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REVIEW

The multiple facets of Cajal-Retzius neurons

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ABSTRACT

Cajal-Retzius neurons (CRs) are among the first-born neurons in the developing cortex of reptiles, birds and mammals, including humans. The peculiarity of CRs lies in the fact they are initially embedded into the immature neuronal network before being almost completely eliminated by cell death at the end of cortical development. CRs are best known for controlling the migration of glutamatergic neurons and the formation of cortical layers through the secretion of the glycoprotein reelin. However, they have been shown to play numerous additional key roles at many steps of cortical development, spanning from patterning and sizing functional areas to synaptogenesis. The use of genetic lineage tracing has allowed the discovery of their multiple ontogenetic origins, migratory routes, expression of molecular markers and death dynamics. Nowadays, single-cell technologies enable us to appreciate the molecular heterogeneity of CRs with an unprecedented resolution. In this Review, we discuss the morphological, electrophysiological, molecular and genetic criteria allowing the identification of CRs. We further expose the various sources, migration trajectories, developmental functions and death dynamics of CRs. Finally, we demonstrate how the analysis of public transcriptomic datasets allows extraction of the molecular signature of CRs throughout their transient life and consider their heterogeneity within and across species.

KEY WORDS: Cajal-Retzius neurons, Development, Cortex, Hippocampus, Molecular profiling, Single-cell transcriptomics

Introduction

Cajal-Retzius cells (CRs) constitute a neuronal type that presents an array of very specific features. They are pioneer neurons of the cerebral cortex; they migrate over very long distances and produce signals that are essential to coordinate brain development. Their most fascinating aspect is undoubtedly their transient lifetime: in rodents, the large majority of CRs undergo apoptosis once cortical layering has taken place. To date, it has been shown that CRs are required for multiple processes throughout development, ranging from cortical layering to functional area formation, dendritogenesis and radial migration of glutamatergic neurons, GABAergic neurons and oligodendrocyte progenitor cells (Barber et al., 2015; Caronia-Brown and Grove, 2011; D'Arcangelo et al., 1995; de Frutos et al., 2016; Griveau et al., 2010; Ogawa et al., 1995; Ogino et al., 2020; Supèr et al., 2000). Their timed death is also crucial for the proper wiring of cortical circuits (Riva et al., 2019). In parallel to the discovery of new developmental functions, accumulating evidence points towards the heterogeneity of this neuronal population in terms of molecular profile, ontogenetic origins, migration routes and cell death fate. The recent development of single cell technologies now enables us to understand what is common and what distinguishes CR subpopulations, to discover how they fulfil a repertoire of functions across development in a subtype-dependent manner, and to tackle the question of their phylogenic conservation and implication in brain complexification.

In this Review, we first summarise the morphological, physiological, molecular and genetic characteristics of CRs. We next describe the ontogenetic origins, migration routes, death dynamics and developmental functions of the various CR subtypes. In the final section, we highlight how public transcriptomic datasets from embryonic to adult brains allow better definition of the molecular signature of CR subtypes and we address their contribution to brain development and evolution.

Morphology and physiology of CR neurons

Location, birth and death of CR neurons

In the late 19th century, Santiago Ramón y Cajal and Gustaf Retzius observed that the outermost layer of the developing cerebral cortex of small mammals and humans is populated by a neuronal population with a dense axonal plexus of horizontal nerve fibres (Ramón y Cajal, 1909; Retzius, 1893). Since then, this neuronal population has been named after these two neuroscientists. CRs are found in the marginal zone (MZ, the future layer I) of the neocortex, in the hippocampus and in the dentate gyrus (Fig. 1A,B). They are identified by their peculiar morphology: they display an elongated soma, a thin axon stemming from one pole of the soma and one thick tapered dendrite from the opposite pole (Fig. 1C) (Bradford et al., 1977; Del Río et al., 1995; Hestrin and Armstrong, 1996; von Haebler et al., 1993).

In the neocortex, their dendrites lie parallel to the pia (Fig. 1C) and often give rise to distal secondary and tertiary dendrites oriented vertically towards the pial surface (Del Río et al., 1995; Radnikow et al., 2002). The axons extend over long distances to form a dense network of horizontal processes covering the entire surface of the neocortex (Anstötz et al., 2014; Sun et al., 2019). A certain degree of confusion exists in the literature, as one can find references to 'Cajal cells', 'Retzius Cells' or 'CR-like cells' (Meyer and Goffinet, 1998; Meyer and Wahle, 1999; Meyer et al., 1999). This is not only due to the scarcity of molecular markers initially available but also related to species- and stage-specific features of CRs (Meyer and González-Gómez, 2018a; Meyer et al., 1999). For example, unlike rodents, the human foetal MZ contains two kinds of morphologically distinct but molecularly undistinguishable CRs: an early born population, initially bipolar but possibly becoming polymorphic and vertically oriented, that disappears around midgestation; and a second subtype of smaller and more superficial CRs that appears around the time the early born population disappears (Meyer and González-Gómez, 2018b; Meyer

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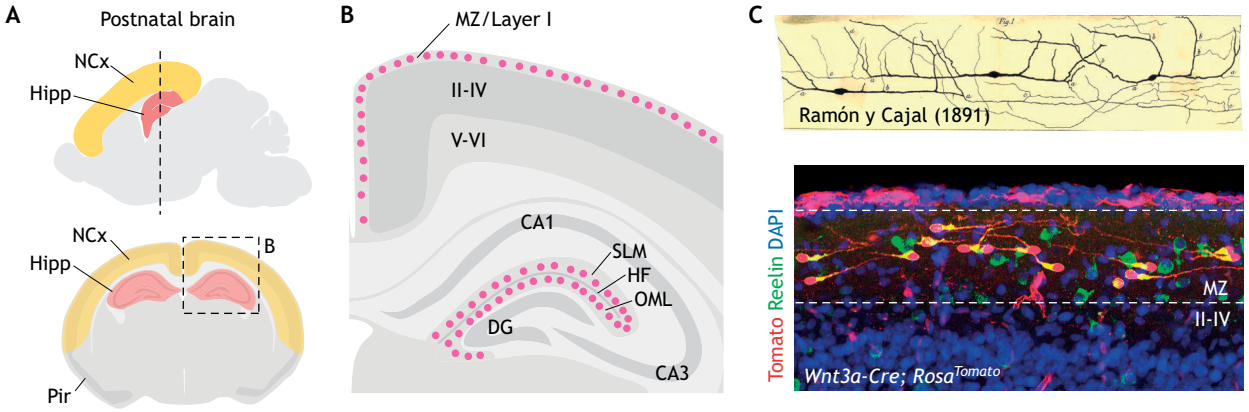

Fig. 1. CRs in the postnatal brain. (A) Sagittal (upper panel) and coronal (lower panel) sections of the postnatal mouse brain indicating the position of the neocortex, hippocampus and piriform cortex. (B) Expanded view of the area outlined in A, indicating the position of CRs (pink dots) at the surface of the cortex and in the hippocampus. (C) Morphology of CRs in the cortex. In the upper panel, a drawing from Santiago Ramón y Cajal depicts the typical bipolar morphology of CRs (Ramón y Cajal, 1891). Axons and primary dendrites are oriented along the pial surface, whereas secondary and tertiary dendrites extend perpendicularly, towards the pial surface. In the lower panel, a representative picture of the marginal zone of a P7 mouse pup shows the localization of CRs in the marginal zone of the neocortex. CRs are identified by Wnt3a-Cre genetic tracing (red) together with reelin immunolabelling (green). Nuclei are stained with DAPI (blue). CA, cornu ammonis; DG, dentate gyrus; HF, hippocampal fissure; Hipp, hippocampus; MZ, marginal zone; NCx, neocortex; OML, outer molecular layer; Pir, piriform cortex; SLM, stratum lacunosum moleculare. Lower panel in C provided by O.B.

and González-Hernández, 1993). As most of the experimental work on CR has been performed in mice, all subsequent discussion refers to this species unless specified otherwise.

In the hippocampal formation, CRs are mainly found in the MZ bordering the hippocampal fissure, the future stratum lacunosum moleculare (SLM) of the hippocampus and outer molecular layer (OML) of the dentate gyrus (Fig. 1B) (Ceranik et al., 2000; Del Río et al., 1995; von Haebler et al., 1993). Their dendrites are confined in the same layer as the somata, whereas their long-range axons sometimes cross the hippocampal fissure to reach sub-regions of the hippocampal formation, such as the subiculum or layer I of the entorhinal cortex (Anstötz et al., 2016; Ceranik et al., 2000).

Birth-dating experiments conducted in rats indicated that CRs are among the first cortical neurons generated, between E12 and E14 (König et al., 1977; Lavdas et al., 1999; Raedler and Raedler, 1978; Valverde et al., 1995). In mice, the peak of CR neurogenesis occurs at E10.5-E12.5 (Hevner et al., 2003; Takiguchi-Hayashi, 2004). In humans, CRs are observed as early as 5 gestational weeks (GW) (Meyer et al., 2000). CRs therefore appear to be an exception to the 'inside-out' model of neocortical development stipulating that early-born neurons occupy deeper positions (Angevine and Sidman, 1961). In rodents, the majority of CRs disappear rapidly after cortical lamination has taken place, during the first 3 postnatal weeks in mice (Causeret et al., 2018). In humans, massive CR demise is observed around GW 23 to 28, although subtype specificities exist (Meyer and González-Gómez, 2018a,b). The early generation and early disappearance of CRs led scientists to refer to them as 'transient early-born' or 'transient pioneer neurons'. Whether their disappearance is due to cell dilution in an expanding cortex (Martin et al., 1999), to morphological transformation (Parnavelas and Edmunds, 1983) or to cell death (Chowdhury et al., 2010; Del Río et al., 1995; Derer and Derer, 1990) was unclear for decades. It is only relatively recently that cell death has been validated as the primary fate of CRs and quantified using genetic tracing (Ledonne et al., 2016).

Neuronal properties

Establishing the neuronal nature of CRs was initially challenging given the difficulty of distinguishing their thin axon, a morphological characteristic that is essential to define a cell as a neuron. Immunological and electrophysiological studies later allowed verification of their neuronal identity: CRs are negative for glial markers and have the ability to fire action potentials (Hestrin and Armstrong, 1996; König and Schachner, 1981). Patch clamp experiments provided an additional tool to characterize the electrophysiological properties of CRs. They display a high input resistance (1 to 3 GΩ) and a depolarized threshold for action potentials (−20 to −40 mV) (Anstötz and Maccaferri, 2020; Ceranik et al., 2000; Luhmann et al., 2000; Mienville and Pesold, 1999; Radnikow et al., 2002; Sava et al., 2010; Sun et al., 2019). Although all these studies reported a membrane potential of −40 mV to −60 mV, these values do not take into consideration the short circuit effects of the leak conductance between cell membrane and patch pipette. After correction, Achilles et al. (2007) reported a resting membrane potential of −80 mV. Upon depolarization, CRs fire repetitive action potentials with a particularly long duration, small amplitude, low discharge frequency and a prominent voltage sag upon hyperpolarization (Ceranik et al., 2000; Kilb and Luhmann, 2000, 2001; Radnikow et al., 2002). Several studies have reported a slight shift towards more mature values of these properties after birth: hyperpolarization of resting membrane potential, decreasing input resistance and threshold for action potential (Sun et al., 2019; Zhou and Hablitz, 1996). CR excitability seems to increase during postnatal life until the time of their demise (Kirmse et al., 2005; Sun et al., 2019), although some groups have failed to observe any maturation of their electrophysiological parameters (Mienville and Pesold, 1999; Radnikow et al., 2002) – a discrepancy possibly due to differences in experimental set-up.

CRs are glutamatergic neurons (Del Río et al., 1995; Hevner et al., 2003; Quattrocolo and Maccaferri, 2014); they express a variety of receptors, including $GABA_A$, NMDA, AMPA/kainate, mGlu and serotoninergic receptors (de Frutos et al., 2016; López-Bendito et al., 2002; Martínez-Galán et al., 2001; Schwartz et al., 1998); and receive synaptic contacts from both GABAergic and non-GABAergic neurons onto their somata and dendrites, as demonstrated by electron microscopy and immunohistology (Anstötz et al., 2014; Radnikow et al., 2002). Although CRs undeniably exhibit GABAergic post-synaptic currents (PSCs), the

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existence of functional glutamatergic synapses remains unclear. Several groups performing whole-cell recordings in the mouse postnatal neocortex have reported that evoked excitatory PSCs are insensitive to the NMDA and AMPA/kainate receptor antagonists but fully abolished by $GABA_A$ receptor blockade, indicating that evoked PSCs are strictly GABAergic (Kirmse et al., 2007; Soda et al., 2003; Sun et al., 2019). In terms of spontaneous PSCs, hippocampal and neocortical CRs have only been reported to display spontaneous GABAergic PSCs, confirming that glutamatergic synapses are most likely physiologically silent (Kilb and Luhmann, 2001; Kirmse et al., 2007; Marchionni et al., 2010; Quattrocolo and Maccaferri, 2013; Soda et al., 2003; Sun et al., 2019). However, some studies performed in the mouse, rat or human neocortex reported the existence of weak glutamatergic inputs (Ceranik et al., 2000; Lu et al., 2001; Radnikow et al., 2002; Sun et al., 2019). In these studies, discrepancies further appear as to whether these excitatory PSCs are mediated via NMDA receptors alone or via both NMDA and AMPA/kainate receptors. It seems that these differences are due to the fact that the expression of functional NMDA and AMPA/kainate receptors is highly dependent on the species or the genetic background, as demonstrated by comparative studies between rat and humans or between C57BL/6J and ICR mouse strains (Chan and Yeh, 2003; Lu et al., 2001).

