HARW Beta 15.0

Dummy Title

--Manuscript Draft--

Manuscript Number:	
Full Title:	Dummy Title
Article Type:	Original Article
Corresponding Author:	* Amy Author Springer Heidelberg, GERMANY
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Springer
Corresponding Author's Secondary Institution:	
First Author:	* Amy Author
First Author Secondary Information:	
Order of Authors:	* Amy Author
Order of Authors Secondary Information:	
Author Comments:	Test
Suggested Reviewers:	

Subtype specification of cerebral cortical neurons in their immature stages

Koji Oishi and Kazunori Nakajima

Department of Anatomy, Keio University School of Medicine, Tokyo 160-8582, Japan

Address correspondence to: Kazunori Nakajima and Koji Oishi, Department of Anatomy, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

TEL: +81-3-5363-3743. FAX: +81-3-5379-1977.

E-mail: kazunori@keio.jp (K.N.) and k-oishi@keio.jp (K.O.)

Acknowledgements

We thank members of the Nakajima Laboratory for valuable discussions. This work was supported by grants from the MEXT/JSPS KAKENHI (JP 17K07061, JP 16H06482, JP 15H02355), Keio Gijuku Fukuzawa Memorial Fund for the Advancement of Education and Research, Takeda Science Foundation, and the Naito Foundation.

ABSTRACT

The diversification of neuronal subtypes during corticogenesis is fundamental to the establishment of the complex cortical structure. Although subtype specification has been assumed to occur in neural progenitor cells, increasing evidence has begun to reveal the plasticity of subtype determination in immature neurons. Here, we summarize recent findings regarding the regulation of subtype specification during later periods of neuronal differentiation, such as the post-mitotic and post-migratory stages. We also discuss thalamocortical axons as an extra-cortical cue that provides information on the subtype determination of immature cortical neurons.

INTRODUCTION

The mammalian isocortex or neocortex, which forms a six-layered structure histologically, comprises hundreds of different neuronal ‘subtypes’ that differ in cell morphology, axonal/dendritic projection pattern, gene expression profile and others [1-3]. This diversification of neuronal subtypes is the basis for the execution of higher brain functions. However, the full picture of how and to what extent neurons become diversified during corticogenesis is yet to be determined.

Almost all excitatory neocortical neurons arise from common neural progenitor cells (NPCs) in the pallium, while other neuronal populations (e.g., Cajal-Retzius neurons and inhibitory interneurons) arise from different brain regions [4-6]. A diverse repertoire of cortical subtypes is generated from NPCs in a sequential manner; the subtypes that eventually locate in the deepest layer are generated at the earliest stage, followed by the production of subtypes that locate above the deepest layer. This process is repeated until the six-layered structure is formed, resulting in a so-called inside-out pattern of neuronal layering [7].

Because each layer in the neocortex is occupied by the neurons generated at around the same time [8,9], a model whereby NPCs change their potential over time, thereby determining the eventual subtype of newly generated neurons, has been widely accepted

(Figure 1A) [10]. In fact, this type of mechanism is utilized in some cases of subtype specification in the *Drosophila* nervous system [11]. In mammals, seminal studies from McConnell's group involving heterochronic transplantation experiments have demonstrated that cortical NPCs undergo a progressive restriction of subtype-producing potential during cortical development; early NPCs, which produce layer 6 neurons, can differentiate into superficial/upper layer neurons when transplanted into the late VZ, while late NPCs, which produce layer 2/3 neurons, transplanted into the early VZ do not differentiate into deep layer neurons [12,13]. However, only a few fate-determining factors with temporally controlled expression profiles in NPCs have been identified in the mammalian nervous system [14-17]; thus, the molecular mechanisms that regulate the temporal changes of NPCs remain largely unknown.

