Development of neuronal lineages in the mammalian cerebral cortex

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SUMMARY

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Neuronal circuits in the cerebral cortex arise through the assembly of two main neuronal elements: inhibitory GABAergic interneurons and excitatory glutamatergic pyramidal cells. Each group of neurons is highly diverse and play a different role during cortical function. Pyramidal cells are primarily involved in transmitting long-range information while interneurons mostly shape the activity of pyramidal cells via local inhibition. Pyramidal cells and interneurons are generated in distinct and distant proliferative regions in the embryonic brain and adopt different strategies to reach the cortex and organize through layers and columns, the main histological and functional hallmarks of cortical cytoarchitecture. Thus, functional circuits arise from early developmental processes, such as neurogenesis and cell migration that guide the precise assembly of cortical neurons.

In this Thesis, we have investigated the contribution of progenitor cells to the generation of cell diversity and how lineage relationships influence the organization of cortical neurons by developing a new method to tag individual progenitors and trace their progenies with regional/subtype specificity *in vivo*. Analyses of cell distributions revealed that all three major classes of interneurons have a strong tendency to organize in discrete cellular clusters in the adult cortex. Individual clusters are composed by either the same or different interneurons subtypes and largely consist of isochronic interneurons that primarily arrange along the laminar dimension of the neocortex. Moreover, our results point out to the existence of interneuron progenitor cell classes that are fate-restricted to generate interneurons for specific cortical layers, in a model that links progenitor cell heterogeneity with the laminar restriction of interneuron lineages.

Similar fate-mapping analyses of pyramidal cells progenitors suggest that multipotent and fate-restricted progenitor cells coexist during cortical development, perhaps with a different contribution of progenitors types respect to interneuron lineages. All together our findings open new perspectives for our understanding of the development of inhibitory and excitatory neurons and their assembly into nascent cortical circuits.

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Los circuitos neuronales de la corteza cerebral contienen dos elementos principales, interneuronas GABAérgicas inhibitorias y células piramidales glutamatérgicas excitatorias. Cada grupo de neuronas es muy heterogéneo y juega un papel distinto en la función cortical. Las células piramidales principalmente transmiten señales de largo alcance, mientras que las interneuronas modulan la actividad de las células piramidales a través de inhibición local. Las células piramidales y las interneuronas se generan en el cerebro embrionario en distintas y distantes regiones proliferativas y adoptan diferentes estrategias para llegar a la corteza y organizarse a través de capas y columnas, las dos principales características histológicas y funcionales de la citoarquitectura cortical. Los circuitos funcionales se originan a partir de procesos del desarrollo temprano, como la neurogénesis y la migración celular que guían el ensamblaje preciso de las neuronas corticales.

En esta Tesis, hemos investigado la contribución de las células progenitoras a la generación de la diversidad celular y cómo las relaciones de linaje influyen en la organización de las neuronas corticales, mediante el desarrollo de un nuevo método para marcar progenitores individuales y seguir sus progenies con especificidad regional y subtipo *in vivo*. Los análisis de la distribución de linajes celulares demostraron que las principales clases de interneuronas tienen una fuerte tendencia a organizarse en agrupamientos celulares discretos en la corteza adulta. Estos grupos de neuronas están individualmente compuestos por uno o varios subtipos de interneuronas y en gran medida por interneuronas isocrónicas que se organizan principalmente a lo largo de la dimensión laminar de la neocorteza. Por otra parte, nuestros resultados indican la existencia de diferentes clases de células progenitoras de interneuronas, restringidas para generar interneuronas de capas corticales específicas, en un modelo que relaciona la diversidad de las células progenitoras con la restricción laminar de los linajes de interneuronas.

Análisis similares de los progenitores de las células piramidales sugieren la existencia simultánea de células progenitoras multipotentes y con capacidad restringida, lo que sugiere una contribución distinta de los progenitores comparado con los linajes de interneuronas. De forma global nuestros resultados abren nuevas perspectivas para el estudio del desarrollo de las neuronas inhibidoras y excitadoras y de los mecanismos que controlan su ensamblaje en circuitos corticales.

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INTRODUCTION

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1. The cerebral cortex: general overview of organization

The mammalian cerebral cortex is the outermost structure of the brain. It mediates high-level functions such as cognition, perception, language and decision-making and it plays a fundamental role in integrating information derived from multiple sensory modalities. At the anatomical level, the cerebral cortex is subdivided into the following different regions: the *archicortex* (hippocampal formation), *paleocortex* (also known as piriform cortex or olfactory cortex) and *isocortex* (also called neocortex). This subdivision reflects the degree of complexity as well as the phylogenetic specialization of the different regions, with the neocortex being the most recent evolutionary acquisition. As result of evolutionary processes, the neocortex represents the largest part as well as perhaps the most complex structure of the mammalian brain. So, the neocortex account for most of the overall increase in brain size and degree of specialization in the more phylogenetic recent species (Krubitzer and Kaas 2005).

The neocortex is further organized in areas, specialized functional subdivisions of cortical territories that are characterized by relatively different cytoarchitectures, specific input and output connections as well as different patterns of gene expression. There are many specialized regions including for instance the primary somatosensory (S1), motor (M1), visual (V1) and auditory (A1) areas, as well as association cortices that integrate information derived from primary sensory regions. Each of these regions connects with other brain structures (e.g. thalamus, basal ganglia etc.) as well as with other cortical areas through projection, commissural and association fibers, and serves different functions related to specific sensory modalities (Sur and Rubenstein 2005; O'Leary et al. 2007).

1.1 Structural organization of the neocortex: layers and columns

Despite the functional and anatomical differences that characterize distinct cortical areas, one of the underlying histological hallmarks of the neocortex is its peculiar cytoarchitecture. On the tangential plane, the neocortex is subdivided in up to six different layers whose thickness and boundaries vary slightly depending on the cortical region. Cortical layers are functionally classified in supragranular (layers I-II/II), granular (layer IV) and infragranular (layers V/VI). Supragranular layers are highly developed in humans and are the primary source and termination of association and commissural connections. The most superficial layer (layer I) is also called plexiform layer and it is largely devoided

of cell somas and particularly enriched in cellular projections. Instead, layer II/III is characterized by high cell density and represents the primary place of input and output for inter-hemispheric connections. Layer IV is the primary target of thalamo-cortical and it is particularly prominent in primary sensory cortices. In contrast, layers V and VI are the principal output stations for subcortical connections, with layer V giving rise mainly to efferents toward the basal ganglia, spinal cord and brain stem and layer VI projecting to the thalamus. Because of this pattern of connectivity, infragranular layers are most prominent in motor and visual cortices. Therefore, as general principle, the laminar identity of cortical neurons reflects their pattern of connectivity.

Another hallmark of cortical cytoarchitecture is represented by columns, which are functional units that are arranged along the entire radial dimension of the neocortex encompassing the six cortical layers. Cortical columns have been described as *"... neurons which lie in narrow vertical columns, or cylinders, extending from layer II through layer VI make up an elementary unit of organization, for they are activated by stimulation of the same class of peripheral receptors…"* (Mountcastle et al. 1957) and are commonly referred as the basic functional unit of cortical processing, evolution and development (Mountcastle 1997; Jones and Rakic 2010). During the past decade, this concept has been refined and now different types of columns are recognised based on physiological, structural and developmental criteria. For example, regions of neurons in the visual cortex that respond to the same stimulation from either the left or right eye have been defined as ocular dominance columns (Hubel and Wiesel 1969; Hensch 2005). In the visual cortex, another type of column has been described as composed by neurons with similar selectivity for the orientation of visual stimuli and has been called 'orientation column' (Hubel and Wiesel 1968). Instead, microcolumns are spatially defined by the dendrites of 3–20 large pyramidal cells of layer V that form clusters that ascend together through layer IV (Fleischhauer et al. 1972; Peters and Walsh 1972).

From a developmental perspective, cortical columns have been related to the development of ontogenic units and defined as 'ontogenic or embryonic columns'. In particular, it has been proposed that the location of cohorts of sibling cortical neurons is not random but is largely predictable and that neurons in columns derive from single progenitor cells or from small polyclonal units (Rakic 1988). According to this model (named 'radial unit hypothesis'), ontogenic units arrange radially as result of development, thereby providing the structural basis for the establishment of the functional elements of

cortical processing and computation. Thus, it has been proposed that the cerebral cortex is a modular structure, composed by basic functional units that are reiterated through the entire cortical volume. Ontogenic columns have been proposed as the basic modular element of the neocortex, but it is still unclear if they correspond to the functional columns observed in the adult cortex. Therefore, although much progress has been made in the identification of cortical columns, their definition primarily rely on functional properties and their anatomical correlate remains still controversial.

1.2 Cytoarchitecture of the neocortex

The cerebral cortex contains hundreds of different cell types including neurons and glial cells. In broad terms, cortical neurons are classified into two main categories: excitatory neurons and inhibitory interneurons. Both classes of neurons form specific circuits that distribute through the laminar and columnar structure of the neocortex. Below I summarize their main characteristics and their classification in the adult neocortex.

1.2.1 Pyramidal neurons

Pyramidal cells represent the most abundant class of cortical neurons and account for approximately 80% of the total neuronal population. Also known as projection neurons (PNs), pyramidal cells send their axons to many distant brain regions, establishing connections with intracortical, subcortical and subcerebral targets. They use glutamate as neurotransmitter and they are therefore excitatory cells, because they elicit membrane depolarization onto postsynaptic neurons mainly through chemical synapses. Morphologically, projection neurons are characterized by stereotypical pyramidal-shaped somas and spiny dendrites. Different projection neuron subtypes populate the mature neocortex and serve specific functions. Distinct subtypes have different laminar and areal allocations, dendritic morphologies and physiological features, and they express unique combinations of molecular markers (Molyneaux et al. 2007).

One remarkable feature of pyramidal cells is their pattern of axonal projections. The hodology (i.e. the path followed by axons to reach their targets) is directly related to function and is commonly used as the main criteria for pyramidal neuron classification (**Figure 1**). Moreover, pyramidal neurons connectivity is roughly linked to their laminar position in the cortex (Greig et al. 2013).

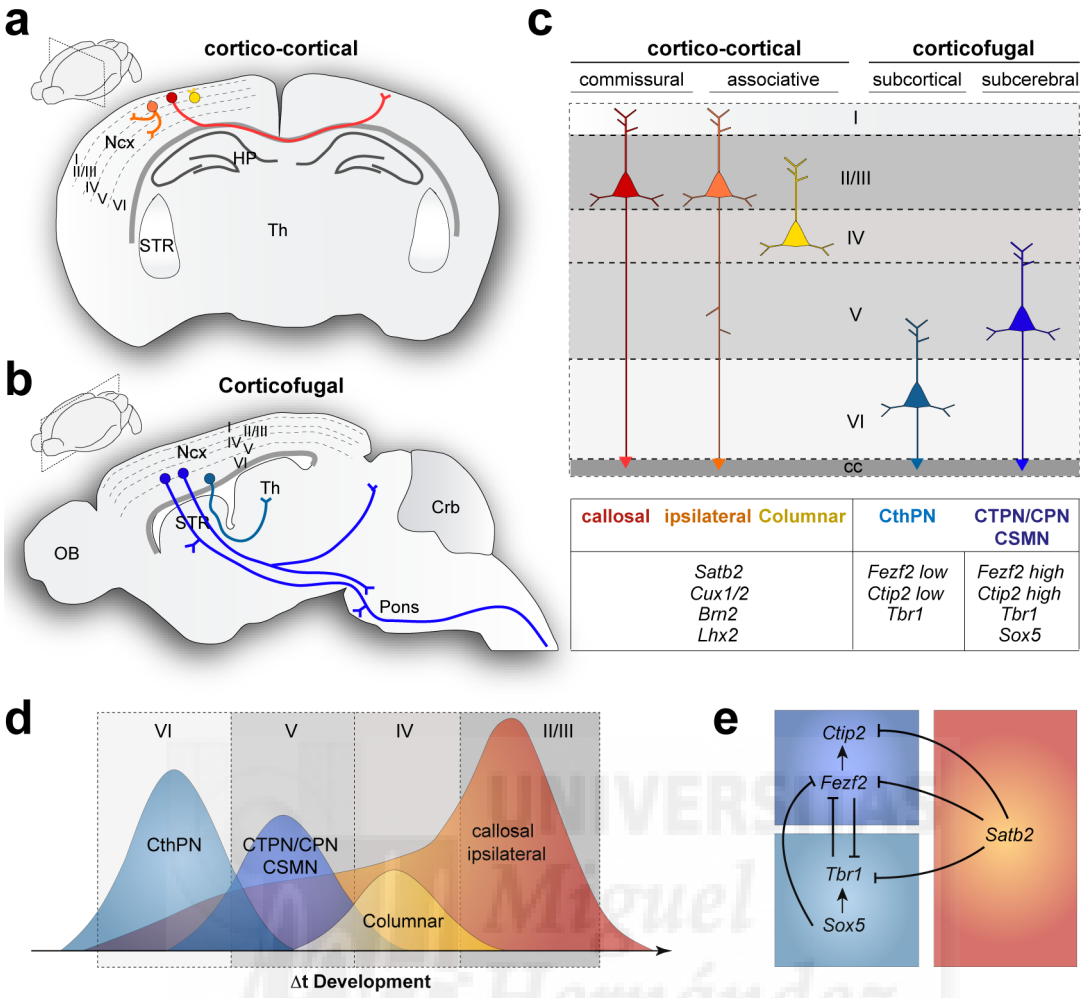

Figure 1. Classification and molecular specification of cortical projection neuron subtypes. (**a, b**) Schematic of a coronal and sagittal sections through the adult brain showing the pattern of connectivity of cortico-cortical and corticofugal PN subtypes respectively, classified according to their axonal projections. (**c**) Magnified view of the laminar localization and molecular characterization of PN subtypes shown in (a) and (b). PN are broadly classified into cortico-cortical and corticofugal subtypes depending if they project to cortical of subcortical structures respectively. Cortico-cortical PN are further subdivided into commissural (neurons that connect with the contralateral hemisphere via the corpus callosum or anterior commissure) and associative (neurons that connect ipsilaterally) subtypes. Cortico-cortical PN typically express Satb2, Cux1 and other molecular markers and localize mostly in superficial cortical layers. CfuPN comprehend subcortical and subcerebral subtypes that project to the thalamus and to other subcerebral structure (e.g. pons and spinal cord) respectively. They are characterized primarily by the expression of Fezf2, Ctip2 and Tbr1 transcription factors and localize in deep cortical layers. (**d**) Temporal specification of PN subtypes during embryonic development. PN subtypes are produce in partially overlapping sequential waves. CThPN are the first followed by subcerebral, columnar and commissural types. (**e**) Main molecular pathways involved in the specification of PNs during development. Arrows indicate transcriptional activation or repression. Cc, corpus callosum; Crb, cerebellum; HP, hippocampal formation; Ncx, neocortex; OB, olfactory bulbs; STR, striatum; Th, thalamus; I-VI, cortical layers I to VI. CTPN, cortico-tectal projection neuron; CPN, cortico-pontine projection neuron; CSMN, cortico-spinal motor neurons. Adapted from Greig et al. 2013.

PNs that target subcortical and subcerebral regions are broadly classified as corticofugal projection neurons (CfuPN) and are primarily allocated in infragranular cortical layers (**Figure 1b, c**). They are further subdivided into corticothalamic (CThPN) and subcerebral (SCPN) projection neurons. CThPNs localize in layer VI and include different subtypes that target specific thalamic nuclei, while SCPNs are mainly found in layer V and project to the spinal cord, superior colliculus, pons and other structures in the brainstem. Based on their target structure, SCPNs are further subdivided into corticotectal (CTPN), corticospinal (CSMN) and other projection neuron subtypes. Another broad class includes PNs that extend their axon across the midline and target the controlateral hemisphere. Neurons in this class, known as commissural projection neurons, include PNs that send their axons across the midline through the corpus callosum (callosal projection neurons, CPNs) as well as a small population that project controlaterally through the anterior commissure (**Figure 1a, c**). CPNs primarily populate layer II/III and, to a minor extent, layers V and VI, and project to mirror positions in the contralateral hemisphere. Finally, the third class is represented by associative PNs that project ipsilaterally and are particularly abundant in layer IV. In addition, there are neurons that send projections to multiple targets and that according to this classification fall into more than one category. For example, intra-telencephalic corticostriatal projection neurons (CstrPNi) project both to the controlateral hemisphere (via *corpus callosum*) and to the ipsilateral striatum (Sohur et al. 2014). In addition, neurons of the same subtype that are localized in different cortical areas connect with functionally distinct regions (e.g., CthPN in somatosensory and visual cortex connect with distinct nuclei in the thalamus) and likely have different properties.

In the last years, much effort has been made to identify molecular markers that are specific to cortical layers and pyramidal neuron subtypes. Some of these markers are not only expressed in mature neurons but are also involved in their developmental specification, acting in molecular pathways of reciprocal cross-repression or activation. For instance, the transcription factor Fez family zinc finger 2 (*Fezf2*) is crucial for the specification of SCPNs and is expressed at high levels by SCPNs and at and low levels by CthPNs (Inoue et al. 2004; Arlotta et al. 2005; Chen et al. 2005b; Molyneaux et al. 2005). *Citp2* (COUP-TF-interacting protein 2) acts downstream of *Fezf2* to specify SCPN identities (Arlotta et al. 2005), while *Tbr1* (T-box brain protein 1) instead repress Fezf2 and Ctip2 and specify CthPNs (Bedogni et al. 2010; McKenna et al. 2011). In contrast, *Satb2* is necessary for CPN specification and is highly expressed by commissural and

associative pyramidal cells (Alcamo et al. 2008; Britanova et al. 2008). Some of the main factors that define PNs subtypes are listed in **Figure 1c**.

1.2.2 Cortical interneurons

Cortical interneurons numerically represent about 20% of the total neuronal population in the cortex. They use the γ-aminobutyric acid (GABA) as their main neurotransmitter, which in the adult brain elicits membrane hyperpolarization in postmitotic neurons. Consequently, GABAergic interneurons are typically inhibitory. Although long range projecting GABAergic neurons have been described (Melzer et al. 2012; Caputi et al. 2013), in this Thesis I will use the term interneuron to refer to GABAergic cells that primarily shape the activity of pyramidal neurons via local, short-range inhibition (Isaacson and Scanziani 2011). Cortical interneurons are an extremely diverse group of cells and therefore their classification requires a combination of morphological, neurochemical and physiological properties (Group et al. 2008; Defelipe et al. 2013). More than 20 different interneurons types have been recognized, but for the purpose of this Thesis, I have broadly classified them into five categories (**Figure 2**): (1) Fast-spiking interneurons that typically contain the calcium binding protein parvalbumin (PV) and are morphologically represented by basket and chandelier cells (Markram et al. 2004; Taniguchi et al. 2013). (2) Interneurons that express the neuropeptide somatostatin (SST). This class comprises intrinsic-burst-spiking and adapting non fast-spiking physiological subtypes. Morphologically, the most abundant class of SST+ interneurons consists of Martinotti cells, which have a typical morphology with the axon projecting toward layer I (Ma et al. 2006). A second class of SST+ interneurons have axons that branch abundantly around the cell soma and primarily synapse onto PV+ expressing interneurons (Xu et al. 2013). (3) Rapidly adapting interneurons with bipolar or double-bouquet morphologies, which typically express the vasointestinal peptide (VIP) and may also contain the calcium binding protein calretinin (CR) (Rudy et al. 2011). (4) Neurogliaform cells expressing nNOS, Reelin and the Serotonin receptor 3a (*5HTR-3a*), with highly branched dendrites and a late-spiking firing pattern (Armstrong et al. 2012; Jaglin et al. 2012). (5) Multipolar cells with irregular or rapidly adapting electrophysiological properties that often contain the neuropeptide Y (NPY) (Lee et al. 2010).

As described for pyramidal cells, differet interneuron subclasses show peculiarities in their pattern of distribution and connectivity (**Figure 2c**). For example, PV-expressing

interneurons are absent from layer I (Rymar and Sadikot 2007), and chandelier cells populate almost exclusively cortical layers II/III and V (Taniguchi et al. 2013). Marinotti cells are particularly abundant in layers II/III, V and VI, but are almost absent from layer IV (Ma et al. 2006). VIP+ bipolar cells almost exclusively populate supragranular cortical layers (Rymar and Sadikot 2007).

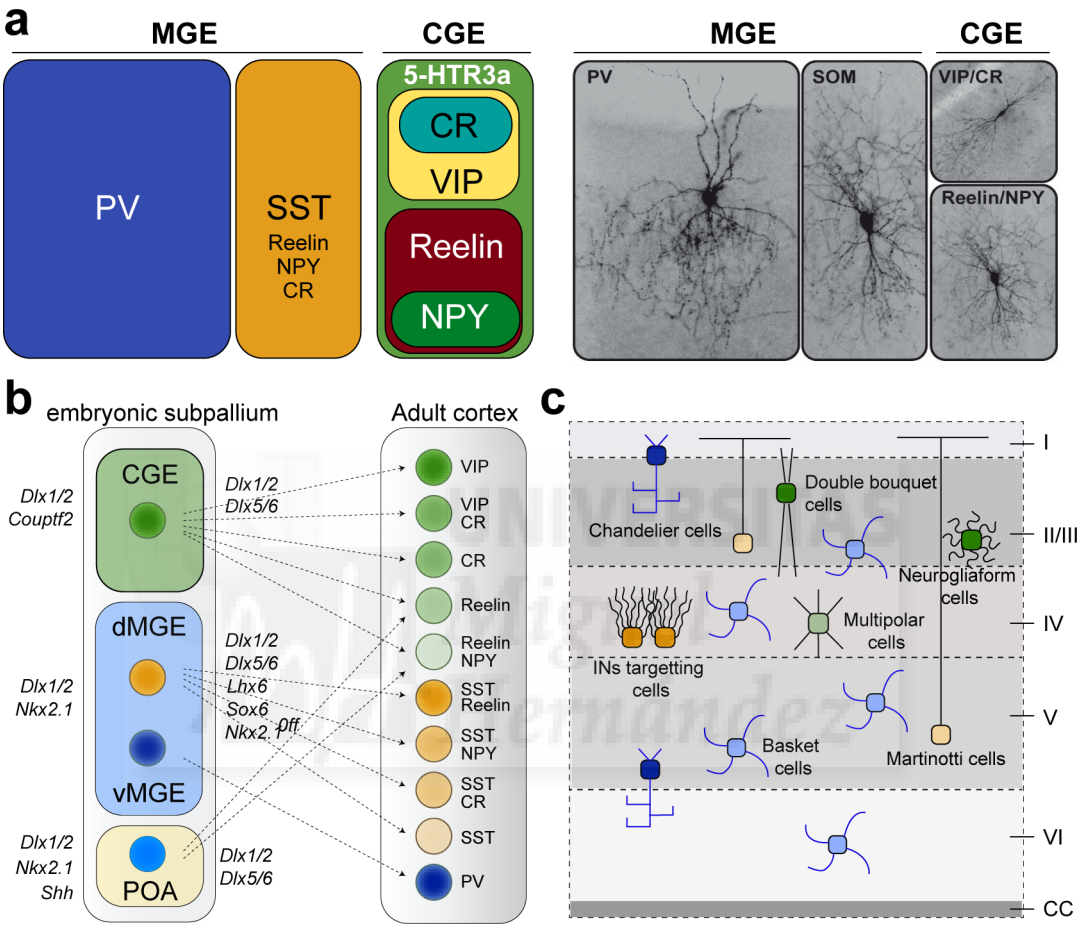

Figure 2. Classification and molecular specification of cortical inhibitory interneuron subtypes. (**a**) Characterization of cortical interneuron subtypes according to the expression of neurochemical markers and morphology. Cortical GABAergic interneurons belong to three main non-overlapping groups. PV and SST – expressing interneurons are embryonically originated in the medial ganglionic eminences while the 5HTR3a – expressing ones derived from the CGE. Each of these classes comprehends different subtypes that are specified through a complex network of transcription factors. The main molecules involved in their developmental specification are shown in (**b**) while the laminar distribution of the different subtypes in the adult cortex is depicted in (**c**). Cc, corpus callosum; MGE, medial ganglionic eminence; CGE, caudal ganglionic eminence; POA, preoptic area; I-VI, cortical layers I to VI. Adapted from Gelman and Marín 2010 and Bartolini et al. 2013.

On top of their laminar distribution, interneuron subtypes target and establish synapses with specific subcellular compartments of pyramidal cells as well as with specific subtypes

of interneurons, which indicate that they are differentially engaged in particular cortical circuit motifs (Markram et al. 2004). Consequently, GABAegic interneurons are also classified based on their pattern of connectivity. For instance, PV+ basket cells innervate perisomatic regions of pyramidal cells, while chandelier cells specifically target the axon initial segment of pyramidal neurons, where the action potentials are generated (Huang et al. 2007). Martinotti cells, double bouquet cells and neurogliafrom cells primarily target the dendrites of pyramidal neurons. Moreover, on top of targeting specifically subcellular compartments of excitatory neurons, interneurons connect among them in specific inhibitory microcircuits. For example, fast-spiking PV+ interneurons preferentially inhibit one another and are also electrically coupled through gap junctions (Galarreta and Hestrin 1999; Fukuda and Kosaka 2003). In contrast, SST+ interneurons preferentially target VIP+ and PV+ interneurons, but not other SST+ interneurons (Pfeffer et al. 2013). Finally, VIP+ interneurons contact preferentially SST+ interneurons and are engaged in disinhibitory circuits in different systems (Lee et al. 2013; Pfeffer et al. 2013; Fu et al. 2014).

2. The development of the cerebral cortex

2.1 Specification and patterning of the telencephalon

Despite the complex cytoarchitecture of the cerebral cortex, this entire structure originates from a simple sheet of neuroepithelium in the most rostral part of the neural tube. Comparative embryological studies have revealed that during early embryogenesis the neural tube is patterned in different vesicles along the anterior-posterior (AP) axis: the prosencephalon (or forebrain), the mesencephalon (or midbrain) and the romboencephalon (or hindbrain) (**Figure 3a**). The prosencephalon consists of diencephalon and telencephalic vesicles, with the later being the anlagen for the cerebral cortex and basal ganglia in the adult brain. According to the prosomeric model (Rubenstein et al. 1994; Rubenstein et al. 1998; Puelles and Rubenstein 2003), the forebrain is subdivided into six different segments called prosomeres, and the telencephalon arises from the alar domain of the secondary prosencephalon (prosomere 1-4). This subdivision is established by early patterning events that take place along the AP axis of the neural tube and appear to be mediated by extrinsic cues (Hébert and Fishell 2008). Subsequentially, prior to cell migration, the telencephalon becomes further subdivided dorso-ventrally in several territories and progenitor domains through the action of morphogenes (Rallu et al. 2002). So, along the dorso-ventral (DV) axis, the telencephalon has two main regions: the pallium (the roof of the telencephalon also called dorsal telencephalon) and the subpallium (also called ventral telencephalon)(Campbell 2003) **(Figure 3b, c)**.

The pallium gives rise to the cerebral cortex while the subpallium is the origin of basal ganglia structures such as the striatum and globus pallidus. The embryonic dorsal telencephalon can be further divided into anterior/lateral, posterior and medial parts that generate glutamatergic excitatory neurons for the neocortex, hippocampus and cortical hem respectively. In contrast, the embryonic subpallium can be divided in three main territories based on morphology and differential pattern of gene expression: the medial ganglionic eminence (MGE), the lateral ganglionic eminence (LGE) and more caudally the caudal ganglionic eminence (CGE, also known as the caudal extension of the dorsal LGE). In addition, the preoptic area (POA) and a large part of the septum are part of the subpallium (**figure 3b, c**). The LGE produces GABAergic projection neurons and interneurons for the striatum and olfactory bulb respectively, while the MGE and CGE give rise to GABAergic interneuron subtypes for cortical structures and basal ganglia

(Wichterle et al. 2001; Wonders and Anderson 2006; Gelman and Marín 2010). Recently, the POA has been shown to be another source of cortical interneurons (Gelman et al. 2009), while the septum do not seem to contribute to the cortical interneuron population (Rubin et al. 2010).

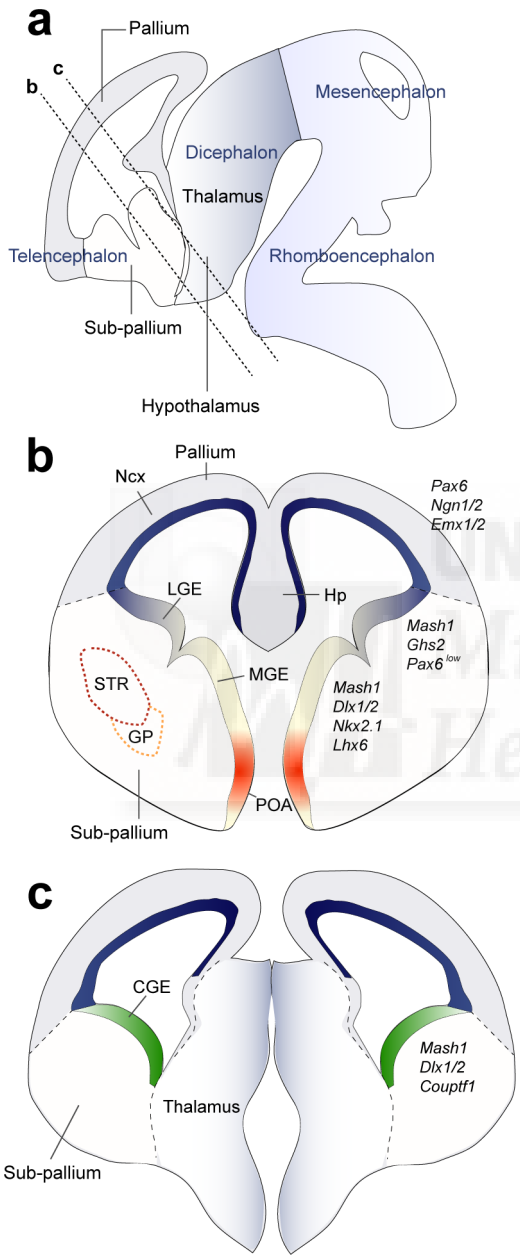

Figure 3. Patterning of the embryonic telencephalon.
(**a**) Schematic of a sagittal section through the developing mouse brain showing the main subdivision along the rostro-caudal axis into prosencephalon (diencephalon and telencephalon), mesencephalon and romboencephalon. (**b, c**) Schematics of coronal sections through the telencephalon showing the main subdivisions along the dorso-ventral axis at two representative levels. The embryonic telencephalon is broadly patterned into dorsal (pallium) and ventral (subpallium) forebrain through the action of morphogenes. Subpallial territories are further divided into LGE, MGE, CGE and POA according to the differential expression of transcription factors. The main transciprion factors involved in the patterning of the different forebrain subdivision are listed at the side of each developing structure. LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; CGE, caudal ganglionic eminence; POA, preoptic area; Hp, hippocampal formation.

The establishment of telencephalic territories relies on the spatial and temporal action of factors known as morphogens that are secreted by signalling centers generally called organizers. Morphogens induce the differential expression of genetic programs and progressively confer positional identity to proliferating cells. At earliest stages, the neural tube is composed of a single layer of proliferating cells and through these events, the

proliferative territories become progressively constrain into particular cell fates and consequently regional boundaries are established (Campbell 2003; Hébert and Fishell 2008). The main molecules involved in the early DV patterning of the telencephalon include bone morphogenic proteins (such as *Bmp4*), members of the fibroblast growth factor (FGF, among which *Fgf8* and *Fgf17* are the most studied) and wingrless-type MMTV integration site families (*Wnt3a*) and sohic hedgedog (*shh*) (Hébert and Fishell 2008) (Rallu et al. 2002).

The early broad DV subdivision of the telencephalon in pallial and subpallial territories is linked to the generation of glutamatergic and GABAergic neurons, respectively, the two main types of neurons that populate the cerebral cortex. So, progenitor cells in the pallium give rise to excitatory glutamatergic neurons that reach their final position in the neocortex through radial migration (Marín and Rubenstein 2003). In contrast, progenitors cells within subpallial territories produce GABAergic neurons for both the basal ganglia and cortical structures. GABAergic interneurons reach the different telencephalic structures through tangential migration (Marín and Rubenstein 2001). Below I will describe in more detail the subsequent specification steps followed by these two territories and the genetic programs that regulate the production of the different neuronal subtypes of the neocortex.

2.2 Molecular control of cell specification in the telencephalon

2.2.1 Basic mechanisms of dorsal forebrain patterning and arealization

Early patterning events in the developing telencephalon specify the DV axis and establish pallial and subpallial territories. Through the release of morphogens from specific signalling centers, different germinal zones acquire their specific positional and molecular identities and give rise to different telencephalic structures (Rubenstein et al. 1998). The formation of the DV axis occurs as neocortical arealization begins and some of the early morphogens (e.g. Fgf8) on top of inducing early downstream specification programs, directly control cortical arealization (Fukuchi-Shimogori and Grove 2001). While the regionalization of the subpallium is at least in part related to the production of specific cell types, the regionalization of pallial territories primarily reflects cortical arealization. Many transcription factors have been described to control the acquisition of areal identities in the developing pallium. Among them, paired box gene 6 (*Pax6)*, empty spiracle homeobox 2 (*Emx2*), *Sp8,* and *Couptf1* are the most studied. For instance, *Pax6* is expressed at the

neural plate stage throughout the telencephalic vescicle, and it interacts with *Nkx2.1* and *Ghs2* transcription factors to first define the pallial boundaries and then become restricted to the pallial progenitors (Corbin et al. 2003). In *Pax6* mouse mutants, the pallial territories upregulate the expression of typical subpallial markers (e.g. *Dlx2*, *Mash1*) and are re-specified toward subpallial fates (Muzio et al. 2002a). Reciprocal genetic interactions within the pallium are further reiterated during cortical arealization through the formation of gradients along the dorso-ventral and rostro-caudal axes (O'Leary and Sahara 2008). For instance, *Pax6* and *Emx2* are not uniformly expressed but instead show complementary gradients of gene expression: *Pax6* is expressed in rostrocaudal and ventrodorsal high to low gradient while *Emx2* show high expression at more caudal levels. These opposite gradients have been shown to impact on the establishment of cortical area identities with *Pax6* and *Emx2* being implicated in specifying frontal/motor and caudal sensory/visual areas respectively (Bishop et al. 2000; Mallamaci et al. 2000; Muzio et al. 2002b). Similarly, gain and loss of function studies have shown that *Sp8* is involved in the specification of rostral cortices (Sahara et al. 2007; Zembrzycki et al. 2007). On the other hand, *Couptf1* is highly expressed at caudal cortical levels, where it functionally represses the specification of frontal/motor cortices in favour of somatosensory and visual area identities (Armentano et al. 2007). How this graded transcription factor expression is translated into the formation of discrete domains that reflect areas boundaries is still an open question. A recent study identified many enhancer sequences that show sharped spatial restricted patterns of activity in pallial territories (Visel et al. 2013). Interestingly, some of these enhancers seems to integrate the information of transcription factors gradients (e.g. Pax6) and translate them into the formation of protodomains that define future cortical areas (Pattabiraman et al. 2014). As result of these processes, progenitor cells are constrained forming a so-called embryonic proto-map that is then transmitted to postmitotic neurons and ultimately reflect the functional organization of the adult neocortex.

2.2.2 Origins and molecular specification of projection neurons

One remarkable feature of PN subtypes is their peculiar laminar identity. Although, this is not an absolute rule (e.g. CPNs are found in different proportions in both supra- and infra-granular layers), as previously discussed, PNs that project to the same target structures are typically confined to particular layers. In addition, the laminar localization of PNs subtypes reflects the temporal order in which they are produced. So, at the population level, PNs are

generated in an inside-out pattern that correlated with their birthdate, which means that early-born neurons populate deep cortical layers while late-born neurons progressively allocate into relatively more superficial positions. Therefore, PN subtypes are generated in partially overlapping sequential waves with layer VI CfuPNs being the earliest and layer II/III CPNs the last ones (**Figure 1d**). The cellular mechanisms of neurogenesis and migration will be discussed in the next paragraphs.

In the last years much efforts have been made to identify the genetic programs controlling the specification of PNs, as well as markers of their laminar identity. Based on their hodology, PN subtypes have been selectively traced using retrograde labelling strategies such as for instance the injection of microspheres into their axonal projection fields. The purification of backtraced PNs sub-populations by fluorescent-activated cell sorting (FACS) followed by transcriptomic analyses, has led to the identification of the main molecular pathways involved in the specification of subtypes identities and, many others are now beginning to be revealed. These analyses point out to the existence of complex molecular networks that involve reciprocal cross-repression mechanisms for the delineation of PN subtypes. In addition, the temporal profiling of PN subtypes during development has revealed an initial overlapping expression of the main molecular controls that are progressively refined as neurons differentiate.

Specification of CfuPN subtype identity

Corticofugal projection neurons include CthPN and SCPN subclasses that are primarily allocated in cortical layers VI and V. Both classes are sequentially generated during early neurogenesis, with picks of neuronal production around embryonic day (E) 12.5 and 13.5 respectively. One of the main players controlling the specification of CfuPNs is the transcription factor *Fezf2*. In particular, *Fezf2* promote the specification of the SCPN subtypes (e.g. CSMNs) by downstream activation of the transcription factor *Ctip2* (Arlotta et al. 2005; Chen et al. 2005b; Molyneaux et al. 2005). In *Fezf2* knockout mice, the presumptive layer V PNs mis-projects to the thalamus and its neurons are molecularly re-specified toward CthPN fates, ectopically expressing *Tbr1* (Chen et al. 2005a; Molyneaux et al. 2005). Conversely, loss of function studies have shown that *Tbr1* is essential for the specification of CthPN, because in *Tbr1* mutant cortices *Fezf2* becomes upregulated (Bedogni et al. 2010). Therefore, *Tbr1* regulate the development of CthPNs by directly repressing the transcription of *Fezf2* and indirectly *Ctip2* (McKenna et al. 2011) (**Figure 1e**). Recently, several lines of evidence further supported a primary role of *Fezf2* in

instructing CfuPN identities in a cell-autonomous manner. Indeed, ectoptic expression of *Fezf2* in the LGE seems sufficient to redirect striatal medium spiny neurons toward CfuPN fates (Rouaux and Arlotta 2010), while *Fezf2* expression in early post-mitotic layer IV spiny neurons and layer II/III CPN reprogrammes these neurons to acquire marker expression and connectivity equivalent to CfuPNs *in vivo* (De la Rossa et al. 2013; Rouaux and Arlotta 2013). CfuPNs include CthPN and SCPN subtypes that are generated in sequential order and populate mainly layer VI and V, respectively. In this context, the expression levels of *Fezf2* have been shown to be important for the temporal production of the different CfuPN subtypes. In particular, transcription factors belonging to the SRY-box (Sox)-containing gene family regulate *Fezf2* expression through directly binding to an enhancer sequence specific for *Fezf2*. In this way, *Sox5* inhibits *Fezf2* gene expression while *Sox4* and *Sox11* activates *Fezf2* by competing with Sox5 for the binding site of the enhancer sequence (Lai et al. 2008; Shim et al. 2012).

Specification of CPN subtype identity

Callosal PNs are late-born (between E14.5 and E16.5 in mouse) commissural neurons that connect homotopically with the contralateral hemisphere by extending their axon through the corpus callosum. Approximately 80% of the CPNs in rodents are located in layers II/III while a about 20% are also found in layer V and VI (Fame et al. 2011). According to the inside-out pattern of migration, infragranular CPNs are produce around the same time than CfuPN subtypes. As *Fezf2* is critical for the specification of CfuPN subtypes, the transcription factor *Satb2* molecularly defines CPN identities for all cortical layers by directly repressing *Ctip2* expression (Alcamo et al. 2008; Britanova et al. 2008) (**Figure 1e**). *Satb2*-deficient neurons upregulate Ctip2 expression, fail to extend axons through the corpus callosum and instead project subcortically. Conversely, the expression of typical CPNs markers (e.g. cut-like homeodomain transcription factor Cux1) is affected in Satb2 mutant mice. CPNs represent a heterogeneous population, and gene expression studies have revealed that superficial and deep-layer CPNs are molecularly distinct (Molyneaux et al. 2009). Moreover, PNs with axons that project toward multiple targets (cortico-cortical and corticofugal) share some molecular features. One example is represented by the intratelencephalic corticostriatal projection neurons (CstrPNi) that project both to the controlateral hemisphere (via *corpus callosum*) and to the ipsilateral striatum. CstrPNi share some features with both CfuPN and CPN identities such as the co-expression of *Satb2* and *Sox5* transcription factors (Sohur et al. 2014).

2.2.3 Origins and molecular specification of cortical GABAergic interneurons

Telencephalic GABAergic interneurons originate from proliferative territories in the embryonic subpallium and migrate tangentially long distances to reach the neocortex (de Carlos et al. 1996; Anderson et al. 1997a; Tamamaki et al. 1997). These evidences derived primarily from studies in rodents and have been recently confirmed in human and non-human primates (Hansen et al. 2013; Ma et al. 2013). Early embryological studies identify the ganglionic eminences based on anatomical criteria. However, while the LGE and MGE are clearly morphologically separated by a sulcus, there is no clear anatomical boundary between these two regions and the CGE. In addition, the subpallium undergoes substantial morphological changes during embryonic development, further complicating the identification of the different eminences based exclusively on anatomical criteria. Nowadays, regional differences within the embryonic subpallium are primarily based on the differential expression of transcription factors that define territories specialized in the production of different types of GABAergic interneurons (**figure 3b, c**). Many genes have been identified as specifically expressed in the subpallium. Among them, members of the *Dlx* family are expressed broadly in all the subpallial territories and have been shown to be essential for the specification of telencephalic interneurons. Mice with mutations in the *Dlx1/2* homeodomain genes show a dramatic impairment of tangential migration and a severe reduction in the number of cortical GABAegic interneurons (Anderson et al. 1997a) as well as abnormal development of the striatum and olfactory bulb (Anderson et al. 1997b). Moreover, it is now well established that virtually all cortical GABAergic interneurons derive from cells expressing *Dlx5/6* (Stühmer et al. 2002). Similarly, the proneural bHLH (basic helix-loop-helix) transcription factor *Mash1* (also known as *Ascl1*) is widely expressed in the subpallium and is essential for maintaining the subpallial identity through a cross-repressive mechanism with Neurogenin1/2 that instead control neuronal production within pallial territories (Fode et al. 2000; Schuurmans and Guillemot 2002).

The Medial Ganglionic Eminence

In addition to *Dlx* family and *Mash1* genes that broadly define the ventral telencephalon, other transcription factors show relatively restricted patterns of expression within the ganglionic eminences. Among them, the homeobox transcription factor *Nkx2.1* is specifically expressed by MGE and POA proliferative zones (Sussel et al. 1999) and is rapidly downregulated in cortical interneurons before they enter the neocortex, while it is

maintained in a subset of striatal interneurons (Marin et al. 2000). In *Nkx2.1* mutant mice, MGE/POA progenitor cells are re-specified to more dorsal fates, and there is a dramatic reduction (~50-60%) in the total number of cortical GABAergic cells (Sussel et al. 1999). Thus, the MGE represents the main source of cortical interneurons. Furthermore, several lines of evidences revealed that the MGE gives rise to two main non-overlapping classes of cortical interneurons: PV-expressing and SST-expressing interneurons (**Figure 2a, b**). These results, first derived from *in vitro* cultures experiments (Xu et al. 2004) and transplantation studies *in vivo* (Butt et al. 2005; Cobos et al. 2007; Butt et al. 2008; Wonders et al. 2008), has been more directly confirmed by genetical fate-mapping studies of *Nkx2.1*-expressing progenitor cells (Fogarty et al. 2007; Xu et al. 2008). Interestingly, *shh* signalling acts upstream of *Nkx2.1* in the specification of the MGE territory (Xu et al. 2005) and modulates the specific production of MGE-derived interneuron subtypes in a dose-dependent manner (Xu et al. 2010). *Nkx2.1* specifies PV+ and SST+ cortical interneuron subtypes by directly activating the LIM-homeobox transcription factor *Lhx6* (Du et al. 2008), which is maintained during migration and in the adult cortex (Lavdas et al. 1999). In the absence of Lhx6, cortical interneurons fail to express PV and SST and show an abnormal allocation in the neocortex (Liodis et al. 2007). Recently, the Sry-related HMG-box-containing transcription factor *Sox6* has been shown to act downstream of *Lhx6* (Batista-Brito et al. 2009) and regulate the position and final maturation of PV+ interneurons and, to a minor extent, SST+ interneurons (Batista-Brito et al. 2008; Azim et al. 2009; Batista-Brito et al. 2009). These results suggest a basic sequential molecular pathway that involves *Nkx2.1*, *Lhx6* and *Sox6* transcription factors and provide a possible genetic imprinting mechanism for the specification of the MGE-derived PV- and SST-expressing interneurons. However, it is still unclear whether both interneuron subtypes share a common progenitor and/or derive from segregated pools of progenitor cells. In this context, transplantation studies have revealed a spatial bias for the origin of interneuron subtypes within the MGE and suggested that PV+ interneurons mainly derive from the ventral MGE, while SST+ interneurons primarily emerge from dorsal MGE progenitors (Wonders et al. 2008). In addition, fate-mapping using the Shh-cre mouse line, in which Cre is mainly expressed by the ventral MGE have shown that this region primarily produces interneurons for the globus pallidus and striatum (Flandin et al. 2010), which suggests that interneurons for the basal ganglia and cortex may indeed derive from segregated pools of progenitor cells.

Gene expression studies have revealed a substantial molecular diversity of MGE progenitor cells based on a differential expression of transcription factors that in combination define putative proliferative sub-domains (Flames et al. 2007; Tucker et al. 2008). In particular, our lab some years ago proposed a subdivision of the embryonic subpallium based on the combinatorial expression of different transcriptional codes (Flames et al. 2007). For instance, the homeodomain transcription factor Nkx6.2 is expressed in a high to low gradient from the LGE/MGE boundary to more ventral regions. Genetic fate mapping and loss of function studies shown that Nkx6.2 generate a highly diverse population of cortical interneuron subtypes (Flames et al. 2007; Fogarty et al. 2007; Sousa et al. 2009). In addition, similarly to what it was shown for the pallium, the discovery of genetic enhancers specifically active in sub-territories in the embryonic subpallium (Visel et al. 2013) and the possibility to fate map these progenitors in a temporal manner will help to characterize the precise contribution of progenitor cell subpopulations to the different interneuron classes.

The Caudal Ganglionic Eminence

Although the precise contribution of the CGE region to the cortical GABAergic interneuron population was initially controversial, it is now well established that the CGE produces about 30-40% of all cortical GABAergic interneurons (Rudy et al. 2011). The identification of this region was initially based only on morphological references, and the lack of clear anatomical boundaries with the LGE complicated its study. The CGE appears relatively late during development compared for example to the MGE and, at the molecular level it shares some patterns of gene expression (e.g. *Gsh2*) with the LGE, thereby making more difficult the design of specific fate-mapping experiments. The family of Coup-tf transcription factors represents one of the main players in the specification of CGE interneurons. Conditional *Coup-tf1* loss of function results in the respecification of CGE interneurons subtypes to MGE fates (Lodato et al. 2011b), while transplantation studies have revealed a role for *Coup-tf2* in directing the caudal stream of migration adopted by CGE-derived interneurons (Kanatani et al. 2008). In addition, similarly to the MGE (Flames et al. 2007), microarray studies of the CGE territory are beginning to reveal the existence of unique molecular profiles within this region (Willi-Monnerat et al. 2008). Many additional markers with unknown function have been recently discovered, such as for example *sp8* and *Prox1* (Ma et al. 2012; Rubin and Kessaris 2013).

The first direct evidence of a substantial contribution of CGE progenitor cells to particular subpopulations of telencephalic interneurons derived from gene expression and cell transplantation studies (Nery et al. 2002). These early observations were subsequently confirmed and expanded by other *in vitro* and *in vivo* studies and it is now well established that the CGE generates bipolar, double-bouquet and some neurogliaform interneurons that express the ionotropic serotonin receptor 3a (5-HT3a) (Lee et al. 2010). Using a transgenic Mash1CRE^{ERT2} line that selectively label the CGE but not the MGE, direct fate-mapping experiments have shown that CGE-derived interneurons primarily localize to superficial cortical layers and consist of approximately 50% of VIP^{+} and/or CR^{+} (but not SST^{+}) interneurons, while the other half part express the glycoprotein Reelin (Miyoshi et al. 2010) (**Figure 2**). Physiologically, both groups are late-spiking interneurons (Miyoshi et al. 2010), but the molecular pathways that underlie the proper specification of these interneuron classes remain largely unknown.

The Preoptic Area

The MGE and CGE together give rise to more than 90% of the entire cortical interneuron population. The remaining seems to derive from the POA, a small domain ventral to the MGE (Gelman et al. 2009). A clear anatomical boundary between POA and MGE is not visible at rostral levels but the molecular profile of this region reveals a unique composition. Although the POA shares with the MGE the expression of *Nkx2.1*, it additionally expresses *shh, Nkx6.2* as well as *Nkx5.1* and *Dbx1*. Interestingly, POA-derived interneurons do not express *Lhx6,* a constant feature of MGE-derived interneurons. Conditional fate mapping studies of Nkx5.1- and Dbx1- expressing domains have demonstrated that the POA give rise to PV+, SST+, Reelin+ and NPY+ cortical interneurons with heterogeneous electrophysiological properties (Gelman et al. 2009; Gelman et al. 2011) (**Figure 2**).

2.3. Neuronal migration in the developing cerebral cortex

Pyramidal neurons (PN) and interneurons are born in different anatomical places in the developing telencephalon: pyramidal cells are born locally in the pallium, the embryonic anlagen of the neocortex, while cortical interneurons are produced in the subpallium, far away from their final position. For this reason, these two cell types adopt different migration strategies to reach the neocortex. PNs migrate radially to form the cortical layers, while interneurons migrate tangentially from the subpallium to the cortex. Once

there, interneurons undergo a complex intracortical migration that involves changes in their direction of migration from tangential to radial together with the acquisition of their laminar identity (Marín and Rubenstein 2003) (**Figure 4a, b**).

The early developmental processes of cell migration directly shape and impact the formation of cortical cytoarchitecture and are intimately linked to the proper assembly of PNs and interneurons into functional circuits. Therefore, despite the very different migration strategies, the final allocation of both cell types must require some degree of coordination (Bartolini et al. 2013). Below I describe some basic concepts for radial and tangential migration, with a particular emphasis on those mechanisms relevant for the structural organization of the neocortex. I then discuss some of common principles involved in the allocation of PNs and interneurons into the cortex.

2.3.1 Migration of pyramidal neurons

During development, the structure of the embryonic cortex undergoes substantial morphological changes, with a sizable increase in thickness that is paralleled by the emergence of different zones along the tangential plane. These morphological changes derive from a complex series of coordinated events, form cell proliferation and neurogenesis to cell migration. At early stages, a thin layer of proliferating cells composes the developing neuroepithelium. Starting ~ E10.5 in mice, the first cohort of postmitotic neurons migrate radially and form a transient layer called preplate (PP). This primordial layer consists of Cajal-Retzius cells and the first cohort of pyramidal neurons. Cajal-Retzius cells constitute a transient population generated by discrete pallial structures (for instance, the cortical hem) that localize all throughout the cortical surface where they play an important role regulating the migration of pyramidal cells (Bielle et al. 2005; Yoshida et al. 2006; Villar-Cerviño and Marín 2012). After the first pyramidal neurons are born, multiple waves of neuroblasts are generated from progenitor cells in the ventricular (VZ) and subventricular zone (SVZ) and migrate radially splitting the existing PP into the marginal zone (MZ) superficially and more deeply the subplate (SP), and form the cortical plate (CP). During development, consecutive waves of post-mitotic PNs migrate radially toward the CP, passing over previously generated neurons and forming in this manner the six cortical laminae of the neocortex in an inside-out fashion. Birthdating studies have shown that, as result of this migration pattern, early-born pyramidal cells primarily occupy deep cortical layers, while the late-born neurons progressively form the more superficial ones (Angevine and Sidman 1961; Fairén et al. 1986) (**Figure 4**). So, while the areal

identity of a PN is determined by the rostro-caudal position of progenitor cells, the laminar allocation strongly correlates with birthdate.

Two modes of PN radial migration have been described: somal translocation and glial-guided locomotion. During somal translocation, neuroblasts first extend a radially oriented and typically long leading process that is attached to the pial surface (or MZ), and subsequently translocate the nucleus within the leading process until they reach the target position. PNs adopt this mode of migration primarily during early development; as development progresses and the cortical thickness increases, PNs migrate mostly using locomotion. Locomotion refers to the migration of neuroblasts along radial glia fibers. Migrating neurons undergoing locomotion show a relatively short leading processes and a trailing process that will eventually become the future axon. During this type of migration, neuroblasts move radially in close proximity to the basal processes of radial glia cells (RGCs). RGCs are the main class of progenitor cells located in the VZ of the developing cortex and are morphologically characterized by having a short apical process that touches the ventricular lumen and a long radial process that extend basally to anchor at surface of the brain. In other words, RGCs are not only the progenitors of PNs but also serve as a physical scaffold that is used by locomoting neurons to move radially toward the CP (Noctor et al. 2001; Noctor et al. 2004).

Radial migration represents an efficient mechanism to maintain topographical relationships between the relative position of progenitor cells in the VZ and the final position of newborn neurons in the mantle (Rakic 1988). According to the 'radial unit hypothesis', neurons derived from the same proliferative unit migrate along radial glia fibers and arrange radially to form ontogenic columns (Rakic 1988). During locomotion, migrating cells undergo substantial morphological changes. Newborn neurons first detach from the VZ and move radially into the SVZ where they transiently acquire a highly dynamic and multipolar morphology, before attaching to radial glia fibers and migrating toward the CP with the typical bipolar morphology (Nadarajah and Parnavelas 2002; Kriegstein and Noctor 2004). During this transition state, multipolar neuroblasts can move tangentially for a short distance and intermingle laterally with other pyramidal neurons (Tabata and Nakajima 2003). Recently, ephrin guidance factors and their Eph receptors have been shown to control the lateral dispersion of pyramidal neurons and impact in the structural organization of cortical columns architecture (Torii et al. 2009; Dimidschstein et al. 2013).

Many secreted signalling molecules and intracellular proteins (mainly microtubule associated proteins) have been shown to regulate pyramidal neuron migration and the formation of cortical layers (Marín et al. 2010). Among them, the signalling pathway elicited by *reelin* is one of the best characterized. *Reelin* is a glycoprotein secreted by Cajal-Retzius cells at the cortical surface that binds to ApoER2 and VLDLR receptors expressed by migrating PN (D'Arcangelo et al. 1999; Hiesberger et al. 1999) and signals through the intracellular adaptor protein Dab1 (Howell et al. 1999). Both spontaneous mutation of *Reelin* (the *reeler* mouse) and genetic loss of function of the intracellular adaptor Dab1 causes severe defects in cortical cytoarchitecture characterized by inverted lamination pattern, as revealed by birthdating analyses (Caviness 1982; Franco et al. 2011). Interestingly, the Reelin pathway seem to interact with other signalling molecules, such as ephrins to regulate the migration and the position of cortical PN (Sentürk et al. 2011).

2.3.2 Migration of cortical interneurons

Cortical interneurons arise from transient structures in the embryonic subpallium and so they migrate very long distances to reach the neocortex (Marín and Rubenstein 2001). Interneuron migration can be subdivided in different phases: (1) Tangential migration to the pallium; (2) Intracortical dispersion and formation of stereotyped migratory stream; and (3) CP invasion and laminar allocation (Marín 2013). The tangential migration of cortical interneurons relies primarily on their ability to differentially respond to guidance factors expressed in distinct telencephalic territories. To properly read the environment, migrating interneurons show highly dynamic leading processes that branch continuously in response to chemoattractant or chemorepellent cues and ultimately determine the direction of migration (Martini et al. 2009; Yanagida et al. 2012). Primary cilia and the leading processes of migrating interneurons are thought to be the primary domains containing guidance receptors (Martini et al. 2009; Baudoin et al. 2012; Higginbotham et al. 2012).

Many factors, including neurotrophins (e.g. GDNF, BDNF and NT4) (Powell et al. 2001; Polleux et al. 2002), neurotransmitters (Cuzon et al. 2006; Bortone and Polleux 2009; Inada et al. 2011) and typical and atypical guidance cues (e.g. semaphorins and neuregulins) (Marín et al. 2001; Flames et al. 2004) contribute to the guidance of interneurons throughout the subpallium and into the cortex. Interestingly, interneurons fated to occupy different telencephalic structures (e.g. striatum and cortex) migrate through a very similar environment but respond differentially to guidance cues, as they hold a different complement of receptors the expression of which is ultimately determined by

intrinsic genetic programs (Nóbrega-Pereira et al. 2008; Nóbrega-Pereira and Marín 2009; van den Berghe et al. 2013). For instance, the Nkx2.1 transcription factor represses the expression of Neuropilin1 and Neuropilin2, receptors for the repulsive molecules Sema3A and Sema3F, respectively, that are expressed in the developing striatum. MGE-derived striatal interneurons continue to express Nkx2.1 during their tangential migration, which downregulates neuropilins and allow the colonization of the striatum by this population of interneurons (Nóbrega-Pereira et al. 2008). Conversely, MGE-derived cortical interneurons downregulate Nkx2.1 expression as they begin migration. This leads to the expression of neuropilins and makes cortical interneurons sensitive to semaphorins expressed in the striatum, which they avoid in their way to the cortex (Marín et al. 2001). In addition, MGE-derived cortical interneurons also follow a gradient of chemoattractive signals generated by different isoforms of Neuregulin1 that act as short- and long-range attractants for cortical interneurons in their way toward the cortex (Flames et al. 2004).

Although most of our knowledge on the migration of interneurons stems from the MGE-derived population, different classes of interneurons follow different routes of migration in the subpallium and therefore likely respond to different cues. For example, CGE-derived interneurons migrate almost exclusively through a caudal stream in the subpallium (Yozu et al. 2005). Independently of their origin and route of migration, once in the pallium cortical interneurons initially avoid the CP and migrate tangentially using two main streams through the MZ and the SVZ/IZ (Lavdas et al. 1999; Wichterle et al. 2001) (**Figure 4b**). This process allows the dispersion of interneurons throughout the entire cortical surface and primarily involved the signalling elicited by the chemokine *Cxcl12*. *Cxcl12* is expressed by the meninges and by intermediate progenitor cells in the SVZ and acts as a strong chemoactractant for migrating interneurons (Tham et al. 2001; Stumm et al. 2003; Daniel et al. 2005; Tiveron et al. 2006). Interneurons respond to Cxcl12 through the G-protein coupled receptors Cxcr4 and Cxcr7 and this signalling pathway has been shown to be key for the tangential dispersion of interneurons throughout the cortex. In absence of the Cxcr4 or Cxcr7 receptors, many interneurons fail to organize through the MZ and SVZ paths and prematurely invade the CP (Stumm et al. 2003; Tiveron et al. 2006; Li et al. 2008; López-Bendito et al. 2008; Sánchez-Alcañiz et al. 2011; Wang et al. 2011b). Interestingly, the two chemokine receptors functionally cooperate during interneuron migration by dynamically adapting interneuron chemoresponsivennes to Cxcl12. Cxcr7 regulate the levels of Cxcr4 at the plasma membrane of migrating

interneurons by buffering the levels of Cxcl12 in the environment and ultimately preventing the desentitization of Cxcr4 (Sánchez-Alcañiz et al. 2011). This mechanism allows the intracortical tangential dispersion of interneurons prior to CP invasion and laminar allocation. Finally, at perinatal stages, interneurons turn to radial their direction of migration and populate the CP in a process that has been suggested to be largely linked to the lost of interneuron chemoresponsivness toward Cxcl12.