Connectivity of CRs

Many experiments have addressed the question of CR integration into the developing cortical network but their input-output connectivity remains poorly understood. In the neocortex, monosynaptic retrograde tracing experiments using Frizzled10-CreERT2 mice (see Table 1) have revealed that layer VI reelin-positive neurons, layer V $Ctip2^+$ pyramidal neurons and layer I inhibitory neurons establish synaptic inputs on CRs (Cocas et al., 2016). Functionally, electrical stimulation of the transient subplate GABAergic neurons and pharmacological activation of mGluR1/5-expressing interneurons, possibly Martinotti cells, elicit monosynaptic PSCs in neocortical CRs (Cosgrove and Maccaferri, 2012; Myakhar et al., 2011).

Regarding their outputs, labelling experiments suggest that neocortical CRs preferentially innervate layer II-III and V pyramidal neurons and, to a lesser extent, other layer I neurons (Anstötz et al., 2014; Radnikow et al., 2002). When CR death is impaired, e.g. in *Bax*-conditional mutants (discussed further below), their increased survival promotes an activity-dependent exuberance of dendrites in layer II/III pyramidal neurons, consistent with a functional link between CRs and upper layer pyramidal neurons (Riva et al., 2019). However, the mechanism involved remains elusive as the photoactivation of CRs in these mutants does not appear to lead to the stimulation of neurons in cortical layers II-III (Riva et al., 2019).

In the hippocampus, optogenetics and electrophysiological experiments have established SLM neurogliaform and oriens-lacunosum-moleculare interneurons as functional presynaptic inputs for CRs (Quattrocolo and Maccaferri, 2013) and SLM interneurons as post-synaptic targets (Anstötz et al., 2018a,b; Quattrocolo and Maccaferri, 2014). Nevertheless, despite recent advances in understanding the hippocampal and neocortical circuits involving CRs, the synaptic function of these cells remains poorly understood and will require further investigation.

Molecular characteristics of CRs

CRs gained significant attention after the discovery that they express high levels of reelin (Reln), a secreted extracellular matrix protein necessary for the lamination of the neocortex (D'Arcangelo et al., 1995; Ogawa et al., 1995). This discovery shed light on an important developmental role of CRs and provided scientists with a sensitive immunological marker. Reln has since then been widely used to immunolabel CRs. At embryonic stages, the onset of expression appears slightly later in certain subpopulations of CRs than others (Griveau et al., 2010). In addition, although all CRs express Reln, it is important to emphasise that Reln expression is not specific to CRs (Anstötz et al., 2016; Chowdhury et al., 2010; Frade-Pérez et al., 2017; Moreau et al., 2020 preprint). For example, *Reln* mRNA can be detected in the cortical plate below the MZ from E12.5 onwards (Yoshida et al., 2006). In addition, from late embryonic stages, the number of interneurons positive for *Reln* within the MZ itself progressively increases (Alcántara et al., 1998; Anstötz and Maccaferri, 2020), which makes it inappropriate to rely on Reln positivity and localization in the MZ to ascertain CR identity (see Fig. 1C).

Two additional markers have been widely used. The calcium-binding protein calretinin has been found to label CRs throughout their life (Del Río et al., 1995; Ogawa et al., 1995; Soriano et al., 1994). However, calretinin expression varies depending on the ontogenetic origins of CRs, developmental stages and species (Bielle et al., 2005; Martínez-Galán et al., 2014; Meyer and Goffinet, 1998; Meyer et al., 1998; Moreau et al., 2020 preprint). In rat, for example, it was reported that virtually no neurons co-express Reln and calretinin at neonatal stages (Martínez-Galán et al., 2014). The transcription factor *p73* (*Trp73*), a paralog of the tumour-suppressor gene *p53* (*Trp53*) is another marker that has been commonly used to label CRs and arguably represents one of the best CR markers available (Cabrera-Socorro et al., 2007; Meyer et al., 2002; Yang et al., 2000; Yoshida et al., 2006). However, not all CR subpopulations are $p73^+$ (Griveau et al., 2010; Hanashima et al., 2007; Moreau et al., 2020 preprint) (see below for the detailed molecular signature of CR subtypes).

Despite the variety of tools available to distinguish CRs, it is striking that none is sufficient to unequivocally identify all of them when used alone, pointing to the existence of multiple subtypes – if not cell types – of CRs. In the following two sections, we will describe how CRs can be distinguished in subpopulations according to their origins, migratory behaviours and cell death fates.

CR subtypes differ in their origins, migration routes and distribution

The origins of CRs long remained enigmatic, or at least debated. Although never formally proven, the idea that CRs could be generated in the neocortical ventricular zone and find their marginal position by radial migration has always been present, perhaps simply because CRs are glutamatergic and the dogma stipulated that glutamatergic cells migrate radially, whereas GABAergic interneurons follow tangential routes (Rubenstein and Rakic, 1999). Currently, human is the only species in which there is some support for this model, with the observation of dispersed Reln-positive columns at early stages (Meyer and González-Gómez, 2018a; Meyer et al., 2000). The retrobulbar area was proposed as a possible origin in humans as CRs appear to migrate tangentially in the cortex from this region (Meyer and Wahle, 1999; Meyer et al., 1998). The medial ganglionic eminence (MGE) was initially suggested to be a source of CRs based on DiI tracing in rats (Lavdas et al., 1999), while another study concomitantly concluded the opposite after analysing MGE-deficient *Nkx2.1* mutants (Sussel et al., 1999). Now, the consensus is that CRs have a pallial origin, at least in rodents. In support of this view, mice lacking *Emx2*

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Table 1. Mouse lines that allow CR cell tracing

Mouse line	Details	Targeted CRs	Other targets	References	Remarks
Wnt3a-Cre	Insertion of IRES-Cre in the 3′UTR	Hem-derived CRs	Hem and choroid plexus	Wnt3a$^{tm1(cre)Eag}$ (Yoshida et al., 2006)	An equivalent line is available: Wnt3a$^{tm1.1(cre)Mull}$ (Gil-Sanz et al., 2013)
ΔNp73-Cre	Knock-in of Cre-IRES-EGFP at the start codon of ΔNp73	Hem-, septum- and thalamic eminence-derived CRs	Choroid plexus, GnRH neurons, vomeronasal neurons, and uncharacterized cells in the preoptic area and retrobulbar area	Trp73$^{tm1(cre)Agof}$ (Tissir et al., 2009)	
p73-LacZ	Insertion of IRES-LacZ downstream exon 4 of *p73*	Hem- and most likely septum- and thalamic eminence-derived CRs	Ependymal cells and choroid plexus	Trp73$^{tm1a(KOMP)Wtsi}$ (Fujitani et al., 2017; Skarnes et al., 2011)	
Dbx1-Cre	Insertion of IRES-Cre in the 3'UTR	VP- and septum-derived CRs	**Interneurons and oligodendrocyte precursor cells from the preoptic area; glutamatergic transient neurons from the PSB; lateral area and arcuate nucleus of the hypothalamus**	Dbx1$^{tm2(cre)Apie}$ (Bielle et al., 2005)	Two similar lines also exist, Dbx1$^{tm1(cre/ERT2)Jcor}$ (Hirata et al., 2009) and Dbx1$^{tm1.1(cre)Mull}$ (Hua et al., 2014), but CR-targeting is unknown A Dbx1-LacZ allele Dbx1^{tm1Tmj} (Pierani et al., 2001) also allows short-term labelling of VP- and septum-derived CRs
Fzd10-CreER^{TM}	Transgene containing 6.7 kb upstream *Fzd10* followed by CreER^{TM}	Hem-derived CRs	Dorsal thalamus, cerebellum, dorsal spinal cord and dorsal root ganglia	Tg(Fzd10-cre/Esr1*)1Chzh (Gu et al., 2009; Gu et al., 2011)	A Fzd10-LacZ transgenic line also labels CR cells (Zhao et al., 2006)
Ndnf-Cre	Insertion of IRES2-dgCre (destabilized EGFP/Cre fusion) in the 3′UTR	CRs that persist in adults Unknown at embryonic or early postnatal stages	**Layer 1 interneurons**	Ndnf$^{tm1.1(folA/cre)Hze}$ (Tasic et al., 2016; Tasic et al., 2018)	Two related lines exist, Ndnf$^{tm1.1(cre/ERT2)Ispgl}$ (Abs et al., 2018) and Ndnf$^{tm1.1(cre)Rudy}$, (Schuman et al., 2019) but CR targeting is unknown
IG17	Transgene containing 18 kb upstream *Grm2* (mGluR2) followed by GFP-fused *IL2RA* (human interleukin 2 receptor alpha)	Not precisely documented Numerous CRs in the neocortex and hippocampus	Granule cells of the olfactory bulb, cerebellar Golgi cells and pontine neurons	Tg(Grm2-IL2RA/GFP)1Nak (Soda et al., 2003; Watanabe et al., 1998)	
Cxcr4-GFP	GENSAT BAC transgene of ∼200 kb (RP23-9A20) with EGFP inserted at the start codon	Not precisely documented Numerous CRs in the postnatal neocortex and hippocampus	Neural progenitor cells in the hippocampus Unknown in the embryonic cortex	Tg(Cxcr4-EGFP)CD73Gsat (Anstötz and Maccaferri, 2020; Gong et al., 2003; Marchionni et al., 2010)	
Pde1c-Cre	GENSAT BAC transgene of ∼50 kb (RP23-142L8) with Cre inserted at the start codon	Probably medial CRs but not precisely documented	Preplate/subplate neurons at embryonic stages Some hilar interneurons in the hippocampus	Tg(Pde1c-Cre)IT146Gsat (Anstötz et al., 2018a; Gong et al., 2003; Osheroff and Hatten, 2009)	A GENSAT Pde1c-GFP transgenic line also exists: Tg(Pde1c-EGFP)S45Gsat (Gong et al., 2003)
Ebf2-GFP	GENSAT BAC transgene of ∼170 kb (RP24-283N8) with EGFP inserted at the start codon	Possibly all CRs subtypes	**Preplate neurons** Weak GFP expression in the cortical plate at E14	Tg(Ebf2-EGFP)FB58Gsat (Chowdhury et al., 2010; Chuang et al., 2011; Gong et al., 2003)	
Ebf3-LacZ	Knock-in of LacZ at the start codon of Ebf3	Hem-, septum- and thalamic eminence-derived CRs		de Frutos et al. (2016); Jin et al. (2014)	A similar line exists, Ebf3^{tm1Reed} (Wang, 2004), but CR targeting is unknown A GENSAT Ebf3-GFP transgenic line also exists: Tg(Ebf3-EGFP)LP183Gsat (Gong et al., 2003)

Mouse lines reported to enable labelling or targeting of CRs. The precise cell types that are targeted (specifically or non-specifically) are not always very well described. The cell types indicated in bold are present in the cerebral cortex and could be mistaken for CRs depending on the experimental paradigm.

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(required for normal pallial development) display a loss of both Reln and calretinin expression in the MZ, suggesting that CRs derive from the *Emx2*-positive pallial anlage (Mallamaci et al., 2000). Moreover, almost all calretinin-expressing cells located in the neocortical MZ of P4 mice derive from *Emx1*-expressing pallial progenitors (Gorski et al., 2002), and CRs express the pallial marker Tbr1, even when located in subpallial regions of the embryonic brain (Hevner et al., 2001, 2003).

It is the use of genetic fate-mapping in mice in combination with *in utero* electroporation that has allowed the precise sites of CR production to be pinpointed. In Table 1, we provide a list of all the transgenic mouse lines currently available that can be used to follow CRs through the expression of Cre recombinase, GFP or β-galactosidase. To date, four sources at the borders of the pallium have been precisely described (Fig. 2): the cortical hem (Takiguchi-Hayashi, 2004), the ventral pallium (VP, also referred to as PSB, pallial-subpallial boundary) (Bielle et al., 2005), the pallial septum (Bielle et al., 2005) and the thalamic eminence (TE) (Meyer et al., 2002; Ruiz-Reig et al., 2017; Tissir et al., 2009). It is generally accepted that the hem provides the majority of CRs, especially in the dorsal pallium. From these focal sites at the border, but within the pallial anlage, CRs reach the neocortical surface by tangential, not radial, migration, at least in rodents.

In humans, analysis of p73 expression (which labels all but VP-derived CRs) suggests that the hem, septum and TE are conserved sources among mammals (Meyer et al., 2002). In sauropsids (birds, turtles and lizards), *Reln*-positive cells have been observed at the pallial surface, but little is known regarding the origins and migratory behaviour of these putative CRs (Bar et al., 2000; Goffinet, 2017). The pattern of p73 expression in lizards suggests that most CRs are located in the ventral telencephalon and derive from the septum and TE, whereas only few hem-derived CR invade the pallium dorsally (Cabrera-Socorro et al., 2007). In birds, whether CRs populate the cortex by tangential migration remains a matter of debate. Although electroporation experiments in quails initially suggested that both the hem and septum give rise to dorsally migrating CRs (Nomura et al., 2008), a recent report challenged this view after failing to detect any tangentially migrating glutamatergic neurons in the developing chick pallium (García-Moreno et al., 2018). It remains to be clarified whether discrepancies between these studies are related to the avian species used, the timing of electroporation (as CR progenitors can only be targeted at very early developmental stages) or any other difference in the experimental paradigm. A possible breakthrough in understanding the evolution of CRs origins and migration among sauropsids and mammals could come from the recent development of single-cell technologies (see below).

Once generated, CRs migrate tangentially following a complex choreography (Fig. 2): VP and septum-derived CRs (identified by the *Dbx1-Cre* mouse line) follow both dorsal and ventral flows (Bielle et al., 2005); hem-derived CRs (identified by the *Wnt3a-Cre* mouse line) migrate anteriorly and laterally but seem to encounter a semi-permeable lateral boundary, possibly corresponding to the lateral olfactory tract (LOT), resulting in most of them being eventually distributed above hippocampal and neocortical territories (Yoshida et al., 2006); and TE-derived CRs (identified by the *ΔNp73-Cre* mouse line together with septum- and hem-derived CRs) follow an anterior stream along the LOT, together with other neurons migrating towards the olfactory bulb (Ruiz-Reig et al., 2017).

