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3D modelling, gene expression mapping and post-mapping image analysis in the developing human brain

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Abstract

As human brain development proceeds, there are complex changes in size and shape, most notably in the developing forebrain. Molecular technologies enable us to characterise the gene expression patterns that underlie these changes. To interpret these patterns the location of expression must be identified and, often, gene expression patterns compared for several genes or across several developmental stages. To facilitate interpretation we have generated a set of three-dimensional models using a recently developed technique, optical projection tomography. The models act as a framework onto which gene expression patterns are mapped and anatomical domains identified using custom-designed software, MAPaint. Here, we demonstrate their use to compare forebrain development at two embryonic stages (Carnegie stages 18 and 21; 44 and 52 days post conception, respectively) and as a means of recording, storing and visualising gene expression data for three example genes *EMX1*, *EMX2* and *OTX2*. Anatomical domains were also mapped to the models and the comparison of gene expression and anatomical data is demonstrated at Carnegie stage 21. The three-dimensional models and sophisticated software facilitate the analysis and visualisation of morphological changes and gene expression patterns during early brain development and can be applied to the development of other complex structures.

Keywords: Mammalian CNS; Telencephalon; Forebrain development; Anatomical domains; Optical projection tomography (OPT); Human

1. Introduction

The telencephalon, the rostral-most part of the neural tube, is the largest and most complex part of the mammalian CNS and shows numerous functional subdivisions, which correlate with distinct gene expression patterns [8]. In order to understand the mechanisms underlying the development of these subdivisions, multiple gene expression patterns need to be analysed both within individual developmental stages and across developmental time. An integral part of the analysis is to identify the specific location(s) where genes are expressed. As complex changes are taking place in brain size and shape, most spectacularly in the telencephalon during human development [6] this can be a difficult task. To facilitate this task, three-dimensional (3D) models of the developing human brain have been generated spanning the major period of organogenesis, Carnegie Stage 12 (CS12; approximately 26 days post conception (dpc)) to CS23 (approximately 56 dpc). The models were generated using optical projection tomography [9], a novel technique, which can rapidly create digital 3D models from unstained intact specimens. Internal and external morphology can be visualised in the models and they provide a 3D framework onto which both anatomical domains and gene expression patterns can be mapped using MAPaint, a set of software developed

Abbreviations: CC, cerebral cortex; Ch, choroid plexus; CS, Carnegie stage; dpc, days post conception; DRG, dorsal root ganglion; LGE, lateral ganglionic eminence; LV, lateral ventricle; MGE, medial ganglionic eminence; OE, olfactory epithelium; OG, optic groove; OPT, optical projection tomography; SC, spinal cord

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as a part of the Edinburgh Mouse Atlas Project [1,2] (http://genex.hgu.mrc.ac.uk/).

Telencephalon development has been compared in OPT models of two developmental stages, CS18 (approximately 44 dpc) and CS21 (approximately 52 dpc) and sample gene expression studies carried out for three genes *EMX1*, *EMX2* and *OTX2*, in order to test the usefulness of the models for comparisons and image-analysis of gene expression patterns and comparisons between gene expression patterns and anatomical domains. The murine orthologues of *EMX1*, *EMX2* and *OTX2*, are known to play key roles in forebrain development [10]. *Otx2* is involved in early specification of the head and at later stages it is also involved with *Emx2* in the specification of the diencephalon [11], *Emx1* is expressed in all pallial regions except the ventral pallium [4] while *Emx2* is expressed strongly in the medial pallium and in a gradient in other pallial areas [4].

2. Materials and methods

2.1. Embryo collection

Human embryos were collected from termination of pregnancy material, with appropriate written consent, approval from the Newcastle and North Tyneside NHS Health Authority Joint Ethics Committee and following national guidelines [7]. Embryos were staged, fixed overnight in 4% paraformaldehyde at 4 °C and either stored in 70% ethanol prior to OPT imaging or wax embedded.

3. OPT

Intact, unstained specimens were rehydrated through a graded series of ethanol and embedded in a block of 1% low melting point agarose. They were dehydrated and cleared and 400 digital images were captured while the specimens were rotated in a full circle with 0.9° steps between each image. The signal corresponded to the weak autofluorescence originating from the paraformaldehyde-fixed tissue and was detected using a wideband FITC filter with excitation at 465–500 nm and emission from 515 to 560 nm. The images were then assembled to recreate the 3D shape of the embryo, using modified tomography algorithms [9].