Laminar allocation of cortical interneurons

The molecular signalling and the cellular events that regulate the precise allocation of interneuron classes into the six cortical layers are still largely unknown. Several lines of evidence suggest a primary role of the neurotransmitter GABA as a stop signal for cortical interneuron migration. It is now well established that GABA, the primary inhibitory neurotransmitter of the CNS, is excitatory during early development. The switch of its action from depolarizing to hyperpolarizing is largely controlled by the upregulation of the potassium/chloride exchanger KCC2, which regulates the reversal potential of the chloride ion (Ben-Ari 2002). Interestingly, experimental evidence suggests that interneurons upregulate the expression of KCC2 while migrating in the cortex and use the hyperpolarizing action of GABA as a stop signal for their migration (Bortone and Polleux 2009). Consistently, interneurons upregulate the KCC2 expression at the time of radial sorting (Miyoshi and Fishell 2011), but it is presently unclear if these events directly regulate their specific laminar allocation.

The allocation of MGE-derived interneurons in cortical layers follows an inside-out pattern that largely recapitulates the mechanism described for pyramidal cells, although both classes of cells seem to use different strategies. Indeed, birthdating and transplantation studies have shown that early-born MGE-derived interneurons preferentially populate infragranular cortical layers whereas late-born interneurons allocate primarily in the superficial layers of the cortex (Miller 1985; Fairén et al. 1986; Valcanis and Tan 2003; Pla et al. 2006). In contrast, CGE-derived interneurons do not follow this principle and preferentially settle in the superficial cortical layers (Miyoshi et al. 2010) (**Figure 4**). In addition, recent work have shown that even coetaneous MGE- and CGE-derived interneurons segregate into different layers despite their concurrent tangential migration (Miyoshi and Fishell 2011), suggesting that birthdate is not the primary factor that determines laminar allocation.

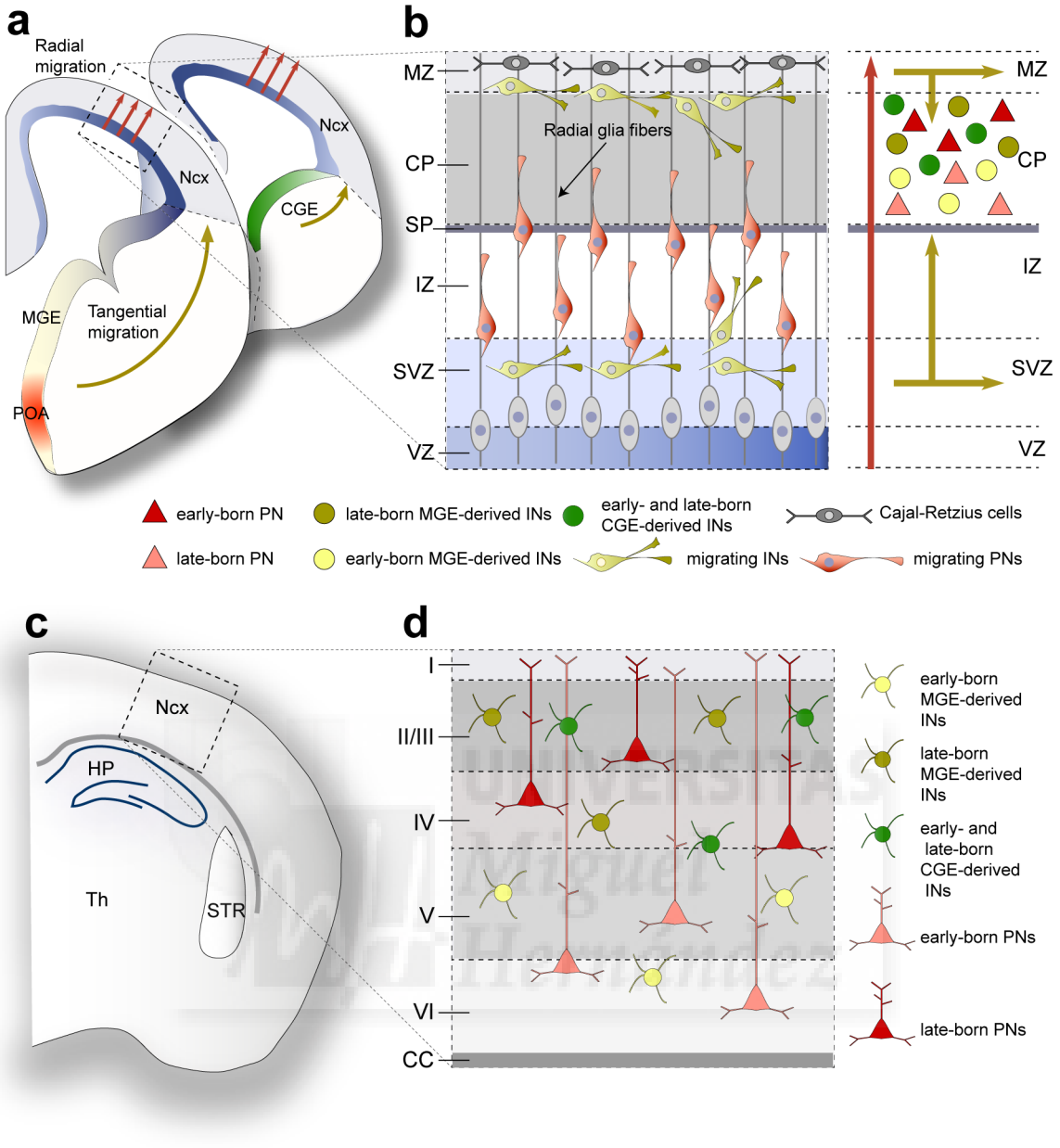

Figure 4. Migration and laminar allocation of cortical neurons. (**a-d**) Schematic representations of the migration strategies used by cortical PNs and INs and their pattern of laminar allocation in the adult brain. PNs migrate radially toward the cortical plate (CP) mainly using the scaffold of radial glia fibers. Cortical INs undergo a long tangential migration through the subpallium, before invading the neocortex through the marginal zone (MZ) and the subventricular zone (SVZ). After a tangential dispersion using these two routes, INs switch from tangential to radial their mode of migration and colonize the developing CP. PNs and MGE-derived interneurons populated the CP in an inside-out pattern that correlated with their birthdate. This pattern is maintained in the adult brain, where early-born cells localize in deep cortical layers while late-born cells populate the more superficial ones. In contrast, INs originated in the CGE primarily populate supragranular layers independently on birthdate (c, d). Arrow lines in (a) and (b) represent the trajectories of migration. Cc, corpus callosum; HP, hippocampal formation; Ncx, neocortex; STR, striatum; Th, thalamus; I-VI, cortical layers I to VI; MGE, medial ganglionic eminence; CGE, caudal ganglionic eminence; POA, preoptic area; SP, subplate; IZ, intermediate zone; VZ, ventricular zone; INs, interneurons; PNs, projection neurons.

Interestingly, cortical interneurons seem to match the distribution of pyramidal cells even in those conditions of abnormal layering (e.g. the *reeler* mouse) (Hevner et al. 2004; Pla et al. 2006). However, in contrast to pyramidal neurons, the intracortical migration and the final laminar allocation of MGE-derived interneurons has been shown to be largely independent on Reelin signalling (Pla et al. 2006). Altogether, current evidence strongly suggest that the laminar allocation of cortical interneurons is hierarchically linked to the position of pyramidal cells, in a model in which cues provided by projection neurons guide cortical interneurons to their appropriate layer. Indeed, recent work has shown that the ectopic placement of PN is sufficient to recruit interneurons to ectopic locations and suggest that specific PN identities selectively attract specific interneuron subtypes (Lodato et al. 2011a).

3. Cellular control of neurogenesis and fate specification in the developing cerebral cortex

The generation of cell diversity involves different types of progenitor cells that are lineally related through distinct modes of cell division (Götz and Huttner 2005; Kriegstein and Alvarez-Buylla 2009) (**Figure 5**). Different progenitor types coexist during development and their patterns of division are finely regulated to produce the different subtypes of neurons and glial cells that form the cortex. In this Section, I will summarize our current knowledge of the main cellular mechanisms of neurogenesis using the developing pallium as a model.

3.1 Modes of cell division and neurogenesis

Before the onset of neurogenesis, the neural tube is mainly composed by neural stem cells with typical epithelial features known as neuroepithelial cells (NECs). NECs are highly polarized along the apico-basal axis and are anchored at the ventricular and pial surfaces through tight and adherent junctions (Götz and Huttner 2005). NECs primarily undergo proliferative symmetric cell divisions and self-renew to produce identical cell types, expanding in this way the pool of neural stem cells. As development progress, NECs lose some of their epithelial characteristics and convert into another type of progenitor cells know as radial glia cells (RGCs). RGCs maintain the apico-basal polarity, but their soma becomes strictly confined within the ventricular zone (VZ) while they continuously extend a pia-directed process that serves as a scaffold used by neuroblasts during radial locomotion. Both NECs and RGCs move the cell body up and down during cell division; a process known as interkinetic nuclear migration (Taverna and Huttner 2010). The morphological changes that occur during NECs to RGCs conversion are paralleled by the expression of several astroglial markes, such as the astrocyte-specific glutamate transporter (GLAST), the brain-lipid-binding protein (BLBP), the glial fibrillary acidic protein (GFAP) as well as intermediate filament proteins including nestin and vimentin (Hartfuss et al. 2001; Noctor et al. 2002). RGCs are thought to undergo mainly asymmetric cell division, a mitotic event that generate daughter cells with different cell fates (Fishell and Kriegstein 2003). RGCs serve as primary progenitor cells for most neurons and macroglia in the cerebral cortex, and during asymmetric mitosis they self-renew and generate non-RGC daughter cells (Malatesta et al. 2000; Hartfuss et al. 2001; Noctor et al. 2001;

Tamamaki et al. 2001; Anthony et al. 2004; Noctor et al. 2004). RGCs specification requires the expression of the transcription factor Pax6 that furthermore seems to control the neurogenic potential of this progenitor class (Götz et al. 1998; Heins et al. 2002; Asami et al. 2011).

The introduction of methods for labelling cells with fluorescent markers (e.g. GFP, green fluorescent protein) and the possibility to trace their behaviour over time represented a real breakthrough for our understanding of the lineage relationships between progenitor cell types and neurons. Several studies have now shown that RGCs produce neurons directly only in ~10% of the cases, while they primarily produce neurons indirectly through another class of mitotically active cells called intermediate or basal progenitors (IPCs) (Haubensak et al. 2004; Miyata et al. 2004; Noctor et al. 2004; Englund et al. 2005). IPCs increase their number dramatically during development and this expansion establishes a new proliferative layer called the subventricular zone (SVZ). In contrast to NECs and RGCs, IPCs show a multipolar morphology without any attachment to the pia or ventricular surfaces and do not undergo interkinetic nuclear migration during cell division. In addition, IPCs downregulate Pax6 and specifically express the transcription factor Tbr2 (Englund et al. 2005). IPCs represent a transient amplifying population that undergo one or two more rounds of symmetrical cell divisions in the SVZ before ultimately producing post-mitotic neurons (Haubensak et al. 2004; Miyata et al. 2004; Noctor et al. 2004). Thus, cortical neurogenesis seems to heavily rely in the expansion of cell lineages through IPCs.

Two additional types of progenitor cells have been recently identified in the developing cerebral cortex: basal radial glia cells (bRGCs) and short neural precursors (SNPs). Initially discovered in humans and ferrets (Hansen et al. 2010; Reillo et al. 2011), bRGCs (also named outer or intermediate RGCs) have also been identified in rodents (Wang et al. 2011a). Mouse bRGCs derive from apical RGCs but unlike them they localize in the upper SVZ and lose the apical process with the ventricle while maintaining the basal process attached to the pial surface. The number of bRGCs increases across the mammalian phylogenetic lineages and for this reason this progenitor type has been linked with evolutionary mechanisms of cortical expansion and folding (Florio and Huttner 2014). SNPs are located in the VZ and are anchored to the ventricular surface through an apical process. They also have a radial process of variable length that extend basally but does not attach to the pia (Gal et al. 2006). Although SNPs are thought to derived from RGCs, they show distinct and unique molecular features and cell cycle kinetics (Stancik et al. 2010).

Interestingly, unlike RGCs, SNPs primarily produce neurons directly without a previous expansion through IPCs (Tyler and Haydar 2013).

Although progenitor cell diversity (in relation on morphology and place of cell division) has been mostly studied in the pallium, recent work identified different types of precursors, including SNPs, IPCs and sub-apical progenitors (SAPs) in the ganglionic eminences (Pilz et al. 2013). This suggests that a similar heterogeneity of progenitor cells exist for interneurons born in the subpallium.

3.2 How to achieve cell diversity

Cortical progenitors produce highly heterogeneous cells including neurons and glial cells. Moreover, cortical pyramidal cells are extremely diverse and subtypes are generated in a remarkably precise developmental sequential order that organize them in layers and columns. How the different types of progenitor cells are lineally related to each other to generate such an astonishing diversity represents a major unanswered question in cortical development. In addition, do the same progenitor cells generate neurons, astrocytes and oligodendrocytes? These important questions have been addressed extensively during the last decades, not only in the mammalian cerebral cortex but also for other structures of the CNS (e.g. the spinal cord and the retina; (Livesey and Cepko 2001; Alaynick et al. 2011) as well as in different organisms (e.g. *drosophila melanogaster*; (Kohwi and Doe 2013).

The development of new lineage tracing methods together with the use of novel genetic tools to label specific cohorts of cells have brought new possibilities to investigate more precisely these issues (Buckingham and Meilhac 2011; Kretzschmar and Watt 2012). One of the most commonly used approaches in the analysis of cell lineages is the retroviral tracing technology. In this method, replication-incompetent retroviruses encoding for reporter proteins are used to infect proliferating cells in the embryonic brain. After infection, the viral genome carrying the reporter gene integrates in the genome of dividing progenitor cells. So, the reporter protein serves as inheritable marker to analyse the lineage relationships between sibling cells (Cepko et al. 1998). Interestingly, similar experimental approaches have been applied to the study of cortical lineages with opposites results. For example, several studies based on the use of ß-galactosidase encoding retroviral vectors revealed the existence of progenitor cells that are fate-restricted to produce either neurons or glial cells in rat and mouse cortices (Price and Thurlow 1988; Grove et al. 1993; Krushel et al. 1993; Luskin et al. 1993; Williams and Price 1995; McCarthy et al. 2001).

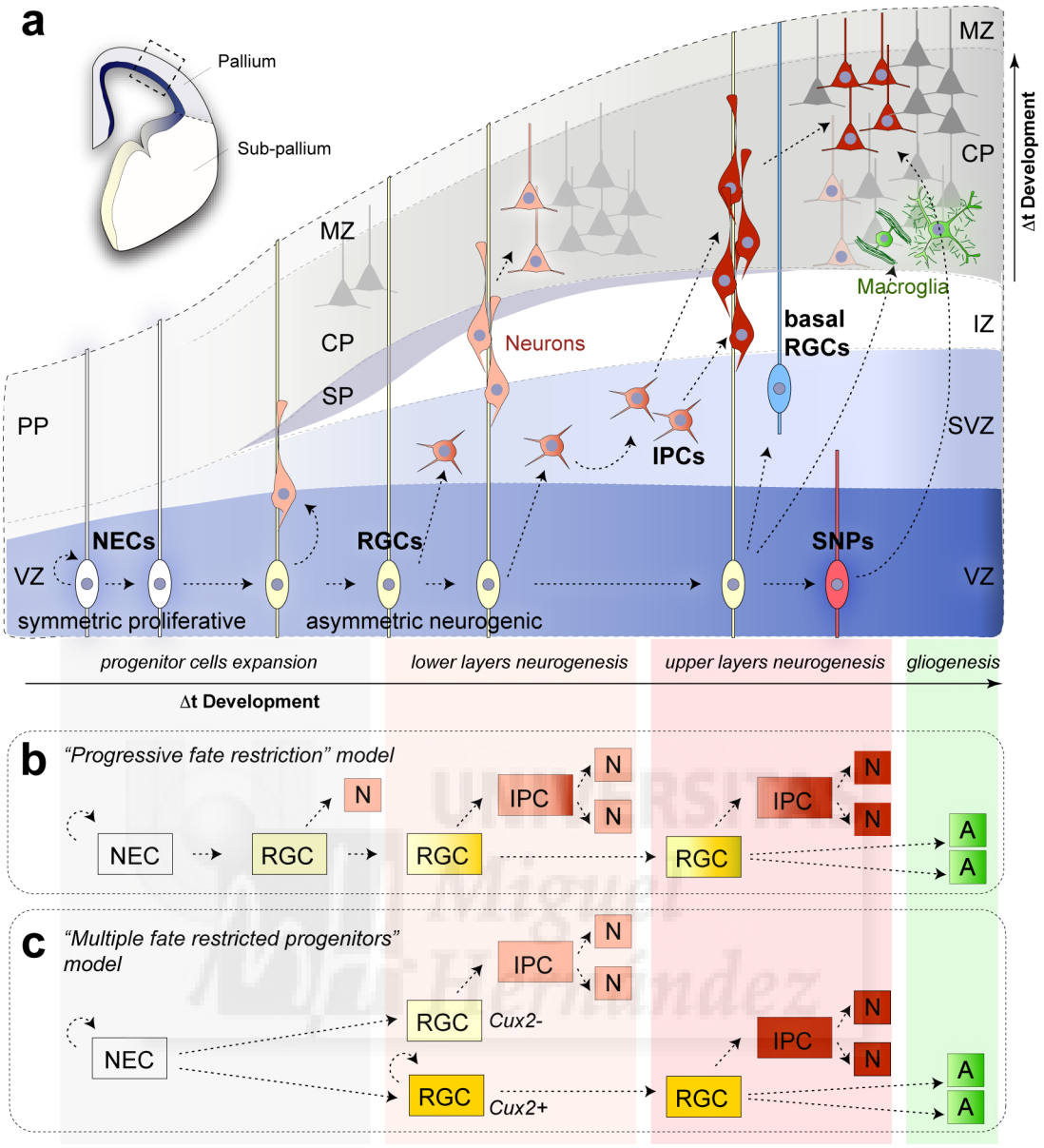

Figure 5. Progenitor cell types and modalities of cell division in the developing cerebral cortex. (**a**) During early development neuroepithelial cells (NECs) divide symmetrically in the ventricular zone (VZ) expanding the pool of NECs. As development progress, NECs convert into radial glia cells (RGCs), VZ progenitors that divide mainly asymmetrically to self-renew and produce either postmitotic neurons (N) or intermediate progenitor cells (IPCs). IPCs are located in the subventricular zone (SVZ) and divide symmetrically to ultimately produce Ns. Short neural precursors (SNPs) are VZ progenitors that produce directly Ns for the superficial layers. Basal radial glia (bRGCs) has been linked to evolutionary expansion of the cortex. At the latest stages, RGCs are thought to differentiate into macroglia. (**b, c**) Models of lineage progression for the generation of cortical pyramidal neurons. (**b**) In the classical model the same progenitor produces deep and superficial –layer neurons, by changing its competence (depicted by the colour gradients) and becoming progressively restricted to produce superficial cells. (**c**) Deep and superficial layers neurons derived from segregated fate-restricted progenitor cells. In this model two lineages (identified by the expression of Cux2) coexist during development with different proliferative behaviours. $Cux2^{+}$ RGCs would be mostly self-expanding when $Cux2^{-}$ RGCs produce deep layer neurons and would become neurogenic later on producing neurons for the supragranular layers. A, astrocytes; CP, cortical plate; IZ, intermediate zone; MZ, marginal zone; PP, preplate; SP, subplate.

Similar results were obtained by lineage tracing experiments in the spinal cord (Wu et al. 2006; Battiste et al. 2007). In contrast, other studies found neurons and astrocytes or oligodendrocytes as part of the same lineage, which suggest the existence of a common progenitor for both types of cells (Walsh and Cepko 1988; Williams et al. 1991; Walsh and Cepko 1992).

Cortical PNs show a peculiar configuration into columns and layers and both types of organization have important functional implications. In addition, PN identity is strongly linked to the laminar position that ultimately reflects its temporal production, and so it has been unclear whether different PN identities arise from the same progenitor cells or from different fate-restricted precursors. Gene expression studies of molecular markers that are specific of progenitor cells types initially suggested that infragranular and supragranular PN were differentially generated by VZ (RGCs) and SVZ (IPCs) progenitor cells, respectively (Tarabykin et al. 2001). The discovery that RGCs and IPCs are lineally related and that RGCs produce neurons mostly through IPCs (Englund et al. 2005) discarded this possibility. Similarly, SNPs have been shown to give rise almost exclusively to superficial-layer neurons, although they also seem to derive from RGCs (Tyler and Haydar 2013). Do RGCs represent a homogeneous type of progenitor cells or they are diverse? Extensive efforts, using a wide range of approaches including genetic fate mapping, retroviral tracing, cell transplantation and stem cell reprogramming have been used to understand the origins of PN diversity. The results so far are fairly contradictory, and led to the elaboration of two main models of corticogenesis (Marín 2012; Franco and Müller 2013; Marín and Müller 2014) (**Figure 5b,c**), known as the *progressive fate-restriction model* and the *multiple fate-restricted progenitors model*.

3.2.1. The progressive fate-restriction model of cortical neurogenesis

According to the classical model of corticogenesis, the same progenitor cell produces PN subtypes for all the six cortical layers in a particular temporal order, giving rise to deep layers neurons first and later on to cells allocated to progressively more superficial layers. This model implies that progenitor cells are multipotent (i.e. able to take on more than one fate) and go through sequential competence states, defined by the ability of a progenitor cell to generate a particular cell fate in response to spatial or temporal factors.

According to this model, progenitor cells become progressively restricted to produce superficial-layer neuronal fates and generate in sequence CthPNs, SCPNs and CPNs. As a

consequence of the inside-out pattern of migration, PN clones arrange radially forming cortical columns. Consequently, the classical model links the development of ontogenic units with the formation of cortical columns ("radial unit hypothesis"). Some experimental evidence in support of a progressive fate-restriction of neuronal progenitors derives from *in vivo* transplantations studies in ferret. The main conclusion that emerged from these studies is that at the time of the last mitotic events, neurons have already acquired the information for their laminar fate. Indeed, isochronic transplantations largely reproduce the pattern of lamination of native cells, while heterochronic transplantations suggest a progressive fate restriction of progenitor cells. Late-born progenitor cells transplanted into a younger environment give rise primarily to superficial layer fates, which indicates that they have already lost the multipotency for the generation of deep-layer PNs (McConnell 1988; McConnell and Kaznowski 1991; Frantz and McConnell 1996; Desai and McConnell 2000). In addition, *in vitro* cultures of embryonic pallial progenitors and embryonic stem cells give rise to the major subtypes of PNs in a sequential order that largely recapitulate the temporal specification observed *in vivo*, as revealed by the expression of specific markers (Shen et al. 2006; Eiraku et al. 2008; Gaspard et al. 2008). Of note, even though these culture systems generate a wide-range of cortical pyramidal cells types, their quantitative representations favours deep layers subtypes with an under-representation of superficial PNs. More direct evidence of a common multipotent progenitor cell able to generate all the major cortical PN subtypes came from pioneer retroviral tracing experiments (Luskin et al. 1988; Price and Thurlow 1988; Walsh and Cepko 1988). More recently, a fate mapping study identified Fezf2-expressing RGCs as multipotent cortical progenitors (Guo et al. 2013).

3.2.2. The multiple fate-restricted progenitors model of cortical neurogenesis

The progressive fate restriction model points out to the existence of a common precursor for all cortical PN subtypes as a basic mechanism of corticogenesis. However, the findings of the studies supporting this model would also be consistent with the existence of lineage-committed progenitors, whose abundance change over time. So, an alternative model proposes that PN subtypes arise during development from independent lineages of pre-specified progenitor cells. The observation that progenitor cells express some of the molecular markers that define adult PN populations (e.g. Cux2 and Fezf2) raised the possibility that RGCs could be heterogeneous and committed to produce specific cell types. Direct support for this hypothesis arrived only recently with the discovery of a

subtype of RGCs committed to produce supragranular PN independently of their birthdate (Franco et al. 2012). In this study, the authors found that the homeobox transcription factor Cux2 (a marker of cortico-cortical PNs mainly in layer II/III and IV) is expressed not only by IPCs in the SVZ (Nieto et al. 2004) but also by a subset of RGCs in the embryonic VZ. To trace the progeny of Cux2+ RGCs, Franco and collagues used a knock-in mouse line in which Cre is fused with a mutated ligand-binding domain for the human estrogen receptor (Cre^{ERT2}), and its expression is driven by the Cux2 promoter sequence. The administration of tamoxifen to pregnant mice allow the translocation of Cre into the nucleus and the recombination of a Cre-dependent reporter allele exclusively in Cux2+ cells in a temporally controlled manner. Using this strategy, the authors shown that Cux2+ RGCs produce primarily upper-layer cells even when they are fate-mapped at early embryonic stages. The analysis of proliferation markers *in vivo* as well as the study of cell cycle dynamics in progenitor cell cultures also suggest that Cux2+ progenitor are mainly proliferative at early stages, while Cux2- progenitors are already neurogenic. According to the finding of this study, the inside-out order in the production of PN subtypes is primarily linked to the cell cycle dynamics of different classes of RGCs instead of being directly dictated by birthdate. In this scenario, Cux2+ and Cux2- progenitor cells are intrinsically specified to produce cortico-cortical and corticofugal PNs, respectively. Both classes of progenitors coexist during development but they become neurogenic at different times, giving rise to subtypes that mainly localize in different layers as determined by their inside-out radial migration.

OBJECTIVES

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The general aim of my Thesis is to understand the role of cell lineages during the development of the cerebral cortex. In particular, we would like to understand how progenitor cells influence the specification of neuronal identities and contribute to generate cell diversity. To this aim, I have focused my research on cortical inhibitory interneurons and excitatory projection neurons and addressed the following specific aims:

1. To develop a method to fate-map progenitor cells *in vivo* with regional and subtype specificity.

2. To study the subtype composition of neuronal lineages in the neocortex.

3. To analyse the distribution and organization of neuronal lineages through the cytoarchitecture of the cerebral cortex.

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RESULTS

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Part 1.

Development of interneurons lineages in the mammalian cerebral cortex

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Chapter 1. Lineage-specific laminar organization of cortical GABAergic interneurons (Nat Neurosci. 2013 16:1199-210)

Chapter 2. Developmental segregation of cortical interneuron cell lineages

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Chapter 1. Lineage-specific laminar organization of cortical GABAergic interneurons

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Lineage-specific laminar organization of cortical GABAergic interneurons

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In the cerebral cortex, pyramidal cells and interneurons are generated in distant germinal zones, and so the mechanisms that control their precise assembly into specific microcircuits remain an enigma. Here we report that cortical interneurons labeled at the clonal level do not distribute randomly but rather have a strong tendency to cluster in the mouse neocortex. This behavior is common to different classes of interneurons, independently of their origin. Interneuron clusters are typically contained within one or two adjacent cortical layers, are largely formed by isochronically generated neurons and populate specific layers, as revealed by unbiased hierarchical clustering methods. Our results suggest that different progenitor cells give rise to interneurons populating infra- and supragranular cortical layers, which challenges current views of cortical neurogenesis. Thus, specific lineages of cortical interneurons seem to be produced to primarily mirror the laminar structure of the cerebral cortex, rather than its columnar organization.

Brain circuitries have evolved as complex networks of excitatory and inhibitory neurons. In the cerebral cortex, excitatory glutamatergic pyramidal cells and inhibitory GABAergic interneurons constitute the main cellular elements of the individual circuits. Pyramidal cells specialize in transmitting information between different cortical regions and to other regions of the brain. Interneurons comprise a heterogeneous group of neurons that shape various forms of collective activity and that primarily contribute to local assemblies, where they provide inhibitory inputs that modulate the responses of pyramidal neurons[1,2].

Pyramidal cells and interneurons are organized along two main axes in the cortex. A first axis divides the cortex into a variable number of layers, depending on cortical area. Neurons within the same cortical layer share important features, including general patterns of connectivity[3]. The second axis reflects the vertical organization of neuronal circuits within a column of cortical tissue. Neurons within a given column are stereotypically interconnected in the vertical dimension, share extrinsic connectivity and function as basic units underlying cortical operations[4,5]. Thus, any cortical area consists of a sequence of columns in which the main cellular constituents, pyramidal cells and interneurons, share a common organization[6]. Understanding the mechanisms that control the distribution of excitatory and inhibitory neurons in each of these repetitive elements is essential to comprehending the fundamental organization of the cortex.

One challenge for elucidating the mechanisms underlying the formation of neural assemblies in the cortex is that pyramidal cells and interneurons are born in separate and distant germinal zones. Thus, while pyramidal cells are produced in the ventricular zone of the pallium and migrate radially to reach their final position[7,8], interneurons are generated in the subpallium and migrate to the cortex via a long tangential migration before acquiring their definitive laminar position[9,10]. The challenge is further complicated by the large diversity of cortical interneurons, which are generated from various progenitor pools[11–15], migrate through partially different routes[16–18] and adopt their final laminar position following distinct rules[19,20].

Cortical interneurons originate from three main sources in the developing subpallium: (i) the medial ganglionic eminence (MGE), which gives rise to fast-spiking (FS), parvalbumin-expressing (PV^+) interneurons and to non-fast-spiking (NFS), somatostatin-expressing (SST^+) interneurons; (ii) the caudal ganglionic eminence (CGE), which is the source of rapidly adapting, typically bipolar interneurons that express calretinin and/or vasointestinal peptide (VIP^+ neurons) and of multipolar interneurons that contain neuropeptide Y and/or reelin but not SST; and (iii) the preoptic area (POA), which produces a small but very heterogeneous fraction of cortical interneurons[15,21]. All main classes of interneurons are found throughout the cortex, but their respective laminar allocation varies. For example, while PV^+ and SST^+ interneurons colonize more or less evenly the entire thickness of the cortex, VIP^+ interneurons are primarily found in superficial layers[19,22–24]. The laminar distribution of cortical interneurons correlates with the birthdate of MGE-derived cells but not that of CGE-derived interneurons[19,23,25,26]. Thus, it is unlikely that the same mechanisms could govern the integration of different classes of GABAergic interneurons into each of the repetitive columnar microcircuits of the cortex.

It has been suggested that interneurons may distribute throughout the cortex following random patterns of migration[27,28]. In contrast, recent work indicated that interneurons derived from the MGE or POA (MGE/POA-derived) do not distribute randomly but rather form spatially organized clusters[29]. It is unclear, however, whether this is a general behavior for all main classes of cortical interneurons and, if so, whether they adopt a predictable pattern of organization in relation to the laminar or columnar organization of the cortex.

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RESULTS

Region-specific labeling of progenitor cells in the subpallium

To study the organization of interneurons in the cortex, we explored the distribution of small cohorts of interneurons derived from a limited number of progenitor cells. Replication-incompetent retroviral vectors encoding reporter genes have been widely used for the analysis of lineage relationships during brain development[30]. These viral vectors can infect mitotic cells only, and so this approach allows the identification of the progeny of cells. One limitation of this method, however, is that it marks progenitor cells indiscriminately, which limits its potential in clonal analyses. To circumvent this limitation, we combined retroviral labeling with the Cre-*lox*P system, a genetic method that allows the irreversible labeling of cells with a specified gene expression history. In brief, we generated a conditional expression system consisting of an enhanced green fluorescent protein gene (*Gfp*) that is silent in the absence of Cre activity but that leads to the expression of the reporter protein after Cre-mediated recombination (**Supplementary Fig. 1a**). Retroviral vectors based on this system (rv::dio-Gfp) likewise infect progenitor cells indiscriminately, but only those expressing Cre recombinase and their progeny will ultimately express *Gfp*. Consistently, the brain of wild-type mice infected with retroviral particles encoding the conditional cassette did not contain GFP-expressing (GFP^+) cells (**Supplementary Fig. 1b–d**), while infection of mouse embryos with ubiquitous Cre expression throughout the ventricular zone led to widespread labeling of GFP^+ clones (**Supplementary Fig. 1e–g**).

We used this method to specifically label progenitor cells in the MGE/POA region using mouse embryos expressing Cre recombinase under the control of the promoter sequence of *Nkx2-1*, which encodes a transcription factor expressed by progenitor cells in these regions[31] (**Fig. 1a**). Delivery of a high-titer retroviral stock into the lateral ventricle of *Nkx2-1-Cre* embryos at embryonic day (E) 11.5 using ultrasound-guided imaging led to robust but highly specific labeling of MGE/POA progenitor cells throughout the rostrocaudal extent of the telencephalon (**Fig. 1b,c**). Immunohistochemical analysis confirmed that labeled cells were confined to $Nkx2\text{-}1^+$ territories, even though no limitations exist for viral infection ($n = 10$, **Fig. 1b–e**). This method therefore allows the specific labeling of progenitor cells with high fidelity.

Tagging interneuron progenitor cells at clonal density

We then carried out experiments at progressively more diluted concentrations to achieve labeling of MGE/POA progenitor cells at clonal density (**Fig. 1f**). When low-titer injections at E11.5 were examined 2 d later, we observed a limited number of clusters containing GFP^+ cells in the MGE and POA (**Fig. 1g**). These clusters were randomly scattered throughout these territories, with no particular spatial bias. They typically contained a single cell with radial glia–like morphology and a variable number of other cells with no apparent contact with the ventricle (**Fig. 1h**). In addition, we also observed some GFP^+ cells that were not close to a cell with radial glia-like morphology. Because retroviruses integrate randomly into one of the daughter cells of the infected progenitor, we reasoned that these isolated cells were likely the result of an integration event in a postmitotic neuron or an intermediate progenitor cell (IPC) (**Fig. 1a**).

The observation of isolated clusters suggested that they contained clonally related cells. To confirm this, we infected E11.5 *Nkx2-1-Cre* mouse embryos with a low-titer mixture of two conditional reporter retroviral vectors, encoding GFP or the red fluorescent protein

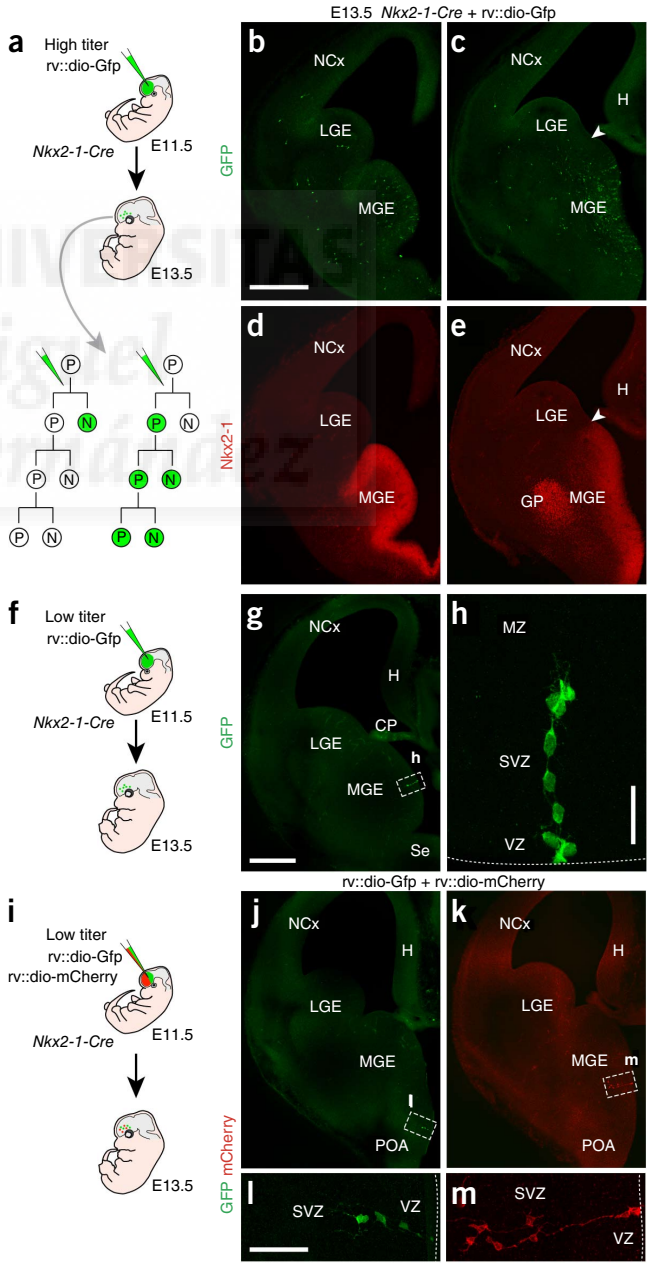

Figure 1 Region-specific labeling of progenitor cells with conditional retroviruses. (**a**) The experimental model for **b**–**e**. Retroviruses randomly integrate into one of the daughter cells, which may lead to the incomplete or complete labeling of the clone. P, progenitor cell; N, neuron. (**b**–**e**) Coronal sections through the telencephalon of an E13.5 *Nkx2-1-Cre* embryo infected with high-titer conditional reporter retroviruses at E11.5 and stained with antibodies against GFP (**b**,**c**) and Nkx2-1 (**d**,**e**). (**f**) The experimental model for **g**,**h**. (**g**,**h**) Coronal section through the telencephalon of an E13.5 *Nkx2-1-Cre* embryo infected with low-titer conditional reporter retroviruses at E11.5 and stained with antibodies against GFP. Panel **h** is a high-magnification image of an individual clone of GFP-labeled cells. (**i**) The experimental model for **j**–**m**. (**j**–**m**) Coronal sections through the telencephalon of an E13.5 *Nkx2-1-Cre* embryo infected with GFP and mCherry low-titer conditional reporter retroviruses at E11.5 and stained with antibodies against GFP and mCherry. Panels **l** and **m** are high magnification images of the boxed areas in **j** and **k**, respectively. CP, choroid plexus; GP, globus pallidus; H, hem; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; NCx, neocortex; POA, preoptic area; Se, septum; Str, striatum; VZ, ventricular zone; SVZ, subventricular zone. Dashed lines define the ventricular surface. Scale bars, 300 μm (**b**–**e**), 250 μm (**g**,**j**,**k**), 50 μm (**h**), 100 μm (**l**,**m**).

mCherry (**Fig. 1i**). Analysis of these experiments at E13.5 revealed that GFP$^+$ and mCherry$^+$ cells typically segregated into distinct clusters (**Fig. 1j–m**).

MGE/POA-derived interneurons cluster in the cortex

We used this method to examine whether distinct classes of interneurons use different strategies to achieve their final distribution in the cerebral cortex. We performed low-titer injections of conditional reporter retroviral stocks in the lateral ventricle of E11.5 *Nkx2-1-Cre* embryos and examined the distribution of labeled interneurons in the neocortex of postnatal day (P) 20 mice, a stage at which interneurons have adopted their final position in the cortex (**Fig. 2a**). These long-term experiments yielded a relatively small number of labeled cells (ranging from ~30 to ~300 cells, depending on the experiment) in the entire neocortex of infected mice. We examined more than 100 cells from three different mice and confirmed that the GFP$^+$ or mCherry$^+$ cells had morphological features of interneurons and were immunoreactive for GABA (data not shown).

We plotted the position of each population of labeled cells in the neocortex using serial section reconstructions and analyzed their distribution and spatial relationships (**Fig. 2b**). Labeled interneurons were always distributed throughout the rostrocaudal extent of the cortex, with no particular spatial bias. However, we observed that many interneurons appeared in discrete clusters of cells that were spatially segregated from one another (**Fig. 2c,d**). To determine whether labeled interneurons were randomly distributed, we calculated the distance from each interneuron to its closest neighbor (nearest neighbor distance, or NND) and constructed the resulting distribution of NNDs for each experimental population. For each data set, we then built NND distributions for 100 computer-simulated populations of randomly distributed neurons, generated using the same number of neurons in the same region of tissue as the real population. Comparison of NND distributions revealed strong differences in the spatial organization of experimental and simulated data sets (**Fig. 2e**; 93% of the comparisons between experimental and simulated data sets had a $P < 0.01$, Kolmogorov-Smirnov test, $n = 14$). Neurons in the experimental data sets were separated by shorter NNDs than neurons in the simulated, randomly distributed data sets (**Fig. 2e**). Thus, interneurons arising from a limited number of progenitor cells in the MGE/POA region do not distribute randomly throughout the neocortex.

Our empirical observations revealed that many interneurons grouped in clusters that spanned roughly 3×10^7 µm^3 of cortical volume. However, we also observed isolated interneurons throughout the cortex, which might correspond to the incomplete labeling of progenitor clones (**Fig. 1a**). To investigate the spatial distribution of interneuron clusters, we developed an unbiased and unsupervised

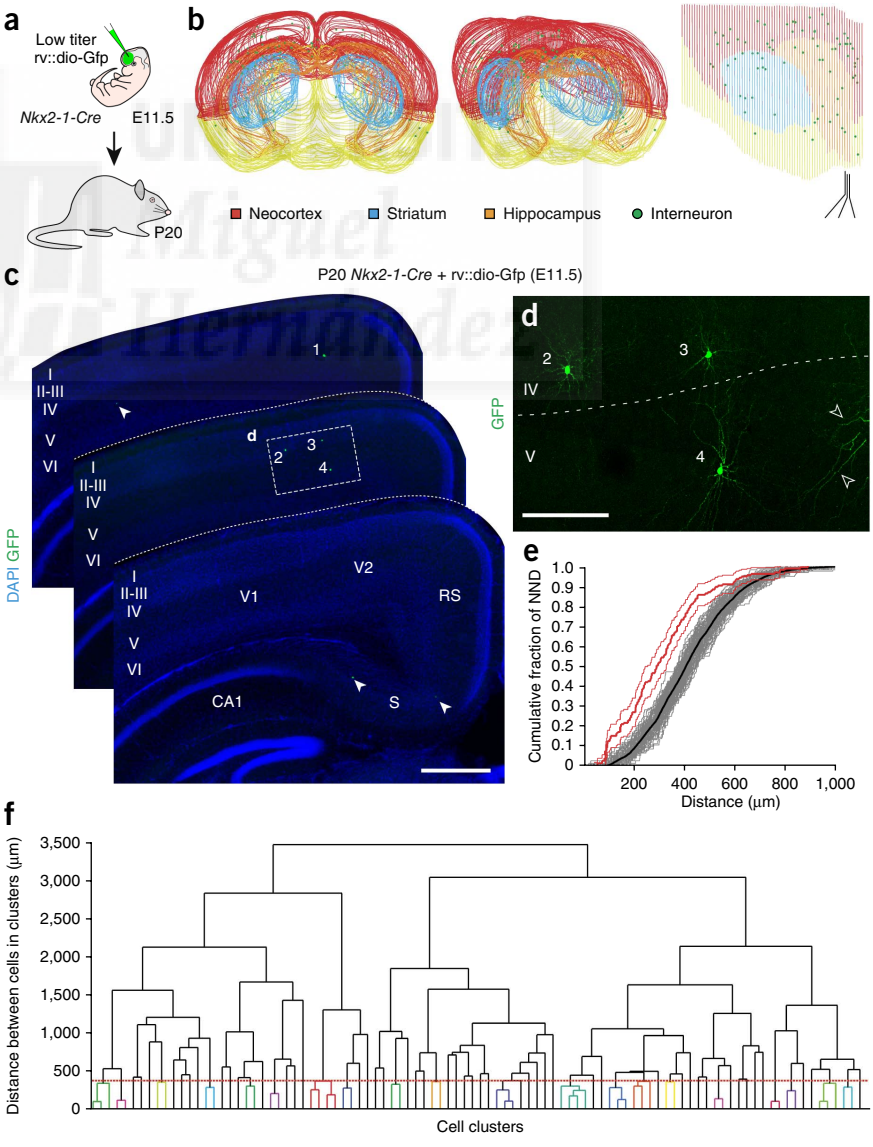

Figure 2 Clustering of MGE/POA-derived interneurons in the cerebral cortex. (**a**) The experimental model. (**b**) Three-dimensional reconstruction of the distribution of cortical interneurons in a P20 *Nkx2-1-Cre* mouse infected with low-titer conditional reporter retroviruses at E11.5. (**c**,**d**) Serial coronal sections (100 µm thick) through the telencephalon of a P20 *Nkx2-1-Cre* mouse infected with low-titer conditional reporter retroviruses at E11.5 and stained with DAPI and antibodies against GFP. Four labeled interneurons (labeled 1 to 4) form a small and compact cluster that spans two adjacent sections. Neurons 2 to 4 are shown at high magnification in **d**. Solid arrowheads in **c** point to other interneurons; open arrowheads in **d** indicate the dendrites of interneuron 1. Dashed lines define external brain boundaries in **c** and cortical layers in **d**. (**e**) Cumulative NNDs for an example population taken from one *Nkx2-1-Cre* mouse, with experimental (heavy red line) and simulated (black line) distributions. Light red lines delineate a $P = 0.05$ confidence interval for the experimental distribution; gray lines depict 100 random repetitions for the simulated distribution. Kolmogorov-Smirnov two-sample test, $P = 5.06 \times 10^{-25}$. (**f**) Dendrogram showing hierarchical relationships between labeled interneurons grouped according to their distances. The red dotted line indicates the threshold value that defines clustering for this experiment. I–VI, cortical layers I to VI; CA1, hippocampal CA1 area; RS, retrosplenial cortex; S, subiculum, V1, primary visual cortex; V2, secondary visual cortex. Scale bars, 300 µm (**c**), 100 µm (**d**).

approach to group labeled cells into individual clusters on the basis of agglomerative hierarchical clustering methods. In brief, for each experimental condition, interneurons were grouped according to proximity relationships (average distance between neurons) and the result displayed in a dendrogram (**Fig. 2f**). The number of clusters in the experiment was then calculated using a threshold distance value that maximized the difference between the number of clusters observed in the experimental data set and the mean number of clusters in 100 simulated populations of randomly distributed neurons, using the same number of neurons in the same region. One advantage of the algorithm is that it is dimensionality-unbiased; that is, no particular dimension bias is introduced in the identification of interneuron clusters, and only the distance between cells factors into their grouping. Using this method, we found that a large fraction of labeled interneurons clustered in the cortex in all experimental data sets (68.23 ± 3.56% of interneurons in clusters, mean ± s.e.m., $n = 10$). These experiments yielded ~50 clusters per data set (49.92 ± 10.29, $n = 12$). The average distance between interneurons in a cluster was remarkably consistent across different brains (mean threshold distance value 389 ± 18 µm, $n = 10$), which suggested that our analysis consistently identified cell clusters in an unsupervised manner.

Interneuron clustering might be linked to lineage relationships or arise as a non–cell-autonomous property of cortical interneurons. Although our short-term analyses suggested that low-titer retroviral infections typically labeled individual clones (**Fig. 1j–m**), ultracentrifugation of retroviral stocks may produce viral clumps resulting in focal infections that resemble clones, even at limiting dilutions. To directly test this, we analyzed the distribution of labeled interneurons in the cortex of P20 *Nkx2.1-Cre* mice that were simultaneously infected with low-titer stocks of conditional retroviruses encoding green (GFP) and red (mCherry) reporters, mixed before ultracentrifugation[32]. As before, we used agglomerative hierarchical clustering to identify interneuron groups labeled with each of the reporter proteins independently. We next used the smaller threshold distance value obtained for these populations to identify interneuron clusters independently of the reporter protein, as if GFP$^+$ and mCherry$^+$ interneurons were derived from the same progenitor cells. Under these conditions, we quantified the fraction of interneuron clusters that contained GFP$^+$ and mCherry$^+$ interneurons. We found that approximately 60% of clusters contained at least one interneuron labeled with a different fluorescent protein than the dominant one for that particular cluster (66.83 ± 1.40% mixed clusters, $n = 6$; **Supplementary Fig. 2**). Thus, although clusters contained clonally related interneurons, most clusters were likely to include cells from a different progenitor, even at very limiting dilutions. This strongly suggested that lineage relationships are not exclusive determinants of interneuron clustering.

Spatial organization of MGE/POA-derived interneuron clusters

We used the dendrograms derived from the agglomerative hierarchical clustering analysis to examine the spatial organization of individual interneurons within clusters. We found that most cell clusters contained interneurons located within a single layer of the cortex or in two adjacent layers (intralaminar, 46.85 ± 4.06%; adjacent interlaminar, 39.45 ± 2.14%; $n = 10$; **Fig. 3a–d**). In contrast, the percentage of clusters with cells that spanned two nonadjacent layers or three or more layers was very small (nonadjacent, 8.22 ± 2.20%; three or more, 6.60 ± 2.33%; $n = 10$; **Fig. 3a–d**).

We next analyzed the laminar distribution of interneuron clusters. We found that intralaminar clusters labeled with retroviral infections at E11.5 did not distribute evenly throughout the cortex but rather concentrated in deep layers (clusters in layer V or VI, 91.18 ± 2.97%; $n = 10$; **Fig. 3e**). Even those clusters that spanned two adjacent layers resided

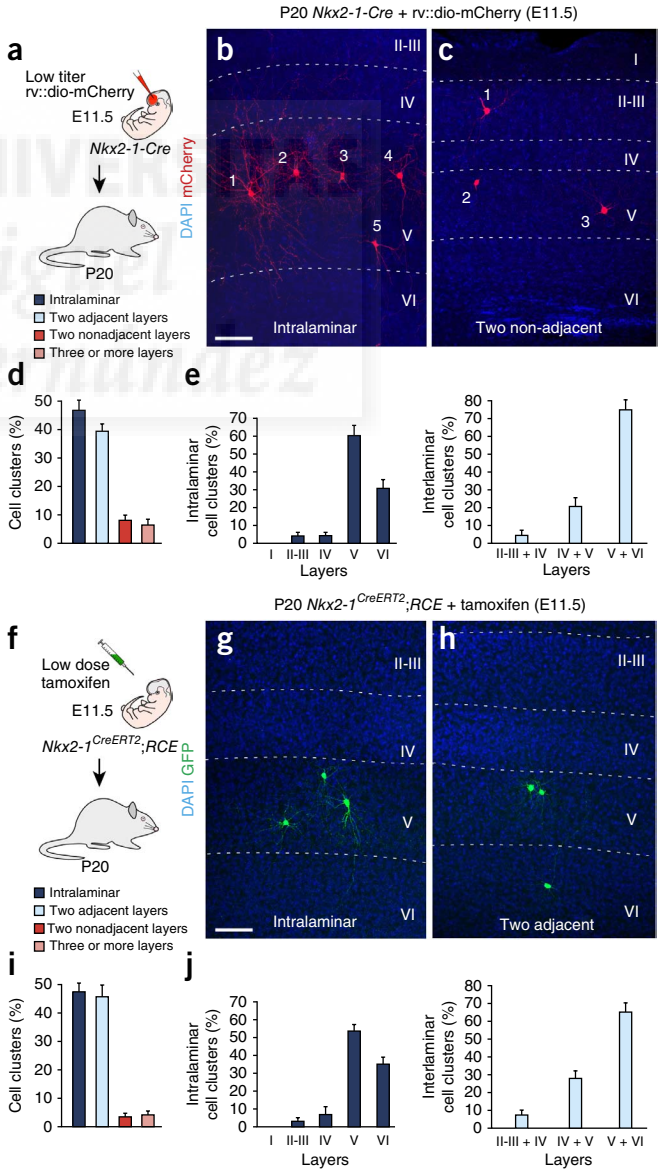

Figure 3 Spatial organization of MGE/POA-derived interneuron clusters. (**a**) The experimental model for **b**–**e**. (**b**,**c**) Coronal sections through the motor (**a**) and visual (**b**) cortices of a P20 *Nkx2-1-Cre* mouse infected with low-titer conditional reporter retroviruses at E11.5 and stained with the nuclear indicator DAPI and antibodies against mCherry. The images show examples of intralaminar (**b**) and interlaminar (**c**) interneuron clusters (cells labeled 1 to 5 and 1 to 3, respectively). (**d**) Quantification of the relative abundance of intralaminar and interlaminar (two adjacent, two nonadjacent, and three or more layers) interneuron clusters. (**e**) Quantification of the laminar distribution of intralaminar and interlaminar (two adjacent layers only) interneuron clusters. (**f**) The experimental model for **g**–**i**. (**g**,**h**) Coronal sections through the neocortex of a P20 *Nkx2-1*^{CreERT2};*RCE* mouse induced with a low tamoxifen dose at E11.5 and stained with DAPI and antibodies against GFP. The images show examples of intralaminar (**g**) and interlaminar (**h**) interneuron clusters. (**i**) Quantification of the relative abundance of intralaminar and interlaminar (two adjacent, two nonadjacent, and three or more layers) interneuron clusters. (**j**) Quantification of the laminar distribution of intralaminar and interlaminar (two adjacent layers only) interneuron clusters. I–VI, cortical layers I to VI. Dashed lines define cortical layers. Scale bars, 100 µm. Histograms depict mean ± s.e.m.

preferentially in the deep layers of the cortex (layer V or VI clusters, 74.97 ± 5.21%; $n = 10$; **Fig. 3e**). These results suggested that progenitor cells infected at E11.5 do not produce many interneurons that populate the superficial layers of the cerebral cortex. Instead, most of the lineages labeled in the MGE/POA at E11.5 seem to give rise almost exclusively to interneurons that occupy the infragranular layers of the cortex.

It is at least theoretically conceivable that we failed to observe interneurons in the superficial layers of the neocortex owing to viral silencing. To rule out this possibility, we used a different method to label interneuron lineages at clonal density. In brief, we crossed mice expressing a tamoxifen-inducible form of Cre (CreERT2) under the control of the *Nkx2-1* promoter region (*Nkx2-1*^{CreERT2} mice) with a general reporter allele (*RCE*, Rosa26 reporter CAG-boosted EGFP) and titrated the amount of tamoxifen required to elicit Cre recombination in MGE/POA progenitors at clonal density (**Supplementary Fig. 3a–d**). Subsequently, we induced Cre expression in E11.5 embryos and analyzed the distribution of labeled interneurons at P20 (**Fig. 3f**).

Analysis of the neocortex of P20 *Nkx2-1*^{CreERT2}*;RCE* mice exposed to low tamoxifen doses at E11.5 again revealed interneuron clustering in the neocortex (**Supplementary Fig. 3e,f**). NND comparisons between experimental and simulated data sets confirmed that the distribution of clonally related MGE/POA-derived interneurons was not random (100% of the comparisons between experimental and simulated data sets had a $P < 0.01$, Kolmogorov-Smirnov, $n = 8$; **Supplementary Fig. 3g**). Agglomerative hierarchical clustering methods revealed that the organization of individual clusters in *Nkx2-1*^{CreERT2}*;RCE* mice induced with low tamoxifen doses was very similar than in *Nkx2-1-Cre* mice infected with retroviruses (78.06 ± 3.16% of interneurons in clusters; 37.38 ± 7.54 clusters per data set, $n = 8$; mean threshold value 485 ± 39 µm; $n = 8$; **Supplementary Fig. 3h**). Most notably, interneuron clusters in *Nkx2-1*^{CreERT2}*;RCE* mice were largely restricted to one or two adjacent layers of the cortex (intralaminar, 47.49 ± 4.33%; adjacent interlaminar, 45.28 ± 4.37; $n = 8$; **Fig. 3g–i**) and were typically confined to deep layers of the neocortex (clusters in layer V or VI, 89.39 ± 3.98; $n = 8$; **Fig. 3j**). These experiments confirmed that the MGE/POA at E11.5 primarily contains progenitor cells that generate interneurons for the infragranular layers of the cortex.

Most interneuron clusters contain coetaneous neurons

There is a strong correlation between time of birth and final laminar distribution in MGE-derived interneurons[25,26,33,34]. To test this idea at clonal levels, we analyzed the spatial organization of the clusters in relation to the timing of neurogenesis of their constituent interneurons. Specifically, we carried out birth-dating analyses at two different embryonic stages (E12.5 and E15.5) in retroviral lineage tracing experiments ($n = 43$ clusters from three different experiments; **Supplementary Fig. 4a**). In these experiments, we defined isochronic clusters as those containing a majority of cells born in the same stage and no cells born in the alternative age, and heterochronic clusters as those containing at least one cell born at E12.5 and another one at E15.5. We found that all intralaminar clusters were isochronic ($n = 28$ of 28 clusters; **Supplementary Fig. 4b,d**), whereas all clusters that spanned several layers were heterochronic ($n = 4$ of 4 clusters; **Supplementary Fig. 4c,d**). The organization of clusters spanning two adjacent layers was more diverse, with roughly half of the clones classified as isochronic ($n = 6$ of 11 clusters) and the other half heterochronic ($n = 5$ of 11 clusters; **Supplementary Fig. 4d**).

The finding of multiple neurons with highly correlated birthdates in most intralaminar clusters suggested that these clones might be generated through the expansion of cell lineages by way of intermediate progenitor cells (IPCs), which would become neurogenic at roughly the same time. To test this hypothesis, we first examined the expression of

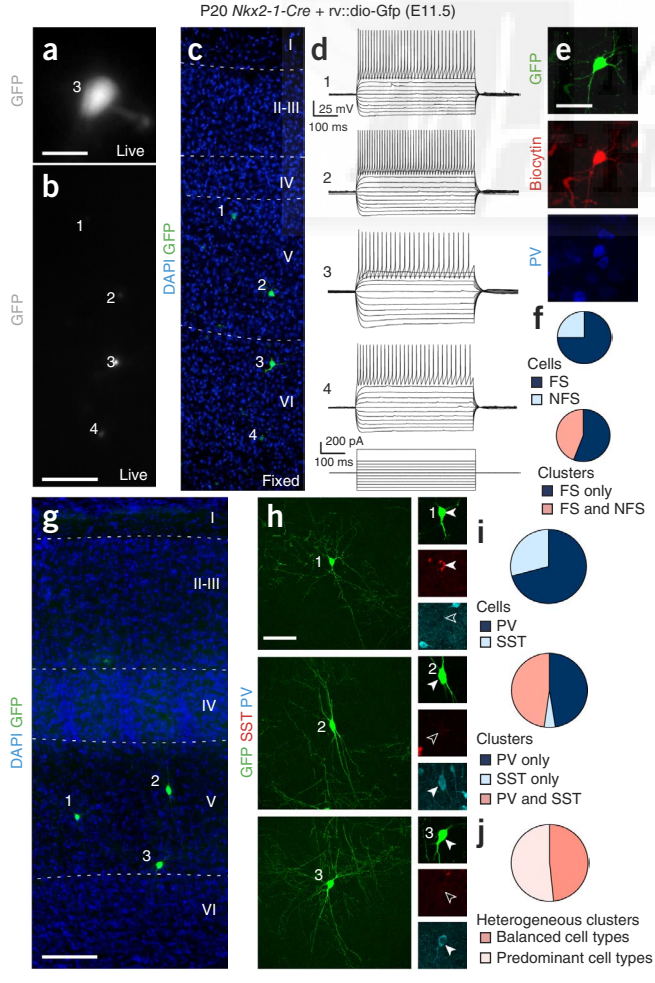

Figure 4 Electrophysiological and neurochemical characterization of MGE/POA-derived interneuron clusters. (**a**,**b**) Live imaging of a coronal section through the somatosensory cortex of a P20 *Nkx2-1-Cre* mouse infected with low-titer conditional reporter retroviruses at E11.5 showing a cluster of four GFP$^+$ interneurons (labeled 1 to 4). The high-magnification image (**a**) was acquired during patch-clamp recording. (**c**) The same coronal section shown in **b** stained with DAPI and antibodies against GFP. (**d**) Current-clamp recordings in whole-cell configuration reflecting the response of interneurons (labeled 1 to 4) in the cluster to current steps. Cells were recorded using a pipette containing biocytin. (**e**) Expression of GFP and PV and biocytin fluorescence in one of the interneurons in the cluster shown in **b** and **c**. (**f**) Quantification of the percentage of FS and NFS cells in interneuron clusters, as well as the fraction of clusters that contained only FS cells or both FS and NFS cells. (**g**,**h**) Coronal section through the somatosensory cortex of a P20 *Nkx2-1-Cre* mouse infected with low-titer conditional reporter retroviruses at E11.5 and stained with DAPI and antibodies against GFP, showing an intralaminar cluster (cells labeled 1 to 3). High-magnification images (**h**) show the morphology of these interneurons, two of which (cells 2 and 3) express PV and the other of which (cell 1) expresses SST. (**i**) Quantification of the percentage of PV$^+$ and SST$^+$ cells in interneuron clusters, as well as the fraction of clusters that contained only PV$^+$ cells, only SST$^+$ cells or both PV$^+$ and SST$^+$ cells. (**j**) Quantification of the fraction of heterogeneous clusters that contained a predominant cell type or similar proportions of PV$^+$ and SST$^+$ cells. I–VI, cortical layers I to VI. Dashed lines define cortical layers. Scale bars, 20 µm (**a**,**e**), 50 µm (**h**), 100 µm (**b**,**c**,**g**; **b** and **c** have the same magnification).

the proliferation marker Ki67 in individual clones 40 h after retroviral infection (**Supplementary Fig. 5a**). We found that many GFP$^+$ cells in each individual clone expressed Ki67 at this stage (**Supplementary Fig. 5b–e**). This observation suggested that most interneuron clusters are formed through the expansion of individual lineages by way of IPCs. To confirm this idea, we infected E11.5 mouse embryos with retroviruses simultaneously encoding mCherry and a fluorescent, ubiquitylation-based cell cycle indicator (Fucci) that accumulates in the nucleus during S, G2 and M phases (**Supplementary Fig. 5f**). Consistent with the Ki67 results, we observed that interneuron clones contained a high percentage of IPCs 40 h after retroviral infection (**Supplementary Fig. 5g–l**). Taken together, these results suggest that most interneuron clones expand through IPCs that divide concurrently, which might explain the abundance of coetaneous interneurons in the clusters (**Supplementary Fig. 4**).