During their migration, CRs remain confined to the marginal zone through the chemoattractive action of Cxcl12 (formerly known as SDF-1) secreted from the meninges and of cognate receptors Cxcr4 and Cxcr7 on CRs (Borrell and Marín, 2006; Paredes, 2006; Trousse et al., 2015). Furthermore, it was recently shown that CRs from the TE and/or septum accumulate in the LOT region during early steps of corticogenesis to eventually undergo a second migratory phase directed towards the neocortex a few days later (de

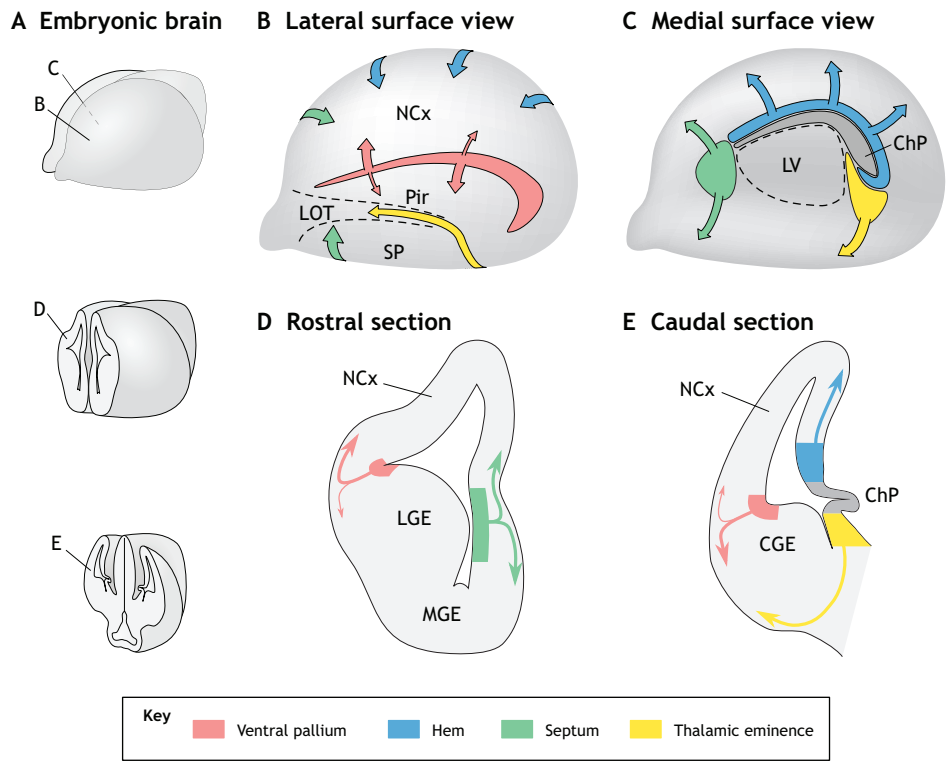

Fig. 2. Origins and migratory routes of CR subtypes. (A) 3D views of the embryonic brain depicting the surface views shown in B and C, and the sections shown in D and E. (B, C) Lateral (B) and medial (C) surface views of the developing forebrain indicating the migratory paths (arrows) of CRs from the ventral pallium (red), septum (green), hem (blue) and thalamic eminence (yellow). (D,E) CR migratory streams (arrows) on rostral (D) and caudal (E) coronal sections. CGE, caudal ganglionic eminence; ChP, choroid plexus; LGE, lateral ganglionic eminence; LOT, lateral olfactory tract; LV, lateral ventricle; MGE, medial ganglionic eminence; NCx, neocortex; Pir, piriform cortex; SP, subpallium.

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Frutos et al., 2016). The formation of such a reservoir of cells followed by their redistribution would allow the density of CRs at the surface of the cortex to be maintained and would compensate for its increase in size during development without the need to generate additional CRs.

CRs are highly motile cells, and eventually cover the entire telencephalon. Their distribution, however, is not homogenous but rather highly reminiscent of their place of birth and migration path. Thus, VP-derived CRs are mostly located in the rostrolateral pallium, septum-derived CRs in the rostromedial pallium, hem-derived CRs in the caudomedial and dorsal pallium, and TE-derived CRs in the caudal ventral telencephalon and the prospective piriform cortex (Bielle et al., 2005; Griveau et al., 2010; Ruiz-Reig et al., 2017; Takiguchi-Hayashi, 2004; Tissir et al., 2009; Yoshida et al., 2006). Such a preferential distribution has been shown to be maintained at postnatal stages (Ledonne et al., 2016). This is achieved by contact repulsion between migrating CRs (Borrell and Marín, 2006), a process modulated by Eph/ephrin interactions and by extracellular Pax6 (Kaddour et al., 2020; Villar-Cerviño et al., 2013) that ensures complete coverage of the telencephalic vesicle. Consistent with this, modulation of the number of CRs or the speed of migration of some CR subsets has been reported to result in the redistribution of the others (Barber et al., 2015; Griveau et al., 2010).

The diversity of CRs does not end in their origin, migration pattern and function. Cell death occurs in CRs at a surprisingly early time and is differentially regulated depending on their origin, molecular identity and localization (Ledonne et al., 2016). In the following section, we describe in detail the most recent discoveries in cell death of CRs and its underlying mechanisms.

CR death: subtype-specific mechanisms and timing

Apoptotic cell death is a physiological process that eliminates about one-third of all immature cortical neurons (Wong and Marín, 2019). This process depends on neuronal activity: silencing neuronal networks increases apoptosis, whereas intensifying the firing frequency promotes neuronal survival (Blanquie et al., 2017b; Ikonomidou, 1999; Priya et al., 2018). Accumulating evidence indicates that this process serves to select functional circuits (Blanquie et al., 2017b) and to match the number of inhibitory neurons to that of excitatory neurons, thus preserving a physiological excitation/inhibition balance (Denaxa et al., 2018; Wong et al., 2018). As noted above, CRs are transient: the vast majority disappear at the end of cortical development and only a small fraction survive until adulthood in rodents, mostly in the hippocampus and to a lesser extent in the neocortex. In humans, the situation is slightly different, as a transient subpopulation disappears around GW 23 to 28, concomitant with the appearance of a second subpopulation that persists until postnatal stages, and to a certain extent in adults (Meyer and González-Gómez, 2018a; Meyer and González-Hernández, 1993).

Morphological studies and permanent labelling in mice have formally and clearly established in a quantitative manner that CRs do not disappear because of migration, transformation or dilution in an expanding cortex, but through programmed cell death (Chowdhury et al., 2010; Derer and Derer, 1990; Ledonne et al., 2016). Quantification of CR apoptosis in different cortical areas shows heterogeneities in their cell death fate. In the mouse hippocampus, which is populated almost exclusively by hem-derived CRs (Louvi et al., 2007; Yoshida et al., 2006), up to 85% of CRs undergo apoptosis (Anstötz and Maccaferri, 2020; Anstötz et al., 2016), whereas this proportion reaches more than 95% in the neocortical MZ (Anstötz and Maccaferri, 2020; Chowdhury et al., 2010; Ledonne et al., 2016). The kinetics of disappearance are also temporally shifted between the two regions: neocortical CRs, even hem-derived ones, are mostly eliminated during the first two postnatal weeks, whereas hippocampal CRs predominantly disappear during the third and fourth postnatal weeks (Anstötz et al., 2016; Del Río et al., 1996; Ledonne et al., 2016).

Within the hippocampal formation, the time course of cell death of CRs appears delayed in the hippocampal fissure and dentate gyrus compared with the subiculum and entorhinal cortex, which display neocortical-like kinetics (Anstötz et al., 2016). The differences go further than a simple variation between the neocortex and hippocampal formation. Comparing the density of ΔNp73-derived (hem/septum) versus Dbx1-derived (VP/septum) CRs along the rostrocaudal axis of the neocortex reveals that the extent of cell death depends on the cortical territories but also on the subtype of CRs: Dbx1-derived CRs decline in numbers as early as P4, especially in rostral regions, whereas ΔNp73-derived CRs mostly decline between P4 and P10 (Ledonne et al., 2016). Moreover, CRs in the medio-rostral cortex undergo death with delayed kinetics with respect to their ontogenetically related equivalents in the dorso-lateral neocortex. These heterogeneities raise the question of the mechanisms underlying CR cell death. Which environmental cues and/or genetic programs control the fate of CRs? Can we predict cell death fate based on the molecular signature, the embryonic origin or the identity acquired throughout development?

The time course and rate of apoptosis of hem-derived CRs depends on their final location (hippocampus versus cortex) (Anstötz and Maccaferri, 2020), indicating that the environment represents an important factor controlling their cell death. Although the mechanisms are not yet fully understood, experimental data indicate that electrical activity is a major factor. In the 1990s, *in vitro* pharmacological studies revealed the pro-apoptotic role of electrical activity on CRs (Del Río et al., 1996; Mienville and Pesold, 1999), an effect that contrasts with its pro-survival role on other cortical neurons during the same period. Another line of evidence is the observation that CRs survive when cultured in pure hippocampal explants but degenerate in entorhino-hippocampal co-cultures, a model that preserves the afferent inputs into CRs (Del Río et al., 1996). These data led to the hypothesis that CR death results from an overload of intracellular calcium via the direct stimulation of their glutamatergic receptors or a global increase in neuronal network activity.

CRs receive $GABA_A$ receptor-mediated inputs (Kilb and Luhmann, 2001; Soda et al., 2003), express the chloride inward transporter NKCC1 throughout their life but do not express the chloride outward transporter KCC2 (Achilles et al., 2007; Pozas et al., 2008). This expression profile, which is typical of immature neurons, renders $GABA_A$ receptor-mediated inputs excitatory. Furthermore, it has been shown that the excitatory component of $GABA_A$-receptor inputs on cultured neocortical CRs triggers their cell death (Blanquie et al., 2017a). This $GABA_A$ receptor -mediated depolarization leads to an increase in expression of the p75 neurotrophic receptor ($p75^{NTR}$), a member of the TNF receptor superfamily that usually causes cell death upon binding of pro-neurotrophins (Dekkers et al., 2013). *In vivo*, deletion of the NKCC1 transporter diminishes the rate and speed of CR death but does not allow a full rescue of CRs (Blanquie et al., 2017a), indicating that additional mechanisms further tune their fate. Whether the increase in $p75^{NTR}$ receptor levels upon $GABA_A$-receptor stimulation is differentially modulated according to the

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ontogenetic origin of CRs or to a particular molecular signature is a question that remains to be addressed. Increasing evidence indicates that intrinsic mechanisms also further underlie the heterogeneities in the cell death of CRs.

ΔNp73, the N-terminally truncated version of p73, is specifically expressed by CRs from the septum, TE and cortical hem (Meyer et al., 2002; Tissir et al., 2009). Whereas full-length p73 expression is associated with apoptotis, ΔNp73 is necessary for maintaining neuronal survival (Pozniak et al., 2000) and seems to control the lifetime of CRs as its genetic inactivation leads to a strong reduction in CR numbers at birth (Tissir et al., 2009). This could explain the apparent faster decline of VP-derived CRs, as these cells never express ΔNp73 (Ledonne et al., 2016). More recently, the observation that septum-derived CRs – but not hem-derived CRs – survive upon genetic overexpression of the hyperpolarizing potassium channel Kir2.1 demonstrates that a similar input can differentially affect the survival fate of CRs depending on their ontogenetic identity (Riva et al., 2019). The downstream pathway also distinguishes the two subpopulations: genetic inactivation of the pro-apoptotic factor Bax rescues septum-derived CRs from apoptosis but does not affect the survival of hem-derived CRs (Ledonne et al., 2016). Altogether, these data demonstrate that a combination between environmental cues and intrinsic factors ultimately control when CRs die, and that subtype-specific mechanisms of death mediate CRs demise.

Programmed cell death of CRs has been shown to be crucial for cortical wiring. Indeed, the survival of septum-derived CRs by genetic inactivation of *Bax* leads to an exuberance of dendrites and spine density in layer II/III pyramidal neurons, resulting in an excitation/inhibition imbalance due to increased excitatory drive (Riva et al., 2019). Thus, proper cortical arealization and wiring depends not only on a specific density of CR subtypes in the neocortex and hippocampus during embryonic and early postnatal development (Barber et al., 2015; de Frutos et al., 2016; Griveau et al., 2010), but also on the appropriate cell death dynamics (Riva et al., 2019). Two major issues in the future will be to disentangle which environmental cues affect the survival of which subpopulations and to better understand whether the subtype-specific mechanisms of cell death are associated with distinct developmental functions and/or alterations in neurodevelopmental disorders.

CR functions during development

The principal function attributed to CRs is the control of radial migration through secretion of Reln (D'Arcangelo et al., 1995; Ogawa et al., 1995). Reln is a large glycoprotein of about 400 kDa. Upon binding to one of its two cognate receptors, the very low density lipoprotein receptor (VLDLR) and the apolipoprotein-E receptor type 2 (ApoER2), the disabled 1 (Dab1) adaptor is phosphorylated and subsequently activates various downstream pathways (D'Arcangelo et al., 1999; Hiesberger et al., 1999). Mouse mutants for Reln (reeler) or Dab1 (scrambler), have an abnormal layering in the hippocampus, neocortex and cerebellum (D'Arcangelo et al., 1995; Howell et al., 1997; Sheldon et al., 1997). Consistent with this, CR depletion in the hippocampus by genetic manipulation of p73 leads to severe morphological defects (Amelio et al., 2020; Meyer et al., 2004, 2019).

CRs further regulate neurite outgrowth, neurite complexity, proliferation and distribution of oligodendrocyte progenitor cells via the Reln/Dab1 signalling pathway (Borrell et al., 2007; Del Río et al., 1997; Niu et al., 2004; Niu et al., 2008; Ogino et al., 2020). In humans, the preferential localization of persistent CRs in sulci raised the hypothesis that they might be involved in cortical folding (Meyer and González-Gómez, 2018a,b). A variety of additional developmental functions have been uncovered, including regulation of the identity and function of radial glia (Supèr et al., 2000), timing of GABAergic interneuron migration (Caronia-Brown and Grove, 2011) and dendritogenesis of cortical pyramidal neurons (de Frutos et al., 2016). Furthermore, CR subtype-specific presence and distribution at the surface of the developing cortex participates in early patterning, and in the control of the size of cortical areas (Barber et al., 2015; Griveau et al., 2010). This function does not rely on a Reln-dependent mechanism but rather subtype-specific signalling (see below). Importantly, decreased CR density at embryonic stages affects cortical circuits even after CRs have disappeared, supporting a long-term function for CRs despite their transient lifespan (de Frutos et al., 2016).

