Cortical development: the art of generating cell diversity

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Summary

The fascinating question of how the enormous diversity of neuronal and glial cells in the cerebral cortex is generated during development was recently discussed at a meeting on cortical development and stem cells in Greece. What emerged from this meeting is an equally fascinating answer, namely that precursor diversity at rather early stages of development anticipates later cell type diversity.

Introduction

The steeply cliffed Aegean island of Santorini was the venue for a recent meeting on neural stem cells and cortical development that was organized by John Parnavelas (University College, London, UK) and Arnold Kriegstein (University of California, San Francisco, CA, USA). The stimulating atmosphere and success of the meeting was fortunately not affected by a pan-Hellenic strike on 11 May, which caused many of the meeting's participants to have their own personal experience of the never-ending travels of Odysseus, as described in ancient Greek mythology.

The ground plan

The cerebral cortex covers almost the entire human brain, owing to its enormous growth and increase in surface area during mammalian evolution. It forms from the most rostral region of the neural tube, the prosencephalon, that later divides into the telencephalon and diencephalon. The dorsal region of the telencephalon gives rise to the cerebral cortex, which comprises the neocortex, paleocortex (piriform cortex) and archicortex (hippocampus), while the ventral telencephalon differentiates into the basal ganglia. These dorsoventral, and also rostrocaudal, differences are established by diffusible morphogens, which are released from distinct locations. For example, fibroblast growth factor 8 (Fgf8) is produced rostrally, while Wnt and bone morphogenetic proteins are released caudally and dorsally, and sonic hedgehog (Shh) is released ventrally (Fig. 1A) (Shimogori et al., 2004). These factors instruct the surrounding tissue to acquire a 'dorsal', 'rostral' or 'ventral' identity, as is evident when Wnt signalling is activated or deleted and causes consequent changes in dorsal patterning. For example, as presented by Corinne Houart (King's College, London, UK), constitutively activating Wnt signalling in zebrafish leads to a dorsalization of the telencephalon. Wnt signalling in the caudodorsal region of the telencephalon does not rely on a single ligand, but rather on several members of the Wnt family, most of which are expressed in a small region located in the caudomedial telencephalon called the cortical hem (Grove et al., 1998). The cortical hem is intercalated between the medial cortex, which will later develop into the hippocampal anlage, and the medial most choroid plexus anlage (Fig. 1A). Besides the richness of Wnt gene expression in the cortical hem, its precise role and progeny have been unknown. However, Liz Grove (University of Chicago, Chicago, IL, USA) shed new light on this at the meeting from her fate-mapping studies of the hem and its progeny. When these researchers crossed a *Wnt3a-Cre* mouse line to reporter strains, they found that reelin-positive cells spread over the dorsal telencephalon in the uppermost layer of the cerebral cortex (layer 1) were labelled as the main progeny of the cortical hem, consistent with previous data (Takiguchi-Hayashi et al., 2004). It is these cells that are largely lost if the hem is ablated at embryonic day (E) 10 by hem-specific diphtheria toxin expression. Notably, however, no obvious patterning defects occur upon hem ablation, nor is cortical layering abnormal, despite the severe reduction of reelin-positive cells (the absence of reelin in the *reeler* mouse mutant leads to defects in cortical layering, as detailed below) (for a review, see Tissir and Goffinet, 2003). A possible explanation for the absence of patterning defects in the dorsal telencephalon, despite ablation of the cortical hem, may be timing. When β-catenin is deleted at E8 in the mouse, the prospective dorsal telencephalon becomes at least partially ventralized, whereas this is not the case when β-catenin is deleted at E11 (Backman et al., 2005). Thus, the absence of gross dorsalization defects in the telencephalon upon hem deletion at E10 might mean that either the crucial time window for Wnt signalling from the cortical hem takes place prior to E10 or that there are other sources of Wnt signalling outside the cortical hem that are sufficient to maintain the dorsal identity of the dorsal telencephalon, at least at these latter stages. Indeed, signalling centres are often most crucial at the earliest developmental stages, and lose their importance when a tissue grows and regional cell identity has been intrinsically fixed by cell autonomous mechanisms (Li et al., 2005). Taken together, at mostly early stages of telencephalic development, local sources of signalling molecules are crucial in setting up the ground plan of the telencephalon and are crucially required for its subsequent differentiation into the cerebral cortex dorsally and the basal ganglia ventrally. As discussed below, new insights were presented at the meeting as to how cell biological processes regulate such signalling events.

