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Developmental Mechanisms Underlying the Generation of Cortical Interneuron Diversity

Minireview

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GABAergic interneurons are critical components of cortical circuits. However, understanding their function has become extremely challenging because they constitute one of the most diverse groups of cells in the central nervous system. Indeed, cortical GABAergic interneurons are heterogeneous in so many different ways—morphology, molecular profiling, electrical properties—that even attempts to discern what parameters should be used to identify cortical interneuron subtypes have failed to generate broad consensus among experts in the field. The extent to which cortical interneuron diversity emerges during development is largely unknown, but it is likely that insights on how this process takes place may help us understand their role as integrative and synchronizing elements in cortical function. Here, we review recent data on how the large variety of distinct classes of cortical interneurons may arise during development.

Despite making up a relatively small percentage of the entire neuronal population in the cerebral cortex (~20%), γ-aminobutyric acid-producing (GABAergic) interneurons represent fundamental modulatory and integrative elements for cortical function. For example, GABAergic interneurons synchronize and shape several types of cortical oscillations underlying various brain functions and prevent the development of hyperexcitability and epileptiform activity (McBain and Fisahn, 2001). GABAergic interneurons are inhibitory in the adult cortex and have a number of common features, including aspiny dendrites and local projections, typically within the same cortical column or to adjacent columns in the cortex.

In contrast to the excitatory pyramidal cells, which comprise a rather homogeneous group of cortical neurons with relatively stereotyped attributes, GABAergic interneurons are extremely diverse in their morphological, physiological, molecular, and synaptic characteristics (DeFelipe, 1997; Kawaguchi and Kondo, 2002; Markram et al., 2004). Understanding this diversity is a daunting task, and attempts to systematically classify interneurons have generated much controversy among scientists in the field. In the hippocampus alone, for example, nearly 20 different types of interneurons have been recognized (Somogyi and Klausberger, 2005).

Here, we review current views on how the enormous variety of morphologically and functionally distinct classes of cortical interneurons may arise during development. In our opinion, discerning how interneuron diversity emerges during development may contribute to the design of a standardized classification scheme for interneurons, as well as clarify their role as integrative and synchronizing elements in cortical function. In view of the phenomenal mosaic that interneuron diversity builds in the cortex, understanding how this multitude of different classes of interneurons arise during development is certainly not going to be a walk in the park. Nevertheless, small pieces of this puzzle are starting to fall into place, and attempts to trace the origin of distinct classes of cortical interneurons are underway.

A Taste of Cortical Interneuron Diversity

Different parameters have been used to classify cortical interneurons into specific subtypes, including morphology, connectivity (afferent and efferent projections and synapse targeting), immunohistochemical characterization, molecular profiling, and single- and multiple-cell electrophysiological analysis. Because of the intrinsic complexity of the mammalian cortex, however, these approaches have rendered an astonishing amount of data that have largely failed to become integrated in a single comprehensive scheme. Despite this drawback, these characterizations of interneuron subtypes have proved to be useful in distinguishing among major interneuron groups. For instance, analysis of the expression of calcium binding proteins such as parvalbumin (PV), calbindin (CB), or calretinin (CR) and neuropeptides such as somatostatin (SST), vasoactive intestinal peptide (VIP), neuropeptide Y (NPY), or cholecystokinin (CCK) in GABAergic neurons have been used to define distinct classes of interneurons in the isocortex and hippocampus on the basis of their neurochemical content. Using this criterion, both the frontal and the visual cortex of rodents contain three largely independent populations of interneurons: (1) PV, (2) SST/CB, and (3) CR/VIP interneurons (Gonchar and Burkhalter, 1997; Kawaguchi and Kondo, 2002).