The diversity of CRs in terms of ontogenic origins, migration routes, cell death and survival fate raises the issue of whether each subtype fulfils a specific function. Patch-clamp experiments followed by biocytin labelling in Dbx1-Cre mice failed to demonstrate any electrophysiological or morphological difference between VP- and septum-derived CRs compared with the remaining subtypes (Sava et al., 2010). In addition, the Reln-dependent control of cortical lamination is not severely affected upon ablation of specific CR subtypes (Bielle et al., 2005; Griveau et al., 2010; Yoshida et al., 2006), consistent with the fact that all CRs secrete Reln. However, mouse mutants in which septum-derived CRs are ablated display a redistribution of other subtypes, regional changes in cortical neurogenesis and, ultimately, an increase in the size of the motor cortex associated with a displacement of somatosensory areas (Griveau et al., 2010). Furthermore, modulation of CR subtypes distribution without affecting their total numbers was shown to affect the size and positioning of higher-order cortical areas (Barber et al., 2015). These data demonstrate that CR diversity is relevant to cortical organization in the tangential dimension and support the hypothesis that CRs send signals to ventricular zone progenitors, either through contact with the glial end-feet in the MZ, or via secreted factors. Although the nature of such signals remains to be established, transcriptomic analyses indicated that it possibly corresponds to the release of subtype-specific morphogens and growth factors (Griveau et al., 2010). The link between CR subpopulations and cortical regionalization is most likely bidirectional, as the patterning genes *Emx1*, *Emx2* and *Pax6* have been shown to be important for CR specification (reviewed by Barber and Pierani, 2016), and the analysis of *Fgf8*, *Emx2*, *Gli3* and *Pax6* mutants revealed that defects differed between CR subtypes (Zimmer et al., 2010). These observations led to the proposal that CRs behave as 'transient signalling units', secreting a subtype-specific combination of factors throughout their migration, thus conveying positional information over long distances in the growing cortex and acting as a complement to the well-described signalling centres releasing morphogens that diffuse over shorter distances (Borello and Pierani, 2010; Griveau et al., 2010). The respective and relative contribution of CR-derived and signalling centre-derived morphogens to cortical regionalization remains to be determined.

In the adult hippocampus, the physiological role of persistent Cajal-Retzius is not yet understood. The Reln/Dab1 signalling pathway is required for synaptic plasticity and memory (Herz and Chen, 2006). However, as the secretion of Reln is progressively taken over by inhibitory neurons throughout development it is reasonable to hypothesize that the role of adult hippocampal CRs is not restricted to the secretion of Reln. Anstötz et al. observed that mice raised in enriched cages, an environment associated with

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enhanced hippocampal neurogenesis, display a higher density of surviving hippocampal CRs than animals maintained in classical cages (Anstötz et al., 2018a). The authors propose that hippocampal CRs might play a role in the regulation of postnatal neurogenesis. The role of adult CRs remains to be elucidated, but the observation that modifying the environment promotes the survival of hippocampal CRs without affecting the survival of neocortical CRs underlines the existence of subtype-specific functions.

Given the accumulating evidence for specific roles for different subtypes, understanding the heterogeneity of CRs is clearly important. As discussed in the next section, deep transcriptomic characterization of CRs is likely to invigorate our understanding of their production, migration, function and disappearance.

Molecular profiling redefines CRs

Although lineage tracing in mice has proved very useful to study the origins, functions and properties of CRs during development, only a few studies have undertaken detailed characterization of CR identities or the mechanisms involved in their production. In addition, a comprehensive molecular definition of CR subtype diversity is still lacking. Indeed, during the past two decades, the molecular toolkit available to identify CRs has been limited to a handful of marker genes and mouse lines. A first attempt to perform unbiased molecular profiling of CRs was reported by the group of Nakanishi using cDNA microarrays on cells sorted by FACS expressing GFP under the regulation of the hem-derived CR-specific metabotropic glutamate receptor subtype 2 (*Grm2*) promoter (Yamazaki et al., 2004) (see Table 1). Although their experimental design successfully led to the identification of CR-specific genes at E13.5 and P2 stages, these bulk data could not reveal subtype-specific facets of CR identity. Transcriptional diversity among CR subtypes was first suggested by performing microarray experiments on FACS-sorted Dbx1-derived neurons isolated from anterior medial and lateral regions of the developing brain at E12.5 (Griveau et al., 2010). Despite the bulk approach, the differential enrichment in CR subtypes in the two fractions analysed led to the proposal that each subset express its own repertoire of signalling molecules.

The recent advances in single cell RNA-sequencing (scRNAseq) technologies offer new opportunities to better characterize CRs among the diversity of cortical neuron types. Using this approach, Tasic and collaborators (Tasic et al., 2018) reported that the few CRs surviving in adult mice classify outside of the major glutamatergic branch, highlighting the very 'special' nature of CRs in the cortex, as foreseen by Ramón y Cajal (Gil et al., 2014). The increased availability of published and public scRNAseq datasets allows profiling of CRs from distinct regions or stages. A first attempt was made by Iacono and colleagues (Iacono et al., 2018) using the '1.3 million brain cells' dataset from10X Genomics (https://support.10xgenomics.com/single-cell-gene-expression/datasets/1.3.0/1M_neurons) that contains several thousand E18.5 CRs but their classification of neocortical CRs into eight clusters awaits validation *in vivo* to understand how these clusters relate to the distinct CR subtypes that can be identified on biological grounds. Interestingly some of the clusters display an apoptotic signature, suggesting that the CR demise program is already initiated by late embryonic stages. However, the absence of *p73*-negative clusters indicate that not all CR diversity was sampled, probably due to the cortical region dissected. More recently, scRNAseq experiments performed at E12.5 allowed profiling of *p73*-positive and -negative CRs, and indicated that the main distinction among subtypes is actually related to their VP versus medial (hem/septum/TE) origin (Moreau et al., 2020 preprint). Interestingly, VP-derived cells appear to lack a strong specific signature and differ from other CR subtypes by the absence of a complete module of genes usually considered as CR markers, such as *Lhx1*, *Ebf3* and *Cdkn1a* (*p21*), in addition to *p73*.

An issue scientists are currently facing is the multiplicity of studies: choosing the relevant datasets and exploring them requires analytical skills and is time consuming. An additional challenge regarding CRs is related to their low density, which, in the absence of enrichment methods, may lead to very low sampling. Here, we give examples of two mouse datasets – in addition to the aforementioned '1.3 million brain cells' dataset – that we believe are suited to the study of CRs as they contain a sufficient number of cells sequenced at a sufficient depth: one from E14.5 cortex (Loo et al., 2019, Fig. 3A) and the '9k brain cells' dataset from 10X genomics (Fig. 3B and https://support.10xgenomics.com/single-cell-gene-expression/datasets/2.1.0/neuron_9k). We also draw the attention of the reader to a very recent preprint containing nearly 300,000 cells collected from E7 to E18 brains that will certainly prove itself helpful in the deep characterization of CRs (La Manno et al., 2020 preprint). As a proof of concept that valuable information can be retrieved from such datasets, we present some analysis focusing on CRs and their heterogeneity (see Supplementary information). We illustrate in Fig. 3A-C the very peculiar clustering of CRs, apart from all other neurons. Lists of genes enriched in CRs can be extracted to characterize in a comprehensive manner what makes CRs distinct from other neuronal types (Fig. 3D). The high sampling of CRs in the '1.3 million brain cells' dataset (more than 14,000 cells) additionally allows CR diversity to be investigated, although it only contains cells from the neocortex and hippocampus (Fig. 3E).

A notable and defining feature of CRs is that they lack expression of the forebrain specifier *Foxg1*. The observation that *Foxg1* KO mice generate an excess of CRs prompted the hypothesis that CRs could represent a default fate (Hanashima, 2004; Muzio and Mallamaci, 2005). Accordingly, CRs would be produced by default in the cortex unless *Foxg1* is expressed and redirects cells towards a pyramidal fate, reminiscent of the repression of alternative fates observed in the spinal cord (Kutejova et al., 2016). The underlying mechanisms were partially unravelled as *Foxg1* inhibition was shown to directly repress key transcription factors required for the acquisition and/or maintenance of CR traits, and especially their migratory behaviour, including Ebf and Dmrt family members (Chiara et al., 2012; Chuang et al., 2011; de Frutos et al., 2016; Kikkawa et al., 2020; Kumamoto et al., 2013). Furthermore, conditional *Foxg1* deletion at E13.5, after the end of normal CR production, leads to ectopic production of *p73*-negative VP-derived, but not Hem-derived, CRs (Hanashima et al., 2007), consistent with the idea that VP-derived CRs are very distinct from other subtypes.

One longstanding question regards the conservation of CRs during cortical evolution. Important differences have been reported between rodents and humans (Meyer and González-Gómez, 2018a). At the molecular level, species-specific differences also appear. For example, human CRs specifically express HAR1A (also known as HAR1F), a non-coding RNA subjected to accelerated evolution in hominoids (Pollard et al., 2006). Nevertheless, scRNAseq data of the developing human cortex at gestational weeks 22-23 (prior to transient CR demise, Fan et al., 2018) confirms that mouse and human CRs are indeed a homologous cell type (Fig. 3F). Although human CRs have been well described, a thorough comparison between human and rodent CRs in terms of developmental profile and subtype cannot yet be drawn because of the lack of longitudinal studies at all stages in human. Because human embryonic or foetal

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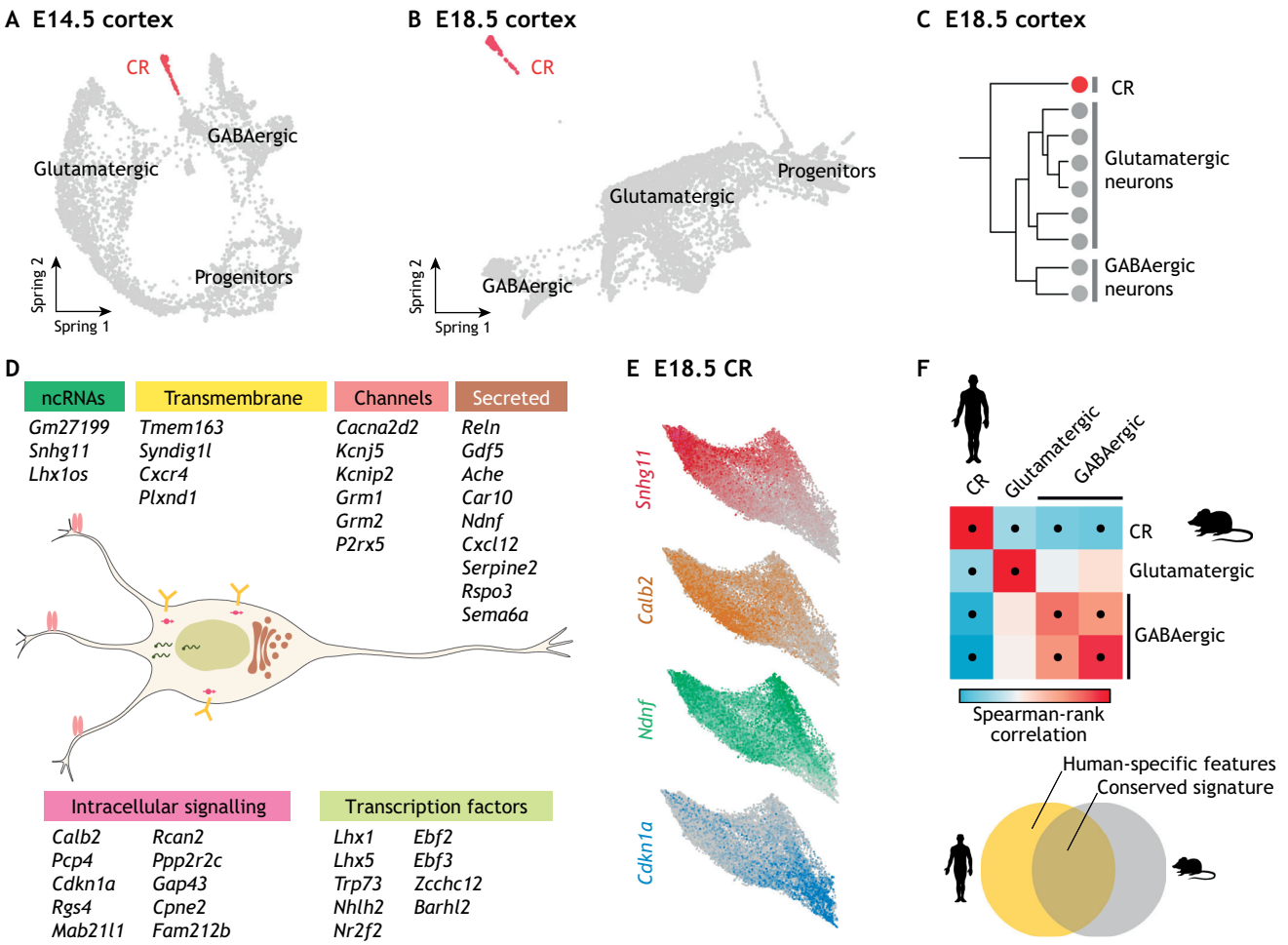

Fig. 3. scRNAseq for the study of CRs. (A,B) scRNAseq SPRING plots show that CRs (highlighted in red) are always positioned away from both glutamatergic and GABAergic neurons. Data from Loo et al. (2019) E14.5 mouse embryos (A) or 10X Genomics E18.5 mouse embryos (B) are analysed. (C) Hierarchical clustering (here from the E18.5 10X Genomics dataset) shows that CRs branch away from all other neuronal types. (D) Identification of genes with particular functions/subcellular localizations that are specifically enriched in CRs. Such factors constitute candidates for CR markers and for key regulators of CR development and function. (E) Examples of CR-enriched genes differentially expressed within the CR cluster from the 10X Genomics E18.5 dataset. Whether these differences reflect CR origins, localization, progression towards apoptosis or any other process is currently unknown. (F) Comparisons between human (Fan et al., 2018) and mouse (E18.5 10X Genomics) datasets demonstrate a significant (black dot) positive correlation between gene modules defining CRs in each species and a negative correlation when comparing CRs with other cortical neurons (inhibitory and excitatory). Such a strategy allows identification of species-specific features or conserved signatures, as pictured by the theoretical Venn diagram. Links to the datasets and details regarding the pipelines used for data analysis can be found in the Supplementary information.

tissue is difficult to obtain, organoids represent an interesting alternative with which to gain access to human CR signatures, especially when combined with scRNAseq (Birey et al., 2017). We predict that deepening our knowledge of the species-specific features of CR will soon allow the assessment of CR contribution to brain evolution, especially in primates.