Characterization of MGE/POA-derived interneuron clusters

We wondered whether cortical clusters contain interneurons with similar electrophysiological and neurochemical properties. To address this question, we identified interneuron clusters in acute 300-µm serial sections through the cerebral cortex of P20–P30 *Nkx2-1-Cre* mice that were infected at E11.5, and recorded the intrinsic electrophysiological properties of interneurons in each of these clusters (**Fig. 4a–c**). Consistent with previous results[11], recorded cells exhibited one of the two main profiles found in MGE/POA-derived interneurons, FS and NFS firing in response to a suprathreshold current injection. We found that a large proportion of clusters exclusively contained FS interneurons (n = 5 of 9 clusters; **Fig. 4d–f** and **Supplementary Fig. 6**). As expected, correlative immunohistochemical analysis revealed that these interneurons contained PV (**Fig. 4e,f**). We also found that many of the recorded clusters contained both FS and NFS interneurons (n = 4 out of 9 clusters; **Fig. 4f** and **Supplementary Fig. 6**). NFS interneurons typically contained the neuropeptide SST and were electrophysiologically heterogeneous (**Supplementary Fig. 6**).

We next analyzed the expression of PV and SST in a large cohort of clusters identified through agglomerative hierarchical clustering analysis (n = 143 clusters from two different experiments). Irrespective of their organization in clusters, we found that interneurons expressing PV outnumbered those expressing SST by a 3:1 proportion (70.99 ± 14.04% and 29.01 ± 7.52%, respectively; **Fig. 4g–i**), a finding that matches the normal proportion of cortical PV$^+$ and SST$^+$ interneurons[35]. Analysis of the neurochemical profile of interneurons within individual clusters revealed that approximately half of the clusters exclusively contained PV$^+$ interneurons (n = 68 out of 143 clusters, **Fig. 4i**), while only a very small fraction were uniformly made of SST$^+$ interneurons (n = 7 out of 143 clusters, **Fig. 4i**). The remaining clusters contained both PV$^+$ and SST$^+$ interneurons (n = 68 out of 143 clusters; **Fig. 4g–i**). Approximately half of the heterogeneous clusters contained a predominant interneuron class (n = 32 out of 68

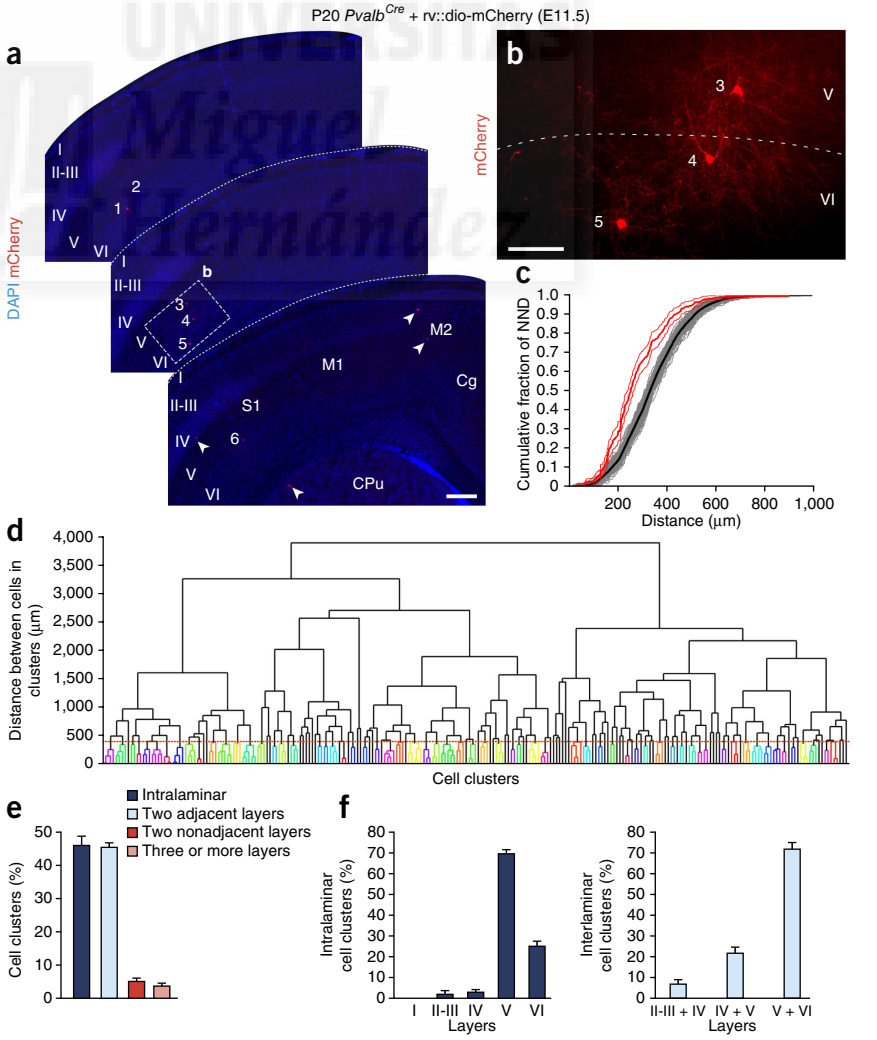

Figure 5 Clustering of PV$^+$ interneurons in the cerebral cortex. (**a**,**b**) Serial coronal sections (100 µm thick) through the telencephalon of a P20 *Pvalb^{Cre}* mouse infected with low-titer conditional reporter retroviruses at E11.5 and stained with DAPI and antibodies against mCherry. Six labeled interneurons (1 to 6) form a large cluster that spans three adjacent sections. Neurons 3 to 5 are shown at high magnification in **b**. Solid arrowheads in **a** point to other labeled interneurons. Dashed lines define external brain boundaries in **a** and cortical layers in **b**. (**c**) Cumulative NNDs for an example population taken from one *Pvalb^{Cre}* mouse, with experimental (heavy red line) and simulated (black line) distributions. Light red lines delineate a $P = 0.05$ confidence interval for the experimental distribution; gray lines depict 100 random repetitions for the simulated distribution. Kolmogorov-Smirnov two-sample test, $P = 6.14 \times 10^{-21}$. (**d**) Dendrogram showing the hierarchical relationship between labeled PV$^+$ interneurons grouped according to their distances using agglomerative hierarchical clustering. The red dotted line indicates the threshold value that defines clustering for this experiment. (**e**) Quantification of the relative abundance of intralaminar and interlaminar (two adjacent, two nonadjacent, and three or more layers) interneuron clusters. (**f**) Quantification of the laminar distribution of intralaminar and interlaminar (two adjacent layers only) interneuron clusters. I–VI, cortical layers I to VI; Cg, cingulate cortex; CPu, caudoputamen nucleus; M1, primary motor cortex; M2, secondary motor cortex; S1, primary somatosensory cortex. Scale bars, 300 µm (**a**), 100 µm (**b**). Histograms depict mean ± s.e.m.

clusters; **Fig. 4j**), while the rest contained similar proportions of both interneuron classes ($n = 36$ out of 68 clusters; **Fig. 4j**).

Reverse lineage tracing of MGE/POA interneurons

One advantage of the method that we developed for lineage analysis is that the combination of retroviral tracing and Cre-*lox*P technology can be extended using Cre lines that are unique to certain classes of interneurons, such as *Pvalb*^{Cre} and *Sst*^{Cre}. In these experiments, progenitor cells are indistinctly infected throughout the telencephalon, but only those that give rise to neurons expressing PV (*Pvalb*^{Cre} infections) or SST (*Sst*^{Cre} infections) will ultimately express the corresponding reporter fluorescent protein (GFP or mCherry). Because Cre expression is postmitotic in both *Pvalb*^{Cre} and *Sst*^{Cre} mice, clones cannot be monitored at embryonic stages, but their ability to cluster in the cortex can be assessed in P20 mice.

We first examined whether PV$^+$ interneurons cluster in the cerebral cortex, as predicted by our experiments in *Nkx2-1-Cre* mice. Analysis of the neocortex of P20 *Pvalb*^{Cre} mice infected with conditional retroviruses at E11.5 revealed that many labeled interneurons were indeed grouped in small clusters throughout the neocortex (**Fig. 5a**,**b**). Consistently, NND comparisons between experimental and simulated data sets confirmed that the distribution of PV$^+$ interneurons was not random (**Fig. 5c**; 93.3% of the comparisons between experimental and simulated data sets had a $P < 0.01$, Kolmogorov-Smirnov, $n = 15$). Identification of individual clusters in each brain using agglomerative hierarchical clustering methods revealed that clusters of PV$^+$ interneurons had an organization very similar to that found in *Nkx2-1-Cre* mice (72.80 ± 2.04% of interneurons in clusters, $n = 10$; 70.50 ± 3.70 clusters per data set, $n = 10$). For example, the average distance between PV$^+$ interneurons in a cluster was also around 400 µm (**Fig. 5d**; mean threshold value 393 ± 11 µm, $n = 14$). In addition, most cell clusters contained interneurons confined to one or, at most, two adjacent layers of the cortex (intralaminar, 45.90 ± 2.71%; adjacent interlaminar, 45.07 ± 1.84%; **Fig. 5e**) and were typically located in deep layers of the neocortex (clusters in layer V or VI, 95.36 ± 1.05%; $n = 10$; **Fig. 5f**).

Analysis of the neocortex of P20 *Sst*^{Cre} mice infected with conditional retroviruses at E11.5 yielded very similar results. We found that many labeled SST$^+$ interneurons clustered throughout the neocortex (**Fig. 6a–c**). NND analyses confirmed that the distribution of clonally related SST$^+$ interneurons was not random (**Fig. 6d**; 87.5% of the comparisons between experimental and simulated data sets had a $P < 0.01$, Kolmogorov-Smirnov, $n = 16$). Clustering analysis revealed a large fraction of clustered interneurons (69.33 ± 2.81% of interneurons in clusters, $n = 7$; 70.29 ± 12.44 clusters per data set,

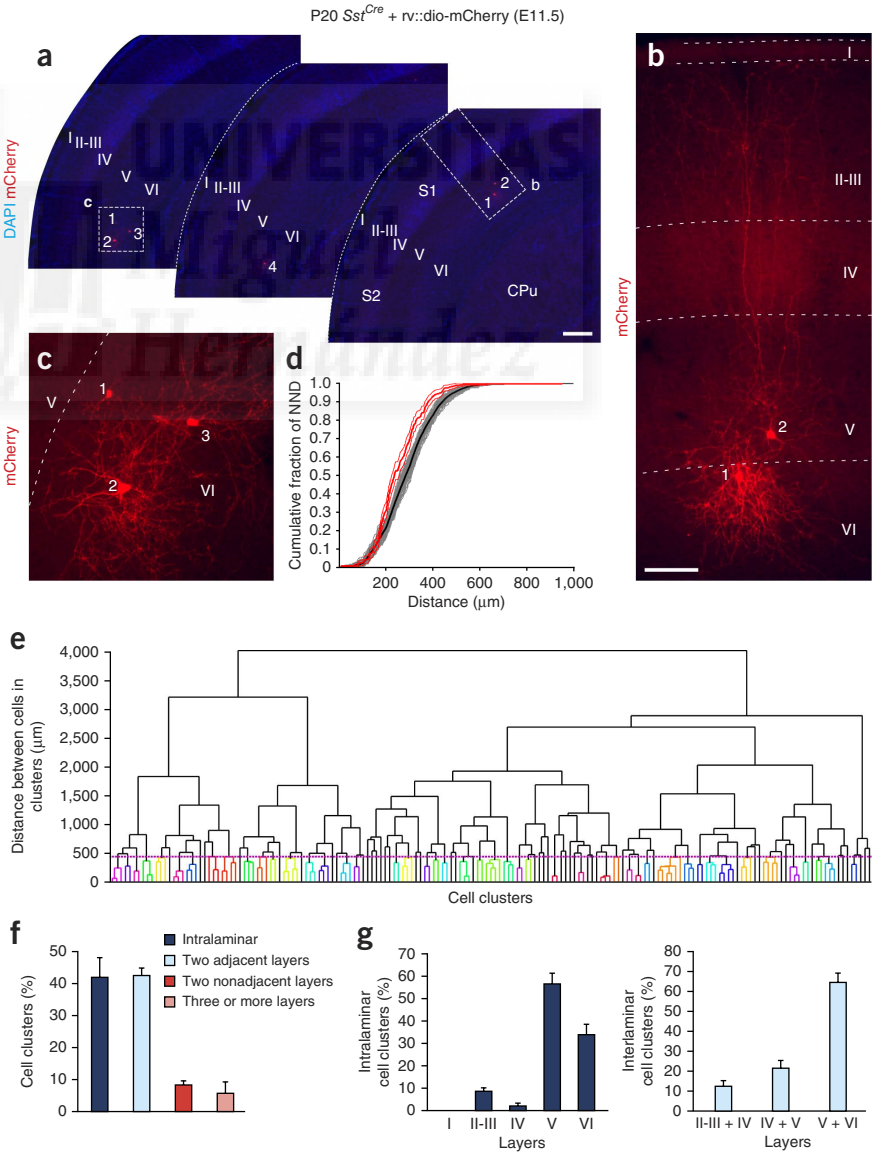

Figure 6 Clustering of SST$^+$ interneurons in the cerebral cortex. (**a**–**c**) Serial coronal sections (100 µm thick) through the telencephalon of a P20 *Sst*^{Cre} mouse infected with low-titer conditional reporter retroviruses at E11.5 and stained with DAPI and antibodies against mCherry. Two clusters are shown (**b** and **c**), containing two (labeled 1 and 2; **b**) and four (labeled 1 to 4; **c**) interneurons, respectively. Dashed lines define external brain boundaries in **a** and cortical layers in **b**,**c**. (**d**) Cumulative NNDs for an example population taken from one *Sst*^{Cre} mouse, with experimental (heavy red line) and simulated (black line) distributions. Light red lines delineate a $P = 0.05$ confidence interval for the experimental distribution; gray lines depict 100 random repetitions for the simulated distribution. Kolmogorov-Smirnov two-sample test, $P = 3.04 \times 10^{-10}$. (**e**) Dendrogram showing the hierarchical relationship between labeled SST$^+$ interneurons grouped according to their distances using agglomerative hierarchical clustering. The purple dotted line indicates the threshold value that defines clustering for this experiment. (**f**) Quantification of the relative abundance of intralaminar and interlaminar (two adjacent, two nonadjacent, and three or more layers) interneuron clusters. (**g**) Quantification of the laminar distribution of intralaminar and interlaminar (two adjacent layers only) interneuron clusters. I–VI, cortical layers I to VI; CPu, caudoputamen nucleus; S1, primary somatosensory cortex; S2, secondary somatosensory cortex. Scale bars, 300 µm (**a**), 100 µm (**b**,**c**; same magnification). Histograms depict mean ± s.e.m.

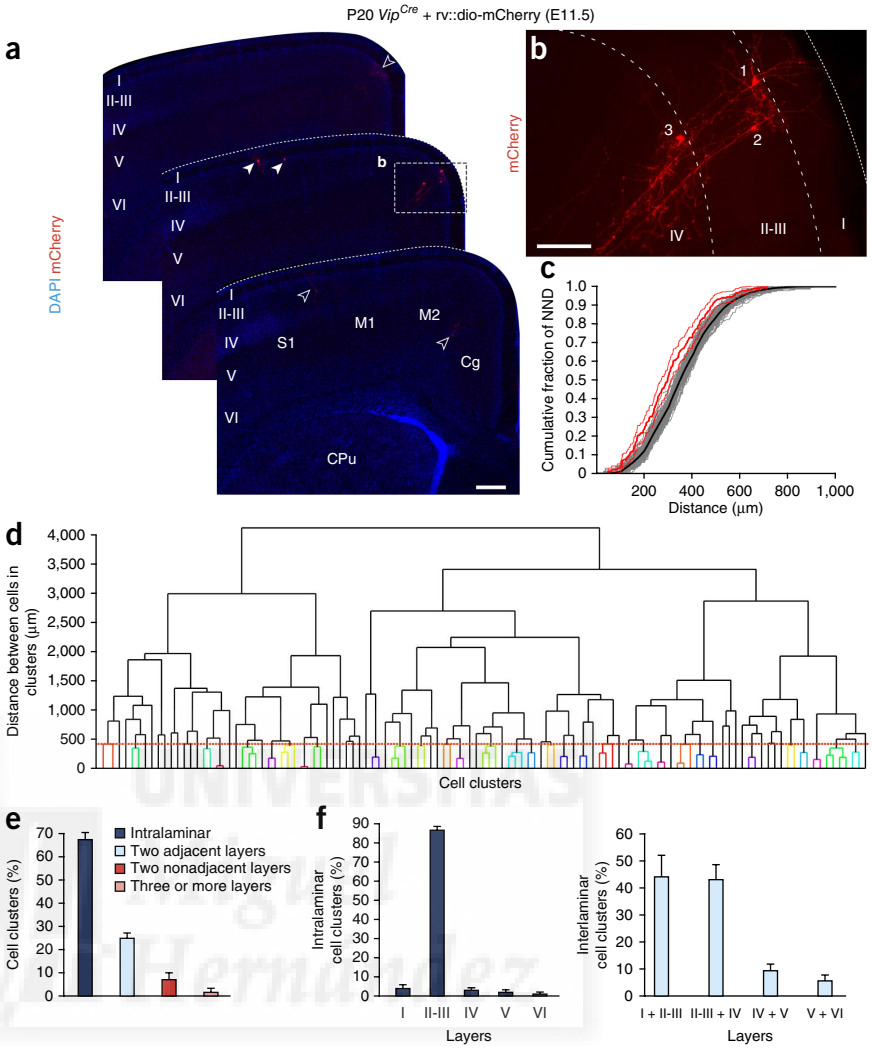

Figure 7 Clustering of VIP+ interneurons in the cerebral cortex. (**a**,**b**) Serial coronal sections (100 μm thick) through the telencephalon of a P20 *Vip*^{Cre} mouse infected with low-titer conditional reporter retroviruses at E11.5 and stained with DAPI and antibodies against mCherry. Three labeled interneurons (labeled 1 to 3) form a cluster confined to a single section. These neurons are shown at high magnification in **b**. Solid arrowheads in **a** point to other labeled interneurons; open arrowheads indicate the dendrites of interneurons labeled in other sections. Dashed lines define external brain boundaries in **a** and cortical layers in **b**. (**c**) Cumulative NNDs for an example population taken from one *Vip*^{Cre} mouse, with experimental (heavy red line) and simulated (black line) distributions. Light red lines delineate a $P = 0.05$ confidence interval for the experimental distribution; gray lines depict 100 random repetitions for the simulated distribution. Kolmogorov-Smirnov two-sample test, $P = 3.22 \times 10^{-8}$. (**d**) Dendrogram showing the hierarchical relationship between labeled PV+ interneurons grouped according to their distances using agglomerative hierarchical clustering. The red dotted line indicates the threshold value that defines clustering for this experiment. (**e**) Quantification of the relative abundance of intralaminar and interlaminar (two adjacent, two nonadjacent, and three or more layers) interneuron clusters. (**f**) Quantification of the laminar distribution of intralaminar and interlaminar (two adjacent layers only) interneuron clusters. I–VI, cortical layers I to VI; Cg, cingulate cortex; CPu, caudoputamen nucleus; M1, primary motor cortex; M2, secondary motor cortex; S1, primary somatosensory cortex. Scale bars, 300 μm (**a**), 100 μm (**b**). Histograms depict mean ± s.e.m.

$n = 7$), with an average distance between SST+ interneurons in a cluster in the same range as that of PV+ interneurons (**Fig. 6e**; mean threshold value 395 ± 23 μm, $n = 13$). As in the case of PV+ interneuron clusters, most SST+ interneuron clusters were confined to one or, at most, two adjacent layers of the cortex (intralaminar, 42.59 ± 5.77%; adjacent interlaminar, 43.26 ± 2.24%, $n = 7$; **Fig. 6f**) and were preferentially distributed through the deep layers of the neocortex (clusters in layer V or VI, 90.77 ± 1.73%; $n = 7$; **Fig. 6g**). Altogether, these experiments demonstrate that lineages of the two main classes of MGE/POA-derived cortical neurons, PV+ and SST+ interneurons, cluster in the cerebral cortex.

CGE-derived interneurons also cluster in the cortex

We wondered whether this phenomenon is exclusive to MGE/POA-derived cortical interneurons. To explore this idea, we carried out a new set of experiments in *Vip*^{Cre} mouse embryos at E11.5. VIP+ interneurons derive from the CGE[36], so the lineage analysis of these interneurons should address that question.

Analysis of the neocortex of P20 *Vip*^{Cre} mice infected with conditional retroviruses at E11.5 revealed that labeled VIP+ interneurons also had a tendency to cluster throughout the neocortex (66.09 ± 2.65% of interneurons in clusters, $n = 9$; **Fig. 7a**,**b**). Accordingly, NND comparisons between experimental and simulated data sets confirmed that the distribution of VIP+ interneurons was not random (**Fig. 7c**; 69% of the comparisons between experimental and simulated data sets had a $P < 0.01$, Kolmogorov-Smirnov, $n = 13$). The average number of clusters was relatively consistent with the other experiments (46.09 ± 10.25 clusters per data set, $n = 11$). Clustering analysis indicated that the average distance between VIP+ interneurons in a cluster was approximately 400 μm (**Fig. 7d**; mean threshold value 374 ± 25 μm, $n = 9$), in the same range as that of MGE/POA-derived interneurons. Furthermore, VIP+ interneurons in most clusters were confined to one or, less frequently, two adjacent layers of the cortex (intralaminar, 66.68 ± 2.84%; adjacent interlaminar, 24.35 ± 1.78%; $n = 11$; **Fig. 7e**). However, in contrast to MGE/POA-derived clusters, VIP+ interneurons accumulated preferentially in superficial layers of the neocortex (clusters in layer II-III, 88.92 ± 1.99%; $n = 11$; **Fig. 7f**). These results revealed that interneuron clustering is common to distinct classes of cortical interneurons, independently of their developmental origin.

Late-born MGE/POA-derived interneurons cluster in upper layers

Finally, we tested whether interneuron clustering was a specific property of early lineages in the subpallium. To this end, we performed low-titer injections of conditional reporter retroviral stocks into the lateral ventricle of E14.5 *Nkx2-1-Cre* embryos and examined the distribution of labeled interneurons in the neocortex of P20 mice. We found that most labeled interneurons clustered throughout the

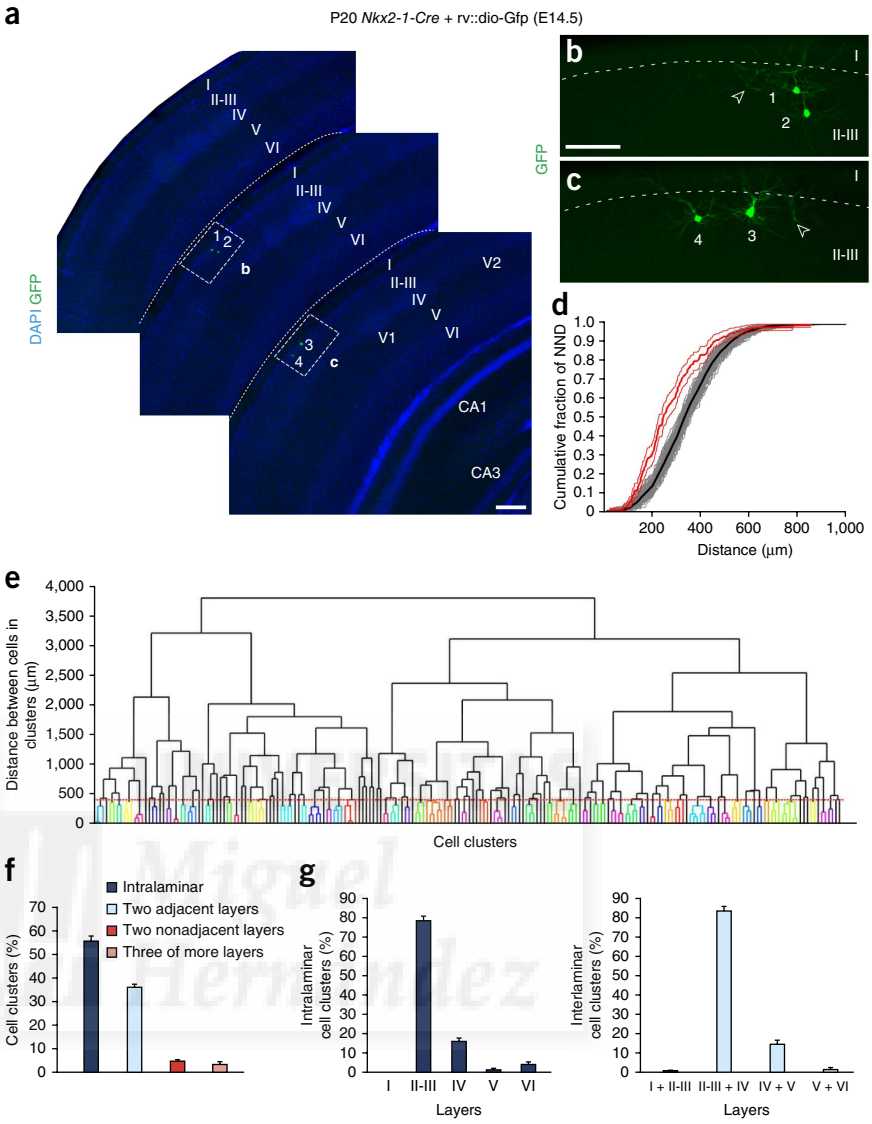

Figure 8 Late born MGE/POA-derived interneurons cluster in superficial cortical layers. (**a**–**c**) Serial coronal sections (100 μm thick) through the telencephalon of a P20 *Nkx2-1-Cre* mouse infected with low-titer conditional reporter retroviruses at E14.5 and stained with DAPI and antibodies against GFP. Four labeled interneurons (labeled 1 to 4) form a compact cluster that spans two adjacent sections. These neurons are shown at high magnification in **b**,**c**. Dashed lines define external brain boundaries in **a** and cortical layer in **b**. (**d**) Cumulative NNDs for an example population taken from one *Nkx2-1-Cre* mouse, with experimental (heavy red line) and simulated (black line) distributions. Light red lines delineate a $P = 0.05$ confidence interval for the experimental distribution; gray lines depict 100 random repetitions for the simulated distribution. Kolmogorov-Smirnov two-sample test, $P = 1.52 \times 10^{-16}$. (**e**) Dendrogram showing the hierarchical relationship between labeled interneurons grouped according to their distances using agglomerative hierarchical clustering. The red dotted line indicates the threshold value that defines clustering for this experiment. (**f**) Quantification of the relative abundance of intralaminar and interlaminar (two adjacent, two nonadjacent, and three or more layers) interneuron clusters. (**g**) Quantification of the laminar distribution of intralaminar and interlaminar (two adjacent layers only) interneuron clusters. I–VI, cortical layers I to VI; CA1, hippocampus CA1 area; CA3, hippocampus CA3 area; V1, primary visual cortex; V2, secondary visual cortex. Scale bars, 300 μm (**a**), 100 μm (**b**,**c**). Histograms depict mean ± s.e.m.

neocortex (**Fig. 8a**–**c**). NND analyses confirmed that the distribution of these interneurons was not random (**Fig. 8d**; 100% of the comparisons between experimental and simulated data sets had a $P < 0.01$, Kolmogorov-Smirnov, $n = 11$). Clustering analysis revealed a large fraction of clustered interneurons (76.49 ± 1.36% of interneurons in clusters, $n = 11$; 86.82 ± 6.62 clusters per data set, $n = 11$), with an average distance between late-born interneurons in a cluster within the same range as that of early-born interneurons (**Fig. 8e**; mean threshold value 386.36 ± 15 μm, $n = 11$). Late-born interneuron clusters were also primarily confined to one or, at most, two adjacent layers of the cortex (intralaminar, 55.77 ± 2.02%; adjacent interlaminar, 35.96 ± 1.81%, $n = 11$; **Fig. 8f**). However, in contrast to those in E11.5 infections, interneuron clusters were preferentially distributed through the superficial layers of the neocortex (clusters in layer II-III or IV: 94.58 ± 1.79%; $n = 11$; **Fig. 8g**). These experiments reinforced the view that at least two separated lineages of progenitor cells exist in MGE/POA, devoted respectively to the generation of interneurons for deep and superficial layers of the cortex.

DISCUSSION

Our results demonstrate that GABAergic interneurons do not distribute randomly throughout the cortex. Instead, interneurons have a tendency to form clusters that occupy a relatively small volume of cortical tissue, a behavior that seems universal across all three major classes of interneurons (PV$^+$, SST$^+$ and VIP$^+$). Although the mechanisms underlying the clustering of interneurons in the cortex remain unclear, our experiments suggest that this process is not univocally linked to their shared clonal origin. Our analysis further indicate that different progenitor cells in the subpallium are committed to generate lineages of interneurons with restricted laminar distributions, which provides an unexpected view of cortical neurogenesis (**Supplementary Fig. 7**).

Methodological considerations

One caveat concerning retroviral tracing analyses is the lack of regional or cellular specificity. Here we have developed an approach to achieve clonal labeling with regional and cellular specificity that exclusively relies on Cre-expressing strains. To this end, we generated conditional retroviruses in which expression of a reporter protein requires Cre-mediated recombination. Our experiments demonstrate that this method can be used to perform lineage analyses for identified populations of progenitor cells or even entire cohorts of postmitotic neurons.

The retrovirus used in this study randomly integrates into one daughter cell after infection, which means that in roughly 50% of the cases infected clones will be incompletely labeled. We would predict

that this 'noise' labeling should interfere with the identification of interneuron clusters. Consistent with this idea, we observed in each experiment that a relatively large fraction (~30%) of interneurons did not seem to associate with any particular cluster. Despite this experimental limitation, our statistical analyses revealed highly significant differences with random distributions for all classes of cortical interneurons, which suggests that interneuron clustering is a very robust process.

Clustering is a property of different types of interneuron

Our experiments suggest that interneuron lineages have a strong tendency to reside roughly in the same location within the cerebral cortex. This finding independently replicates recent work on the organization of MGE/POA-derived interneurons in the neocortex[29] but, in addition, generalizes this concept to all main classes of cortical inhibitory cells. These results indicate that interneuron clustering is a universal phenomenon for cortical GABAergic interneurons, independently of their embryonic origin.

Clustering did not seem to be equally robust for the three main classes of interneurons (**Supplementary Fig. 8**). Clustering of PV^+ interneurons seemed stronger than for SST^+ and VIP^+ interneurons. While this may simply reflect intrinsic differences in the behavior of distinct interneuron lineages, alternative explanations may exist. For example, it is conceivable that both SST^+ and VIP^+ cells are generated as part of larger, mixed clones containing other classes of interneurons. This seems to be the case for SST^+ interneurons, which are often found in mixed clones with PV^+ interneurons, and it could also be the case for VIP^+ interneurons, as other classes of interneurons are simultaneously generated in the CGE[11,17,36,37]. In contrast, many PV^+ interneurons seem to arise from homogeneous clones, which are therefore larger and easier to identify.

It has been previously suggested that interneuron clustering is directly related to lineage relationships[29]. However, our 'cocktail' experiments using GFP^+ and $mCherry^+$ retroviruses indicated that many interneuron clusters might contain cells derived from two adjacent progenitor cells. These results suggest that interneuron clustering might not be strictly determined by lineage relationships but rather by the non–cell-autonomous sharing of migratory cues. This hypothesis is supported by the observation that most cells in a cluster tended to be synchronously generated and might therefore migrate to the cortex using the same guidance mechanisms. Obviously, clonally related interneurons born roughly at the same time will also share the same environment and therefore will have a strong tendency to cluster, but lineage *per se* might not be the key factor controlling their clustering.

The mechanisms leading to the formation of interneuron clusters remains to be addressed experimentally, but on the basis of our observations it is tempting to speculate that interneurons born roughly at the same time from adjacent progenitor cells would end up in approximately the same region of the neocortex. This solution implies that there is some sort of topographical organization relating the subpallium and the neocortex, which is preserved during the tangential migration of interneurons to the cortex. Radial migration is an effective mechanism for preserving topographical relationships between the ventricle and the mantle[38], but it is unclear how tangential migration would guarantee this process.

Layer-specific interneuron lineages

One of the most intriguing findings of our lineage analysis is that interneurons labeled after E11.5 and E14.5 viral infections consistently clustered in the same or in two adjacent layers, either in infragranular layers (E11.5 MGE/POA-derived interneurons) or in superficial layers of the cortex (E14.5 MGE/POA-derived interneurons and CGE-derived interneurons). In other words, interneuron clusters did not typically extend through the entire thickness of the cerebral cortex. This is in sharp contrast with recent findings from the Shi laboratory[29], which concluded that the clonal production of interneurons is somehow linked to the vertical, columnar organization of the neocortex. We believe that the origin of this discrepancy is methodological and due to the use of different criteria for the classification of interneuron clusters. On the basis of the observation that most interneuron clusters cover a range of roughly 300 µm (a measurement that is within the range of our observations), Brown and colleagues[29] defined a cuboidal matrix to identify horizontal and vertical interneuron clusters, which measured 300 µm on the side of the tangential plane but used the entire thickness of the cortex in the radial dimension (~1 mm). This method is biased in favor of scoring groups of interneurons as vertical clusters because it brings together neurons that are more distant in the radial dimension (up to 1 mm) than in the tangential plane (300 µm). In contrast, we used an unbiased approach that is completely adimensional, as it relies only on the net distances between neurons to report clustering preferences. Using this method, we came to the conclusion that most interneurons group in one or two adjacent layers. This is not simply a semantic discrepancy. We recognized that many of the clusters we observed had a 'vertical' appearance even when including cells within the same layer. However, we believe that what is biologically relevant in this context is that clusters consistently mapped into infragranular or supragranular layers of the cortex but very rarely expanded across these layers.

The restricted laminar organization of interneuron clusters is not entirely surprising for VIP^+ interneurons because it is well established that most CGE-derived interneurons end up in superficial layers of the cortex[36]. However, MGE/POA-derived interneurons colonize the entire thickness of the cortex[13,14], and so it was unexpected to find that interneuron lineages labeled by E11.5 viral injections were largely confined to deep layers of the cortex. Notably, this observation is not influenced by our clustering analysis because laminar distributions are independent of whether labeled cells are classified as part of a cluster or not (**Supplementary Fig. 9**).

These results have important implications for the organization of cortical GABAergic interneurons (**Supplementary Fig. 7**). First, they imply that the progenitor cells that are present in the MGE/POA at E11.5 do not contribute much to the generation of interneurons for superficial layers of the cortex, which in turn suggests that different progenitor cells must exist to produce interneurons for deep and superficial cortical layers. This idea is further supported by the observation that layer I interneurons are exclusively generated from progenitor cells located in the POA and CGE but not in the MGE[13–15,36]. We favor the hypothesis that interneuron lineages devoted to deep and superficial layers of the cortex segregate before E11.5 and have different proliferation dynamics. Accordingly, both types of progenitors would coexist in the ventricular zone at E11.5, but in very different proportions. This possibility would imply that the distinct types of progenitor cells would lead to partly overlapping waves of neurogenesis for the generation of deep and superficial layer interneurons. This hypothesis is compatible with the observed shift in the production of particular interneuron subtypes in the MGE during development[39].

Excitatory and inhibitory neuronal assemblies in the cortex

Proper functioning of the neocortex depends critically on the balance between excitatory pyramidal cells and inhibitory GABAergic interneurons[40,41]. Considering the tremendous heterogeneity of both pyramidal cells and interneurons[42,43], precise mechanisms must exist

that enable the formation of excitatory and inhibitory cell assemblies in the cerebral cortex in a layer-to-layer and region-to-region basis.

Neocortical pyramidal cells are organized along two main axes, horizontal layers and vertical columns. Distinct classes of pyramidal cells occupy different layers, and layers are vertically linked into columns through coherent sets of functional connections[4,5]. These layer-specific classes of cortical excitatory neuron derive from radial glial progenitor cells in sequential order, with neurons destined for deep layers being generated first, followed by superficial layer neurons. It is commonly accepted that the fate of pyramidal cells is related to their birthdate, which progressively restricts the neurogenic potential of a common radial glial progenitor[8,38]. The radial unit hypothesis also establishes a basis for the generation of functional columns in the cortex because clonally related pyramidal cells seem to establish preferential connections and respond to common sensory stimuli[44–46]. However, this view has been recently challenged by the discovery of distinct lineages of ventricular zone progenitor cells that are intrinsically specified to generate pyramidal cells for different cortical layers[47,48]. In line with this notion, our study suggests that deep- and superficial-layer interneurons also derive from separate lineages of progenitor cell. Thus, a common theme is emerging on the organization of the cerebral cortex that links its laminar dimension to the fate specification of progenitor cells.

It has been thought that cortical GABAergic interneurons may somehow be organized in relation to pyramidal cells[20,34,49,50]. Our results support the idea that progenitor cells in the subpallium generate interneurons whose spatial organization reflects the laminar distribution of pyramidal cells. This observation is consistent with the idea that the laminar position of different cortical interneurons, at least those emerging from the MGE, is regulated by layer-specific or even cell type–specific cues produced by pyramidal cells[34,49,50]. Although our results support a general model for the matching of deep- and superficial-layer interneurons and pyramidal cells, the existence of distinct lineages of cortical interneurons in the developing subpallium might allow for an even more specific coordination in the assembly of excitatory and inhibitory neuronal populations in the cerebral cortex.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHORS CONTRIBUTIONS

G.C., M.M. and O.M. designed the project; G.C., N.D. and I.S. performed the research and analyzed the results; Z.J.H., M.M. and O.M. provided analytical tools, reagents and transgenic mice. G.C., M.M. and O.M. interpreted the data and wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

1. Klausberger, T. & Somogyi, P. Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science* **321**, 53–57 (2008).
2. Isaacson, J.S. & Scanziani, M. How inhibition shapes cortical activity. *Neuron* **72**, 231–243 (2011).
3. DeFelipe, J. & Fariñas, I. The pyramidal neuron of the cerebral cortex: morphological and chemical characteristics of the synaptic inputs. *Prog. Neurobiol.* **39**, 563–607 (1992).
4. Mountcastle, V.B. The columnar organization of the neocortex. *Brain* **120**, 701–722 (1997).
5. Jones, E.G. & Rakic, P. Radial columns in cortical architecture: it is the composition that counts. *Cereb. Cortex* **20**, 2261–2264 (2010).
6. Silberberg, G., Gupta, A. & Markram, H. Stereotypy in neocortical microcircuits. *Trends Neurosci.* **25**, 227–230 (2002).
7. Rakic, P. Guidance of neurons migrating to the fetal monkey neocortex. *Brain Res.* **33**, 471–476 (1971).
8. Noctor, S.C., Flint, A.C., Weissman, T.A., Dammerman, R.S. & Kriegstein, A.R. Neurons derived from radial glial cells establish radial units in neocortex. *Nature* **409**, 714–720 (2001).
9. Anderson, S.A., Eisenstat, D.D., Shi, L. & Rubenstein, J.L.R. Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. *Science* **278**, 474–476 (1997).
10. Marín, O. & Rubenstein, J.L.R. A long, remarkable journey: tangential migration in the telencephalon. *Nat. Rev. Neurosci.* **2**, 780–790 (2001).
11. Butt, S.J. *et al.* The temporal and spatial origins of cortical interneurons predict their physiological subtype. *Neuron* **48**, 591–604 (2005).
12. Flames, N. *et al.* Delineation of multiple subpallial progenitor domains by the combinatorial expression of transcriptional codes. *J. Neurosci.* **27**, 9682–9695 (2007).
13. Fogarty, M. *et al.* Spatial genetic patterning of the embryonic neuroepithelium generates GABAergic interneuron diversity in the adult cortex. *J. Neurosci.* **27**, 10935–10946 (2007).
14. Xu, Q., Tam, M. & Anderson, S.A. Fate mapping Nkx2.1-lineage cells in the mouse telencephalon. *J. Comp. Neurol.* **506**, 16–29 (2008).
15. Gelman, D.M. *et al.* The embryonic preoptic area is a novel source of cortical GABAergic interneurons. *J. Neurosci.* **29**, 9380–9389 (2009).
16. Wichterle, H., Turnbull, D.H., Nery, S., Fishell, G. & Alvarez-Buylla, A. In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. *Development* **128**, 3759–3771 (2001).
17. Nery, S., Fishell, G. & Corbin, J.G. The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations. *Nat. Neurosci.* **5**, 1279–1287 (2002).
18. Yozu, M., Tabata, H. & Nakajima, K. The caudal migratory stream: a novel migratory stream of interneurons derived from the caudal ganglionic eminence in the developing mouse forebrain. *J. Neurosci.* **25**, 7268–7277 (2005).
19. Rymar, V.V. & Sadikot, A.F. Laminar fate of cortical GABAergic interneurons is dependent on both birthdate and phenotype. *J. Comp. Neurol.* **501**, 369–380 (2007).
20. Miyoshi, G. & Fishell, G. GABAergic interneuron lineages selectively sort into specific cortical layers during early postnatal development. *Cereb. Cortex* **21**, 845–852 (2011).
21. Wonders, C.P. & Anderson, S.A. The origin and specification of cortical interneurons. *Nat. Rev. Neurosci.* **7**, 687–696 (2006).
22. Hendry, S.H., Jones, E.G. & Emson, P.C. Morphology, distribution, and synaptic relations of somatostatin- and neuropeptide Y-immunoreactive neurons in rat and monkey neocortex. *J. Neurosci.* **4**, 2497–2517 (1984).
23. Cavanagh, M.E. & Parnavelas, J.G. Development of vasoactive-intestinal-polypeptide-immunoreactive neurons in the rat occipital cortex: a combined immunohistochemical-autoradiographic study. *J. Comp. Neurol.* **284**, 637–645 (1989).
24. Ma, Y., Hu, H., Berrebi, A.S., Mathers, P.H. & Agmon, A. Distinct subtypes of somatostatin-containing neocortical interneurons revealed in transgenic mice. *J. Neurosci.* **26**, 5069–5082 (2006).
25. Miller, M.W. Cogeneration of retrogradely labeled corticocortical projection and GABA-immunoreactive local circuit neurons in cerebral cortex. *Brain Res.* **355**, 187–192 (1985).
26. Fairén, A., Cobas, A. & Fonseca, M. Times of generation of glutamic acid decarboxylase immunoreactive neurons in mouse somatosensory cortex. *J. Comp. Neurol.* **251**, 67–83 (1986).
27. Ang, E.S. Jr., Haydar, T.F., Gluncic, V. & Rakic, P. Four-dimensional migratory coordinates of GABAergic interneurons in the developing mouse cortex. *J. Neurosci.* **23**, 5805–5815 (2003).
28. Tanaka, D.H. *et al.* Random walk behavior of migrating cortical interneurons in the marginal zone: time-lapse analysis in flat-mount cortex. *J. Neurosci.* **29**, 1300–1311 (2009).
29. Brown, K.N. *et al.* Clonal production and organization of inhibitory interneurons in the neocortex. *Science* **334**, 480–486 (2011).
30. Walsh, C. & Cepko, C.L. Clonally related cortical cells show several migration patterns. *Science* **241**, 1342–1345 (1988).

31. Sussel, L., Marín, O., Kimura, S. & Rubenstein, J.L. Loss of Nkx2.1 homeobox gene function results in a ventral to dorsal molecular respecification within the basal telencephalon: evidence for a transformation of the pallidum into the striatum. *Development* **126**, 3359–3370 (1999).
32. Cepko, C.L. *et al.* Lineage analysis using retroviral vectors. *Methods* **14**, 393–406 (1998).
33. Valcanis, H. & Tan, S.S. Layer specification of transplanted interneurons in developing mouse neocortex. *J. Neurosci.* **23**, 5113–5122 (2003).
34. Pla, R., Borrell, V., Flames, N. & Marín, O. Layer acquisition by cortical GABAergic interneurons is independent of Reelin signaling. *J. Neurosci.* **26**, 6924–6934 (2006).
35. Kawaguchi, Y. & Kubota, Y. GABAergic cell subtypes and their synaptic connections in rat frontal cortex. *Cereb. Cortex* **7**, 476–486 (1997).
36. Miyoshi, G. *et al.* Genetic fate mapping reveals that the caudal ganglionic eminence produces a large and diverse population of superficial cortical interneurons. *J. Neurosci.* **30**, 1582–1594 (2010).
37. Xu, Q., Cobos, I., De La Cruz, E., Rubenstein, J.L. & Anderson, S.A. Origins of cortical interneuron subtypes. *J. Neurosci.* **24**, 2612–2622 (2004).
38. Rakic, P. Specification of cerebral cortical areas. *Science* **241**, 170–176 (1988).
39. Miyoshi, G., Butt, S.J., Takebayashi, H. & Fishell, G. Physiologically distinct temporal cohorts of cortical interneurons arise from telencephalic Olig2-expressing precursors. *J. Neurosci.* **27**, 7786–7798 (2007).
40. Hensch, T.K. Critical period plasticity in local cortical circuits. *Nat. Rev. Neurosci.* **6**, 877–888 (2005).
41. Turrigiano, G. Too many cooks? Intrinsic and synaptic homeostatic mechanisms in cortical circuit refinement. *Annu. Rev. Neurosci.* **34**, 89–103 (2011).
42. Molyneaux, B.J., Arlotta, P., Menezes, J.R. & Macklis, J.D. Neuronal subtype specification in the cerebral cortex. *Nat. Rev. Neurosci.* **8**, 427–437 (2007).
43. Markram, H. *et al.* Interneurons of the neocortical inhibitory system. *Nat. Rev. Neurosci.* **5**, 793–807 (2004).
44. Yu, Y.C., Bultje, R.S., Wang, X. & Shi, S.H. Specific synapses develop preferentially among sister excitatory neurons in the neocortex. *Nature* **458**, 501–504 (2009).
45. Li, Y. *et al.* Clonally related visual cortical neurons show similar stimulus feature selectivity. *Nature* **486**, 118–121 (2012).
46. Ohtsuki, G. *et al.* Similarity of visual selectivity among clonally related neurons in visual cortex. *Neuron* **75**, 65–72 (2012).
47. Stancik, E.K., Navarro-Quiroga, I., Sellke, R. & Haydar, T.F. Heterogeneity in ventricular zone neural precursors contributes to neuronal fate diversity in the postnatal neocortex. *J. Neurosci.* **30**, 7028–7036 (2010).
48. Franco, S.J. *et al.* Fate-restricted neural progenitors in the mammalian cerebral cortex. *Science* **337**, 746–749 (2012).
49. Hevner, R.F., Daza, R.A., Englund, C., Kohtz, J. & Fink, A. Postnatal shifts of interneuron position in the neocortex of normal and reeler mice: evidence for inward radial migration. *Neuroscience* **124**, 605–618 (2004).
50. Lodato, S. *et al.* Excitatory projection neuron subtypes control the distribution of local inhibitory interneurons in the cerebral cortex. *Neuron* **69**, 763–779 (2011).

ONLINE METHODS

Mouse strains. *Nestin-Cre* (Jackson labs 003771), *Nkx2-1-Cre* (ref. 14), *Nkx2-1*^{CreERT2}, *Sst*^{Cre} and *Vip*^{Cre} (ref. 51), *Pvalb*^{Cre} (ref. 52), Rosa26 reporter CAG-boosted EGFP (*RCE*)[53] mice were kept at the Instituto de Neurociencias. All animal procedures were approved by the corresponding ethical committees (IN-CSIC and CEEA-PRBB) and were performed in accordance with Spanish (law 32/2007) and European regulations (EU directive 86/609, EU decree 2001-486). The day of vaginal plug was considered to be embryonic day (E) 0.5 and the day of birth postnatal day (P) 0.

DNA construct and retrovirus production. Cre-dependent conditional reporter retroviral constructs (rv::dio) were generated by subcloning a modified version of a double-floxed inverted open reading frame cassette[54] into BamH1 and PmeI restriction sites of a retroviral backbone kindly provided by F.H. Gage. This vector contains an internal CAG chicken β-actin promoter and the woodchuck hepatitis post-transcriptional regulatory element (WPRE), and encodes either GFP (enhanced) or membrane-bound (palmitoylation tag) mCherry as a reporter fluorescent protein. To visualize proliferating cells within interneuron clones, we used a fluorescent ubiquitylation-based cell cycle indicator system[55]. This Fucci indicator consists of a modified Azami Green fluorescent protein fused with a fragment of human geminin, which accumulates in the nucleus during S-G2-M phases but is rapidly degraded when cells progress to G1. In brief, we generated viruses encoding mCherry and a fragment of human geminin (1–110) fused with Azami Green (AM-V9014; MBL International Corporation) by using the P2A self-cleaving peptide as linker. Moloney murine leukemia viruses (MoMLV) were produced by transfecting HEK293T cells with the corresponding vectors along with CMV-vsvg and CMV-gagpol helper plasmids, and concentrated as previously described[56]. In the two-marker experiments (simultaneous injection of the GFP- and mCherry-encoding conditional reporter retroviruses), the two vectors were produced in the same plates and mixed before concentration by ultracentrifugation.

***In utero* retroviral infection.** Pregnant females were deeply anesthetized with isoflurane and E11.5 or E14.5 embryos, depending on the experiment, were individually injected using an ultrasound backscattering microscope (Visualsonic), as described previously[34]. Low-titer conditional retroviruses were released into the telencephalic ventricles using a nanoliter injector[57]. In birthdating experiments, pregnant females received intraperitoneal injections (three injections in 12 h) at E12.5 with 30 mg/kg EdU (5-ethynyl-2′-deoxyuridine, A10044 Invitrogen) and at E15.5 with 50 mg/kg BrdU (5-bromo-2′-deoxyuridine, B5002 Sigma-Aldrich). *Nkx2-1*^{CreERT2}*;RCE* pregnant females received a single intraperitoneal injection of tamoxifen (2–3 mg/kg) diluted in corn oil at E11.5.

Immunohistochemistry. Postnatal mice were perfused transcardially with 4% paraformaldehyde (PFA) in PBS and the dissected brains were postfixed for 2 h at 4 °C in the same solution. Embryonic brains were dissected out in cold PBS and fixed in 4% PFA for 2–12 h. Postnatal brains were sectioned at 100 µm on a vibratome (VT1000S, Leica), while embryonic brains were sectioned at 50–60 µm. Free-floating coronal sections were then subsequently processed for immunohistochemistry as previously described[34]. The following primary antibodies were used: rabbit anti-GFP (1:1,000; A11122, Molecular Probes), chicken anti-GFP (1:1,000; GFP-1020, Aves Labs), rabbit anti-DsRed (1:500; 632496, Clontech), rat anti-SST (1:200; MAB354, Millipore), mouse anti-PV (1:750; PARV-19, Sigma-Aldrich), rabbit anti-PV (1:4,000; PV-28, Swant), rabbit anti-GABA (1:1,000; A2052, Sigma-Aldrich), mouse anti-BrdU conjugated with Alexa Fluor 647 (1:1,000; B35133, Invitrogen), rabbit anti-Nkx2-1 (1:1,000; PA 0100, Biopat), and rabbit anti-Ki67 (1:200, NCL-Ki67p, Novocastra). Cell nuclei were stained with 5 µM 4′-6-diamidino-2-phenylindole (DAPI) in PBS and sections mounted with Mowiol (Sigma) with NPG (Calbiochem). For mCherry/EdU/BrdU triple stainings, sections were first processed for mCherry immunohistochemistry, then fixed 20 min with 4% PFA in PBS and subsequently processed for EdU and BrdU staining. Tissue treatment for BrdU staining was performed as previously described[34]. EdU$^+$ cells were detected using the Click-iT EdU Imaging kit (Molecular Probes) with Alexa Fluor 488. For immunohistochemistry after electrophysiological recordings, slices were fixed immediately after recording in 4% PFA at 4 °C from 2–12 h. Biocytin was revealed using ABC complex (1:100; Abcys). mAG-hGem protein expression was visualized without antibody staining.

Serial section reconstruction. To obtain a three-dimensional reconstruction of the telencephalon, each slice was plotted in serial order from rostral to caudal using a fluorescence microscope coupled to a Neurolucida system (MBF Bioscience). The external boundaries of each slice, as well as the neocortex, hippocampus and striatum, were traced. Each interneuron was identified with a unique code that reflected its precise position. Serial reconstructions were then obtained by aligning each section of the brain with the previous traces. The Cartesian coordinates (x, y, z) of each cell and the cell contour were then extracted and used for the analyses of cell distributions and clustering.

Electrophysiological recordings. P20–P30 *Nkx2-1-Cre* mice that were injected at E11.5 with rv:dio-Gfp were deeply anesthetized with isoflurane, decapitated and the brain rapidly removed and placed in ice-cold oxygenated solution consisting of (in mM) 248 sucrose, 3 KCl, 0.5 $CaCl_2$, 4 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$ and 10 glucose, saturated with 95% O_2 and 5% CO_2. Coronal slices (300 µm) were cut through the somatosensory cortex using a vibratome (Leica). The slices were then maintained at room temperature in artificial cerebrospinal fluid (ACSF) consisting of (in mM) 124 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$ and 10 glucose, saturated with 95% O_2 and 5% CO_2. For patch-clamp recordings in whole-cell configuration, slices were transferred to a chamber and continuously superfused with ACSF at 32 °C. Cells were visualized with infrared-differential interference optics (Hamamatsu camera controller) viewed through a 40× water-immersion objective (Olympus). Patch microelectrodes (6–10 MΩ) were pulled from borosilicate glass (1.5 mm outer diameter, 0.86 mm inner diameter; Harvard Apparatus) using a vertical P10 puller (Narishige) and filled with a potassium gluconate–based intracellular solution containing (in mM) 140 potassium gluconate, 10 HEPES, 2 NaCl, 4 KCl, 4 ATP, 0.4 GTP and 0.6 Alexa 555. Biocytin (2–5 mg/ml) was added for post-recording immunocytochemistry. For targeting, GFP$^+$ cells were excited at 488 nm. Interneurons were kept under current-clamp configuration with an Axoclamp 200A amplifier operating in a fast mode. Data were filtered online at 2 kHz and were acquired at a 20 kHz sampling rate using pClamp 6.0.2 software (Molecular Devices). Resting membrane potential, membrane resistance and membrane conductance were rapidly measured after patching the GFP$^+$ interneurons. We analyzed offline in Clampfit 10.2 the action potential traces and calculated the following parameters: spike threshold potential, action potential amplitude, duration of the action potential at its half amplitude, AHP amplitude, maximum firing frequency (average frequency elicited by the maximum 500 ms current injection), onset frequency (first or second inter-spike interval), steady-state frequency (average of the last five inter-spike intervals), percentage of spike frequency adaptation (percentage of reduction between the first inter-spike frequency and the steady-state frequency). Data from cells with no action potentials were discarded. For the interneuron classification, we used the nomenclature proposed by the Petilla group[58]. We considered as "adapting" (AD) a cell with a percentage of spike frequency adaptation greater than 25%.

Imaging. Images were acquired using fluorescence microscopes (DM5000B/CTR5000 and DMIRB; Leica) coupled to digital cameras (DC500 or DFC350FX, Leica; OrcaR2, Hamamatsu) or a confocal microscope (DMIRE2/ CTRMIC/TCS SP2; Leica). Neurolucida software (MBF Bioscience) was used for serial section reconstructions using a fluorescence microscope coupled to a digital camera (QICAM Fast 1394; QImaging).

Quantification of cell position and age. For the spatial distribution of interneuron clusters, the laminar position of each cell in the neocortex (identified by Cartesian coordinates) was recorded. Clusters of interneurons were classified according to the following categories: intralaminar cluster when all cells in the cluster were located in the same layer, and interlaminar cluster when cells in the cluster were localized in different layers. Interlaminar clusters were further subdivided into three groups: clusters spanning two adjacent layers, two nonadjacent layers, or three or more layers. In birthdating experiments, clusters were defined as isochronic when most cells in the cluster (>50%) were labeled with one of the markers (EdU or BrdU) and no cells were labeled with the other marker, or heterochronic when the cluster contained cells labeled with the two markers (EdU and BrdU). In the electrophysiological experiments, homogeneous and heterogeneous clusters were defined on the basis of intrinsic physiological properties

(FS and AD). Predominant and balanced cell clusters were defined on the basis of their relative composition of PV- and SST-expressing interneurons.

Statistical analysis of cell distributions and clustering. We first analyzed the distribution of interneurons in the neocortex by calculating nearest neighbor distances (NNDs). After determining the distance between each interneuron and the closest one, we compared these distances to the values expected for a sample of neurons located at random in the same volume (that is, the same neocortical hemisphere). Specifically, for each experiment, the experimental interneuron distribution was compared with 100 simulated distributions, generated by positioning the same number of elements at random locations distributed uniformly in the exact same region of tissue as the experimental condition. NNDs were displayed as cumulative distributions.

Interneuron clusters were defined and identified by applying an unsupervised agglomerative hierarchical clustering analysis[59], whereby interneurons were grouped according to their proximity. Results were displayed as hierarchical cluster trees or dendrograms. Specifically, dendrograms were built to visualize the hierarchical relationship of neurons according to their spatial distance. Starting from the initial condition in which each interneuron was an individual object, the closest objects were successively merged into clusters until only one cluster remained (the entire set of neurons). In the dendrogram, each node represented a merging point of cells into clusters or clusters into higher-level clusters. Two objects (cells or clusters) were merged into a single one depending on the average spatial distance between all their constituent neurons. The clustering analysis performed was exclusive (meaning that each cell was assigned to a single cluster). To identify experimental clusters, the results of the analysis for interneurons were compared to the same analysis applied to the 100 random simulated data sets. Clusters were accepted if the average distance between their constituent neurons (determined as above) fell below a threshold, determined as the distance that maximized the difference between the number of clusters in the experimental data set and the average number of clusters in the 100 simulated repetitions.

