

Introduction & Objectives

There are a number of molecules involved in the migration of interneurons to the cerebral cortex during mouse corticogenesis. The ephrins are the principle family of guidance molecules that either attract or repel interneurons to their desired destination. Literature in this field has recently begun to blossom, however, little has been identified in the role of ephrinA2 with interneuronal migration. We therefore decided to set out three major techniques to investigate this: **1)** Expression profiles to view migratory pathways of ephrinA2/GAD positive cells in an embryonic mouse brain. **2)** Investigate the presence of interneurons in the cortex of wild type and ephrinA2 knock-out mice. **3)** Use siRNA to degrade ephrinA2 RNA and investigate the location of interneurons. It is important to discover more about interneurons, as well as study the molecules involved with their migration, as problems with this have been identified in a number of diseases, including Autism and Schizophrenia.

- Using an embryonic mouse at the age of 15.5 days (E15.5), we dissected the brain away from the head.

We then froze the brain and cut it into 15µm coronal sections using a cryostat, whilst immediately slide-mounting the sections. We collected 3 series consisting of two slides per series.

Following this, we stained one series for **ephrinA2 and GAD65/67** (a vital enzyme involved in GABA synthesis during neuronal development). We stained using a fluorescently labelled secondary antibody and viewed under the microscope.

- Our results showed (see Figure 1) that ephrinA2 positive interneurons migrate from the preoptic area to the cortex in a similar way to other ephrin positive interneurons (see Figure 2).

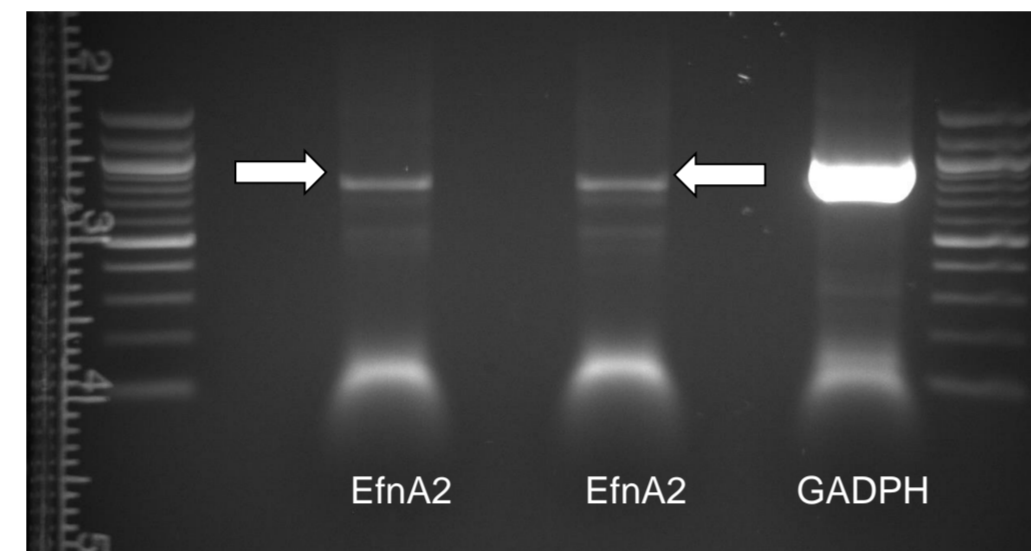


Figure 3: An example of a gel showing the ephrinA2 cDNA band following a PCR reaction containing cDNA and an efnA2 primer.

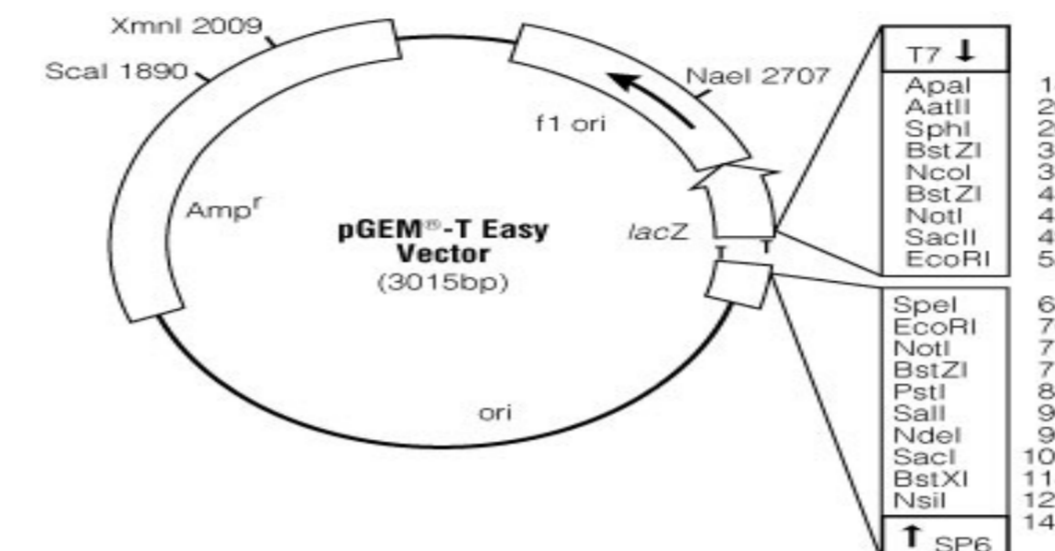


Figure 4: A vector map of the pGEMT plasmid used to clone an ephrinA2 product containing a 'sticky end' restriction site and 'blunt end' restriction site.