In sauropsids, only a handful of studies have addressed the matter and the issue is not as clear. As noted above, *Reln* expression has been described in the telencephalic MZ of turtles, lizards and birds (Bar et al., 2000; Goffinet, 2017). By contrast, expression of *p73* was reported to be very limited in lizards (Cabrera-Socorro et al., 2007), whereas in crocodiles most subpial *Reln*$^{+}$ cells were also found to be *p73*$^{+}$ (Tissir et al., 2003). In chick, *Lhx1* and *Lhx5* (two genes enriched in mouse CR, see Fig. 3) are expressed in the septum and thalamic eminence, while Lhx5 is also found in the hem, but neither gene seems to be expressed in MZ cells of the cortex (Abellan et al., 2010a,b). Whether these structures can generate CRs in avian embryos remains an unresolved issue. An unequivocal assessment of the degree of CR conservation in sauropsids will likely come from scRNAseq experiments, but the only data available so far come from adult specimens (Tosches et al., 2018), which, as in mice, appear largely devoid of CRs.

Overall, molecular profiling has revealed the extent to which CRs distinguish themselves from all other cortical neurons and has exposed their heterogeneity. These data have also opened new perspectives on their generation, elimination and contribution to brain development. As the number and quality of such datasets improve, and as data from more species are added, we expect single cell profiling to further contribute to our understanding of CRs.

Future challenges and perspectives

Over the past 15 years, thanks to the development of genetic tracing tools and more recently single cell technologies, we have made tremendous progress in exposing the multiple facets of CRs during cortical development. The nature of subtype- and species-specific features have begun to be unravelled. However, the molecular

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mechanisms underpinning CR diversity and their relevance for the construction of functional or dysfunctional cortical networks is still largely unclear. For example, the topology of the gene regulatory networks (GRNs) controlling the acquisition and the maintenance of CR identity remains unknown. Further efforts will be necessary to identify the terminal selector transcription factors (Hobert, 2016) for CRs. New attempts to map the GRN of CRs will likely take advantage of scATACseq approaches that allow the identification of regulatory regions of the genome that are specifically accessible in CRs compared with other cortical neurons. New technologies such as spatial transcriptomics could prove helpful, especially in humans, to precisely map CR subsets and pinpoint their origins.

Besides a better classification of CRs, the precise architecture of transient cortical networks in which CRs are embedded is still lacking. Trans-synaptic viral tracing of the different subpopulations of CRs will contribute to the better characterization of such networks. This will pave the way to understanding how CRs contribute to shape mature cortical networks during development and whether CRs subtypes are assigned to specific functions. In terms of brain evolution, CRs are a highly relevant cell type, as their numbers and diversity increases from sauropsids to mammals. They control key processes in brain morphogenesis and emerge as potential players in cortical gyrification. How CRs have evolved across species and their contribution to the growing complexity of the brain will be one of the questions to solve for the next decade. Another future challenge will be to determine the contribution of CRs to neurodevelopmental disorders in humans. Abnormal CR survival has been suggested to occur in individuals with polymicrogyria, focal cortical dysplasia or temporal lobe epilepsy, pathologies associated with seizures (Blümcke et al., 1999; Eriksson, 2001; Garbelli et al., 2001). The study of mouse models with extended CR survival (Ledonne et al., 2016; Riva et al., 2019) will certainly prove useful in this perspective.

The most fascinating aspect of CRs is certainly their almost purely developmental role. They are a unique example of a cell type that is produced very early during corticogenesis (the migration and positioning of which all over the developing forebrain is tightly regulated), that performs essential functions in the establishment of brain architecture, and that is almost completely eliminated once development is over. The very few CRs surviving until adulthood might reveal themselves necessary for a physiological cortical processing but until then, CRs remind us that there are important functions for neurons beyond synaptic transmission.

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References

Abellan, A., Menuet, A., Dehay, C., Medina, L. and Retaux, S. (2010a). Differential expression of LIM-homeodomain factors in Cajal-Retzius cells of primates, rodents, and birds. *Cereb. Cortex* **20**, 1788-1798. doi:10.1093/cercor/bhp242

Abellan, A., Vernier, B., Rétaux, S. and Medina, L. (2010b). Similarities and differences in the forebrain expression of Lhx1 and Lhx5 between chicken and mouse: Insights for understanding telencephalic development and evolution. *J. Comp. Neurol.* **518**, 3512-3528. doi:10.1002/cne.22410

Abs, E., Poorthuis, R. B., Apelblat, D., Muhammad, K., Pardi, M. B., Enke, L., Kushinsky, D., Pu, D.-L., Eizinger, M. F., Conzelmann, K.-K. et al. (2018). Learning-related plasticity in dendrite-targeting layer 1 interneurons. *Neuron* **100**, 684-699.e6. doi:10.1016/j.neuron.2018.09.001

Achilles, K., Okabe, A., Ikeda, M., Shimizu-Okabe, C., Yamada, J., Fukuda, A., Luhmann, H. J. and Kilb, W. (2007). Kinetic properties of Cl- uptake mediated by Na +-dependent K+-2Cl- cotransport in immature rat neocortical neurons. *J. Neurosci.* **27**, 8616-8627. doi:10.1523/JNEUROSCI.5041-06.2007

Alcántara, S., Ruiz, M., D'Arcangelo, G., Ezan, F., de Lecea, L., Curran, T., Sotelo, C. and Soriano, E. (1998). Regional and cellular patterns of reelin mRNA expression in the forebrain of the developing and adult mouse. *J. Neurosci.* **18**, 7779-7799. doi:10.1523/JNEUROSCI.18-19-07779.1998

Amelio, I., Panatta, E., Niklison-Chirou, M. V., Steinert, J. R., Agostini, M., Morone, N., Knight, R. A. and Melino, G. (2020). The c terminus of p73 is essential for hippocampal development. *Proc. Natl. Acad. Sci. USA* **117**, 15694-15701. doi:10.1073/pnas.2000917117

Angevine, J. B. and Sidman, R. L. (1961). Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature* **192**, 766-768. doi:10.1038/192766b0

Anstötz, M. and Maccaferri, G. (2020). A toolbox of criteria for distinguishing cajal–retzius cells from other neuronal types in the postnatal mouse hippocampus. *eNeuro* **7**, ENEURO.0516–19.2019. doi:10.1523/ENEURO.0516-19.2019

Anstötz, M., Cosgrove, K. E., Hack, I., Mugnaini, E., Maccaferri, G. and Lübke, J. H. R. (2014). Morphology, input–output relations and synaptic connectivity of Cajal–Retzius cells in layer 1 of the developing neocortex of CXCR4-EGFP mice. *Brain Struct. Funct.* **219**, 2119-2139. doi:10.1007/s00429-013-0627-2

Anstötz, M., Huang, H., Marchionni, I., Haumann, I., MacCaferri, G. and Lübke, J. H. R. (2016). Developmental profile, morphology, and synaptic connectivity of Cajal-Retzius cells in the postnatal mouse hippocampus. *Cereb. Cortex* **26**, 855-872. doi:10.1093/cercor/bhv271

Anstötz, M., Lee, S. K., Neblett, T. I., Rune, G. M. and Maccaferri, G. (2018a). Experience-dependent regulation of Cajal-Retzius cell networks in the developing and adult mouse hippocampus. *Cereb. Cortex* **28**, 672-687. doi:10.1093/cercor/bhx153

Anstötz, M., Lee, S. K. and Maccaferri, G. (2018b). Expression of TRPV1 channels by Cajal-Retzius cells and layer-specific modulation of synaptic transmission by capsaicin in the mouse hippocampus. *J. Physiol.* **596**, 3739-3758. doi:10.1113/JP275685

Bar, I., Lambert de Rouvroit, C. and Goffinet, A. M. (2000). The evolution of cortical development. An hypothesis based on the role of the Reelin signaling pathway. *Trends Neurosci.* **23**, 633-638. doi:10.1016/S0166-2236(00)01675-1

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

Barber, M., Arai, Y., Morishita, Y., Vigier, L., Causeret, F., Borello, U., Ledonne, F., Coppola, E., Contremoulins, V., Pfrieger, F. W. et al. (2015). Migration speed of Cajal-Retzius cells modulated by vesicular trafficking controls the size of higher-order cortical areas. *Curr. Biol.* **25**, 2466-2478. doi:10.1016/j.cub.2015.08.028

Bielle, F., Griveau, A., Narboux-Nême, N., Vigneau, S., Sigrist, M., Arber, S., Wassef, M. and Pierani, A. (2005). Multiple origins of Cajal-Retzius cells at the borders of the developing pallium. *Nat. Neurosci.* **8**, 1002-1012. doi:10.1038/nn1511

Birey, F., Andersen, J., Makinson, C. D., Islam, S., Wei, W., Huber, N., Fan, H. C., Metzler, K. R. C., Panagiotakos, G., Thom, N. et al. (2017). Assembly of functionally integrated human forebrain spheroids. *Nature* **545**, 54-59. doi:10.1038/nature22330

Blanquie, O., Liebmann, L., Hübner, C. A., Luhmann, H. J., Sinning, A., Genetics, H., Schiller, F. and Jena, D. (2017a). NKCC1-mediated GABAergic signaling promotes postnatal cell death in neocortical Cajal-Retzius cells. *Cereb. Cortex* **25**, 1644-1659. doi:10.1093/cercor/bhw004

Blanquie, O., Yang, J.-W., Kilb, W., Sharopov, S., Sinning, A. and Luhmann, H. J. (2017b). Electrical activity controls area-specific expression of neuronal apoptosis in the mouse developing cerebral cortex. *Elife* **6**, e27696. doi:10.7554/eLife.27696

Blümcke, I., Beck, H., Suter, B., Hoffmann, D., Födisch, H. J., Wolf, H. K., Schramm, J., Elger, C. E. and Wiestler, O. D. (1999). An increase of hippocampal calretinin-immunoreactive neurons correlates with early febrile seizures in temporal lobe epilepsy. *Acta Neuropathol.* **97**, 31-39. doi:10.1007/s004010050952

Borrell, V. and Marín, O. (2006). Meninges control tangential migration of hem-derived Cajal-Retzius cells via CXCL12/CXCR4 signaling. *Nat. Neurosci.* **9**, 1284-1293. doi:10.1038/nn1764

Borello, U. and Pierani, A. (2010). Patterning the cerebral cortex: traveling with morphogens. *Curr. Opin. Genet. Dev*. **20**, 408-415. doi:10.1016/j.gde.2010.05.003

Borrell, V., Pujadas, L., Simó, S., Durà, D., Solé, M., Cooper, J. A., Del Río, J. A. and Soriano, E. (2007). Reelin and mDab1 regulate the development of hippocampal connections. *Mol. Cell. Neurosci*. **36**, 158-173. doi:10.1016/j.mcn.2007.06.006

Bradford, R., Parnavels, J. G. and Lieberman, A. R. (1977). Neurons in layer I of the developing occipital cortex of the rat. *J. Comp. Neurol.* **176**, 121-132. doi:10.1002/cne.901760108

Cabrera-Socorro, A., Hernandez-Acosta, N. C., Gonzalez-Gomez, M. and Meyer, G. (2007). Comparative aspects of p73 and Reelin expression in Cajal-Retzius cells and the cortical hem in lizard, mouse and human. *Brain Res.* **1132**, 59-70. doi:10.1016/j.brainres.2006.11.015

Caronia-Brown, G. and Grove, E. A. (2011). Timing of cortical interneuron migration is influenced by the cortical hem. *Cereb. Cortex* **21**, 748-755. doi:10.1093/cercor/bhq142

Causeret, F., Coppola, E. and Pierani, A. (2018). Cortical developmental death: selected to survive or fated to die. *Curr. Opin. Neurobiol.* **53**, 35-42. doi:10.1016/j.conb.2018.04.022

Ceranik, K., Zhao, S. and Frotscher, M. (2000). Development of the entorhino-hippocampal projection: guidance by Cajal-Retzius cell axons. *Ann. N. Y. Acad. Sci.* **911**, 43-54. doi:10.1111/j.1749-6632.2000.tb06718.x

Chan, C.-H. and Yeh, H. H. (2003). Enhanced GABA a receptor–mediated activity following activation of NMDA receptors in cajal–retzius cells in the developing mouse neocortex. *J. Physiol*. **550**, 103-111. doi:10.1113/jphysiol.2003.042556

Chiara, F., Badaloni, A., Croci, L., Yeh, M. L., Cariboni, A., Hoerder-Suabedissen, A., Consalez, G. G., Eickholt, B., Shimogori, T., Parnavelas, J. G. et al. (2012). Early B-cell factors 2 and 3 (EBF2/3) regulate early migration of Cajal-Retzius cells from the cortical hem. *Dev. Biol*. **365**, 277-289. doi:10.1016/j.ydbio.2012.02.034

