NEUROLOGY AND PRECLINICAL NEUROLOGICAL STUDIES - ORIGINAL ARTICLE

Embryonic mouse medial neocortex as a model system for studying the radial glial scaffold in fetal human neocortex

Samir Vaid[1] · Oskari Heikinheimo[2] · Takashi Namba[3]

Abstract

Neocortex is the evolutionarily newest region in the brain, and is a structure with diversified size and morphology among mammalian species. Humans have the biggest neocortex compared to the body size, and their neocortex has many foldings, that is, gyri and sulci. Despite the recent methodological advances in in vitro models such as cerebral organoids, mice have been continuously used as a model system for studying human neocortical development because of the accessibility and practicality of in vivo gene manipulation. The commonly studied neocortical region, the lateral neocortex, generally recapitulates the developmental process of the human neocortex, however, there are several important factors missing in the lateral neocortex. First, basal (outer) radial glia (bRG), which are the main cell type providing the radial scaffold to the migrating neurons in the fetal human neocortex, are very few in the mouse lateral neocortex, thus the radial glial scaffold is different from the fetal human neocortex. Second, as a consequence of the difference in the radial glial scaffold, migrating neurons might exhibit different migratory behavior and thus distribution. To overcome those problems, we propose the mouse medial neocortex, where we have earlier revealed an abundance of bRG similar to the fetal human neocortex, as an alternative model system. We found that similar to the fetal human neocortex, the radial glial scaffold, neuronal migration and neuronal distribution are tangentially scattered in the mouse medial neocortex. Taken together, the embryonic mouse medial neocortex could be a suitable and accessible in vivo model system to study human neocortical development and its pathogenesis.

Keywords Radial glial scaffold · Neuronal migration · Evolution · Mouse model

Introduction

Brain has experienced enormous changes in its size and morphology during human evolution (Rakic 2009). The most significant changes are observed in the neocortex, the evolutionarily newest brain region which is responsible for higher order brain functions such as cognition (Rakic 2009). One of the prominent evolutionary changes of neocortex is folding, in the other words, gyri and sulci (Heuer et al. 2019; Namba et al. 2019). To accomplish a properly folded brain, neurons need to migrate in a coordinated manner, and disorganization of neuronal migration will result in neocortical malformations such as lissencephaly (Klingler et al. 2021).

Neurons are born in the germinal zones (ventricular zone, VZ; subventricular zone, SVZ) in the developing neocortex. The newly generated neurons then migrate toward the cortical plate (CP), which underlies the surface of the neocortex. Neuronal migration is known to be controlled by many cell-intrinsic and -extrinsic factors (Kawauchi and Hoshino 2008; Namba et al. 2015). Of several cell-extrinsic factors, radial glial scaffolds are thought to play a critical role in guiding neurons from the place of their birth to the final destination, the CP (Casingal et al. 2022). The radial glial scaffold is a structure transiently observed in the developing brain, and is mainly consisted of the basal process of the apical (ventricular) radial glia (aRG) and basal (outer) radial glia (bRG), which are located in the VZ and SVZ, respectively, and act

✉ Takashi Namba
takashi.namba@helsinki.fi

1 Department of Basic Neurosciences, University of Geneva, 1211 Geneva, Switzerland

2 Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, P.O. 140, 00029 Helsinki, Finland

3 Neuroscience Center, HiLIFE - Helsinki Institute of Life Science, University of Helsinki, P.O. 63, 00014 Helsinki, Finland

as neural stem and progenitor cells (NPCs) (Casingal et al. 2022; Namba and Huttner 2017). The trajectory of the radial glial scaffold in the developing neocortex is known to be different between gyrencephalic (e.g., human, macaque and ferret) and lissencephalic species (e.g., mouse) (Gadisseux et al., 1992; Misson et al. 1991; Rakic 2003; Reillo et al. 2011). In the gyrencephalic species, the radial glial scaffold scatters more tangentially than in the lissencephalic species (Reillo et al. 2011). The tangentially scattered radial glial scaffold is thought to navigate the tangential dispersion of neurons, which finally leads to the folding of the neocortex.

Embryonic mouse neocortex has the advantage of accessibility and practicality of gene manipulation to examine the role of factors/genes influencing the radial glial scaffold and neuronal migration as a model system of human neocortex. However, there are two major disadvantages that might hinder the understanding of the human radial glial scaffold. First, the number of bRG is significantly smaller in the embryonic mouse neocortex than the fetal human neocortex (Kelava et al. 2012; Vaid et al. 2018; Wang et al. 2011), thus the majority of the radial glial scaffold consists of the aRG. Second, the radial glial scaffold is scattered less tangentially in the embryonic mouse neocortex, than the fetal human neocortex (Mission et al. 1991; Rakic 2003). We have previously shown that there is a region in the embryonic mouse neocortex where the proportion of bRG to other basal progenitors is similar to the fetal human neocortex at a specific developmental time point (Vaid et al. 2018). The region is the medial neocortex at E18.5. In the present study, we examined whether the structure of the radial glial scaffold in the embryonic mouse medial neocortex is similar to that in the fetal human neocortex to seek the possible utility of the embryonic mouse medial neocortex as a model system to study the human neocortical radial glial scaffold.

Here, we found that the directionality of the radial glial scaffold in the mouse medial neocortex exhibit a tangentially scattered pattern. Furthermore, neurons generated in the medial neocortex migrate tangentially to form a fan-shaped structure. Taken together, these results corroborate the idea that the mouse medial neocortex is a more suitable model system to study human neocortical development than the commonly used mouse lateral neocortex.

Materials and methods

Mice

C57BL/6J mice were used for all experiments using mouse tissue. Mice were maintained in pathogen-free conditions at the animal facility of the University of Helsinki, the University of Geneva, and the Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG). All experiments were performed in accordance with Finnish, Swiss and German animal welfare legislation and were overseen by the Institutional Animal Welfare Officer. Necessary licenses were obtained from the regional ethical commission for animal experimentation in Helsinki (Aluehallintovirasto), in Geneve (Service de la consommation et des affaires vétérinaires), and in Dresden, Germany (Tierversuchskommission, Landesdirektion Dresden). Embryonic mouse brain tissues were dissected in PBS and immediately fixed with 4% PFA overnight at 4 °C, and then washed with PBS followed by 30% sucrose in PBS. The tissues were embedded in OCT compound (Sakura Finetek USA) and frozen using either liquid nitrogen or dry ice. The frozen samples were kept at − 80 °C for long-term storage.