Spotlight on cilia and microvilli

A mutagenesis screen designed to identify molecular mechanisms that regulate telencephalic patterning (Zarbalis et al., 2004) revealed two mouse mutants with somewhat paradoxical defects in dorsoventral patterning: an early loss of ventral structures but an apparent ventralization of the cortex over time. The defects were traced to a loss of the retrograde motor for intraflagellar transport, and the telencephalon in these mutant mice had neuroectodermal cells with abnormally thick cilia filled with intraflagellar transport particles. Andy

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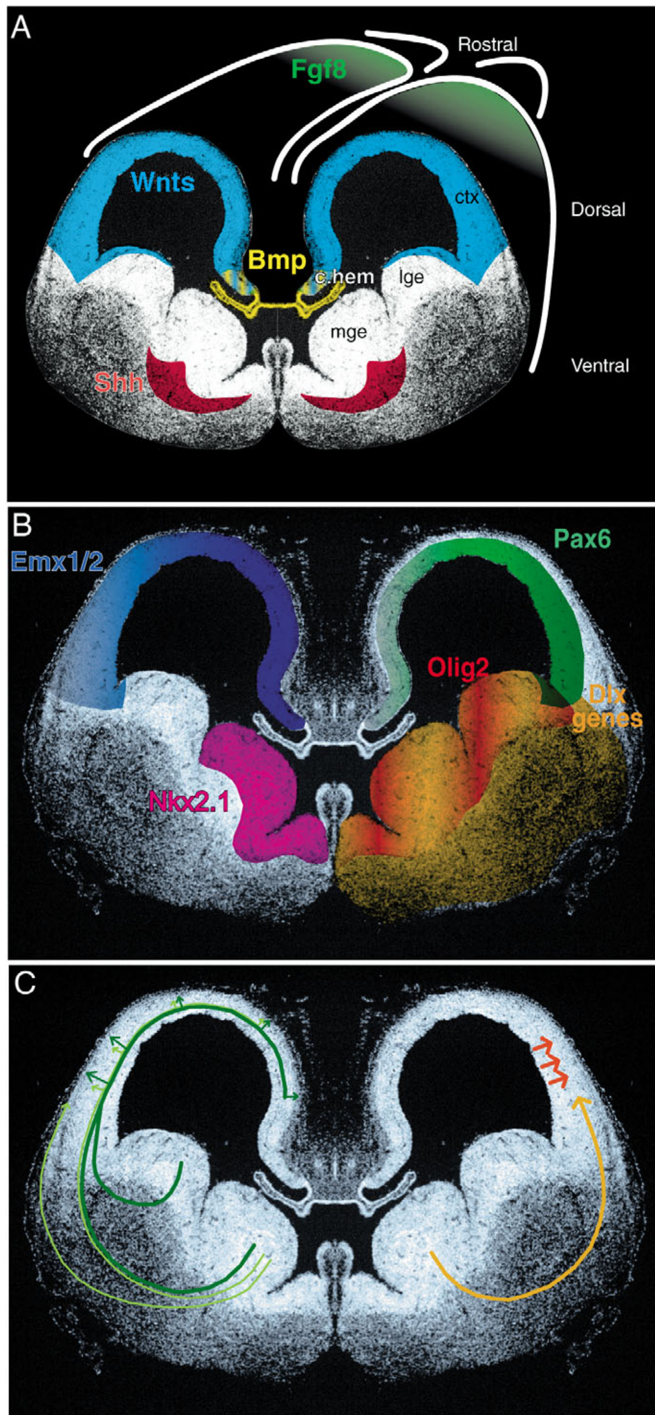

Fig. 1. Key gene expression and migration patterns in telencephalic development. (A) The key morphogens, (B) the patterning transcription factors and (C) the main pathways of ventrodorsal cell migration in a frontal section of a DAPI-labelled telencephalon of the developing mouse telencephalon at embryonic day 13. Many signalling and migrational pathways, as well as transcription factors, have been omitted, as we have illustrated here just the morphogens, transcription factors and migrational pathways discussed in the text. Dark green, late migrating interneurons; light green, early migrating interneurons; red, late generated oligodendrocytes; yellow, early generated oligodendrocytes. c. hem, cortical hem; ctx, cerebral cortex adjacent to the choroid plexus (yellow); lge, lateral ganglionic eminence; mge, medial ganglionic eminence.

