Introduction
Parkinson’s disease is an upper motor neuron disease that affects 1 in 500 people in the UK[1]. In patients with Parkinson’s disease, there is a degeneration of the nerve cells in the brain (mainly in the substantia nigra) that produce dopamine[2]. The cause for this degeneration is currently unknown. The ability to differentiate mouse embryonic stem cells (mESC) into dopaminergic neurons will open further avenues of research into why these neurons degenerate in Parkinson’s.

Aims
• Establish a protocol for differentiating mouse embryonic stem cells (mESC) into dopaminergic neurons.
• Perform immunofluorescence staining to confirm neuronal and dopaminergic differentiation.

Cell Culture

Stage 1. mESC proliferation
Figure 1. mESC of the CGR8 cell line were cultured in a media containing Leukaemia inhibitory factor (LIF) to prevent spontaneous differentiation. (x10 magnification)

Stage 2. General differentiation
Figure 2. The cells were grown as a monolayer in a media containing 10µg/ml N2 and 20µg/ml B27 supplements (N2B27 media) for 12 days. (x20 magnification)

Stage 3. Neuronal expansion and dopaminergic priming
Figure 3. These cells had been cultured in a N2B27 media containing 400ng/ml sonic hedgehog, 20ng/ml bFGF and 100ng/ml FGF for 6 days. (x20 magnification)

Stage 4. Neuronal and dopaminergic differentiation
Figure 4. These cells had been cultured in a N2B27 media containing 200µM Ascorbic acid and 1µg/ml laminin and were kept in a hypoxia incubator at 37ºC for 5 days. (x20 magnification)

Immuno-fluorescence for neuronal markers
The cells were labelled with TuJ1 (Neuromics) and anti-Tyrosine Hydroxylase (Abcam) antibodies, using standard protocols. TuJ1 (stained green) is a marker for all neurons and Tyrosine Hydroxylase (stained red) is a specific marker for dopaminergic neurons. DAPI (stained blue) stains the nuclei of all cells.

Stage 2. General differentiation
Figure 5. No TuJ1 or TH positive cells after general differentiation at x20 magnification

Stage 3. Neuronal Expansion
Figure 6. TuJ1 positive cells but no TH positive cells after neuronal expansion at x20 magnification

Stage 4. Dopaminergic Differentiation
Figure 7. Many TuJ1 positive cells and some TH positive cells after final differentiation stage at x20 magnification

Figure 8. Many TuJ1 positive cells and some TH positive cells after final differentiation stage at x40 magnification

Conclusion
• We successfully differentiated mESC into dopaminergic neurons.
• However, the protocol needs to be refined for higher yields of dopaminergic neurons.

References