# Differentiation of Mouse Embryonic Stem Cells into Dopaminergic Neurons

Rebecca Webb-Mitchell* ▪ Gabriele Saretzki
*100359077 ▪ Programme of study: MBBS ▪ email: r.webb-mitchell@ncl.ac.uk

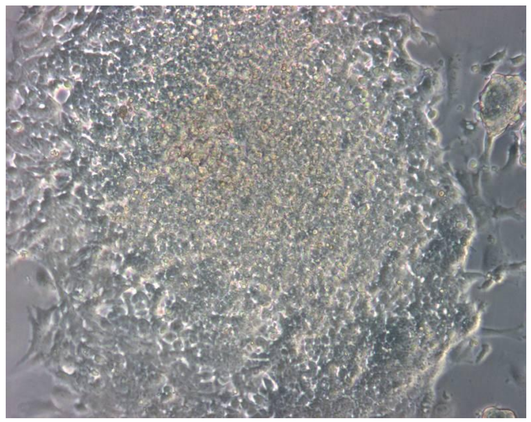
## Introduction

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

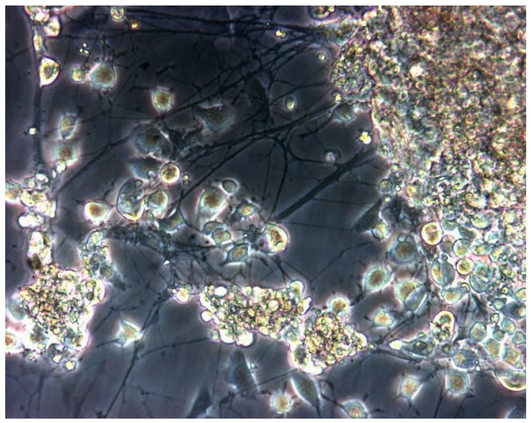
•Establish a protocol for differentiating mouse embryonic stem cells (mESC) into dopaminergic neurons.
•Perform immunofluorescence staining to confirm neuronal and dopaminergic differentiation.

## Cell Culture



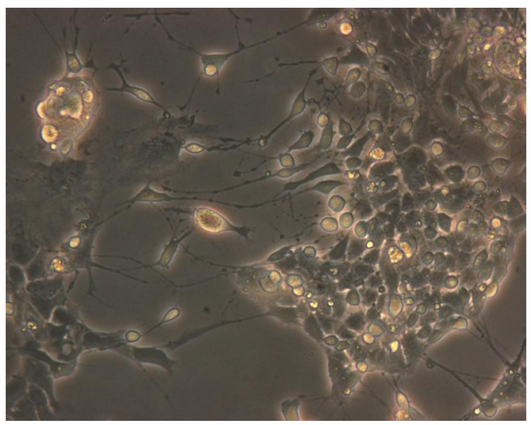
### Stage 1. mESC proliferation

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



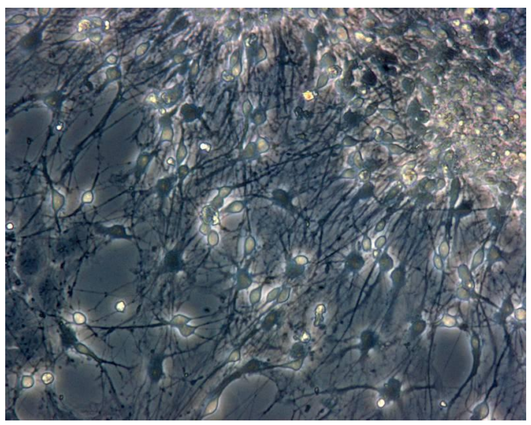
### Stage 2. General differentiation

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

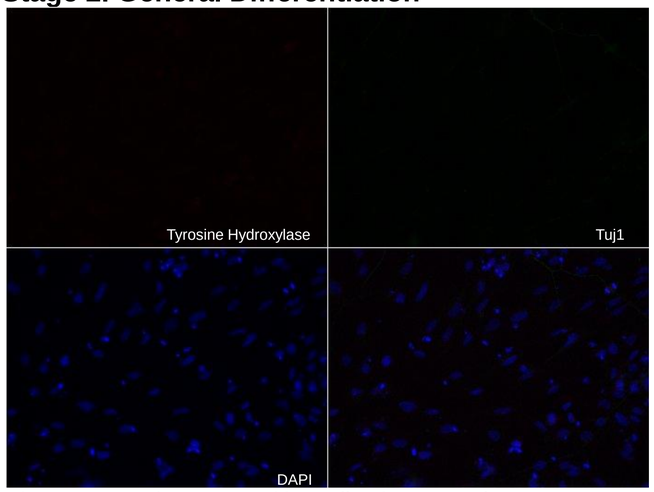



Figure 5. No Tuj1 or TH positive cells after general differentiation at x20 magnification