Electrophysiological analysis suggests that these chemically identified interneuron populations also differ in their firing characteristics. Thus, PV interneurons are typically fast-spiking (FS) cells with low input resistances, spikes of short duration, and abrupt episodes of nonadapting repetitive discharges. In contrast, SST/CB interneurons largely correspond to burst-spiking nonpyramidal (BSNP) cells, also known as low-threshold (LTS) interneurons. BSNP cells exhibit bursting activity from hyperpolarized potentials. Finally, CR/VIP interneurons frequently have firing characteristics found in regular-spiking nonpyramidal (RSNP) cells. Of note, this large categorization of interneuron subclasses may have little functional value because interneuron function is known to depend also on many other characteristics, such as the inputs received by the distinct classes of interneurons based on their position in the circuit, the specific targeting of their axonal projections (e.g., axonal or dendritic synapses), the ever-expanding repertoire of voltage-gated channels present in each inhibitory neuron, or even the brain state (Klausberger et al., 2003). Acknowledging this limitation, this chemical

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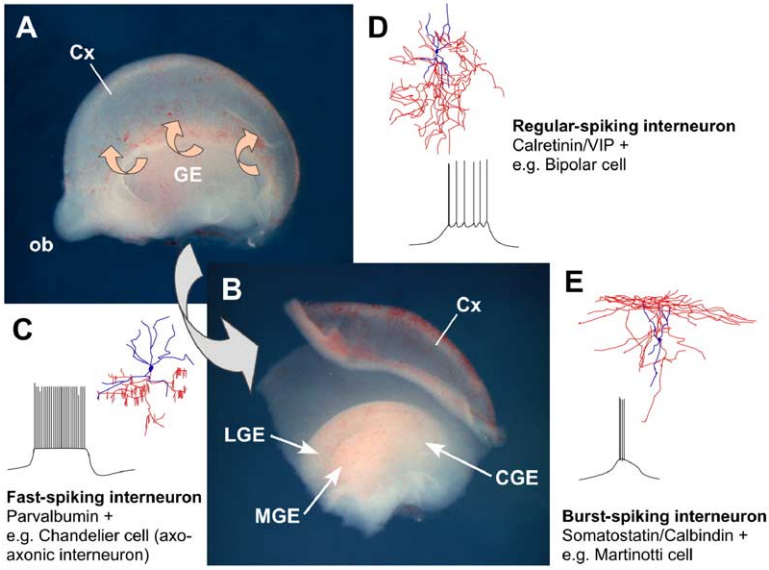

Figure 1. Major Subclasses of Cortical Interneurons and Their Origin in the Embryonic Telencephalon

(A and B) Medial views of a telencephalic hemisphere from an E13.5 mouse embryo before (A) or after (B) removing part of the cortex (Cx) to show the spatial arrangement of the ganglionic eminences (GE). The olfactory bulb (ob) and the septum have been removed in (B). Note that the lateral, medial, and caudal ganglionic eminences (LGE, MGE, and CGE, respectively) form a morphological continuum within the subpallium. (C–E) Morphological (axons in red and dendritic arbors and soma in blue) and firing pattern schematic representations of three major groups of cortical interneurons. Fast-spiking (FS) cells are typically parvalbumin+, such as basket cells and chandelier cells (C). Burst-spiking nonpyramidal (BSNP) interneurons are frequently somatostatin+ (some also positive for calbindin D28k), like Martinotti cells with ascending axonal arbors to layer I (D). Regular-spiking nonpyramidal (RSNP) interneurons, such as bipolar cells with descending axonal arbors, typically express VIP and calretinin (E).

and electrophysiological characterization allows the identification of at least three major groups of interneurons in the isocortex. So, how does this heterogeneity emerge during development?

Origin of Cortical GABAergic Interneurons

Research over the past few years has provided compelling evidence that a large number of cortical GABAergic neurons are born in the subpallial telencephalon (reviewed in Corbin et al., 2001; Marín and Rubenstein, 2001) (Figure 1). Such a conclusion is based on a number of different experimental approaches, including analysis of neuronal migration in slice cultures, genetic manipulations, and in vivo fate mapping of cortical GABAergic interneurons. GABAergic interneurons born in the subpallium migrate tangentially to populate the entire cortex, including the piriform cortex, isocortex (e.g., neocortex), and hippocampus.