In addition to the aforementioned model, it is also conceivable that cortical lamination or appropriate cell positioning in the cortical plate play a role in the full differentiation or maturation of the neurons. According to this model, the positioning of immature neurons is primarily regulated by a mechanism utilizing information corresponding to their birthdate, followed by the determination of eventual subtypes via surrounding environmental signals (Figure 1B). This hypothesis has not attracted much attention because

the neocortex of *reeler* mice (*reelin* deficient mice), which exhibits a highly-disorganized layer structure [18], possesses an almost normal set of neuronal subtypes [19,20]. However, the *reeler* neocortex still has inverted or mirror-imaged layer structures [21], thereby potentially retaining laminar information, leaving it an open question as to whether all laminar fates are determined intrinsically in NPCs or whether they can be modulated during later stages. Here, we will summarize progress that has begun to reveal the regulation of subtype specification of cortical neurons during later periods, such as the post-mitotic and post-migratory stages of immature neurons.

Identification of subtype-determining genes

Recent progress towards understanding the mechanisms of cortical subtype specification involves the identification of a dozen subtype-determining genes, most of which are transcription factors. For example, Macklis' group identified several subtype determinants using an elegant system, revealing gene expression profiles in different cortical subtypes that were separately collected based on different axonal projection patterns [22,23]. Our group reported that two types of transcription factors, Rorb and Brn1/2, regulate the specification of

layer 4 and layer 2/3 neurons, respectively [24] (see below).Other factors were also identified and have been reviewed elsewhere [1-3].

The scenario for the temporal specification of NPCs assumes that different fate determinants are expressed sequentially in NPCs, similar to the *Drosophila* nervous system [11]. However, many of the identified fate determinants, such as Tbr1, Satb2, Bhlhb5, and Zfpm2, begin to be expressed in post-mitotic migrating neurons or post-migratory neurons [25-28,23], albeit with a few exceptions (e.g., Fezf2 [17,29] and Brn factors [15]), implying that subtype determination generally takes place in post-mitotic immature neurons. An alternative explanation is that these expression profiles are caused by a 'timer'-like mechanism, whereby the expression onset of fate determinants in immature neurons is already programmed in NPCs [30]. Further studies will be needed to reveal the exact timing of when neuronal subtypes are determined and the underlying molecular mechanisms.

Plasticity of immature neurons in subtype specification

Recent findings on cortical subtype determinants have opened the gate to understanding plasticity in NPCs and immature neurons. For example, ectopically expressed subtype determinants convert transfected cells to the subtypes that these factors normally control.

The ectopic expression of the cortical deep layer determinant Fezf2 in ventral NPCs, which are normally fated to generate striatal neurons, is sufficient to instruct these cells to become cortical deep layer-like neurons [31]. Notably, this type of fate conversion was also observed in post-mitotic immature neurons. Ectopically expressed Fezf2 in post-mitotic superficial layer neurons reprograms the cells into deep layer-like neurons, including their molecular identify, morphology, physiology and functional input/output connectivity, indicating a high level of plasticity in post-mitotic cortical neurons [32]. Interestingly, this fate conversion does not occur in mature neurons (i.e., after postnatal day 10), suggesting a 'critical period' in this fate conversion process.

Extrinsic control of subtype specification in immature neurons

The aforementioned fate conversion occurs when a subtype determinant is forcedly expressed; thus, whether the fate determination of immature neurons is controlled by endogenous extracellular signals remains uncertain. To examine the events that occur in immature neurons and their possible involvement in subtype specification, our group screened for genes that are highly upregulated in immature neurons that have just finished radial migration beneath the marginal zone [33] or within the primitive cortical zone (PCZ) [34].

Among them, some genes were eventually expressed in a certain subtype of neurons [33]. Protocadherin20 (Pcdh20), which belongs to the cadherin superfamily, was identified as a gene that fulfills these criteria. Pcdh20 begins to be expressed in post-migratory neurons destined to become layer 4 neurons (‘future’ layer 4 neurons) and is expressed in layer 4 of the mature neocortex. Pcdh20 knockdown experiments revealed that a reduction in Pcdh20 expression causes the future layer 4 neurons to be located in layer 2/3, with barely, if any, effect on the proliferation of NPCs and the migratory behaviors of neurons. Surprisingly, these ectopically positioned neurons acquire the features of the surrounding neurons (i.e., layer 2/3 neurons) and lose their original characteristics (i.e., layer 4 neurons). Moreover, the disruption of neuronal positioning using another method recapitulates this fate conversion. These results indicate that the subtype determination of layer 4 neurons occurs during post-mitotic stages, at least in part, and can be influenced by extracellular signals [35]. On the other hand, another study reported that when future layer 2/3 neurons are forced to be placed into layer 5, they do not acquire layer 5 features [36]. These results suggest that a variety of extracellular signals may modify fate specification in a subtype-specific manner. In fact, we found that the maturation of layer 4 neurons is controlled by thalamocortical axons (TCAs), which mainly project to the layer 4 neurons in mature neuronal circuits (see below).