Human tissues

Fetal human brain tissue (GW19) was obtained from the Helsinki University Hospital, with approval by the ethics committee of the Hospital district of Helsinki and Uusimaa (HUS/1170/2021). Fetal human brain tissues were dissected in PBS and immediately fixed with 4% PFA overnight at 4 °C, and then washed with PBS followed by 30% sucrose in PBS. The tissues were embedded in OCT compound (Sakura Finetek USA) and frozen using liquid nitrogen. The frozen samples were kept at − 80 °C for long-term storage.

Antibodies

Antibodies used in this study were as follows; anti-GFP (chicken IgY, GFP-1020, Aves Labs), anti-GLAST (guinea pig IgG, GLAST-GP-Af1000, Frontier institute, Japan), anti-nestin (mouse IgG, 611659, BD biosciences), anti-chicken IgY-Alexa Fluor 488 (donkey, 703–545-155, Jackson ImmunoResearch), anti-guinea pig IgG-Aexa Fluor 647 (donkey, 706–605-148, Jackson ImmunoResearch), anti-mouse IgG-Cy3 (donkey, 715–165-151, Jackson ImmunoResearch).

In utero electroporation (IUE) of embryonic mouse neocortex

IUE was performed as previously described (Namba et al. 2020; Vaid et al. 2018). Briefly, pregnant mice with E13.5 embryos were anesthetized with isofluorane, followed by subcutaneous injection with the analgesic (0.1 ml, Metamizol, 200 mg/kg). In some cases Tamgesic (0.1 ml, Tamgesic, 200 mg/kg) was used as the analgesic and was given as one-shot 30 min before the surgery and second shot immediately after the surgery. Using a glass micropipette the embryos were injected intraventricularly with a solution containing 0.1% Fast Green (Sigma) in sterile 154 mM NaCl, 0.3 µg/µl of the pCAGGS-EGFP or pCAGGS-nlsRFP, followed by electroporation (24 V, five 50 ms pulses with 950 ms

intervals). After surgery, when analgesic used during the surgery was Metamizol, mice received additional Metamizol for one day via drinking water (1.33 mg/ml). Mice were sacrificed either by cervical dislocation or by IP injection with pentobarbitol and embryos were harvested 72 h post-electroporation, and the embryonic brains were dissected and PFA-fixed for immunohistochemistry (see below) or processed for time-lapse imaging (see below).

Immunohistochemistry

For nestin and GLAST immunofluorescence, 40 µm-thick coronal cryosections were stained as described previously (Vaid et al. 2018). Briefly, antigen retrieval was performed by incubating the sections, on the glass slide, in 0.01 M Na-citrate buffer for 60 min at 70 °C, followed by incubation for 20 min at room temperature. Sections were further permeabilized with 0.3% (wt/vol) Triton X-100 in PBS for 30 min and quenched with 2 mM glycine in PBS for 30 min, followed by blocking with a solution containing 0.2% (wt/vol) gelatin, 300 mM NaCl and 0.3% (wt/vol) Triton X-100 in PBS (blocking buffer). Primary antibodies were diluted in blocking buffer and sections incubated with primary antibodies overnight at 4 °C. After washing the section with PBS, the sections were incubated at room temperature with the appropriate secondary donkey antibodies and were counterstained with DAPI (Sigma, 1:1000). Sections were then mounted in Mowiol (Merck Biosciences).

Time-lapse imaging

The slice culture was prepared as previously described (Namba et al. 2021). The electroporated brains with RFP were sectioned either manually or by tissue chopper into 250 µm slices. Then the slices were embedded in a collagen gel on a glass-bottom dish. The time-lapse images were taken by laser scanning confocal microscope LSM710 (Zeiss) with a Plan-apochromat 20× objective. The images were taken every 15 min, for 30 h. During imaging, the slices were kept in an atmosphere of 5% CO_2 + 40% O_2 + 55% N_2.

Image acquisition and quantification

The fluorescent images were acquired using LSM710, LSM780 and LSM800, with 10x, 20x and 40x objectives. When the images were taken as tile scans, the images were stitched together using the ZEN software (Zeiss).

Quantifications were performed using the ZEN software and Fiji. Time-lapse imaging data were analyzed by TrackMate plugin of Fiji. The directionality of the nestin-positive fibers and tracks of RFP-positive cells was calculated by Fiji.

Single cell RNA sequencing data analysis

Single cell RNA sequencing data processed in Vaid et al., (GSE120976) (Vaid et al. 2018) were analyzed and visualized using R 4.1.2 and R package Seurat v4.