In our analyses, the sample size for each data set (hemisphere) represents the entire population of labeled interneurons in that experiment, with no exclusions. No statistical methods were used to predetermine sample size per experiment.

When the difference between NND distributions for experimental and simulated data sets was not significant, the result was scored as such; **Supplementary Figure 8** reports the dependence of test statistical significance on sample size. Biological replicates (*n* values are different populations derived from different brains from different litters) were analyzed to assess biological variability and reproducibility of data. The population size for biological replicates was similar to that generally employed in the field. NND cumulative distributions for experimental and simulated data sets were compared using the Kolmogorov-Smirnov two-sample test. This non-parametric test depends on differences in the shape of the empirical cumulative distribution. Testing was two-sided (the null hypothesis was no difference between the distributions), and statistical significance was set at $P < 0.01$. Results of clustering analyses were displayed as dendrograms and quantitative measurements were displayed as mean ± s.e.m.

NND and clustering analyses were performed using Matlab software (MathWorks).

51. Taniguchi, H. *et al.* A resource of Cre driver lines for genetic targeting of GABAergic neurons in cerebral cortex. *Neuron* **71**, 995–1013 (2011).
52. Hippenmeyer, S. *et al.* A developmental switch in the response of DRG neurons to ETS transcription factor signaling. *PLoS Biol.* **3**, e159 (2005).
53. Sousa, V.H., Miyoshi, G., Hjerling-Leffler, J., Karayannis, T. & Fishell, G. Characterization of Nkx6–2-derived neocortical interneuron lineages. *Cereb. Cortex* **19** (suppl. 1), i1–i10 (2009).
54. Cardin, J.A. *et al.* Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature* **459**, 663–667 (2009).
55. Sakaue-Sawano, A. *et al.* Visualizing spatiotemporal dynamics of multicellular cell-cycle progression. *Cell* **132**, 487–498 (2008).
56. Tashiro, A., Sandler, V.M., Toni, N., Zhao, C. & Gage, F.H. NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus. *Nature* **442**, 929–933 (2006).
57. Gaiano, N., Kohtz, J.D., Turnbull, D.H. & Fishell, G. A method for rapid gain-of-function studies in the mouse embryonic nervous system. *Nat. Neurosci.* **2**, 812–819 (1999).
58. Ascoli, G.A. *et al.* Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. *Nat. Rev. Neurosci.* **9**, 557–568 (2008).
59. Carugo, O. Clustering criteria and algorithms. *Methods Mol. Biol.* **609**, 175–196 (2010).

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Corrigendum: Lineage-specific laminar organization of cortical GABAergic interneurons

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In the version of this article initially published online, author Z. Josh Huang's name was misspelled Josh Z. Huang. The error has been corrected for the print, PDF and HTML versions of this article.

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Supplementary Information

Lineage-specific laminar organization of cortical GABAergic interneurons

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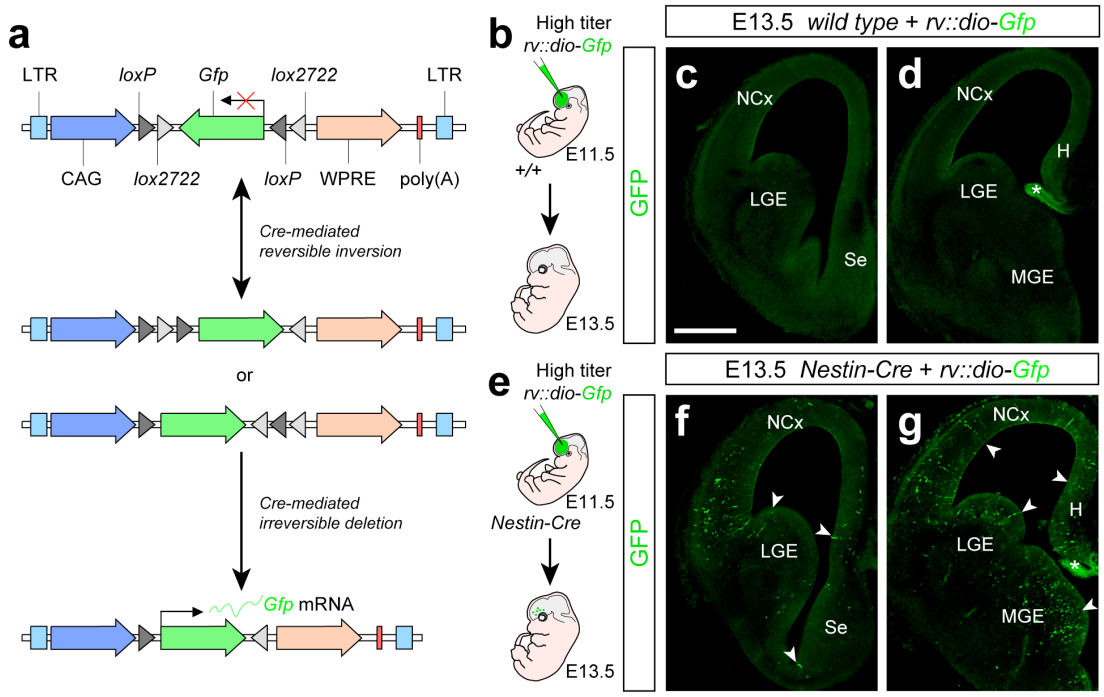
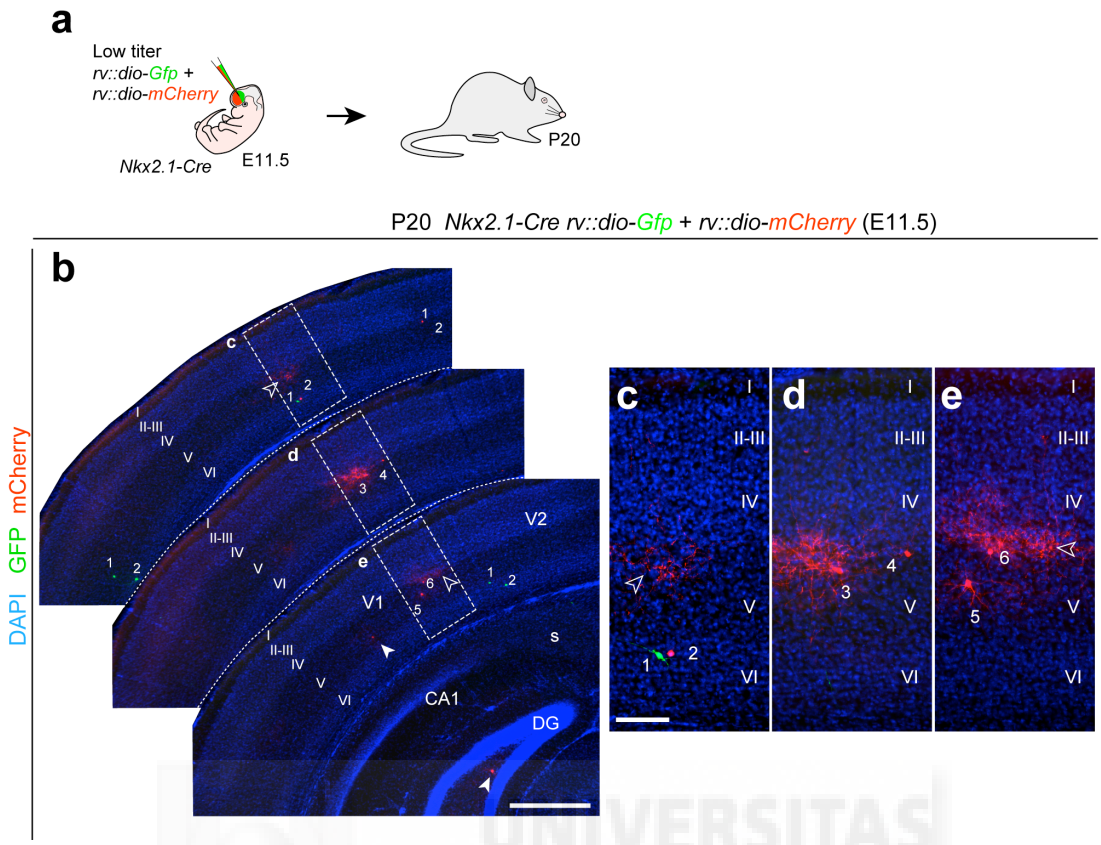


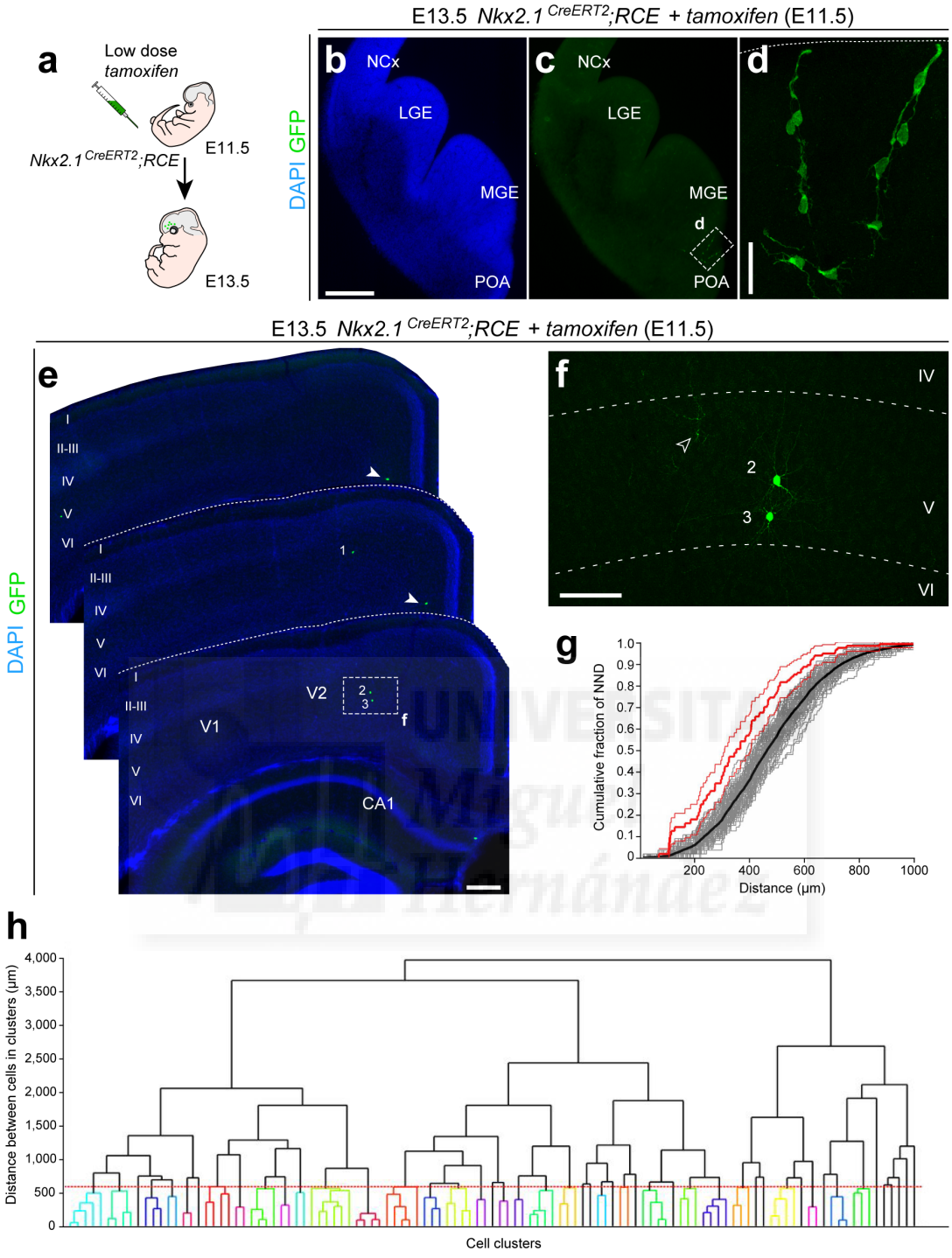
Supplementary Figure 1. Genetic strategy for the generation of conditional reporter retroviruses.

(a) Schematic showing the structure of the viral Cre recombinase-dependent expression system carrying a reversed and doubled-floxed cDNA sequence encoding enhanced green fluorescent protein (*Gfp*) under the control of the strong chicken ß-actin promoter CAG. Cre-mediated inversion and subsequent recombination of compatible loxP sites leads to the expression of GFP. A similar construct was built for the expression of mCherry. (**b**) Schematic of the experimental paradigm. (**c,d**) Coronal sections through the telencephalon of an E13.5 wild type embryo infected with high titer conditional reporter retroviruses at E11.5 and stained with antibodies against GFP. The asterisk denotes autofluorescence present in the choroid plexus. (**e**) Schematic of the experimental paradigm. (**f,g**) Coronal sections through the telencephalon of an E13.5 *Nestin-Cre* embryo infected with high titer conditional reporter retroviruses at E11.5 and stained with antibodies against GFP. Arrowheads point to individual clones in the neocortex (NCx), hippocampus (H), septum (Se), lateral and medial eminences (LGE and MGE, respectivey). Scale bar: 300 μm (**c,d,f,g**).



Supplementary Figure 2. Non cell-autonomous clustering of interneuron lineages.

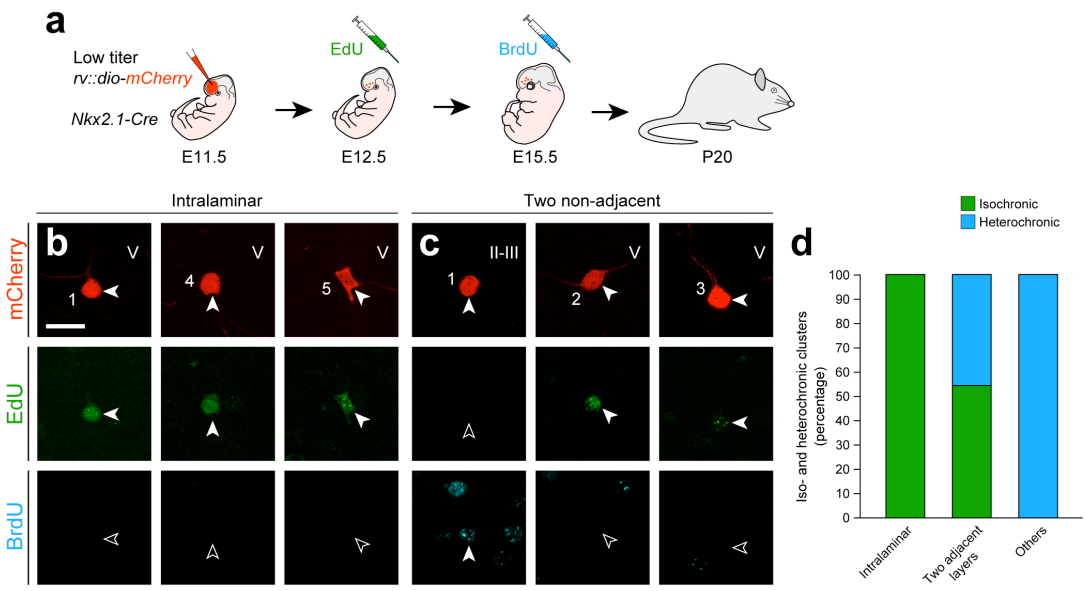
(**a**) Schematic of the experimental paradigm. (**b–e**) Serial coronal sections (100 μm thick) through the telencephalon of a P20 *Nkx2-1-Cre* mouse infected with both low titer GFP and mCherry conditional reporter retroviruses at E11.5 and stained with DAPI and antibodies against GFP and mCherry. Six labeled interneurons (labeled 1 to 6) form a large cluster that span through three adjacent sections in the primary visual cortex. These neurons are shown at high magnification in (c–e). Neurons 2 to 5 are labeled with mCherry, but neuron 1 in this cluster is labeled with GFP. Three additional clusters, one labeled with mCherry and two with GFP are also found in these sections. Solid arrowheads point to other isolated interneurons, while the open arrowhead indicates the dendrites of others mCherry interneurons whose cell body is located in adjacent sections. I-VI, cortical layers I to VI; CA1, hippocampus CA1 area; DG, dentate gyrus; V1, primary visual cortex; V2, secondary visual cortex; S, subiculum. Scale bars: 300 μm (**b**), 100 μm (**c–e**).



Supplementary Figure 3. Genetic lineage tracing of MGE/POA-derived interneurons with an inducible Cre strain.

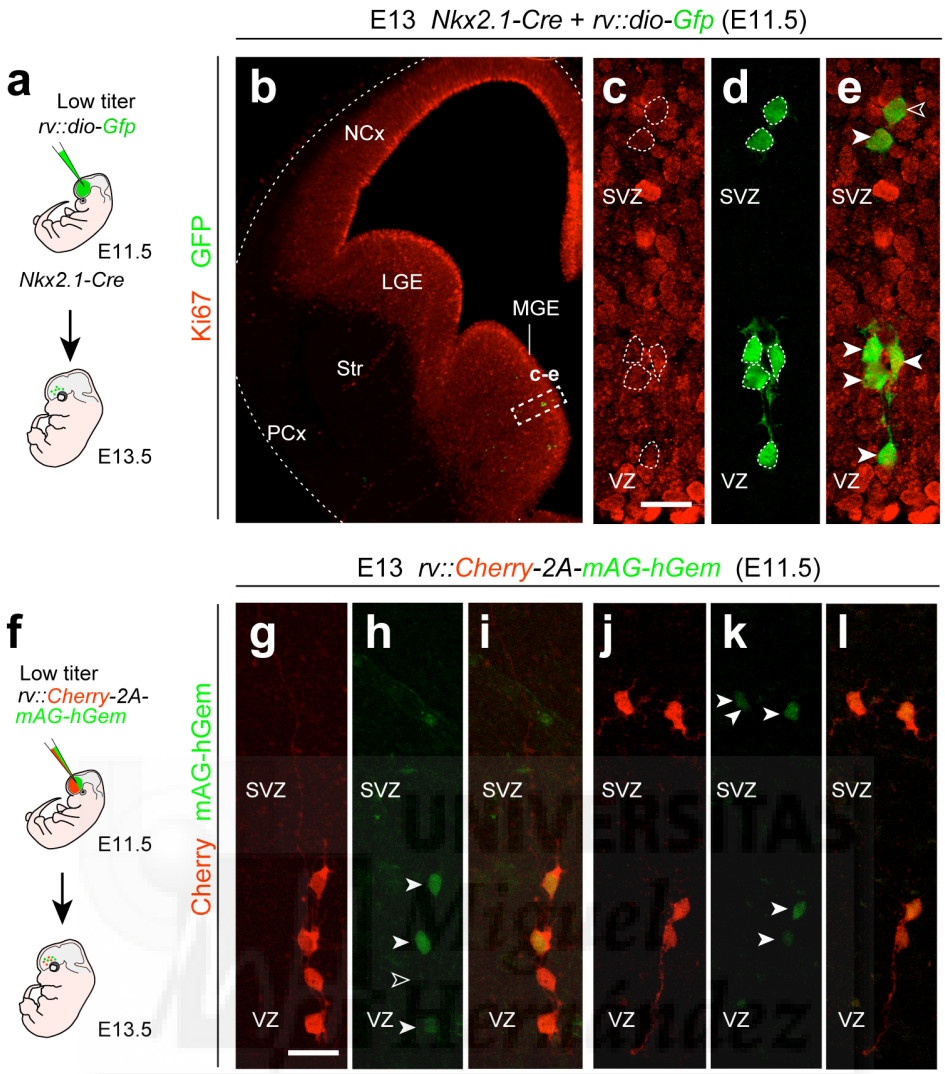
(**a**) Schematic of the experimental paradigm. (**b–d**) Coronal sections through the telencephalon of an E13.5 *Nkx2-1-Cre^{ERT2};RCE* reporter embryo induced with a low tamoxifen dose at E11.5 and stained with DAPI (b) and antibodies against GFP (c,d).

The high magnification image (d), corresponding to the boxed region in (c), displays individual clones of GFP-labeled cells. (**e,f**) Serial coronal sections (100 μm thick) through the telencephalon of a P20 *Nkx2-1^{CreERT2}; RCE* reporter mouse induced with low dose tamoxifen at E11.5 and stained with DAPI and antibodies against GFP. Three labeled interneurons (labeled 1 to 3) form a compact cluster that span through two adjacent sections. Two of these neurons (2 and 3) are shown at high magnification in (f). Solid arrowheads in (e) point to other interneurons, while open arrowheads in (f) indicate the dendrites of the interneuron labeled 1. (**g**) Cumulative functions of nearest neighbor distances (NDD) for all experimental all interneuron pairs in the experimental (red line) and simulated (black line) distributions. The light red lines delineate a $P = 0.05$ confidence interval for the experimental distribution, whereas the gray lines depict a hundred random repetitions for the simulated distribution. Kolmogorov-Smirnov two-sample test, $P = 5{,}07 \times 10^{-7}$. (**h**) Dendrogram showing the hierarchical relationship between labeled interneurons that are grouped according to their distances using agglomerative hierarchical clustering. The red dotted line indicates the threshold value that defines clustering for this experiment. LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; POA, preoptic area; I-VI, cortical layers I to VI; CA1, hippocampus CA1 area; V1, primary visual cortex; V2, secondary visual cortex; NCx, neocortex. Scale bars equal 300 μm (b, c and e), 100 μm (f) and 50 μm in (d).



Supplementary Figure 4. Birthdate of MGE/POA-derived interneuron clusters.

(**a**) Schematic of the experimental paradigm. (**b,c**) High magnification images of some of the interneurons shown in Fig. 3b and 3c, respectively, stained with antibodies against EdU and BrdU. The numbers refer to individual interneurons shown in Fig. 3b,c. Solid and open arrowheads point to EdU or BrdU labeled and unlabeled interneurons, respectively. (**d**) Quantification of the fraction of isochronic and heterochronic clusters among those defined as intralaminar, two adjacent layers and others (two non-adjacent and three or more layers). I-VI, cortical layers I to VI. Scale bars: 50 μm (**b,c**).



Supplementary Figure 5. Abundance of intermediate progenitors in MGE/POA-derived clones.

(**a**) Schematic of the experimental paradigm. (**b–e**) Coronal section through the telencephalon of an E13 *Nkx2-1-Cre* embryo infected with low titer conditional reporter retroviruses at E11.5 and stained with antibodies against GFP and Ki67. The high magnification images (c–e) show a single clone (boxed rectangle in b) in which most of the labeled cells are progenitors (solid arrowheads). The open arrowhead indicates a GFP+/Ki67- cell, which is likely a newborn neuron. (**f**) Schematic of the experimental paradigm. (**g–l**) Coronal section through the telencephalon of E13 embryos infected with low titer mCherry-2A-mAG-hGeminin coding retroviruses at E11.5 and stained with antibody against mCherry. The images show two examples of

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MGE clones (g-i and j-l respectively) labeled with mCherry in which most of the cells express the mAG-hGeminin protein marker of cell proliferation (S, G2 and M phases of the cell cycle; solid arrowheads). The open arrowhead indicates a mCherry+/mAG-hGem- cell, which have exited the cell cycle. The green signal of the mAG-hGem fusion protein in (h-i and k-l) represents the endogenous AG fluorescence. LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; NCx, neocortex; PCx, piriform cortex; Str, striatum; SVZ, subventricular zone; VZ, ventricular zone. Scale bars: 300 μm (**b**), 50 μm (**c–e,g–l**).

a

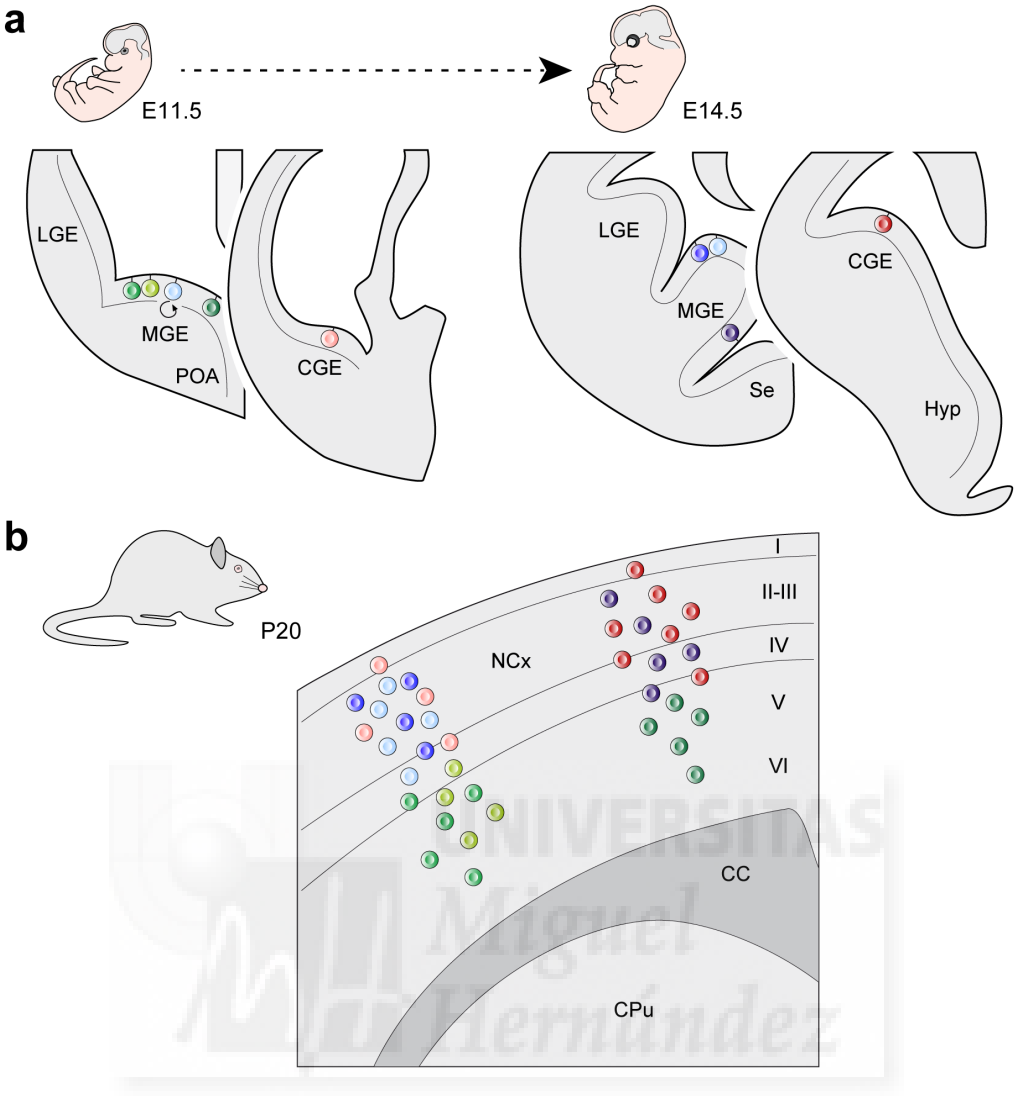
	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5
Cluster 1	FS	FS	FS	FS	-
Cluster 2	FS	FS	FS	-	-
Cluster 3	FS	FS	FS	-	-
Cluster 4	AD	AD	AD	FS	-
Cluster 5	FS	FS	FS	-	-
Cluster 6	AD	FS	FS	AD	FS
Cluster 7	AD	FS	AD	FS	FS
Cluster 8	FS	FS	FS	FS	-
Cluster 9	AD	AD	AD	FS	-

b

	FS	AD
RMP (mV)	-64.1 ± 1.1	-68.6 ± 8.1
Rm (MΩ)	198.3 ± 16.0	265.1 ± 47.7
C_m (pF)	37.5 ± 2.4	43.7 ± 7.0
APT (mV)	-36.7 ± 1.1	-42.6 ± 3.1
APA (mV)	57.6 ± 1.8	63.1 ± 3.4
APD (ms)	0.42 ± 0.03	0.85 ± 0.11
AHP (mV)	-18.4 ± 1.1	-7.1 ± 1.3
MFF(Hz)	89.1 ± 4.9	35.0 ± 5.2
Fr_1 (Hz)	124-364	40-240
Fr_{SS} (Hz)	95-303	27-163
Freq adaption (%)	21.9 ± 2.1	54.3 ± 7.3
n	24	11

Supplementary Figure 6. Electrophysiological analysis of interneuron clusters in Nkx2-1-Cre experiments.

(**a**) Cellular composition of interneuron clusters analyzed with electrophysiology. (**b**) Quantification of the electrophysiological properties of clustered interneurons in the cortex. RMP, Resting membrane potential; Rm, Membrane resistance; AD, adapting; APT, Action potential threshold; APA, Action potential amplitude; APD, Action potential duration at its half amplitude; AHP, After hyperpolarization amplitude; Cm, Membrane capacitance; FS, Fast spiking; MFF, Maximum firing frequency; Fr1, First spike frequency; Frss, Steady state frequency; Freq adaptation, Spike frequency adaptation.



Supplementary Figure 7. Lineage-specific laminar organization of cortical GABAergic interneurons

(**a,b**) The schematic drawings summarize the main conclusions of the study. At E11.5, the embryonic subpallium contains a heterogeneous pool of progenitor cells. The most abundant progenitors cells in the MGE at this stage (greenish circles) give rise to interneurons that populate the deep layers of the cortex at P20. Progenitor cells that give rise to interneurons for the superficial layers of the cortex (blue circle) are very scarce and probably self-expanding at this age. At E14.5, however, the progenitor cells that produce interneurons for the superficial cortical layers (bluish circles) are the predominant lineage in the MGE. Sibling interneurons and, most likely, synchronous interneurons derived from adjacent progenitor cells end up in approximately the same spatial location in the cerebral cortex. Progenitor cells in the

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CGE (reddish circles) primarily give rise to interneurons that populate the superficial layers of the cortex.

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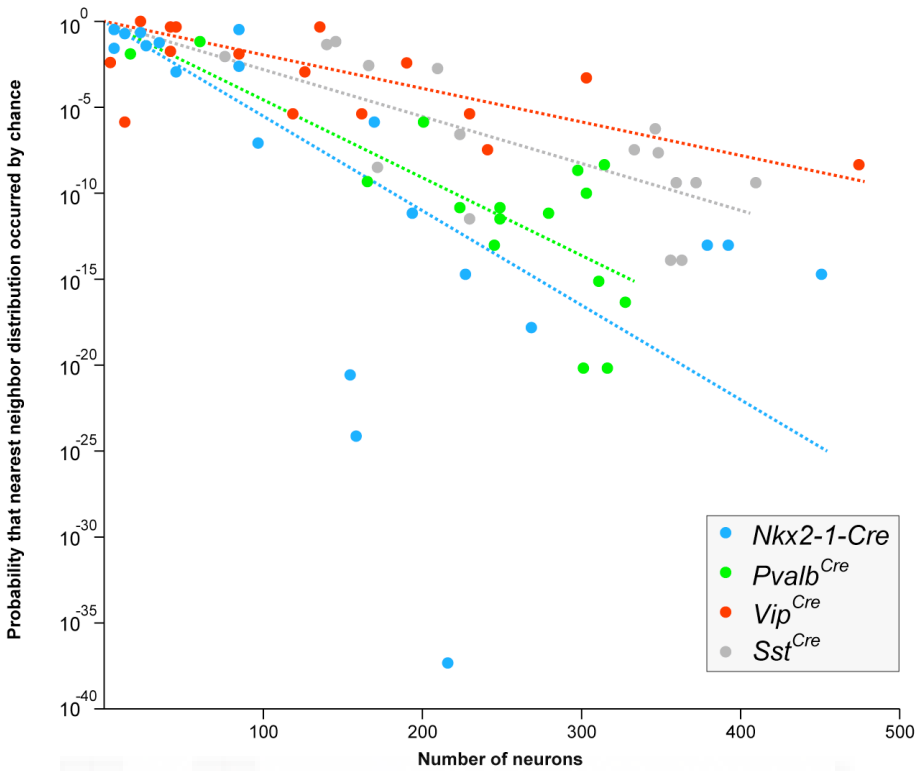
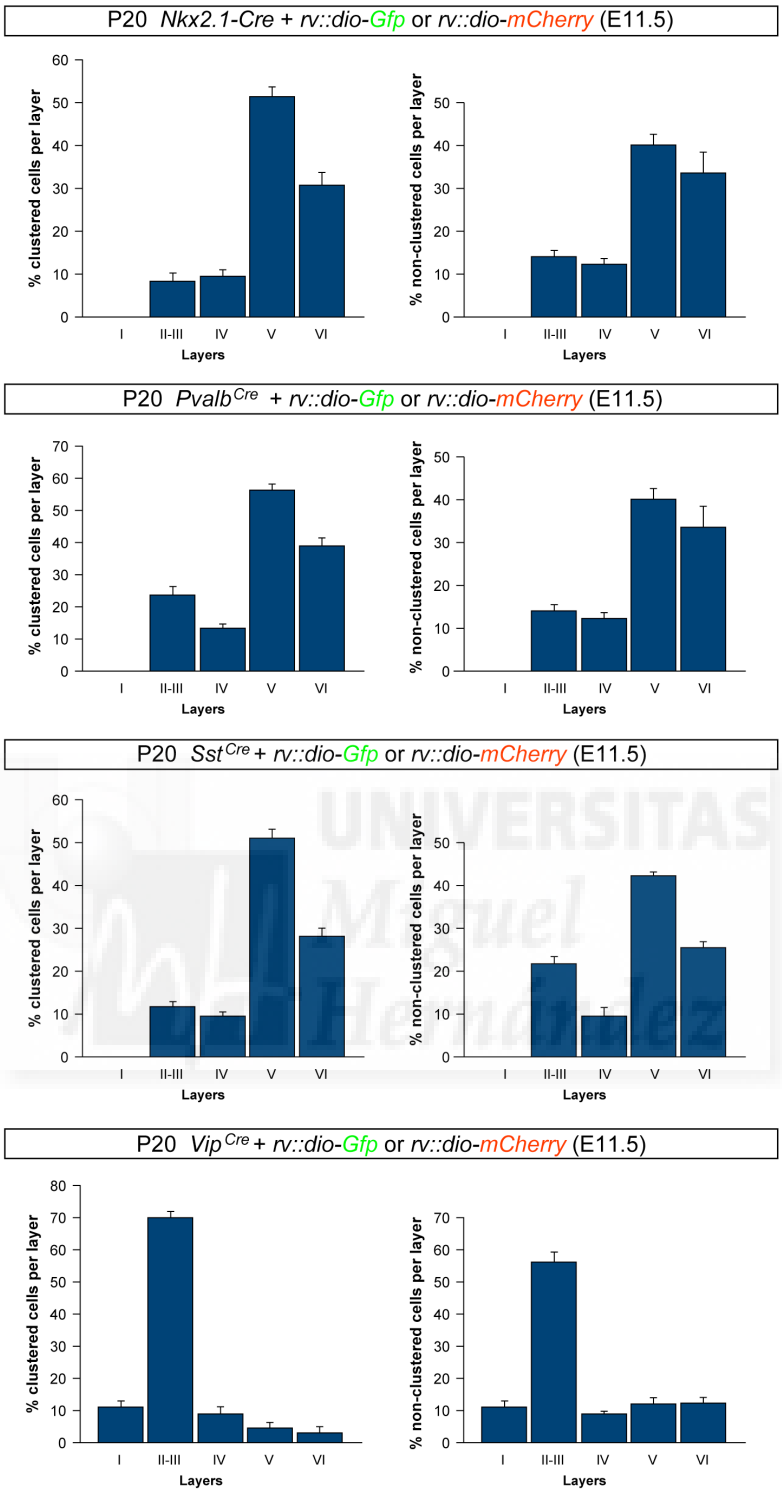


Supplementary Figure 8. Statistical significance of nearest neighbor distance (NND) analyses in the different classes of experiments.

The probability that each experimental population of labeled neurons had randomly distributed NNDs is plotted against the number of neurons in the population. Different plots correspond to different classes of experiments (*Nkx-2-1-Cre*, *Pvalb*^{Cre}, *Vip*^{Cre}, *Sst*^{Cre}). P-values were generated by comparing the real experimental data set with 100 iterations of simulated randomly distributed neuronal populations, as described in the main text. Larger populations yielded statistically stronger evidence that interneurons do not distribute randomly. For a given population size, *Pvalb*^{Cre} populations clustered more robustly than *Vip*^{Cre} and *Sst*^{Cre} populations, as seen in the much greater level of statistical significance (note logarithmic scaling of probability values).



Supplementary Figure 9. Laminar distribution of clonally related interneurons in the different classes of experiments.

Quantification of the percentage of clustered and non-clustered interneurons per layer in the different classes of experiments.

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Chapter 2. Developmental segregation of cortical interneuron cell lineages

In the previous chapter, we described a new method to tag specific populations of progenitor cells with fluorescent proteins and trace their progenies. By combining classic retroviral tracing methods with cre/lox mouse genetics, we were able to specifically target telencephalic progenitor cells giving rise to interneurons lineages in the mouse cerebral cortex (Ciceri et al. 2013). In order to specifically label progenitor cells in the MGE/POA region, one of the main sources of cortical interneurons, we used mouse embryos expressing the cre recombinase under the control of the promoter sequence of *Nkx2-1*, which encodes a transcription factor expressed by progenitor cells in these regions (Xu et al. 2008). By analysing the spatial organization of labelled interneurons, others and we found that cortical interneurons have a strong tendency to cluster in the cerebral cortex according to lineage relationships (Brown et al. 2011; Ciceri et al. 2013). In addition, by infecting different cre-expressing mouse strains with conditional reporter retroviruses, we were able to further extend our analysis to different interneuron subtypes labelled at clonal density. Collectively, our results indicate that the clustering behaviour of interneuron lineages is a common property of different classes of interneurons derived for molecularly distinct proliferative regions, as shown for MGE-derived PV+ and SST+ interneurons and for CGE-derived VIP+ interneurons. Therefore, interneuron clustering is a property that seems independent of embryonic origin and the genetic programs of cell fate specification. Our experiments also revealed that lineage relationships are not the exclusive determinant of interneuron clustering. This suggests that non-cell autonomous factors, such as the sharing of guidance responsiveness by coetaneous cells born in nearby locations, may also influence this process. Further investigation of this issue will provide a more detailed description of the mechanisms underlying this intriguing phenomenon.

One of the most interesting findings of our study is the existence of fate-restricted lineages for cortical interneurons, which are linked to specific laminar locations. For example, fate mapping of MGE/POA progenitor cells at early developmental stages (E11.5) revaled that these cells predominantly give rise to interneuron clusters that localize in deep layers of the neocortex (layer V and VI). By contrast, retroviral tracing experiments at later stages (E14.5) preferentially labelled progenitor cells that produce interneurons located in the superficial layers of the neocortex (layer II/III and IV). These

results suggest that MGE/POA progenitor cells are diverse in nature and that this heterogeneity might be linked to the laminar allocation of the interneurons that they generate (Marín and Müller 2014). Thus, the inside-out pattern adopted by MGE-derived interneurons while populating the cortex does not seem to be caused by the columnar distribution of individual interneuron lineages, as previously proposed, but rather as a consequence of the existence of distinct progenitor cells with different laminar potential at different stages of development. In other words, interneuron lineages primarily mirror the laminar structure of the neocortex rather than its columnar organization.

To explain the preferential generation of deep and superficial layer interneurons at different stages of embryonic development, we hypothesized that these segregated pools of precursors may coexist in the proliferative neuroepithelium during most of the neurogenic period, but that their relative abundance would change during development. According to this hypothesis, the predominant class of progenitor cells at early embryonic stages (E11.5) would generate deep layer interneurons. A second pool of progenitor cells would then progresivelly replace deep layer interneuron progenitors at late embryonic stages (E14.5), thereby generating superficial layer interneurons. In this chapter, we provide additional evidence supporting the existence of at least two different lineages of MGE/POA progenitor cells in the embryonic subpallium, and we identify the timing of their segregation as independent lineages.

Laminar-commited interneuron progenitors segregate prior to E10.5

Our experiments strongly support the existence of at least two main classes of laminar-commited interneuron progenitor cells in the embryonic subpallium (Ciceri et al., 2013), but it is remains unclear when these progenitor cells segregate during early development and how they dynamically evolve during neurogenesis. The most likely possibility is that these two progenitor types arise early during development from uncommitted precursor cells. If this were the case, then our retroviral tracing approach should allow the identification at some point during development of uncommitted progenitor cells that give rise to interneurons located in similar proportions across all layers of the neocortex. To test this hypothesis, we performed a novel series of retroviral tracing experiments in which we injected conditional reporter retroviruses in *Nkx2-1-Cre* embryos at progressively earlier stages of development. As in previous experiments, we quantify the proportion of interneurons across cortical layers for each experimental condition.

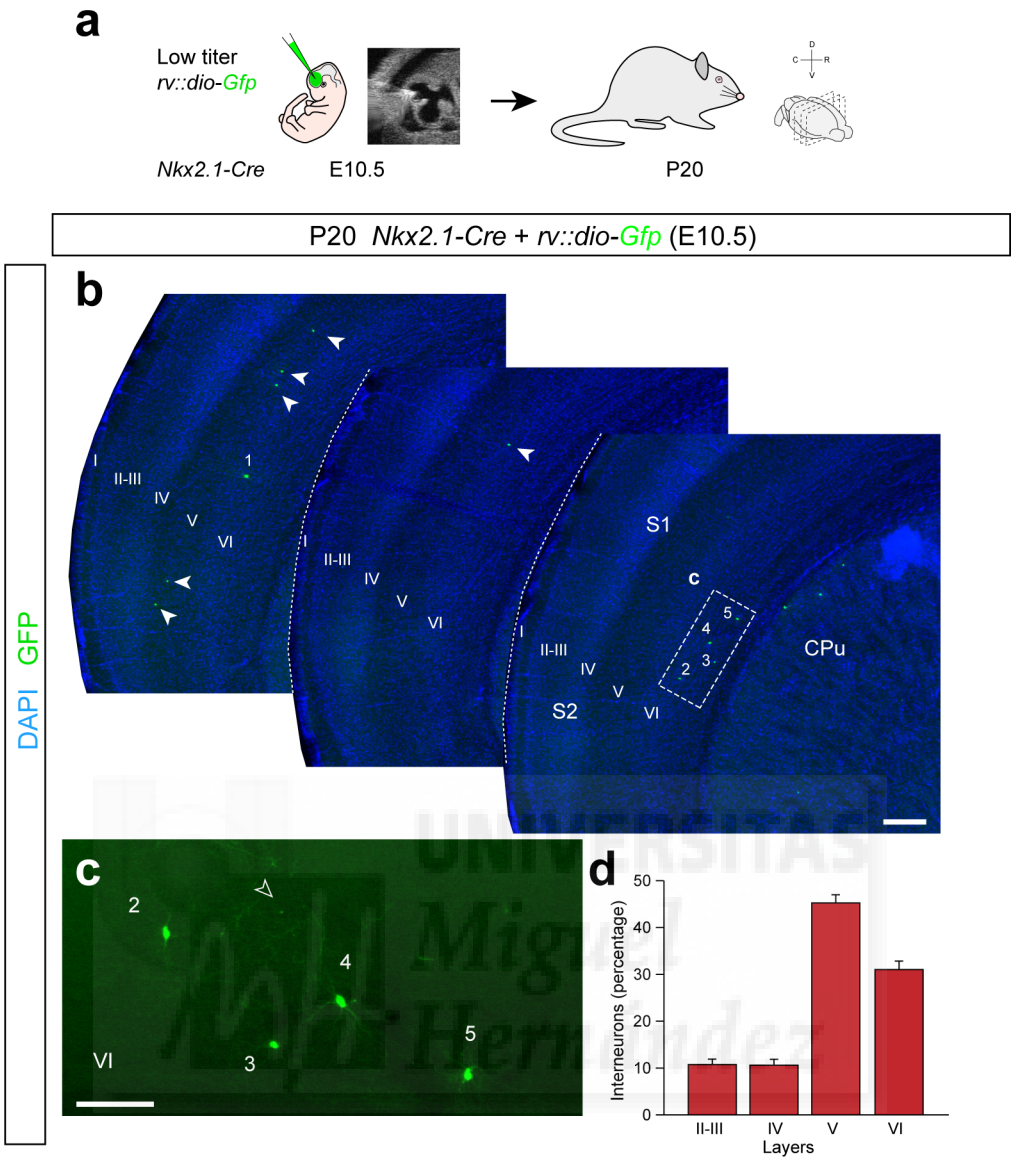

Figure 1. Distribution of Interneurons lineages labelled at E10.5 (a) Schematic of the experimental paradigm. (**b**) Serial coronal sections (100 μm thick) through the telencephalon of a P20 Nkx2.1-cre mouse infected with low titer conditional reporter retroviruses at E10.5, stained with DAPI and antibodies against gfp. (**c**) High magnification of region boxed in (b), gfp channel only. (**d**) Quantification of the distribution of labeled interneurons through cortical layers. I-VI, cortical layers I to VI; Cpu, caudatum putamen; S1, primary somatosensory cortex; S2, socondary somatosensory cortex. Scale bars: 300 μm (b), 100 μm (c, d). Error bars represent s.e.m.

Analysis of *Nkx2-1-Cre* brains injected at E10.5 with low titer conditional reporter retroviral stocks revealed that most MGE/POA-derived interneurons generated at this stage are preferentially distributed through the deep layers of the neocortex (layer V: 46.00 ± 1.64%; layer VI: 31.71 ± 1.67%; n = 12; **Fig. 1a-d** and **Fig. 5a,c**), wheras only a small fraction was found in superficial layers (layer II/III: 11.19 ± 1.00%; layer IV: 11.10 ± 1.14%; n = 12; **Fig. 1d** and **Fig. 5a-c**). Thus, the laminar distribution of cortical interneurons labelled by E10.5 retroviral infections largely matches that following

injections performed at E11.5 (**Fig. 5c**), which indicates that the two main lineages of corical MGE/POA interneuron are already segregated at E10.5. We wondered whether this strong bias in the allocation of interneurons in the infragranular layers of the cortex is caused by the non-uniform distribution of interneurons along the rostro-caudal extent of the neocortex. To rule out this possibility, we plotted the relative laminar fraction of cells by section, serially in rostro-caudal order. This analysis revealed that the strong bias toward deep layer interneurons is homogeneous along the rostro-caudal extent of the neocortex and occurs independently of the cortical area considered (**Fig. 2**).

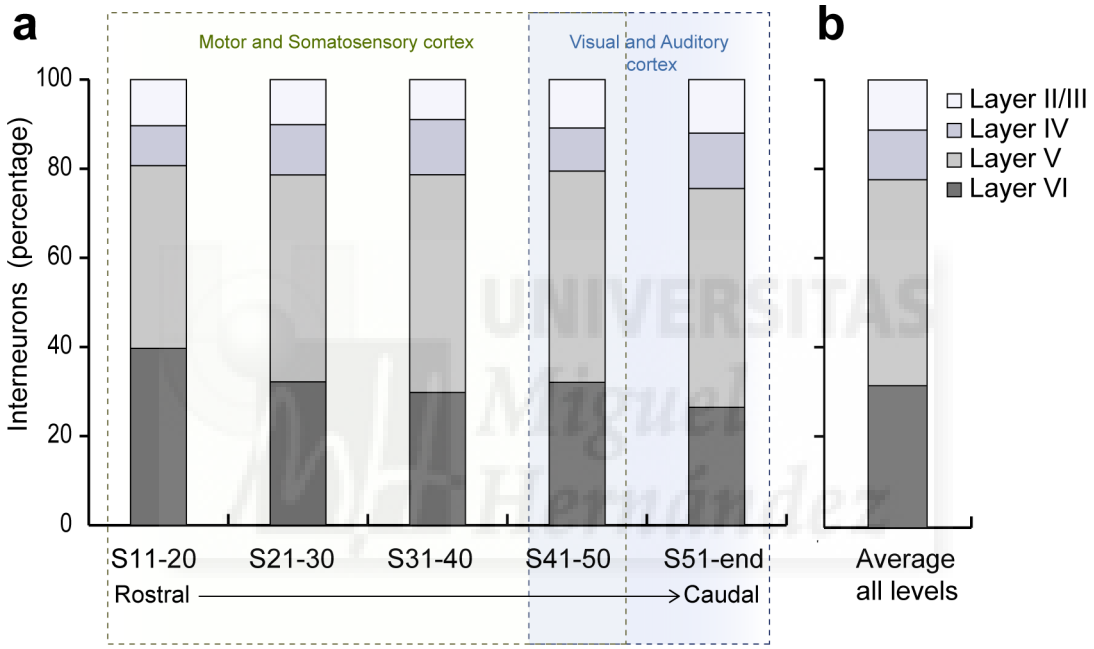

Figure 2. Laminar distribution of interneurons labelled at clonal density across the rostro-caudal extent of the cortex. Graphs show the laminar distribution of P20 cortical interneurons labelled by conditional reporter retroviral infection of E10.5 Nkx2.1-cre mouse embyos. (**a**) Data are plotted grouping brain section in rostro-caudal order. (**b**) Graph showing the average laminar distribution of cortical interneurons labelled by conditional reporter retroviral infection of E10.5 Nkx2.1-cre mouse embyos. S, brain sections. Error bars represent s.e.m.

We next performed retroviral-tracing experiments at E9.5 and quantified the distribution of interneurons per layer, as before. Interestingly, retroviral injections at this stage labeled a similar fraction of interneurons throughout layers II-VI of the neocortex (layer II/III: 23.40 ± 3.57%; layer IV: 22.97 ± 2.73%; layer V: 30.50 ± 3.00%; layer VI: 23.13 ± 3.84%; n = 13; **Fig. 3a-k** and **Fig. 5a,c)**. Of note, preliminary analysis of the distribution of cells in these datasets suggests that interneurons derived from E9.5 progenitors also cluster in the cerebral cortex (**Fig 3b-e** and **Fig 3f-j**).

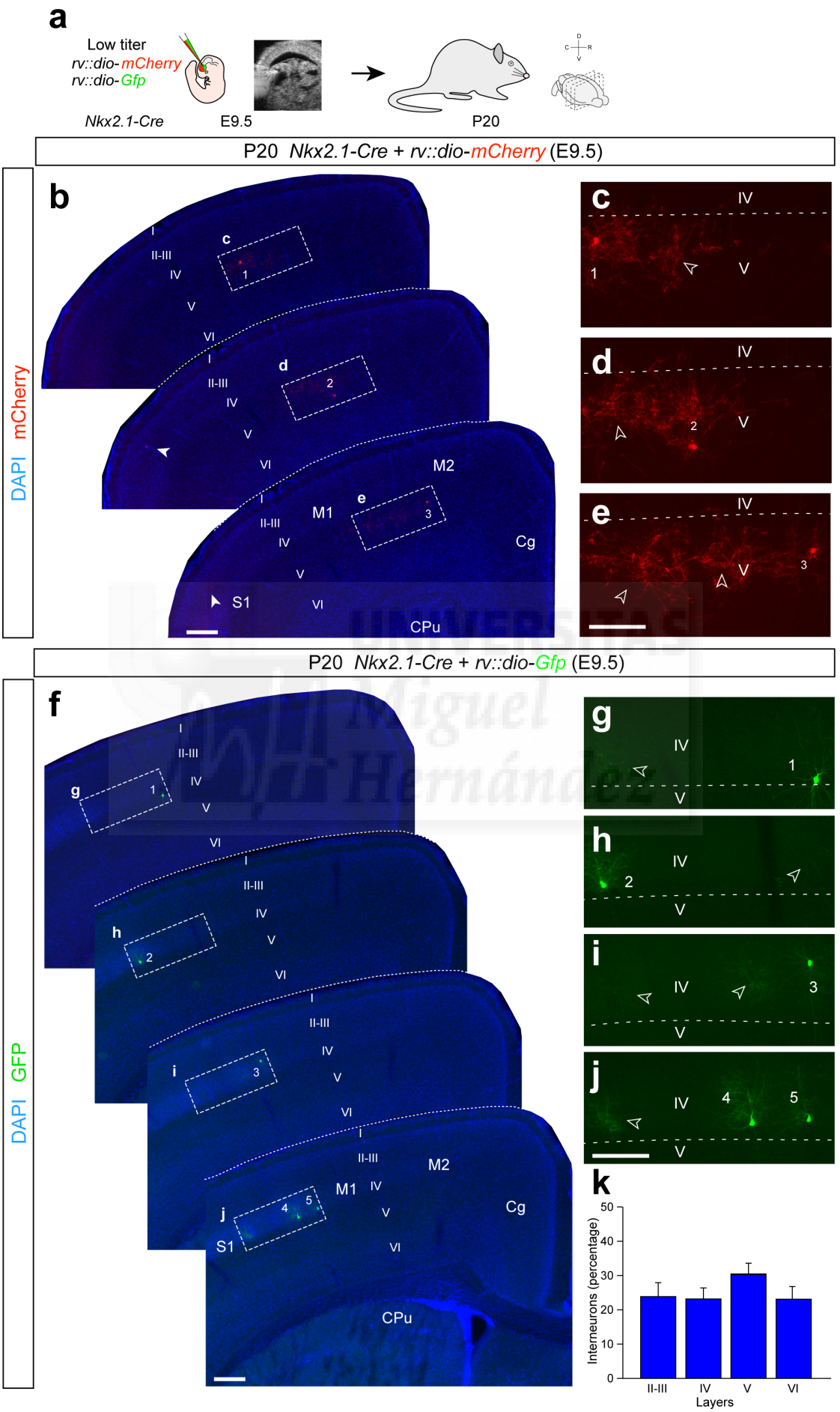
a
Low titer
rv::dio-mCherry
rv::dio-Gfp
Nkx2.1-Cre
E9.5
P20
P20 Nkx2.1-Cre + rv::dio-mCherry (E9.5)
DAPI mCherry
b
c
d
e
M2
M1
Cg
S1
CPu
P20 Nkx2.1-Cre + rv::dio-Gfp (E9.5)
DAPI GFP
f
g
h
i
j
k
Interneurons (percentage)
Layers
II-III
IV
V
VI

Figure 3. Distribution of interneurons lineages labelled at E9.5. (**a**) Schematic of the experimental paradigm. (**b**) Serial coronal sections (100 μm thick) through the telencephalon of a P20 Nkx2.1-cre mouse infected with low titer conditional reporter retroviruses at E9.5, stained with DAPI and antibodies against mCherry. (**c-e**) High magnification of region boxed in (b), mCherry channel only. (**f**) Serial coronal sections (100 μm thick) through the telencephalon of a P20 Nkx2.1-cre mouse infected with low titer conditional reporter retroviruses at E9.5, stained with DAPI and antibodies against gfp. (**g-j**) High magnification of region boxed in (f), gfp channel only (**k**) Quantification of the distribution of labeled interneurons through cortical layers. I-VI, cortical layers I to VI; Cg, cingulate cortex; Cpu, caudatum putamen; M1, primary motor cortex; M2, secondary motor cortex S1, primary somatosensory cortex; S2, secondary somatosensory cortex. Scale bars: 300 μm (b, f), 100 μm (c-d, g-j). Error bars represent s.e.m.

In summary, these experiments suggest that the progenitor cells that give rise to interneurons found in deep and superifical layers of the cortex, respectively, derive from uncommitted MGE/POA precursor cells that face the lateral ventricles at E9.5. These evidences further reinforced our working model and identify the transition between E9.5 and E10.5 as the developmental time window for the segregation of deep and superficial layers-committed MGE/POA lineages.

Late induction of early progenitor cells confirms segregated interneuron lineages

According to our model, the relative proportion of deep layer-committed progenitors in the MGE/POA venricular zone (VZ) should decrease over time, following a trend that would fit with the known relationship between interneuron birthdate and laminar allocation (Miller 1985; Fairén et al. 1986; Valcanis and Tan 2003; Pla et al. 2006). In other words, the quantitative contribution of deep layer-committed progenitors to the overall population of cortical interneurons should decrease progressively, although these progenitors should continue to produce deep layer interneurons even at late developmental stages (**Fig. 6**). To directly test this hypothesis, we designed an experiment to label an enriched proportion of deep layer-committed progenitors at late neurogenesis by combining our conditional retroviral labelling method with the tamoxifen inducible cre recombinase system (CreERT2). CreERT2 consists of a form of cre fused to a mutated human estrogen receptor binding protein. CreERT2 is restricted to the cytoplasm and can only access the nucleus and drive genetic recombination after exposure to tamoxifen. In brief, we performed low titer injections of conditional reporter retroviruses in the lateral ventricle of *Nkx2-1*^{CreERT2} embryos at E11.5 (a developmental stage at which, according to our hypothesis, deep layer-committed progenitors represent the majority of progenitor cells present in the subpallium) and induced the fluorescent tagging of these progenitors by administering a

high dose of tamoxifen to pregnant mice at E14.5 (**Fig. 4a**). Because Nkx2-1 is expressed by MGE/POA progenitor cells but it is rapidly down regulated in early post-mitotic cortical interneurons (Marin et al. 2000), only those cells infected with retroviruses at E11.5 that remain as progenitor cells at E14.5 will be labelled in this experiment, thereby revealing the neurogenic potential of early progenitor cells at late developmental stages.

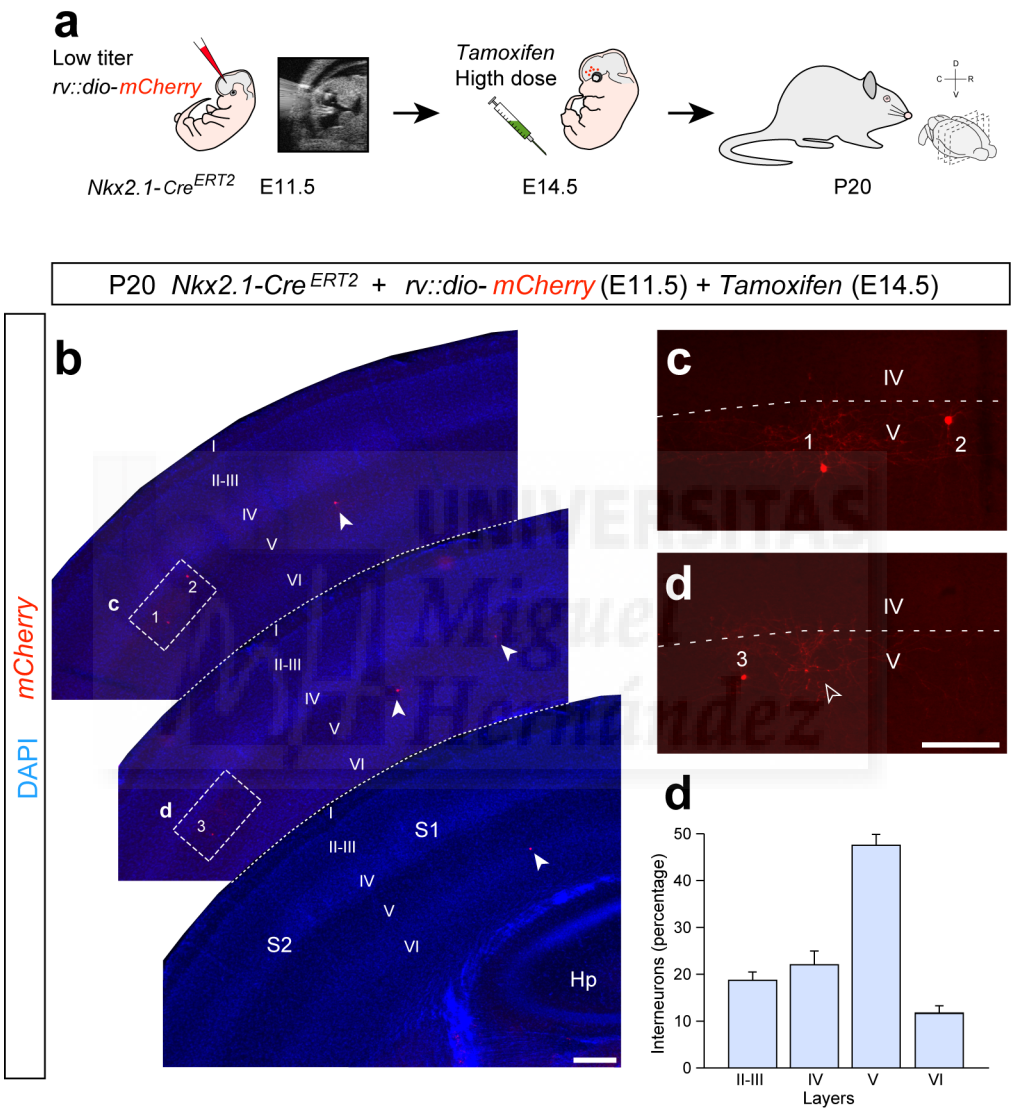

Figure 4. Late induction of early retroviral labelled progenitors confirmed lineages laminar restrictions. (**a**) Schematic of the experimental paradigm. (**b**) Serial coronal sections (100 μm thick) through the telencephalon of a P20 Nkx2.1-creERT2 mouse infected with low titer conditional reporter retroviruses at E11.5 and induced with tamoxifen at E14.5, stained with DAPI and antibodies against mCherry. (**c, d**) High magnification of region boxed in (a), mCherry channel only. (**e**) Quantification of the distribution of labeled interneurons through cortical layers. I-VI, cortical layers I to VI; Hp, hippocampus; S1, primary somatosensory cortex; S2, socondary somatosensory cortex. Scale bars: 300 μm (b), 100 μm (c, d). Error bars represent s.e.m.