The occurrence of the fate conversion mentioned above raises the possibility that future layer 2/3 and layer 4 neurons have common characteristics in their immature stages. Indeed, both immature layer 2/3 and layer 4 neurons that have just finished radial migration express the transcription factor Brn2, which is eventually expressed in layer 2/3 but not in layer 4 [24]. In future layer 4 neurons, the expression of another transcription factor, Rorb, is sharply increased during maturation, while Brn2 is down-regulated. Further investigation of the regulatory mechanisms involving Brn2 and Rorb revealed a mutually repressive interaction, which could work as a positive feedback loop (e.g., an increase in Rorb expression causes a decrease in Brn1/2 expression, leading to the further upregulation of Rorb), and an essential role in the specification of layer 2/3 and layer 4 neurons. These results strongly support the notion that the eventual specification of superficial layer subtypes is modified in post-mitotic immature neurons [24]. Of special note, the onset of Rorb expression was observed in developing layer 4 neurons a few days after birth, while Rorb expression during embryonic stages was confined to future layer 5 neurons. Thus, Rorb cannot be used as a layer 4 marker in analyses of embryonic brains, as is often the case when analyzing mutant mice that show perinatal lethality.

Regulation of subtype specification by thalamocortical axons

The cerebral cortex comprises dozens of functional areas such as motor, somatosensory and visual areas [37]. Cortical areas differ in several aspects including histological organization, which mainly reflects differences in the thicknesses of layers, patterns of connectivity and molecular properties, indicating that the number and characteristics of the components (i.e., neuronal subtypes) vary in different areas. Therefore, the arealization process, especially when accompanied by changes in gene expression profiles, can be regarded as a diversification of neuronal subtype specification.

Two hypotheses have been postulated for the process of arealization of the neocortex. One is a proto-map model, in which regional differences are predetermined at early developmental stages [38]. This model is supported by the findings that the gradient expression of several fate determinants in NPCs along the rostrocaudal, mediolateral and dorsoventral axes play critical roles in arealization (e.g., expression patterns of Pax6 [39], Emx2 [40], Sp8 [41,42] and Coup-TF1 [43]). The other is a proto-cortex model, in which input/influence from outside of the neocortex regulates arealization while NPCs have equivalent potential [44]. This model requires the regulation of differentiation in immature neurons, which is of interest in this review.

TCAs have been postulated as an extra-cortical cue in the proto-cortex model because of their region-specific projection to the cortex from a given thalamic nucleus (e.g., projection to the primary somatosensory area from the ventral posterior medial nucleus [VPM] and to the primary visual area from the lateral geniculate nucleus [LGN]) [37]. This notion has been supported by several studies. Surgically induced ectopic axonal projection from the LGN (the axons of which normally project to the visual area) to the auditory area is sufficient to convert this area into a visual-like area [45]. Another study showed that the engraftment of visual areal tissues into the somatosensory area can result in the acquisition of some ‘somatosensory’ characteristics [46].

Recent genetic studies also support the notion of TCAs as an extra-cortical source for arealization. The genetic alteration of the LGN-visual area projection resulted in the dysregulation of the delineation of primary and high-order visual areas as well as areal size [47,48]. Moreover, Jabaudon’s group showed that the identity of layer 4 neurons is controlled by modality-specific thalamocortical signals [49]. Through the genetic disruption of the VPM, the primary somatosensory area, which receives input from the VPM, alternatively receives input from the posterior nucleus, a non-specific nucleus, which normally sends axons to layer 4 neurons in the secondary somatosensory area. This change instructs the primary

somatosensory area to differentiate into the secondary somatosensory-like structure, which eventually responds to nociceptive stimuli, which normally activate the secondary somatosensory area [49].