Statistical analysis

Data were analyzed with Excel (Microsoft, WA), Statcel3 (OMS, Japan), and MYSTAT (Systat Software, CA). Shapiro–Wilk normality test or the F test was used for testing normality or homoscedasticity of all samples, respectively. Two-tailed Student's t-test was used for all two-group comparison. Results were interpreted as statistically significant when $p < 0.05$.
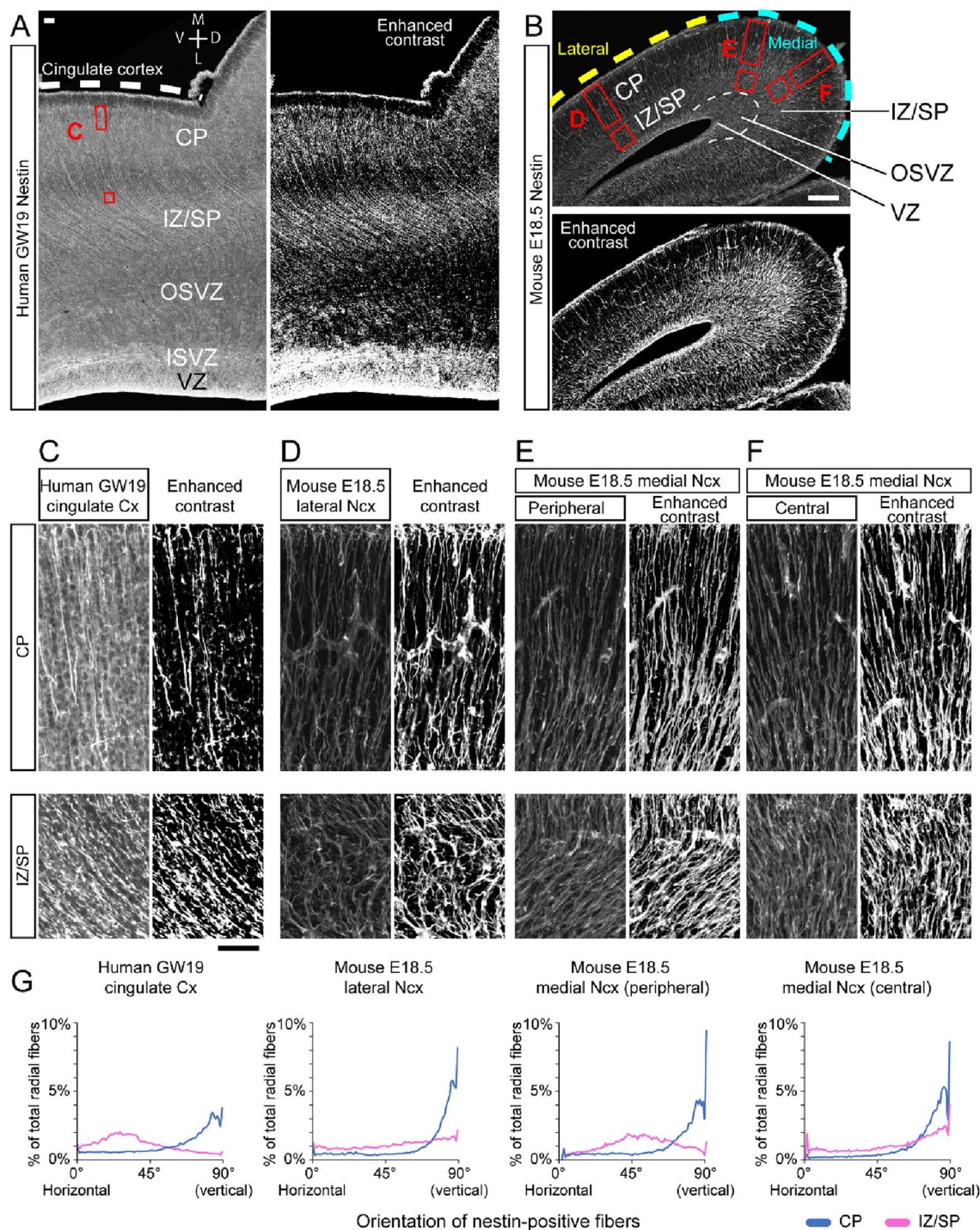
Results

Radial glial scaffold in the mouse medial neocortex at E18.5 exhibit a tangentially scattered pattern

The radial glial scaffolds in the fetal human neocortex at gestational week (GW) 19 and embryonic mouse neocortex at E18.5 were visualized by nestin (Fig. 1) and GLAST (Supplementary Fig. 1) immunostaining. Since mouse medial neocortex will give rise to the cingulate and retrosplenial cortex, the human cingulate cortex was examined. In addition, since GW19 is the earliest stage at which cingulate cortex exhibit a sulcus (Chi et al. 1977; Namba et al. 2019), the cingulate cortex at GW19 is the best region to compare and analyze the ontogeny of the radial glial scaffolds in the gyrencephalic neocortex. The nestin-positive fibers in the CP of human neocortex were aligned largely parallel to each other and perpendicular to the surface of the neocortex (i.e., the basal surface) (Fig. 1A, C, G), whereas the fibers in the intermediate zone and subplate (IZ/SP) were aligned tangentially compared to the CP (Fig. 1A, C, G). Similar organization of the radial glial scaffold was also observed by the GLAST immunostaining (Supplementary Fig. 1). These results, especially the finding of tangential orientation in the regions apical to the CP, suggest that the radial glial scaffold in the human tissue exhibits a tangentially scattered pattern, which is consistent with the previous studies from macaque and ferret (Reillo et al. 2011).

In the mouse lateral neocortex at E18.5, the nestin-positive fibers in the CP were aligned perpendicular to the basal surface (Fig. 1B, D, G) as same as the fibers in the human fetal neocortex. In contrast, the nestin-positive fibers in the IZ/SP of the mouse lateral neocortex had a randomly but yet mostly vertical alignment (Fig. 1D, G), suggesting that the scaffold in the mouse lateral neocortex largely keeps radially oriented-manner with some

Human GW19 cingulate Cx | Mouse E18.5 lateral Ncx | Mouse E18.5 medial Ncx (peripheral) | Mouse E18.5 medial Ncx (central)

Orientation of nestin-positive fibers

CP — IZ/SP

randomness in the IZ/SP. The randomness observed in the IZ/SP might partially due to the branching of radial fibers (Takahashi et al. 1990). The nestin-positive fibers in the medial neocortex, however, exhibited distinct features. In the medial neocortex close to the lateral neocortex (we called as the peripheral medial neocortex), while the nestin-positive fibers aligned perpendicular to the basal surface in the CP as shown in the previous studies (Mission

et al. 1991a; Mission et al. 1991b), the fibers in the IZ/SP bent toward the OSVZ-like structure in the medial neocortex (Fig. 1B, E, G). Therefore, similar to what is observed in the fetal human neocortex (Fig. 1C, G), the radial glial scaffold runs tangentially in the mouse medial neocortex at E18.5. Interestingly, the central part of the medial neocortex contained the nestin-positive fibers perpendicular to the basal surface all the way down to the OSVZ-like zone