The generation of cortical GABAergic interneurons extends through a lengthy period of embryonic neurogenesis in the telencephalon. In mice, for example, interneurons are generated during a period that extends roughly from embryonic day (E) 12.5 to birth. GABAergic interneurons tangentially migrating to the neocortex and hippocampus arise from multiple proliferative regions of the subpallial telencephalon, including the lateral, medial, and caudal ganglionic eminences (LGE, MGE, and CGE, respectively), and perhaps the anterior entopeduncular region (AEP) and the area around the base of the olfactory bulb (reviewed in Corbin et al., 2001; Marín and Rubenstein, 2001). All of these progenitor regions express the *Dlx1*, *Dlx2*, and *Mash1* genes, which control their timing of differentiation and contribute to the acquisition of a "proto-GABAergic" phenotype by subpallial-derived cortical interneurons (Anderson et al., 1997; Fode et al., 2000). In addition, recent reports suggest that a subpopulation of cortical interneurons derives from DLX-expressing progenitors located in the pallium, the primordium of the cortex itself (Bellion et al., 2003; Letinic et al., 2002).

There are two possible models of how interneuron diversity arises in the telencephalon. One possible scenario would be that DLX-expressing progenitors produce "protointerneurons" in which differentiation depends primarily on epigenetic factors present in the cortex. A second possibility would be that distinct types of interneurons derive from spatially or temporally segregated pools of DLX-expressing progenitors. The differentiation of distinct classes of interneurons undoubtedly depends on epigenetic factors present in the cortex, such as neurotrophins and activity (Marty et al., 1997). However, recent data suggests that interneuron diversity is established early during neurogenesis, with both spatial and temporal differences contributing to the development of distinct classes of interneurons.

Spatial Segregation of Cortical Interneuron Precursors in the Subpallium

The existence of regional differences in the specification of neural progenitors has been shown to mediate the generation of neuronal diversity in different structures of the CNS. In the spinal cord, the best-characterized system so far, graded signaling by morphogenetic molecules defines distinct domains of progenitor cells characterized by the expression of a specific combination of homeodomain proteins. Subsequently, the homeodomain proteins expressed by progenitor cells seem to specify the identity of each of the classes of postmitotic neurons that derive from individual progenitor domains (Jessell, 2000). A similar situation has been described in the telencephalon, where at least three progenitor domains—LGE, MGE, and CGE—are distinguished in the subpallium. Early evidence supporting the division of the subpallium into these three domains comes from gene expression studies and analysis of mouse embryos with mutations in homeodomain proteins expressed in the subpallium. For example, the LGE (which is further subdivided into dorsal and ventral domains) is characterized by the expression of *Pax6* (high levels in the dLGE and low levels in the vLGE),

whereas the MGE distinctly expresses *Titf1* (a.k.a. *Nkx2-1*). Moreover, MGE-derived cells exhibit more prominent migratory properties than LGE-derived cells (Nery et al., 2001). No distinct molecular markers have been identified so far for the CGE (both LGE and MGE markers are found in the CGE), although the differential behavior of CGE-derived cells in mouse embryos with mutations in homeodomain proteins that affect the development of the LGE or the MGE suggests that this region contains progenitor cells that are distinct from those in the LGE and MGE (Nery et al., 2002).

Pioneer in vitro experiments suggested that cells migrate from these three sources to the developing cortex in mice (Anderson et al., 2001). However, recent fate-mapping studies using ultrasound-guided transplantation methods have shown that only the MGE and CGE seem to generate cortical interneurons in vivo (Nery et al., 2002; Wichterle et al., 2001), and the contribution of the LGE to the population of cortical interneurons remains controversial. Now, do MGE and CGE progenitors generate distinct types of cortical interneurons?