Peterson (University of California, San Francisco, CA, USA) presented evidence that it is the intraflagellar transport directed out of the cilia that fails in these mouse mutants. He presented additional evidence that the smoothened (Smo) protein, an essential component of the Shh signal transduction pathway, is normally localized to cilia. The inability of the mutants to localize Smo to the cilia causes a loss of both transcriptional activation and transcriptional repression by the Gli transcription factors (Liu et al., 2005). Shh is expressed in the ventral most regions of the developing telencephalon (Fig. 1A) and acts via Gli activators to regulate Nkx2.1 and specify ventral regions (Fig. 1B), while Gli-repressor activity is required for dorsal telencephalic regions to develop. Thus, intraflagellar transport in apically located cilia is crucial for a specific signalling pathway, an important demonstration of a role for these enigmatic organelles.

In support of a broad role of such apically located signalling mechanisms, Wieland Huttner (Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany) presented a novel mechanism of shedding of apical fate determinants. CD133 (also known as prominin 1) is contained on membrane protrusions of many diverse stem cells, including neuroepithelial cells, where it locates to the tips of microvilli in a distinct cholesterol-based lipid microdomain (Corbeil et al., 2000; Kosodo et al., 2004; Weigmann et al., 1997). Although its precise functional role is not yet well understood, Huttner presented strong evidence that cells discard the prominin-containing domain of the apical membrane in a novel regulated manner (Marzesco et al., 2005). Huttner and colleagues detected two classes of prominin-containing vesicles, named prominosomes, in the ventricular fluid (the apical axis of neuroepithelial or radial glia cells are exposed to the ventricular surface) that do not contain clathrin and hence are not released by exocytosis. The relative proportion of small and large prominosomes in the ventricular fluid changes during development. The presence of microvilli on neuroepithelial cells correlates with a predominance of small prominosomes in the ventricular fluid, while at later stages, large protrusions on neuroepithelial/radial glial cells seemingly give rise to the large prominosomes in the ventricle. Intriguingly, the release of prominosomes correlates with the reduction of the apical plasma membrane from 2% in symmetrically dividing cells to 1% in asymmetrically dividing cells (Kosodo et al., 2004), suggesting that the regulated release of prominosomes may prime the cells for asymmetric cell division during neurogenesis.

Diversity of cortical precursors

The findings presented above highlight the importance of signalling within the ventricular fluid and the role of the apical membrane domain in signalling in cell division and cortical development. Indeed, elegant time-lapse studies of individual precursors in cortical slice preparations have demonstrated that an important difference exists between the precursors that divide at the apical surface (ventricular zone precursors) or within the parenchyma (abventricular mitoses, basal precursors): only the former divide asymmetrically; the latter nearly always divide symmetrically and in most cases only once (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004). Thus, cell fate and the number of cell divisions are rather limited in the absence of access to apical fate

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determinants. As the apical membrane surface is so small (1-2% of the total membrane, see above), its unequal or equal distribution does not readily correlate with the orientation of cell division (Kosodo et al., 2004). Vertically oriented cell division can either divide the apical membrane patch equally, in which case both daughters remain as precursors, or unequally, resulting in the generation of two unequal daughters: a neuron and a precursor cell. Thus, it is the membrane fusion mediated by the cleavage furrow, rather than the gross orientation of cell division, that seems to predict the mode of cell division – at least in the mammalian cerebral cortex (reviewed by Götz and Huttner, 2005). Consistent with this concept, Steven Noctor (University of California, San Francisco, CA, USA) presented evidence that the basally located precursors undergo randomly oriented divisions with a bias towards a horizontal plane of cell division, consistent with previous observations (Smart, 1973). Thus, the orientation of cell division can not serve as an indicator of symmetric or asymmetric daughter cell fate. A riddle not yet solved, however, is why abventricular cell divisions are neurogenic in the embryonic cortex, while at some point, glial precursors also divide at this position. In fact, glial precursors also divide mostly symmetrically with the notable exception of some adult neural stem cells that are characterized by access to the ventricle (Alvarez-Buylla et al., 2001). Indeed, signalling from the ventricle also continues to exert crucial influence during adult neurogenesis. Arturo Alvarez-Buylla (University of California, San Francisco, CA, USA) presented evidence for a signalling gradient that is established by the secretion of diffusible molecules from the choroid plexus into the cerebrospinal fluid and by the directed beating of ependymal cilia, which influences the directed migration of neuroblasts in the adult subependymal zone.