- We firstly designed the **siRNA** complimentary to the ephrinA2 RNA product.
- We then carried out RNA extraction on an adult mouse brain, followed by reverse transcriptase PCR using ephrinA2 specific primers, forming **ephrinA2 cDNA**. We ran this cDNA on a gel (see Figure 3) and carried out gel extraction on the ephrinA2 cDNA band.
- We then used a hybrid plasmid (pCAGGS derivative) to insert the ephrinA2 cDNA using restriction endonucleases. Following an incubation period, a Miniprep Test and further cutting with restriction endonucleases, we discovered that the plasmid was very poor at integrating the cDNA insert.
- Therefore, we developed a new method of inserting the cDNA into a plasmid vector. We used the **pGEMT** plasmid (see Figure 4) and cut both the plasmid and the cDNA using two different restriction endonucleases to produce one blunt end and one sticky end. This means that the plasmid cannot re-ligate on itself, increasing the chance of incorporating the cDNA insert.
- Due to the time limitations of my project, I did not progress further on this. However, our aim was to produce a vector with the cDNA insert and a separate vector with the siRNA insert. We could then inject these vectors into **HEL cells** to test if the siRNA removes the ephrinA2 RNA product. If it did, then we could test the siRNA on mouse tissue via electroporation of the vector into one hemisphere of a developing mouse brain. We would then let the brain develop and dissect the brain away, identifying the location of the interneurons (in a similar way as described in the right hand column).

- Taking a number of adult wild type and ephrinA2 **knock-out** mouse brains, we could carry out immunohistochemistry upon coronal sections cut at **40µm**.

We would stain for a number of markers using either **fluorescently labelled secondary antibodies**, or using **DAB**.

- Our results show (see Figure 5) that in the adult wild type mouse cortex, there is a much greater density of ephrinA2/calbindin (a calcium binding protein present in some types of interneurons) positive interneurons than seen in the efnA2 knock-out mice in layers 2 and 3.

However, we are still undertaking the cell counts on these sections to quantify the results. These results show us that ephrinA2 is an important molecule in the migration of interneurons to the cerebral cortex during corticogenesis.

Furthermore, we can see clearly in the wild type mouse a distinction of the **barrel field** in layer 4, which is not seen clearly in the knock-out mouse. This may indicate problems with sensory input for these mice.