Several lines of evidence suggest that this is the case, although in vivo verification is still outstanding. First, migrating cells from the subpallium to the cortex constitute a very heterogeneous population regarding their neurochemical content. Second, GABAergic interneurons invade the isocortex and hippocampus following various migratory routes (marginal zone, subplate and subventricular zone), illustrating the differential behavior of distinct groups of immature interneurons (Ang et al., 2003; Tanaka et al., 2003). Thus, it seems likely that distinct types of GABAergic interneurons respond differentially to distinct sets of guidance molecules, despite the fact that all migrating interneurons may use similar mechanisms to reach the cortex (reviewed in Marín and Rubenstein, 2003). For example, interneurons expressing the Neuregulin-1 receptor ErbB4 preferentially invade the cortex through the subventricular zone, reinforcing the view that genetically different classes of cortical interneurons respond to different cues and migrate along different routes to their final destination (Flames et al., 2004). Third, in vitro experiments showed that cultured cells derived from the MGE predominantly differentiate to PV- and SST-expressing GABAergic interneurons, whereas cultured cells obtained from the CGE give rise to CR-expressing GABAergic interneurons (Xu et al., 2004). These experiments suggest that, out of the three major groups of cortical GABAergic interneurons, both PV+ fast-spiking interneurons and SST/CB+ burst-spiking interneurons may derive from the MGE, whereas CR/VIP+ regular-spiking interneurons may come from the CGE. In line with this view, a recent study using GAD65-GFP transgenic mice as a tool to study the migration of a fraction of cortical interneurons suggested that calretinin-expressing interneurons derive primarily from the CGE (López-Bendito et al., 2004). These observations are also supported by genetic data, since cell cultures derived from dissociated cortices obtained from E18.5 *Titf1* mutant embryos do not contain PV- or SST-expressing neurons, whereas the number of CR interneurons is similar to that found in cell cultures derived from control cortices (Xu et al., 2004). This genetic evidence reinforces the notion that CR-expressing interneurons derive from a region lacking *Titf1* expression in the CGE. In addition, it suggests that PV- and SST-expressing interneurons depend on *Titf1* expression to develop and therefore are likely to derive from the MGE.

Temporal Differences Contributing to the Generation of Interneuron Diversity

In the spinal cord, progenitor cells that occupy the same dorsoventral and rostrocaudal position generate phenotypically different sets of neurons that differ in their birthdates (Jessell, 2000). Thus, environmental changes during development also influence the output of progenitor cells, and different types of neurons may derive from common precursors.

The time of neurogenesis also appears to be an important factor in the generation of cortical interneuron diversity. For example, GABAergic interneurons in the hippocampus are generally born earlier than those destined for the isocortex (Soriano et al., 1989), suggesting that temporal differences in the generation of local circuit neurons are important to define distinct sets of GABAergic interneurons destined for different regions of the cerebral cortex. Moreover, perturbation of the function of factors affecting the timing of differentiation of interneurons, such as DLX1 and DLX2, has a greater impact on the development of hippocampal neurons than in other cortical regions (Pleasure et al., 2000), reinforcing the notion that the time of neurogenesis may significantly influence the fate of cortical interneurons. Thus, time of neurogenesis appears to be an important factor contributing to the diversification of interneuron populations in different regions of the cerebral cortex.

The acquisition of a laminar identity by cortical interneurons also appears to be determined at the time of neurogenesis, as it is the case for cortical projection neurons (Rakic, 1974). Thus, in agreement with classical birthdating studies, recent transplantation experiments have shown that interneurons born in the MGE or CGE at different times populate specific layers of the neocortex in an inside-out order (Nery et al., 2002; Valcanis and Tan, 2003). Interestingly, GABAergic interneurons tend to adopt the same cortical layer as projection neurons born roughly at the same time, even though migrating interneurons require some "extra time" to reach the pallium and they appear to enter the developing cortical plate both from superficial and deep routes. Similar to projection neurons, laminar acquisition is also dependent on interneuron progenitor receptivity to environmental cues during their last round of cell division, suggesting that interneurons are already specified with respect to their future layer destination before the initiation of their long-distance tangential migration (Valcanis and Tan, 2003).

As described above, current data suggest that birth date may influence regional and laminar differences in cortical interneuron development, but the extent to which there is a relationship between birth dates and adult interneuron phenotypes is presently unclear. Birth date periods largely overlap among the major interneuron subtypes (PV, SST/CB, and CR/VIP), suggesting that a relation between neurogenesis timing and interneuron specification may not exist. Nevertheless, cortical CR/VIP interneurons appear to be born in a relatively narrow window of time within the CGE (Xu et al., 2004), suggesting that this region may give rise to a

different type of interneuron afterward. Moreover, laminar fate of CR/VIP interneurons in the visual cortex does not seem to follow the typical inside-out order described for other interneuron types (Yozu et al., 2004). These results suggest that the alignment of interneurons may be regulated differentially at least for some interneuron subtypes. The definition of distinct subclasses within the three major groups of cortical interneurons and the subsequent detailed analysis of their birthdates may shed some light on this question.