Analysis of P20 *Nkx2-1*^{CreERT2} brains infected with conditional reporter retroviruses at E11.5 and induced with tamoxifen at E14.5 revealed that most labelled interneurons were located in deep layers of the neocortex (layer V and VI: 59.15 ± 3.20 %; n = 12). In particular, we observed a large proportion of interneurons in cortical layer V (47.72 ± 3.13 %; n = 12; **Fig. 4a-d** and **Fig. 5c**), with the remaining cells localized mainly in superficial layers of the neocortex (layer II/III: 18.58 ± 1.99 %; layer IV: 22.27 ± 2.75 %; n=12; **Fig. 4d** and **Fig. 5c**).

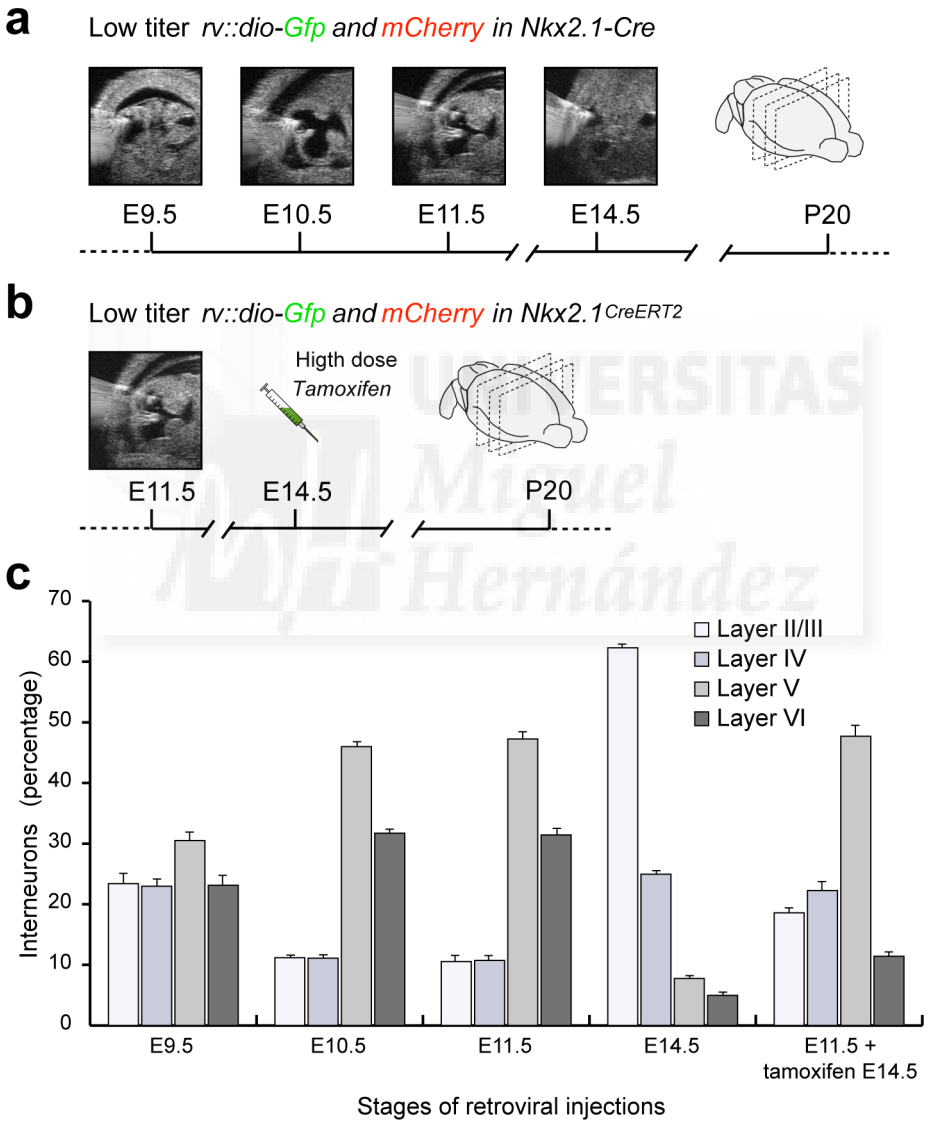

Figure 5. The segregation of deep vs. superficial interneuron lineages. (**a, b**) Schematic summary of the experimental paradigms used in the study. (**b**) Quantification of the laminar distribution of MGE/POA derived interneurons labelled by low-titer conditional reporter retroviruses across the different types of experiments.

It is important to note that these experiments yielded a different interneuron distribution than that obtained when infections were performed at E14.5 in *Nkx2-1-Cre* embryos, the same stage we used for the tamoxifen induction (layer II/III: 62.31 ± 0.93 %; layer IV: 24.97 ± 0.82 %; layer V: 7.75 ± 0.59 %; layer VI: 4.97 ± 0.47 %; *n*= 11; **Fig. 5a-c**). Figure 5 contains a summary of the fraction of cells found in each cortical layer in the different types of experiments, for an easy comparison. These results strongly support the multiple lineages model for the generation of deep and superficial layer interneurons. In fact, if MGE/POA progenitors were to behave according to the classical model of neurogenesis (i.e. multipotent progenitors whose potential to generate interneurons is progressively restricted towards superficial layer interneurons; see Introduction, **Fig. 5b**), one would have expected that the induction of Cre-mediated recombination at E14.5 would have yielded almost exclusively interneurons in the superficial layers of the cortex. In contrast, these experiments revealed interneuron distributions similar to those observed after retroviral targeting of Nkx2-1-expressing progenitor cells at E11.5 (i.e. most interneurons in infragranular layers, **Fig. 5c**). Thus, using this strategy we were able to specifically target an enriched proportion of deep layer-committed progenitor cells, which are likely present in relatively small numbers at E14.5, as revealed by the experiments we carried out in *Nkx2-1-Cre* embryos.

Of note, we also observed an increased proportion of superficial layer interneurons in the experiments with E11.5 infections in *Nkx2-1*^{CreERT2} embryos and tamoxifen induction at E14.5 (layer II/III: 18.58 ± 1.99 %; layer IV: 22.27 ± 2.75 %; *n*=12; **Fig. 4d** and **Fig. 5c**) compared to the retroviral injections at E11.5 in *Nkx2-1-Cre* embryos (layer II/III: 10.57 ± 1.90%; layer IV: 10.75 ± 1.38%; *n* = 12; **Fig. 5c**). Our interpretation of these differences is that the few superficial layer-committed interneuron progenitors that we targeted at E11.5 are likely to be self-expanding, and therefore they would generate a larger fraction of labeled superficial layer interneurons after tamoxifen administration at E14.5. The same comparison also revealed that deep layer-committed progenitors become progressively restricted to produce layer V cells during development (layer VI: 31.43 ± 2.45% in E11.5 infections in *Nkx2-1-Cre*; 11.44 ± 1.43 in E11.5 infections in *Nkx2-1*^{CreERT2} and tamoxifen induction at E14.5). Thus, within each of the two main lineages of MGE/POA-derived interneurons, progenitor cells seem to behave as multipotent progenitor cells (giving rise to layer V and layer VI interneurons, for example), whose potential becomes progressively restricted over time.

Altogether, our results reinforce the view that multiple segregated lineages contribute differentially to the production of interneurons for deep and superficial layers of the cortex, respectively. Moreover, our experimental evidence indicates that birthdate is not the primary factor that instructs cell positioning.

Toward a model of lineage progression for MGE/POA-derived cortical interneurons

Based on the results presented in Chapter 1 and 2, we proposed a new model of neurogenesis for MGE/POA-derived cortical interneurons (**Fig. 6**).

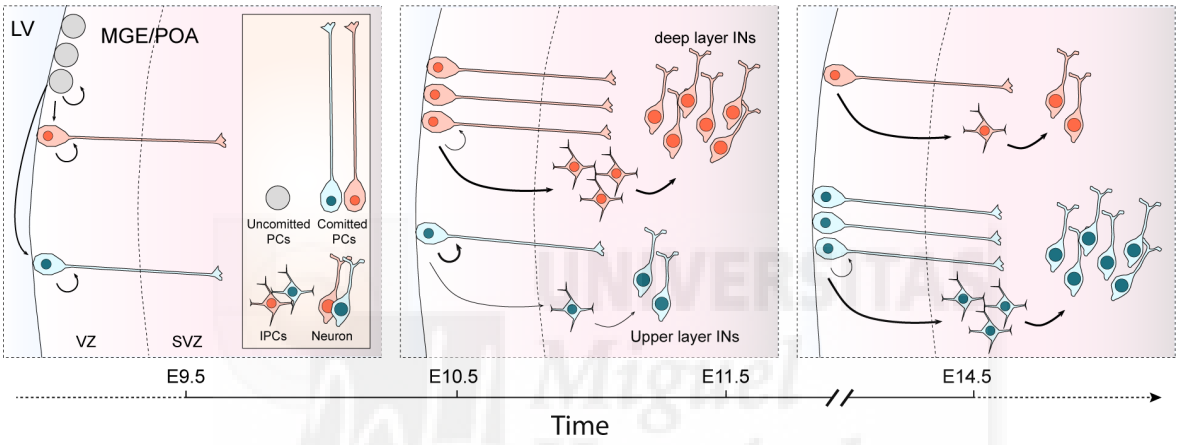

Figure 6. Model of lineage progression for MGE/POA interneurons progenitor cells. The schematic drawings summarize the main conclusion of the study in relation to the generation of MGE-derived cortical interneurons. At E9.5, the embryonic MGE contains pools of uncommitted progenitor cells (gray circles), able to generate interneurons for all the six cortical layers. In between E9.5 and E10.5 progenitor cells segregate in two different lineages: deep layer- (reddish shapes) and superficial-layers (blueish shapes) committed progenitors cells. During development, the embryonic MGE contains both classes of progenitors. At E10.5 and E11.5 the most abundant class produce interneurons for the deep cortical layers at P20, while the other class is very scarce and/or less neurogenic at these stages. Superficial layer committed progenitors are likely self-expanding at early stages to become the predominat progenitor class facing the lateral ventricle at E14.5. Both progenitor classes produce neurons for deep and superficial layers respectively primarily through intermediate progenitor cells that exit the cell cycle routhly sincronously. As result of these processes, MGE-derived interneurons at population level colonize the cerebral cortex in an inside-out pattern that relect at lineage level the relative proportion of the two laminar-restricted classes of progenitor cells.

In our model, the neuroepithelium before E9.5 consists of a pool of uncommitted progenitors able to produce interneurons for all cortical layers. Uncommitted precursors subsequently segregate into two different classes of progenitor cells between E9.5 and E10.5. These two classes are largely pre-specified to produce interneurons for deep and

superficial layers, respectively. The factor(s) that promote the transition between uncommitted to committed interneuron progenitor cells remain to be identified. According to our view, the two classes of progenitor cells co-exist in the embryonic subpallium, but their relative proportions vary during development. Deep layers-committed progenitors are particularly abundant at early stages of development and enter neurogenesis earlier than superficial layers-committed progenitors, which instead constitute the most abundant progenitor class at later stages. Each of these two lineages might go trough different windows of competence in producing different interneuron subtypes within infra- and supragranular layers, respectively. Consequently, at the population level interneurons colonize the neocortex in an inside-out pattern that largely correlates with their birthdate. Our model does not support the link between ontogenic units and the formation of cortical columns (as it has been proposed for example in the classical model of cortical pyramidal cell neurogenesis), but instead relate the inside-out pattern adopted by MGE-derived interneurons in populating the neocortex to the relative ratio of progenitor classes in the germinal zone.

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Part 2.

Development of pyramidal cell lineages in the mammalian cerebral cortex

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Chapter 3. Multipotent and fate restricted lineages coexist in the developing neocortex (manuscript in preparation)

In Part 1 of the Thesis we investigated how lineage relationships influence the final properties and organization of inhibitory interneurons in the mammalian neocortex (Ciceri et al. 2013). Using a combination of the retroviral tracing method and Cre/lox mouse genetics, we targeted interneurons progenitors at different embryonic stages and fate mapped their progenies in the adult necortex. Based on the results described in Part 1 of the Thesis, we proposed a model for the development of *Nkx2-1*-derived interneurons lineages that is based on the existence of committed progenitors cells (**Fig. 6,** Chapter2). In particular, we found that two distinct progenitors classes are pre-specified to produce superficial and deep layers cortical interneurons, respectively, in a model that link progenitor cell diversity with laminar allocation.

Chapter 3: Multipotent and fate restricted lineages coexist in the developing neocortex

INTRODUCTION

The other neuronal class that populate the neocortex consist of excitatory pyramidal cells. Pyramidal neurons represent around 70-80% of the total neuronal populations in the cortex and are involved in regulating the laminar allocation of interneurons (Hevner et al. 2004; Pla et al. 2006; Lodato et al. 2011a). However, it is presently unclear how individual lineages of pyramidal cells organise in the cortex, and how they may influence the distribution of interneurons.

The generation and migration of pyramidal neurons have been extensively studied over the last fifty years (Kriegstein and Noctor 2004). It is well established that cortical pyramidal neurons allocate in the cortex following an inside-out pattern of migration that correlates with their birthdate (Miller 1985; Fairén et al. 1986). This pattern of migration is responsible of the formation of the six layers of the neocortex, with early generated pyramidal cells populating the deep layers and late born progressively occupying more superficial layers. As described in the Introduction, different classes of cortical pyramidal neurons are classified according to the expression of several molecular markers as well as to their axonal projections (Molyneaux et al. 2007). To some extent, the lamination patterns of projection neurons reflect their hodology and marker expression, and therefore the subtype identities. For example, superficial layers primarily contain cortico-cortical pyramidal cells (such as callosal projection neurons, for example), while deep layers are mainly enriched in corticofugal pyramidal cells (Molyneaux et al. 2007). The

transcriptional mechanisms involved in the specification of the different identities of pyramidal cells have been extensively studied over the past few years. These mechanisms function both at the level of progenitor cells as well as during the post-mitotic refinement of neuronal fates (Greig et al. 2013).

During the last years, many different molecular and morphological classes of progenitor cells have been identified. In brief, it is well established that, projection neurons in the neocortex arise first from neuroepitelial cells and later on from radial glial cells (RGCs) that undergo symmetric and asymmetric cell divisions, respectively. RGCs produce neurons primarily through intermediate progenitor cells (IPCs) (Noctor et al. 2001; Noctor et al. 2004) (Englund et al. 2005; Götz and Huttner 2005; Kriegstein and Alvarez-Buylla 2009). However, the lineage progression and the molecular mechanisms that lead to such a peculiar temporal order in the generation of projection neurons remain largely unknown.

One of the most widely accepted models for the generation of neocortical pyramidal cells (referred here as the classical model of neurogenesis) proposes that the same progenitor cell is able to produce excitatory neurons for all the cortical layers, from layer II to layer VI (Rakic 1988). According to this model, the particular temporal order in the production of pyramidal cell subtypes (which, at population level, reflects the inside-out patter of migration and it is responsible for the normal pattern of cortical lamination) reflects the change in potentiality within individual progenitor cells. The classical model implies that progenitor cells are multipotent *in nature* and go through sequential temporal windows of competence; producing first deep layer neurons and later on progressively more superficial ones (Shen et al. 2006). In other words, progenitor cells are progressively restricted during development to give rise to neurons with more superficial layer fates, and thereby generate ontogenic units that organize forming cortical columns.

The observation that molecular markers that identify pyramidal cells subtypes and are often used to define layers in the adult cortex are also expressed at early stages by progenitor cells in embryonic pallium led to the suggestion that subtype identity might be specified at the progenitor cells stage. According to this alternative model, projection neuron diversity arises from pools of pre-specified progenitor cells restricted to produce a particular cell lineage in the cortex, independently on birthdate. Direct evidence supporting the existence of restricted lineages in the neocortex derive from a recent fate-mapping study of Cux2-expressing progenitor cells (Franco et al. 2012). Cux2 is a transcription factor that is highly enriched in layers II/III and IV of the adult cortex, which is also

expressed by SVZ pallial progenitor cells (Nieto et al. 2004; Cubelos et al. 2008);(Franco et al. 2012). Recently, Cux2 expression has also been detected in a subset of Pax6+ RGCs from early stages of neurogenesis onward (Franco et al. 2012). Using in utero electroporation and inducible $Cux2^{CreERT2}$ fate mapping strategies, Franco and colleagues showed that Cux2+ RGCs are fate-restricted *in nature* before the onset of neurogenesis to produce primarily superficial-layer callosal projection neurons. According to these results, at the early stages of development Cux2+ progenitors would primarily divide symmetrically to self-expand and become neurogenic later on during development. In contrast, Cux2- progenitors would be neurogenic at early stages and mainly produce projection neurons for deep layers (Franco and Müller 2013; Marín and Müller 2014). These results have been recently challenged by a study that analysed the distribution of projection neurons derived from Fezf2+ progenitor cells. By using inducible $Fezf2^{CreERT2}$ transgenic mice, the authors of this study conclude that projection neuron progenitors cells are multipotent and give rise to different neuronal subtypes that distribute all along the cortical thickness (Guo et al. 2013). Thus, it is presently unclear whether progenitor cells in the pallium are mulipotent, and generate all classes of excitatory neurons, or some fate-restricted progenitor cells exist that give rise to specific classes of pyramidal cells. Here, we carried out a systematic fate-mapping study of pallial progenitor cells using retroviral-tracing strategies at different time points during development to characterize the contribution of individual pallial progenitor cells to the six layers of the neocortex.

RESULTS

Retroviral labelling of projection neurons lineages in the developing pallium

To study the organization of piramidal cell lineages in the developing neocortex, we used the conditional reporter retroviral tools (rv::dio-gfp and rv::dio-mCherry) that we developed to trace cortical interneuron lineages (Ciceri et al. 2013). As explained in Chapter 1, this method is based on the fact that retroviruses after infection integrate their genome only into mitotic cells, thereby labelling progenitor cells and their progenies with fluorescent reporters. In our system, the expression of the reporter is dependent on Cre recombination, and therefore the injection of rv::dio in Cre-espressing mice allow the specific targeting of different cell populations. In this scenario, the injection of Cre-dependent retroviruses in the lateral ventricle of embyonic brains leads to widespread infection but highly specific labelling exclusively of Cre-expressing cells. To specifically target pyramidal cells, we used *Nex-Cre* mice in which the expression of Cre is driven by

the promoter region of the transcription factor Math2, which is confined to dorsal telencephalic SVZ progenitors and virtually all early cortical pyramidal neurons as soon as they become postmitotic (Goebbels et al. 2006). In these mice, Cre is not expressed in RGCs, and therefore piramidal cells clones cannot be fully visualized at early time points, but their organization can be efficiently assessed in adult brains. To achieve sparse labelling of individual clones in the neocortex, we performed injections using very low titer retroviral stocks at different developmental stages and analyzed the spatial organization of individual clones at P20.

Developmental time-course of projection neuron lineages

To characterize how piramidal cell clones develop in the cerebral cortex, we performed ultrasound-guided low titer injections of conditional reporter retroviruses in *Nex-Cre* embryos at the following embryonic stages: E10.5, E11.5, E12.5 and E14.5. We systematically analysed these experiments by collecting P20 brain sections in serial order and studied the distribution of labelled cells in the entire brain. As expected, labelled cells in these experiments shown the characteristic morphology of projection neurons, with typical pyramidal shaped somas and spiny apical dendrites projecting toward layer I. In P20 Nex-Cre brains injected embryonically with low titer rv::dio-gfp and/or rv::dio-mCherry, pyramidal cells were tipically organized in a few cell clusters spatially segregated one from another that were likely derived from the infection of individual progenitor cells. In other words, the pyramidal cell clusters observed in the P20 brains likely represent ontogenic cortical units. Cells clusters were typically found throughout the entire rostrocaudal extent of the cortex, across different cortical areas without any particular spatial bias.

We first quantified the average size (number of neurons/clone) of individual cell clusters labelled at the different stages of development. We though that this information might be usefull to infer the type of cell divisions (proliferative *vs*. neurogenic) that progenitor cells might use at each particular stage. Retroviral injections at E10.5 labelled clones formed at P20 by ~60 neurons (59.62 ± 8.03; n = 21 clones from 3 different brains; average neuron number/clone ± s.e.m). The cluster size dramatically decreases when progenitors are labelled one or two day laters (E11.5: 13.27 ± 1,03; *n* = 37 clones from 3 different brains; E12.5: 5.74 ± 0.3; *n* = 57 clones from 4 different brains; average neuron number/clone ± s.e.m) indicating that E10.5/E11.5 progenitor cells are likely

symmetrically self-expanding, while between E11.5 and E12.5 RGCs become neurogenic and produce an average of 5 or 6 neurons (**Fig. 1a**).

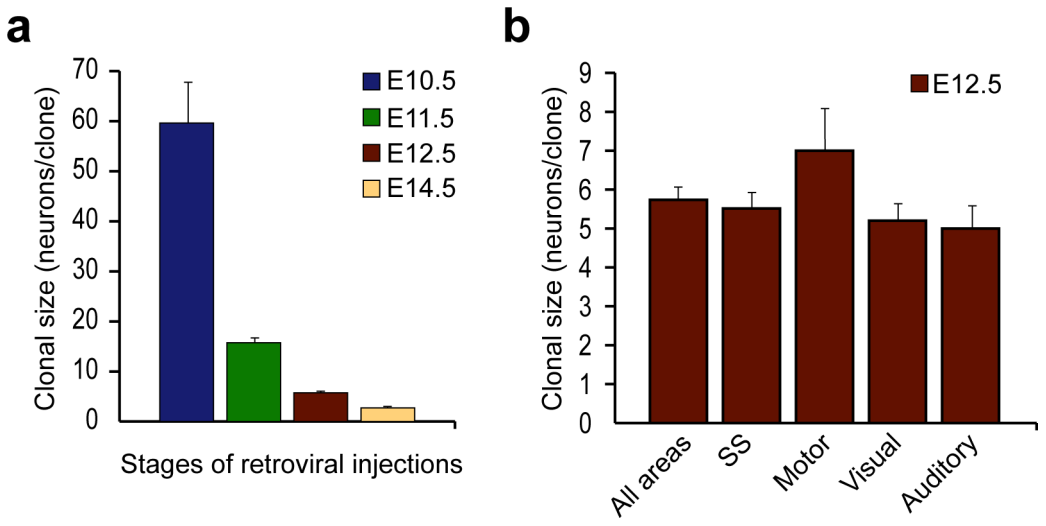

Figure 1. Size of pyramidal cells clones labelled at different time points during embryonic development. (**a**) Quantification of the size (number of neurons/clone) of pyramidal cell clones in P20 Nex-cre mice labelled with conditional reporter retroviruses at the following embryonic stages: E10.5, E11.5, E12.5 and E14.5. (**b**) Quantification of the size (number of neurons/clone) of pyramidal cell clones in P20 Nex-cre mice labelled with conditional reporter retroviruses at E12.5. The graph shows the clonal size across different cortical areas. SS; somatosensory cortex. Histograms depict average ± s.e.m

No differences were observed across different cortical areas (**Fig. 1b**). These results suggest that retroviral tracing at E12.5 mainly labels sibling cells derived from individual neurogenic RGCs, while injections at earlier time points result in the labelling ontogenic units derived from multiple sibling RGCs. We are currently performing a series of short-term experiments injecting conventional Gfp-expressing retroviruses in wild type mice at each developmental stage to directly analize the proliferation dynamics and clonal composition in Pax6+ RGCs, Tbr2+ IPCs and Tuj1+ young neurons.

Spatial organization of projection neuron lineages

We next analyzed the spatial organization of piramidal neuron clusters labelled at different times during developmental stages by quantifying the laminar position of individual cells within segregated cell clusters at P20. We first analysed P20 *Nex-Cre* brains injected with conditional reportes retroviruses at E10.5. In the particular example shown in figure **2a-c**, the only ontogenic unit labelled in that brain is the one shown. Overall, our analysis

revealed that virtually all clones labelled at this stage distribute through the entire cortical thickness and populate all cortical layers from layer VI to layer II/III (**Fig. 2d**).

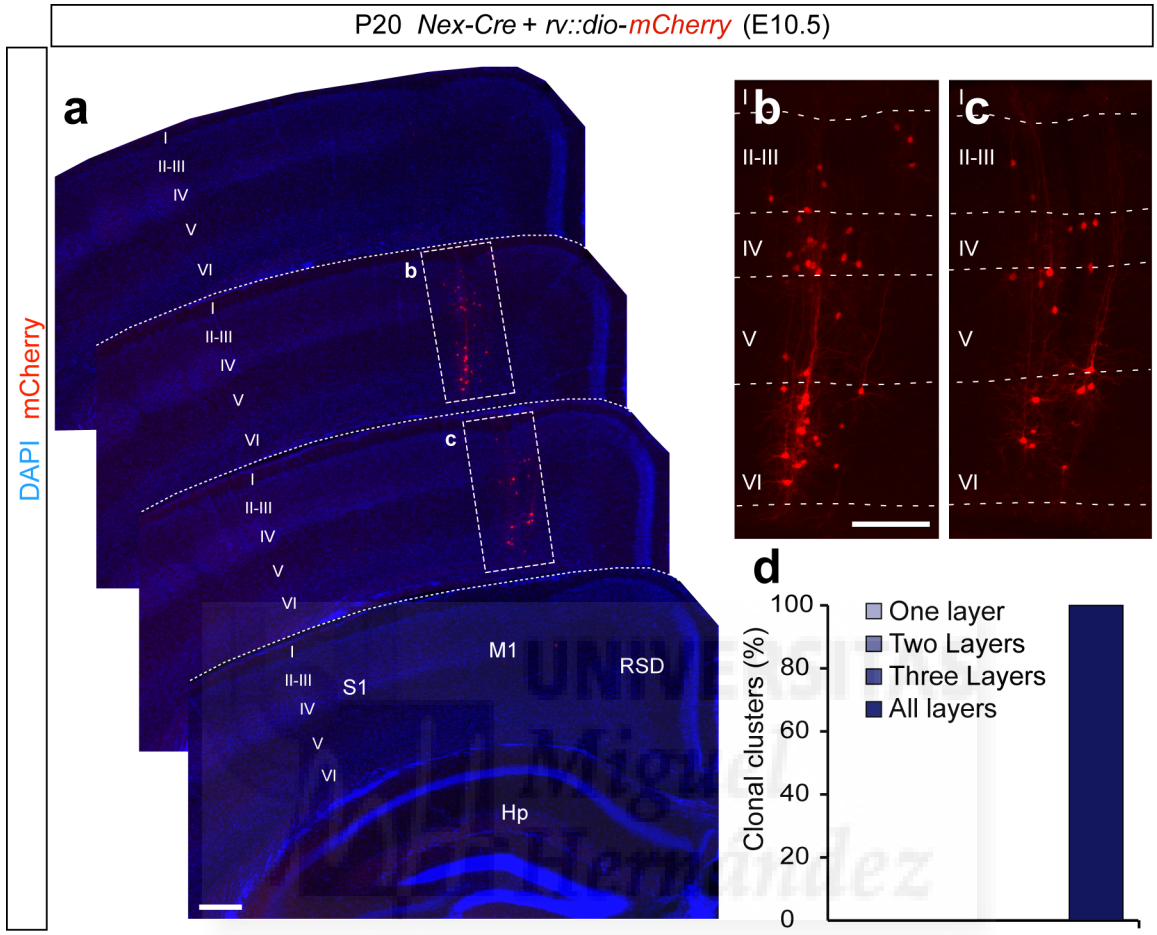

Figure 2. Distribution of PCs lineages labelled at E10.5. (**a**) Serial coronal sections (100 μm thick) through the telencephalon of a P20 Nex-cre mouse infected with low titer conditional reporter retroviruses at E10.5, stained with DAPI and antibodies against mCherry. (**b, c**) High magnification of region boxed in (a), mCherry channel only. (**d**) Quantification of the relative abundance of labeled clonal cell clusters classified according to the laminar position of labelled cells. Clusters are classified in one-layer, two-layers, three-layers and all layers, depending on the distribution of labelled cells. Histograms depict average ± s.e.m. I-VI, cortical layers I to VI; Hp, hippocampus; M1, primary motor cortex; RSD, retrospinal cortex; S1, primary somatosensory cortex. Scale bars: 300 μm (a), 100 μm (b, c).

In contrast, analysis of clones labelled after retroviral infections at E11.5 revealed diverse configurations, with ~50% of the clusters containing neurons that span through all cortical layers (56.62 ± 12.38 %; clusters spanning layers VI to II/II; n = 37 clones from 3 different brains **Fig. 3a, f-j**), while ~35% of the clusters contained neurons that distribute only through three cortical layers (36.24 ± 7.42 %, clusters spanning three cortical layers; n = 37 clones from 3 different brains; **Fig. 3a-e, j**).

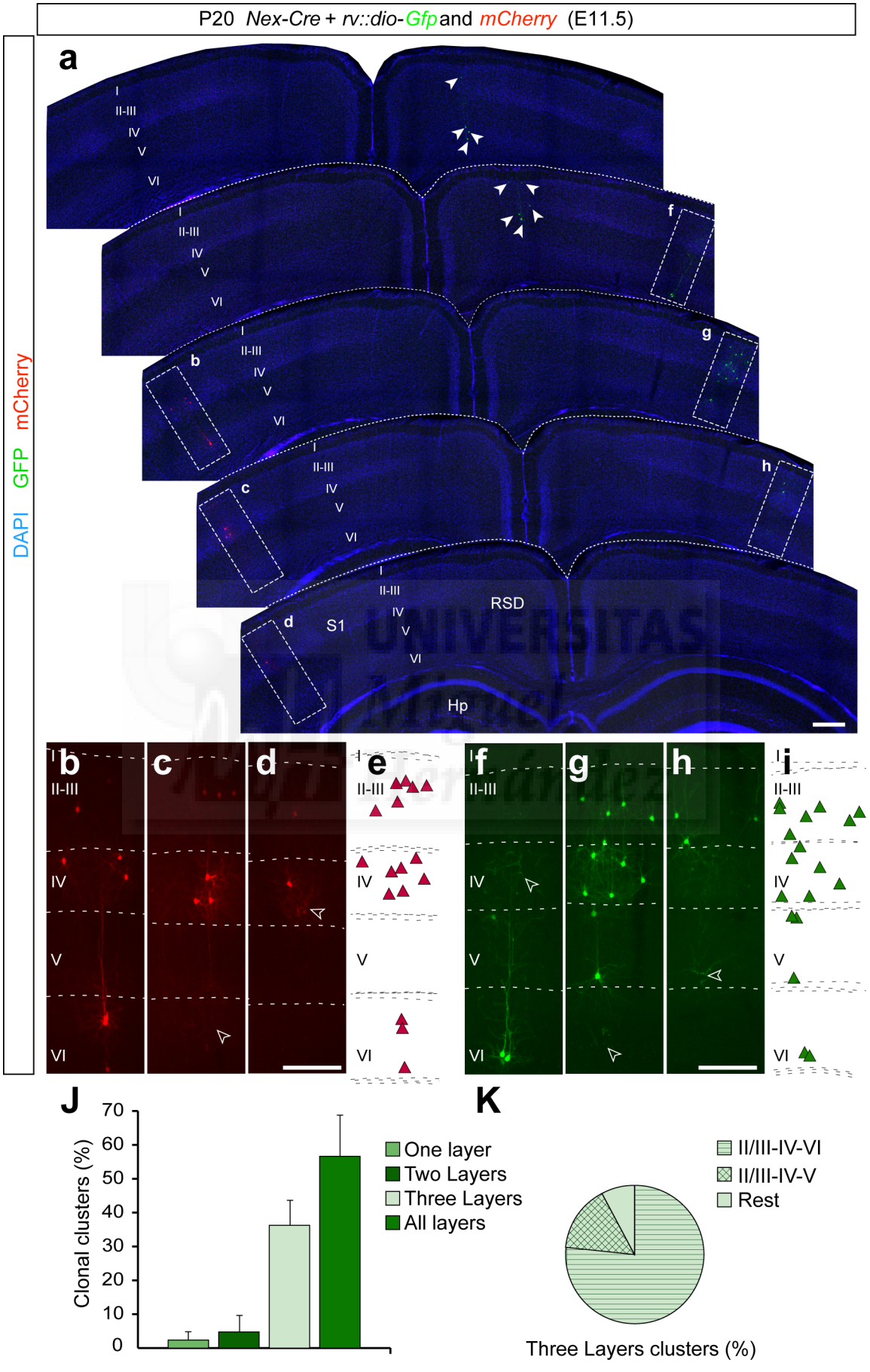
P20 Nex-Cre + rv::dio-Gfp and mCherry (E11.5)
DAPI GFP mCherry
a
I
II-III
IV
V
VI
b
c
d
f
g
h
RSD
S1
Hp
b
c
d
e
f
g
h
i
J
Clonal clusters (%)
70
60
50
40
30
20
10
0
One layer
Two Layers
Three Layers
All layers
K
II/III-IV-VI
II/III-IV-V
Rest
Three Layers clusters (%)

Figure 3. Distribution of PCs lineages labelled at E11.5. (**a**) Serial coronal sections (100 μm thick) through the telencephalon of a P20 Nex-cre mouse infected with low titer conditional reporter retroviruses at E11.5, stained with DAPI and antibodies against mCherry and gfp. (**b-c, f-h**) High magnifications of region boxed in (a) that show two examples of clonal clusters labelled at E11.5. mCherry (b-c) and gfp (f-h) channel only. (**e, i**) Schematic representation of the clonal clusters obtained by the superposition of cells found in the serial sections shown in (b-d) and (f-h) respectively. (**j**) Quantification of the relative abundance of labeled clonal cell clusters classified according to the laminar position of labelled cells. Clusters are classified in one-layer, two-layers, three-layers and all layers, depending on the distribution of labelled cells. (**k**) Quantification of the laminar distribution of three-layers pyramidal cell clusters. Histograms depict average ± s.e.m. I-VI, cortical layers I to VI; Hp, hippocampus; M1, primary motor cortex; RSD, retrospinal cortex; S1, primary somatosensory cortex. Scale bars: 300 μm (a), 100 μm (b-i).

Interestingly, of these clusters, ~77% (corresponding to ~27% of the total number of the E11.5 clusters) contained cells populating layer VI, IV and II/III, but not layer V (n = 10/37 clusters from 3 different brain; **Fig. 3b-e, k**). These results are unexpected, because they imply that almost a third of the RGCs labelled at E11.5 does not produce layer V pyramidal cells, while they are able to produce cells for the other cortical layers.

We then analysed the spatial organization of ontogenic units labelled by retroviral infection at E12.5, stage at which our previous results suggested that RGCs are primarily neurogenic. Analysis of P20 *Nex-cre* mice injected at E12.5 revealed an even more diverse picture in the laminar distribution of cell clusters. About 40% of the clusters contain neurons populating three different layers (43.34 ± 7.07 %; clusters spanning three cortical layers**;** *n* = 57 clones from 4 different brains **Fig 4a-d; Fig. 5a**). Insterestingly, and consistently with the fate-mapping experiments at E11.5, ~56% of these clones (corresponding to ~23% of the total clones labelled at E12.5) lack layer V neurons (n = 13/57 clones from 4 different brain; **Fig. 5d**). In addition, ~22% of the clones labelled at E12.5 were confined within a single cortical layer and another ~20% was composed by cells spanning two different cortical layers (22.15 ± 8.38 %; clusters confined to one cortical layer; 21.12 ± 4.58 %, clusters spanning two cortical layers; *n* = 57 clones from 4 different brains **Fig. 4e-l; Fig 5a**). It is worth noting, that intralaminar and two-layer clones were mostly restricted to either infragranular (layer VI and/or V) or supragranular (layer II/III and/or IV) cortical layers (**Fig 4 e-l; Fig. 5 a-c**).

Finally we analized the spatial organization of pyramidal cell lineages labelled by retroviral infection at E14.5. These analyses revealed, that virtually all pyramidal lineages labelled at this state populate the same or two adjacent layers (**Fig. 6a-d**). Moreover, according to the inside-out pattern of pyramidal cells migration, these lineages were entirely confined to the superficial cortical layers (**Fig. 6e-f**).

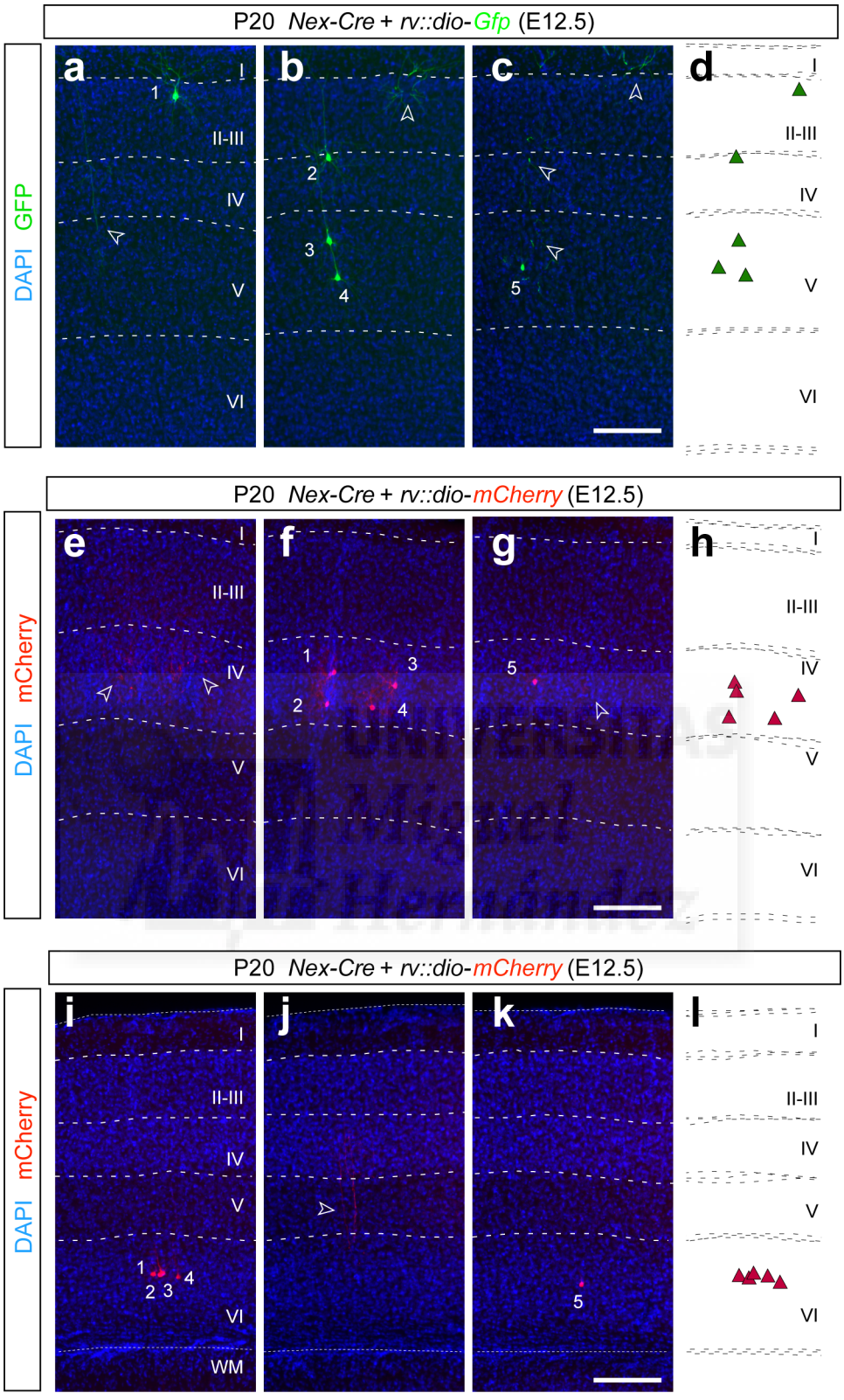

Figure 4. Distribution of PCs lineages labelled at E12.5. (**a-c; e-g; i-k**) Serial coronal sections (100 μm thick) through the telencephalon of P20 Nex-cre mice infected with low titer conditional reporter retroviruses at E12.5, stained with DAPI and antibodies against mCherry and gfp. (d, h, l) Schematic representations of the three different clonal clusters shown in (a-c), (e-g) and (i-k) respectively, obtained by the superposition of cells found in the cortical sections. I-VI, cortical layers I to VI; WM, white matter. Scale bars: 100 μm.

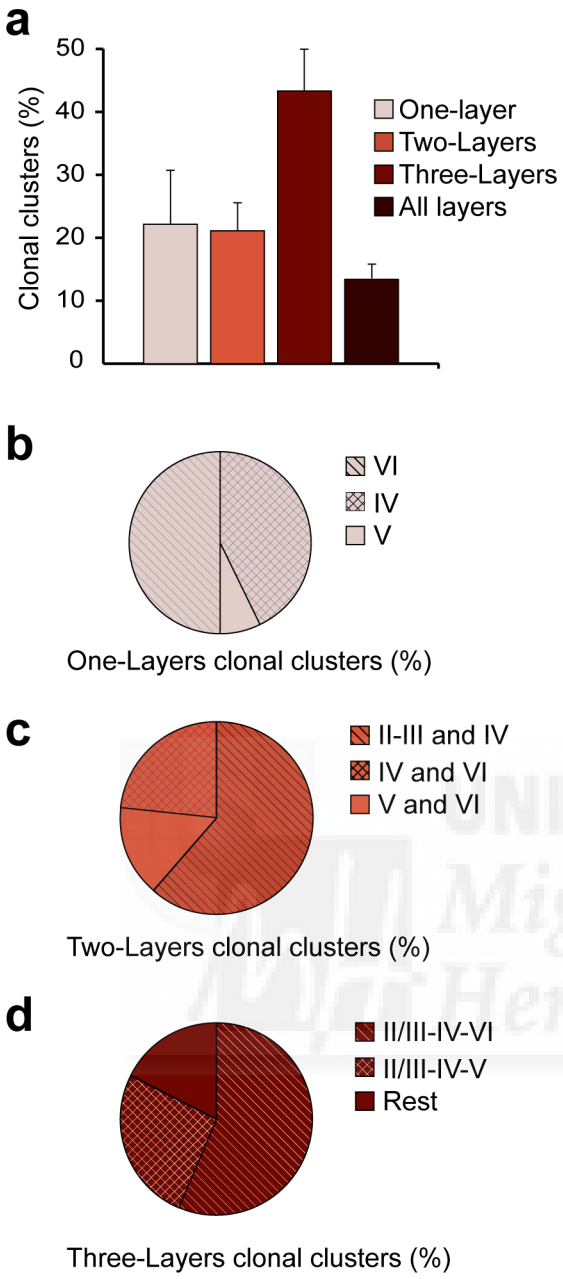

Figure 5. Quantification Distribution of PCs lineages labelled at E12.5.
(**a**) Quantification of the relative abundance of P20 clonal cell clusters labelled by contional reporter retroviral infections in E12.5 Nex-cre embryos. Clonal clusters are classified in one-layer, two-layers, three-layers and all layers, depending on the distribution of labelled cells.
(**b**) Quantification of the laminar distribution of one-layers pyramidal cell clusters.
(**c**) Quantification of the laminar distribution of two-layers pyramidal cell clusters.
(**d**) Quantification of the laminar distribution of three-layers pyramidal cell clusters. Histograms depict average ± s.e.m. I-VI, cortical layers I to VI.

DISCUSSION

Pallial progenitor cell diversity: possible models of lineage progression

Overall, the retroviral tracing experiments of pyramidal neurons progenitors reveal a substantial diversity in their behaviour and laminar outcome during development. Our results suggest that E10.5 retroviral injections primarily labelled neuroepithelial cells or early self-renewing RGCs. Therephore, at this stage our tracing targets lineages derived from multiple sibling RGCs that distribute in all the six cortical layers.

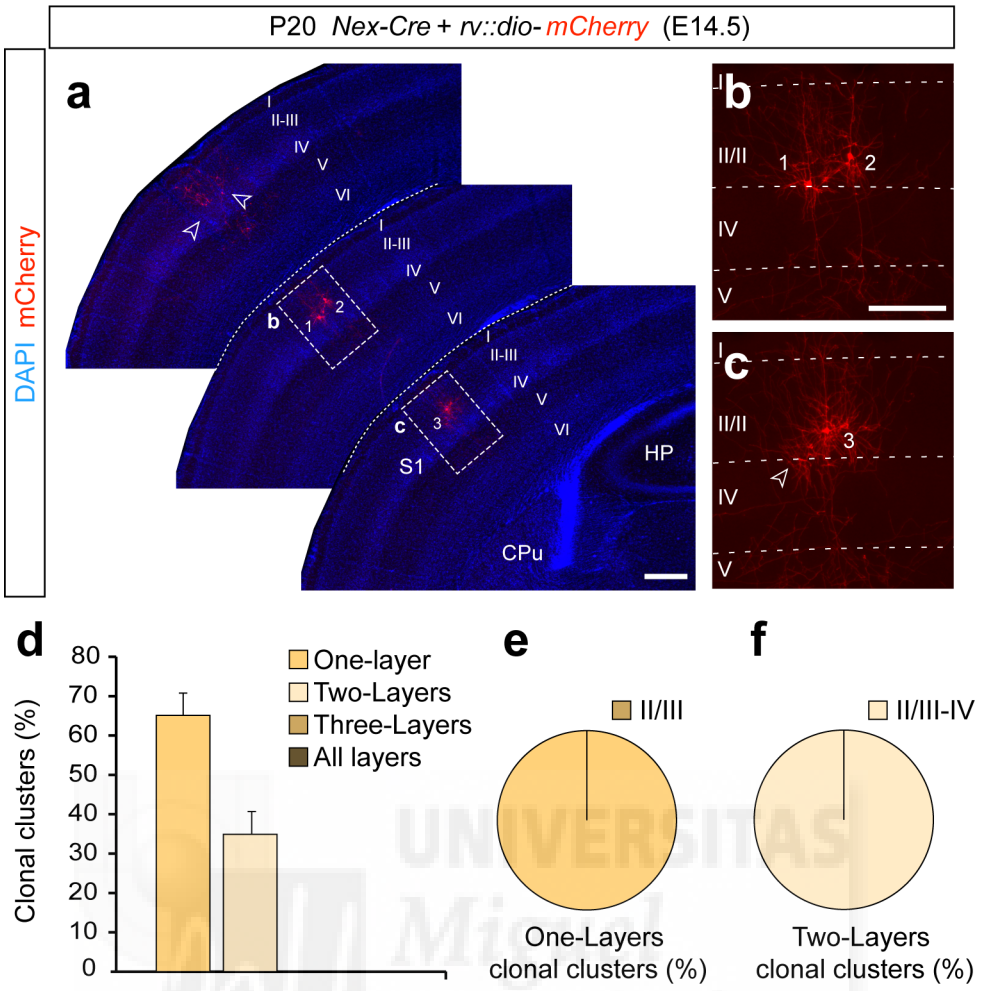

Figure 6. Distribution of PCs lineages labelled at E14.5. (**a**) Serial coronal sections (100 μm thick) through the telencephalon of a P20 Nex-cre mouse infected with low titer conditional reporter retroviruses at E14.5, stained with DAPI and antibodies against mCherry. (**b,c**) High magnifications of region boxed in (a). mCherry channel only. (**d**) Quantification of the relative abundance of labeled clonal cell clusters classified according to the laminar position of labelled cells. Clusters are classified in one-layer, two-layers, three-layers and all layers, depending on the distribution of labelled cells. (**e, f**) Quantification of the laminar distribution of one- and two-layers pyramidal cell clusters respectively. Histograms depict average ± s.e.m. I-VI, cortical layers I to VI; Cpu, caudatum putamen; Hp, hippocampus; S1, primary somatosensory cortex. Scale bars: 300 μm (a), 100 μm (b, c).

Starting from E11.5, lineage distribution across layers shown a more diverse picture. In particular, the existence of lineages lacking layer V neurons reveals a new pattern of pyramidal cells clonal distribution that was not reported before. This pattern is still present at E12.5. Therefore, these results suggest that at least in some cases, RGCs may skip the production of layer V cells, which provide a clear "exception" to the classical model of neurogenesis. Alternatively, the layer V cells of these clones underwent selective cell death at some point during developmet. Further analyses will clarify this issue. Moreover, the

analysis of the pyramidal cell subtypes will provide a more detailed description of these clones.

Retroviral injections at E12.5 label also neuronal lineages that are restricted to either infragranular of supragranular cortical layer. These evidences are consistent with previous data in litterature (Franco et al. 2012) and suggest that at least the half part of the pallial progenitors at early neurogenesis (E12.5) give rise to pyramidal cell lineages confined to either infra- or supra-granular layers. However, as previously mention, retroviruses integrate their genome into the host cells genome during the first round of cell division. Because, theoretically, the events of superinfection of the same cell by multiple viruses are really rare, each retroviral infection results in labelling one of the two newborn cells. This implies that during asymmetric neurogenic cell division the labelling with the fluorescent reporter occurs in either the self-renewing RGCs or the IPCs/post-mitotic cells with the same probability (in 50% of the cases). For this reason, it is still unclear if the laminar restriction of pyramidal cell lineages observed in fate-mapping experiments at E12.5 derived from labelling of primary RGCs and consequently represents entire clones; or if this laminar restriction derived from labelling of committed IPCs that represents only a part of the clone. The development of new tools for lineage tracing as well as further study on the contribution of the different progenitor types on pyramidal neuron diversity will answer this important question.

EXPERIMENTAL PROCEDURES

mouse strains. Nex-cre mice (Goebbels et al. 2006) were kept at the Instituto de Neurociencias. All animal procedures were approved by the corresponding ethical committees (IN-CSIC and CEEA-PRBB) and were performed in accordance with Spanish (law 32/2007) and European regulations (EU directive 86/609, EU decree 2001-486). The day of vaginal plug was considered to be embryonic day (E) 0.5 and the day of birth postnatal day (P) 0.

DNA construct and retrovirus production. Cre-dependent conditional reporter retroviral constructs (rv::dio) were generated by subcloning a modified version of a double-floxed inverted open reading frame cassette into BamH1 and PmeI restriction sites of a retroviral backbone kindly provided by F.H. Gage. This vector contains an internal CAG chicken -actin promoter and the woodchuck hepa- titis post-transcriptional regulatory element

(WPRE), and encodes either GFP (enhanced) or membrane-bound (palmitoylation tag) mCherry as a reporter fluorescent protein (Ciceri et al. 2013). Moloney murine leukemia viruses (MoMLV) were produced by transfecting HEK293T cells with the corresponding vectors along with CMV-vsvg and CMV-gagpol helper plasmids, and concentrated as previously described (Tashiro et al. 2006).

In utero **retroviral infection.** Pregnant females were deeply anesthetized with isoflurane and E10.5, E11.4, E12.5 and E14.5 embryos, depending on the experiment, were individually injected using an ultrasound backscattering microscope (Visualsonic), as described previously (Pla et al. 2006). Low-titer conditional retroviruses were released into the telencephalic ventricles using a nanoliter injector (Gaiano et al. 1999).

Immunohistochemistry. Postnatal mice were perfused transcardially with 4% paraformaldehyde (PFA) in PBS and the dissected brains were postfixed for 2 h at 4 °C in the same solution. Brains were sectioned at 100 μm on a vibratome (VT1000S, Leica) and free-floating coronal sections were then subsequently processed for immunohistochemistry as previously described (Pla et al. 2006). The following primary antibodies were used: rabbit anti-GFP (1:1,000; A11122, Molecular Probes), chicken anti-GFP (1:1,000; GFP-1020, Aves Labs), rabbit anti-DsRed (1:500; 632496, Clontech). Cell nuclei were stained with 5 μM 4′-6-diamidino-2-phenylindole (DAPI) in PBS and sections mounted with Mowiol (Sigma) with NPG (Calbiochem).

Imaging. Images were acquired using fluorescence microscopes (DM5000B/ CTR5000 and DMIRB; Leica) coupled to digital cameras (DC500 or DFC350FX, Leica; OrcaR2, Hamamatsu) or a confocal microscope (DMIRE2/ CTRMIC/TCS SP2; Leica).

Part 3.

Integration of inhibitory and excitatory neurons in cortical structures

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Chapter 4. Integration of GABAergic interneurons into cortical cell assemby: Lessons from embryos and adult (Neuron. 2013 79:849-64)

Chapter 4. Integration of GABAergic interneurons into cortical cell assemby: Lessons from embryos and adult

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Neuron
Review

Integration of GABAergic Interneurons into Cortical Cell Assemblies: Lessons from Embryos and Adults

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In the forebrain, cortical structures consist of networks of excitatory and inhibitory neurons born in distant locations. Understanding how these two major classes of neurons integrate into unique functional cell assemblies may shed light on the organization of cortical circuits. In this review, we provide an overview of the mechanisms used by GABAergic interneurons to reach their final position, with an emphasis on the final steps of this process. To this end, we analyze similarities and differences between the integration of GABAergic interneurons in the developing cerebral cortex and in the postnatal brain, using the neocortex and the olfactory bulb as model systems.

Introduction

From a reductionistic perspective, many brain circuits have evolved as hierarchical networks of excitatory glutamatergic neurons and γ-aminobutyric acid-containing (GABAergic) interneurons. In the telencephalon, for example, cortical structures consist of excitatory and inhibitory neuronal assemblies independent of their complexity and function. Accordingly, functional circuits in regions as disparate as the olfactory bulb, hippocampus, and neocortex rely on relatively similar cell assemblies of glutamatergic neurons and GABAergic interneurons. Glutamatergic neurons are the main excitatory units in these networks, typically linked through multiple recurrent connections that are critical for computational performance (Binzegger et al., 2004; Somogyi et al., 1998). GABAergic interneurons, on the other hand, comprise a highly heterogeneous group of neurons that maintain the stability of cortical networks through synaptic inhibition. In addition, interneurons modulate network activity by shaping the spatiotemporal dynamics of different forms of synchronized oscillations (Klausberger and Somogyi, 2008).

The organization of neuronal assemblies in the cortex seems to obey certain rules that guarantee a critical balance between excitation and inhibition while maximizing their computational ability. In the cerebral cortex, for example, the ratio between excitatory and inhibitory neurons is relatively constant across regions and species (Fishell and Rudy, 2011; Hendry et al., 1987; Sahara et al., 2012). In the adult olfactory bulb, where interneurons are continuously added throughout life, the proportion of newborn neurons that integrates into the mature network is tightly regulated (Kohwi et al., 2007; Winner et al., 2002). In addition, GABAergic interneurons in the cerebral cortex and olfactory bulb come in a rich variety of classes, each having highly stereotypical laminar arrangements, unique patterns of connectivity, and functions (Fishell and Rudy, 2011; Klausberger and Somogyi, 2008; Lledo et al., 2008). This enormous variety of interneuron classes provides cortical circuits with the required flexibility to carry out complex computational operations during information processing.

Considering the highly stereotypical organization of cortical networks, the most striking aspect of their assembly is that their cellular ingredients are born in separate locations. While glutamatergic neurons of the olfactory bulb and the cerebral cortex are generated locally by progenitor cells in the developing pallium (Molyneaux et al., 2007; Rakic, 2007), GABAergic interneurons populating these structures derive from the subpallium, the base of the telencephalon (Batista-Brito and Fishell, 2009; Gelman and Marín, 2010; Wonders and Anderson, 2006). Consequently, glutamatergic neurons and GABAergic interneurons follow very different strategies to reach their final destination. Glutamatergic neurons migrate radially to form the different layers of cortical structures (Rakic, 2006). In contrast, interneurons first migrate tangentially from their birthplace to the cerebral cortex and olfactory bulb and subsequently switch their mode of migration to radial to adopt their final position in these structures (Marín and Rubenstein, 2001). How these apparently disconnected processes synchronize during development is arguably one of the most fascinating questions on the assembly of neuronal circuits in the mammalian brain.

The purpose of this review is to summarize our current understanding of the mechanisms controlling the coordinated integration of glutamatergic neurons and GABAergic interneurons into cortical networks. The emphasis is on those aspects related to the final settlement of GABAergic interneurons in the cerebral cortex and olfactory bulb, and not so much on the mechanisms controlling their tangential migration to their target structures (reviewed in Belvindrah et al., 2009; Marín, 2013). The developing neocortex is used here as a model for the coordinated integration of glutamatergic neurons and GABAergic interneurons into nascent cortical circuits, while the adult olfactory bulb illustrates the ability of newborn GABAergic interneurons to integrate into fully mature networks.

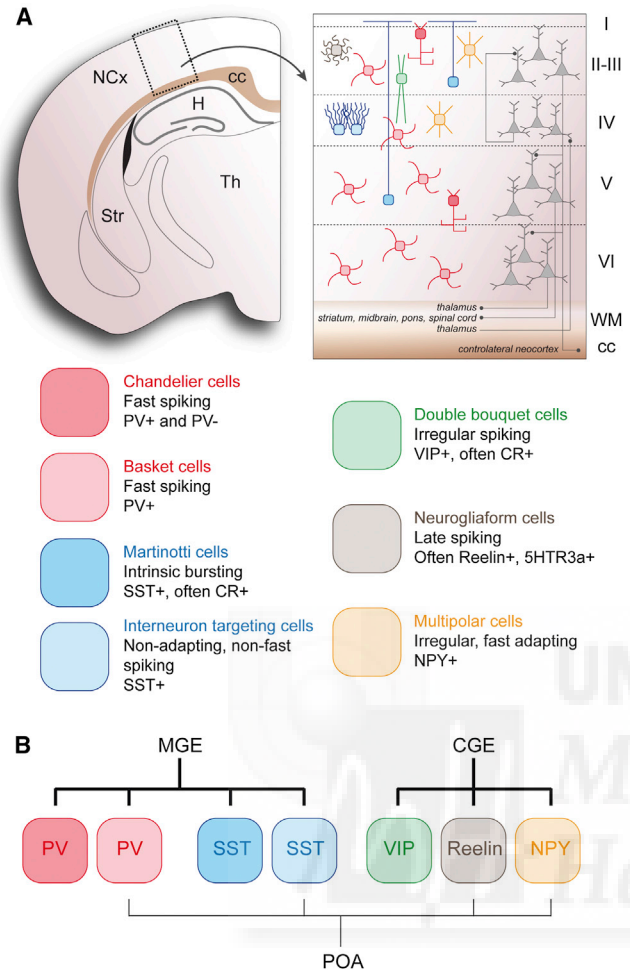

Figure 1. Major Classes of Neocortical Interneurons and Their Developmental Origins

(A) Schematic of a coronal section through the mouse cerebral cortex showing the main classes of GABAergic interneurons and their respective laminar allocation. Fast-spiking PV^+ basket cells are distributed throughout all cortical layers except for layer I. Chandelier cells localize primarily to the border between layers I and II/III, and in layer V. SST^+ Martinotti cells are mainly found in layers II/III and V and extend their axon toward layer I. Non-fast-spiking, nonadapting SST^+ interneurons are restricted to layer IV. Rapidly adapting VIP^+ interneurons and late-spiking neurogliaform cells are particularly abundant in layer II/III. Finally, multipolar cells that often contain NPY are found through layers II/III and IV. The laminar organization of pyramidal cells is also schematically represented.

