td>
<td>14q22</td>
<td>Familial + sporadic</td>
</tr>
<tr>
<td>FUS</td>
<td>AD</td>
<td>Adult</td>
<td>16q12</td>
<td>Normal lifespan</td>
</tr>
<tr>
<td>TAR</td>
<td>AD</td>
<td>Adult</td>
<td>1q41</td>
<td>Familial + sporadic</td>
</tr>
<tr>
<td>ALS1</td>
<td>AD</td>
<td>Adult</td>
<td>21q22.1</td>
<td>Normal lifespan</td>
</tr>
</tbody>
</table>

- 90% sporadic: cause unknown
- 10% familial

### Loss of motor neurones in ALS

Normal

ALS

(Tsukagoshi et al, 1979; J Neurol Sci 41, 307-307)

### Muscle fibre-type grouping in ALS

Normal

ALS

(Source: unknown - slide courtesy Brian Dickie, MNDA)
Summary of 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 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 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 licensed for treatment is riluzole (suppressor of glutamate release); prolongs life by ca. 3 months with no effect on quality of life

SOD1-dependent ALS

- 1993: ~2% of total cases have mutations in SOD1
- 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.
**SOD1**
- (Cu/Zn) superoxide dismutase
- Catalyses reaction:
  \[ 2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2 \]
- Ubiquitously expressed
- 100 different mutations found throughout protein
- Many have normal enzyme activity
- Gain of toxic function?

Cu\(^{2+}\) + SOD + O\(_2\) \rightarrow Mn\(^{2+}\) + SOD + O\(_2\)
Cu\(^{2+}\) + SOD + O\(_2\) \rightarrow Cu\(^{3+}\) + SOD + H\(_2\)O\(_2\)

Cu\(^{2+}\) + SOD + O\(_2\) \rightarrow Mn\(^{2+}\) + SOD + O\(_2\)
Cu\(^{2+}\) + SOD + O\(_2\) \rightarrow Cu\(^{3+}\) + SOD + H\(_2\)O\(_2\)


**SOD1\(^{G93A}\) mice develop progressive hindlimb paralysis**

“Symptomatic” Endstage


FIG. 1. Change in weight and spinal lengths of G93A male and female mice with enzymes compared to age-matched, wild-type control mice. Each symbol and line represents an individual mouse. The arrow indicates the median age at onset of illness in G93A mice (91 days).

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

Synapses degenerate before axons in SOD1G93A 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)


Robert Hartley

thy1.2::YFP16/SOD1G93A

SOD1G93A muscle NMJs : synaptic "autotomy"?
Synapses degenerate before axons in SOD1<sup>G93A</sup> mice

Robert Hartley

Caveats
- Both onset and progression of disease in SOD1<sup>G93A</sup> mutants are proportional to transgene copy number
- No human cases are due to overexpression of SOD1
- Neither knockout nor overexpression of normal SOD1 in mice produces ALS
- Mechanism of ALS in SOD1 mice is still unknown (best hypothesis: toxic ‘gain of function’ affecting intracellular organelles and axonal transport)
- No convincing treatments that ‘rescue’ SOD1 mice or substantially delay onset or progression of disease
- e.g. reported benefits of minocycline in SOD1 mice; but ineffective or harmful in human clinical trials

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


Findings
ALSFRS-R score deterioration was faster in the minocycline group than in the placebo group (-4.3 vs -1.8; p=0.005). Patients on minocycline also had non-significant tendencies towards better decline in FVC (-1.4 vs -0.5; p=0.10) and MMT score (-0.3 vs -0.2; p=0.11) and greater mortality during the 9-months treatment phase (hazard ratio=1.32, 95% CI 0.83 to 2.10; p=0.23). The difference in mortality was not significant between the groups. No differences were seen in non-serious gastrointestinal and neurological adverse events.

Interpretation
Our findings that minocycline has a harmful effect on patients with ALS have implications for trials of minocycline as a potential neuroprotective agent for patients with other neurological disorders.
The available evidence suggests that historically there has been significant publication bias in that there is an overrepresentation of small studies reporting beneficial results with a relative paucity of published small studies showing no treatment effect. This may generate an overly optimistic impression of the utility of the experimental model for identifying potentially useful therapeutic agents for human clinical trials.

Most treatment trials in the mouse model of ALS are of limited methodological quality. Legitimate questions surround the use of treatment success in the SOD1 mouse to guide the selection of therapeutic agents for evaluation in human clinical trials.

SOD1 mice may nevertheless be good test-beds for developing new diagnostic methods.

We (clinicians, scientists and MND patients) urgently need:
- Better understanding of risk factors for MND, including genetic risk factors
- Better ‘biomarkers’ for early detection, permitting accurate and early diagnosis; monitoring of disease progression; and efficacy of treatment
- Better animal models of MND
- Better ways to preserve and protect motor neurones from degeneration
- Better ways to promote rapid and accurate compensation or regeneration of axons and their neuromuscular connections

**In vivo CME showing degeneration of distal axons and NMJ in SOD1G93A mice**

**The "Cell Vizio" confocal microendoscope**

- Proximal end of ProFlex fibre bundle
- x,y resolution: ~4 µm
- z resolution: ~15 µm (WD=0)

- Probe holder
- Laser beam tuning
- Laser power
- To the best of my knowledge
Possible defects and therapeutic targets in ALS

- Neuroinflammation
- Aberrant Axonal Transport
- Cytoskeletal Defects
- Mitochondrial Defects
- Neurotrophic Insufficiency
- Protein Misfolding & Aggregation
- Excitotoxicity

**Synaptic Degeneration precedes axonal degeneration after axotomy in WldS mice**

**Synapses degenerate in WldS homozygotes over 3-10 days**

(YFP does not interfere with the WldS phenotype)

*YFP16: WldS - 5d axotomy*

**Synaptic protection by WldS is lost with age**

*Graphs: D. Thomson*

**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?