Our finding that layer 4 subtype specification requires the correct positioning of immature neurons prompted us to examine whether TCAs provide a positional cue for the differentiation of superficial layer neurons. The analysis of mutant mice in which virtually all TCAs were absent in the neocortex revealed a decrease in layer 4 neurons and an increase in layer 2/3 neurons instead, suggesting that TCAs are essential for determining the number of neurons in superficial layers [35]. We speculated that TCAs induce the expression of Rorb or reduce that of Brn1/2 in future layer 4 neurons, unbalancing the mutual repressive interaction between these factors and leading to the establishment of Rorb-high and Brn1/2-low expression in layer 4 (Figure 2). The influences of TCAs on other layers have also been described [47,48,50], implying that TCAs may act on multiple layers to establish area-dependent features.

Although several TCA-derived molecules that affect the dendritic development of cortical neurons have been identified [51], the overall molecular mechanisms of how TCAs regulate cortical subtypes remain to be determined. Because the timing of synapse formation

between TCAs and layer 4 neurons corresponds to that of the maturation process of layer 4 neurons (e.g., the onset of Rorb expression and the morphological change into stellate cells), a neuronal activity-dependent mechanism has been assumed. Although several studies have shown that TCA input is critical for the formation of a barrel structure, a specific feature of the mature primary somatosensory area in rodents [52,53], the effect of synaptic TCA input on subtype specification has remained unclear. A recent study using conditional knockout mice that showed no synaptic transmission from TCAs to cortical neurons due to removal of all the glutamate transporters from thalamocortical neurons revealed that synaptic input is required for the maintenance of the cortical layer structure as well as that of subtype specific marker expressions [50]. Importantly, although barrel structures are missing in this mutant line similar to the observations made in previous studies, the specification of layer 4 neurons, as judged by the expression of molecular markers, occurred correctly until around one week after birth. Therefore, we speculate that the initial specification of layer 4 neurons does not require synaptic transmission but rather is instructed via molecular mechanisms evoked by direct cell-cell interactions or secreted molecules. Molecular interactions accompanied by synaptic transmission may also play a role. During the maturation stages of layer 4 neurons, synaptic input through TCAs would induce barrel structures [52,53] and then maintain the

layer structure and neuronal subtypes after maturation [50]. More generally, this kind of mechanism can control subtype specification not only in the barrel structure, but also in the radially oriented columnar structure, which is a feature of the cerebral cortex [38,54].

Concluding remarks

One of the fundamental questions to be solved in cortical development is how the eventual 'layer', which is composed of several subtypes of neurons, is formed during development. A prevailing idea was that the formation of cortical layers is not regulated by precise mechanisms, but by a process whereby neurons finish radial migration and accumulate. However, some studies suggest that neurons are actively regulated so that they become located in their eventual positions irrespective of radial migration processes. An in vitro study has shown that cortical neurons acquire a birth-date-dependent segregation mechanism before they finish radial migration [55]. In Pcdh20-knockdown neurons in vivo, the process by which the post-migratory future layer 4 neurons begin to locate to the lower part of the superficial cortical plate is specifically disrupted [35]. Another study has reported that Robo1-knockdown neurons do not reach their final position correctly, mainly because of a defect in the process of cell positioning after radial migration [56]. The accumulation of cortical neurons in the PCZ, the outermost cell-dense region of the CP, has also been

proposed to be involved in proper laminar formation via mechanisms such as cell sorting among the neurons [57,58].

Much attention has been paid to understanding the proliferation/differentiation of NPCs and the migration of neurons during corticogenesis. However, processes after radial migration, including subtype specification and cell positioning, also play important roles in establishing the highly organized cerebral cortex. What, then, is the advantage of forming a highly organized structure? Recently, Reelin, an essential extracellular matrix protein that regulates cortical lamination, has been reported to play a role in the formation of neuronal networks [59]. Although the paper also claimed that the inside-out positioning of neurons is essential for network formation, the *reeler* neocortex has a markedly disorganized cortical structure; thus, whether inside-out positioning is indeed involved in this process remains an unsolved question. Nevertheless, this result strongly suggests that an organized cortical structure is pivotal to the proper formation of neuronal networks, thereby enabling the execution of proper neuronal functions.

