Motor Units and Motor Neuron Disease

1. a) What is a Motor Unit?

1 alpha neuron and all of the muscle fibres it innervates.

b) What is a Motor Pool?

All the motor units which give rise to the innervation of one muscle (e.g. biceps)

c) What is a Motor Neuron?

A multipolar neuron which has one axon that branches into muscle fibres and causes a contraction of all the muscle fibres in its motor unit.

Question 1 notes

Upper Motor Neurons  
Brain → Spinal Cord  
Lower Motor Neurons  
Spinal Cord → Muscles

Larger human muscles can contain around 1000 motor units in one motor pool. The mouse models used in the 2 papers we looked at would have around 10 motor units in the motor pool.

2. What is Amyotrophic Lateral Sclerosis (ALS)?

a) What are its causes?

90-95% - sporadic/spontaneous.
   i) Trauma etc.

5-10% - inherited
   i) 20% SOD1 (Superoxide Dismutase 1) mutation (This accounts for only 1-2% of all ALS cases)
   ii) juvenile ALS2 mutation

b) Pathophysiology

i) Accumulation of harmful superoxide radicals. SOD1 is involved in the metabolism of these free radicals, therefore a mutation in SOD1 leads to free radical build-up.

ii) Microglial activation → inflammatory response involving cytokines and positive feedback. Schwann cells are a type of glial cell found in the PNS whereas oligodendrocytes & astrocytes are glial cells found in the CNS.

iii) Toxicity of proximal cells affects the rate of degeneration as well as the toxicity of the neuron itself.

iv) VEGF dysregulation. VEGF promotes the growth of neurons and protects them from hypoxia (low levels of oxygen) induced injury. In
normal patients VEGF is upregulated in the face of hypoxia. In
diseased patients the response to hypoxia is to downregulate VEGF
implying VEGF dysregulation in ALS.

v) *Glutamate concentrations in synapses.*

**Normal** – Glutamate is removed from the synapses of upper motor
neurons → lower motor neurons by the enzyme EAAT2.

**Disease** – Mutation in EAAT2 means that glutamate is not removed
from the synapse. This leads to the degeneration of the motor
neron by the following pathway:-

Increase in synaptic Glutamate concentration (↓ EAAT2)
↓
Activation of Ca\(^{2+}\) permeable Glutamate receptors
↓
↑↑[Ca\(^{2+}\)] intracellular
↓
Excitotoxicity
↓
Neuronal Death

2. b) i) notes

**SOD1**: - The protein encoded by this gene binds copper and zinc ions and is
one of two isoymes responsible for destroying free superoxide radicals in the
body. The encoded isozyme is a soluble cytoplasmic protein, acting as a
homodimer to convert naturally-occurring but harmful superoxide radicals to
molecular oxygen and hydrogen peroxide. The other isozyme is a
mitochondrial protein. Mutations in this gene have been implicated as causes
of familial ALS. Rare transcript variants have been reported for this gene.

c) **Clinical Symptoms** 5 in 100,000 are affected

1) Twitching and cramping
2) Slow onset (Age of onset – 50-60 years, also cases of juvenile onset.
   Diagnosis dependant (!). Fatality usually 3-5 years after diagnosis)
3) Speech and coordination of fine movement impaired.
4) Increased reflexes (and some abnormal ones e.g. Babinski’s reflex –
   instead of moving down, the big toes moves upwards indicating upper
   motor neuron damage)
5) Spasticity
6) Difficulty swallowing
7) Bladder control and eye movements are spared (research into these
   motor neurons for future therapies?)
8) Failure of respiratory muscles → fatal
9) Majority of cases the senses remain unimpaired. Intelligence and
   personality remain.
10) Some cases of ALS can effect Cognitive capability – misdiagnosis?
2c) notes

ALS affects 3 different types of muscle fibres with varying degrees of susceptibility: -

1st affected – Fast twitch (fatigable)
2nd affected – Fast twitch (fatigue resistant)
3rd affected – Slow twitch

d) Clinical Analysis

Animal Model
Stain for axon loss. Staining myelin with YFP. Anti-green FP is used to enhance visuals.

Human
- EMG recordings show enlarged spikes when a neuron is showing compensatory growth.
- Testing reflexes and for muscle fasiculations.
- Loss of sensory function rules out ALS
- MRIs.

3. a) What is the model used in the paper?