◄**Fig. 1** Radial glial scaffold in fetal human and embryonic mouse neocortex. (**A**) Nestin-positive fibers in human neocortex at GW19. The cingulate cortex is indicated by the dashed line. The regions indicated by the red boxes are shown at higher magnification in C. The right panel is an image with enhanced contrast. M: medial, L: lateral, D: dorsal, V: ventral. (**B**) Nestin-positive fibers in mouse neocortex at E18.5. The lateral and medial neocortex are indicated by the yellow and light blue dashed lines, respectively. The regions indicated by the red boxes are shown at higher magnification in D, E, and F. The dashed line in the medial neocortex indicates the border between IZ/SP and OSVZ-like structure. The bottom panel is an image with enhanced contrast. (**C–F**) Nestin-positive fibers in the CP (top panels) and the IZ/SP (bottom panels) of GW19 human (**C**), E18.5 mouse lateral neocortex (**D**), and E18.5 mouse medial neocortex (peripheral: **E**, central: **F**). The right panels are images with enhanced contrast. (**G**) Orientation of nestin-positive fibers in the CP (blue) and in the IZ/SP (magenta) of human cingulate cortex at GW19, mouse lateral and medial neocortex. The proportion of nestin-positive fibers at a specific angle to the total fibers is calculated every 1° from 0° (horizontal to the basal surface) to 90° (vertical: perpendicular to the basal surface). $n = 4$ for each region. *CP* cortical plate, *ISVZ* inner subventricular zone, *IZ/SP* intermediate zone/subplate, *OSVZ* outer subventricular zone, *VZ* ventricular zone. Scale bars: 200 µm in A and B, 50 µm in C applies to D-F

through the IZ/SP (Fig. 1B, F, G). Similar organization of the radial glial scaffold was also observed by the GLAST immunostaining (Supplementary Fig. 1). Therefore, the mouse medial neocortex possesses the fan-shaped radial glial scaffold, which is tangentially projected from the germinal zones toward the basal surface.

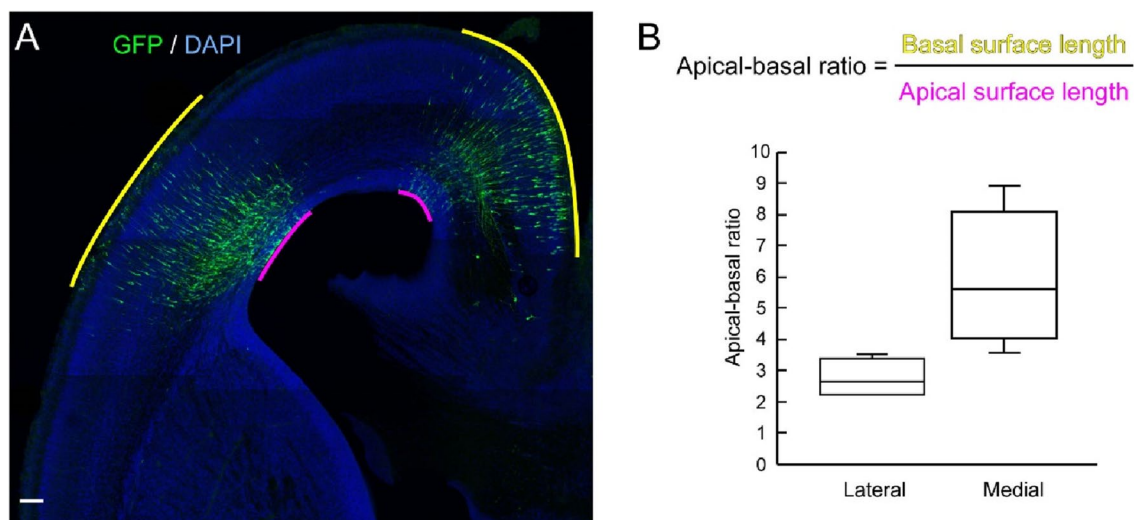## Electroporated region is dispersed tangentially in the embryonic mouse medial neocortex

More tangentially scattered radial glial scaffold in the mouse medial neocortex than mouse lateral neocortex could affect the distribution of electroporated cells and their progenies. To test this hypothesis, embryonic mouse neocortex at E15.5 was electroporated with cytoplasmic GFP under the control of a ubiquitous promoter CAG to label both NPCs and neurons, and the electroporated area at E18.5 was analyzed to show to what extent the electroporated cells and their progenies are distributed. We compared the length of the apical surface and basal surface of the electroporated regions (we termed the ratio apical-basal ratio; Fig. 2A, B). If the ratio is close to 1, there is no tangential dispersion. While the apical-basal ratio in the lateral neocortex is approximately 2, the ratio in the medial neocortex is nearly 6 (Fig. 2B), showing that the progeny of the electroporated cells distribute tangentially three times more in the medial neocortex than the lateral neocortex.

Neurons migrate tangentially in the embryonic mouse medial neocortex

The tangential dispersion of the electroporated region in the mouse medial neocortex rises the possibility that the newly generated neurons migrate tangentially in the medial neocortex. There are three major migratory modes of neurons in the developing neocortex (Kawauchi and Hoshino 2008; Namba et al. 2015). Locomotion and somal translocation are the characteristic radial glial scaffold-dependent migration of neurons, which allow neurons to migrate long distances (Kawauchi and Hoshino 2008; Namba et al. 2015). The other mode is so-called multipolar migration, which is the radial glial scaffold-independent and typically exhibits slow speed and thus short distance travel in a given period (Kawauchi and Hoshino 2008; Namba et al. 2015; Tabata and Nakajima 2003). We electroporated the medial neocortex of mouse embryos at E15.5 with nuclear-localized RFP under the control of the CAG promoter, and then the neocortical tissues were processed for slice culture and time-lapse imaging (Fig. 3A). RFP-positive cells in the CP and IZ/SP without cell division are considered neurons. To focus on the radial glial scaffold-dependent neuronal migration, this study only analyzed the neurons that migrate more than 40 µm in direct distance between the start and end of the track during the imaging period (13 h; Fig. 3B). There are no differences in the speed and directional change rate of RFP-positive neurons in the CP and IZ/SP (Fig. 3C, D). However, the RFP-positive neurons in the peripheral IZ/SP migrate more tangentially than the neurons in the CP or in the central IZ/SP (Fig. 3B, E). These results suggest that the neurons in the medial neocortex migrate tangentially, prominently in the peripheral part, to accomplish the tangential distribution of neurons.

Discussion

To analyze human neocortical development, there are currently several options. Each option has advantages and disadvantages (Table 1). For example, ferret, a gyrencephalic carnivore, is used for studying neocortical expansion and folding (Kalebic et al. 2018; Kawasaki et al. 2013; Reillo and Borrell 2012), however, the accessibility is limited due to the high cost of the animals and their housing. In addition, the gene knockout ferrets have been reported (Johnson et al. 2018) but have not been widely used so far. Cerebral organoids generated from human iPSCs are now widely used because of the established methods for genome editing in iPSCs and the fewer ethical problems (Eiraku et al. 2008; Lancaster et al. 2017). However, it is still on the way to the organoids with the quality comparable to the fetal human neocortical tissue in vivo (Pollen et al. 2019),

Fig. 2 Electroporated region is dispersed tangentially in the embryonic mouse medial neocortex. (**A**) A representative image of electroporated regions at E18.5 mouse neocortex, which has been electroporated with GFP-expressing plasmid at E15.5. Yellow and magenta lines indicate the basal and apical surface of the electroporated regions. (**B**) Apical-basal ratio (a ratio of the basal surface length to the apical surface length) of the electroporated mouse lateral and medial neocortex at E18.5. Data within the boxes represent the 25–75% quartiles, and the line in the middle shows the median value. $n = 4$ embryos for each region

especially the abundance of bRG, neuronal layer formation and gyrification.