(B) Grouping of the main classes of cortical interneurons according to their developmental origins. cc, corpus callosum; HC, hippocampus; NCx, neocortex; Str, striatum; Th, thalamus; WM, white matter; MGE, medial ganglionic eminence; CGE, caudal ganglionic eminence; POA, preoptic area; I–VI, cortical layers I to VI; 5HTR3a, ionotropic serotonin receptor 3a.

Integration of GABAergic Interneurons in the Developing Cortex

Glutamatergic pyramidal cells and inhibitory GABAergic interneurons constitute the main cellular elements of each of the individual modules or microcircuits of the cerebral cortex. Pyramidal cells represent about 80% of the neurons in the cortex and specialize in transmitting information between different cortical areas and to other regions of the brain. GABAergic interneurons, on the other hand, control and orchestrate the activity of pyramidal cells.

Pyramidal cells are a highly heterogeneous group of neurons with different morphological, neurochemical, and electrophysiological features. A basic classification of pyramidal cells is based on their connectivity, which is roughly linked to their laminar location in the cortex (Jones, 1984) (Figure 1). Subcortical projection pyramidal cells are the main neurons in layers V and VI. They target the thalamus (layer VI) and other telencephalic and subcerebral regions, such as the striatum, midbrain, pons, and spinal cord (layer V pyramidal cells). Pyramidal cells in layer IV, the granular layer, are associative neurons that project to pyramidal cells in layers II/III. Finally, callosal projection pyramidal cells project to the contralateral cortex and are particularly abundant in layers II/III. Some of these pyramidal cells are also present in layers V and VI. Layer II/III pyramidal cells also project abundantly to infragranular pyramidal cells.

More than 20 different classes of interneurons have been identified in the hippocampus and neocortex, each of them with distinctive spatial and temporal capabilities to influence cortical circuits (Fishell and Rudy, 2011; Klausberger and Somogyi, 2008). The classification of interneurons is a remarkably complicated task because their unequivocal identification requires a combination of morphological, neurochemical, and electrophysiological properties (Ascoli et al., 2008; DeFelipe et al., 2013). For the purpose of this review, neocortical interneurons can be broadly classified into five categories (Figure 1). The most abundant group consists of interneurons with the electrophysiological signature of fast-spiking neurons. It includes two main classes of interneurons: basket cells and chandelier cells (Markram et al., 2004). Most fast-spiking interneurons express the calcium binding protein parvalbumin (PV), although many chandelier cells do not (Taniguchi et al., 2013). A second group of interneurons is characterized by the expression of the neuropeptide somatostatin (SST). It includes interneurons with intrinsic-burst-spiking or adapting nonfast-spiking electrophysiological profiles and includes at least two different classes of interneurons. Martinotti cells, with a characteristic axon extending into layer I, are the most abundant SST^+ interneurons (Ma et al., 2006; Xu et al., 2013). In addition, a second class of SST^+ interneurons with axons that branch abundantly near the cell soma has been identified (Ma et al., 2006; Xu et al., 2013). The third major group of neocortical interneurons includes rapidly adapting interneurons with bipolar or double-bouquet morphologies, which typically express the vasointestinal peptide (VIP) and may also contain the calcium binding protein calretinin (CR) (Rudy et al., 2011). Neurogliaform cells constitute a fourth large group of neocortical interneurons (Armstrong et al., 2012). They have a very characteristic morphology, with highly branched short dendrites and a defining dense local axonal plexus. Neurogliaform cells have a late-spiking firing pattern, and many express Reelin and the ionotropic serotonin receptor 3a. Finally, a fifth group of interneurons consists of multipolar cells with irregular or rapidly adapting electrophysiological properties that often contain neuropeptide Y (NPY) (Lee et al., 2010). As explained below, the different classes of interneurons distribute through the cerebral cortex following highly specific regional and laminar patterns. This remarkable degree of organization suggests that the functional

integration of interneurons into specific neuronal circuits is largely dependent on their precise positioning within the cortex.

Pyramidal cells and interneurons are organized along two main dimensions in the cerebral cortex. The first axis divides the cortex into a variable number of layers depending on the cortical area. Neurons within the same cortical layer share important features, including general patterns of connectivity (Dantzker and Callaway, 2000; Molyneaux et al., 2007). The second axis reflects the vertical organization of neuronal circuits within a column of cortical tissue. Neurons within a given column are stereotypically interconnected in the radial dimension, share extrinsic connectivity, and function as the basic units underlying cortical operations (Mountcastle, 1997). Thus, any given cortical area consists of a sequence of columns in which their main cellular constituents, pyramidal cells and interneurons, share a common laminar organization. From this perspective, the integration of GABAergic interneurons within the organized matrix of layers and columns that compose the cortex might be better understood as a sequence of events that first determine the specific rostrocaudal and mediolateral coordinates of interneurons in the tangential plane (i.e., regional distribution) and subsequently determine their precise layering within the radial axis (i.e., laminar distribution).

Regional Distribution of Cortical Interneurons

As local circuit neurons, interneurons could be potentially incorporated in any cortical region. The question is whether interneurons are specified to migrate to precise locations or they just colonize the cerebral cortex without being targeted to specific coordinates. In other words, is there a correlation between their site of origin within the subpallium and their distribution along the rostrocaudal and mediolateral dimensions of the cortex?

Multiple lines of evidence suggest that the different classes of cortical interneurons are born in specific regions of the subpallium (Gelman and Marín, 2010; Wonders and Anderson, 2006) (Figure 1). In brief, the embryonic subpallium has five major proliferative regions: the lateral, medial, and caudal ganglionic eminences (LGE, MGE, and CGE, respectively), the preoptic area (POA), and the septum. The large majority of PV^+ and SST^+ interneurons derive from the MGE (Butt et al., 2005; Flames et al., 2007; Fogarty et al., 2007; Inan et al., 2012; Taniguchi et al., 2013; Wichterle et al., 2001; Xu et al., 2004, 2008). In turn, the CGE gives rise to most of the remaining interneurons, including bipolar VIP^+ interneurons, most neurogliaform neurons, and NPY^+ multipolar interneurons (Butt et al., 2005; Miyoshi et al., 2010; Nery et al., 2002; Xu et al., 2004). Finally, the POA generates a small, but diverse, contingent of PV^+, SST^+, and NPY^+ interneurons (Gelman et al., 2009, 2011).

Although the vast majority of cortical interneurons originate in the embryonic subpallium and migrate as postmitotic cells toward the cortex, postnatal sources of cortical interneurons seem to exist. One of these has been identified in the dorsal white matter and comprises what seems to be an expanding pool of progenitor cells possibly derived from the LGE and/or CGE (Riccio et al., 2012; Wu et al., 2011). Interestingly, these interneurons appear to follow a unique specification program and differentiate later than interneurons born in the embryo. Interneurons from this source populate primarily the lower layers of the anterior cingulate cortex. In addition, the adult subventricular zone (SVZ), the main postnatal source of olfactory bulb interneurons, also seems to give rise to some interneurons that populate forebrain structures other than the olfactory bulb, including the neocortex, caudoputamen nucleus, and nucleus accumbens (Inta et al., 2008). Intriguingly, some of the SVZ-derived interneurons that populate the deep layers of the frontal cortex share some morphological and functional features with olfactory bulb interneurons. They are small, axonless neurons that establish dendrodendritic synapses and integrate into the network in an experience-dependent manner (Le Magueresse et al., 2011).

These studies suggest that specific classes of interneurons derive from distinct regions of the subpallium to later colonize multiple cortical structures. Fast-spiking interneurons are a clear example of this circumstance. Transplantation and genetic fate-mapping studies have shown that the MGE is the origin of fast-spiking interneurons found in the amygdala, striatum, piriform cortex, hippocampus, and neocortex (Marín et al., 2000; Pleasure et al., 2000; Tricoire et al., 2011; Wichterle et al., 2001; Xu et al., 2008). Several lines of evidence suggest that distinct pools of progenitor cells within the MGE are specified to produce interneurons for each of these telencephalic structures. For instance, striatal and cortical interneurons seem to derive from different progenitor pools within the MGE (Flandin et al., 2010). Consistent with this notion, striatal and cortical interneurons are specified to reach their targets by expressing different complements of guidance receptors (Marín et al., 2001; Nóbrega-Pereira et al., 2008; van den Berghe et al., 2013). In addition, the hippocampus contains certain classes of interneurons that do not seem to have a clear homolog in the neocortex, such as PV^+/SST^+ bistratified cells (Buhl et al., 1994). Similarly, VIP^+ interneurons populate the cortex and the hippocampus but are absent from the striatum. Thus, it is conceivable that different pools of progenitor cells within the subpallium are specified to generate interneurons that migrate to specific subdivisions of the telencephalon (i.e., striatum, amygdala, neocortex, hippocampus).

Does the same rule apply for different neocortical regions? If this were the case, then one would expect to observe a topographical relationship between the origin of a specific class of interneurons within the subpallium and their final distribution in the neocortex. Transplantation experiments in slices have shown that the mediolateral distribution of GABAergic interneurons in the neocortex is not topographically related to their birthplace. So, irrespective of the site of origin in the MGE, interneurons tend to colonize the neocortex following a lateral to medial progression (Lourenço et al., 2012), in parallel to the normal maturation gradient of pyramidal cells (Bayer and Altman, 1987). Consistent with this notion, PV^+ interneurons within the same layer are, on average, younger in the lateral third of the somatosensory cortex than in the medial third (Rymar and Sadikot, 2007).

The mechanisms that control the regional distribution of neocortical interneurons are presently unclear, but several lines of evidence suggest that this process is related to the transition of interneuron migration from tangential to radial or, more precisely, to its timing (Figure 2). On their entry into the pallium, interneurons do not immediately target the cortical plate, where developing pyramidal cells are beginning to differentiate. Instead, interneurons continue their tangential spread using the

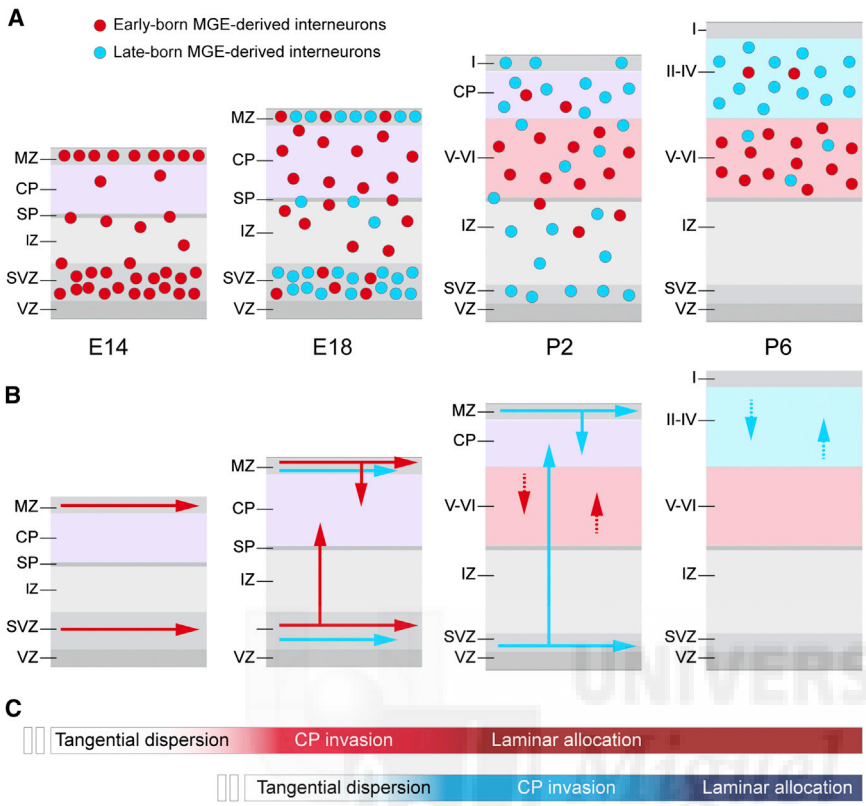

Figure 2. Integration of MGE-Derived Interneurons into Cortical Layers

(A–C**)** Schematic representation of the different phases underlying the integration of GABAergic interneurons in the neocortex. Circles in (A) schematically represent the distribution of MGE-derived interneurons, while arrow lines in (B) represent the migratory trajectories followed by interneurons. Early- and late-born MGE-derived interneurons are depicted in red and blue, respectively. The figure shows schematic representations of the mouse neocortex at different developmental stages (E14, E18, P2, and P6). Three distinct phases can be observed for each cohort of interneurons: tangential dispersion, cortical plate (CP) invasion, and laminar allocation. These consecutive phases seem common to all MGE-derived interneurons, but their timing varies depending on the age of interneurons (C). Sorting of interneurons into different layers of the cortex seems to follow a two-step process. First, interneurons seem generally attracted to the CP (purple); subsequently, they restrict their distribution to particular layers (light blue and light red), so that early-born MGE-derived interneurons primarily settle in infragranular layers, while late-born MGE-derived interneurons populate the superficial layers. This later phase appears to depend on signals released by pyramidal cells. MZ, marginal zone; SP, subplate; IZ, intermediate zone; SVZ, subventricular zone; VZ, ventricular zone; I–VI, cortical layers I to VI.

marginal and subventricular zones of the cortex (Lavdas et al., 1999; Marín and Rubenstein, 2001; Wichterle et al., 2001). Eventually, interneurons switch their mode of migration from tangential to radial and invade the cortical plate, where they take residence. This suggests that the mediolateral and rostrocaudal position of an interneuron during this transition determines its final coordinates in the neocortex.

The chemokine Cxcl12 regulates the tangential dispersion of interneurons throughout the neocortex. This molecule is expressed by the meninges and intermediate progenitor cells in the subventricular zone of the cortex and contributes to maintain interneurons within the tangential migratory streams (Daniel et al., 2005; Stumm et al., 2003; Tham et al., 2001; Tiveron et al., 2006). Interneurons respond to Cxcl12 using two G protein couple receptors, Cxcr4 and Cxcr7. In mouse mutants for these receptors, interneurons leave the migratory streams and enter the cortical plate prematurely, which disrupts their regional distribution within the neocortex (Li et al., 2008; López-Bendito et al., 2008; Meechan et al., 2012; Sánchez-Alcañiz et al., 2011; Tanaka et al., 2010). These studies strongly suggest that the timing of exit from the migratory streams—and so the final distribution of neocortical interneurons—is directly linked at a molecular level with the loss of responsiveness to Cxcl12.

Laminar Allocation of Cortical Interneurons

The laminar organization of pyramidal cells has been studied for several decades, and important progress has been made in understanding the mechanisms controlling their ordered allocation into specific layers. The characteristic six-layered structure of the neocortex emerges during development in an inside-out pattern that is universal among mammalian species (Rakic, 2007). Newborn pyramidal cells always migrate through previous cohorts of pyramidal neurons, so that early-born cells end up located in deep (i.e., infragranular) layers, and late-born cells populate superficial (i.e., supragranular) layers of the cortex. A signaling pathway elicited by Reelin, a glycoprotein expressed by Cajal-Retzius cells at the surface of the cortex, controls the ordered migration of pyramidal cells (Franco and Müller, 2011; Soriano and Del Río, 2005). This pattern of migration allows the organization of particular classes of pyramidal cells into coherent groups with similar functional properties. In other words, pyramidal cells exhibit comparable—although not necessarily identical—patterns of axonal connections within each of the cortical layers, which contribute to the establishment of reproducible circuits within each column of the cerebral cortex.

A superficial analysis of the distribution of GABAergic interneurons may lead to the premature conclusion that these cells distribute uniformly throughout all layers of the cerebral cortex. There is, however, a remarkable degree of sophistication in the laminar distribution of neocortical GABAergic interneurons (Figure 1). For instance, PV^+ interneurons are absent from layer I (Rymar and Sadikot, 2007), while Martinotti cells are particularly abundant in layers V and VI, and to a minor extent in layers II/III, but nearly absent from layer IV (Ma et al., 2006). In addition, most bipolar or double-bouquet interneurons reside in the supragranular layers of the cortex (Rymar and Sadikot, 2007), while chandelier cells are almost exclusively found in layers II and V

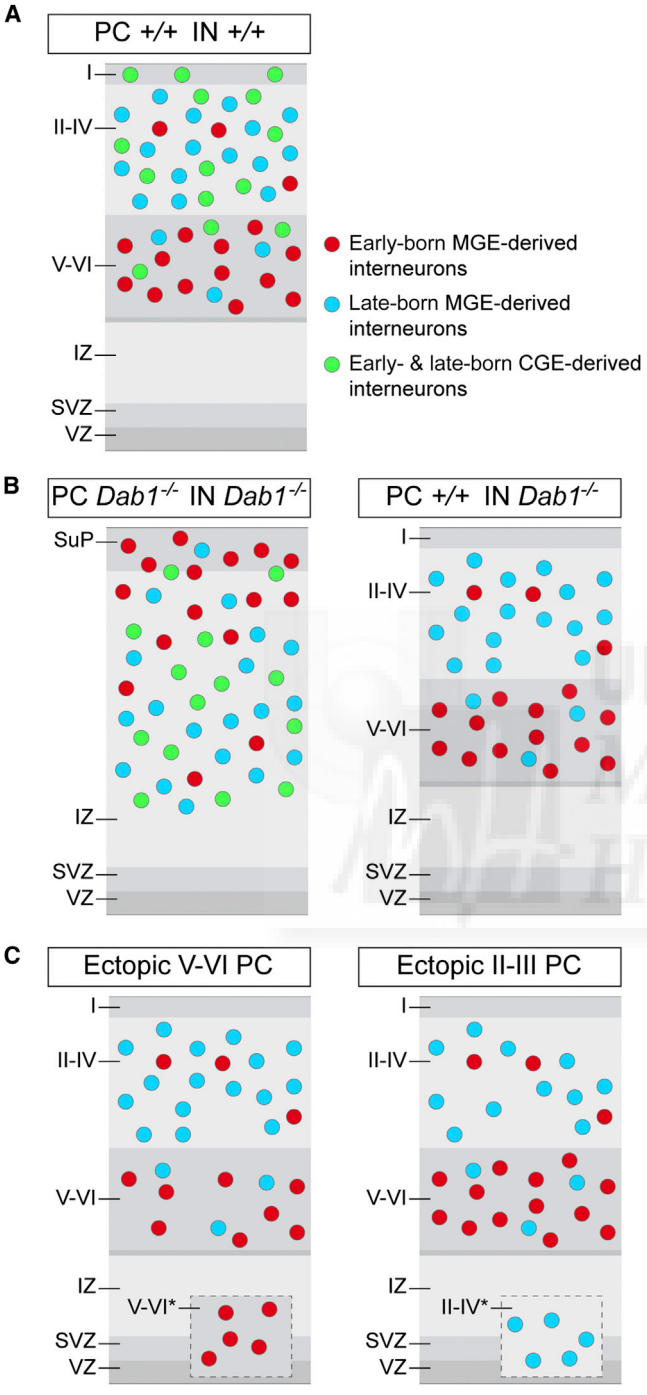

Figure 3. Pyramidal Cells Control the Distribution of GABAergic Interneurons in the Neocortex

(A) Schematic diagram illustrating the laminar distribution of MGE- and CGE-derived interneurons in the neocortex. Similar to pyramidal cells, MGE-derived interneurons distribute in a roughly inside-out pattern: early-born MGE-derived interneurons (red circles) are mainly located in infragranular layers, while late-born MGE-derived interneurons (blue circles) occupy the superficial layers. CGE-derived interneurons (green circles) distribute primarily throughout supragranular layers independently of their birthdate.

(B) Abnormal distribution of pyramidal cells in *Dab1*$^{-/-}$ mice disturbs the laminar organization of MGE-derived interneurons (left panel). This phenotype is due to the abnormal location of PN in *Dab1*$^{-/-}$ mice, because when *Dab1*$^{-/-}$ interneurons are transplanted into wild-type mice, they adopt a normal distribution (right panel).

(C) Pyramidal cells selectively recruit local interneurons based on their subtype-specific identity. The generation of ventricular zone (VZ) ectopias containing infragranular (left panel) or supragranular (right panel) pyramidal cells is sufficient to recruit early- and late-born interneurons, respectively, to this abnormal location. IN, interneurons; IZ, intermediate zone; PC, pyramidal cells; SuP, superplate; SVZ, subventricular zone; VZ, ventricular zone; I–VI, cortical layers I to VI; V–VI* and II–IV*, ectopic infragranular and supragranular pyramidal cells, respectively.

in the rodent neocortex (Taniguchi et al., 2013). Even those interneurons that seem to distribute more or less uniformly through most cortical layers, such as PV^{+} basket cells, display distinct patterns of connectivity according to their laminar position (Tremblay et al., 2010). This remarkable degree of organization suggests that precise developmental mechanisms control the laminar distribution of cortical interneurons.

The laminar distribution of MGE-derived interneurons follows a sequence that is similar to that followed by pyramidal cells. Thus, early-born MGE-derived interneurons primarily populate the infragranular layers of the neocortex, while late-born interneurons colonize the supragranular layers (Fairén et al., 1986; Miller, 1985; Pla et al., 2006; Rymar and Sadikot, 2007; Valcanis and Tan, 2003) (Figure 3). This seems to imply that the time of neurogenesis largely determines the laminar allocation of interneurons. However, several lines of evidence suggest that this is actually not the case. First, CGE-derived interneurons largely concentrate in supragranular layers of the cortex, independently of their birthdate (Miyoshi et al., 2010; Rymar and Sadikot, 2007; Xu et al., 2004). This indicates that the birthdate is not a universal predictor of laminar allocation for interneurons. Second, the distribution of MGE-derived interneurons is directly influenced by the position of pyramidal cells (Hevner et al., 2004; Lodato et al., 2011; Pla et al., 2006). For example, the laminar distribution of interneurons is abnormal in *reeler* mice (Hevner et al., 2004), and this is not due to the loss of Reelin signaling in interneurons (Pla et al., 2006) (Figure 3). These studies led to an alternative hypothesis to explain the laminar distribution of interneurons, according to which interneurons would adopt their laminar position in response to cues provided by specific classes of pyramidal cells. Direct support for this idea derives from experiments in which the laminar position of MGE-derived interneurons was specifically altered by disrupting the laminar distribution of specific classes of pyramidal cells, independently of their birthdate (Lodato et al., 2011) (Figure 3). Thus, MGE-derived interneurons appear to occupy deep or superficial layers of the cortex in response to specific signals provided by pyramidal cells located in these layers. Consequently, this process is perhaps only correlatively, but not causally, linked to the time of neurogenesis.

Recent studies on the generation of cortical lineages have shed further light on the chemical matching hypothesis for the laminar distribution of neocortical interneurons. The classical view of cortical development is based on the premise that pyramidal cells in all layers of the neocortex originate from the same lineage (Woodworth et al., 2012). In other words, cortical progenitors are multipotent and give rise to any class of pyramidal cell, but are gradually restricted to producing neurons for

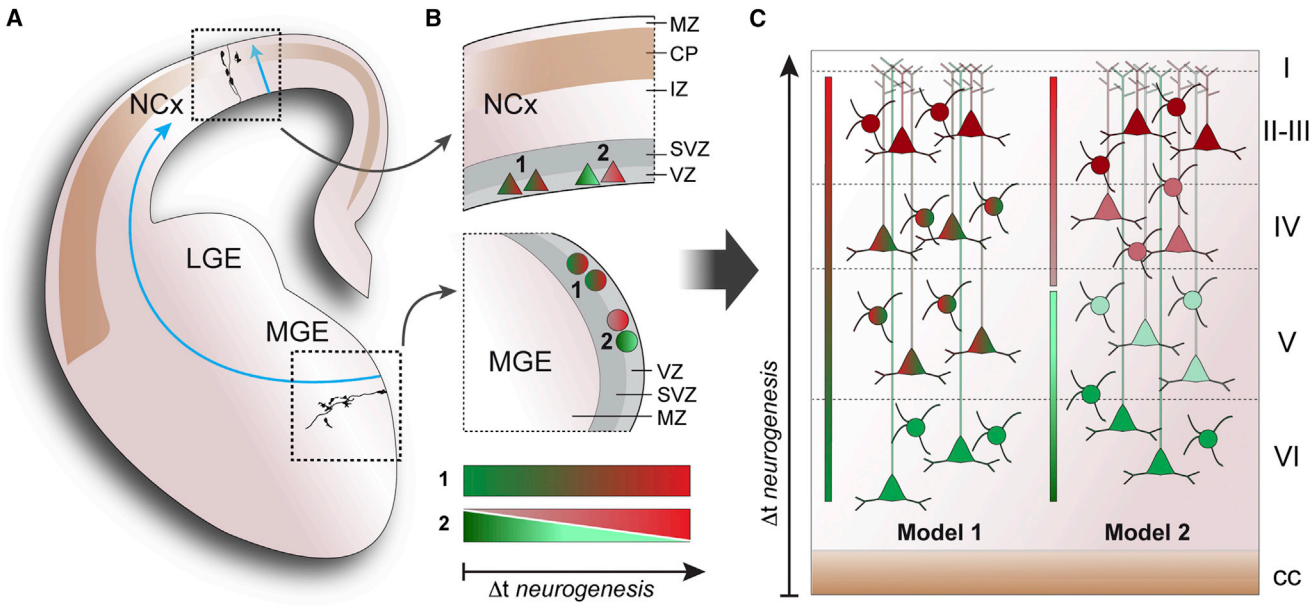

Figure 4. Lineages and Ontogenic Organization of the Neocortex

(A and B) Schematic diagram of a coronal section through the mouse telencephalon during embryonic development. The boxed areas in (A) correspond to the schemas shown in (B), which illustrate two models (1 and 2) of neurogenesis for pyramidal cells and MGE-derived interneurons. According to the classical model (model 1), progenitor cells in the embryonic cortex (triangles) and in MGE (circles) are multipotent. Each progenitor cell in these regions has the potential to generate pyramidal cells and interneurons, respectively, for all cortical layers. The fate potential of progenitor cells is progressively restricted along neurogenesis so that they give rise first to deep cortical neurons and later on to progressively more superficial neurons (transition from green to red in model 1). Model 2 is based on the observation that at least two classes of progenitor cells seem to exist for pyramidal cells (triangles) and interneurons (circles), each one committed to generate neurons with specific laminar allocations. In this model, the two lineages coexist in the proliferative regions, but their relative proportion and/or neurogenic potential changes during development.

(C) Schematic diagram of a coronal section through the adult neocortex, showing lineage relationships and neuron distributions for model 1 (left) and model 2 (right). In model 1, lineages of pyramidal cells and interneurons are organized along the columnar dimension of the neocortex. In model 2, lineages of pyramidal cells and interneurons are primarily organized along the laminar dimension of the neocortex. Color codes in the figure do not represent any developmental program but simply reflect the fate of cells according to their laminar position. In addition, note that both models are not incompatible. cc, corpus callosum; NCx, neocortex; MGE, medial ganglionic eminence; LGE, lateral ganglionic eminence; MZ, marginal zone; CP, cortical plate; IZ, intermediate zone; SVZ, subventricular zone; VZ, ventricular zone; I–VI, cortical layers I to VI.

progressively more superficial layers (Noctor et al., 2001; Rakic, 1988). Recent work on the organization of interneuron lineages also led to the conclusion that MGE-derived interneurons that extend throughout multiple layers of the cortex derive from the same progenitor cells (Brown et al., 2011) (Figure 4, model 1).

This view of cortical neurogenesis has recently been challenged by the identification of different classes of progenitor cells for both pyramidal cells and interneurons (Ciceri et al., 2013; Franco et al., 2012; Stancik et al., 2010) (Figure 4, model 2). In the pallium, two classes of progenitor cells in the neocortex might exist: one largely responsible for the generation of pyramidal cells in deep (V and VI) layers and another one for pyramidal cells in superficial (II and IV) layers (Franco et al., 2012). Similarly, recent work on the organization of progenitor cells in the subpallium suggests that MGE-derived interneurons originate from at least two separate lineages: one that primarily produces interneurons for deep (V and VI) layers of the cortex and another one that generates interneurons for superficial (II and IV) layers (Ciceri et al., 2013) (Figure 4). According to this model, the relative proportion of the different types of progenitor cells varies with time, and this determines the classes of pyramidal cells and interneurons that are being produced at a particular developmental stage. Furthermore, these experiments suggest that MGE-derived interneurons might be generated to mirror the laminar organization of pyramidal cells.

Early Functional Interactions

The distribution of GABAergic interneurons into the cerebral cortex also relies on functional interactions between these cells and the networks into which they integrate. Initially, these interactions rely on the ability of migrating interneurons to sense the combined extracellular levels of GABA and glutamate, and so they precede the onset of synaptogenesis in the cortex. Both neurotransmitters enhance neuronal migration in the embryo because they depolarize the membrane of interneurons and stimulate the generation of calcium transients (Cuzon et al., 2006; Manent et al., 2005). However, the reversal potential for chloride ions changes in interneurons as they mature, and so GABA becomes hyperpolarizing when this occurs. This change turns ambient GABA into a stop signal for migrating interneurons, because hyperpolarizing GABA decreases the frequency of intracellular calcium transients (Bortone and Polleux, 2009). The potassium/chloride exchanger KCC2 mediates the reversal potential of chloride ions in maturing neurons (Ben-Ari, 2002), and so the mechanisms controlling the upregulation of this transporter are likely linked to the termination of migration (Bortone and Polleux, 2009). Consistently, interneurons upregulate

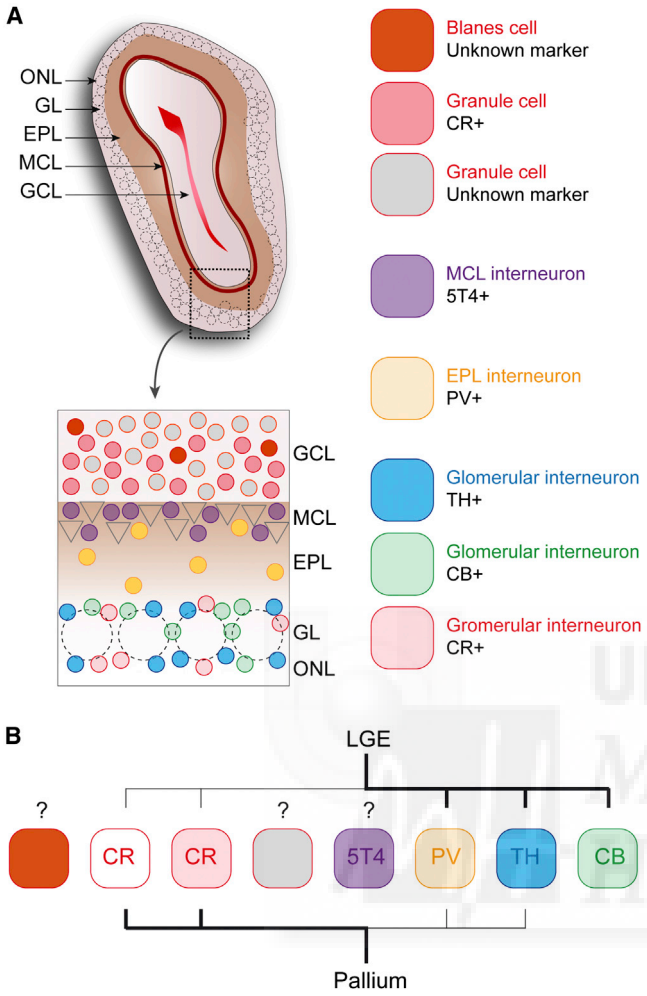

Figure 5. Major Classes of Olfactory Bulb Interneurons and Their Developmental Origins

(A) Schematic of a coronal section through the mouse olfactory bulb showing the main classes of GABAergic interneurons and their respective laminar allocation. Granule cells include at least three different classes: Blanes cells, CR^+ granule cells preferentially located in the most superficial aspect of the granule cell layer, and granule cells without a known specific marker. The mitral cell layer contains interneurons that express the glycoprotein 5T4. The external plexiform layer contains PV^+ interneurons. Periglomerular interneurons comprise at least three classes based on their neurochemical content: TH^+, CB^+, and CR^+ cells.

(B) Grouping of the main classes of cortical interneurons according to their developmental origins. ONL, olfactory nerve layer; GL, glomerular layer: EPL, external plexiform layer; MCL, mitral cell layer; GCL, granule cell layer; LGE, lateral ganglionic eminence.

KCC2 expression during their radial sorting in the cortex (Miyoshi and Fishell, 2011); however, it is presently unclear how this process is integrated with the laminar allocation of interneurons. One possibility is that interneurons get preferentially immobilized in layers with increased network activity, in which modification of calcium dynamics might be more prominent (de Lima et al., 2009). Alternatively, the layer-specific cues that are thought to control the final distribution of interneurons might also regulate the expression of KCC2 in these cells. In agreement with this hypothesis, factors released by cortical cells decrease the mobility of embryonic interneurons in culture (Inamura et al., 2012). In any case, early patterns of activity seem to play a clear role in the final settlement of interneurons, independently of their origin (Bortone and Polleux, 2009; De Marco García et al., 2011).

Integration of GABAergic Interneurons in the Adult Olfactory Bulb

The adult olfactory bulb represents a good model to study the ability of newly generated GABAergic interneurons to integrate into mature networks. Similar to the cerebral cortex, the olfactory bulb is organized as an assembly of excitatory and inhibitory neurons distributed through layers (Zou et al., 2009). However, olfactory interneurons outnumber excitatory neurons in an ~100:1 proportion, perhaps because the primary function of the olfactory bulb is to discriminate sensory information. In addition, neural circuits in the olfactory bulb are continuously remodeled by the addition of new GABAergic interneurons, generated through the process of adult neurogenesis. This circumstance makes the adult olfactory bulb an ideal model for studying how GABAergic interneurons integrate into mature neuronal circuits. Transplantation experiments have shown that embryonic cortical interneurons also have the ability to migrate and functionally integrate in the adult cortex (Alvarez-Dolado et al., 2006; Wichterle et al., 1999), which suggests that this might be a rather general characteristic of GABAergic interneurons.

Two classes of excitatory neurons are present in the olfactory bulb, mitral cells and tufted cells, which are confined to a single layer that lies between the external plexiform and granule cell layers (Figure 5). Both classes of neurons are glutamatergic, but they comprise several different populations that diverge in the spatial organization of their connections and molecular markers (Mizuguchi et al., 2012; Mori and Sakano, 2011). Mitral cells and tufted cells send their primary dendrites into single glomeruli, where they receive inputs from olfactory sensory neurons. In turn, they convey this information to other brain centers in the telencephalon through the lateral olfactory tract (Igarashi et al., 2012). Hence, as in the cortex, excitatory neurons are the main projection neurons in the olfactory bulb.

The olfactory bulb contains several classes of GABAergic interneurons, grouped in three main populations: granule cells, external plexiform layer interneurons, and periglomerular cells (Figure 5) (Batista-Brito et al., 2008). It is worth noting that olfactory bulb interneurons have not been as extensively characterized as cortical interneurons, and so their classification largely relies on marker analyses at this point. Granule cells are the most abundant GABAergic neurons in the olfactory bulb. They have a small soma and make dendrodendritic connections with excitatory neurons (Price and Powell, 1970). Several classes of neurons have been identified within the granule cell layer, including external granule cells, whose soma is located within the mitral cell layer and expresses the glycoprotein 5T4, CR^+ granule cells located in the external aspect of the granule cell layer, and Blanes cells (Imamura et al., 2006; Pressler and Strowbridge, 2006). This later population of interneurons is specialized in inhibiting granule cells, thereby controlling the strength of inhibition on the excitatory neurons (Pressler and Strowbridge, 2006). Many granule cells do not express any known markers,

which suggests an even larger diversity within this population. The most common population of interneurons in the external plexiform layer contains PV (Kosaka and Kosaka, 2008), but several other classes of interneurons seem to exist in this layer (Huang et al., 2013; Krosnowski et al., 2012; Liberia et al., 2012). Interneurons in this layer are thought to provide inhibition to mitral and tufted cells (Huang et al., 2013), probably by targeting their apical dendrites. Finally, three distinct subtypes of interneurons have been identified in the glomerular layer of the mouse, based on the expression of tyrosine hydroxylase (TH), calbindin (CB), and CR, respectively (Kohwi et al., 2007; Kosaka and Kosaka, 2005). These interneurons receive direct input from olfactory receptor neuron axons and synapse with the dendrites of mitral and tufted cells (Kosaka and Kosaka, 2005).

The organization of olfactory bulb interneurons into distinct layers is directly related to their function in the neural circuit, processing olfactory information (Zou et al., 2009). Interneurons in the glomerular layer receive synapses from olfactory receptor neuron axons and, in turn, synapse with the dendrites of mitral cells and tufted cells. In turn, granule cells established dendrodendritic synapses with excitatory neurons in the external plexiform layer. Consequently, the laminar allocation of interneurons largely determines their function within the neural circuits that underlie the processing of sensory information in the olfactory bulb.

Sources of Adult-Born Olfactory Bulb Interneurons

Olfactory interneurons are born remotely in the subpallium and reach their final destination through tangential migration (Altman, 1969; Belvindrah et al., 2009; Luskin, 1993). During embryonic stages, the olfactory bulb emerges as a protrusion of the rostral tip of the telencephalon that is continuous with the region of the subpallium that gives rise to its interneurons (Gong and Shipley, 1995). As development proceeds, however, interneurons must migrate increasing distances to reach their destination. Importantly, many interneurons continue to be generated through adulthood (Lois and Alvarez-Buylla, 1994), which poses a notable challenge for the transit of new inhibitory neurons to the olfactory bulb.

The origin of olfactory interneurons has been classically associated with the LGE, a region that was shown to contribute to the SVZ of the lateral ventricles in the postnatal telencephalon (Stenman et al., 2003; Wichterle et al., 2001). However, recent evidence indicates that the diversity of OB interneurons derives from a more extensive and heterogeneous germinal region than previously thought (Lledo et al., 2008). Genetic fate-mapping analyses have confirmed that the LGE is the main contributor to the adult SVZ. Thus, the majority of dividing cells in the SVZ derive from lineages expressing the subpallial marker Gsh2, and nearly 70% of the olfactory bulb interneurons emerge from these progenitors (Young et al., 2007). The remaining interneurons derive from a lineage of progenitor cells that express the transcription factor Emx1 and are therefore classically considered pallial derivatives (Young et al., 2007). However, this should be interpreted with caution because LGE progenitors may also contain low levels of Emx1 (Waclaw et al., 2009). Independently of their origin, Emx1$^+$ progenitors in the adult are located in the regions of the lateral ventricular wall facing the corpus callosum, from where neurosphere-forming stem cells have been obtained (Ventura and Goldman, 2007; Willaime-Morawek et al., 2006). Finally, a very small fraction of olfactory bulb interneurons (~1%) seem to derive from a lineage of SVZ progenitor cells that express the transcription factor Nkx2-1 (Young et al., 2007), a marker of the MGE.

LGE and pallial progenitors contribute differently to the diversity of olfactory bulb interneurons (Figure 5). For instance, periglomerular cells are produced by both sets of progenitors, although in different proportions. LGE-derived progenitors contribute many TH$^+$ interneurons and the large majority of CB$^+$ cells, whereas pallium-derived progenitors produce most CR$^+$ neurons (Kohwi et al., 2007; Stenman et al., 2003; Young et al., 2007). PV$^+$ interneurons in the external plexiform layer are also generated from both classes of progenitors, although most seem to derive from the LGE (Li et al., 2011). In the granular cell layer, most CR$^+$ interneurons develop from pallial progenitors, while the remaining cells are likely derived from the LGE (Kohwi et al., 2007; Merkle et al., 2007; Young et al., 2007).

Each population of olfactory bulb interneurons is produced in a unique temporal pattern and turnover rate (Lledo et al., 2008). This suggests that the neurogenic processes occurring during development and in the adult are not directly equivalent (De Marchis et al., 2007; Lemasson et al., 2005). Interestingly, bromodeoxyuridine (BrdU) labeling experiments revealed that the relative ratio of the different subtypes of olfactory bulb interneurons remains relatively constant from birth to adulthood, although they seem to be produced at different rates. For instance, CR$^+$ cells make up the largest proportion of newborn neurons in adult mice (Batista-Brito et al., 2008), while TH$^+$ and CB$^+$ periglomerular interneurons are produced to a lesser extent, and PV$^+$ interneurons are not significantly turned over in the adult (Kohwi et al., 2007; Li et al., 2011). It is presently unclear what physiological circumstances determine the precise turnover of the different classes of olfactory bulb interneurons in the adult.

Regional and Laminar Distribution of Adult-Born Olfactory Bulb Interneurons

The mechanisms controlling the migration of embryonic interneurons to the olfactory bulb resemble in many aspects that of cortical interneurons (Long et al., 2007) and will not be considered here in detail. However, the migration of interneurons to the olfactory bulb changes dramatically as the brain matures, because the brain parenchyma becomes progressively less permissive for migration. Adult-born interneurons migrate to the olfactory bulb through the rostral migratory stream (RMS), a highly specialized structure in which chains of migrating neuroblasts are ensheathed by astrocytes (Doetsch and Alvarez-Buylla, 1996; Jankovski and Sotelo, 1996; Lois et al., 1996; Thomas et al., 1996) (Figure 6). Interneurons migrate, crawling into each other in a process that is known as chain migration (Wichterle et al., 1997). Many factors have been shown to influence the tangential migration of olfactory neuroblasts through the RMS (reviewed in Belvindrah et al., 2009), but very little is known on the mechanisms that control the final distribution of newborn interneurons in the olfactory bulb.

Newborn interneurons seem to distribute uniformly throughout the rostrocaudal extent of the olfactory bulb (Lemasson et al., 2005). In contrast, interneurons target a specific layer within the olfactory bulb, according to their fate, in a process that is likely determined at the time of their specification. In agreement

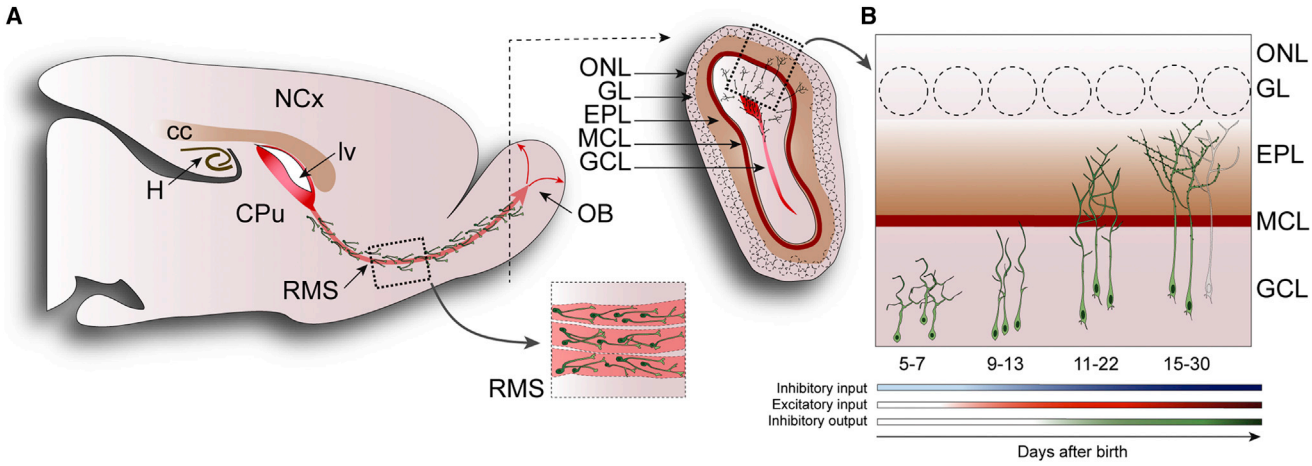

Figure 6. Integration of Adult-Born Interneurons into the Olfactory Bulb

(A) Schematic of sagittal section through the mouse brain illustrating the migration and integration of adult-born GABAergic interneurons into the olfactory bulb. Olfactory bulb interneurons are produced in the SVZ and reach the olfactory bulb though the rostral migratory stream (RMS).

(B) Schematic of a coronal section depicting the laminar organization of the adult olfactory bulb. The inset illustrates different stages in the maturation of granule cells, from their arrival to the olfactory bulb to their integration into functional circuits. The numbers refer to their approximate age in days. ONL, olfactory nerve fiber layer; GL, glomerular layer: EPL, external plexiform layer; MCL, mitral cell layer; GCL, granule cell layer; cc, corpus callosum; CPu, caudoputamen nucleus; H, hippocampus; lv, lateral ventricle; NCx, neocortex.

with this notion, overexpression of the transcription factor Pax6 in migrating neuroblasts promotes their differentiation to periglomerular TH^+ cells at the expense of other interneuron classes (Hack et al., 2005). These results reinforce the view that the laminar allocation is largely linked to the fate of cells originating from different progenitor cells. Since granular and periglomerular interneurons play very distinct roles in the processing of olfactory information (Chen and Shepherd, 2005; Shepherd et al., 2007), the precise targeting of these cells to their appropriate layer seems critical for the function of the olfactory bulb.

Important differences seem to exist in the mechanisms underlying the laminar distribution of cortical and olfactory bulb interneurons. First, olfactory bulb interneurons reside in layers that lack projection neurons, which is in sharp contrast to most of their neocortical counterparts (with the exception of cortical layer I). This suggests that the hypothetical mechanism proposed to regulate the allocation of most neocortical interneurons is unlikely to apply in the olfactory bulb. Second, adult-born interneurons reach their final position by traversing a territory that is largely populated by fully mature, differentiated neurons. This indicates that the mechanisms regulating the integration of interneurons into their appropriate target layer in the olfactory bulb are maintained through adulthood, at least for periglomerular and granule cells.

Reelin is the only factor identified to date that seems to influence the laminar positioning of olfactory bulb interneurons. In contrast to the cerebral cortex, where Reelin regulates the distribution of pyramidal cells and only affects the location of GABAergic interneurons in a non-cell-autonomous manner (Pla et al., 2006), this glycoprotein seems to directly control the migration of olfactory bulb interneurons. Indeed, mitral and tufted cells adopt their final position independently of this signaling system (Devor et al., 1975). Conversely, Reelin produced by these cells is required for interneurons to detach from the RMS and adopt their normal laminar position (Hack et al., 2002; Hellwig et al., 2012). In *reeler* mutants, for example, some TH^+ and CB^+ interneurons fail to reach the glomerular layer and instead reside in the external plexiform layer; some defects have also been reported in the distribution of CR^+ interneurons in the granular layer (Hellwig et al., 2012). Nevertheless, the position of PV^+ interneurons in the external plexiform layer, and most periglomerular interneurons, is unaffected by the loss of Reelin signaling, which suggests that the correct laminar distribution of olfactory bulb interneurons depends on additional factors. Consistent with this idea, a population of glial cells located in the olfactory nerve layer, the olfactory ensheathing cells, releases a chemoattractive activity that attracts migrating neuroblasts in vitro (Zhu et al., 2010). This suggests that olfactory ensheathing cells may contribute to regulate the radial distribution of interneurons in the surface of the olfactory bulb.

Functional Integration of Adult-Born Interneurons

As in the developing cortex, the integration of interneurons in the olfactory bulb also seems under the influence of activity-dependent mechanisms. Migrating neuroblasts are sensitive to the action of neurotransmitters, although they seem to exert different effects than in the cortex. There are no specific studies on the expression of chloride transporters in adult-born interneurons, but analysis of their expression in early postnatal stages suggests that interneurons lack KCC2 when they arrive to the olfactory bulb (Mejia-Gervacio et al., 2011). Consequently, interneurons terminate their migration in the olfactory bulb in an environment with a high concentration of ambient GABA and under depolarizing conditions. Intriguingly, neuroblast migration is reduced by the tonic depolarizing action of GABA acting on $GABA_A$ receptors (Bolteus and Bordey, 2004; Mejia-Gervacio et al., 2011). These results, which contrast the proposed role for hyperpolarizing GABA as a stop signal for cortical interneurons, reveal that the function of ambient neurotransmitters in

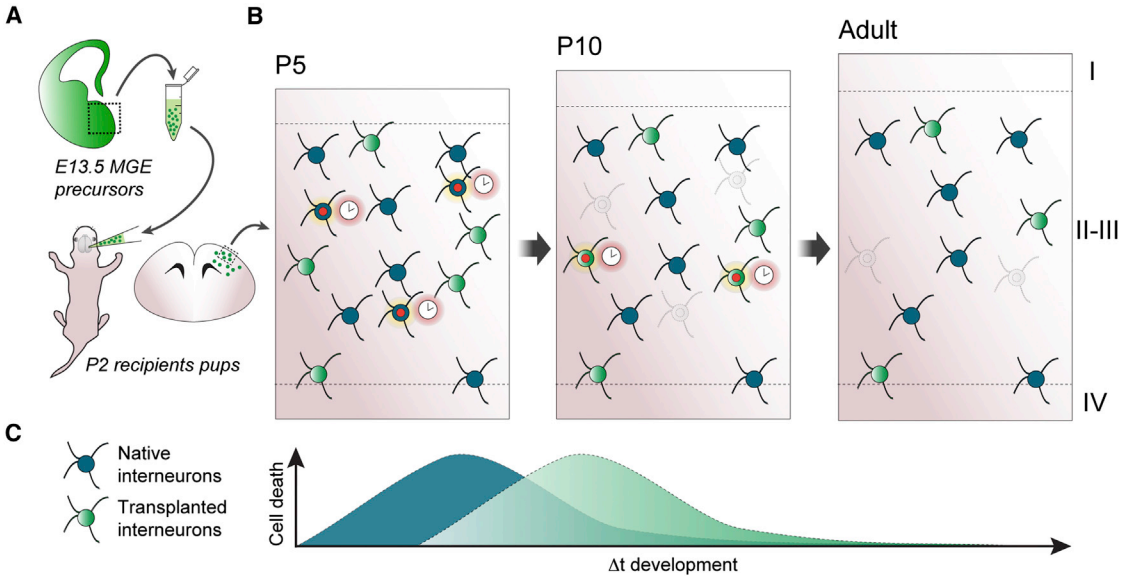

Figure 7. Intrinsic Developmental Cell Death of Cortical GABAergic Interneurons

(A) Schematic diagrams of the experimental paradigm used to study the programmed cell death of cortical interneurons (Southwell et al., 2012). MGE donor cells from GFP-expressing embryos were transplanted into the neocortex of early postnatal recipient mice, and their number and distribution were analyzed several days later, together with the native interneuron population.

(B) Schematic diagrams of coronal sections through the neocortex of transplanted mice at three different time points during postnatal development. Approximately 40% of interneurons (native and transplanted) undergo programmed cell death during early postnatal development. However, each population of interneurons undergoes cell death (red nucleus indicates active caspase-3) according to an internal clock that depends on the actual age of the interneurons, rather than according to environmental influence. Since transplanted interneurons (dark green) were moved forward in development, they undergo programmed cell death several days later than the native population (light green). The time window of cell death largely overlaps with the period of intense synaptogenesis, suggesting that the survival of interneurons might be linked to their recruitment into circuits.

(C) The temporal windows of neuronal cell death for the native (blue) and transplanted (green) interneurons are out of phase due to heterochronic transplantation. MGE, medial ganglionic eminence; I–IV, cortical layers I to IV.

the functional integration of GABAergic interneurons is more complex than previously thought.

Several studies have analyzed in detail the maturation and integration of adult-born interneurons into the olfactory bulb (Figure 6). The synaptic integration of newborn interneurons occurs over a period of approximately 3 weeks (Petreanu and Alvarez-Buylla, 2002), although newborn neurons already receive glutamatergic and GABAergic synapses within 24 hr after leaving the RMS (Katagiri et al., 2011; Panzanelli et al., 2009). As interneurons progressively settle into their final position, they acquire functional properties that make them indistinguishable from preexisting neurons (Belluzzi et al., 2003; Carleton et al., 2003). Interestingly, the majority of functional outputs from newborn interneurons at the end of their integration period and their characteristics do not seem to change over time (Bardy et al., 2010). In contrast, glutamatergic inputs onto newborn interneurons display enhanced plasticity during this period of maturation (Nissant et al., 2009), which may provide a basis for adult neurogenesis-dependent olfactory learning.

General Principles in the Integration of Embryonic and Adult GABAergic Interneurons

There are a number of emerging concepts that can be extracted from our current understanding of the mechanisms controlling the integration of GABAergic interneurons into the developing neocortex and in the mature olfactory bulb. In particular, it seems clear that many of the features that distinguish the different classes of GABAergic interneurons, such as their intrinsic properties and perhaps even their final allocation, are intrinsically determined.

Intrinsic Developmental Programs

Several stages in the development of GABAergic interneurons, both in the cerebral cortex and the olfactory bulb, seem to be regulated by the execution of a maturational program intrinsic to inhibitory neurons. In other words, the behavior of interneurons at any given time in development is better predicted by their cellular age than by changes in the local environment. Since interneurons are born asynchronously, this implies that the developing cerebral cortex contains a mixture of interneurons at diverse stages of maturation. These differences are obviously exaggerated in the olfactory bulb, where adult-born interneurons coexist with interneurons that were generated in the embryo.

The existence of an intrinsic maturational program in GABAergic interneurons predicts that interneurons born at different times would behave differently within the same environment. This has been observed, for example, in relation to the settlement of interneurons in the cortical plate. Birthdating analyses have shown that not all interneurons switch from tangential to radial migration simultaneously in response to a common trigger. Instead, interneurons invade the cortical plate when they are between 6 and 8 days old; therefore, early-born interneurons enter the cortical plate before late-born interneurons (López-Bendito

et al., 2008) (Figure 2). This indicates that the switch from tangential to radial migration is largely determined by the age of interneurons. Consistent with this idea, many late-born (embryonic day 15.5, E15.5) interneurons transplanted into E12.5 embryos settle in deep layers of the cortex instead of their normal superficial location (Pla et al., 2006), probably because under these circumstances they stop responding to the cues that support their tangential migration at the same time as early-born (12.5) interneurons, which settle in deep layers of the cortex. The intrinsic developmental program may therefore influence the settlement of interneurons in the cortex by regulating the responsiveness of each cohort of interneurons to cues present in the cortex.

Transplantation experiments have also revealed that the death of cortical interneurons in the early postnatal cortex might also be under intrinsic control (Figure 7). Southwell and colleagues (2012) observed that many cortical interneurons undergo programmed cell death in vivo between postnatal day 7 (P7) and P11 in vivo, when interneurons are between 11 and 18 days old. When transplanted into older cortices (P3), interneurons undergo programmed cell death later than normal (~P15), which demonstrates that this process is intrinsically linked to the cellular age of interneurons. Consistently, cortical interneurons undergo programmed cell death in vitro with the same temporal dynamics as in vivo (Southwell et al., 2012). In the adult olfactory bulb, interneurons also die within a well-defined temporal window, approximately 15–30 days after birth (Petreanu and Alvarez-Buylla, 2002).

Further evidence supporting the existence of an intrinsic clock that controls the maturation of these cells comes from the analysis of their modulation of ocular dominance plasticity. During a critical period in the postnatal development of the visual cortex, visual experience influences the organization of thalamocortical axon terminals to produce alternating ocular dominance domains (Hensch, 2005). Occlusion of one eye during this period triggers a rapid reorganization of thalamic terminals in the cortex, a process that is regulated by inhibitory neurotransmission. In mice, ocular dominance plasticity peaks between P26 and P28, when interneurons are roughly between 33 and 35 days of age. Transplantation of interneuron precursors into the postnatal cortex reopens the critical period of ocular dominance plasticity when transplanted interneurons reach a cellular age equivalent to that of endogenous inhibitory neurons during the normal critical period (Southwell et al., 2010).

Recent efforts to derive cortical interneurons from human pluripotent stem cells (hPSCs) or human-induced pluripotent stem cells (hiPSCs) have also emphasized the ability of these cells to differentiation according to an intrinsic program of maturation. Both in vitro and after transplantation into the rodent cortex, human GABAergic interneurons derived from hPSCs or hiPSCs mature following a protracted timeline of several months, thereby mimicking the endogenous human neural development (Maroof et al., 2013; Nicholas et al., 2013). Altogether, these findings suggest that multiple aspects of the integration of interneurons in cortical networks are regulated by the execution of a maturational program intrinsic to inhibitory neurons.

Adjusting Inhibition

Several mechanisms dynamically adjust the balance between excitation and inhibition in the adult brain (Haider et al., 2006; Turrigiano, 2011). However, it is likely that developmental programs are also coordinated to play an important role in this process. Indeed, the relative density of pyramidal cells and interneurons remains relatively constant from early stages of corticogenesis, when both classes of neurons are still migrating to their final destination (Sahara et al., 2012). One possibility is that the generation of both classes of neurons is coordinated through some kind of feedback mechanism that balances proliferation in the pallium and subpallium. Alternatively, the production of factors controlling the migration of GABAergic interneurons to the cortex might be proportional to the number of pyramidal cells generated. For example, it has been shown that cortical intermediate progenitor cells (IPCs) produce molecules that are required for the normal migration of interneurons (Tiveron et al., 2006), and mutants with reduced numbers of IPCs have a deficit in cortical interneurons (Sessa et al., 2010).

Cell death is another prominent factor regulating neuronal incorporation during development (Katz and Shatz, 1996; Voyvodic, 1996). It has long been appreciated that a sizable proportion of inhibitory neurons is eliminated from the cerebral cortex through apoptosis during the period of synaptogenesis (Miller, 1995), and recent work estimated that approximately 40% of the interneurons in the cortex perish around this time (Southwell et al., 2012). Similarly, only about half of the adult-born granule cells survive more than a few days after reaching the olfactory bulb (Petreanu and Alvarez-Buylla, 2002).

The mechanisms controlling the death of newborn olfactory bulb interneurons have been studied with some detail. There seems to exist a critical period during which sensory activity influences the survival of newborn interneurons (Kelsch et al., 2009; Yamaguchi and Mori, 2005), which largely overlaps with the period when interneurons become synaptically integrated into the olfactory bulb (15–30 days after birth). During this period, interneurons arriving to the olfactory bulb (i.e., roughly born at the same time) compete for survival, probably because newborn interneurons are more sensitive to the overall activity of nearby circuits than mature olfactory interneurons. In agreement with this idea, interneurons that survived this period tend to persist for life (Winner et al., 2002). Thus, both the synaptic integration and the survival of newborn interneurons seem to depend on sensory activity mechanisms, which are intrinsically linked to the cell excitability. Consistent with this, synaptic development and survival of newly generated neurons are dramatically impaired in anosmic mice (Corotto et al., 1994; Petreanu and Alvarez-Buylla, 2002), while sensory enrichment promotes the survival of newborn olfactory interneurons (Bovetti et al., 2009; Rochefort et al., 2002). Moreover, increasing cell-intrinsic excitability in maturing granule cells enhances their synaptic integration and partially rescues neuronal survival in a sensory-deprived olfactory bulb (Kelsch et al., 2009; Lin et al., 2010), while forced hyperpolarization decreases survival (Lin et al., 2010). Since most interneurons have already matured and received connections by the time they die, it has been hypothesized that only interneurons connected to active circuits would ultimately survive (Petreanu and Alvarez-Buylla, 2002), an idea that has

obtained experimental support in the adult dentate gyrus (Kee et al., 2007). Thus, the death of adult-born interneurons seems to be intimately linked to mechanisms of structural plasticity in the olfactory bulb.