The transgenic mouse model called G93A-SOD1 was used in the first paper as a model for human ALS. The SOD1 gene contains a mutation at position 93 where Glycine is replaced by Alanine (hence G93A). Yim et. Al (1996) found that the ALS symptoms observed in G93A transgenic mice were not caused by the reduction of SOD1 activity, but induced a gain-of-function result – an enhancement of the free radical generating function. Crystallographic studies showed that the active channel for the G93A mutant is larger than that of the wildtype, thus it’s more accessible to peroxide.

b) Is it a good model? Why?

It is a good model because it successfully represents the gain-of-function of SOD1 found in some familial cases of ALS. However since this only affects 1-2% of all ALS sufferers it is not a good model for studying ALS in its entirety, but with no other significant leads for sporadic cases, research into SOD1 mutations remains one of the few ways we can further our understanding of ALS.

c) Is there a better model that could be used?

The mutation of A4V (position 4 Alanine → Valine) in exon 1 (of 5 exons) was found to be the most common of all SOD1 mutations (of which there are 20-50 depending on which literature you look at). The A4V mutation is the most clinically severe. G93A and A4V mutations act in similar ways. An A4V-SOD1 transgenic model would have also been a good choice, but since more
work has been done with the G93A-SOD1, it would have been a ‘safer’ choice.

In the second paper, a new model for ALS was stumbled on by accident. They found that after axotomy, Wld$^S$ axons were protected from degeneration at all ages, but synapses were not. This finding provides the potential for a good model for the synaptic degeneration observed in ALS.

What is interesting is that the Wld protein is localised to the cell nucleus, so the protein itself cannot be directly responsible for the protection of axons after axotomy. This provides new avenues for researchers to explore with regards to other factors involved in Wld expression.

4. What triggers degeneration and compensation and how do they occur?

Neuronal degeneration:
Neuronal cell death is mediated by the process of apoptosis (‘cell suicide’). Cell death signals are modulated by a family of proteins known as BCL-2. These are stimulated by various death signals (including DNA damage, growth factor deprivation, death receptor activation etc.) and act on the mitochondria to cause Bax and Bak to become inserted in the outer mitochondrial membrane, therefore leading to the release of apoptotic factors. The most significant apoptotic factor is cytochrome C, which acts to activate the enzyme caspase-9 and so lead to cell death.

As mentioned in the pathophysiology section, there are a wide variety of triggers implicated in the motor neurone degeneration seen in ALS. The main two implicated currently implicated in ALS are:

Oxidative damage – as a result of a mutant SOD1, superoxide radicals accumulate hence cause damage. This is an intrinsic death signal, hence stimulating the process described above. There is also new information that shows the presence of SOD1 on the inner mitochondrial surface? (IMS), hence the mutation in the SOD1 can also lead to direct damage of the mitochondria.

Excitotoxicity - free radical damage to the mitochondria and age can both decrease the levels of ATP produced (see below). This in turn increases the sensitivity of the cell to glutamate, hence causes an accumulation of Ca ions. A defective EAAT2 transporter can also lead to the activation of the NMDA receptors shown, also causing an increase in intracellular Ca. In both cases increased Ca levels result in toxicity of the mitochondria, which leads to activation of apoptotic pathways, hence cell death and neurodegeneration.
Neuronal regeneration:
The process of neuronal compensation is different in ALS than regeneration in a nerve after direct damage, as the neurone that is doing the compensation is a different one than the one that is degenerating:

The target cell (in this case muscle) releases something called neuronal growth factor (NGF). This binds to the neurone and both it and its receptor is internalised. It then acts directly on the nucleus to increase production of neurotransmitters and to stimulate axonal growth. Growth then occurs by the standard method, i.e. by filaments of the cytoskeleton pushing out the membrane as the axon lengthens.

-NB I couldn’t find much on neural regeneration in ALS, all the stuff I found was about after injury and that seemed to be different, but I hope this stuff is relevant…

5. Current and future treatments for ALS.

1. **Riluzole** (antibiotic) – Glutamate antagonist. Only increases survival by a matter of months. Unsure at to *when* it is most effective. It acts best on Bulbar patients suffering from swallowing difficulties.
2. **Minocycline** (antibiotic) – blocks microglial activation.
3. **Ceftiaxone** (antibiotic) – increases the concentrations of naturally occurring transporter proteins taking Glutamate away from the synapse.
4. **Treat Effects**
   a. Getting rid of saliva
b. Preventing depression  
c. Delaying respiratory failure  
d. Feeding tubes for patients with swallowing problems

Future treatments?
1. **VEGF treatment** – to promote the regrowth of neurons.
2. Trying to find ways to stimulate endogenous stem cells to stimulate new motor neurons to grow.