Mice have decades of history as an in vivo experimental model system which allows us to analyze neocortical development to cognitive functions associated with the neocortex upon gene manipulations. In many studies, the lateral neocortex has been chosen as a region of interest, therefore a large amount of accumulated knowledge on brain development is available (Coquand et al. 2021; Kawauchi et al. 2010; Nakagawa et al. 2019; Namba et al. 2014; Shitamukai et al. 2011). However, there are considerable differences in the developmental process between the mouse lateral neocortex and human neocortex. First, the abundance of bRG is significantly lower in the embryonic mouse lateral neocortex (approximately 10% of total basal progenitors) compared to the fetal human neocortex (approximately 50% of total basal progenitors) (Kelava et al., 2012; Vaid et al. 2018; Wang et al. 2011). Second, less tangential dispersion of the radial glial scaffold (Gadisseux et al. 1992; Rakic, 2003), thus neurons (Cortay et al., 2020; Gertz and Kriegstein 2015; Hara et al. 2016; Martinez-Martinez et al. 2018), are observed throughout the embryonic period. It is important to consider these features when we study neocortical development using mice as a model.

We have previously proposed that the mouse medial neocortex at E18.5 is a better suited model system for studying human neocortex, since the mouse medial neocortex contains an abundance of bRG (approximately 50% of total basal progenitors) which is comparable to the fetal human neocortex (Vaid et al. 2018). Since then, several groups have

started to use the mouse medial neocortex as a model system of human neocortical bRG (Bertacchi et al. 2020; Kerimoglu et al. 2021; Pinson et al. 2022; Wang et al. 2022). In the present study, we aim to further expand the utility of the mouse medial neocortex to studying radial glial scaffold which regulates neuronal migration. Since newly generated neurons are known to migrate more tangentially in the IZ/SP of gyrencephalic species, and reduction of tangential migration has been shown to affect the gyrus volume (Reillo et al. 2010), tangentially scattered radial glial scaffold and neuronal migration are important features to be addressed in the context of examining cortical malformation-associated genes in mice. Interestingly, the mouse medial neocortex is prone to develop folding upon genetical manipulations (Wang et al. 2016, 2022), suggesting that the tangentially scattered radial glial scaffold and neuronal migration described in the present study could be involved in the ectopic gyrification in the mouse medial neocortex.

The tangential neuronal migration in gyrencephalic neocortex is known to be regulated by cell-to-cell interaction (Del Toro et al. 2017). Expression of the fibronectin leucine rich-repeat transmembrane protein (FLRT), a cell adhesion molecule, is higher in the embryonic mouse neocortex than the fetal human neocortex. The higher FLRTs expression in the mouse lateral neocortex is required for the radial migration of neurons, and their reduction induces tangential neuronal dispersion, thus neocortical folding. Interestingly, *Flrt1*, *Flrt2* and *Flrt3* gene expression has been shown to be highest in the lateral neocortex, and lower in the medial neocortex of embryonic mouse (Del Toro et al. 2017;
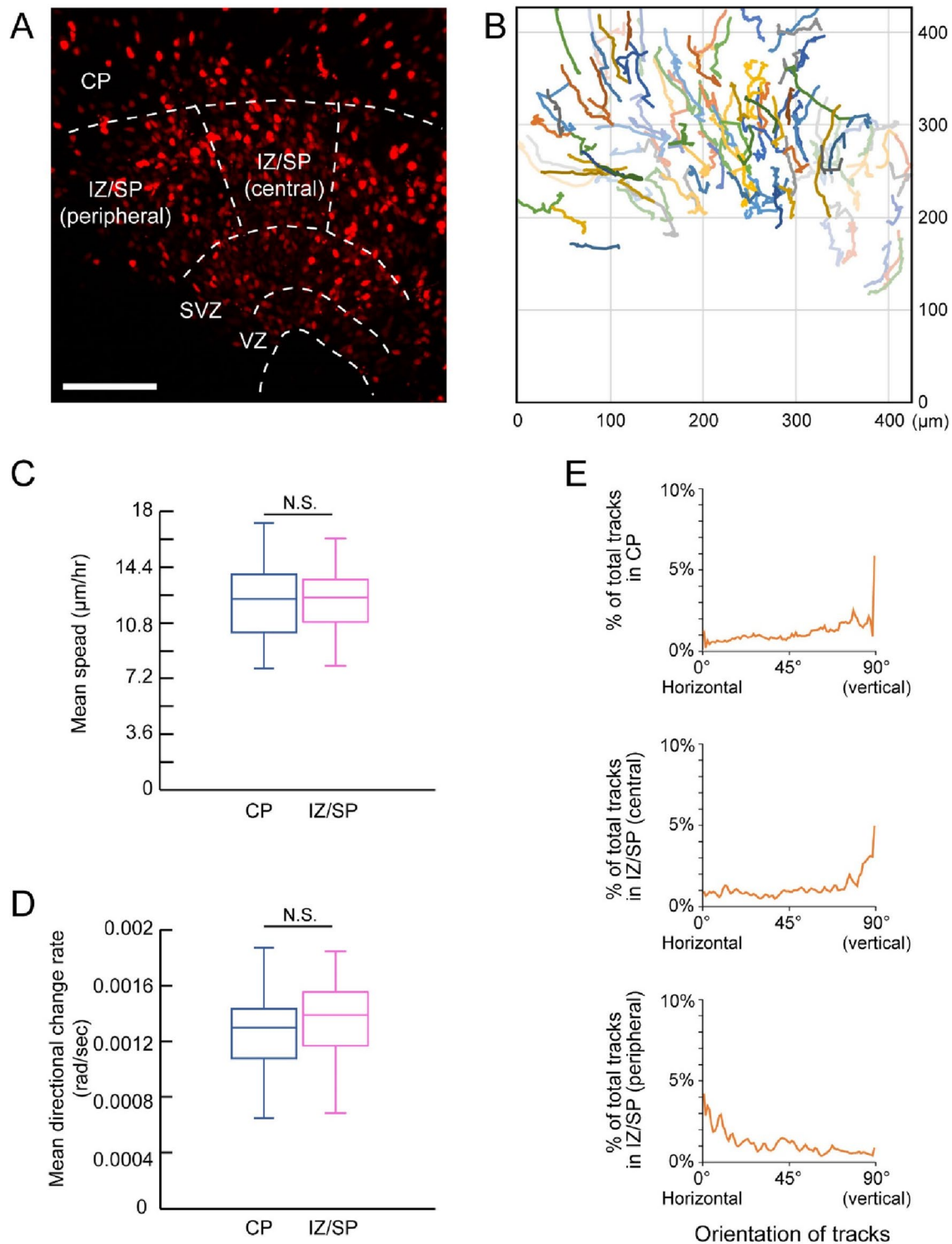
Fig. 3 Neurons migrate more tangentially in the peripheral part of the IZ/SP in the mouse medial neocortex. (**A**) A representative image of electroporated regions at the beginning of time-lapse imaging of mouse neocortical slice, which has been electroporated with nslRFP-expressing plasmid at E15.5 and processed for imaging at E18.5. Dashed lines indicate borders of different regions labeled on the image. Scale bar: 100 μm. (**B**) Tracks of the RFP-positive cells in the CP and IZ/SP. (**C**, **D**) Mean speed (**C**) and mean directional change rate (**D**) of the RFP-positive cell migration in the CP and in the IZ/ SP. Data within the boxes represent the 25–75% quartiles, and the line in the middle shows the median value. Total 58 cells and 63 cells were analyzed for the CP and IZ/SP, respectively. (**E**) Orientation of the tracks of RFP-positive cell migration in the CP, and in the central and peripheral part of IZ/SP. The proportion of the tracks at a specific angle to the total tracks is calculated every 1° from 0° (horizontal to the basal surface) to 90° (vertical: perpendicular to the basal surface). Total 121 cells from 3 experiments were analyzed