Chowdhury, T. G., Jimenez, J. C., Bomar, J. M., Cruz-Martin, A., Cantle, J. P. and Portera-Cailliau, C. (2010). Fate of Cajal-Retzius neurons in the postnatal mouse neocortex. *Front. Neuroanat.* **4**, 10. doi:10.3389/neuro.05.010.2010

Chuang, S.-M., Wang, Y., Wang, Q., Liu, K.-M. and Shen, Q. (2011). Ebf2 marks early cortical neurogenesis and regulates the generation of Cajal-Retzius neurons in the developing cerebral cortex. *Dev. Neurosci*. **33**, 479-493. doi:10.1159/000330582

Cocas, L. A., Fernandez, G., Barch, M., Doll, J., Diaz, I. Z. and Pleasure, S. J. (2016). Cell type-specific circuit mapping reveals the presynaptic connectivity of developing cortical circuits. *J. Neurosci.* **36**, 3378-3390. doi:10.1523/JNEUROSCI.0375-15.2016

Cosgrove, K. E. and Maccaferri, G. (2012). mGlu1α-dependent recruitment of excitatory GABAergic input to neocortical Cajal-Retzius cells. *Neuropharmacology* **63**, 486-493. doi:10.1016/j.neuropharm.2012.04.025

D'Arcangelo, G., Miao, G. G., Chen, S. C., Scares, H. D., Morgan, J. I. and Curran, T. (1995). A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. *Nature* **374**, 719-723. doi:10.1038/374719a0

D'Arcangelo, G., Homayouni, R., Keshvara, L., Rice, D. S., Sheldon, M. and Curran, T. (1999). Reelin is a ligand for lipoprotein receptors. *Neuron* **24**, 471-479. doi:10.1016/S0896-6273(00)80860-0

de Frutos, C. A., Bouvier, G., Arai, Y., Thion, M. S., Lokmane, L., Keita, M., Garcia-Dominguez, M., Charnay, P., Hirata, T., Riethmacher, D. et al. (2016). Reallocation of olfactory Cajal-Retzius cells shapes neocortex architecture. *Neuron* **92**, 435-448. doi:10.1016/j.neuron.2016.09.020

Dekkers, M. P. J., Nikoletopoulou, V. and Barde, Y.-A. (2013). Death of developing neurons: new insights and implications for connectivity. *J. Cell Biol.* **203**, 385-393. doi:10.1083/jcb.201306136

del Río, J. A., Martinez, A., Fonseca, M., Auladell, C. and Soriano, E. (1995). Glutamate-like immunoreactivity and fate of Cajal-Retzius cells in the murine cortex as identified with calretinin antibody. *Cereb. Cortex* **5**, 13-21. doi:10.1093/cercor/5.1.13

Del Río, J. A., Heimrich, B., Supèr, H., Borrell, V., Frotscher, M. and Soriano, E. (1996). Differential survival of Cajal-Retzius cells in organotypic cultures of hippocampus and neocortex. *J. Neurosci.* **16**, 6896-6907. doi:10.1523/JNEUROSCI.16-21-06896.1996

Del Río, J. A., Heimrich, B., Borrell, V., Förster, E., Drakew, A., Alcántara, S., Nakajima, K., Miyata, T., Ogawa, M., Mikoshiba, K. et al. (1997). A role for Cajal-Retzius cells and reelin in the development of hippocampal connections. *Nature* **385**, 70-74. doi:10.1038/385070a0

Denaxa, M., Neves, G., Rabinowitz, A., Kemlo, S., Liodis, P., Burrone, J. and Pachnis, V. (2018). Modulation of apoptosis controls inhibitory interneuron number in the cortex. *Cell Rep.* **22**, 1710-1721. doi:10.1016/j.celrep.2018.01.064

Derer, P. and Derer, M. (1990). Cajal-retzius cell ontogenesis and death in mouse brain visualized with horseradish peroxidase and electron microscopy. *Neuroscience* **36**, 839-856. doi:10.1016/0306-4522(90)90027-2

Eriksson, S. H. (2001). Persistent reelin-expressing Cajal-Retzius cells in polymicrogyria. *Brain* **124**, 1350-1361. doi:10.1093/brain/124.7.1350

Fan, X., Dong, J., Zhong, S., Wei, Y., Wu, Q., Yan, L., Yong, J., Sun, L., Wang, X., Zhao, Y. et al. (2018). Spatial transcriptomic survey of human embryonic cerebral cortex by single-cell RNA-seq analysis. *Cell Res.* **28**, 730-745. doi:10.1038/s41422-018-0053-3

Frade-Pérez, M. D., Miquelajáuregui, A. and Varela-Echavarría, A. (2017). Origin and migration of olfactory Cajal-Retzius cells. *Front. Neuroanat.* **11**, 97. doi:10.3389/fnana.2017.00097

Fujitani, M., Sato, R. and Yamashita, T. (2017). Loss of p73 in ependymal cells during the perinatal period leads to aqueductal stenosis. *Sci. Rep*. **7**, 12007. doi:10.1038/s41598-017-12105-z

Garbelli, R., Frassoni, C., Ferrario, A., Tassi, L., Bramerio, M. and Spreafico, R. (2001). Cajal-Retzius cell density as marker of type of focal cortical dysplasia. *Neuroreport* **12**, 2767-2771. doi:10.1097/00001756-200108280-00034

García-Moreno, F., Anderton, E., Jankowska, M., Begbie, J., Encinas, J. M., Irimia, M. and Molnár, Z. (2018). Absence of tangentially migrating glutamatergic neurons in the developing avian brain. *Cell Rep*. **22**, 96-109. doi:10.1016/j.celrep.2017.12.032

Gil-Sanz, C., Franco, S. J., Martinez-Garay, I., Espinosa, A., Harkins-Perry, S. and Müller, U. (2013). Cajal-retzius cells instruct neuronal migration by coincidence signaling between secreted and contact-dependent guidance cues. *Neuron* **79**, 461-477. doi:10.1016/j.neuron.2013.06.040

Gil, V., Nocentini, S. and del RÃo, J. A. (2014). Historical first descriptions of Cajalâ€"Retzius cells: from pioneer studies to current knowledge. *Front. Neuroanat.* **8**, 1-9. doi:10.3389/fnana.2014.00032

Goffinet, A. M. (2017). The evolution of cortical development: the synapsid-diapsid divergence. *Development* **144**, 4061-4077. doi:10.1242/dev.153908

Gong, S., Zheng, C., Doughty, M. L., Losos, K., Didkovsky, N., Schambra, U. B., Nowak, N. J., Joyner, A., Leblanc, G., Hatten, M. E. et al. (2003). A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* **425**, 917-925. doi:10.1038/nature02033

Gorski, J. A., Talley, T., Qiu, M., Puelles, L., Rubenstein, J. L. R. and Jones, K. R. (2002). Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the Emx1-expressing lineage. *J. Neurosci*. **22**, 6309-6314. doi:10.1523/JNEUROSCI.22-15-06309.2002

Griveau, A., Borello, U., Causeret, F., Tissir, F., Boggetto, N., Karaz, S. and Pierani, A. (2010). A novel role for Dbx1-derived Cajal-Retzius cells in early regionalization of the cerebral cortical neuroepithelium. *PLoS Biol.* **8**, e1000440. doi:10.1371/journal.pbio.1000440

Gu, X., Yan, Y., Li, H., He, D., Pleasure, S. J. and Zhao, C. (2009). Characterization of the Frizzled10 -CreER™ transgenic mouse: an inducible Cre line for the study of Cajal-Retzius cell development. *Genesis* **47**, 210-216. doi:10.1002/dvg.20472

Gu, X., Liu, B., Wu, X., Yan, Y., Zhang, Y., Wei, Y., Pleasure, S. J. and Zhao, C. (2011). Inducible genetic lineage tracing of cortical hem derived Cajal-Retzius cells reveals novel properties. *PLoS ONE* **6**, 1-9. doi:10.1371/journal.pone.0028653

Hanashima, C. (2004). Foxg1 suppresses early cortical cell fate. *Science* **303**, 56-59. doi:10.1126/science.1090674

Hanashima, C., Fernandes, M., Hebert, J. M. and Fishell, G. (2007). The role of Foxg1 and dorsal midline signaling in the generation of Cajal-Retzius subtypes. *J. Neurosci.* **27**, 11103-11111. doi:10.1523/JNEUROSCI.1066-07.2007

Herz, J. and Chen, Y. (2006). Reelin, lipoprotein receptors and synaptic plasticity. *Nat. Rev. Neurosci.* **7**, 850-859. doi:10.1038/nrn2009

Hestrin, S. and Armstrong, W. E. (1996). Morphology and physiology of cortical neurons in layer I. *J. Neurosci*. **16**, 5290-5300. doi:10.1523/JNEUROSCI.16-17-05290.1996

Hevner, R. F., Shi, L., Justice, N., Hsueh, Y.-P., Sheng, M., Smiga, S., Bulfone, A., Goffinet, A. M., Campagnoni, A. T. and Rubenstein, J. L. (2001). Tbr1 regulates differentiation of the preplate and layer 6. *Neuron* **29**, 353-366. doi:10.1016/S0896-6273(01)00211-2

Hevner, R. F., Neogi, T., Englund, C., Daza, R. A. . and Fink, A. (2003). Cajal–Retzius cells in the mouse: transcription factors, neurotransmitters, and birthdays suggest a pallial origin. *Dev. Brain Res.* **141**, 39-53. doi:10.1016/S0165-3806(02)00641-7

Hiesberger, T., Trommsdorff, M., Howell, B. W., Goffinet, A., Mumby, M. C., Cooper, J. A. and Herz, J. (1999). Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. *Neuron* **24**, 481-489. doi:10.1016/S0896-6273(00)80861-2

Hirata, T., Li, P., Lanuza, G. M., Cocas, L. A., Huntsman, M. M. and Corbin, J. G. (2009). Identification of distinct telencephalic progenitor pools for neuronal diversity in the amygdala. *Nat. Neurosci*. **12**, 141-149. doi:10.1038/nn.2241

Hobert, O. (2016). Terminal selectors of neuronal identity. *Curr. Top. Dev. Biol*. **116**, 455-475. doi:10.1016/bs.ctdb.2015.12.007

Howell, B. W., Hawkes, R., Soriano, P. and Cooper, J. A. (1997). Neuronal position in the developing brain is regulated by mouse disabled-1. *Nature* **389**, 733-737. doi:10.1038/39607

Hua, Z. L., Jeon, S., Caterina, M. J. and Nathans, J. (2014). Frizzled3 is required for the development of multiple axon tracts in the mouse central nervous system. *Proc. Natl. Acad. Sci. USA* **111**, E3005-E3014. doi:10.1073/pnas.1406399111

Iacono, G., Mereu, E., Guillaumet-Adkins, A., Corominas, R., Cusco, I., Rodríguez-Esteban, G., Gut, M., Pérez-Jurado, L. A., Gut, I. and Heyn, H. (2018). Bigscale: an analytical framework for big-scale single-cell data. *Genome Res.* **28**, 878-890. doi:10.1101/gr.230771.117