As evident from the above discussion, both the location and time of cell division have to be tightly controlled during cortical development to ensure the generation of proper neuronal and non-neuronal cell numbers, but how this is molecularly regulated still remains unknown. The Notch-signalling effectors Hes1, Hes3 and Hes5 might be crucial players in this process, as nicely illustrated by Ryoichiro Kageyama (Kyoto University, Kyoto, Japan), who presented the effects of disrupting Hes genes in mice. Their loss, he showed, interfered with the capacity of radial glia to undergo asymmetric cell division, thereby causing radial glia cell depletion and premature neurogenesis (Hatakeyama et al., 2004). In addition, both apical intercellular junctions and the basal lamina are lost in these mutants, resulting in the scattering of neuronal cells and in the disorganization of brain and spinal cord structures. These data also raise the intriguing possibility that Notch signalling acts via, or may require, adherens junction complexes, thereby providing yet another link between cell fate and apicobasal membrane specializations (as also evident in the numb and numb-like mutant cortex) (Li et al., 2003). According to this idea, Notch-signalling via Hes maintains neuroepithelial or radial glia identity (Gaiano et al., 2000), while Hes expression is not maintained in abventricular progenitors, allowing these cells to differentiate into neurons. Hes proteins have a similar function in maintaining radial glial properties in brain compartment boundaries and in signalling centres, where *Hes1* expression is high and persistent, resulting in the permanent suppression of neurogenesis. Strikingly, although Hes factors can promote cell proliferation by repressing cyclin-dependent kinase inhibitors (Castella et al., 2000; Murata et al., 2005), persistently high levels of Hes (as found in boundary cells) reduce cell proliferation, while still preventing neurogenesis. This role is reminiscent of a general mechanism for maintaining stem cells in a quiescent state – a notion that is supported by the fact that some boundary cells maintain undifferentiated or stem-cell-like properties into adulthood (Alvarez-Buylla et al., 2001; Geling et al., 2004).

Specificity in origin relates to subtype diversity

Once a multi- or bi-potent precursor cell has chosen to generate further differentiated progeny, such as neuronal or glial precursors in the neuroepithelium, how is neuronal and glial subtype heterogeneity established? During telencephalon development, GABAergic interneurons and oligodendrocyte precursors migrate from ventral regions into dorsal territories (Fig. 1C). However, not all oligodendrocyte precursors originate at ventral positions, but rather originate in various telencephalic (and spinal cord) regions. This was beautifully demonstrated by Nic Kessaris (University College, London, UK), who used a rich collection of mice expressing the Cre recombinase in specific regions of the developing brain [and spinal cord, see Fogarty et al. (Fogarty et al., 2005)]. Kessaris and colleagues' fate-mapping of the progeny of cells from the *Nkx2.1*-expressing medial ganglionic eminence (Fig. 1B) (which they mapped by crossing a *Nkx2.1*::*Cre* line to a reporter line) confirmed that all oligodendrocyte precursors in the dorsal telencephalon and the cerebral cortex originally derived from ventral sources at most embryonic stages. However, their numbers dwindled at late embryonic stages, until only 4% of all oligodendrocytes were labelled in the postnatal and adult brain. Indeed, the converse fate-mapping experiment, using *Emx1* to drive *Cre* in the dorsal telencephalon (Fig. 1B), revealed that oligodendrocytes originating from dorsal regions take over around birth, when 30% of all oligodendrocytes are derived from dorsal precursors in the cortex, reaching 50% at later stages. Thus, oligodendrocyte precursors from ventral regions dominate at embryonic stages throughout the telencephalon, while regional sources of oligodendrocytes take over postnatally (see also Ivanova et al., 2003; Spassky et al., 2001). A similar scenario occurs in the subplate and marginal zone, where transient sources of neurons function during development, while other neuronal subtypes take over in the adult cortex. However, by using the Cre-lines to ablate specific oligodendrocyte precursors, Kessaris showed that each population of oligodendrocyte precursors can compensate for the other, and, so far, no oligodendrocyte precursor that has a region-specific origin has been observed to have a specific function. As ventrally derived precursors generate probably both interneurons and oligodendrocytes (He et al., 2001; Yung et al., 2002), the ventrally derived precursors might be bipotent neuro-oligo precursors, while the dorsally derived oligodendrocyte precursors may be glial-restricted precursors. This would explain why the number of bi-potent oligodendrocyte precursors drops postnatally (Belachew et al., 2003) and would predict that most oligodendrocyte precursors maintain their bipotent fate in the *Emx1-Cre*-depleted oligodendrocyte precursor pool.

