This paper has two parts:

Section A: Short Answer / Problem / Experimental Design questions (50 marks)
- Answer all questions in the spaces provided on the examination paper

Section B: Essay question (marked out of 100, weighted 50 marks)
- Answer either question A or question B or question C
- Write your answer on the separate paper provided and attach it to the back of the question paper using the treasury tag provided
- The marks available for each question are indicated on the paper

For marker use only:

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SECTION A: Short Answer / Problem / Experimental Design questions

Answer all questions in the spaces provided

Mark total for this section: 50

1) A newborn shows arthrogryposis multiplex congenita and, as a fetus, it was observed to have fewer spontaneous contractions than expected. The mother does not show any neuromuscular transmission symptoms. Hypothesize a mechanism that explains these observations. (3 marks)

**LO:** Articulate the role of key players in neuromuscular transmission and NMJ development.

The mother produces antibodies against the gamma subunit of the AChR (1), which is only expressed in the embryo but declines and is replaced by the epsilon subunit from birth, so the mother would lack any symptoms (1). During pregnancy, anti-gamma subunit antibodies from mum would cross the placenta and would render the receptor inefficient in the fetus, hence the reduced contractions (1).

2) Briefly describe the rationale and molecular basis for treating patients with a mutation causing AChR clustering defects at the neuromuscular junction. (4 marks)

**LO:** Articulate the rationale of therapeutic treatments for neuromuscular transmission disorders.

Lack of clustering will result in reduced transmission because of the lower concentration of AChRs (1). Any treatment that enhances neuromuscular transmission may be applied (1). This include cholinesterase inhibitors to increase the number of AChR activated by each quantum such as pyridostigmine (1) or Voltage-gated K+ channels blockers to increase the number of quanta released by the nerve impulse such as 3,4-DAP or ephedrine (1). Marks will not be awarded for treatments to counteract autoantibodies, as these would not apply to a genetic condition.

3) a. Below is a diagram of the tetanus holotoxin. For the domains named in the figure, describe their function with regard to toxin action (3 marks)
Light chain: enzymatic digestion of target synaptic protein, blocking synaptic transmission

Hₐ domain: binding of ganglioside target on synapse, facilitates uptake

Hₙ domain: translocation of the light chain from the synaptic vesicle to the cytoplasm via a pore formed in the vesicle membrane - translocates toxic enzyme to cytoplasm

b). Both toxins attach to the motorneuron initially. Where is the critical site of action for: (2 marks)

i) Botulinal toxins

   Motorneuron pre-synaptic terminal (1 mark)

ii) Tetanus toxin

   Presynaptic terminal of the spinal interneuron (1 mark)

Generally done well. Most students could assign a function to each domain correctly showing an understanding of toxin mechanism
c. In the figure below, hippocampal cultures were treated with Tetanus Toxin and either Tetrodotoxin (TTX, blocks action potentials) or high potassium (high K+, generates action potentials). Cultures were then fixed and stained for the synaptic vesicular glutamate receptor (vGlut1) and synaptobrevin (syb II).

![Image of hippocampal neurons with vGLUT1 and syb II staining](image)

a) Provide an explanation for the difference in staining intensity for synaptobrevin (syb II) in the TTX and high K+ conditions. (2 marks)

Synaptic activity (endocytosis) is required for TeTx entry (1 mark) and degradation of syb II (1 mark).

Very few students were able to make the inference between activity and uptake of the toxin. Much guessing was done on the stability of the SNARE complex and accessibility to the toxin. Quite a discriminatory question.

b) What is the functional consequence of the reduction in synaptobrevin? (1 mark)
Loss of synaptic transmission

Majority of students answered this correctly.

**LO: Understand the mechanisms by which Tetanus Toxin and Botulinal Toxin bind to target cells, enter them and incapacitate synaptic function**

4. Dominant mutations in the SOD1 gene cause Familial Amyotrophic Lateral Sclerosis (FALS). Describe the sequence of events that occur at the neuromuscular junction in a mouse globally expressing the G93A-SOD1 protein. (4 marks)

In young mice, pre- and post-synaptic components of the NMJ will look wildtype (1 mark). As SOD1 mice age, the neuron will fragment and retract leaving the post-synaptic material essentially intact (1 marks). A subpopulation of motoneurons are resistant to death and will generate ‘compensatory growth’ that re-populate the end-plate (2 marks)

Many students appeared to miss the specific leading point of the ‘events that occur at the neuromuscular junction’. There was much information that was not specific to the NMJ: mitochondrial dysfunction, glial dysfunction, excess glutamate causing excitotoxicity. The mammalian NMJ is cholinergic, therefore the excess glutamate is happening at the other end of the neuron from the synapse.

5a. What are the barriers and limitations to treating various Lysosomal Storage Diseases with Enzyme Replacement Therapy? (2 marks)

Blood Brain Barrier will prevent enzymes crossing to the brain. This therapy can only work for soluble proteins, not membrane proteins, and so will only work for diseases affecting somatic tissue, not neuronal. Can be got around via a stent.

Majority of students mentioned BBB, and impermeability to large molecules such as proteins.