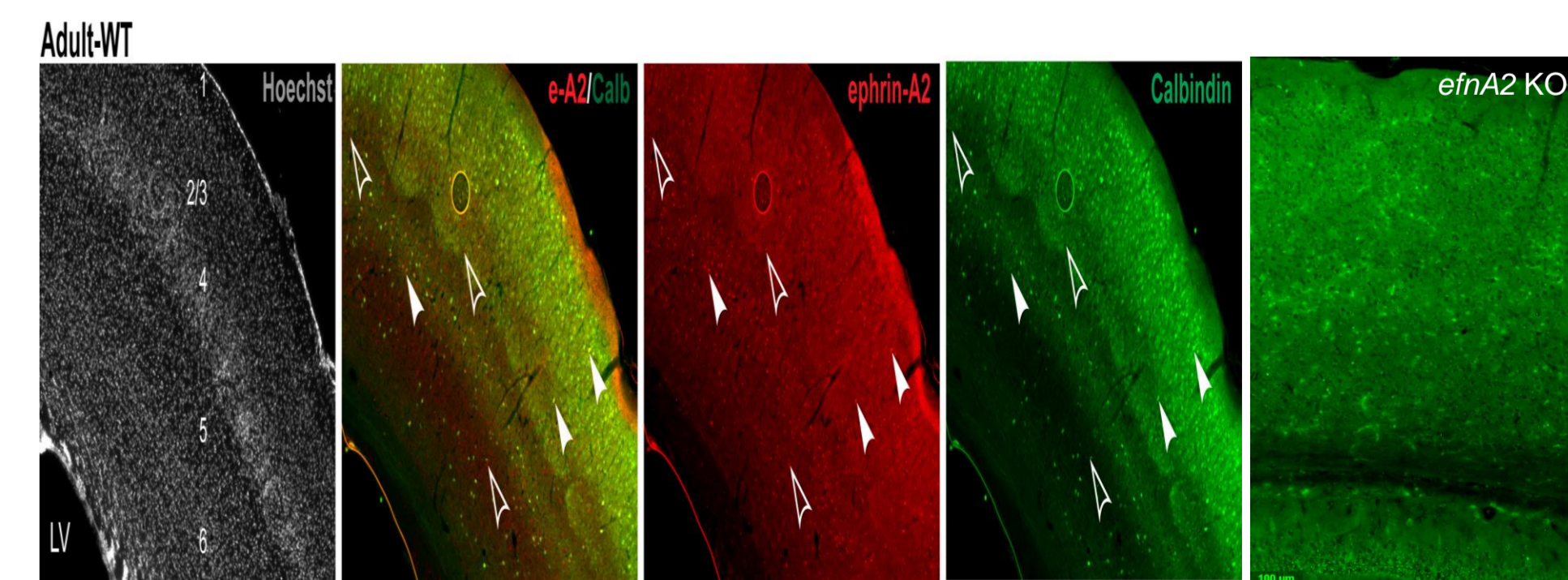


Figure 5: Images showing the presence of calbindin and ephrinA2 positive cells in the cortex of an adult wild type mouse. Also shows the presence of calbindin positive cells (interneurons) in the cortex of an adult ephrinA2 knock out mouse.

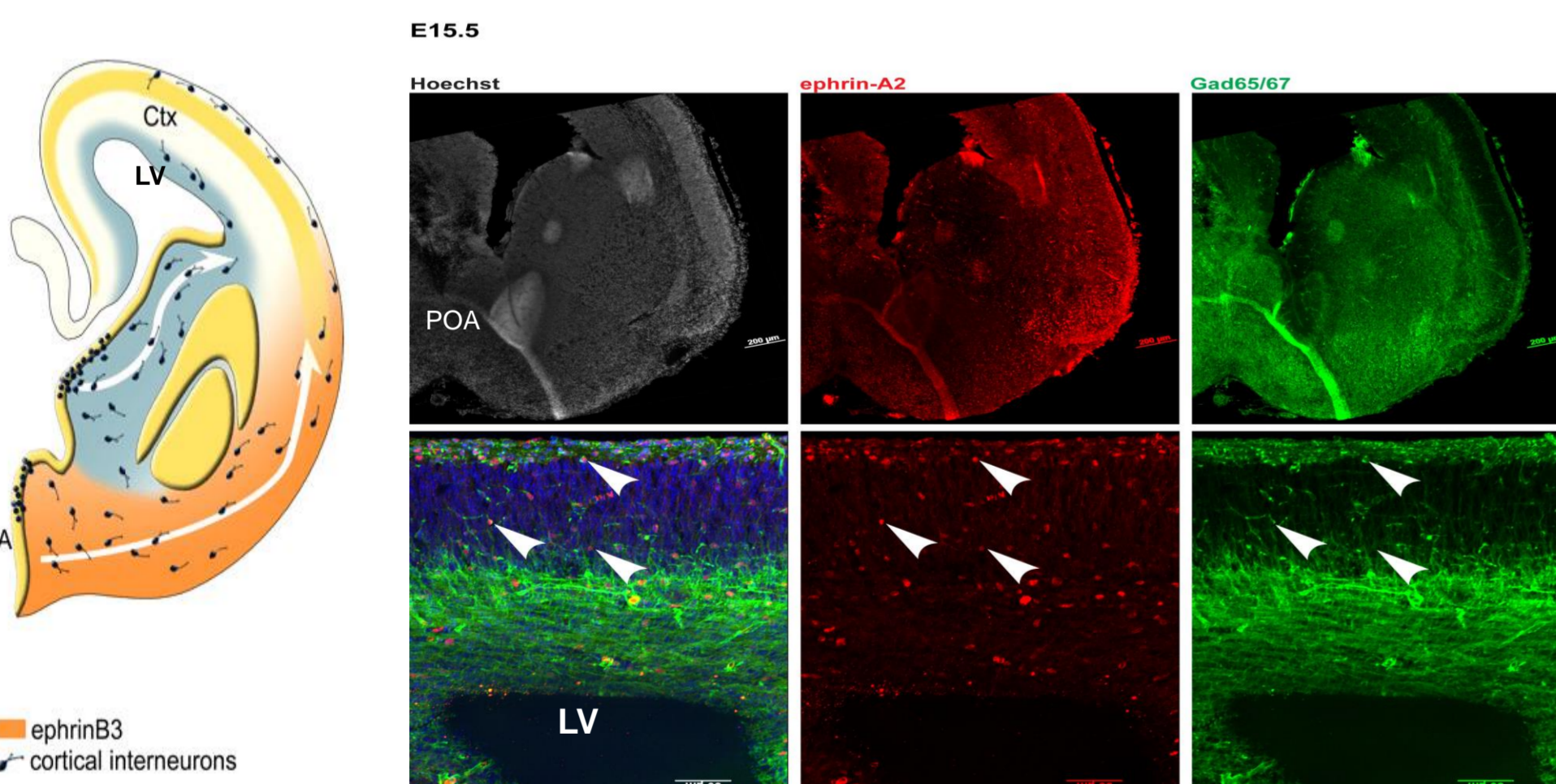


Figure 2: A diagram showing the migration pathway of ephrinB3 interneurons. (Adapted from Zimmer et al.)

Figure 1: Expression profile of ephrinA2 positive and GAD positive cells in an embryonic (E.15.5) mouse brain. Also shows an image combining the two, with a Hoechst stain identifying all cells in the cortex.