Ikonomidou, C. (1999). Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. *Science* **283**, 70-74. doi:10.1126/science.283.5398.70
Jin, S., Kim, J., Willert, T., Klein-Rodewald, T., Garcia-Dominguez, M., Mosqueira, M., Fink, R., Esposito, I., Hofbauer, L. C., Charnay, P. et al. (2014). Ebf factors and MyoD cooperate to regulate muscle relaxation via Atp2a1. *Nat. Commun.* **5**, 3793. doi:10.1038/ncomms4793
Kaddour, H., Coppola, E., Di Nardo, A. A., Le Poupon, C., Mailly, P., Wizenmann, A., Volovitch, M., Prochiantz, A. and Pierani, A. (2020). Extracellular Pax6 regulates tangential Cajal-Retzius cell migration in the developing mouse neocortex. *Cereb. Cortex* **30**, 465-475. doi:10.1093/cercor/bhz098
Kikkawa, T., Sakayori, N., Yuuki, H. and Katsuyama, Y. (2020). Dmrt genes participate in the development of Cajal-Retzius cells derived from the cortical hem in the telencephalon. *Dev. Dyn.* **249**, 698-710. doi:10.1002/dvdy.156
Kilb, W. and Luhmann, H. J. (2000). Characterization of a hyperpolarization-activated inward current in Cajal-Retzius cells in rat neonatal neocortex. *J. Neurophysiol.* **84**, 1681-1691. doi:10.1152/jn.2000.84.3.1681
Kilb, W. and Luhmann, H. J. (2001). Spontaneous GABAergic postsynaptic currents in Cajal-Retzius cells in neonatal rat cerebral cortex. *Eur. J. Neurosci.* **13**, 1387-1390. doi:10.1046/j.0953-816x.2001.01514.x
Kirmse, K., Grantyn, R. and Kirischuk, S. (2005). Developmental downregulation of low-voltage-activated Ca2+ channels in Cajal-Retzius cells of the mouse visual cortex. *Eur. J. Neurosci.* **21**, 3269-3276. doi:10.1111/j.1460-9568.2005.04171.x
Kirmse, K., Dvorzhak, A., Henneberger, C., Grantyn, R. and Kirischuk, S. (2007). Cajal-Retzius cells in the mouse neocortex receive two types of pre- and postsynaptically distinct GABAergic inputs. *J. Physiol.* **585**, 881-895. doi:10.1113/jphysiol.2007.145003
König, N. and Schachner, M. (1981). Neuronal and glial cells in the superficial layers of early postnatal mouse neocortex: Immunofluorescence observations. *Neurosci. Lett.* **26**, 227-231. doi:10.1016/0304-3940(81)90137-3
König, N., Valat, J., Fulcrand, J. and Marty, R. (1977). The time of origin of Cajal-Retzius cells in the rat temporal cortex. An autoradiographic study. *Neurosci. Lett.* **4**, 21-26. doi:10.1016/0304-3940(77)90118-5
Kumamoto, T., Toma, K.-i., Gunadi, McKenna, W. L., Kasukawa, T., Katzman, S., Chen, B. and Hanashima, C. (2013). Foxg1 Coordinates the switch from nonradially to radially migrating glutamatergic subtypes in the neocortex through spatiotemporal repression. *Cell Rep.* **3**, 931-945. doi:10.1016/j.celrep.2013.02.023
Kutejova, E., Sasai, N., Shah, A., Gouti, M. and Briscoe, J. (2016). Neural progenitors adopt specific identities by directly repressing all alternative progenitor transcriptional programs. *Dev. Cell* **36**, 639-653. doi:10.1016/j.devcel.2016.02.013
La Manno, G., Siletti, K., Furlan, A., Gyllborg, D., Vinsland, E., Langseth, C. M., Khven, I., Johnsson, A., Nilsson, M., Lönnerberg, P. et al. (2020). Molecular architecture of the developing mouse brain. *bioRxiv* 2020.07.02.184051. doi:10.1101/2020.07.02.184051
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. doi:10.1523/JNEUROSCI.19-18-07881.1999
Ledonne, F., Orduz, D., Mercier, J., Vigier, L., Grove, E. A., Tissir, F., Angulo, M. C., Pierani, A. and Coppola, E. (2016). Targeted inactivation of bax reveals a subtype-specific mechanism of Cajal-Retzius neuron death in the postnatal cerebral cortex. *Cell Rep.* **17**, 3133-3141. doi:10.1016/j.celrep.2016.11.074
Loo, L., Simon, J. M., Xing, L., McCoy, E. S., Niehaus, J. K., Guo, J., Anton, E. S. and Zylka, M. J. (2019). Single-cell transcriptomic analysis of mouse neocortical development. *Nat. Commun.* **10**, 1-11. doi:10.1038/s41467-018-07882-8
López-Bendito, G., Shigemoto, R., Fairén, A. and Luján, R. (2002). Differential distribution of group I metabotropic glutamate receptors during rat cortical development. *Cereb. Cortex* **12**, 625-638. doi:10.1093/cercor/12.6.625
Louvi, A., Yoshida, M. and Grove, E. A. (2007). The derivatives of theWnt3a lineage in the central nervous system. *J. Comp. Neurol.* **504**, 550-569. doi:10.1002/cne.21461
Lu, S.-M., Zecevic, N. and Yeh, H. H. (2001). Distinct NMDA and AMPA receptor–mediated responses in mouse and human Cajal-Retzius cells. *J. Neurophysiol.* **86**, 2642-2646. doi:10.1152/jn.2001.86.5.2642
Luhmann, H. J., Reiprich, R. A., Hanganu, I. and Kilb, W. (2000). Cellular physiology of the neonatal rat cerebral cortex: Intrinsic membrane properties, sodium and calcium currents. *J. Neurosci. Res.* **62**, 574-584. doi:10.1002/1097-4547(20001115)62:4<574::AID-JNR12>3.0.CO;2-0
Mallamaci, A., Mercurio, S., Muzio, L., Cecchi, C., Pardini, C. L., Gruss, P. and Boncinelli, E. (2000). The lack of Emx2 causes impairment of Reelin signaling and defects of neuronal migration in the developing cerebral cortex. *J. Neurosci.* **20**, 1109-1118. doi:10.1523/JNEUROSCI.20-03-01109.2000
Marchionni, I., Takács, V. T., Nunzi, M. G., Mugnaini, E., Miller, R. J. and Maccaferri, G. (2010). Distinctive properties of CXC chemokine receptor 4-expressing Cajal-Retzius cells versus GABAergic interneurons of the postnatal hippocampus. *J. Physiol.* **588**, 2859-2878. doi:10.1113/jphysiol.2010.190868
Martin, R., Gutiérrez, A., Peniafiell, A., Marin-Padilla, M. and de la Calle, A. (1999). Persistence of Cajal-Retzius cells in the adult human cerebral cortex. An immunohistochemical study. *Histol. Histopathol.* **14**, 487-490.
Martínez-Galán, J. R., López-Bendito, G., Luján, R., Shigemoto, R., Fairén, A. and Valdeolmillos, M. (2001). Cajal-Retzius cells in early postnatal mouse cortex selectively express functional metabotropic glutamate receptors. *Eur. J. Neurosci.* **13**, 1147-1154. doi:10.1046/j.0953-816x.2001.01494.x
Martínez-Galán, J. R., Moncho-Bogani, J. and Caminos, E. (2014). Expression of calcium-binding proteins in layer 1 reelin-immunoreactive cells during rat and mouse neocortical development. *J. Histochem. Cytochem.* **62**, 60-69. doi:10.1369/0022155413509381
Meyer, G. and Goffinet, A. M. (1998). Prenatal development of reelin-immunoreactive neurons in the human neocortex. *J. Comp. Neurol.* **397**, 29-40. doi:10.1002/(SICI)1096-9861(19980720)397:1<29::AID-CNE3>3.0.CO;2-K
Meyer, G. and González-Gómez, M. (2018a). The heterogeneity of human Cajal-Retzius neurons. *Semin. Cell Dev. Biol.* **76**, 101-111. doi:10.1016/j.semcdb.2017.08.059
Meyer, G. and González-Gómez, M. (2018b). The subpial granular layer and transient versus persisting Cajal-Retzius neurons of the fetal human cortex. *Cereb. Cortex* **28**, 2043-2058. doi:10.1093/cercor/bhx110
Meyer, G. and González-Hernández, T. (1993). Developmental changes in layer I of the human neocortex during prenatal life: a DiI-tracing and AChE and NADPH-d histochemistry study. *J. Comp. Neurol.* **338**, 317-336. doi:10.1002/cne.903380302
Meyer, G. and Wahle, P. (1999). The paleocortical ventricle is the origin of reelin-expressing neurons in the marginal zone of the foetal human neocortex. *Eur. J. Neurosci.* **11**, 3937-3944. doi:10.1046/j.1460-9568.1999.00818.x
Meyer, G., Soria, J. M., Martínez-Galán, J. R., Martín-Clemente, B. and Fairén, A. (1998). Different origins and developmental histories of transient neurons in the marginal zone of the fetal and neonatal rat cortex. *J. Comp. Neurol.* **397**, 493-518.<493::AID-CNE4>3.0.CO;2-X
Meyer, G., Goffinet, A. M. and Fairén, A. (1999). What is a Cajal-Retzius cell? A reassessment of a classical cell type based on recent observations in the developing neocortex. *Cereb. Cortex* **9**, 765-775. doi:10.1093/cercor/9.8.765
Meyer, G., Schaaps, J. P., Moreau, L. and Goffinet, A. M. (2000). Embryonic and Early Fetal Development of the Human Neocortex. *J. Neurosci.* **20**, 1858-1868. doi:10.1523/JNEUROSCI.20-05-01858.2000
Meyer, G., Perez-Garcia, C. G., Abraham, H. and Caput, D. (2002). Expression of p73 and reelin in the developing human cortex. *J. Neurosci.* **22**, 4973-4986. doi:10.1523/JNEUROSCI.22-12-04973.2002
Meyer, G., Cabrera Socorro, A., Perez Garcia, C. G., Martinez Millan, L., Walker, N. and Caput, D. (2004). Developmental roles of p73 in Cajal-Retzius cells and cortical patterning. *J. Neurosci.* **24**, 9878-9887. doi:10.1523/JNEUROSCI.3060-04.2004
Meyer, G., González-Arnay, E., Moll, U., Nemajerova, A., Tissir, F. and González-Gómez, M. (2019). Cajal-Retzius neurons are required for the development of the human hippocampal fissure. *J. Anat.* **235**, 569-589. doi:10.1111/joa.12947
Mienville, J. M. and Pesold, C. (1999). Low resting potential and postnatal upregulation of NMDA receptors may cause Cajal-Retzius cell death. *J. Neurosci.* **19**, 1636-1646. doi:10.1523/JNEUROSCI.19-05-01636.1999
Moreau, M. X., Saillour, Y., Cwetsch, A. W., Pierani, A. and Causeret, F. (2020). Single-cell transcriptomics of the early developing mouse cerebral cortex disentangles the spatial and temporal components of neuronal fate acquisition. *bioRxiv*. doi:10.1101/2020.11.27.401398
Muzio, L. and Mallamaci, A. (2005). Foxg1 confines Cajal-Retzius neuronogenesis and hippocampal morphogenesis to the dorsomedial pallium. *J. Neurosci.* **25**, 4435-4441. doi:10.1523/JNEUROSCI.4804-04.2005
Myakhar, O., Unichenko, P. and Kirischuk, S. (2011). GABAergic projections from the subplate to Cajal-Retzius cells in the neocortex. *Neuroreport* **22**, 525-529. doi:10.1097/WNR.0b013e32834888a4
Niu, S., Renfro, A., Quattrocchi, C. C., Sheldon, M. and D'Arcangelo, G. (2004). Reelin promotes hippocampal dendrite development through the VLDLR/ApoER2-Dab1 pathway. *Neuron* **41**, 71-84. doi:10.1016/S0896-6273(03)00819-5
Niu, S., Yabut, O. and D'Arcangelo, G. (2008). The Reelin signaling pathway promotes dendritic spine development in hippocampal neurons. *J. Neurosci.* **28**, 10339-10348. doi:10.1523/JNEUROSCI.1917-08.2008
Nomura, T., Takahashi, M., Hara, Y. and Osumi, N. (2008). Patterns of neurogenesis and amplitude of reelin expression are essential for making a mammalian-type cortex. *PLoS ONE* **3**, e1454. doi:10.1371/journal.pone.0001454
Ogawa, M., Miyata, T., Nakajimat, K., Yagyu, K., Seike, M., Ikenaka, K., Yamamoto, H. and Mikoshibat, K. (1995). The reeler gene-associated antigen on Cajal-Retzius neurons is a crucial molecule for laminar organization of cortical neurons. *Neuron* **14**, 899-912. doi:10.1016/0896-6273(95)90329-1
Ogino, H., Nakajima, T., Hirota, Y., Toriuchi, K., Aoyama, M., Nakajima, K. and Hattori, M. (2020). The secreted glycoprotein reelin suppresses the proliferation and regulates the distribution of oligodendrocyte progenitor cells in the embryonic neocortex. *J. Neurosci.* **40**, 7625-7636. doi:10.1523/JNEUROSCI.0125-20.2020
Osheroff, H. and Hatten, M. E. (2009). Gene expression profiling of preplate neurons destined for the subplate: genes involved in transcription, axon extension, neurotransmitter regulation, steroid hormone signaling, and neuronal survival. *Cereb. Cortex* **19**, i126-i134. doi:10.1093/cercor/bhp034