Table 1 Summary of advantages and disadvantages of each model systems

	Human fetal neocortex	Human cerebral organoids	Ferret embryonic neocortex	Mouse embryonic lateral neocortex	Mouse embryonic medial neocortex
Gyrification	Yes	Needs improvement	Yes	No	No
bRG abundance	High	Needs improvement	High	Low	High
Fan-shaped RG scaffold	Yes	No data	Yes	No	Yes
Tangential migration of pyramidal neurons	Yes	No data	Yes	Less	Yes
Highly annotated genome	Yes	Yes	Less annotated	Yes	Yes
Gene KO/manipulation	Yes (ex vivo electroporation)	Yes	Yes (mainly in utero electroporation)	Yes (KO mice and in utero electroporation)	Yes (KO mice and in utero electroporation)
RNA sequencing data	Many	Many	No	Many	Not many
Accessibility	Low	High	Low-mid	High	High

Yamagishi et al. 2011). Consistent with this previous study, only a minor population of neurons in the mouse medial neocortex express *Flrt1* (Supplementary Fig. 2), suggesting a possibility that the lower expression of FLRTs in the mouse medial neocortex enable the neurons migrate tangentially along the fan-shaped radial glial scaffold.

Unfortunately, mice are not the perfect model system to study human neocortical development, and never will be, because there are significant contributions of human- and primate-specific genes in human neocortical development (Fiddes et al. 2018; Florio et al. 2015, 2018; Liu et al. 2017; Namba et al. 2020; Pinson et al. 2022; Suzuki 2020; Suzuki et al. 2018; Vaid and Huttner 2020; Van Heurck et al. 2022). However, using the mouse medial neocortex as a model system appears to be a good compromise in terms of bRG abundance, and the tangentially scattered radial glial scaffold and neuronal migration. Therefore, the mouse medial neocortex is a "poor man's Porsche", an alternative, cheaper and more accessible way to study human neocortical development and its pathogenesis.

Declarations

References

Bertacchi M, Romano AL, Loubat A, Tran Mau-Them F, Willems M, Faivre L, Khau van Kien P, Perrin L, Devillard F, Sorlin A et al (2020) NR2F1 regulates regional progenitor dynamics in the mouse neocortex and cortical gyrification in BBSOAS patients. EMBO J. https://doi.org/10.15252/embj.2019104163

Casingal CR, Descant KD, Anton ES (2022) Coordinating cerebral cortical construction and connectivity: unifying influence of radial progenitors. Neuron 110:1100–1115. https://doi.org/10.1016/j.neuron.2022.01.034

Chi JG, Dooling EC, Gilles FH (1977) Gyral development of the human brain. Ann Neurol 1:86–93. https://doi.org/10.1002/ana.410010109

Coquand L, Victoria GS, Tata A, Carpentieri JA, Brault JB, Guimiot F, Fraisier V, Baffet AD (2021) CAMSAPs organize an acentrosomal microtubule network from basal varicosities in radial glial cells. J Cell Biol. https://doi.org/10.1083/jcb.202003151

Cortay V, Delaunay D, Patti D, Gautier E, Doerflinger N, Giroud P, Knoblauch K, Huissoud C, Kennedy H, Dehay C (2020) Radial migration dynamics is modulated in a laminar and area-specific manner during primate corticogenesis. Frontiers Cell Develop Bio 8:588814. https://doi.org/10.3389/fcell.2020.588814

Del Toro D, Ruff T, Cederfjall E, Villalba A, Seyit-Bremer G, Borrell V, Klein R (2017) Regulation of cerebral cortex folding by controlling neuronal migration via FLRT adhesion molecules. Cell. https://doi.org/10.1016/j.cell.2017.04.012

Eiraku M, Watanabe K, Matsuo-Takasaki M, Kawada M, Yonemura S, Matsumura M, Wataya T, Nishiyama A, Muguruma K, Sasai Y (2008) Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. Cell Stem Cell 3(5):19–532. https://doi.org/10.1016/j.stem.2008.09.002