References

1. Greig LC, Woodworth MB, Galazo MJ, Padmanabhan H, Macklis JD (2013) Molecular logic of neocortical projection neuron specification, development and diversity. Nat Rev Neurosci 14 (11):755-769. doi:nrn3586 [pii]
10.1038/nrn3586

2. Lodato S, Arlotta P (2015) Generating neuronal diversity in the mammalian cerebral cortex. Annu Rev Cell Dev Biol 31:699-720. doi:10.1146/annurev-cellbio-100814-125353

3. Kwan KY, Sestan N, Anton ES (2012) Transcriptional co-regulation of neuronal migration and laminar identity in the neocortex. Development 139 (9):1535-1546. doi:10.1242/dev.069963

4. Marin O, Rubenstein JL (2001) A long, remarkable journey: tangential migration in the telencephalon. Nat Rev Neurosci 2 (11):780-790. doi:10.1038/35097509

5. Barber M, Pierani A (2016) Tangential migration of glutamatergic neurons and cortical patterning during development: Lessons from Cajal-Retzius cells. Dev Neurobiol 76 (8):847-881. doi:10.1002/dneu.22363

6. Nakajima K (2007) Control of tangential/non-radial migration of neurons in the developing cerebral cortex. Neurochem Int 51 (2-4):121-131. doi:10.1016/j.neuint.2007.05.006

7. Takahashi T, Goto T, Miyama S, Nowakowski RS, Caviness VS, Jr. (1999) Sequence of neuron origin and neocortical laminar fate: relation to cell cycle of origin in the developing murine cerebral wall. J Neurosci 19 (23):10357-10371

8. Angevine JB, Jr., Sidman RL (1961) Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. Nature 192:766-768

9. Rakic P (1974) Neurons in rhesus monkey visual cortex: systematic relation between time of origin and eventual disposition. Science 183 (123):425-427

10. Dehay C, Kennedy H (2007) Cell-cycle control and cortical development. Nat Rev Neurosci 8 (6):438-450. doi:nrn2097 [pii]
10.1038/nrn2097

11. Kohwi M, Doe CQ (2013) Temporal fate specification and neural progenitor competence during development. Nat Rev Neurosci 14 (12):823-838

12. McConnell SK, Kaznowski CE (1991) Cell cycle dependence of laminar determination in developing neocortex. Science 254 (5029):282-285

13. Frantz GD, McConnell SK (1996) Restriction of late cerebral cortical progenitors to an upper-layer fate. Neuron 17 (1):55-61. doi:S0896-6273(00)80280-9 [pii]

14. Alsio JM, Tarchini B, Cayouette M, Livesey FJ (2013) Ikaros promotes early-born

neuronal fates in the cerebral cortex. Proc Natl Acad Sci U S A 110 (8):E716-725. doi:10.1073/pnas.1215707110

15. Dominguez MH, Ayoub AE, Rakic P (2013) POU-III transcription factors (Brn1, Brn2, and Oct6) influence neurogenesis, molecular identity, and migratory destination of upper-layer cells of the cerebral cortex. Cereb Cortex 23 (11):2632-2643. doi:bhs252 [pii] 10.1093/cercor/bhs252

16. Frantz GD, Weimann JM, Levin ME, McConnell SK (1994) Otx1 and Otx2 define layers and regions in developing cerebral cortex and cerebellum. J Neurosci 14 (10):5725-5740

17. Hirata T, Suda Y, Nakao K, Narimatsu M, Hirano T, Hibi M (2004) Zinc finger gene fez-like functions in the formation of subplate neurons and thalamocortical axons. Dev Dyn 230 (3):546-556. doi:10.1002/dvdy.20068

18. Honda T, Kobayashi K, Mikoshiba K, Nakajima K (2011) Regulation of cortical neuron migration by the Reelin signaling pathway. Neurochem Res 36 (7):1270-1279. doi:10.1007/s11064-011-0407-4

19. Hevner RF, Daza RA, Rubenstein JL, Stunnenberg H, Olavarria JF, Englund C (2003) Beyond laminar fate: toward a molecular classification of cortical projection/pyramidal neurons. Dev Neurosci 25 (2-4):139-151. doi:10.1159/000072263 DNE20030252_4139 [pii]