Paredes, M. F. (2006). Stromal-derived factor-1 (CXCL12) regulates laminar position of Cajal-Retzius cells in normal and dysplastic brains. *J. Neurosci.* **26**, 9404-9412. doi:10.1523/JNEUROSCI.2575-06.2006
Parnavelas, J. G. and Edmunds, S. M. (1983). Further evidence that Retzius-Cajal cells transform to nonpyramidal neurons in the developing rat visual cortex. *J. Neurocytol.* **12**, 863-871. doi:10.1007/BF01258156
Pierani, A., Moran-Rivard, L., Sunshine, M., Littman, D., Goulding, M. and Jessell, T. (2001). Control of interneuron fate in the developing spinal cord by the progenitor homeodomain protein Dbx1. *Neuron* **29**, 367-384. doi:10.1016/S0896-6273(01)00212-4
Pollard, K. S., Salama, S. R., Lambert, N., Lambot, M.-A., Coppens, S., Pedersen, J. S., Katzman, S., King, B., Onodera, C., Siepel, A. et al. (2006). An RNA gene expressed during cortical development evolved rapidly in humans. *Nature* **443**, 167-172. doi:10.1038/nature05113
Pozas, E., Paco, S., Soriano, E. and Aguado, F. (2008). Cajal-Retzius cells fail to trigger the developmental expression of the Cl-extruding co-transporter KCC2. *Brain Res.* **1239**, 85-91. doi:10.1016/j.brainres.2008.08.058
Pozniak, C. D., Radinovic, S., Yang, A., McKeon, F., Kaplan, D. R. and Miller, F. D. (2000). An anti-apoptotic role for the p53 family member, p73, during developmental neuron death. *Science* **289**, 304-306. doi:10.1126/science.289.5477.304
Priya, R., Paredes, M. F., Karayannis, T., Yusuf, N., Liu, X., Jaglin, X., Graef, I., Alvarez-Buylla, A. and Fishell, G. (2018). Activity regulates cell death within cortical interneurons through a calcineurin-dependent mechanism. *Cell Rep.* **22**, 1695-1709. doi:10.1016/j.celrep.2018.01.007
Quattrocolo, G. and Maccaferri, G. (2013). Novel GABAergic circuits mediating excitation/inhibition of Cajal-Retzius cells in the developing hippocampus. *J. Neurosci.* **33**, 5486-5498. doi:10.1523/JNEUROSCI.5680-12.2013
Quattrocolo, G. and Maccaferri, G. (2014). Optogenetic activation of Cajal-Retzius cells reveals their glutamatergic output and a novel feedforward circuit in the developing mouse hippocampus. *J. Neurosci.* **34**, 13018-13032. doi:10.1523/JNEUROSCI.1407-14.2014
Radnikow, G., Feldmeyer, D., Lübke, J. and Lü Bke, J. (2002). Axonal projection, input and output synapses, and synaptic physiology of Cajal-Retzius cells in the developing rat neocortex. *J. Neurosci.* **22**, 6908-6919. doi:10.1523/JNEUROSCI.22-16-06908.2002
Raedler, E. and Raedler, A. (1978). Autoradiographic study of early neurogenesis in rat neocortex. *Anat. Embryol. (Berl).* **154**, 267-284. doi:10.1007/BF00345657
Ramón y Cajal, S. (1891). Sur la structure de l'écorce cérébrale de quelques mammifères. *Cellule* **7**, 123-176.
Ramón y Cajal, S. (1909). *Histologie du système nerveux de l'homme des vertébrés* (ed. **França**). Paris: Maloine.
Retzius, R. (1893). Die Cajal'schen Zellen der Grosshirnrinde beim Menschen und bei Saügetieren. *Biol. Untersuchungen* **5**, 1-8.
Riva, M., Genescu, I., Habermacher, C., Orduz, D., Ledonne, F., Rijli, F. M., López-Bendito, G., Coppola, E., Garel, S., Angulo, M. C. et al. (2019). Activity-dependent death of transient Cajal-Retzius neurons is required for functional cortical wiring. *Elife* **8**, 1-18. doi:10.7554/eLife.50503
Rubenstein, J. L. R. and Rakic, P. (1999). Genetic control of cortical development. *Cereb. Cortex* **9**, 521-523. doi:10.1093/cercor/9.6.521
Ruiz-Reig, N., Andrés, B., Huilgol, D., Grove, E. A., Tissir, F., Tole, S., Theil, T., Herrera, E. and Fairén, A. (2017). Lateral thalamic eminence: a novel origin for mGluR1/Lot cells. *Cereb. Cortex* **27**, 2841-2856.
Sava, B. A., Dávid, C. S., Teissier, A., Pierani, A., Staiger, J. F., Luhmann, H. J. and Kilb, W. (2010). Electrophysiological and morphological properties of Cajal-Retzius cells with different ontogenetic origins. *Neuroscience* **167**, 724-734. doi:10.1016/j.neuroscience.2010.02.043
Schuman, B., Machold, R. P., Hashikawa, Y., Fuzik, J., Fishell, G. J. and Rudy, B. (2019). Four unique interneuron populations reside in neocortical layer 1. *J. Neurosci.* **39**, 125-139. doi:10.1523/JNEUROSCI.1613-18.2018
Schwartz, T. H., Rabinowitz, D., Unni, V., Kumar, V. S., Smetters, D. K., Tsiola, A., Yuste, R. and Rabinowitz, D. (1998). Networks of coactive neurons in developing layer 1. *Neuron* **20**, 541-552. doi:10.1016/S0896-6273(00)80993-9
Sheldon, M., Rice, D. S., D'Arcangelo, G., Yoneshima, H., Nakajima, K., Mikoshiba, K., Howell, B. W., Cooper, J. A., Goldowitz, D. and Curran, T. (1997). Scrambler and yotari disrupt the disabled gene and produce a reeler-like phenotype in mice. *Nature* **389**, 730-733. doi:10.1038/39601
Skarnes, W. C., Rosen, B., West, A. P., Koutsourakis, M., Bushell, W., Iyer, V., Mujica, A. O., Thomas, M., Harrow, J., Cox, T. et al. (2011). A conditional knockout resource for the genome-wide study of mouse gene function. *Nature* **474**, 337-342. doi:10.1038/nature10163
Soda, T., Nakashima, R., Watanabe, D., Nakajima, K., Pastan, I. and Nakanishi, S. (2003). Segregation and Coactivation of Developing Neocortical Layer 1 Neurons. *J. Neurosci.* **23**, 6272-6279. doi:10.1523/JNEUROSCI.23-15-06272.2003
Soriano, E., Del Río, J. A., Martínez, A. and Supèr, H. (1994). Organization of the embryonic and early postnatal murine hippocampus. I. Immunocytochemical characterization of neuronal populations in the subplate and marginal zone. *J. Comp. Neurol.* **342**, 571-595. doi:10.1002/cne.903420406
Sun, L., Chen, R., Bai, Y., Li, J., Wu, Q., Shen, Q. and Wang, X. (2019). Morphological and physiological characteristics of Ebf2-EGFP-expressing Cajal-Retzius cells in developing mouse neocortex. *Cereb. Cortex* **29**, 3864-3878. doi:10.1093/cercor/bhy265
Supèr, H., Del Río, J. A., Martínez, A., Pérez-Sust, P. and Soriano, E. (2000). Disruption of neuronal migration and radial glia in the developing cerebral cortex following ablation of Cajal-Retzius cells. *Cereb. Cortex* **10**, 602-613. doi:10.1093/cercor/10.6.602
Sussel, L., Marin, O., Kimura, S. and Rubenstein, J. L. R. (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. *Development* **126**, 3359-3370. doi:10.1242/dev.126.15.3359
Takiguchi-Hayashi, K. (2004). Generation of reelin-positive marginal zone cells from the caudomedial wall of telencephalic vesicles. *J. Neurosci.* **24**, 2286-2295. doi:10.1523/JNEUROSCI.4671-03.2004
Tasic, B., Menon, V., Nguyen, T. N., Kim, T. K., Jarsky, T., Yao, Z., Levi, B., Gray, L. T., Sorensen, S. A., Dolbeare, T. et al. (2016). Adult mouse cortical cell taxonomy revealed by single cell transcriptomics. *Nat. Neurosci.* **19**, 335-346. doi:10.1038/nn.4216
Tasic, B., Yao, Z., Graybuck, L. T., Smith, K. A., Nguyen, T. N., Bertagnolli, D., Goldy, J., Garren, E., Economo, M. N., Viswanathan, S. et al. (2018). Shared and distinct transcriptomic cell types across neocortical areas. *Nature* **563**, 72-78. doi:10.1038/s41586-018-0654-5
Tissir, F., Lambert De Rouvroit, C., Sire, J.-Y., Meyer, G. and Goffinet, A. M. (2003). Reelin expression during embryonic brain development in Crocodylus niloticus. *J. Comp. Neurol.* **457**, 250-262. doi:10.1002/cne.10573
Tissir, F., Ravni, A., Achouri, Y., Riethmacher, D., Meyer, G. and Goffinet, A. M. (2009). DeltaNp73 regulates neuronal survival in vivo. *Proc. Natl. Acad. Sci. USA* **106**, 16871-16876. doi:10.1073/pnas.0903191106
Tosches, M. A., Yamawaki, T. M., Naumann, R. K., Jacobi, A. A., Tushev, G. and Laurent, G. (2018). Evolution of pallium, hippocampus, and cortical cell types revealed by single-cell transcriptomics in reptiles. *Science* **360**, 881-888. doi:10.1126/science.aar4237
Trousse, F., Poluch, S., Pierani, A., Dutriaux, A., Bock, H. H., Nagasawa, T., Verdier, J. M. and Rossel, M. (2015). CXCR7 receptor controls the maintenance of subpial positioning of Cajal-Retzius cells. *Cereb. Cortex* **25**, 3446-3457. doi:10.1093/cercor/bhu164
Valverde, F., De Carlos, J. A. and López-Mascaraque, L. (1995). Time of origin and early fate of preplate cells in the cerebral cortex of the rat. *Cereb. Cortex* **5**, 483-493. doi:10.1093/cercor/5.6.483
Villar-Cerviño, V., Molano-Mazón, M., Catchpole, T., Valdeolmillos, M., Henkemeyer, M., Martínez, L. M., Borrell, V. and Marín, O. (2013). Contact repulsion controls the dispersion and final distribution of cajal-retzius cells. *Neuron* **77**, 457-471. doi:10.1016/j.neuron.2012.11.023
von Haebler, D., Stabel, J., Draguhn, A. and Heinemann, U. (1993). Properties of horizontal cells transiently appearing in the rat dentate gyrus during ontogenesis. *Exp. Brain Res.* **94**, 33-42. doi:10.1007/BF00230468
Wang, S. S. (2004). Genetic disruptions of O/E2 and O/E3 genes reveal involvement in olfactory receptor neuron projection. *Development* **131**, 1377-1388. doi:10.1242/dev.01009
Watanabe, D., Inokawa, H., Hashimoto, K., Suzuki, N., Kano, M., Shigemoto, R., Hirano, T., Toyama, K., Kaneko, S., Yokoi, M. et al. (1998). Ablation of cerebellar Golgi cells disrupts synaptic integration involving GABA inhibition and NMDA receptor activation in motor coordination. *Cell* **95**, 17-27. doi:10.1016/S0092-8674(00)81779-1
Wong, F. K. and Marín, O. (2019). Developmental cell death in the cerebral cortex. *Annu. Rev. Cell Dev. Biol.* **35**, 523-542. doi:10.1146/annurev-cellbio-100818-125204
Wong, F. K., Bercsenyi, K., Sreenivasan, V., Portalés, A., Fernández-Otero, M. and Marín, O. (2018). Pyramidal cell regulation of interneuron survival sculpts cortical networks. *Nature* **557**, 668-673. doi:10.1038/s41586-018-0139-6
Yamazaki, H., Sekiguchi, M., Takamatsu, M., Tanabe, Y. and Nakanishi, S. (2004). Distinct ontogenic and regional expressions of newly identified Cajal-Retzius cell-specific genes during neocorticogenesis. *Proc. Natl. Acad. Sci. USA* **101**, 14509-14514. doi:10.1073/pnas.0406295101
Yang, A., Walker, N., Bronson, R., Kaghad, M., Oosterwegel, M., Bonnin, J., Vagner, C., Bonnet, H., Dikkes, P., Sharpe, A. et al. (2000). p73-deficient mice have neurological, pheromonal and inflammatory defects but lack spontaneous tumours. *Nature* **404**, 99-103. doi:10.1038/35003607
Yoshida, M., Assimacopoulos, S., Jones, K. R. and Grove, E. A. (2006). Massive loss of Cajal-Retzius cells does not disrupt neocortical layer order. *Development* **133**, 537-545. doi:10.1242/dev.02209
Zhao, C., Guan, W. and Pleasure, S. J. (2006). A transgenic marker mouse line labels Cajal–Retzius cells from the cortical hem and thalamocortical axons. *Brain Res.* **1077**, 48-53. doi:10.1016/j.brainres.2006.01.042
Zhou, F. M. and Hablitz, J. J. (1996). Postnatal development of membrane properties of layer I neurons in rat neocortex. *J. Neurosci.* **16**, 1131-1139. doi:10.1523/JNEUROSCI.16-03-01131.1996
Zimmer, C., Lee, J., Griveau, A., Arber, S., Pierani, A., Garel, S. and Guillemot, F. (2010). Role of Fgf8 signalling in the specification of rostral Cajal-Retzius cells. *Development* **137**, 293-302. doi:10.1242/dev.041178

Supplementary information

The datasets used to produce Fig. 3 were as follows.

Fig. 3A
Dataset generated by Loo et al. (2019) corresponding to E14.5 cortex, retrieved from GEO under the accession number GSE123335.

Fig. 3B,C
'9k Brain Cells from an E18 Mouse' dataset from 10X Genomics, corresponding to cells from cortex, hippocampus and subventricular zone of an E18 mouse, retrieved from https://support.10xgenomics.com/single-cell-gene-expression/datasets/2.1.0/neuron_9k

Fig. 3E
'1.3 Million Brain Cells from E18 Mice' dataset from 10X Genomics, corresponding to cells from cortex, hippocampus and subventricular zone of two E18 mice, retrieved from https://support.10xgenomics.com/single-cell-gene-expression/datasets/1.3.0/1M_neurons

Fig. 3F
Dataset generated by Fan et al. (2018) corresponding to cells from the entire human cortex of one 22 gestational week and two 23 gestational week embryos, retrieved from GEO under the accession number GSE103723.

Cell quality control, normalization and scaling were conducted under R (v 3.6.3) using Seurat (v 2.3.4) (Butler et al., 2018), while dimentionality reduction was performed using the SPRING tool (Weinreb et al., 2018) . In all datasets, medial CR cells clusters were readily identified by their unique co-expression of *Trp73*, *Cacna2d2* and *Reln*. Hierarchical clustering in Fig. 3C was obtained using Seurat function «BuildClusterRree». Genes indicated in Fig. 3D were manually picked when consistent between the datasets mentioned above and ours (Moreau et al., 2020). The SPRING plot in Fig. 3E represents 14k CR cells corresponding to CRs identified by Iacono et al. (2018) using the '1.3 Million Brain Cells' dataset and further filtered to remove doublets. The cross-species comparison between human and mouse data was conducted according to the approach developed by Tosches et al. (2018) and using the '9k Brain Cells' and Fan et al. (2018) datasets.

References

Butler, A., Hoffman, P., Smibert, P., Papalexi, E. and Satija, R. (2018). Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nat. Biotechnol.* **36**, 411-420. https://doi.org/10.1038/nbt.4096

Fan, X., Dong, J., Zhong, S., Wei, Y., Wu, Q., Yan, L., Yong, J., Sun, L., Wang, X., Zhao, Y., et al. (2018). Spatial transcriptomic survey of human embryonic cerebral cortex by single-cell RNA-seq analysis. *Cell Res.* **28**, 730-745. doi: 10.1038/s41422-018-0053-3

Iacono, G., Mereu, E., Guillaumet-Adkins, A., Corominas, R., Cusco, I., Rodríguez-Esteban, G., Gut, M., Pérez-Jurado, L. A., Gut, I. and Heyn, H. (2018). Bigscale: An analytical framework for big-scale single-cell data. *Genome Res.* **28**, 878-890. doi:10.1101/gr.230771.117

Loo, L., Simon, J. M., Xing, L., McCoy, E. S., Niehaus, J. K., Guo, J., Anton, E. S. and Zylka, M. J. (2019). Single-cell transcriptomic analysis of mouse neocortical development. *Nat. Commun.* **10**, 1-11. https://doi.org/10.1038/s41467-018-08079-9

Moreau, M. X., Saillour, Y., Cwetsch, A. W., Pierani, A. and Causeret, F. (2020). Single-cell transcriptomics of the early developing mouse cerebral cortex disentangles the spatial and temporal components of neuronal fate acquisition. *bioRxiv* doi: https://doi.org/10.1101/2020.11.27.401398

Tosches, M. A., Yamawaki, T. M., Naumann, R. K., Jacobi, A. A., Tushev, G. and Laurent, G. (2018). Evolution of pallium, hippocampus, and cortical cell types revealed by single-cell transcriptomics in reptiles. *Science* **360**, 881-888. DOI: 10.1126/science.aar4237

Weinreb, C., Wolock, S. and Klein, A. M. (2018). SPRING: a kinetic interface for visualizing high dimensional single-cell expression data. *Bioinformatics* **34**, 1246-1248. DOI: 10.1093/bioinformatics/btx792