Fiddes IT, Lodewijk GA, Mooring M, Bosworth CM, Ewing AD, Mantalas GL, Novak AM, van den Bout A, Bishara A, Rosenkrantz JL et al (2018) Human-specific NOTCH2NL genes affect notch signaling and cortical neurogenesis. Cell 173:1356–1369. https://doi.org/10.1016/j.cell.2018.03.051

Florio M, Albert M, Taverna E, Namba T, Brandl H, Lewitus E, Haffner C, Sykes A, Wong FK, Peters J et al (2015) Human-specific gene ARHGAP11B promotes basal progenitor amplification and neocortex expansion. Science 347:1465–1470. https://doi.org/10.1126/science.aaa1975

Florio M, Heide, M., Pinson, A., Brandl, H., Albert, M., Winkler, S., Wimberger, P., Huttner, W.B., and Hiller, M. (2018) Evolution and cell-type specificity of human-specific genes preferentially expressed in progenitors of fetal neocortex. eLife https://doi.org/10.7554/eLife.32332

Gadisseux JF, Evrard P, Mission JP, Caviness VS Jr (1992) Dynamic changes in the density of radial glial fibers of the developing murine cerebral wall: a quantitative immunohistological analysis. J Comp Neurol 322:246–254. https://doi.org/10.1002/cne.903220210

Gertz CC, Kriegstein AR (2015) Neuronal migration dynamics in the developing ferret cortex. J Neurosci 35:14307–14315. https://doi.org/10.1523/JNEUROSCI.2198-15.2015

Hara, Y., Fukaya, M., Hayashi, K., Kawauchi, T., Nakajima, K., and Sakagami, H. (2016) ADP Ribosylation Factor 6 Regulates Neuronal Migration in the Developing Cerebral Cortex through FIP3/Arfophilin-1-dependent Endosomal Trafficking of N-cadherin. eNeuro https://doi.org/10.1523/ENEURO.0148-16.2016

Heuer K, Gulban OF, Bazin PL, Osoianu A, Valabregue R, Santin M, Herbin M, Toro R (2019) Evolution of neocortical folding: a phylogenetic comparative analysis of MRI from 34 primate species. Cortex; a J Devoted Study Nervous System Behavior 118:275–291. https://doi.org/10.1016/j.cortex.2019.04.011

Johnson MB, Sun X, Kodani A, Borges-Monroy R, Girskis KM, Ryu SC, Wang PP, Patel K, Gonzalez DM, Woo YM et al (2018) Aspm knockout ferret reveals an evolutionary mechanism governing cerebral cortical size. Nature 556:370–375. https://doi.org/10.1038/s41586-018-0035-0

Kalebic, N., Gilardi, C., Albert, M., Namba, T., Long, K.R., Kostic, M., Langen, B., and Huttner, W.B. (2018). Human-specific ARHGAP11B induces hallmarks of neocortical expansion in developing ferret neocortex. eLife https://doi.org/10.7554/eLife.41241.

Kawasaki H, Toda T, Tanno K (2013) In vivo genetic manipulation of cortical progenitors in gyrencephalic carnivores using in utero electroporation. Biology Open 2:95–100. https://doi.org/10.1242/bio.20123160

Kawauchi T, Hoshino M (2008) Molecular pathways regulating cytoskeletal organization and morphological changes in migrating neurons. Dev Neurosci 30:36–46. https://doi.org/10.1159/000109850

Kawauchi T, Sekine K, Shikanai M, Chihama K, Tomita K, Kubo K, Nakajima K, Nabeshima Y, Hoshino M (2010) Rab GTPases-dependent endocytic pathways regulate neuronal migration and maturation through N-cadherin trafficking. Neuron 67:588–602. https://doi.org/10.1016/j.neuron.2010.07.007

Kelava I, Reillo I, Murayama AY, Kalinka AT, Stenzel D, Tomancak P, Matsuzaki F, Lebrand C, Sasaki E, Schwamborn JC et al (2012) Abundant occurrence of basal radial glia in the subventricular zone of embryonic neocortex of a lissencephalic primate, the common marmoset Callithrix jacchus. Cereb Cortex 22:469–481. https://doi.org/10.1093/cercor/bhr301

Kerimoglu C, Pham L, Tonchev AB, Sakib MS, Xie Y, Sokpor G, Ulmke PA, Kaurani L, Abbas E, Nguyen H et al (2021) H3 acetylation selectively promotes basal progenitor proliferation and neocortex expansion. Sci Adv. https://doi.org/10.1126/sciadv.abc679

Klingler E, Francis F, Jabaudon D, Cappello S (2021) Mapping the molecular and cellular complexity of cortical malformations. Science. https://doi.org/10.1126/science.aba4517

Lancaster MA, Corsini NS, Wolfinger S, Gustafson EH, Phillips AW, Burkard TR, Otani T, Livesey FJ, Knoblich JA (2017) Guided self-organization and cortical plate formation in human brain organoids. Nat Biotechnol 35:659–666. https://doi.org/10.1038/nbt.3906

Liu J, Liu W, Yang L, Wu Q, Zhang H, Fang A, Li L, Xu X, Sun L, Zhang J et al (2017) The primate-specific gene tmem14b marks outer radial glia cells and promotes cortical expansion and folding. Cell Stem Cell 21(635–649):e638. https://doi.org/10.1016/j.stem.2017.08.013

Martinez-Martinez MA, Ciceri G, Espinos A, Fernandez V, Marin O, Borrell V (2018) Extensive branching of radially-migrating neurons in the mammalian cerebral cortex. J Comp Neurol. https://doi.org/10.1002/cne.24597

Mission JP, Takahashi T, Caviness VS Jr (1991) Ontogeny of radial and other astroglial cells in murine cerebral cortex. Glia 4:138–148. https://doi.org/10.1002/glia.440040205

Mission JP, Austin CP, Takahashi T, Cepko CL, Caviness VS Jr (1991) The alignment of migrating neural cells in relation to the murine neopallial radial glial fiber system. Cereb Cortex 1:221–229. https://doi.org/10.1093/cercor/1.3.221

Nakagawa N, Plestant C, Yabuno-Nakagawa K, Li J, Lee J, Huang CW, Lee A, Krupa O, Adhikari A, Thompson S et al (2019) Memo1-mediated tiling of radial glial cells facilitates cerebral cortical development. Neuron 103:836–852. https://doi.org/10.1016/j.neuron.2019.05.049