3.1. Anatomical domain painting

Anatomical domains on the OPT models were ‘painted’ using MAPaint software which was developed in Edinburgh Mouse Atlas project (http://genex.mrc.ac.uk; [1,2]) and then visualised using a 3D rendering program called AVS-Express.

3.2. Probe preparation and tissue in situ hybridization

Sense and antisense probes for *OTX2*, *EMX1* and *EMX2* were synthesized by transcribing linearized plasmid (pGEM3Z) containing 300, 350 and 1200 bp fragment [nucleotides 374–674 of GenBank accession no. NM_021728 (*OTX2*), nucleotides 730–1080 of GenBank accession no. NM_004097 (*EMX1*) and nucleotides 730–1930 of GenBank

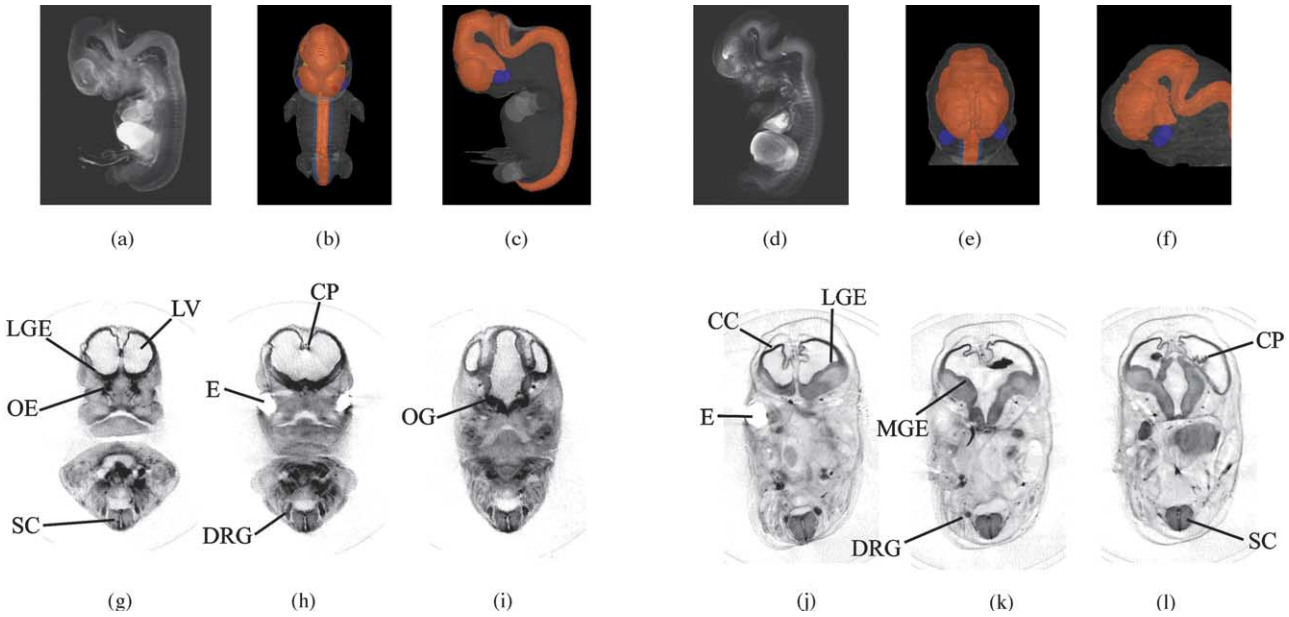

Fig. 1. Comparison of telencephalon development in the CS18 and CS21 models: (a and d) show the volume rendered OPT models of CS18 and CS21. Frontal view (b and e) and lateral view (c and f) of the painted domains of CS18 and CS21 OPT models are shown. The painted domains are neural tube, orange; dorsal root ganglion, light blue (b and e); eye, dark blue; otic pit, yellow (b). Transverse digital OPT sections viewed in MAPaint of the CS18 (g–i) and CS21 (j–l) models. The most rostral section from each model is on the left. CC, cerebral cortex; CP, choroid plexus; DRG, dorsal root ganglion; E, eye; LGE, lateral ganglionic eminence; LV, lateral ventricle; MGE, medial ganglionic eminence; OE, olfactory epithelium; OG, optic groove; SC, spinal chord.