### Stage 3. Neuronal Expansion

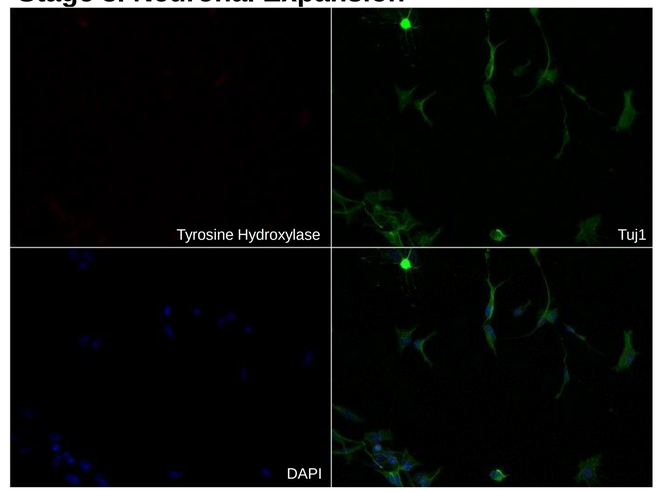



Figure 6. Tuj1 positive cells but no TH positive cells after neuronal expansion at x20 magnification

### Stage 4. Dopaminergic Differentiation

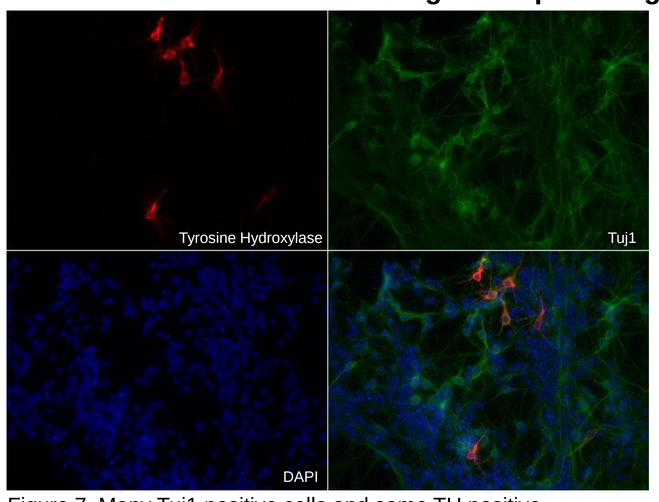



Figure 7. Many Tuj1 positive cells and some TH positive cells after final differentiation stage at x20 magnification

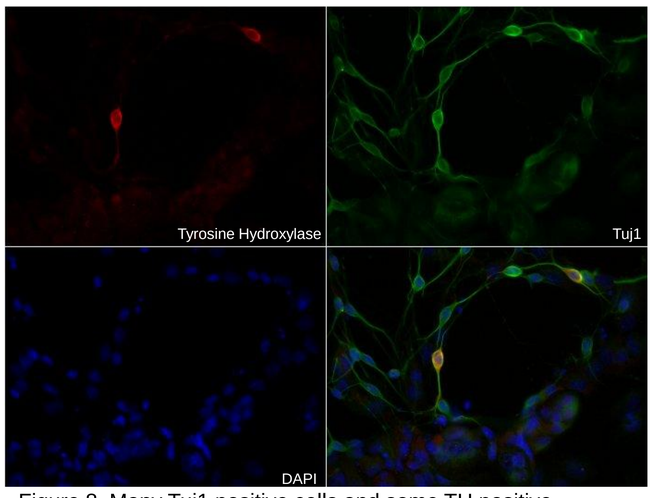



Figure 8. Many Tuj1 positive cells and some TH positive cells after final differentiation stage at x40 magnification

## Conclusion

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

## References

1.Parkinson's UK. *What is Parkinson's?* 2012 [17/08/2012]; Available from: http://www.parkinsons.org.uk/about_parkinsons/what_is_parkinsons.aspx.
2.Meissner, W.G., et al., *Priorities in Parkinson's disease research.* Nat Rev Drug Discov, 2011. **10**(5): p. 377-393.