20. Dekimoto H, Terashima T, Katsuyama Y (2010) Dispersion of the neurons expressing layer specific markers in the reeler brain. Dev Growth Differ 52 (2):181-193. doi:DGD1153 [pii] 10.1111/j.1440-169X.2009.01153.x

21. Boyle MP, Bernard A, Thompson CL, Ng L, Boe A, Mortrud M, Hawrylycz MJ, Jones AR, Hevner RF, Lein ES (2011) Cell-type-specific consequences of Reelin deficiency in the mouse neocortex, hippocampus, and amygdala. J Comp Neurol 519 (11):2061-2089. doi:10.1002/cne.22655

22. Arlotta P, Molyneaux BJ, Chen J, Inoue J, Kominami R, Macklis JD (2005) Neuronal subtype-specific genes that control corticospinal motor neuron development in vivo. Neuron 45 (2):207-221. doi:S0896627304008530 [pii] 10.1016/j.neuron.2004.12.036

23. Galazo MJ, Emsley JG, Macklis JD (2016) Corticothalamic Projection Neuron Development beyond Subtype Specification: Fog2 and Intersectional Controls Regulate Intraclass Neuronal Diversity. Neuron 91 (1):90-106. doi:10.1016/j.neuron.2016.05.024

24. Oishi K, Aramaki M, Nakajima K (2016) Mutually repressive interaction between Brn1/2 and Rorb contributes to the establishment of neocortical layer 2/3 and layer 4. Proc Natl

Acad Sci U S A 113 (12):3371-3376. doi:10.1073/pnas.1515949113

25. Hevner RF, Shi L, Justice N, Hsueh Y, Sheng M, Smiga S, Bulfone A, Goffinet AM, Campagnoni AT, Rubenstein JL (2001) Tbr1 regulates differentiation of the preplate and layer 6. Neuron 29 (2):353-366. doi:S0896-6273(01)00211-2 [pii]

26. Alcamo EA, Chirivella L, Dautzenberg M, Dobreva G, Farinas I, Grosschedl R, McConnell SK (2008) Satb2 regulates callosal projection neuron identity in the developing cerebral cortex. Neuron 57 (3):364-377. doi:S0896-6273(07)01017-3 [pii]
10.1016/j.neuron.2007.12.012

27. Britanova O, de Juan Romero C, Cheung A, Kwan KY, Schwark M, Gyorgy A, Vogel T, Akopov S, Mitkovski M, Agoston D, Sestan N, Molnar Z, Tarabykin V (2008) Satb2 is a postmitotic determinant for upper-layer neuron specification in the neocortex. Neuron 57 (3):378-392. doi:S0896-6273(08)00033-0 [pii]
10.1016/j.neuron.2007.12.028

28. Joshi PS, Molyneaux BJ, Feng L, Xie X, Macklis JD, Gan L (2008) Bhlhb5 regulates the postmitotic acquisition of area identities in layers II-V of the developing neocortex. Neuron 60 (2):258-272. doi:S0896-6273(08)00674-0 [pii]
10.1016/j.neuron.2008.08.006

29. Molyneaux BJ, Arlotta P, Hirata T, Hibi M, Macklis JD (2005) Fezl is required for the birth and specification of corticospinal motor neurons. Neuron 47 (6):817-831. doi:S0896-6273(05)00732-4 [pii]
10.1016/j.neuron.2005.08.030

30. Azim E, Shnider SJ, Cederquist GY, Sohur US, Macklis JD (2009) Lmo4 and Clim1 progressively delineate cortical projection neuron subtypes during development. Cereb Cortex 19 Suppl 1:i62-69. doi:10.1093/cercor/bhp030

31. Rouaux C, Arlotta P (2010) Fezf2 directs the differentiation of corticofugal neurons from striatal progenitors in vivo. Nat Neurosci 13 (11):1345-1347. doi:10.1038/nn.2658

32. De la Rossa A, Bellone C, Golding B, Vitali I, Moss J, Toni N, Luscher C, Jabaudon D (2013) In vivo reprogramming of circuit connectivity in postmitotic neocortical neurons. Nat Neurosci 16 (2):193-200. doi:nn.3299 [pii]
10.1038/nn.3299