These observations also highlight the need to re-examine

with these more accurate tools whether indeed all GABAergic neurons – as currently thought – originate at ventral positions. Many certainly do (reviewed by Marin and Rubenstein, 2001), and another focus of the meeting was on how early subtypes of GABAergic interneurons are specified. Transplantation experiments have directly demonstrated that the birth date of a cortical interneuron in the ganglionic eminence predicts which cortical layer it will later occupy, such that early-born GABAergic neurons settle in the deeper cortical layers, whereas later-born GABAergic neurons settle largely in the upper cortical layers (Valcanis and Tan, 2003). As heterochronic transplants show a mixed phenotype (i.e. some transplanted cells behave according to the host environment, while others behave according to their intrinsic birth date), both intrinsic mechanisms of specification, as well as extrinsic factors that regulate layer-specific cell migration, seem to play a role. Seong-Seng Tan (Howard Florey Institute, Melbourne, Australia) presented intriguing evidence that one of the extrinsic cues regulating interneuron layer-specific targeting is reelin, the same factor that also governs the radial migration of cortical projection neurons (reviewed by Tissir and Goffinet, 2003). Interneurons deficient in one of the essential intracellular mediators of reelin signalling, Dab1, end up in the wrong layer position, just as pyramidal cells do in *reeler* mutant mice (Polleux et al., 1998). As late-born interneurons are seen to delve into deeper positions of the cortical wall where pyramidal neurons are about to commence their radial journey, these data suggest that both pyramidal and non-pyramidal neurons react to the same extrinsic signals directing their layer-specific migration. Neuronal migration is, however, also governed by intrinsic cues, such as the transcription factor neurogenin 2 (Ngn2). Data from Franck Polleux (University of North Carolina, Chapel Hill, NC, USA) and Francois Guillemot (National Institute of Medical Research, London, UK) revealed a novel mechanism by which Ngn2 affects the radial migration of cortical neurons independently of its DNA-binding domain but dependent on its phosphorylation state. This mechanism also provides a basis for integrating extrinsic and intrinsic neuronal migration regulatory mechanisms – an obvious requirement for matching the position of diverse cell types in the same layer.

In this regard it is amazing how many detailed aspects of the phenotype of a neuron seem to be already prefigured at its birth date. For example, new data from the laboratory of Gord Fishell (Skirball Institute, New York, NY, USA) demonstrate that both the laminar position and the physiological characteristics of an interneuron subtype correlate with the birthday of these interneurons, as beautifully demonstrated by the use of the tamoxifen-inducible form of Cre (CreERT2) under region-specific control elements. The fate-mapping of interneurons by inducing the nuclear localization of Cre in different regions of the ventral telencephalon (using the mouse lines, Olig2-CreERT2, Dlx2-CreERT2 and Dlx5-6-CreERT2, see Fig. 1B) revealed a fascinating degree of specificity. For example, interneurons generated at E10 from the *Olig2*-expressing domain were almost exclusively interneurons that fire trains of action potentials at high rates (fast-spiking), whereas those generated from the same domain at E15 were mostly of the regular-spiking phenotype. These results show that rather sophisticated electrophysiological features of interneuron subtypes correlate with their much earlier spatial and temporal origin, many weeks before these features develop.

