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Assembling layer 1 of the neocortex: roles of Cajal-Retzius cells

Ioana Genescu

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THÈSE DE DOCTORAT
DE L'UNIVERSITÉ PSL

Préparée à l'Institut de Biologie de l'École Normale Supérieure

**L'assemblage de la couche 1 du néocortex : Rôles des
cellules de Cajal-Retzius**

Assembling layer 1 of the neocortex: Roles of Cajal-Retzius cells

Soutenue par

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LIST OF ABBREVIATIONS

5HT3R	Serotonin receptor 3A
A1	Primary Auditory cortex
AIS	axon initial segment
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPAr	AMPA receptors
AP	antero-posterior
ApoER2	Apolipoprotein E receptor 2
ASD	Autistic Spectrum Disorders
Bak	Bcl2 antagonist/killer 1
Bax	Bcl2 associated X protein
BDNF	Brain-Derived Neurotrophic Factor
BMP	Bone Morphogenic protein
Casp	Caspase
Cbln4	Cerebellin 4
CC	Corpus Callosum
cENO	cortical Early Network Oscillations
CGE	Caudal Ganglionic Eminence
cKO	conditional Knock-Out
CNO	Clozapine – N - oxide
CNS	Central Nervous System
CoupTF1	NR2F1, COUP transcription factor 1
CoupTF2	Chicken ovalbumin upstream promoter-transcription factor 2
CP	Cortical Plate
CRc	Cajal-Retzius cell
Ctip2	Chicken ovalbumin upstream promoter transcription factor-interacting protein 2
Dab1	Disabled 1
Dbx1	Developing brain homeobox 1
Dlx	Distal less homeobox
DS	Down Syndrome
DV	dorso-ventral
E	embryonic day
E/I	Excitation/Inhibition ratio
Emx2	Empty spiracle homeobox 2
EPSC	Excitatory postsynaptic currents
Er81	Ets variant gene 1
Fezf2	FEZ family zinc finger 2
Fgf	Fibroblast growth factor
Fgf13	Fibroblast growth factor 13
GABA	Gamma amino-butiric acid
GDP	Giant Depolarizing Potential
GluN1	N-methyl-D-aspartate receptor subunit NR1
IGF	Insulin Growth Factor
Igf1	Insulin like growth factor 1

ION	Infraorbital Nerve
IPSC	Inhibitory postsynaptic currents
KCC2	Potassium-chloride transporter member 5
L1	Cortical layer 1
L2/3	Cortical layer 2/3
L4	Cortical layer 4
L5/6	Cortical layer 5/6
Lgi2	Leucine-rich repeat LGI family member 2
LOT	Lateral Olfactory Tract
M1	Primary Motor cortex
MGE	Medial Ganglionic Eminence
MZ	Marginal Zone
NDNF	Neuron Derived Neurotrophic Factor
NGF	Nerve Growth Factor
NGFc	Nurogliaform cells
NKCC1	Na-K-Cl cotransporter isoform 1
NMDA	Acid N-methyl-D-aspartic
NMDAR	NMDA receptors
NPY	Neuropeptide Y
Nr2f2	Nuclear receptor subfamily 2 group F member 2
NT-3	Neurotrophin-3
NT-4	Neurotrophin-4
P	postnatal day
Pax6	Paired box gene 6
PCD	Programmed Cell Death
pCRc	human persistent Cajal-Retzius cells
PNS	Peripheral Nervous System
POA	Preoptic Area
Prox 1	Prospero homeobox 1
PrV	Rostral principal nucleus in the brainstem
PV	Parvalbumin
RA	Retinoic Acid
RELN	Reelin protein
RGc	Radial glia cell
S1	Primary Somatosensory cortex
Satb2	Special AT-rich sequence-binding protein 2
SBc	Single bouquet cells
SE	Septum
Sema	Semaphorin
Shh	Sonic Hedgehog
SNP	Single nucleotide polymorphism
SP	Subplate
SP8	Specficity protein 8
SPA	Synchronous Plateau Assemblies
SPn	Subplate neurons
SST	Somatostatin
SVZ	Subventricular Zone
TCA	Thalamocortical axons

tCRc	human transient Cajal-Retzius cells
TTX	Tetrodotoxin
V1	Primary Visual cortex
VGlut 1	Vesicular Glutamate transporter 1
VGlut 2	Vesicular Glutamate transporter 2
VIP	Vasoactive Intestinal peptide
VLDLR	Very low density lipoprotein receptor
VPM	Ventral Posteromedial Nucleus of the thalamus
VZ	Ventricular zone
Wnt3A	Wingless – type MMTV integration site families
ZI	Zona Incerta