5b. What are the potential alternative therapies that may be employed to overcome these barriers and how do they work? (6 marks)
Substrate reduction therapy - small molecules cross the BBB, reduce the amount of potential substrate that would build up in the lysosome. (2 marks)
Small molecule chaperone therapy - imino sugars help mutant enzymes fold and evade the ERAD system restoring some function in the lysosome (2 marks)
Bone marrow therapy - cells carrying normal copies of LSD related membrane proteins can infiltrate the CNS (2 marks)

BMT, Substrate reduction therapy, chaperone therapy were all mentioned and generally described well. Where errors crept in was when the ‘drugs used to silence ERAD’ (not true). Stent was also mentioned and accepted because it was a working therapy. Many failed to hit a full answer by omitting to mention that SRT and chaperone therapy employed small molecules that could cross the BBB. Neuronal stem cells were not accepted as correct for the reason that the BBB is still a barrier (BMT delivers to the brain directly).

LO: Appreciate that the routes to therapeutic intervention have to be based on nature of the lysosomal protein deficit and the major organs affected by the disease

6. It was recently reported that mutations in the Fbxo7 gene are linked to autosomal recessive Parkinson’s. It has been proposed that the FBXO7 protein has a mitochondrial localisation signal, but others suggest it acts at the nucleus. Outline an experimental program to determine the cellular actions of FBXO7, and how its role may fit with other proteins associated with Parkinson’s. (10 marks)

Raise an antibody; determine its subcellular localisation in control and patient tissue (eg fibroblasts) and/or use GFP tags (3 marks). Create animal model to mimic human mutations and examine whether models recapitulate cellular distribution ie same is true for fly neurons (1 marks). Marks also given to those who sensibly suggested looking at how manipulating the mitochondrial localization sequence affected this.

State MT hypothesis: Use genetic manipulations and look for epistasis to show if it is linked to pink1/parkin, will pink/parkin/other MT gene rescue loss of Fbxo7 / Will expression of Fbxo7 rescue PINK/parkin. (3 marks)
State Nuclear hypothesis: Is level of asynuclein affected? Binding to promoter of asynuclein ? Level of acetylation affected? Interaction with UDCA or salbutamol ? (3 marks) Marks also for microarrays to look for changes in transcription, or CHIP.

Ideas around Western blot and Co-IP also got marks - there were a lot of
inventive ideas.

LO: Draw together information ... cellular, molecular, physiological and behavioural approaches to the understanding the (dys)function of the brain.

7. Explain how astrocytes support ongoing neuronal activity. (3 marks)

Release of neurotransmitter leads to increased [glutamate] uptake (1 mark) by astrocytes, which requires Na, and ATP, so increasing glial metabolism (1 mark). This increases glucose consumption by astrocytes leading to lactate production (1 mark) by replenishing the ATP. Lactate is taken up by neurons and used as a substrate, so providing them with energy (1 mark)

LO: Draw together information ... to provide a cohesive view of anatomical, cellular, molecular, physiological and behavioural approaches to the understanding the (dys)function of the brain.

Generally good answers, but needed to emphasise the link to neural activity - glutamate release - glutamate uptake

8. Deep brain stimulation (DBS) has proven successful for the treatment of Parkinson’s disease. A researcher has seen a report that DBS may also be beneficial for Alzheimer’s disease (AD). The researcher now wants to fully evaluate its efficacy for the treatment of late-onset AD.

a) Outline a research program to test whether DBS improves AD symptoms, or whether it can be a disease modifying therapy. You may use an animal model for these experiments (6 marks)

Answers should identify a relevant model or identify a human sample population(1 mark)- The model could be a rodent or non-human primate model based on AD mutations in APOe4 (1 mark). To evaluate whether DBS improves symptoms answers should propose a DBS protocol and brain region to receive stimulation (1 mark) and cognitive testing using learning paradigms to evaluate short-term and long-term memory (1 mark) while checking for undesirable side effects using sensory/motor tests (1 mark). Answers should include longitudinal analysis and evaluate long-term prognosis (1 mark).

Many answers were focused on early onset models (a-beta or tau) which were disappointing. Few people assessed if DBS was detrimental in wildtype mice.
Lots of good detail about Morris water maze. Key point is to separate symptomatic and disease modifying therapy - time course of atrophy, or test learning of each mouse with DBS on and off. A few people suggested testing acquisition of memory and retention separately - nice idea! Some suggested systematic testing of different regions, but most focused on the hippocampus and cortex, and gave a reason for doing so - well done. I think the paper in the lecture notes focused on frontal cortex.

b) How could the researcher investigate the cellular and molecular basis for any improvements in disease pathogenesis. (4 marks)

Answers could identify ways to examine the effects of DBS on neuronal viability/death (1 mark) in fixed brain sections using relevant markers (1 mark) . This means focusing on APOe4 or cholesterol as markers. Doing investigations into cholinergic signalling was a really good idea. Students could suggest looking at synaptic structures/dendritic spines (1 mark) by staining using presynaptic and postsynaptic markers (1 mark).

