Investigating the expression profile of *EphA5, EphA6* and *EphA7* in the developing marmoset visual cortex

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The neocortex is the outermost region of the brain and is responsible for processing the sensory information. The neocortex is organised into functionally unique and cytoarchitecturally distinct areas. To understand how the borders between cortical areas arise during development, we investigated the role of the Eph/ephrin family of guidance cue molecules in selectively guiding cortical neurons to specific regions, in a primate model. RNA in situ hybridisation revealed the homogenous expression of the receptors EphA5, -A6 and –A7 across the visual cortex. After birth, EphA5 adopted a distinct profile across the cortex, suggesting an area-specific migration.

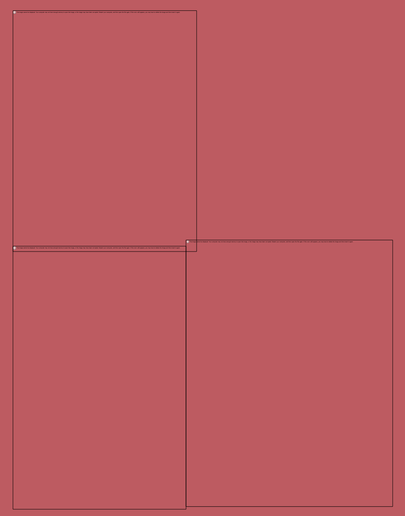
Introduction

The neocortex is responsible for the processing of sensory information and it consists of many functional areas with the number of areas increases as the species become more complex. Mice rely primarily on olfaction whereas primates rely heavily on vision and hearing and so the cortex is divided up according to the importance of these senses to the organisms' survival. The primate brain consists of two main types of neurons: pyramidal dopaminergic neurons which are excitatory and can project their axons to distant parts of the nervous system, and GABAergic interneurons which are inhibitory and whose axons are only projected within a localised area. The cortex has 6 morphologically distinct layers which are seen throughout all of the areas, with layer 1 being the outermost layer, and layer 6 being the innermost layer. During development, before these layers form, transient layers exist including the ventricular zone (VZ) and subventricular zone (SVZ) where neurogenesis occurs. Neural progenitor cells proliferate to form these neurons, which will then migrate radially to the outer layers, but it is not known what causes these newly-born neurons to decide where to migrate to, or when to stop migrating. In this study we are going to look at the Eph/ephrin family of guidance molecules, as these have previously been shown to have a role in morphogenesis and axon guidance. In particular, we are going to look at the EphA5, EphA6 and EphA7 receptors to see whether they are present in the developing cortex. Studies have previously been carried out in the rhesus monkey (*Macaca mulatta* and *Macaca fascicularis*) however perhaps a more suitable model is the common marmoset (*Callithrix jacchus*) as it has a smaller body size and a faster development to sexual maturity. In addition, the surface of the cortex in the marmoset is smooth (lysencephalic) and this will make it easier to study in development, as there will be less folding of the cortex as it develops. In the marmoset, corticogenesis has begun by embryonic day 80 and by embryonic day 130, all 6 cortical layers are present. We will therefore look at these two stages, in addition to 2 postnatal stages to study the differences in expression of *EphA5, EphA6* and *EphA7* mRNA in the cortex.

Methods

- The brains of four marmoset monkeys (*Callithrix jacchus*) aged embryonic day (ED) 80 (one animal), ED130 (one animal), postnatal day (PD) 5 (one animal) and PD14 (one animal) were used in this study.
- The marmosets were injected with a lethal dose of sodium pentobarbital, perfused transcardially and warmed to 37°C, followed by a paraformaldehyde fixative solution. The brains were then harvested, postfixed in the same fixative solution for 24 hours, cryoprotected in sucrose, frozen in liquid nitrogen and stored at -80°C. Coronal sections were cut at 40µm using a cryostat, collected in series and immediately mounted onto glass microscope slides (SuperFrost Plus).
- The template used to generate the probes in this study are those designed by Mashiko et al. in their paper, Comparative Anatomy of Marmoset and Mouse Cortex from Genomic Expression (Mashiko, Yoshida et al. 2012).
- RNA *In-situ* hybridisation was carried out on the sections under RNase free conditions. After various washing steps and permeabilising the cell membrane with Proteinase K, the sections were then incubated with one of the riboprobes in hybridisation buffer at 60°C for 16 hours. The sections were washed again and treated with RNase to remove any unbound riboprobe to reduce background noise. The sections were then incubated with anti-DIG antibody overnight at 4°C before being incubated in NBT/BCIP staining solution.

Results



- *EphA5, EphA6* and *EphA7* expression is seen at ED130 and PD5 and 14 and restricted to certain layers, particularly layer 4 and VZ
- After birth, *EphA5* expression is seen in different layers in the different areas.
- Expression is more intense in the primary visual area of MT and in V2, compared to V1 and V3

Discussion

- A distinct profile of *EphA5* was seen in the postnatal brain, suggesting that there is an area-specific migration
- We were unable to determine the expression profile of *EphA6* and *EphA7* in the developing brain and would need to repeat these experiments

All experiments were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the Monash University Animal Ethics Committee, which also monitored the welfare of the animals.

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