It is presently unclear whether programmed cell death in developing cortical interneurons depends on similar mechanisms than in the olfactory bulb, but recent experiments pointed out an interesting parallel between both structures. Southwell and colleagues (2012) found that heterochronically transplanted interneurons do not influence cell death dynamics in the endogenous population (Figure 7). This seems to suggest that the competition for survival is normally restricted to cortical interneurons born roughly at the same time, as in the olfactory bulb. Thus, it is conceivable that cell death selectively eliminate inappropriately integrated cortical interneurons within specific lineages, although this hypothesis remains to be experimentally tested. In any case, these results reinforce the view that the integration of interneurons into cortical networks critically depends on a maturational program linked to their cellular age.

A Look Ahead

Much progress has been made over the past years regarding our understanding of the mechanisms regulating the migration of embryonic and adult-born GABAergic interneurons. However, our understanding of the integration of these cells into functional circuits in the cerebral cortex and olfactory bulb, respectively, is very limited. We know basically nothing about the mechanisms through which interneurons adopt their precise laminar distributions and how this process influences functional connectivity patterns between interneurons and pyramidal cells. Recent work has led to the suggestion that SST^+ and PV^+ interneurons connect promiscuously to nearby pyramidal cells (Fino and Yuste, 2011; Packer and Yuste, 2011); therefore, the connectivity maps of interneurons could simply result from the overlap of axonal and dendritic arborizations between both cell types (Packer et al., 2012). According to this principle, the laminar allocation of interneurons might be irrelevant for their functional integration into cortical networks, i.e., similar interneurons located in different layers might be interchangeable. On the other hand, it is well established that different classes of interneurons receive distinct excitatory and inhibitory laminar input patterns (Xu and Callaway, 2009; Yoshimura and Callaway, 2005). In agreement with this notion, a remarkable degree of specificity in the cellular selection of postsynaptic targets for at least some classes of interneurons seems to exist. For example, layer IV neurogliaform and SST^+ interneurons selectively target local PV^+ basket cells while largely avoiding pyramidal cells in this layer (Chittajallu et al., 2013; Xu et al., 2013). In contrast to the promiscuous view of cellular targeting by cortical interneurons (Packer et al., 2012), these observations suggest that the fine-scale connectivity of cortical networks might be directly influenced by the appropriate laminar allocation of interneurons. Future experiments should contribute to solve this apparent paradox.

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REFERENCES

Altman, J. (1969). Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J. Comp. Neurol. *137*, 433–457.

Alvarez-Dolado, M., Calcagnotto, M.E., Karkar, K.M., Southwell, D.G., Jones-Davis, D.M., Estrada, R.C., Rubenstein, J.L., Alvarez-Buylla, A., and Baraban, S.C. (2006). Cortical inhibition modified by embryonic neural precursors grafted into the postnatal brain. J. Neurosci. *26*, 7380–7389.

Armstrong, C., Krook-Magnuson, E., and Soltesz, I. (2012). Neurogliaform and Ivy cells: A major family of nNOS expressing GABAergic neurons. Front Neural Circuits *6*, 23.

Ascoli, G.A., Alonso-Nanclares, L., Anderson, S.A., Barrionuevo, G., Benavides-Piccione, R., Burkhalter, A., Buzsáki, G., Cauli, B., Defelipe, J., Fairén, A., et al.; Petilla Interneuron Nomenclature Group. (2008). Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. Nat. Rev. Neurosci. *9*, 557–568.

Bardy, C., Alonso, M., Bouthour, W., and Lledo, P.M. (2010). How, when, and where new inhibitory neurons release neurotransmitters in the adult olfactory bulb. J. Neurosci. *30*, 17023–17034.

Batista-Brito, R., and Fishell, G. (2009). The developmental integration of cortical interneurons into a functional network. Curr. Top. Dev. Biol. *87*, 81–118.

Batista-Brito, R., Close, J., Machold, R., and Fishell, G. (2008). The distinct temporal origins of olfactory bulb interneuron subtypes. J. Neurosci. *28*, 3966–3975.

Bayer, S.A., and Altman, J. (1987). Directions in neurogenetic gradients and patterns of anatomical connections in the telencephalon. Prog. Neurobiol. *29*, 57–106.

Belluzzi, O., Benedusi, M., Ackman, J., and LoTurco, J.J. (2003). Electrophysiological differentiation of new neurons in the olfactory bulb. J. Neurosci. *23*, 10411–10418.

Belvindrah, R., Lazarini, F., and Lledo, P.M. (2009). Postnatal neurogenesis: from neuroblast migration to neuronal integration. Rev. Neurosci. *20*, 331–346.

Ben-Ari, Y. (2002). Excitatory actions of gaba during development: the nature of the nurture. Nat. Rev. Neurosci. *3*, 728–739.

Binzegger, T., Douglas, R.J., and Martin, K.A. (2004). A quantitative map of the circuit of cat primary visual cortex. J. Neurosci. *24*, 8441–8453.

Bolteus, A.J., and Bordey, A. (2004). GABA release and uptake regulate neuronal precursor migration in the postnatal subventricular zone. J. Neurosci. *24*, 7623–7631.

Bortone, D., and Polleux, F. (2009). KCC2 expression promotes the termination of cortical interneuron migration in a voltage-sensitive calcium-dependent manner. Neuron *62*, 53–71.

Bovetti, S., Veyrac, A., Peretto, P., Fasolo, A., and De Marchis, S. (2009). Olfactory enrichment influences adult neurogenesis modulating GAD67 and plasticity-related molecules expression in newborn cells of the olfactory bulb. PLoS ONE *4*, e6359.

Brown, K.N., Chen, S., Han, Z., Lu, C.H., Tan, X., Zhang, X.J., Ding, L., Lopez-Cruz, A., Saur, D., Anderson, S.A., et al. (2011). Clonal production and organization of inhibitory interneurons in the neocortex. Science *334*, 480–486.

Buhl, E.H., Halasy, K., and Somogyi, P. (1994). Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the number of synaptic release sites. Nature *368*, 823–828.

Butt, S.J., Fuccillo, M., Nery, S., Noctor, S., Kriegstein, A., Corbin, J.G., and Fishell, G. (2005). The temporal and spatial origins of cortical interneurons predict their physiological subtype. Neuron *48*, 591–604.

Carleton, A., Petreanu, L.T., Lansford, R., Alvarez-Buylla, A., and Lledo, P.M. (2003). Becoming a new neuron in the adult olfactory bulb. Nat. Neurosci. *6*, 507–518.

Chen, W.R., and Shepherd, G.M. (2005). The olfactory glomerulus: a cortical module with specific functions. J. Neurocytol. *34*, 353–360.

Chittajallu, R., Pelkey, K.A., and McBain, C.J. (2013). Neurogliaform cells dynamically regulate somatosensory integration via synapse-specific modulation. Nat. Neurosci. *16*, 13–15.

Ciceri, G., Dehorter, N., Sols, I., Huang, J.Z., Maravall, M., and Marín, O. (2013). Lineage-specific laminar organization of cortical GABAergic interneurons. Nat. Neurosci.. http://dx.doi.org/10.1038/nn.3485.

Corotto, F.S., Henegar, J.R., and Maruniak, J.A. (1994). Odor deprivation leads to reduced neurogenesis and reduced neuronal survival in the olfactory bulb of the adult mouse. Neuroscience *61*, 739–744.

Cuzon, V.C., Yeh, P.W., Cheng, Q., and Yeh, H.H. (2006). Ambient GABA promotes cortical entry of tangentially migrating cells derived from the medial ganglionic eminence. Cereb. Cortex *16*, 1377–1388.

Daniel, D., Rossel, M., Seki, T., and König, N. (2005). Stromal cell-derived factor-1 (SDF-1) expression in embryonic mouse cerebral cortex starts in the intermediate zone close to the pallial-subpallial boundary and extends progressively towards the cortical hem. Gene Expr. Patterns *5*, 317–322.

Dantzker, J.L., and Callaway, E.M. (2000). Laminar sources of synaptic input to cortical inhibitory interneurons and pyramidal neurons. Nat. Neurosci. *3*, 701–707.

de Lima, A.D., Gieseler, A., and Voigt, T. (2009). Relationship between GABAergic interneurons migration and early neocortical network activity. Dev. Neurobiol. *69*, 105–123.

De Marchis, S., Bovetti, S., Carletti, B., Hsieh, Y.C., Garzotto, D., Peretto, P., Fasolo, A., Puche, A.C., and Rossi, F. (2007). Generation of distinct types of periglomerular olfactory bulb interneurons during development and in adult mice: implication for intrinsic properties of the subventricular zone progenitor population. J. Neurosci. *27*, 657–664.

De Marco García, N.V., Karayannis, T., and Fishell, G. (2011). Neuronal activity is required for the development of specific cortical interneuron subtypes. Nature *472*, 351–355.

DeFelipe, J., López-Cruz, P.L., Benavides-Piccione, R., Bielza, C., Larrañaga, P., Anderson, S., Burkhalter, A., Cauli, B., Fairén, A., Feldmeyer, D., et al. (2013). New insights into the classification and nomenclature of cortical GABAergic interneurons. Nat. Rev. Neurosci. *14*, 202–216.

Devor, M., Caviness, V.S., Jr., and Derer, P. (1975). A normally laminated afferent projection to an abnormally laminated cortex: some olfactory connections in the reeler mouse. J. Comp. Neurol. *164*, 471–482.

Doetsch, F., and Alvarez-Buylla, A. (1996). Network of tangential pathways for neuronal migration in adult mammalian brain. Proc. Natl. Acad. Sci. USA *93*, 14895–14900.

Fairén, A., Cobas, A., and Fonseca, M. (1986). Times of generation of glutamic acid decarboxylase immunoreactive neurons in mouse somatosensory cortex. J. Comp. Neurol. *251*, 67–83.

Fino, E., and Yuste, R. (2011). Dense inhibitory connectivity in neocortex. Neuron *69*, 1188–1203.

Fishell, G., and Rudy, B. (2011). Mechanisms of inhibition within the telencephalon: "where the wild things are". Annu. Rev. Neurosci. *34*, 535–567.

Flames, N., Pla, R., Gelman, D.M., Rubenstein, J.L., Puelles, L., and Marín, O. (2007). Delineation of multiple subpallial progenitor domains by the combinatorial expression of transcriptional codes. J. Neurosci. *27*, 9682–9695.

Flandin, P., Kimura, S., and Rubenstein, J.L. (2010). The progenitor zone of the ventral medial ganglionic eminence requires Nkx2-1 to generate most of the globus pallidus but few neocortical interneurons. J. Neurosci. *30*, 2812–2823.

Fogarty, M., Grist, M., Gelman, D., Marín, O., Pachnis, V., and Kessaris, N. (2007). Spatial genetic patterning of the embryonic neuroepithelium generates GABAergic interneuron diversity in the adult cortex. J. Neurosci. *27*, 10935–10946.

Franco, S.J., and Müller, U. (2011). Extracellular matrix functions during neuronal migration and lamination in the mammalian central nervous system. Dev. Neurobiol. *71*, 889–900.

Franco, S.J., Gil-Sanz, C., Martinez-Garay, I., Espinosa, A., Harkins-Perry, S.R., Ramos, C., and Müller, U. (2012). Fate-restricted neural progenitors in the mammalian cerebral cortex. Science *337*, 746–749.

Gelman, D.M., and Marín, O. (2010). Generation of interneuron diversity in the mouse cerebral cortex. Eur. J. Neurosci. *31*, 2136–2141.

Gelman, D.M., Martini, F.J., Nóbrega-Pereira, S., Pierani, A., Kessaris, N., and Marín, O. (2009). The embryonic preoptic area is a novel source of cortical GABAergic interneurons. J. Neurosci. *29*, 9380–9389.

Gelman, D., Griveau, A., Dehorter, N., Teissier, A., Varela, C., Pla, R., Pierani, A., and Marín, O. (2011). A wide diversity of cortical GABAergic interneurons derives from the embryonic preoptic area. J. Neurosci. *31*, 16570–16580.

Gong, Q., and Shipley, M.T. (1995). Evidence that pioneer olfactory axons regulate telencephalon cell cycle kinetics to induce the formation of the olfactory bulb. Neuron *14*, 91–101.

Hack, I., Bancila, M., Loulier, K., Carroll, P., and Cremer, H. (2002). Reelin is a detachment signal in tangential chain-migration during postnatal neurogenesis. Nat. Neurosci. *5*, 939–945.

Hack, M.A., Saghatelyan, A., de Chevigny, A., Pfeifer, A., Ashery-Padan, R., Lledo, P.M., and Götz, M. (2005). Neuronal fate determinants of adult olfactory bulb neurogenesis. Nat. Neurosci. *8*, 865–872.

Haider, B., Duque, A., Hasenstaub, A.R., and McCormick, D.A. (2006). Neocortical network activity in vivo is generated through a dynamic balance of excitation and inhibition. J. Neurosci. *26*, 4535–4545.

Hellwig, S., Hack, I., Zucker, B., Brunne, B., and Junghans, D. (2012). Reelin together with ApoER2 regulates interneuron migration in the olfactory bulb. PLoS ONE *7*, e50646.

Hendry, S.H., Schwark, H.D., Jones, E.G., and Yan, J. (1987). Numbers and proportions of GABA-immunoreactive neurons in different areas of monkey cerebral cortex. J. Neurosci. *7*, 1503–1519.

Hensch, T.K. (2005). Critical period plasticity in local cortical circuits. Nat. Rev. Neurosci. *6*, 877–888.

Hevner, R.F., Daza, R.A., Englund, C., Kohtz, J., and Fink, A. (2004). Postnatal shifts of interneuron position in the neocortex of normal and reeler mice: evidence for inward radial migration. Neuroscience *124*, 605–618.

Huang, L., Garcia, I., Jen, H.I., and Arenkiel, B.R. (2013). Reciprocal connectivity between mitral cells and external plexiform layer interneurons in the mouse olfactory bulb. Front Neural Circuits *7*, 32.

Igarashi, K.M., Ieki, N., An, M., Yamaguchi, Y., Nagayama, S., Kobayakawa, K., Kobayakawa, R., Tanifuji, M., Sakano, H., Chen, W.R., and Mori, K. (2012). Parallel mitral and tufted cell pathways route distinct odor information to different targets in the olfactory cortex. J. Neurosci. *32*, 7970–7985.

Imamura, F., Nagao, H., Naritsuka, H., Murata, Y., Taniguchi, H., and Mori, K. (2006). A leucine-rich repeat membrane protein, 5T4, is expressed by a subtype of granule cells with dendritic arbors in specific strata of the mouse olfactory bulb. J. Comp. Neurol. *495*, 754–768.

Inamura, N., Kimura, T., Tada, S., Kurahashi, T., Yanagida, M., Yanagawa, Y., Ikenaka, K., and Murakami, F. (2012). Intrinsic and extrinsic mechanisms control the termination of cortical interneuron migration. J. Neurosci. *32*, 6032–6042.

Inan, M., Welagen, J., and Anderson, S.A. (2012). Spatial and temporal bias in the mitotic origins of somatostatin- and parvalbumin-expressing interneuron subgroups and the chandelier subtype in the medial ganglionic eminence. Cereb. Cortex *22*, 820–827.

Inta, D., Alfonso, J., von Engelhardt, J., Kreuzberg, M.M., Meyer, A.H., van Hooft, J.A., and Monyer, H. (2008). Neurogenesis and widespread forebrain migration of distinct GABAergic neurons from the postnatal subventricular zone. Proc. Natl. Acad. Sci. USA *105*, 20994–20999.

Jankovski, A., and Sotelo, C. (1996). Subventricular zone-olfactory bulb migratory pathway in the adult mouse: cellular composition and specificity as

determined by heterochronic and heterotopic transplantation. J. Comp. Neurol. *371*, 376–396.

Jones, E.G. (1984). Laminar distribution of cortical efferent cells. In Cerebral Cortex, Vol 1: Cellular components of the cerebral cortex, A. Peters and E.G. Jones, eds. (New York: Plenum Press), pp. 521–553.

Katagiri, H., Pallotto, M., Nissant, A., Murray, K., Sassoè-Pognetto, M., and Lledo, P.M. (2011). Dynamic development of the first synapse impinging on adult-born neurons in the olfactory bulb circuit. Neural Syst Circuits *1*, 6.

Katz, L.C., and Shatz, C.J. (1996). Synaptic activity and the construction of cortical circuits. Science *274*, 1133–1138.

Kee, N., Teixeira, C.M., Wang, A.H., and Frankland, P.W. (2007). Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus. Nat. Neurosci. *10*, 355–362.

Kelsch, W., Lin, C.W., Mosley, C.P., and Lois, C. (2009). A critical period for activity-dependent synaptic development during olfactory bulb adult neurogenesis. J. Neurosci. *29*, 11852–11858.

Klausberger, T., and Somogyi, P. (2008). Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. Science *321*, 53–57.

Kohwi, M., Petryniak, M.A., Long, J.E., Ekker, M., Obata, K., Yanagawa, Y., Rubenstein, J.L., and Alvarez-Buylla, A. (2007). A subpopulation of olfactory bulb GABAergic interneurons is derived from Emx1- and Dlx5/6-expressing progenitors. J. Neurosci. *27*, 6878–6891.

Kosaka, T., and Kosaka, K. (2005). Structural organization of the glomerulus in the main olfactory bulb. Chem. Senses *30*(*Suppl 1*), i107–i108.

Kosaka, T., and Kosaka, K. (2008). Heterogeneity of parvalbumin-containing neurons in the mouse main olfactory bulb, with special reference to short-axon cells and betaIV-spectrin positive dendritic segments. Neurosci. Res. *60*, 56–72.

Krosnowski, K., Ashby, S., Sathyanesan, A., Luo, W., Ogura, T., and Lin, W. (2012). Diverse populations of intrinsic cholinergic interneurons in the mouse olfactory bulb. Neuroscience *213*, 161–178.

Lavdas, A.A., Grigoriou, M., Pachnis, V., and Parnavelas, J.G. (1999). The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. J. Neurosci. *19*, 7881–7888.

Le Magueresse, C., Alfonso, J., Khodosevich, K., Arroyo Martín, A.A., Bark, C., and Monyer, H. (2011). "Small axonless neurons": postnatally generated neocortical interneurons with delayed functional maturation. J. Neurosci. *31*, 16731–16747.

Lee, S., Hjerling-Leffler, J., Zagha, E., Fishell, G., and Rudy, B. (2010). The largest group of superficial neocortical GABAergic interneurons expresses ionotropic serotonin receptors. J. Neurosci. *30*, 16796–16808.

Lemasson, M., Saghatelyan, A., Olivo-Marin, J.C., and Lledo, P.M. (2005). Neonatal and adult neurogenesis provide two distinct populations of newborn neurons to the mouse olfactory bulb. J. Neurosci. *25*, 6816–6825.

Li, G., Adesnik, H., Li, J., Long, J., Nicoll, R.A., Rubenstein, J.L.R., and Pleasure, S.J. (2008). Regional distribution of cortical interneurons and development of inhibitory tone are regulated by Cxcl12/Cxcr4 signaling. J. Neurosci. *28*, 1085–1098.

Li, X., Sun, C., Lin, C., Ma, T., Madhavan, M.C., Campbell, K., and Yang, Z. (2011). The transcription factor Sp8 is required for the production of parvalbumin-expressing interneurons in the olfactory bulb. J. Neurosci. *31*, 8450–8455.

Liberia, T., Blasco-Ibáñez, J.M., Nácher, J., Varea, E., Zwafink, V., and Crespo, C. (2012). Characterization of a population of tyrosine hydroxylase-containing interneurons in the external plexiform layer of the rat olfactory bulb. Neuroscience *217*, 140–153.

Lin, C.W., Sim, S., Ainsworth, A., Okada, M., Kelsch, W., and Lois, C. (2010). Genetically increased cell-intrinsic excitability enhances neuronal integration into adult brain circuits. Neuron *65*, 32–39.

Lledo, P.M., Merkle, F.T., and Alvarez-Buylla, A. (2008). Origin and function of olfactory bulb interneuron diversity. Trends Neurosci. *31*, 392–400.

Lodato, S., Rouaux, C., Quast, K.B., Jantrachotechatchawan, C., Studer, M., Hensch, T.K., and Arlotta, P. (2011). Excitatory projection neuron subtypes control the distribution of local inhibitory interneurons in the cerebral cortex. Neuron *69*, 763–779.

Lois, C., and Alvarez-Buylla, A. (1994). Long-distance neuronal migration in the adult mammalian brain. Science *264*, 1145–1148.

Lois, C., García-Verdugo, J.M., and Alvarez-Buylla, A. (1996). Chain migration of neuronal precursors. Science *271*, 978–981.

Long, J.E., Garel, S., Alvarez-Dolado, M., Yoshikawa, K., Osumi, N., Alvarez-Buylla, A., and Rubenstein, J.L. (2007). Dlx-dependent and -independent regulation of olfactory bulb interneuron differentiation. J. Neurosci. *27*, 3230–3243.

López-Bendito, G., Sánchez-Alcañiz, J.A., Pla, R., Borrell, V., Picó, E., Valdeolmillos, M., and Marín, O. (2008). Chemokine signaling controls intracortical migration and final distribution of GABAergic interneurons. J. Neurosci. *28*, 1613–1624.

Lourenço, M.R., Garcez, P.P., Lent, R., and Uziel, D. (2012). Temporal and spatial regulation of interneuron distribution in the developing cerebral cortex—an in vitro study. Neuroscience *201*, 357–365.

Luskin, M.B. (1993). Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. Neuron *11*, 173–189.

Ma, Y., Hu, H., Berrebi, A.S., Mathers, P.H., and Agmon, A. (2006). Distinct subtypes of somatostatin-containing neocortical interneurons revealed in transgenic mice. J. Neurosci. *26*, 5069–5082.

Manent, J.B., Demarque, M., Jorquera, I., Pellegrino, C., Ben-Ari, Y., Aniksztejn, L., and Represa, A. (2005). A noncanonical release of GABA and glutamate modulates neuronal migration. J. Neurosci. *25*, 4755–4765.

Marín, O. (2013). Cellular and molecular mechanisms controlling the migration of neocortical interneurons. Eur. J. Neurosci. *38*, 2019–2029.

Marín, O., and Rubenstein, J.L.R. (2001). A long, remarkable journey: tangential migration in the telencephalon. Nat. Rev. Neurosci. *2*, 780–790.

Marín, O., Anderson, S.A., and Rubenstein, J.L.R. (2000). Origin and molecular specification of striatal interneurons. J. Neurosci. *20*, 6063–6076.

Marín, O., Yaron, A., Bagri, A., Tessier-Lavigne, M., and Rubenstein, J.L. (2001). Sorting of striatal and cortical interneurons regulated by semaphorin-neuropilin interactions. Science *293*, 872–875.

Markram, H., Toledo-Rodriguez, M., Wang, Y., Gupta, A., Silberberg, G., and Wu, C. (2004). Interneurons of the neocortical inhibitory system. Nat. Rev. Neurosci. *5*, 793–807.

Maroof, A.M., Keros, S., Tyson, J.A., Ying, S.W., Ganat, Y.M., Merkle, F.T., Liu, B., Goulburn, A., Stanley, E.G., Elefanty, A.G., et al. (2013). Directed differentiation and functional maturation of cortical interneurons from human embryonic stem cells. Cell Stem Cell *12*, 559–572.

Meechan, D.W., Tucker, E.S., Maynard, T.M., and LaMantia, A.S. (2012). Cxcr4 regulation of interneuron migration is disrupted in 22q11.2 deletion syndrome. Proc. Natl. Acad. Sci. USA *109*, 18601–18606.

Mejia-Gervacio, S., Murray, K., and Lledo, P.M. (2011). NKCC1 controls GABAergic signaling and neuroblast migration in the postnatal forebrain. Neural Dev. *6*, 4.

Merkle, F.T., Mirzadeh, Z., and Alvarez-Buylla, A. (2007). Mosaic organization of neural stem cells in the adult brain. Science *317*, 381–384.

Miller, M.W. (1985). Cogeneration of retrogradely labeled corticocortical projection and GABA-immunoreactive local circuit neurons in cerebral cortex. Brain Res. *355*, 187–192.

Miller, M.W. (1995). Relationship of the time of origin and death of neurons in rat somatosensory cortex: barrel versus septal cortex and projection versus local circuit neurons. J. Comp. Neurol. *355*, 6–14.

Miyoshi, G., and Fishell, G. (2011). GABAergic interneuron lineages selectively sort into specific cortical layers during early postnatal development. Cereb. Cortex *21*, 845–852.

Miyoshi, G., Hjerling-Leffler, J., Karayannis, T., Sousa, V.H., Butt, S.J., Battiste, J., Johnson, J.E., Machold, R.P., and Fishell, G. (2010). Genetic fate mapping reveals that the caudal ganglionic eminence produces a large and

diverse population of superficial cortical interneurons. J. Neurosci. *30*, 1582–1594.

Mizuguchi, R., Naritsuka, H., Mori, K., Mao, C.A., Klein, W.H., and Yoshihara, Y. (2012). Tbr2 deficiency in mitral and tufted cells disrupts excitatory-inhibitory balance of neural circuitry in the mouse olfactory bulb. J. Neurosci. *32*, 8831–8844.

Molyneaux, B.J., Arlotta, P., Menezes, J.R., and Macklis, J.D. (2007). Neuronal subtype specification in the cerebral cortex. Nat. Rev. Neurosci. *8*, 427–437.

Mori, K., and Sakano, H. (2011). How is the olfactory map formed and interpreted in the mammalian brain? Annu. Rev. Neurosci. *34*, 467–499.

Mountcastle, V.B. (1997). The columnar organization of the neocortex. Brain *120*, 701–722.

Nery, S., Fishell, G., and Corbin, J.G. (2002). The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations. Nat. Neurosci. *5*, 1279–1287.

Nicholas, C.R., Chen, J., Tang, Y., Southwell, D.G., Chalmers, N., Vogt, D., Arnold, C.M., Chen, Y.J., Stanley, E.G., Elefanty, A.G., et al. (2013). Functional maturation of hPSC-derived forebrain interneurons requires an extended timeline and mimics human neural development. Cell Stem Cell *12*, 573–586.

Nissant, A., Bardy, C., Katagiri, H., Murray, K., and Lledo, P.M. (2009). Adult neurogenesis promotes synaptic plasticity in the olfactory bulb. Nat. Neurosci. *12*, 728–730.

Nóbrega-Pereira, S., Kessaris, N., Du, T., Kimura, S., Anderson, S.A., and Marín, O. (2008). Postmitotic Nkx2-1 controls the migration of telencephalic interneurons by direct repression of guidance receptors. Neuron *59*, 733–745.

Noctor, S.C., Palmer, S.L., McLaughlin, D.F., and Juliano, S.L. (2001). Disruption of layers 3 and 4 during development results in altered thalamocortical projections in ferret somatosensory cortex. J. Neurosci. *21*, 3184–3195.

Packer, A.M., and Yuste, R. (2011). Dense, unspecific connectivity of neocortical parvalbumin-positive interneurons: a canonical microcircuit for inhibition? J. Neurosci. *31*, 13260–13271.

Packer, A.M., McConnell, D.J., Fino, E., and Yuste, R. (2012). Axo-dendritic overlap and laminar projection can explain interneuron connectivity to pyramidal Cells. Cereb. Cortex. Published online August 31, 2012. http://dx.doi.org/10.1093/cercor/bhs210.

Panzanelli, P., Bardy, C., Nissant, A., Pallotto, M., Sassoè-Pognetto, M., Lledo, P.M., and Fritschy, J.M. (2009). Early synapse formation in developing interneurons of the adult olfactory bulb. J. Neurosci. *29*, 15039–15052.

Petreanu, L., and Alvarez-Buylla, A. (2002). Maturation and death of adult-born olfactory bulb granule neurons: role of olfaction. J. Neurosci. *22*, 6106–6113.

Pla, R., Borrell, V., Flames, N., and Marín, O. (2006). Layer acquisition by cortical GABAergic interneurons is independent of Reelin signaling. J. Neurosci. *26*, 6924–6934.

Pleasure, S.J., Anderson, S., Hevner, R., Bagri, A., Marín, O., Lowenstein, D.H., and Rubenstein, J.L. (2000). Cell migration from the ganglionic eminences is required for the development of hippocampal GABAergic interneurons. Neuron *28*, 727–740.

Pressler, R.T., and Strowbridge, B.W. (2006). Blanes cells mediate persistent feedforward inhibition onto granule cells in the olfactory bulb. Neuron *49*, 889–904.

Price, J.L., and Powell, T.P. (1970). The synaptology of the granule cells of the olfactory bulb. J. Cell Sci. *7*, 125–155.

Rakic, P. (1988). Specification of cerebral cortical areas. Science *241*, 170–176.

Rakic, P. (2006). A century of progress in corticoneurogenesis: from silver impregnation to genetic engineering. Cereb. Cortex *16*(*Suppl 1*), i3–i17.

Rakic, P. (2007). The radial edifice of cortical architecture: from neuronal silhouettes to genetic engineering. Brain Res. Brain Res. Rev. *55*, 204–219.

Riccio, O., Murthy, S., Szabo, G., Vutskits, L., Kiss, J.Z., Vitalis, T., Lebrand, C., and Dayer, A.G. (2012). New pool of cortical interneuron precursors in the early postnatal dorsal white matter. Cereb. Cortex *22*, 86–98.

Rochefort, C., Gheusi, G., Vincent, J.D., and Lledo, P.M. (2002). Enriched odor exposure increases the number of newborn neurons in the adult olfactory bulb and improves odor memory. J. Neurosci. *22*, 2679–2689.

Rudy, B., Fishell, G., Lee, S., and Hjerling-Leffler, J. (2011). Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. Dev. Neurobiol. *71*, 45–61.

Rymar, V.V., and Sadikot, A.F. (2007). Laminar fate of cortical GABAergic interneurons is dependent on both birthdate and phenotype. J. Comp. Neurol. *501*, 369–380.

Sahara, S., Yanagawa, Y., O'Leary, D.D., and Stevens, C.F. (2012). The fraction of cortical GABAergic neurons is constant from near the start of cortical neurogenesis to adulthood. J. Neurosci. *32*, 4755–4761.

Sánchez-Alcañiz, J.A., Haege, S., Mueller, W., Pla, R., Mackay, F., Schulz, S., López-Bendito, G., Stumm, R., and Marín, O. (2011). Cxcr7 controls neuronal migration by regulating chemokine responsiveness. Neuron *69*, 77–90.

Sessa, A., Mao, C.A., Colasante, G., Nini, A., Klein, W.H., and Broccoli, V. (2010). Tbr2-positive intermediate (basal) neuronal progenitors safeguard cerebral cortex expansion by controlling amplification of pallial glutamatergic neurons and attraction of subpallial GABAergic interneurons. Genes Dev. *24*, 1816–1826.

Shepherd, G.M., Chen, W.R., Willhite, D., Migliore, M., and Greer, C.A. (2007). The olfactory granule cell: from classical enigma to central role in olfactory processing. Brain Res. Brain Res. Rev. *55*, 373–382.

Somogyi, P., Tamás, G., Lujan, R., and Buhl, E.H. (1998). Salient features of synaptic organisation in the cerebral cortex. Brain Res. Brain Res. Rev. *26*, 113–135.

Soriano, E., and Del Río, J.A. (2005). The cells of cajal-retzius: still a mystery one century after. Neuron *46*, 389–394.

Southwell, D.G., Froemke, R.C., Alvarez-Buylla, A., Stryker, M.P., and Gandhi, S.P. (2010). Cortical plasticity induced by inhibitory neuron transplantation. Science *327*, 1145–1148.

Southwell, D.G., Paredes, M.F., Galvao, R.P., Jones, D.L., Froemke, R.C., Sebe, J.Y., Alfaro-Cervello, C., Tang, Y., Garcia-Verdugo, J.M., Rubenstein, J.L., et al. (2012). Intrinsically determined cell death of developing cortical interneurons. Nature *491*, 109–113.

Stancik, E.K., Navarro-Quiroga, I., Sellke, R., and Haydar, T.F. (2010). Heterogeneity in ventricular zone neural precursors contributes to neuronal fate diversity in the postnatal neocortex. J. Neurosci. *30*, 7028–7036.

Stenman, J., Toresson, H., and Campbell, K. (2003). Identification of two distinct progenitor populations in the lateral ganglionic eminence: implications for striatal and olfactory bulb neurogenesis. J. Neurosci. *23*, 167–174.

Stumm, R.K., Zhou, C., Ara, T., Lazarini, F., Dubois-Dalcq, M., Nagasawa, T., Höllt, V., and Schulz, S. (2003). CXCR4 regulates interneuron migration in the developing neocortex. J. Neurosci. *23*, 5123–5130.

Tanaka, D.H., Mikami, S., Nagasawa, T., Miyazaki, J., Nakajima, K., and Murakami, F. (2010). CXCR4 is required for proper regional and laminar distribution of cortical somatostatin-, calretinin-, and neuropeptide Y-expressing GABAergic interneurons. Cereb. Cortex *20*, 2810–2817.

Taniguchi, H., Lu, J., and Huang, Z.J. (2013). The spatial and temporal origin of chandelier cells in mouse neocortex. Science *339*, 70–74.

Tham, T.N., Lazarini, F., Franceschini, I.A., Lachapelle, F., Amara, A., and Dubois-Dalcq, M. (2001). Developmental pattern of expression of the alpha chemokine stromal cell-derived factor 1 in the rat central nervous system. Eur. J. Neurosci. *13*, 845–856.

Thomas, L.B., Gates, M.A., and Steindler, D.A. (1996). Young neurons from the adult subependymal zone proliferate and migrate along an astrocyte, extracellular matrix-rich pathway. Glia *17*, 1–14.

Tiveron, M.C., Rossel, M., Moepps, B., Zhang, Y.L., Seidenfaden, R., Favor, J., König, N., and Cremer, H. (2006). Molecular interaction between projection neuron precursors and invading interneurons via stromal-derived factor 1 (CXCL12)/CXCR4 signaling in the cortical subventricular zone/intermediate zone. J. Neurosci. *26*, 13273–13278.

Tremblay, R., Clark, B.D., and Rudy, B. (2010). Layer-specific organization within the fast-spiking interneuron population of mouse barrel cortex. In 2010 Neuroscience Meeting Planner (San Diego: Society for Neuroscience).

Tricoire, L., Pelkey, K.A., Erkkila, B.E., Jeffries, B.W., Yuan, X., and McBain, C.J. (2011). A blueprint for the spatiotemporal origins of mouse hippocampal interneuron diversity. J. Neurosci. *31*, 10948–10970.

Turrigiano, G. (2011). Too many cooks? Intrinsic and synaptic homeostatic mechanisms in cortical circuit refinement. Annu. Rev. Neurosci. *34*, 89–103.

Valcanis, H., and Tan, S.S. (2003). Layer specification of transplanted interneurons in developing mouse neocortex. J. Neurosci. *23*, 5113–5122.

van den Berghe, V., Stappers, E., Vandesande, B., Dimidschstein, J., Kroes, R., Francis, A., Conidi, A., Lesage, F., Dries, R., Cazzola, S., et al. (2013). Directed migration of cortical interneurons depends on the cell-autonomous action of Sip1. Neuron *77*, 70–82.

Ventura, R.E., and Goldman, J.E. (2007). Dorsal radial glia generate olfactory bulb interneurons in the postnatal murine brain. J. Neurosci. *27*, 4297–4302.

Voyvodic, J.T. (1996). Cell death in cortical development: How much? Why? So what? Neuron *16*, 693–696.

Waclaw, R.R., Wang, B., Pei, Z., Ehrman, L.A., and Campbell, K. (2009). Distinct temporal requirements for the homeobox gene Gsx2 in specifying striatal and olfactory bulb neuronal fates. Neuron *63*, 451–465.

Wichterle, H., Garcia-Verdugo, J.M., and Alvarez-Buylla, A. (1997). Direct evidence for homotypic, glia-independent neuronal migration. Neuron *18*, 779–791.

Wichterle, H., Garcia-Verdugo, J.M., Herrera, D.G., and Alvarez-Buylla, A. (1999). Young neurons from medial ganglionic eminence disperse in adult and embryonic brain. Nat. Neurosci. *2*, 461–466.

Wichterle, H., Turnbull, D.H., Nery, S., Fishell, G., and Alvarez-Buylla, A. (2001). In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. Development *128*, 3759–3771.

Willaime-Morawek, S., Seaberg, R.M., Batista, C., Labbé, E., Attisano, L., Gorski, J.A., Jones, K.R., Kam, A., Morshead, C.M., and van der Kooy, D. (2006). Embryonic cortical neural stem cells migrate ventrally and persist as postnatal striatal stem cells. J. Cell Biol. *175*, 159–168.

Winner, B., Cooper-Kuhn, C.M., Aigner, R., Winkler, J., and Kuhn, H.G. (2002). Long-term survival and cell death of newly generated neurons in the adult rat olfactory bulb. Eur. J. Neurosci. *16*, 1681–1689.

Wonders, C.P., and Anderson, S.A. (2006). The origin and specification of cortical interneurons. Nat. Rev. Neurosci. *7*, 687–696.

Woodworth, M.B., Custo Greig, L., Kriegstein, A.R., and Macklis, J.D. (2012). SnapShot: cortical development. Cell *151*, 918–918.e1.

Wu, S., Esumi, S., Watanabe, K., Chen, J., Nakamura, K.C., Nakamura, K., Kometani, K., Minato, N., Yanagawa, Y., Akashi, K., et al. (2011). Tangential migration and proliferation of intermediate progenitors of GABAergic neurons in the mouse telencephalon. Development *138*, 2499–2509.

Xu, X., and Callaway, E.M. (2009). Laminar specificity of functional input to distinct types of inhibitory cortical neurons. J. Neurosci. *29*, 70–85.

Xu, Q., Cobos, I., De La Cruz, E., Rubenstein, J.L., and Anderson, S.A. (2004). Origins of cortical interneuron subtypes. J. Neurosci. *24*, 2612–2622.

Xu, Q., Tam, M., and Anderson, S.A. (2008). Fate mapping Nkx2.1-lineage cells in the mouse telencephalon. J. Comp. Neurol. *506*, 16–29.

Xu, H., Jeong, H.Y., Tremblay, R., and Rudy, B. (2013). Neocortical somatostatin-expressing GABAergic interneurons disinhibit the thalamorecipient layer 4. Neuron *77*, 155–167.

Yamaguchi, M., and Mori, K. (2005). Critical period for sensory experience-dependent survival of newly generated granule cells in the adult mouse olfactory bulb. Proc. Natl. Acad. Sci. USA *102*, 9697–9702.

Yoshimura, Y., and Callaway, E.M. (2005). Fine-scale specificity of cortical networks depends on inhibitory cell type and connectivity. Nat. Neurosci. *8*, 1552–1559.

Young, K.M., Fogarty, M., Kessaris, N., and Richardson, W.D. (2007). Subventricular zone stem cells are heterogeneous with respect to their embryonic origins and neurogenic fates in the adult olfactory bulb. J. Neurosci. *27*, 8286–8296.

Zhu, Y., Cao, L., Su, Z., Mu, L., Yuan, Y., Gao, L., Qiu, Y., and He, C. (2010). Olfactory ensheathing cells: attractant of neural progenitor migration to olfactory bulb. Glia *58*, 716–729.

Zou, D.J., Chesler, A., and Firestein, S. (2009). How the olfactory bulb got its glomeruli: a just so story? Nat. Rev. Neurosci. *10*, 611–618.

GENERAL DISCUSSION

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Functional circuits in the mammalian brain arise from early developmental processes that guide the precise assembly of their cellular components. The cerebral cortex consists of two main classes of neurons with different embryonic origins and genetic programs of cell-fate specification, excitatory pyramidal cells and inhibitory interneurons. In this Thesis, we investigated the role of cell lineages in shaping the specification and organization of different classes of excitatory and inhibitory neurons during the development of the mouse cerebral cortex *in vivo*. We developed a novel approach to label individual progenitor cells and trace their progenies with regional/subtype specificity based on a combination of retroviral tracing technology and Cre/lox mouse genetics. Using this method, we analysed how lineage relationships impact the formation of cortical cytoarchitecture.

1. Lineage specification of cortical inhibitory interneurons

1.1 Mechanisms of interneuron clustering

Our results demonstrate that interneurons lineages do not distribute randomly in the adult cerebral cortex but instead have a strong tendency to form cells clusters that are confined within a relatively small volume of cortical tissue. This finding independently replicates a recent study form the Shi laboratory that first reported the clustering behaviour for MGE/POA-derived interneurons in the neocortex (Brown et al. 2011). In this study, the authors took also advance of a different retroviral technology and used the avian RCAS/TVA viral system (replication-competent avian sarcoma-leukosis virus long terminal repeat with splice acceptor /tumor virus A) in combination with Cre/loxP mouse genetics (Beier et al. 2011; von Werder et al. 2012). Because mammalian cells do not endogenously express the TVA receptor for EnvA carrying-avian viruses, they are not susceptible to RCAS viruses infection (Weiss 1982). The ectopic conditional expression of TVA receptors in mice using tissue-specific Cre drivers confers susceptibility for avian retroviruses infections in defined progenitor populations. Thus, in this system the specificity for labelling interneuron progenitors is based on viral tropisms. In contrast, the expression of fluorescent proteins in our system relies exclusively on Cre-mediated recombination and can be efficiently used to label specific progenitors cells and their progenies, as well as cohorts of post-mitotic neuronal subtypes (derived from infections of mitotic progenitor cells, independently on the time point in which Cre begins to be expressed). We applied our lineage tracing method to the analysis of three main classes of cortical interneurons (SST^+, PV^+ and VIP^+), and we found that clustering behaviour is a

general property of all classes of cortical interneurons, independently on their embryonic origin and program of cell fate specification (as shown for the MGE-derived SST^+ and PV^+ subtypes and for the CGE-derived VIP^+ ones).

The mechanisms that mediate the clustering of interneuron lineages remain unclear. The observation of mitotic cells of subpallial origin in the perinatal cortex led to the suggestion that some cortical interneurons might retain mitotic potential after reaching the neocortex (Wu et al. 2011). Local cell division in the postnatal cortex is important for the generation of astrocytes (Ge et al. 2012), but the quantitative relevance of these events for cortical interneurons is presently unclear. While this mechanism would certainly explain interneuron clustering, it is unlikely that this phenomenon per se accounts for the strong tendency of interneuron clustering observed in the recent studies (Brown et al. 2011; Ciceri et al. 2013). Birth-dating analyses performed at embryonic stages indicate that most interneurons clusters are composed by coetaneous cells and suggest that the expansion of interneuron lineages through IPCs is actually a major mechanism for interneuron production. This finding, together with the observation that non-apical cell division represent about 60% of the mitosis in the ganglionic eminences (Pilz et al. 2013), support the view that lineage expansion take place primarily in the SVZ of the subpallium prior to tangential migration.

An alternative explanation for the clustering behaviour is that lineage-related interneurons may coordinate their migration to the neocortex. This hypothesis would imply that interneurons reiteratively interact with each other during tangential migration and maintain reciprocal spatial relationships through a kind of collective cell migration. Although there are not evidences supporting this hypothesis for interneurons, cell-cell interactions have been shown to be relevant for the migration of other neuronal populations in the embryonic brain (Villar-Cerviño et al. 2013), and may therefore represent a possible mechanism for future investigations.

During the last decades, multiple studies have shown that environmental cues regulate the migration of telencephalic interneurons (Marín 2013). Interneurons read these signals as attractive, permissive or repulsive, depending on the differential expression of receptors for guidance cues. Thus, one interesting explanation of the clustering phenomenon could be that the composition of the receptor machinery might be specified at progenitor cell level and that sibling interneurons may share similar responsiveness to guidance cues, which will in turn determine their final spatial organization. In addition, while lineage

clustering seems a general property of cortical interneurons, the simultaneous injection of retroviruses encoding either Gfp or mCherry revealed that lineage relationships are not an exclusive determinant of interneuron clustering. Based on this, it is tempting to speculate that also non-sibling interneurons born from nearby progenitor cells roughly around the same time window may end up in approximately neighbouring positions in the adult cortex. This hypothesis is consistent with the finding that interneuron clusters typically contained interneurons with highly correlated birthdate. Thus, according to this hypothesis, the spatial and temporal specification of progenitor cells (i.e. the combinatorial expression of transcriptional codes) in the ganglionic eminences would instruct newborn interneurons about their future location. This solution implies the existence of a topographical organization of the embryonic subpallium that relates the position of progenitor cells in the eminences with the allocation of interneurons along the rostro-caudal axis of the cortex. It is well established that the pallium is topographically organized along the tangential axis so that the embryonic position of progenitor cells match the area identity of post-mitotic pyramidal cells (Rakic 1988). This map is efficiently maintained along development through radial migration. However, how long-distance tangential migration can ensure such organization for cortical interneurons represents a clear challenge. Interestingly, a recent study has shown that the coordination between genetic programs of progenitor cells specification and the spatial distribution of cues in the migratory environment allow the topographic tangential migration of precerebellar neurons in the brainstem (Di Meglio et al. 2013). This study suggested a role for epigenetic factors in balancing intrinsic and extrinsic programs during tangential migration, and opened new perspective on this topic.

1.2 Spatial organization of interneuron lineages and models of lineage progression

The analysis of the spatial distribution of interneuron clusters performed in our study and in Brown et al (2011) revealed some apparent discrepancies. Brown and colleagues analysed the organization of interneurons derived from MGE/POA progenitors infected at E12.5 and reported that they primarily adopt a vertical organization, spanning several cortical layers. Instead, we performed retroviral injections at different developmental stages and found that interneurons clusters are largely confined within one or two adjacent cortical layers. Therefore, according to our analyses interneuron clusters organize primarily along the laminar dimension of the neocortex. Interestingly, Brown and colleagues also

reported that a fraction of MGE/POA progenitors labelled at E12.5 produce interneurons that form horizontal clusters that likely correspond to those clusters that we found distributed through one or two adjacent layers in our experiments. Of note, retroviral infections of MGE/POA progenitors at early developmental time points (E10.5 and E11.5) result in the labelling interneurons that primarily populate deep cortical layers (layers V and VI). Conversely, retroviral-tracing experiments performed at E14.5 label MGE/POA progenitor cells that produce interneurons located in superficial layers (layers II/III and IV). While the localization of interneuron lineages labelled at E14.5 through superficial layers was somehow expected, our finding that early-labelled lineages are largely confined within infragranular cortical layers has important implications.

The strong correlation that exists between the time of birth and the laminar position for MGE-derived interneurons through the cortex (Miller 1985; Fairén et al. 1986; Valcanis and Tan 2003; Pla et al. 2006) suggests that the spatial organization of interneurons lineages is of primary importance to infer the pattern of neurogenesis in the MGE. The vertical organization of interneuron lineages reported in Brown at al. support a *progressive fate restriction model* in which a single progenitor cells is constantly active during the entire period of neurogenesis and generates interneurons for all cortical layers in an inside-out fashion. Instead, our evidence that early-labelled MGE/POA progenitors do not generate many interneurons for the superficial layers strongly supports the existence of multiple fate-restricted progenitor pools that generate interneurons for specific cortical layers. Collectively, our results are consistent with a model in which the MGE may contain distinct interneuron progenitors with different lineage potentials, at least in reference to the laminar distribution of their progenies. How can these studies be reconciled? One possibility is that the relative abundance of fate-restricted progenitor pools varies during development. MGE precursors committed to produce infragranular cells might be particularly abundant at early stages of neurogenesis and be progressively depleted during development. By contrast, a second class of progenitors might be primarily self-expanding or slowly dividing at early time points, only to become neurogenic at later stages. In this scenario, the inside-out pattern adopted by MGE-derived interneurons while populating the cortex reflects the laminar potential of distinct progenitor pools during development rather than the columnar distribution of individual interneuron lineages. Therefore, birthdate itself may not the primary factor that instructs interneurons about their laminar position. Consistent with this hypothesis, the early pool of MGE progenitors continue to contribute

to deep layers interneurons even at relatively late stages of development (E11.5 rv::dio::gfp or mCherry + tamoxifen at E14.5 in Nkx2-1^{creErt2} experiment). This reinforces the view that progenitors are largely committed to generate interneurons for specific layers of the cortex (infragranular or granular/supragranular) independently on birthdate. In addition, the contribution of deep layer committed progenitor cells to layer VI interneurons seems to decline as the development progresses, which suggest that *the progressive fate restriction model* may indeed apply to interneuron lineages *within* each of the progenitor classes identified in our study. Thus, it is likely that the remaining deep-layer committed progenitors that are still present at E14.5 do not generate many layer VI interneurons because its fate is already restricted to produce cells for layer V. In this context, it would be interestingly to study the molecular mechanisms that control this progressive restriction. Several elegant studies in *Drosophila* have shown that the sequential expression of different transcription factors within individual progenitor cells lead to the temporally controlled production of different cell types (Bayraktar and Doe 2013; Li et al. 2013) and suggest that sub-nuclear genome reorganization can play a role in fate-restriction (Kohwi et al. 2013). Finally, we cannot exclude that some interneuron lineages adopt a vertical organization and that both models of neurogenesis might coexist, since our analyses also detected some interlaminar interneuron clusters. However, according to our analysis these lineages account for a minor fraction.

How deep- and superficial layer progenitors emerge during development? The retroviral tracing experiments performed at different time points indicate that these two progenitors classes segregate between E9.5 and E10.5. The retroviral tracing of MGE/POA progenitor cells at E9.5 leads to the labelling of interneurons through all cortical layers, which reinforce our working model and suggest the existence of uncommitted precursor cells lining the lateral ventricle at this stage. Preliminary observations also suggest that interneurons labelled at E9.5 adopt a laminar organization. Thus, according to our model of lineage progression in the MGE, at least three different progenitor types populate this region, each with a particular neurogenic potential.

It is presently unclear whether the interneuron precursors that we identified based on their lineage potential correspond to morphologically distinct classes of progenitors cells. Recent work reported a high degree of progenitor cell diversity in the ganglionic eminences based on morphology and place of cell division (Pilz et al. 2013). This includes not only IPCs in SVZ and SNPs equivalent to the ones identified in the pallium, but also

bipolar RGCs that divide preferentially at apical or sub-apical positions. Interestingly, the ratio between the numbers of apical and sub-apical/basal mitosis progressively changes during development (Pilz et al. 2013). In spite of this morphological diversity, the molecular fingerprints of the different progenitor classes remain largely unknown. Analysis of gene-expression profiles at single progenitor and/or single clone level would potentially reveal molecular pathways (i.e. transcription factors) involved in the specification of different interneuron lineages and allow systematic fate-mapping studies of MGE progenitor classes. In this context, not only the presence or absence of particular transcription factors would be relevant but also the dynamic fluctuations of their levels of expression. For instance, it has been recently shown that oscillatory networks of Notch family genes and bHLH transcription factors control cell-fate choice in the ventral telencephalon (Imayoshi et al. 2013). In addition, while the role of epigenetic factors in regulating for instance the transition of pallial progenitor cell from neurogenic to gliogenic has been studied (Hirabayashi et al. 2009; Hirabayashi and Gotoh 2010), little is known about the impact of such mechanisms during neurogenesis in the ganglionic eminences.

The MGE produces neurons and oligodendrocytes not only for the neocortex but also for the hippocampus and basal forebrain structures such as the globus pallidus and the striatum (Marin et al. 2000; Kessaris et al. 2006; Xu et al. 2008; Mckinsey et al. 2013). Fate-mapping studies at the population level indicate that, for instance, Shh-expressing progenitors in the ventral MGE primarily produce interneurons for the basal forebrain, and do not contribute much to the neocortical population (Flandin et al. 2010). This strongly suggests the existence of segregated progenitor pools for different forebrain structures. However, whether interneurons populating different structures share a common clonal origin or derive from segregated precursors remains still an open question. Similarly, it is still unclear whether the same MGE progenitor cell produces both neurons and glia. This issue is particularly relevant also in the context of the lineage potential of deep- and superficial layers committed MGE progenitor cells. For instance, we ignore if the pool of deep layer restricted progenitors is actually depleted as development progress or if there is a switch in their fate-competence (from interneurons to oligodendrocytes) during late developmental stages. Addressing these and other questions will require the development of new lineage tracing technologies with increased single clone resolution. To this end, different methods have been proposed. One interesting strategy is based on viral libraries in which each library member carry a distinct DNA fragment as molecular tag together

with a reporter protein. Lineage restrictions are inferred following the isolation of cells from the tissue and analysis of DNA tags through PCR. This method, originally developed for lineage tracing in the nervous system (Golden et al. 1995; McCarthy et al. 2001), has also been recently applied for lineage tracing in the hematopoietic lineage (Lu et al. 2011). Although this system has high-resolution power, it is very laborious, which limits its application to large histological samples. Based on the similar principle, lineage-tracing methods based on combinatorial labelling with different fluorescent tags has been proposed (García-Marqués and López-Mascaraque 2013; García-Moreno et al. 2014; Loulier et al. 2014). Beyond the need to improve and simplify the imaging and the analysis required for its full exploitation, this system would allow for the imaging of multiple lineages during time-lapse microscopy and slice electrophysiology.

The organization and number of cortical interneurons is not only determined by events that occur at the level of progenitor cells (i.e., progenitor cell diversity and pattern of cell division), but also by events that occur at postnatal stages. Programmed cell death eliminate about 40% of the cortical interneurons during early postnatal development (Southwell et al. 2012). Using transplantation assays, it has been found that developmental cell death largely depends on the cellular age of interneurons. These results suggest that intrinsic genetic programs encoded within individual precursors may instruct interneurons to undergo apoptosis at a specific developmental timing, independently on environmental factors (i.e. trophic factors). Alternatively, cohorts of interneurons may compete for limiting survival signals (e.g. cell-cell interaction) in the cortical environment. In the latter case, competitions would be restricted to roughly coetaneous interneurons that likely undergo similar processes with a similar developmental window. In either case, it would be interesting to test whether programmed cell death occurs randomly with no particular relationship to the spatial organization of individual lineages or if both processes are somehow related. For instance, it is unclear whether entire lineages are eliminated or cell death occurs within each individual lineage. In this context, it is theoretically conceivable that the superficial layers interneurons derived from retroviral infections of MGE progenitor at early time points are eliminated through programmed cell death. Although collectively our data strongly support the *multiple fate-restricted progenitors model*, it remains a formal possibility that selective cell death contribute to the laminar restriction of interneuron lineages.

It is worth noting that the temporal window of interneuron cell death matches the pick of interneuron synaptogenesis, suggesting that interneuron survival might be linked to their recruitment into cortical circuits. In this scenario, early functional interactions between neurons might promote interneuron survival and select those interneurons that will be integrated into future cortical circuits, which might represents a possible mechanism that underpins the sculpting of early cortical assembly motifs. Whether interneuron clustering has physiological implications in cortical circuitry is still an unexplored question. In this direction, the analysis of the functional interaction between interneurons belonging to the same lineage as well as their pattern of pre- and post- synaptic connectivity with pyramidal cells would provide important insights into the development of cortical networks.

2. Lineage specification of cortical pyramidal cells

The generation of pyramidal cells subtypes has been intensively studied during the past few decades. As explained in the introduction, the main events of progenitor cell division as well as the lineage progression through different morphological and molecular progenitor types have been largely elucidated. However, the ontogeny of the different identities of pyramidal neurons (PNs) is still matter of debate and represents a fundamental challenge for our understanding of cortical development. In the attempt of reconstructing the developmental history of pyramidal cell subtypes, different experimental approaches have been used, with controversial results and interpretations. In the more recent literature, two articles addressed the contribution of molecularly defined progenitor classes at population level and reached different conclusions. Franco and colleagues reported the finding of Cux2-expressing RGCs that are restricted to generate superficial layers PNs, which led them to propose that the mouse cerebral cortex develops according to *multiple fate-restricted progenitors model* (Franco et al. 2012). In contrast, Guo and colleagues analysed the contribution of Fezf2-expressing progenitor cells to the PN laminar identities and concluded that pallial RGCs are primarily multipotent and produce all major PN subtypes (Guo et al. 2013), which favours the *progressive fate restriction model*.

In this thesis, we systematically fate-mapped individual pallial progenitor cells using our conditional retroviral-tracing strategy and characterized the contribution of individual progenitor cells to neurons throughout the six layers of the neocortex. According to the general view of cortical development, retroviral injections in the lateral ventricle likely label distinct apical progenitor types (NECs and RGCs) depending on the developmental

time point. Consistently, the number of neurons/clone progressively declines during development likely as result of labelling self-expanding NECs at early time points (E10.5/E11.5) and neurogenic asymmetrically diving RGCs from E12.5 onward. Thus, according to our data, viral injections at E11.5/E12.5 label individual RGCs and allow the study of their contribution to the different cortical layers.

Retroviral tracings experiments at E10.5 labelled PNs distributed throughout all the six cortical layers, which support the view that NECs are indeed multipotent. Thus, in these experiments clonal arrays are likely derived from multiple sibling RGCs. This result is consistent with a study presented during the last meeting of the Society for Neuroscience (Gao et al. 2013) in which the authors performed lineage tracing analysis using MADM (mosaic analysis with double markers) technology (Zong et al. 2005) in combination with tamoxifen-inducible Cre drivers specifically expressed in pallial progenitors. In this method, the coding sequence of two (green and red) reporter genes is split in two parts. The partial coding sequences for green and red fluorescent proteins are separated by a loxP containing intron and reciprocally inserted in the two alleles of specific genomic locus. Cre-mediated inter-chromosomal recombination reconstitutes functional green and red fluorescent proteins coding sequence and results in cell labelling. During cell division, if the recombination occurs during G2 phase of the cell cycle and depending on the pattern of chromatids segregation, there are two possible outcomes: the generation of two daughter cells differentially labelled (green or red), or the production of non-labelled and double-labelled daughter cells. Recombination events during G1 or G0 phases always generate double-labelled cells.

While the laminar distribution of PN derived from E10.5 labelled pallial progenitors was consistent between both studies, similar analyses at later stages reveal some apparent discrepancies. Gao and colleagues performed lineage-tracing experiments from E10.5 to E13.5 and reported that independently on the embryonic stage of labelling, PN lineages consistently distribute through all cortical layers, supporting the *progressive fate-restriction model* of cortical neurogenesis. Instead, our results indicates that about 25% of the lineages labelled at both E11.5 and E12.5 contained cells populating layer VI, IV and II/III, but not layer V. Moreover, about 45% of the lineages labelled at E12.5 were confined within one or two cortical layers and mostly restricted to either infragranular or supragranular layers. The finding of PN lineages lacking layer V neurons reveal a new pattern of distribution and suggests that in some cases RGCs may skip the production of

layer V cells while be able to subsequently produce layer IV and II/III neurons. Alternatively, selective cell death of the layer V PNs during development may also explain this observation. The finding of infragranular and supragranular restricted PN lineages labelled at E12.5 is consistent with the results reported by Franco and colleagues (Franco et al. 2012), and suggest that multipotent and fate-restricted PN lineages may coexist in the mouse cerebral cortex. It is worth mentioning, however, that the integration of transgenes carried by retroviral vectors occurs during cell division so that the fluorescent reporter is inherited by one of the two daughter cells. Thus, the infection of individual RGCs could theoretically result in labelling either the self-renewing RGCs or the IPCs/post-mitotic cells in 50% of the cases. Thus, it is equally possible that the laminar-restricted ontogenic units found in E12.5 fate-mapping experiments actually derive from IPCs rather than primary RGCs. In this scenario, IPCs would be the progenitor type intrinsically committed to produce PN for specific cortical layers and it would be interesting to study in more detail this progenitor class. In any case, it would be important to analyse the subtype composition of individual PN lineages, independently on the laminar localization of PN. In fact, while the laminar position of PNs largely reflects their identity, there are some PN subtypes (e.g. Satb2-expressing CPN) that are more widely distributed through layers. A comprehensive analysis of the markers expression, together with the analysis of the pattern of axonal projections at single clone level will solve this important question.