Namba T, Huttner WB (2017) Neural progenitor cells and their role in the development and evolutionary expansion of the neocortex. Wires Dev Biol. https://doi.org/10.1002/wdev.256

Namba T, Kibe Y, Funahashi Y, Nakamuta S, Takano T, Ueno T, Shimada A, Kozawa S, Okamoto M, Shimoda Y et al (2014)

Pioneering axons regulate neuronal polarization in the developing cerebral cortex. Neuron 81:814–829. https://doi.org/10.1016/j.neuron.2013.12.015

Namba T, Funahashi Y, Nakamuta S, Xu C, Takano T, Kaibuchi K (2015) Extracellular and intracellular signaling for neuronal polarity. Physiol Rev 95:995–1024. https://doi.org/10.1152/physrev.00025.2014

Namba T, Vaid S, Huttner WB (2019) Primate neocortex development and evolution: Conserved versus evolved folding. J Comp Neurol 527:1621–1632. https://doi.org/10.1002/cne.24606

Namba T, Doczi J, Pinson A, Xing L, Kalebic N, Wilsch-Bräuninger M, Long KR, Vaid S, Lauer J, Bogdanova A et al (2020) Human-specific ARHGAP11B acts in mitochondria to expand neocortical progenitors by glutaminolysis. Neuron 105:867–881. https://doi.org/10.1016/j.neuron.2019.11.027

Namba T, Haffner C, Huttner WB (2021) Ex vivo tissue culture protocols for studying the developing neocortex. Bio Protoc 11(10):e4031. https://doi.org/10.21769/BioProtoc.4031

Pinson A, Xing L, Namba T, Kalebic N, Peters J, Oegema CE, Traikov S, Reppe K, Riesenberg S, Maricic T et al (2022) Human TKTL1 implies greater neurogenesis in frontal neocortex of modern humans than Neanderthals. Science. https://doi.org/10.1126/science.abl6422

Pollen AA, Bhaduri A, Andrews MG, Nowakowski TJ, Meyerson OS, Mostajo-Radji MA, Di Lullo E, Alvarado B, Bedolli M, Dougherty ML et al (2019) Establishing cerebral organoids as models of human-specific brain evolution. Cell 176(743–756):e717. https://doi.org/10.1016/j.cell.2019.01.017

Rakic P (2003) Elusive radial glial cells: historical and evolutionary perspective. Glia 43:19–32. https://doi.org/10.1002/glia.10244

Rakic P (2009) Evolution of the neocortex: a perspective from developmental biology. Nat Rev Neurosci 10(10):724–735

Reillo I, Borrell V (2012) Germinal zones in the developing cerebral cortex of ferret: ontogeny, cell cycle kinetics, and diversity of progenitors. Cereb Cortex 22:2039–2054. https://doi.org/10.1093/cercor/bhr284

Reillo I, de Juan Romero C, Garcia-Cabezas MA, Borrell V (2011) A role for intermediate radial glia in the tangential expansion of the mammalian cerebral cortex. Cereb Cortex 21:1674–1694. https://doi.org/10.1093/cercor/bhq238

Shitamukai A, Konno D, Matsuzaki F (2011) Oblique radial glial divisions in the developing mouse neocortex induce self-renewing progenitors outside the germinal zone that resemble primate outer subventricular zone progenitors. J Neurosci 31:3683–3695. https://doi.org/10.1523/JNEUROSCI.4773-10.2011

Suzuki IK (2020) Molecular drivers of human cerebral cortical evolution. Neurosci Res 151:1–14. https://doi.org/10.1016/j.neures.2019.05.007

Suzuki IK, Gacquer D, Van Heurck R, Kumar D, Wojno M, Bilheu A, Herpoel A, Lambert N, Cheron J, Polleux F et al (2018) Human-specific NOTCH2NL genes expand cortical neurogenesis through Delta/Notch regulation. Cell 173:1370–1384. https://doi.org/10.1016/j.cell.2018.03.067

Tabata H, Nakajima K (2003) Multipolar migration: the third mode of radial neuronal migration in the developing cerebral cortex. J Neurosci 23:9996–10001

Takahashi T, Misson JP, Caviness VS Jr (1990) Glial process elongation and branching in the developing murine neocortex: a qualitative and quantitative immunohistochemical analysis. J Comp Neurol 302:15–28. https://doi.org/10.1002/cne.903020103

Vaid S, Huttner WB (2020) Transcriptional regulators and human-specific/primate-specific genes in neocortical neurogenesis. Int J Mol Sci. https://doi.org/10.3390/ijms21134614

Vaid S, Camp JG, Hersemann L, Eugster Oegema C, Heninger AK, Winkler S, Brandl H, Sarov M, Treutlein B, Huttner WB, Namba T (2018) A novel population of Hopx-dependent basal radial glial cells in the developing mouse neocortex. Development. https://doi.org/10.1242/dev.169276

Van Heurck R, Bonnefont J, Wojno M, Suzuki IK, Velez-Bravo FD, Erkol E, Nguyen DT, Herpoel A, Bilheu A, Beckers S et al (2022) CROCCP2 acts as a human-specific modifier of cilia dynamics and mTOR signaling to promote expansion of cortical progenitors. Neuron. https://doi.org/10.1016/j.neuron.2022.10.018

Wang X, Tsai JW, LaMonica B, Kriegstein AR (2011) A new subtype of progenitor cell in the mouse embryonic neocortex. Nat Neurosci 14:555–561. https://doi.org/10.1038/nn.2807

Wang L, Hou S, Han YG (2016) Hedgehog signaling promotes basal progenitor expansion and the growth and folding of the neocortex. Nat Neurosci 19:888–896. https://doi.org/10.1038/nn.4307

Wang L, Park JY, Liu F, Olesen KM, Hou S, Peng JC, Infield J, Levesque AC, Wang YD, Jin H et al (2022) A kinase-independent function of cyclin-dependent kinase 6 promotes outer radial glia expansion and neocortical folding. Proc Natl Acad Sci U S A 119:e2206147119. https://doi.org/10.1073/pnas.2206147119

Yamagishi S, Hampel F, Hata K, Del Toro D, Schwark M, Kvachnina E, Bastmeyer M, Yamashita T, Tarabykin V, Klein R, Egea J (2011) FLRT2 and FLRT3 act as repulsive guidance cues for Unc5-positive neurons. EMBO J 30:2920–2933. https://doi.org/10.1038/emboj.2011.189