Cortex evolution in mice and humans

Intriguingly, the matching of projection and interneuron numbers has apparently been maintained during the expansion of the cerebral cortex in phylogeny even across cortical areas (Winfield et al., 1980), despite prominent differences in the total number of neurons residing in specific layers of distinct cortical areas. In mammals, including in mice and humans, cortical areas take the form of radial domains within the cortex that specialize in specific aspects of information processing. For example, the occipital cortex comprises the visual areas, with the primary visual area V1 receiving direct thalamic input, in contrast to the neighbouring secondary visual cortex. Importantly, many more neurons reside in the upper cortical layers of V1 than of V2, and this feature of cortical organization seems to be specified by early regulatory mechanisms at the precursor cell stage. Colette Dehay (INSERM, Lyon, France) presented evidence that in primates, upper layer neurons in V1 are mostly generated by a layer of precursors that seems to have no analogy in the mouse, the so-called outer subventricular zone (OSVZ) (Smart et al., 2002). This zone contains most of the precursors at the time when upper layer neurons are generated (see Campbell, 2005), while the ventricular zone consists of a cell layer that is just a few cell diameters thick. Surprisingly, the OSVZ contains cells with a radial glia morphology, which is notably distinct from the morphology of the precursors in the SVZ of the mouse cortex that exhibit only short processes with no apparent contact with the apical surface or basement membrane (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004). Importantly for the generation of area-specific differences, precursors in the OSVZ of V1 in the primate cortex have a 30% faster cell cycle than those located in V2 [for cell cycle differences in cortical area of the mouse, see Polleux et al. (Polleux et al., 1997)], and the fastest cycling cells are located in close proximity to thalamic axons that innervate V1 but not V2. These findings suggest that shortening the cell cycle in G1 by decreasing p27 levels and increasing cyclin E causes the area-specific increase in upper layer neurons mediated by the innervating afferents. Thus, regulating cell cycle length specifies area identity, illustrating just one of several links between cell cycle regulation and cell fate.

The striking concept to emerge from this work is that the fine-tuned physiological factors that influence cortical information processing in distinct areas are set up at amazingly early stages of cortical development. Besides the early role of Fgf8 in ventralizing the developing telencephalon, as demonstrated in a beautiful allelic series of *Fgf8* mutant mice (John Rubenstein, University of California, San Francisco, CA, USA) and by conditional deletions of the Fgf receptors *Fgfr1* and *Fgfr2* by BF1-Cre (Jean Hébert, Albert Einstein College of Medicine, New York, USA), the rostral location of Fgf8 (Fig. 1A) also provides the rostral pole that specifies the cortical area in a rostrodorsal and lateromedial Cartesian grid. Indeed, tinkering with Fgf8 also influences cortical area specification (Fukuchi-Shimogori and Grove, 2001), including the appropriate innervation from the thalamus, as presented by Liz Grove. During cortical development, thalamic fibres first innervate the subplate layer according to their specification

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(thalamic fibres from visual relay stations, the lateral geniculate nucleus, innervate the subplate of the later visual cortex), and only later extend towards their final target neurons in layer 4 of the cortex. Grove presented data suggesting that thalamic fibres can re-orient towards layer 4 of their correct area, if area identity has been shifted after the generation of subplate neurons. When area shifts were induced after E11 (by electroporation of additional Fgf8 sources), only neurons generated from then onwards, i.e. those forming cortical layers 5 to 2, are affected. Consequently, thalamic afferents target their normal position in the subplate zone, but when they grow into the later-generated layer 4, they seemingly 'realize' that they are in the wrong cortical area and grow laterally towards layer 4 of the correct area identity. These observations strengthen the idea that the detailed functional aspects of neuronal specificity and connectivity are set up at the time of neuronal birth date. Indeed, Dennis O'Leary (Salk Institute, San Diego, CA, USA) presented evidence that characteristic system properties of visual information processing, which were initiated at the precursor cell stage by the mild overexpression of *Emx2*, also follow the expansion of visual areas (Hamasaki et al., 2004). This expansion of visual areas, however, comes at a price, as the consequent reduction in motor areas apparently leads to significant defects in motor behaviour, suggesting that a specific area size is crucial for appropriate information processing.

Taken together, these and other findings presented at this meeting show that the functional features of cortical neuronal information processing are established at amazingly early stages of development, such as at the precursor cell level, and that already at these early stages neuronal and glial heterogeneity are prefigured. Thus, systems neuroscience moves to the early stages of development.

We thank the organizers, John Parnavelas and Arnold Kriegstein, for a truly inspiring and wonderful meeting that comprised many more highlights than we could discuss here. We therefore apologize to all colleagues who presented fascinating new data that we could not discuss here owing to space constraints. We are also very grateful to Marie-Theres Schmid for preparing the figure for this review.

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