Alternatively, suggestions to examine tau/Abeta plaques are also acceptable. This generally got a mark, but it needed to explain why this was being done, in a late-onset model, when APOe4 should be the main focus.

Since DBS involves electrical signalling it could induce LTP and strengthen synaptic connections. At least one answer proposed using electrophysiology to test LTP - excellent idea.

Generally this part was a bit disappointing as there was little link between DBS and the measures being examined - how might DBS affect cell biology?

LO: Draw together information … cellular, molecular, physiological and behavioural approaches to the understanding the (dys)function of the brain. Design experiments related to brain function in health and disease.

   Explain the major social consequences of neurodegenerative disease;
   Explain the neural basis of neurodegeneration (to expert and lay audiences)..

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SECTION B: Essay question
Answer one question on the separate paper provided

Remember to write your candidate number at the top of the page and indicate whether you have answered question A or B or C

Mark total for this section: 50

EITHER

A) How has the gate control hypothesis illuminated our understanding of pain perception and therapies targeted at pain relief?

Key point: this hypothesis has been particularly useful in devising experiments, identifying the pharmacology of the spinal cord (opioids, tachykinins), understanding the effect of Tens machines, and of psychological stimuli (mummy rub it better). This leads to better understanding of opiate analgesia, and novel therapies addressing Na channels and synaptic transmission by primary afferents.

LO Show understanding of the molecular, cellular, physiological and anatomical basis of pain perception, animal models of nociception, and the action and social consequences of current and future therapies

OR

B) To what extent has the sequencing of the human genome fulfilled its potential towards the understanding and treatment of neurodegenerative diseases?
Key points: genetic understanding has advanced lysosomal disorders, and suggested therapies, but in MS, PD, and AD the link between the genetic models (worms, flies, fish, rodents) and treatment is still tenuous. New GWAS studies are highlighting new genes (now up to 24 in PD) so the reducing cost of gemone sequencing will bring future advances. Role in new gene therapy (gene editing).

Many essays focused on data from human gene studies, rather than human genome sequencing. HGS promised to identify the causes, offer faster diagnostics and personalized medicine, and GWAS surveys do manage to identify genes/chromosomal regions linked to disease. A reference Genome has been crucial in many studies. Showing mutations are causative requires more effort. Once mutations are identified, it opens the way for animal and cell models. HGS data is key to Crispr/CAS9 based genome editing (ensuring no off-target effects), or to microarray or RNA seq approaches. Big, unexpected bonus, is the wide range of bioinformatic data and tools.

Watch out that the presenilins and other genes were identified by positional cloning and other techniques used before HGS.

LO: Draw together information from different lectures to provide a cohesive view of anatomical, cellular, molecular, physiological and behavioural approaches to the understanding the (dys)function of the brain.

- Explain the major social consequences of neurodegenerative disease.

OR

C) TARDP, also known as TDP-43 is found as insoluble intracellular plaques in some forms of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). Mutations in TDP-43 also lead to familial forms of ALS with TDP-43 positive plaque pathology.

Outline a series of experiments that could be used to examine the role of TDP-43 in ALS-FTD progression in vivo.

I have specifically shown the students how to formulate an experimental outline type of question, this essay also covers two lectures and quite bit of the material in them, plus some directed extra reading.

The essay should start with a reiteration of the question with some
additional information, specifically the concept of tdp-43 having prion-like domains of low-complexity. Tdp-43 is an RNA binding protein and is involved in stress granule formation where mRNAs are sequestered. Recent work has suggested that the low complexity domain acts to trigger a 'phase-shift' to segregate the tdp-43 and mRNA into a 'gel'-like compartment. Continued stress causes protein aggregation and deposition. Mutations in tdp-43 accelerate this process. This is the central idea that they should be following. I would then expect three main aims/objectives to test the role of this hypothesis in vivo, using either transgenic mice expressing mutant tdp-43, or heterozygous mutants, and combinations of these in another model of tdp-43 pathology positive FTD/ALS (such as C9ORF72). I have explicitly directed them to explain their thinking as they work through their objectives, and this will be assessed in relation to the outlined hypothesis.

Majority of answers were able to deliver a good background on the disease. Background on TDP-43 was patchy, and the absence of this showed in the subsequent answers - the proposed experiments did not really act from an understanding of TDP-43 function. Few answers proposed decent aims and objectives as directed. Many answers set up a model system, and went on the validate the model. This was good, but often the answers given thereafter were lacking. Giving a mouse ALS-FTD tells us that we can give a mouse ALS-FTD - but is not informative about the disease. Making the model, as a Drosophila or mouse often lacked specifics about the nature of the mutation. TDP-43 mutations in patients are dominant. The model was often ‘introduce mutations into mouse via Cas9/CRISPR’ - but no specifics on the mutation given and the outcomes thereafter made it unclear which type of mutation the author intended. Major deficit overall was the missing information that TDP-43 proteinopathy is seen in ~90% of ALS patients, but mutations only occur in 2-3% of patients. Aggregates occur due to stress generally, mutations predispose to aggregation. Also lack of background on P-bodies/stress granules and the role of TDP-43 in forming ‘membrane-less organelles’ in the form of ‘gel-like’ formations in the cell.