33. Tachikawa K, Sasaki S, Maeda T, Nakajima K (2008) Identification of molecules preferentially expressed beneath the marginal zone in the developing cerebral cortex. Neurosci Res 60 (2):135-146. doi:S0168-0102(07)01797-X [pii]
10.1016/j.neures.2007.10.006

34. Sekine K, Honda T, Kawauchi T, Kubo K, Nakajima K (2011) The outermost region of the

developing cortical plate is crucial for both the switch of the radial migration mode and the Dab1-dependent "inside-out" lamination in the neocortex. J Neurosci 31 (25):9426-9439. doi:31/25/9426 [pii]
10.1523/JNEUROSCI.0650-11.2011
35. Oishi K, Nakagawa N, Tachikawa K, Sasaki S, Aramaki M, Hirano S, Yamamoto N, Yoshimura Y, Nakajima K (2016) Identity of neocortical layer 4 neurons is specified through correct positioning into the cortex. Elife 5. doi:10.7554/eLife.10907
36. Ramos RL, Bai J, LoTurco JJ (2006) Heterotopia formation in rat but not mouse neocortex after RNA interference knockdown of DCX. Cereb Cortex 16 (9):1323-1331. doi:bhj074 [pii]
10.1093/cercor/bhj074
37. O'Leary DD, Chou SJ, Sahara S (2007) Area patterning of the mammalian cortex. Neuron 56 (2):252-269. doi:S0896-6273(07)00772-6 [pii]
10.1016/j.neuron.2007.10.010
38. Rakic P (1988) Specification of cerebral cortical areas. Science 241 (4862):170-176
39. Bishop KM, Rubenstein JL, O'Leary DD (2002) Distinct actions of Emx1, Emx2, and Pax6 in regulating the specification of areas in the developing neocortex. J Neurosci 22 (17):7627-7638
40. Muzio L, Di Benedetto B, Stoykova A, Boncinelli E, Gruss P, Mallamaci A (2002) Conversion of cerebral cortex into basal ganglia in Emx2(-/-) Pax6(Sey/Sey) double-mutant mice. Nat Neurosci 5 (8):737-745. doi:10.1038/nn892
41. Zembrzycki A, Griesel G, Stoykova A, Mansouri A (2007) Genetic interplay between the transcription factors Sp8 and Emx2 in the patterning of the forebrain. Neural Dev 2:8. doi:10.1186/1749-8104-2-8
42. Sahara S, Kawakami Y, Izpisua Belmonte JC, O'Leary DD (2007) Sp8 exhibits reciprocal induction with Fgf8 but has an opposing effect on anterior-posterior cortical area patterning. Neural Dev 2:10. doi:10.1186/1749-8104-2-10
43. Liu Q, Dwyer ND, O'Leary DD (2000) Differential expression of COUP-TFI, CHL1, and two novel genes in developing neocortex identified by differential display PCR. J Neurosci 20 (20):7682-7690
44. O'Leary DD (1989) Do cortical areas emerge from a protocortex? Trends Neurosci 12 (10):400-406
45. Sur M, Leamey CA (2001) Development and plasticity of cortical areas and networks. Nat Rev Neurosci 2 (4):251-262. doi:10.1038/35067562
46. Schlaggar BL, O'Leary DD (1991) Potential of visual cortex to develop an array of

functional units unique to somatosensory cortex. Science 252 (5012):1556-1560

47. Chou SJ, Babot Z, Leingartner A, Studer M, Nakagawa Y, O'Leary DD (2013) Geniculocortical input drives genetic distinctions between primary and higher-order visual areas. Science 340 (6137):1239-1242. doi:10.1126/science.1232806

48. Vue TY, Lee M, Tan YE, Werkhoven Z, Wang L, Nakagawa Y (2013) Thalamic control of neocortical area formation in mice. J Neurosci 33 (19):8442-8453. doi:10.1523/JNEUROSCI.5786-12.2013

49. Pouchelon G, Gambino F, Bellone C, Telley L, Vitali I, Luscher C, Holtmaat A, Jabaudon D (2014) Modality-specific thalamocortical inputs instruct the identity of postsynaptic L4 neurons. Nature 511 (7510):471-474. doi:nature13390 [pii] 10.1038/nature13390