Terminal Differentiation of GABAergic Interneurons in the Cortex

As described above, increasing evidence suggests that early specification of progenitor cells in the subpallium largely accounts for the emergence of cortical interneuron diversity. However, it is also clear that differentiation of cortical interneurons is controlled by environmental factors that act in combination with the patterning mechanisms. For example, both activity and growth factors are required to regulate PV expression in a specific subset of cortical interneurons (Huang et al., 1999; Jones et al., 1994). Interestingly, cells with the morphology of this class of interneurons (e.g., basket and chandelier neurons) develop in the absence of these factors, suggesting that the development of class-specific morphological features might be partly activity independent (Patz et al., 2004). Moreover, one of the most prominent features that distinguish different classes of interneurons, the specific targeting of synapses to distinct subcellular compartments of the postsynaptic neuron, develops independent of thalamic input and probably involves molecular labels and experience-independent forms of activity (Di Cristo et al., 2004).

In the adult cortex, specific interneuron subtypes are differentially distributed in distinct areal positions (DeFelipe, 1997). In addition to specification mechanisms—presently unknown—that may act to restrict the distribution of specific subtypes of interneurons to distinct regions of the cerebral cortex (e.g., through the expression of specific guidance receptors), epigenetic mechanisms appear to contribute to the emergence of a relatively heterogeneous distribution of interneuron subclasses. For example, differential cell death has been proposed as a mechanism to explain the preponderance of NPY cells in layer VI of the rat visual cortex (Cavanagh and Parnavelas, 1990). These observations should be taken into account to develop a comprehensive view of the mechanisms generating the diversity of GABAergic interneurons in the cerebral cortex.

Future Directions

The recent review by Markram et al. (2004) comprehensively illustrates the notion that cortical interneuron diversity is far more complex that outlined here. The combination of morphological, electrophysiological, and molecular traits found in cortical interneurons is so diverse that it has led some authors to suggest that interneurons may form a continuum of cell types rather than individual subclasses. The data reviewed here, however, clearly show that early specification of progenitor cells in the subpallium largely accounts for the generation of at least the major groups of cortical GABAergic interneurons, although additional experiments seem necessary to demonstrate to what extent the differential MGE and CGE potentialities observed in vitro are preserved in vivo. In addition, it remains to be elucidated which factors are responsible for the expression of *Dlx1/2* and *Mash1* genes in the pallium, as well as the specific characteristics that may distinguish pallial from subpallial-derived GABAergic interneurons. In this context, however, it should be noted that it is still unclear to what extent cortical GABAergic interneurons derive from a subset of pallial progenitors in different mammalian species (see Xu et al., 2004, for further discussion).

The spatial and temporal coordinates of progenitor cells in the subpallium appear to be strong predictors of the specific cortical GABAergic interneuron subtype they generate. This highly deterministic view of cortical interneuron development would suggest that, as in other regions of the CNS, the generation of cortical interneuron diversity relies primarily on the developmental expression of specific combinations of homeodomain proteins in different progenitor pools. However, the limited number of transcription factors with restricted temporal or spatial expression in the subpallium seems insufficient to explain the diversity found in the cortex. Moreover, considering the ever-expanding list of distinct cortical GABAergic subtypes that appear to exist in vivo, it is likely that the present subdivision of the subpallium into a few broad progenitor domains only represents a fraction of the progenitor pools present in this region of the telencephalon. The identification of additional transcription factors with such restricted patterns of expression would greatly support the hypothesis that cell type specification mechanisms in the telencephalon match those described in the spinal cord. We anticipate that dissecting further the combinatorial code underlying the segregation of progenitor domains in the subpallium will turn out to be extremely useful for understanding the diversity of cortical interneurons subtypes, a goal that has proved challenging to attain.

Acknowledgments

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