The analysis of connectivity patterns in retroviral labelled lineages of excitatory units revealed a preferential synaptic coupling between pairs of sibling neurons compared to non-sibling neighbours (Yu et al. 2009). Further analysis proposed that such preferential connectivity arise from transient electrical synapsis established preferentially between sibling PNs during development (Yu et al. 2012). While the preferential connection between sibling PNs opens new perspectives for the development of cortical sub-networks, whether this pattern of connectivity reflects the functional organization of the neocortex in columns as suggested by the radial unit hypothesis remains still an open question. Two recent studies addressed more directly this issue by looking at stimulus orientation selectivity between sibling PNs in the visual cortex (Li et al. 2012; Ohtsuki et al. 2012). Neurons in the visual cortex respond differentially to the orientations of visual stimuli. In some species, including primates, neurons with similar responsiveness share pattern of connectivity and are grouped into radially units while in rodents neurons with different selectivity are intermingled among them. In either case, similar orientation responsiveness

has been proposed as a functional unit and called 'orientation columns'(Hubel and Wiesel 1968). Both studies reported that sibling neurons are more likely to have similar orientation preferences than non-sister ones. However, the strength of stimulus preference reported in both studies shows some differences that likely derive form the different stages of lineage labelling and analysis. Li and colleagues analysed pairs of pyramidal cells labelled by retroviral infection at E15-E17 and found strong preferential responsiveness between sibling pyramidal cells. Instead, Ohtsuki and colleagues used a mouse line that label much larger arrays (Magavi et al. 2012), likely derived from progenitor cells at consistently earlier stages of development. While supporting a role of lineages in the development of stimulus orientation selectivity, this study suggested that other factors (e.g. extrinsic activity driven by experiences) could impact on this phenomenon. For example, it would be interesting to study how the distance within the neurogenic three between siblings PNs impact on the establishment of preferential connectivity and responsiveness to similar stimuli. The analysis of the same parameters between superficial-layer cells derived from sibling RGCs as well as between infra- and supra-granular PNs derived from the same RGCs would help to solve these questions.

3. Inhibitory and excitatory neuron assembly

The analysis of the lineage specification of cortical interneurons and pyramidal cells reveal some important differences. The fate-mapping analysis of MGE progenitor cells strongly support the existence of progenitor classes intrinsically specified to produce interneurons for deep and superficial layers starting from early stages of development. Instead, the analysis of PN lineage development reveals a more complicated and still controversial picture. On one side, our results suggest that both multipotent and fate restricted RGC types may coexist in the embryonic pallium and give rise to radial and laminar ontogenic arrays. However, our results are also compatible with the alternative possibility that RGCs are largely multipotent and that the laminar restriction of some PN lineages arises at the level of individual IPCs, which might be committed to produce infragranular or supragranular PNs.

In either case, our findings open new perspectives for the assembly of inhibitory and excitatory neurons into cortical circuits. Multiple evidences suggest that cortical GABAergic interneurons organize in relation to pyramidal cells and that the laminar localization of cortical interneurons is regulated by layer-specific or cell type–specific cues

produced by pyramidal cells (Hevner et al. 2004; Pla et al. 2006; Lodato et al. 2011a; Miyoshi and Fishell 2011). The finding of distinct MGE-derived lineages restricted to populate specific cortical layers independently on birthdate suggests that the differential responsiveness to laminar cues might be intrinsically specified at the level of individual progenitor cells.

CONCLUSIONS

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1. Conditional reporter retroviruses are a useful tool for region- and cell-specific fate mapping analyses.
2. Lineage analyses of GABAergic interneuron progenitor cells revealed that all the major classes of cortical interneurons (the MGE/POA-derived PV+ and SST+ and the CGE-derived VIP+) have a strong tendency to cluster in the cerebral cortex, independently on embryonic origin and cell-fate specification programs.
3. MGE/POA-derived interneuron clusters are either homogeneous or heterogeneous in their composition, based on electrophysiological and neurochemical properties.
4. MGE/POA-derived interneuron clusters primarily distribute in the same or in two adjacent layers.
5. Intra-lamina clusters are composed by largely coetaneous cells, which suggest that interneuron lineages are expanded through intermediate progenitor cells that become neurogenic roughly at the same time.
6. MGE/POA progenitors labelled at E10.5/E11.5 produce interneurons mostly for deep cortical layers, whereas progenitor cells labelled at E14.5 give rise to interneurons that colonize the superficial layers of the cortex. These results suggest the existence of multiple fate-restricted interneuron lineages with different laminar potential.
7. Lineage analyses revealed a substantial diversity of glutamatergic neuron progenitor cells, including the existence of progenitor cells that produce lineages with different laminar distribution patterns. These results suggest that multipotent and fate-restricted projection neuron lineages may coexist in the developing neocortex. Alternatively our results point out a role of intermediate progenitor cells in the laminar restriction of pyramidal cell lineages.

1. Los retrovirus reporteros condicionales representan una herramienta experimental útil para el análisis del destino celular con especificidad tanto regional que de subtipo celular.
2. Los análisis de linaje de progenitores de interneuronas GABAérgicas demuestran que las tres principales clases de interneuronas corticales (SST+ y PV+ derivadas del MGE/POA, y VIP+ derivadas del CGE) tienen una fuerte tendencia a formar agrupamientos en la corteza cerebral, independientemente de su origen embrionario y de los diferentes programas de especificación celular.
3. Los agrupamientos de interneuronas derivados del MGE/POA son homogéneos o heterogéneos en su composición, tanto en sus propiedades electrofisiológicas como neuroquímicas.
4. Los agrupamientos de interneuronas derivados del MGE/POA se distribuyen principalmente en una o dos capas adyacentes de la corteza cerebral.
5. Los agrupamientos intralaminares contienen principalmente células coetáneas, lo que sugiere que los linajes de interneuronas se expanden a través de células progenitoras intermedias que se convierten en neurogénicas aproximadamente al mismo tiempo.
6. Los progenitores del MGE/POA a E10.5/E11.5 producen principalmente interneuronas destinadas a las capas corticales profundas, mientras que los progenitores marcados a E14.5 generan interneuronas que colonizan las capas superficiales. Estos resultados revelan una heterogeneidad de los progenitores de interneuronas con respecto a la localización en capas corticales profundas o superficiales, lo que sugiere la existencia de múltiples linajes de interneuronas restringidos y con diferente potencial laminar.
7. Las análisis de linaje de las células progenitoras de neuronas glutamatérgicas demuestran una diversidad sustancial en su comportamiento. Estos resultados sugieren la co-existencia de linajes de neuronas de proyección multipotentes y restringidos el neocórtex durante el desarrollo. Alternativamente nuestros resultados señalan un papel de las células progenitoras intermedias en la restricción laminar de linajes de células piramidales.

REFERENCES

UNIVERSITAS
Miguel
Hernández

Alaynick WA, Jessell TM, Pfaff SL. 2011. SnapShot: spinal cord development. in *CELL*, pp. 178-178.e171.

Alcamo EA, Chirivella L, Dautzenberg M, Dobreva G, Fariñas I, Grosschedl R, McConnell SK. 2008. Satb2 regulates callosal projection neuron identity in the developing cerebral cortex. in *Neuron*, pp. 364-377.

Anderson SA, Eisenstat DD, Shi L, Rubenstein JL. 1997a. Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. in *Science*, pp. 474-476.

Anderson SA, Qiu M, Bulfone A, Eisenstat DD, Meneses J, Pedersen R, Rubenstein JL. 1997b. Mutations of the homeobox genes Dlx-1 and Dlx-2 disrupt the striatal subventricular zone and differentiation of late born striatal neurons. in *Neuron*, pp. 27-37.

Angevine JB, Sidman RL. 1961. Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. in *Nature*, pp. 766-768.

Anthony TE, Klein C, Fishell G, Heintz N. 2004. Radial glia serve as neuronal progenitors in all regions of the central nervous system. in *Neuron*, pp. 881-890.

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. in *Neuron*, pp. 207-221.

Armentano M, Chou S-J, Tomassy GS, Leingärtner A, O'Leary DDM, Studer M. 2007. COUP-TFI regulates the balance of cortical patterning between frontal/motor and sensory areas. in *Nature neuroscience*, pp. 1277-1286.

Armstrong C, Krook-Magnuson E, Soltesz I. 2012. Neurogliaform and Ivy Cells: A Major Family of nNOS Expressing GABAergic Neurons. in *Front Neural Circuits*, p. 23.

Asami M, Pilz GA, Ninkovic J, Godinho L, Schroeder T, Huttner WB, Götz M. 2011. The role of Pax6 in regulating the orientation and mode of cell division of progenitors in the mouse cerebral cortex. in *Development*, pp. 5067-5078.

Azim E, Jabaudon D, Fame RM, Macklis JD. 2009. SOX6 controls dorsal progenitor identity and interneuron diversity during neocortical development. in *Nature Neuroscience*, pp. 1238-1247.

Bartolini G, Ciceri G, Marín O. 2013. Integration of GABAergic interneurons into cortical cell assemblies: lessons from embryos and adults. in *Neuron*, pp. 849-864.

Batista-Brito R, Close J, Machold R, Fishell G. 2008. The distinct temporal origins of olfactory bulb interneuron subtypes. in *J Neurosci*, pp. 3966-3975.

Batista-Brito R, Rossignol E, Hjerling-Leffler J, Denaxa M, Wegner M, Lefebvre V, Pachnis V, Fishell G. 2009. The cell-intrinsic requirement of Sox6 for cortical interneuron development. in *Neuron*, pp. 466-481.

Battiste J, Helms AW, Kim EJ, Savage TK, Lagace DC, Mandyam CD, Eisch AJ, Miyoshi G, Johnson JE. 2007. Ascl1 defines sequentially generated lineage-restricted neuronal and oligodendrocyte precursor cells in the spinal cord. in *Development*, pp. 285-293.

Baudoin J-P, Viou L, Launay P-S, Luccardini C, Espeso Gil S, Kiyasova V, Irinopoulou T, Alvarez C, Rio J-P, Boudier T et al. 2012. Tangentially migrating neurons assemble a primary cilium that promotes their reorientation to the cortical plate. in *Neuron*, pp. 1108-1122.

Bayraktar OA, Doe CQ. 2013. Combinatorial temporal patterning in progenitors expands neural diversity. in *Nature*, pp. 449-455.

Bedogni F, Hodge RD, Elsen GE, Nelson BR, Daza RAM, Beyer RP, Bammler TK, Rubenstein JLR, Hevner RF. 2010. Tbr1 regulates regional and laminar identity of postmitotic neurons in developing neocortex. in *Proc Natl Acad Sci USA*, pp. 13129-13134.

Beier KT, Samson MES, Matsuda T, Cepko CL. 2011. Conditional expression of the TVA receptor allows clonal analysis of descendents from Cre-expressing progenitor cells. in *Developmental Biology*, pp. 309-320.

Ben-Ari Y. 2002. Excitatory actions of gaba during development: the nature of the nurture. in *Nature Reviews Neuroscience*, pp. 728-739.

Bielle F, Griveau A, Narboux-Nême N, Vigneau S, Sigrist M, Arber S, Wassef M, Pierani A. 2005. Multiple origins of Cajal-Retzius cells at the borders of the developing pallium. in *Nature neuroscience*, pp. 1002-1012.

Bishop KM, Goudreau G, O'Leary DD. 2000. Regulation of area identity in the mammalian neocortex by Emx2 and Pax6. in *Science*, pp. 344-349.

Bortone D, Polleux F. 2009. KCC2 expression promotes the termination of cortical interneuron migration in a voltage-sensitive calcium-dependent manner. in *Neuron*, pp. 53-71.

Britanova O, de Juan Romero C, Cheung A, Kwan KY, Schwark M, Gyorgy A, Vogel T, Akopov S, Mitkovski M, Agoston D et al. 2008. Satb2 is a postmitotic determinant for upper-layer neuron specification in the neocortex. in *Neuron*, pp. 378-392.

Brown KN, Chen S, Han Z, Lu C-H, Tan X, Zhang X-J, Ding L, Lopez-Cruz A, Saur D, Anderson SA et al. 2011. Clonal production and organization of inhibitory interneurons in the neocortex. in *Science*, pp. 480-486.

Buckingham ME, Meilhac SM. 2011. Tracing cells for tracking cell lineage and clonal behavior. in *Developmental Cell*, pp. 394-409.

Butt SJB, Fuccillo M, Nery S, Noctor S, Kriegstein A, Corbin JG, Fishell G. 2005. The temporal and spatial origins of cortical interneurons predict their physiological subtype. in *Neuron*, pp. 591-604.

Butt SJB, Sousa VH, Fuccillo MV, Hjerling-Leffler J, Miyoshi G, Kimura S, Fishell G. 2008. The requirement of Nkx2-1 in the temporal specification of cortical interneuron subtypes. in *Neuron*, pp. 722-732.

Campbell K. 2003. Dorsal-ventral patterning in the mammalian telencephalon. in *Current Opinion in Neurobiology*, pp. 50-56.

Caputi A, Melzer S, Michael M, Monyer H. 2013. The long and short of GABAergic neurons. in *Curr Opin Neurobiol*, pp. 179-186.

Caviness VS. 1982. Neocortical histogenesis in normal and reeler mice: a developmental study based upon [3H]thymidine autoradiography. in *Brain Research*, pp. 293-302.

Cepko C, Ryder E, Austin C, Golden J, Fields-Berry S, Lin J. 1998. Lineage analysis using retroviral vectors. in *Methods*, pp. 393-406.

Chen B, Schaevitz LR, McConnell SK. 2005a. Fezl regulates the differentiation and axon targeting of layer 5 subcortical projection neurons in cerebral cortex. in *Proc Natl Acad Sci USA*, pp. 17184-17189.

Chen J-G, Rasin M-R, Kwan KY, Sestan N. 2005b. Zfp312 is required for subcortical axonal projections and dendritic morphology of deep-layer pyramidal neurons of the cerebral cortex. in *Proc Natl Acad Sci USA*, pp. 17792-17797.

Ciceri G, Dehorter N, Sols I, Huang ZJ, Maravall M, Marín O. 2013. Lineage-specific laminar organization of cortical GABAergic interneurons. in *Nature Neuroscience*, pp. 1199-1210.

Cobos I, Borello U, Rubenstein JLR. 2007. Dlx transcription factors promote migration through repression of axon and dendrite growth. in *Neuron*, pp. 873-888.

Corbin JG, Rutlin M, Gaiano N, Fishell G. 2003. Combinatorial function of the homeodomain proteins Nkx2.1 and Gsh2 in ventral telencephalic patterning. in *Development*, pp. 4895-4906.

Cubelos B, Sebastián-Serrano A, Kim S, Moreno-Ortiz C, Redondo JM, Walsh CA, Nieto M. 2008. Cux-2 controls the proliferation of neuronal intermediate precursors of the cortical subventricular zone. in *Cerebral Cortex*, pp. 1758-1770.

Cuzon VC, Yeh PW, Cheng Q, Yeh HH. 2006. Ambient GABA promotes cortical entry of tangentially migrating cells derived from the medial ganglionic eminence. in *Cereb Cortex*, pp. 1377-1388.

D'Arcangelo G, Homayouni R, Keshvara L, Rice DS, Sheldon M, Curran T. 1999. Reelin is a ligand for lipoprotein receptors. in *Neuron*, pp. 471-479.

Daniel D, Rossel M, Seki T, König N. 2005. Stromal cell-derived factor-1 (SDF-1) expression in embryonic mouse cerebral cortex starts in the intermediate zone close to the pallial-subpallial boundary and extends progressively towards the cortical hem. in *Gene Expr Patterns*, pp. 317-322.

de Carlos JA, López-Mascaraque L, Valverde F. 1996. Dynamics of cell migration from the lateral ganglionic eminence in the rat. in *J Neurosci*, pp. 6146-6156.

De la Rossa A, Bellone C, Golding B, Vitali I, Moss J, Toni N, Lüscher C, Jabaudon D. 2013. In vivo reprogramming of circuit connectivity in postmitotic neocortical neurons. in *Nature Neuroscience*, pp. 193-200.

Defelipe J, López-Cruz PL, Benavides-Piccione R, Bielza C, Larrañaga P, Anderson S, Burkhalter A, Cauli B, Fairén A, Feldmeyer D et al. 2013. New insights into the classification and nomenclature of cortical GABAergic interneurons. in *Nature Reviews Neuroscience*, pp. 202-216.

Desai AR, McConnell SK. 2000. Progressive restriction in fate potential by neural progenitors during cerebral cortical development. in *Development*, pp. 2863-2872.

Di Meglio T, Kratochwil CF, Vilain N, Loche A, Vitobello A, Yonehara K, Hrycaj SM, Roska B, Peters AHFM, Eichmann A et al. 2013. Ezh2 orchestrates topographic migration and connectivity of mouse precerebellar neurons. in *Science*, pp. 204-207.

Dimidschstein J, Passante L, Dufour A, van den Ameele J, Tiberi L, Hrechdakian T, Adams R, Klein R, Lie DC, Jossin Y et al. 2013. Ephrin-B1 controls the columnar distribution of cortical pyramidal neurons by restricting their tangential migration. in *Neuron*, pp. 1123-1135.

Du T, Xu Q, Ocbina PJ, Anderson SA. 2008. NKX2.1 specifies cortical interneuron fate by activating Lhx6. in *Development*, pp. 1559-1567.

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. in *Cell Stem Cell*, pp. 519-532.

Englund C, Fink A, Lau C, Pham D, Daza RAM, Bulfone A, Kowalczyk T, Hevner RF. 2005. Pax6, Tbr2, and Tbr1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. in *J Neurosci*, pp. 247-251.

Fairén A, Cobas A, Fonseca M. 1986. Times of generation of glutamic acid decarboxylase immunoreactive neurons in mouse somatosensory cortex. in *J Comp Neurol*, pp. 67-83.

Fame RM, MacDonald JL, Macklis JD. 2011. Development, specification, and diversity of callosal projection neurons. in *Trends Neurosci*, pp. 41-50.

Fishell G, Kriegstein AR. 2003. Neurons from radial glia: the consequences of asymmetric inheritance. in *Curr Opin Neurobiol*, pp. 34-41.

Flames N, Long JE, Garratt AN, Fischer TM, Gassmann M, Birchmeier C, Lai C, Rubenstein JLR, Marín O. 2004. Short- and long-range attraction of cortical GABAergic interneurons by neuregulin-1. in *Neuron*, pp. 251-261.

Flames N, Pla R, Gelman DM, Rubenstein JLR, Puelles L, Marín O. 2007. Delineation of multiple subpallial progenitor domains by the combinatorial expression of transcriptional codes. in *J Neurosci*, pp. 9682-9695.

Flandin P, Kimura S, Rubenstein JLR. 2010. The progenitor zone of the ventral medial ganglionic eminence requires Nkx2-1 to generate most of the globus pallidus but few neocortical interneurons. in *J Neurosci*, pp. 2812-2823.

Fleischhauer K, Petsche H, Wittkowski W. 1972. Vertical bundles of dendrites in the neocortex. in *Z Anat Entwicklungsgesch*, pp. 213-223.

Florio M, Huttner WB. 2014. Neural progenitors, neurogenesis and the evolution of the neocortex. in *Development*, pp. 2182-2194.

Fode C, Ma Q, Casarosa S, Ang SL, Anderson DJ, Guillemot F. 2000. A role for neural determination genes in specifying the dorsoventral identity of telencephalic neurons. in *Genes & Development*, pp. 67-80.

Fogarty M, Grist M, Gelman D, Marín O, Pachnis V, Kessaris N. 2007. Spatial genetic patterning of the embryonic neuroepithelium generates GABAergic interneuron diversity in the adult cortex. in *J Neurosci*, pp. 10935-10946.

Franco SJ, Gil-Sanz C, Martinez-Garay I, Espinosa A, Harkins-Perry SR, Ramos C, Müller U. 2012. Fate-restricted neural progenitors in the mammalian cerebral cortex. in *Science*, pp. 746-749.

Franco SJ, Martinez-Garay I, Gil-Sanz C, Harkins-Perry SR, Müller U. 2011. Reelin regulates cadherin function via Dab1/Rap1 to control neuronal migration and lamination in the neocortex. in *Neuron*, pp. 482-497.

Franco SJ, Müller U. 2013. Shaping our minds: stem and progenitor cell diversity in the mammalian neocortex. in *Neuron*, pp. 19-34.

Frantz GD, McConnell SK. 1996. Restriction of late cerebral cortical progenitors to an upper-layer fate. in *Neuron*, pp. 55-61.

Fu Y, Tucciarone JM, Espinosa JS, Sheng N, Darcy DP, Nicoll RA, Huang ZJ, Stryker MP. 2014. A cortical circuit for gain control by behavioral state. in *Cell*, pp. 1139-1152.

Fukuchi-Shimogori T, Grove EA. 2001. Neocortex patterning by the secreted signaling molecule FGF8. in *Science*, pp. 1071-1074.

Fukuda T, Kosaka T. 2003. Ultrastructural study of gap junctions between dendrites of parvalbumin-containing GABAergic neurons in various neocortical areas of the adult rat. in *Neuroscience*, pp. 5-20.

Gaiano N, Kohtz JD, Turnbull DH, Fishell G. 1999. A method for rapid gain-of-function studies in the mouse embryonic nervous system. in *Nature Neuroscience*, pp. 812-819.

Gal JS, Morozov YM, Ayoub AE, Chatterjee M, Rakic P, Haydar TF. 2006. Molecular and morphological heterogeneity of neural precursors in the mouse neocortical proliferative zones. in *J Neurosci*, pp. 1045-1056.

Galarreta M, Hestrin S. 1999. A network of fast-spiking cells in the neocortex connected by electrical synapses. in *Nature*, pp. 72-75.

Gao P, Hippenmeyer S, L H, Huang K, Luo L, Shi S-H. 2013. Clonal analysis of excitatory neuron neurogenesis and organization in the neocortex. in *Society for Neuroscience 2013 poster 41105/A5*.

García-Marqués J, López-Mascaraque L. 2013. Clonal identity determines astrocyte cortical heterogeneity. in *Cerebral Cortex*, pp. 1463-1472.

García-Moreno F, Vasistha NA, Begbie J, Molnár Z. 2014. CLoNe is a new method to target single progenitors and study their progeny in mouse and chick. in *Development*, pp. 1589-1598.

Gaspard N, Bouschet T, Hourez R, Dimidschstein J, Naeije G, van den Ameele J, Espuny-Camacho I, Herpoel A, Passante L, Schiffmann SN et al. 2008. An intrinsic mechanism of corticogenesis from embryonic stem cells. in *Nature*, pp. 351-357.

Ge W-P, Miyawaki A, Gage FH, Jan YN, Jan LY. 2012. Local generation of glia is a major astrocyte source in postnatal cortex. in *Nature*, pp. 376-380.

Gelman D, Griveau A, Dehorter N, Teissier A, Varela C, Pla R, Pierani A, Marín O. 2011. A wide diversity of cortical GABAergic interneurons derives from the embryonic preoptic area. in *J Neurosci*, pp. 16570-16580.

Gelman DM, Marín O. 2010. Generation of interneuron diversity in the mouse cerebral cortex. in *Eur J Neurosci*, pp. 2136-2141.

Gelman DM, Martini FJ, Nóbrega-Pereira S, Pierani A, Kessaris N, Marín O. 2009. The embryonic preoptic area is a novel source of cortical GABAergic interneurons. in *J Neurosci*, pp. 9380-9389.

Goebbels S, Bormuth I, Bode U, Hermanson O, Schwab MH, Nave K-A. 2006. Genetic targeting of principal neurons in neocortex and hippocampus of NEX-Cre mice. in *genesis*, pp. 611-621.

Golden JA, Fields-Berry SC, Cepko CL. 1995. Construction and characterization of a highly complex retroviral library for lineage analysis. in *Proc Natl Acad Sci USA*, pp. 5704-5708.

Götz M, Huttner WB. 2005. The cell biology of neurogenesis. in *Nat Rev Mol Cell Biol*, pp. 777-788.

Götz M, Stoykova A, Gruss P. 1998. Pax6 controls radial glia differentiation in the cerebral cortex. in *Neuron*, pp. 1031-1044.

Greig LC, Woodworth MB, Galazo MJ, Padmanabhan H, Macklis JD. 2013. Molecular logic of neocortical projection neuron specification, development and diversity. in *Nat Rev Neurosci*, pp. 755-769.

Group PIN, Ascoli GA, Alonso-Nanclares L, Anderson SA, Barrionuevo G, Benavides-Piccione R, Burkhalter A, Buzsáki G, Cauli B, Defelipe J et al. 2008. Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. in *Nat Rev Neurosci*, pp. 557-568.

Grove EA, Williams BP, Li DQ, Hajihosseini M, Friedrich A, Price J. 1993. Multiple restricted lineages in the embryonic rat cerebral cortex. in *Development*, pp. 553-561.

Guo C, Eckler MJ, McKenna WL, Mckinsey GL, Rubenstein JLR, Chen B. 2013. Fezf2 expression identifies a multipotent progenitor for neocortical projection neurons, astrocytes, and oligodendrocytes. in *Neuron*, pp. 1167-1174.

Hansen DV, Lui JH, Flandin P, Yoshikawa K, Rubenstein JL, Alvarez-Buylla A, Kriegstein AR. 2013. Non-epithelial stem cells and cortical interneuron production in the human ganglionic eminences. in *Nature neuroscience*, pp. 1576-1587.

Hansen DV, Lui JH, Parker PRL, Kriegstein AR. 2010. Neurogenic radial glia in the outer subventricular zone of human neocortex. in *Nature*, p. 554.

Hartfuss E, Galli R, Heins N, Götz M. 2001. Characterization of CNS precursor subtypes and radial glia. in *Dev Biol*, pp. 15-30.

Haubensak W, Attardo A, Denk W, Huttner WB. 2004. Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: a major site of neurogenesis. in *Proc Natl Acad Sci USA*, pp. 3196-3201.

Hébert JM, Fishell G. 2008. The genetics of early telencephalon patterning: some assembly required. in *Nature Reviews Neuroscience*, pp. 678-685.

Heins N, Malatesta P, Cecconi F, Nakafuku M, Tucker KL, Hack MA, Chapouton P, Barde Y-A, Götz M. 2002. Glial cells generate neurons: the role of the transcription factor Pax6. in *Nature neuroscience*, pp. 308-315.

Hensch TK. 2005. Critical period plasticity in local cortical circuits. in *Nat Rev Neurosci*, pp. 877-888.

Hevner RF, Daza RAM, Englund C, Kohtz J, Fink A. 2004. Postnatal shifts of interneuron position in the neocortex of normal and reeler mice: evidence for inward radial migration. in *Neuroscience*, pp. 605-618.

Hiesberger T, Trommsdorff M, Howell BW, Goffinet A, Mumby MC, Cooper JA, Herz J. 1999. Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. in *Neuron*, pp. 481-489.

Higginbotham H, Eom T-Y, Mariani LE, Bachleda A, Hirt J, Gukassyan V, Cusack CL, Lai C, Caspary T, Anton ES. 2012. Arl13b in primary cilia regulates the migration and placement of interneurons in the developing cerebral cortex. in *Developmental Cell*, pp. 925-938.

Hirabayashi Y, Gotoh Y. 2010. Epigenetic control of neural precursor cell fate during development. in *Nature Reviews Neuroscience*, pp. 377-388.

Hirabayashi Y, Suzki N, Tsuboi M, Endo TA, Toyoda T, Shinga J, Koseki H, Vidal M, Gotoh Y. 2009. Polycomb limits the neurogenic competence of neural precursor cells to promote astrogenic fate transition. in *Neuron*, pp. 600-613.

Howell BW, Herrick TM, Cooper JA. 1999. Reelin-induced tyrosine [corrected] phosphorylation of disabled 1 during neuronal positioning. in *Genes Dev*, pp. 643-648.

Huang ZJ, Di Cristo G, Ango F. 2007. Development of GABA innervation in the cerebral and cerebellar cortices. in *Nat Rev Neurosci*, pp. 673-686.

Hubel DH, Wiesel TN. 1968. Receptive fields and functional architecture of monkey striate cortex. in *The Journal of Physiology*, pp. 215-243.

-. 1969. Anatomical demonstration of columns in the monkey striate cortex. in *Nature*, pp. 747-750.

Imayoshi I, Isomura A, Harima Y, Kawaguchi K, Kori H, Miyachi H, Fujiwara T, Ishidate F, Kageyama R. 2013. Oscillatory control of factors determining multipotency and fate in mouse neural progenitors. in *Science*, pp. 1203-1208.

Inada H, Watanabe M, Uchida T, Ishibashi H, Wake H, Nemoto T, Yanagawa Y, Fukuda A, Nabekura J. 2011. GABA regulates the multidirectional tangential migration of GABAergic interneurons in living neonatal mice. in *PLoS ONE*, p. e27048.

Inoue K, Terashima T, Nishikawa T, Takumi T. 2004. Fez1 is layer-specifically expressed in the adult mouse neocortex. in *Eur J Neurosci*, pp. 2909-2916.

Isaacson JS, Scanziani M. 2011. How inhibition shapes cortical activity. in *Neuron*, pp. 231-243.

Jaglin XH, Hjerling-Leffler J, Fishell G, Batista-Brito R. 2012. The origin of neocortical nitric oxide synthase-expressing inhibitory neurons. in *Front Neural Circuits*, p. 44.

Jones EG, Rakic P. 2010. Radial columns in cortical architecture: it is the composition that counts. in *Cerebral Cortex*, pp. 2261-2264.

Kanatani S, Yozu M, Tabata H, Nakajima K. 2008. COUP-TFII is preferentially expressed in the caudal ganglionic eminence and is involved in the caudal migratory stream. in *J Neurosci*, pp. 13582-13591.

Kessaris N, Fogarty M, Iannarelli P, Grist M, Wegner M, Richardson WD. 2006. Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. in *Nature neuroscience*, pp. 173-179.

Kohwi M, Doe CQ. 2013. Temporal fate specification and neural progenitor competence during development. in *Nature Reviews Neuroscience*, pp. 823-838.

Kohwi M, Lupton JR, Lai S-L, Miller MR, Doe CQ. 2013. Developmentally regulated subnuclear genome reorganization restricts neural progenitor competence in Drosophila. in *Cell*, pp. 97-108.

Kretzschmar K, Watt FM. 2012. Lineage tracing. in *Cell*, pp. 33-45.

Kriegstein A, Alvarez-Buylla A. 2009. The glial nature of embryonic and adult neural stem cells. in *Annu Rev Neurosci*, pp. 149-184.

Kriegstein AR, Noctor SC. 2004. Patterns of neuronal migration in the embryonic cortex. in *Trends Neurosci*, pp. 392-399.

Krubitzer L, Kaas J. 2005. The evolution of the neocortex in mammals: how is phenotypic diversity generated? in *Curr Opin Neurobiol*, pp. 444-453.

Krushel LA, Johnston JG, Fishell G, Tibshirani R, van der Kooy D. 1993. Spatially localized neuronal cell lineages in the developing mammalian forebrain. in *Neuroscience*, pp. 1035-1047.

Lai T, Jabaudon D, Molyneaux BJ, Azim E, Arlotta P, Menezes JRL, Macklis JD. 2008. SOX5 controls the sequential generation of distinct corticofugal neuron subtypes. in *Neuron*, pp. 232-247.

Lavdas AA, Grigoriou M, Pachnis V, Parnavelas JG. 1999. The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. in *J Neurosci*, pp. 7881-7888.

Lee S, Hjerling-Leffler J, Zagha E, Fishell G, Rudy B. 2010. The largest group of superficial neocortical GABAergic interneurons expresses ionotropic serotonin receptors. in *J Neurosci*, pp. 16796-16808.

Lee S, Kruglikov I, Huang ZJ, Fishell G, Rudy B. 2013. A disinhibitory circuit mediates motor integration in the somatosensory cortex. in *Nature Neuroscience*, pp. 1662-1670.

Li G, Adesnik H, Li J, Long J, Nicoll RA, Rubenstein JLR, Pleasure SJ. 2008. Regional distribution of cortical interneurons and development of inhibitory tone are regulated by Cxcl12/Cxcr4 signaling. in *J Neurosci*, pp. 1085-1098.

Li X, Erclik T, Bertet C, Chen Z, Voutev R, Venkatesh S, Morante J, Celik A, Desplan C. 2013. Temporal patterning of Drosophila medulla neuroblasts controls neural fates. in *Nature*, pp. 456-462.

Li Y, Lu H, Cheng P-l, Ge S, Xu H, Shi S-H, Dan Y. 2012. Clonally related visual cortical neurons show similar stimulus feature selectivity. in *Nature*, pp. 118-121.

Liodis P, Denaxa M, Grigoriou M, Akufo-Addo C, Yanagawa Y, Pachnis V. 2007. Lhx6 activity is required for the normal migration and specification of cortical interneuron subtypes. in *J Neurosci*, pp. 3078-3089.

Livesey FJ, Cepko CL. 2001. Vertebrate neural cell-fate determination: lessons from the retina. in *Nature Reviews Neuroscience*, pp. 109-118.

Lodato S, Rouaux C, Quast KB, Jantrachotechatchawan C, Studer M, Hensch TK, Arlotta P. 2011a. Excitatory projection neuron subtypes control the distribution of local inhibitory interneurons in the cerebral cortex. in *Neuron*, pp. 763-779.

Lodato S, Tomassy GS, De Leonibus E, Uzcategui YG, Andolfi G, Armentano M, Touzot A, Gaztelu JM, Arlotta P, Menendez de la Prida L et al. 2011b. Loss of COUP-TFI alters the balance between caudal ganglionic eminence- and medial ganglionic

eminence-derived cortical interneurons and results in resistance to epilepsy. in *J Neurosci*, pp. 4650-4662.

López-Bendito G, Sánchez-Alcañiz JA, Pla R, Borrell V, Picó E, Valdeolmillos M, Marín O. 2008. Chemokine signaling controls intracortical migration and final distribution of GABAergic interneurons. in *J Neurosci*, pp. 1613-1624.

Loulier K, Barry R, Mahou P, Le Franc Y, Supatto W, Matho KS, Ieng S, Fouquet S, Dupin E, Benosman R et al. 2014. Multiplex cell and lineage tracking with combinatorial labels. in *Neuron*, pp. 505-520.

Lu R, Neff NF, Quake SR, Weissman IL. 2011. Tracking single hematopoietic stem cells in vivo using high-throughput sequencing in conjunction with viral genetic barcoding. in *Nat Biotechnol*, pp. 928-933.

Luskin MB, Parnavelas JG, Barfield JA. 1993. Neurons, astrocytes, and oligodendrocytes of the rat cerebral cortex originate from separate progenitor cells: an ultrastructural analysis of clonally related cells. in *J Neurosci*, pp. 1730-1750.

Luskin MB, Pearlman AL, Sanes JR. 1988. Cell lineage in the cerebral cortex of the mouse studied in vivo and in vitro with a recombinant retrovirus. in *Neuron*, pp. 635-647.

Ma T, Wang C, Wang L, Zhou X, Tian M, Zhang Q, Zhang Y, Li J, Liu Z, Cai Y et al. 2013. Subcortical origins of human and monkey neocortical interneurons. in *Nature neuroscience*, pp. 1588-1597.

Ma T, Zhang Q, Cai Y, You Y, Rubenstein JLR, Yang Z. 2012. A subpopulation of dorsal lateral/caudal ganglionic eminence-derived neocortical interneurons expresses the transcription factor Sp8. in *Cereb Cortex*, pp. 2120-2130.

Ma Y, Hu H, Berrebi AS, Mathers PH, Agmon A. 2006. Distinct subtypes of somatostatin-containing neocortical interneurons revealed in transgenic mice. in *J Neurosci*, pp. 5069-5082.

Magavi S, Friedmann D, Banks G, Stolfi A, Lois C. 2012. Coincident generation of pyramidal neurons and protoplasmic astrocytes in neocortical columns. in *J Neurosci*, pp. 4762-4772.

Malatesta P, Hartfuss E, Götz M. 2000. Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. in *Development*, pp. 5253-5263.

Mallamaci A, Muzio L, Chan CH, Parnavelas J, Boncinelli E. 2000. Area identity shifts in the early cerebral cortex of Emx2-/- mutant mice. in *Nature neuroscience*, pp. 679-686.

Marín O. 2012. Brain development: The neuron family tree remodelled. in *Nature*, pp. 185-186.

-. 2013. Cellular and molecular mechanisms controlling the migration of neocortical interneurons. in *Eur J Neurosci*, pp. 2019-2029.

Marin O, Anderson SA, Rubenstein JL. 2000. Origin and molecular specification of striatal interneurons. in *J Neurosci*, pp. 6063-6076.

Marín O, Müller U. 2014. Lineage origins of GABAergic versus glutamatergic neurons in the neocortex. in *Current Opinion in Neurobiology*, pp. 132-141.

Marín O, Rubenstein J. 2001. A long, remarkable journey: tangential migration in the telencephalon. in *Nature Reviews Neuroscience*, pp. 780-790.

Marín O, Rubenstein JLR. 2003. C ELLM IGRATION IN THEF OREBRAIN. in *Annu Rev Neurosci*, pp. 441-483.

Marín O, Valiente M, Ge X, Tsai L-H. 2010. Guiding neuronal cell migrations. in *Cold Spring Harbor Perspectives in Biology*, p. a001834.

Marín O, Yaron A, Bagri A, Tessier-Lavigne M, Rubenstein JL. 2001. Sorting of striatal and cortical interneurons regulated by semaphorin-neuropilin interactions. in *Science*, pp. 872-875.

Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C. 2004. Interneurons of the neocortical inhibitory system. in *Nat Rev Neurosci*, pp. 793-807.

Martini FJ, Valiente M, López Bendito G, Szabó G, Moya F, Valdeolmillos M, Marín O. 2009. Biased selection of leading process branches mediates chemotaxis during tangential neuronal migration. in *Development*, pp. 41-50.

McCarthy M, Turnbull DH, Walsh CA, Fishell G. 2001. Telencephalic neural progenitors appear to be restricted to regional and glial fates before the onset of neurogenesis. in *J Neurosci*, pp. 6772-6781.

McConnell SK. 1988. Fates of visual cortical neurons in the ferret after isochronic and heterochronic transplantation. in *J Neurosci*, pp. 945-974.

McConnell SK, Kaznowski CE. 1991. Cell cycle dependence of laminar determination in developing neocortex. in *Science*, pp. 282-285.

McKenna WL, Betancourt J, Larkin KA, Abrams B, Guo C, Rubenstein JLR, Chen B. 2011. Tbr1 and Fezf2 regulate alternate corticofugal neuronal identities during neocortical development. in *J Neurosci*, pp. 549-564.

Mckinsey GL, Lindtner S, Trzcinski B, Visel A, Pennacchio LA, Huylebroeck D, Higashi Y, Rubenstein JLR. 2013. Dlx1&2-dependent expression of Zfhx1b (Sip1, Zeb2) regulates the fate switch between cortical and striatal interneurons. in *Neuron*, pp. 83-98.

Melzer S, Michael M, Caputi A, Eliava M, Fuchs EC, Whittington MA, Monyer H. 2012. Long-range-projecting GABAergic neurons modulate inhibition in hippocampus and entorhinal cortex. in *Science*, pp. 1506-1510.

Miller MW. 1985. Cogeneration of retrogradely labeled corticocortical projection and GABA-immunoreactive local circuit neurons in cerebral cortex. in *Brain Res*, pp. 187-192.

Miyata T, Kawaguchi A, Saito K, Kawano M, Muto T, Ogawa M. 2004. Asymmetric production of surface-dividing and non-surface-dividing cortical progenitor cells. in *Development*, pp. 3133-3145.

Miyoshi G, Fishell G. 2011. GABAergic interneuron lineages selectively sort into specific cortical layers during early postnatal development. in *Cereb Cortex*, pp. 845-852.

Miyoshi G, Hjerling-Leffler J, Karayannis T, Sousa VH, Butt SJB, Battiste J, Johnson JE, Machold RP, Fishell G. 2010. Genetic fate mapping reveals that the caudal ganglionic eminence produces a large and diverse population of superficial cortical interneurons. in *J Neurosci*, pp. 1582-1594.

Molyneaux BJ, Arlotta P, Fame RM, MacDonald JL, MacQuarrie KL, Macklis JD. 2009. Novel subtype-specific genes identify distinct subpopulations of callosal projection neurons. in *J Neurosci*, pp. 12343-12354.

Molyneaux BJ, Arlotta P, Hirata T, Hibi M, Macklis JD. 2005. Fezl is required for the birth and specification of corticospinal motor neurons. in *Neuron*, pp. 817-831.

Molyneaux BJ, Arlotta P, Menezes JRL, Macklis JD. 2007. Neuronal subtype specification in the cerebral cortex. in *Nature Reviews Neuroscience*, pp. 427-437.

Mountcastle VB. 1997. The columnar organization of the neocortex. in *Brain*, pp. 701-722.

Mountcastle VB, DAVIES PW, BERMAN AL. 1957. Response properties of neurons of cat's somatic sensory cortex to peripheral stimuli. in *J Neurophysiol*, pp. 374-407.

Muzio L, Di Benedetto B, DiBenedetto B, Stoykova A, Boncinelli E, Gruss P, Mallamaci A. 2002a. Conversion of cerebral cortex into basal ganglia in Emx2(-/-) Pax6(Sey/Sey) double-mutant mice. in *Nature neuroscience*, pp. 737-745.

-. 2002b. Emx2 and Pax6 control regionalization of the pre-neuronogenic cortical primordium. in *Cereb Cortex*, pp. 129-139.

Nadarajah B, Parnavelas JG. 2002. Modes of neuronal migration in the developing cerebral cortex. in *Nature Reviews Neuroscience*, pp. 423-432.

Nery S, Fishell G, Corbin JG. 2002. The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations. in *Nature Neuroscience*, pp. 1279-1287.

Nieto M, Monuki ES, Tang H, Imitola J, Haubst N, Khoury SJ, Cunningham J, Gotz M, Walsh CA. 2004. Expression of Cux-1 and Cux-2 in the subventricular zone and upper layers II-IV of the cerebral cortex. in *J Comp Neurol*, pp. 168-180.

Nóbrega-Pereira S, Kessaris N, Du T, Kimura S, Anderson SA, Marín O. 2008. Postmitotic Nkx2-1 controls the migration of telencephalic interneurons by direct repression of guidance receptors. in *Neuron*, pp. 733-745.

Nóbrega-Pereira S, Marín O. 2009. Transcriptional control of neuronal migration in the developing mouse brain. in *Cerebral Cortex*, pp. i107-113.

Noctor S, Flint A, Weissman T, Dammerman R, Kriegstein A. 2001. Neurons derived from radial glial cells establish radial units in neocortex. in *Nature*, pp. 714-720.

Noctor SC, Flint AC, Weissman TA, Wong WS, Clinton BK, Kriegstein AR. 2002. Dividing precursor cells of the embryonic cortical ventricular zone have morphological and molecular characteristics of radial glia. in *J Neurosci*, pp. 3161-3173.

Noctor SC, Martínez-Cerdeño V, Ivic L, Kriegstein AR. 2004. Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. in *Nature Neuroscience*, pp. 136-144.

O'Leary DD, Sahara S. 2008. Genetic regulation of arealization of the neocortex. in *Curr Opin Neurobiol*, pp. 90-100.

O'Leary DDM, Chou S-J, Sahara S. 2007. Area patterning of the mammalian cortex. in *Neuron*, pp. 252-269.

Ohtsuki G, Nishiyama M, Yoshida T, Murakami T, Histed M, Lois C, Ohki K. 2012. Similarity of Visual Selectivity among Clonally Related Neurons in Visual Cortex. in *Neuron*, pp. 65-72.

Pattabiraman K, Golonzhka O, Lindtner S, Nord AS, Taher L, Hoch R, Silberberg SN, Zhang D, Chen B, Zeng H et al. 2014. Transcriptional regulation of enhancers active in protodomains of the developing cerebral cortex. in *Neuron*, pp. 989-1003.

Peters A, Walsh TM. 1972. A study of the organization of apical dendrites in the somatic sensory cortex of the rat. in *J Comp Neurol*, pp. 253-268.

Pfeffer CK, Xue M, He M, Huang ZJ, Scanziani M. 2013. Inhibition of inhibition in visual cortex: the logic of connections between molecularly distinct interneurons. in *Nature Neuroscience*, pp. 1068-1076.

Pilz G-A, Shitamukai A, Reillo I, Pacary E, Schwausch J, Stahl R, Ninkovic J, Snippert HJ, Clevers H, Godinho L et al. 2013. Amplification of progenitors in the mammalian telencephalon includes a new radial glial cell type. in *Nat Comms*, p. 2125.

Pla R, Borrell V, Flames N, Marín O. 2006. Layer acquisition by cortical GABAergic interneurons is independent of Reelin signaling. in *J Neurosci*, pp. 6924-6934.

Polleux F, Whitford KL, Dijkhuizen PA, Vitalis T, Ghosh A. 2002. Control of cortical interneuron migration by neurotrophins and PI3-kinase signaling. in *Development*, pp. 3147-3160.

Powell EM, Mars WM, Levitt P. 2001. Hepatocyte growth factor/scatter factor is a motogen for interneurons migrating from the ventral to dorsal telencephalon. in *Neuron*, pp. 79-89.

Price J, Thurlow L. 1988. Cell lineage in the rat cerebral cortex: a study using retroviral-mediated gene transfer. in *Development*, pp. 473-482.

Puelles L, Rubenstein JLR. 2003. Forebrain gene expression domains and the evolving prosomeric model. in *Trends Neurosci*, pp. 469-476.

Rakic P. 1988. Specification of cerebral cortical areas. in *Science*, pp. 170-176.

Rallu M, Corbin JG, Fishell G. 2002. Parsing the prosencephalon. in *Nature Reviews Neuroscience*, pp. 943-951.

Reillo I, de Juan Romero C, García-Cabezas MÁ, Borrell V. 2011. A role for intermediate radial glia in the tangential expansion of the mammalian cerebral cortex. in *Cereb Cortex*, pp. 1674-1694.

Rouaux C, Arlotta P. 2010. Fezf2 directs the differentiation of corticofugal neurons from striatal progenitors in vivo. in *Nature Neuroscience*, pp. 1345-1347.

-. 2013. Direct lineage reprogramming of post-mitotic callosal neurons into corticofugal neurons in vivo. in *Nat Cell Biol*, pp. 214-221.

Rubenstein JL, Martinez S, Shimamura K, Puelles L. 1994. The embryonic vertebrate forebrain: the prosomeric model. in *Science*, pp. 578-580.

Rubenstein JL, Shimamura K, Martinez S, Puelles L. 1998. Regionalization of the prosencephalic neural plate. in *Annu Rev Neurosci*, pp. 445-477.

Rubin AN, Alfonsi F, Humphreys MP, Choi CKP, Rocha SF, Kessaris N. 2010. The germinal zones of the basal ganglia but not the septum generate GABAergic interneurons for the cortex. in *J Neurosci*, pp. 12050-12062.

Rubin AN, Kessaris N. 2013. PROX1: a lineage tracer for cortical interneurons originating in the lateral/caudal ganglionic eminence and preoptic area. in *PLoS ONE*, p. e77339.

Rudy B, Fishell G, Lee S, Hjerling-Leffler J. 2011. Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. in *Devel Neurobio*, pp. 45-61.

Rymar VV, Sadikot AF. 2007. Laminar fate of cortical GABAergic interneurons is dependent on both birthdate and phenotype. in *J Comp Neurol*, pp. 369-380.

Sahara S, Kawakami Y, Izpisua Belmonte JC, O'Leary DDM. 2007. Sp8 exhibits reciprocal induction with Fgf8 but has an opposing effect on anterior-posterior cortical area patterning. in *Neural Dev*, p. 10.

Sánchez-Alcañiz JA, Haege S, Mueller W, Pla R, Mackay F, Schulz S, López-Bendito G, Stumm R, Marín O. 2011. Cxcr7 controls neuronal migration by regulating chemokine responsiveness. in *Neuron*, pp. 77-90.

Schuurmans C, Guillemot F. 2002. Molecular mechanisms underlying cell fate specification in the developing telencephalon. in *Curr Opin Neurobiol*, pp. 26-34.

Sentürk A, Pfennig S, Weiss A, Burk K, Acker-Palmer A. 2011. Ephrin Bs are essential components of the Reelin pathway to regulate neuronal migration. in *Nature*, pp. 356-360.

Shen Q, Wang Y, Dimos JT, Fasano CA, Phoenix TN, Lemischka IR, Ivanova NB, Stifani S, Morrisey EE, Temple S. 2006. The timing of cortical neurogenesis is encoded within lineages of individual progenitor cells. in *Nature Neuroscience*, pp. 743-751.

Shim S, Kwan KY, Li M, Lefebvre V, Sestan N. 2012. Cis-regulatory control of corticospinal system development and evolution. in *Nature*, pp. 74-79.

Sohur US, Padmanabhan HK, Kotchetkov IS, Menezes JRL, Macklis JD. 2014. Anatomic and molecular development of corticostriatal projection neurons in mice. in *Cerebral Cortex*, pp. 293-303.

Sousa VH, Miyoshi G, Hjerling-Leffler J, Karayannis T, Fishell G. 2009. Characterization of Nkx6-2-derived neocortical interneuron lineages. in *Cerebral Cortex*, pp. i1-10.

Southwell DG, Paredes MF, Galvao RP, Jones DL, Froemke RC, Sebe JY, Alfaro-Cervello C, Tang Y, Garcia-Verdugo JM, Rubenstein JL et al. 2012. Intrinsically determined cell death of developing cortical interneurons. in *Nature*, pp. 109-113.

Stancik E, Navarro-Quiroga I, Sellke R, Haydar T. 2010. Heterogeneity in ventricular zone neural precursors contributes to neuronal fate diversity in the postnatal neocortex. in *The Journal of Neuroscience*, pp. 7028-7036.

Stühmer T, Puelles L, Ekker M, Rubenstein JLR. 2002. Expression from a Dlx gene enhancer marks adult mouse cortical GABAergic neurons. in *Cereb Cortex*, pp. 75-85.

Stumm RK, Zhou C, Ara T, Lazarini F, Dubois-Dalcq M, Nagasawa T, Höllt V, Schulz S. 2003. CXCR4 regulates interneuron migration in the developing neocortex. in *J Neurosci*, pp. 5123-5130.

Sur M, Rubenstein JLR. 2005. Patterning and plasticity of the cerebral cortex. in *Science*, pp. 805-810.

Sussel L, Marin O, Kimura S, Rubenstein JL. 1999. Loss of Nkx2.1 homeobox gene function results in a ventral to dorsal molecular respecification within the basal telencephalon: evidence for a transformation of the pallidum into the striatum. in *Development*, pp. 3359-3370.

Tabata H, Nakajima K. 2003. Multipolar migration: the third mode of radial neuronal migration in the developing cerebral cortex. in *J Neurosci*, pp. 9996-10001.

Tamamaki N, Fujimori KE, Takauji R. 1997. Origin and route of tangentially migrating neurons in the developing neocortical intermediate zone. in *J Neurosci*, pp. 8313-8323.

Tamamaki N, Nakamura K, Okamoto K, Kaneko T. 2001. Radial glia is a progenitor of neocortical neurons in the developing cerebral cortex. in *Neurosci Res*, pp. 51-60.

Taniguchi H, Lu J, Huang ZJ. 2013. The spatial and temporal origin of chandelier cells in mouse neocortex. in *Science*, pp. 70-74.

Tarabykin V, Stoykova A, Usman N, Gruss P. 2001. Cortical upper layer neurons derive from the subventricular zone as indicated by Svet1 gene expression. in *Development*, pp. 1983-1993.

Tashiro A, Sandler VM, Toni N, Zhao C, Gage FH. 2006. NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus. in *Nature*, pp. 929-933.

Taverna E, Huttner WB. 2010. Neural progenitor nuclei IN motion. in *Neuron*, pp. 906-914.

Tham TN, Lazarini F, Franceschini IA, Lachapelle F, Amara A, Dubois-Dalcq M. 2001. Developmental pattern of expression of the alpha chemokine stromal cell-derived factor 1 in the rat central nervous system. in *Eur J Neurosci*, pp. 845-856.

Tiveron M-C, Rossel M, Moepps B, Zhang YL, Seidenfaden R, Favor J, König N, Cremer H. 2006. Molecular interaction between projection neuron precursors and invading interneurons via stromal-derived factor 1 (CXCL12)/CXCR4 signaling in the cortical subventricular zone/intermediate zone. in *J Neurosci*, pp. 13273-13278.

Torii M, Hashimoto-Torii K, Levitt P, Rakic P. 2009. Integration of neuronal clones in the radial cortical columns by EphA and ephrin-A signalling. in *Nature*, pp. 524-528.

Tucker ES, Segall S, Gopalakrishna D, Wu Y, Vernon M, Polleux F, Lamantia A-S. 2008. Molecular specification and patterning of progenitor cells in the lateral and medial ganglionic eminences. in *J Neurosci*, pp. 9504-9518.

Tyler WA, Haydar TF. 2013. Multiplex genetic fate mapping reveals a novel route of neocortical neurogenesis, which is altered in the Ts65Dn mouse model of Down syndrome. in *J Neurosci*, pp. 5106-5119.

Valcanis H, Tan S-S. 2003. Layer specification of transplanted interneurons in developing mouse neocortex. in *The Journal of neuroscience*, pp. 5113-5122.

van den Berghe V, Stappers E, Vandesande B, Dimidschstein J, Kroes R, Francis A, Conidi A, Lesage F, Dries R, Cazzola S et al. 2013. Directed migration of cortical interneurons depends on the cell-autonomous action of Sip1. in *Neuron*, pp. 70-82.

Villar-Cerviño V, Marín O. 2012. Cajal-Retzius cells. in *Curr Biol*, p. R179.

Villar-Cerviño V, Molano-Mazón M, Catchpole T, Valdeolmillos M, Henkemeyer M, Martínez Luis M, Borrell V, Marín O. 2013. Contact Repulsion Controls the Dispersion and Final Distribution of Cajal-Retzius Cells. in *Neuron*, pp. 457-471.

Visel A, Taher L, Girgis H, May D, Golonzhka O, Hoch RV, Mckinsey GL, Pattabiraman K, Silberberg SN, Blow MJ et al. 2013. A high-resolution enhancer atlas of the developing telencephalon. in *Cell*, pp. 895-908.

von Werder A, Seidler B, Schmid RM, Schneider G, Saur D. 2012. Production of avian retroviruses and tissue-specific somatic retroviral gene transfer in vivo using the RCAS/TVA system. in *Nat Protoc*, pp. 1167-1183.

Walsh C, Cepko CL. 1988. Clonally related cortical cells show several migration patterns. in *Science*, pp. 1342-1345.

-. 1992. Widespread dispersion of neuronal clones across functional regions of the cerebral cortex. in *Science*, pp. 434-440.

Wang X, Tsai J-W, Lamonica B, Kriegstein AR. 2011a. A new subtype of progenitor cell in the mouse embryonic neocortex. in *Nature Neuroscience*, pp. 555-561.

Wang Y, Li G, Stanco A, Long JE, Crawford D, Potter GB, Pleasure SJ, Behrens T, Rubenstein JLR. 2011b. CXCR4 and CXCR7 have distinct functions in regulating interneuron migration. in *Neuron*, pp. 61-76.

Weiss R. 1982. Experimental biology and assay of RNA tumor viruses. in RNA tumor viruses. in *RNA tumor viruses* (eds. W R., T N., V H., C J.), pp. 209-260. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Wichterle H, Turnbull DH, Nery S, Fishell G, Alvarez-Buylla A. 2001. In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. in *Development*, pp. 3759-3771.

Willi-Monnerat S, Migliavacca E, Surdez D, Delorenzi M, Luthi-Carter R, Terskikh AV. 2008. Comprehensive spatiotemporal transcriptomic analyses of the ganglionic eminences demonstrate the uniqueness of its caudal subdivision. in *Mol Cell Neurosci*, pp. 845-856.

Williams BP, Price J. 1995. Evidence for multiple precursor cell types in the embryonic rat cerebral cortex. in *Neuron*, pp. 1181-1188.

Williams BP, Read J, Price J. 1991. The generation of neurons and oligodendrocytes from a common precursor cell. in *Neuron*, pp. 685-693.

Wonders CP, Anderson SA. 2006. The origin and specification of cortical interneurons. in *Nat Rev Neurosci*, pp. 687-696.

Wonders CP, Taylor L, Welagen J, Mbata IC, Xiang JZ, Anderson SA. 2008. A spatial bias for the origins of interneuron subgroups within the medial ganglionic eminence. in *Dev Biol*, pp. 127-136.

Wu S, Esumi S, Watanabe K, Chen J, Nakamura KC, Nakamura K, Kometani K, Minato N, Yanagawa Y, Akashi K et al. 2011. Tangential migration and proliferation of intermediate progenitors of GABAergic neurons in the mouse telencephalon. in *Development*, pp. 2499-2509.

Wu S, Wu Y, Capecchi MR. 2006. Motoneurons and oligodendrocytes are sequentially generated from neural stem cells but do not appear to share common lineage-restricted progenitors in vivo. in *Development*, pp. 581-590.

Xu H, Jeong H-Y, Tremblay R, Rudy B. 2013. Neocortical somatostatin-expressing GABAergic interneurons disinhibit the thalamorecipient layer 4. in *Neuron*, pp. 155-167.

Xu Q, Cobos I, De La Cruz E, Rubenstein JL, Anderson SA. 2004. Origins of cortical interneuron subtypes. in *J Neurosci*, pp. 2612-2622.

Xu Q, Guo L, Moore H, Waclaw RR, Campbell K, Anderson SA. 2010. Sonic hedgehog signaling confers ventral telencephalic progenitors with distinct cortical interneuron fates. in *Neuron*, pp. 328-340.

Xu Q, Tam M, Anderson SA. 2008. Fate mapping Nkx2.1-lineage cells in the mouse telencephalon. in *J Comp Neurol*, pp. 16-29.

Xu Q, Wonders CP, Anderson SA. 2005. Sonic hedgehog maintains the identity of cortical interneuron progenitors in the ventral telencephalon. in *Development*, pp. 4987-4998.

Yanagida M, Miyoshi R, Toyokuni R, Zhu Y, Murakami F. 2012. Dynamics of the leading process, nucleus, and Golgi apparatus of migrating cortical interneurons in living mouse embryos. in *Proc Natl Acad Sci USA*, pp. 16737-16742.

Yoshida M, Assimacopoulos S, Jones KR, Grove EA. 2006. Massive loss of Cajal-Retzius cells does not disrupt neocortical layer order. in *Development*, pp. 537-545.

Yozu M, Tabata H, Nakajima K. 2005. The caudal migratory stream: a novel migratory stream of interneurons derived from the caudal ganglionic eminence in the developing mouse forebrain. in *J Neurosci*, pp. 7268-7277.

Yu Y-C, Bultje RS, Wang X, Shi S-H. 2009. Specific synapses develop preferentially among sister excitatory neurons in the neocortex. in *Nature*, pp. 501-504.

Yu Y-C, He S, Chen S, Fu Y, Brown KN, Yao X-H, Ma J, Gao KP, Sosinsky GE, Huang K et al. 2012. Preferential electrical coupling regulates neocortical lineage-dependent microcircuit assembly. in *Nature*, pp. 113-117.

Zembrzycki A, Griesel G, Stoykova A, Mansouri A. 2007. Genetic interplay between the transcription factors Sp8 and Emx2 in the patterning of the forebrain. in *Neural Dev*, p. 8.

Zong H, Espinosa JS, Su HH, Muzumdar MD, Luo L. 2005. Mosaic analysis with double markers in mice. in *Cell*, pp. 479-492.