50. Li H, Fertuzinhos S, Mohns E, Hnasko TS, Verhage M, Edwards R, Sestan N, Crair MC (2013) Laminar and columnar development of barrel cortex relies on thalamocortical neurotransmission. Neuron 79 (5):970-986. doi:S0896-6273(13)00559-X [pii] 10.1016/j.neuron.2013.06.043

51. Sato H, Fukutani Y, Yamamoto Y, Tatara E, Takemoto M, Shimamura K, Yamamoto N (2012) Thalamus-derived molecules promote survival and dendritic growth of developing cortical neurons. J Neurosci 32 (44):15388-15402. doi:10.1523/JNEUROSCI.0293-12.2012

52. Mizuno H, Luo W, Tarusawa E, Saito YM, Sato T, Yoshimura Y, Itohara S, Iwasato T (2014) NMDAR-regulated dynamics of layer 4 neuronal dendrites during thalamocortical reorganization in neonates. Neuron 82 (2):365-379. doi:10.1016/j.neuron.2014.02.026

53. Narboux-Neme N, Evrard A, Ferezou I, Erzurumlu RS, Kaeser PS, Laine J, Rossier J, Ropert N, Sudhof TC, Gaspar P (2012) Neurotransmitter release at the thalamocortical synapse instructs barrel formation but not axon patterning in the somatosensory cortex. J Neurosci 32 (18):6183-6196. doi:10.1523/JNEUROSCI.0343-12.2012

54. Prothero J (1997) Cortical scaling in mammals: a repeating units model. J Hirnforsch 38 (2):195-207

55. Ajioka I, Nakajima K (2005) Birth-date-dependent segregation of the mouse cerebral cortical neurons in reaggregation cultures. Eur J Neurosci 22 (2):331-342. doi:EJN4214 [pii] 10.1111/j.1460-9568.2005.04214.x

56. Gonda Y, Andrews WD, Tabata H, Namba T, Parnavelas JG, Nakajima K, Kohsaka S, Hanashima C, Uchino S (2013) Robo1 regulates the migration and laminar distribution of upper-layer pyramidal neurons of the cerebral cortex. Cereb Cortex 23 (6):1495-1508. doi:10.1093/cercor/bhs141

57. Hirota Y, Nakajima K (2017) Control of Neuronal Migration and Aggregation by Reelin

Signaling in the Developing Cerebral Cortex. Front Cell Dev Biol 5:40. doi:10.3389/fcell.2017.00040

58. Sekine K, Kubo K, Nakajima K (2014) How does Reelin control neuronal migration and layer formation in the developing mammalian neocortex? Neurosci Res 86:50-58. doi:10.1016/j.neures.2014.06.004

59. He S, Li Z, Ge S, Yu YC, Shi SH (2015) Inside-Out Radial Migration Facilitates Lineage-Dependent Neocortical Microcircuit Assembly. Neuron 86 (5):1159-1166. doi:10.1016/j.neuron.2015.05.002

Figure legends

Figure 1. Models of subtype specification of cortical neurons.

A. The differentiation potential of NPCs changes over time, resulting in the generation of different neuronal subtypes. The colors of the NPCs represent the different characteristics of the NPCs.

B. The neuronal subtypes are not determined during immature stages (left). After the positioning of the neurons in the cortical plate, they acquire their own characteristics by receiving extracellular signals, such as environmental cues (right).

Figure 2. Subtype specification of post-migratory superficial layer neurons.

Future layer 4 neurons are not yet specified when they reach the outer-most part of the cortical plate. In fact, they express Brn2, a layer 2/3 marker in the mature neocortex (upper left). After radial migration, future layer 4 neurons are positioned in the lower part of the cortical plate in a Pcdh20-dependent manner (upper middle). This positioning allows them to come in contact with thalamocortical axons (TCAs), thereby initiating programs to differentiate into layer 4 neurons. The programs include a mutually repressive interaction

between Rorb and Brn1/2. Future layer 4 and layer 2/3 neurons finally begin to express Rorb and Brn1/2, respectively, with these factors playing critical roles in the differentiation of each neuronal subtype (upper right). If any of these steps is blocked, the future layer 4 neurons do not differentiate into layer 4, but instead acquire layer 2/3 characteristics (bottom).