INTRODUCTION

Preamble

The neocortex, which controls sensory perception, motor behaviors and cognition, relies on complex networks containing both excitatory and inhibitory neurons. The excitation/inhibition (E/I) ratio is essential for circuit function since its imbalance has been linked to the etiology of several neurodevelopmental disorders. A main entry to neocortical circuits is located in the superficial layer 1 (L1), which contains mostly excitatory inputs and inhibitory interneurons, that collectively regulate cerebral functions. In spite of the increasing evidence that L1 plays central functional roles in providing context for sensory processing, cross-modal communication, learning, arousal and consciousness, we still have a very limited understanding of how it wires up during development. In this introduction, I will briefly summarize our current understanding of the physiological roles of L1 in adult stages and focus on how the precise architecture of L1 is established during development, in the global context of neocortical wiring. I will particularly focus on the rodent somatosensory cortex and highlight the contribution of key regulators in the assembly of cortical circuits, the transient early-born Cajal-Retzius (CRc) cells.

1. Overview of the cerebral cortex: architecture and connectivity

1.1. Neuronal composition of the neocortex

The cerebral cortex is essential for sensory perception, motor behavior or cognitive functions and anatomically comprises the archicortex (hippocampus), the paleocortex (olfactory cortex), and the neocortex (isocortex). From an evolutionary point of view, the neocortex is likely the most recent structure of the brain and has undergone a dramatic expansion likely associated with the emergence of multiple and complex functions (Kandel, Schwartz, Jessell, Siegelbaum, & Hudspeth, 2012).

One key aspect of cortical organization relies on the dichotomy between two large classes of functionally distinct neuronal subpopulations: i) excitatory glutamatergic neurons, pyramidal and spiny stellate neurons, which account for approximately 80% of cortical neurons; ii) GABAergic inhibitory interneurons, which represent approximately 20% of the neuronal population. Both excitatory and inhibitory inputs work in strict balance to enable a proper functioning of cortical circuits, since imbalance in the excitation/inhibition (E/I) ratio has been linked to the etiology of several neurodevelopmental disorders including Epilepsy, Autism Spectrum Disorders (ASD) and Schizophrenia (Oscar Marín, 2012; Nelson & Valakh, 2015). Before presenting how excitatory and inhibitory neurons integrate in functional neocortical circuits, it is essential to provide an overview of the general organization of the neocortex.

1.2. Architecture of the neocortex: areas, connectivity, layers and columns

The neocortex is organized into precisely delineated areas characterized by specific roles, patterns of connectivity and molecular markers (Fig. 1A) (O'Leary, Chou, & Sahara, 2007). Primary areas directly integrate sensory information of a single modality which is relayed by thalamic first-order nuclei (Jabaudon & López Bendo, 2012). The main primary areas are the motor cortex (M1), primary somatosensory cortex (S1), auditory cortex (A1) and visual cortex (V1). They are surrounded by higher order and associative areas, which have been classically

involved in the integration of these sensory-motor information and complex cross-modal connections (O'Leary et al., 2007). The primary or higher order "nature" of cortical areas is defined by their pattern of connectivity with the thalamus: thalamic first order nuclei project to primary cortical areas, whereas higher order thalamic nuclei receive inputs from primary cortical areas and target higher order cortical areas in a less specific manner, following a cortico-thalamic-cortical loop (Frangeul et al., 2016; Pouchelon et al., 2014).

1.2.1 Organization of the neocortex: tangential in layers and radial in columns

Despite specific roles, both primary and higher order cortical areas display a similar gross organization along the radial and tangential planes (Fig. 1B). Tangentially, the neocortex is formed by six layers. Starting from the surface or pia, the layer 1 (L1) is the most superficial layer of the neocortex. It presents a limited density of neurons, restricted to GABAergic interneurons, but hosts abundant axonal projections, dendritic processes and glial cells (Feldmeyer, 2012; Harris & Shepherd, 2015; Ibrahim, Schuman, Bandler, Rudy, & Fishell, 2020; Muralidhar, Wang, & Markram, 2014; Tremblay, Lee, & Rudy, 2016). Deeper, layers 2/3 are characterized by a high cellular density of both pyramidal neurons, interneurons and glial cells. Axons of layer 2/3 pyramidal neurons are the main source of interhemispheric (or contralateral) projections, through the Corpus Callosum (CC). Layer 4, in sensory granular primary cortical areas, constitutes the main receiver of the primary thalamic input, driving sensory information into cortical circuits. And last, deep cortical layers are considered the main output of the cortex, targeting the striatum, brainstem and spinal cord other subcerebral structures for layer 5 neurons, and the thalamus for layer 6 neurons (Fig.1B) (Lodato & Arlotta, 2014; Molyneaux, Arlotta, Menezes, & Macklis, 2007). Layer 5 also comprises though also cortico-cortical projection neurons. The pyramidal neurons in each layer are characterized by combination of specific transcription factors which collectively establish their identity (Fig. 1C).

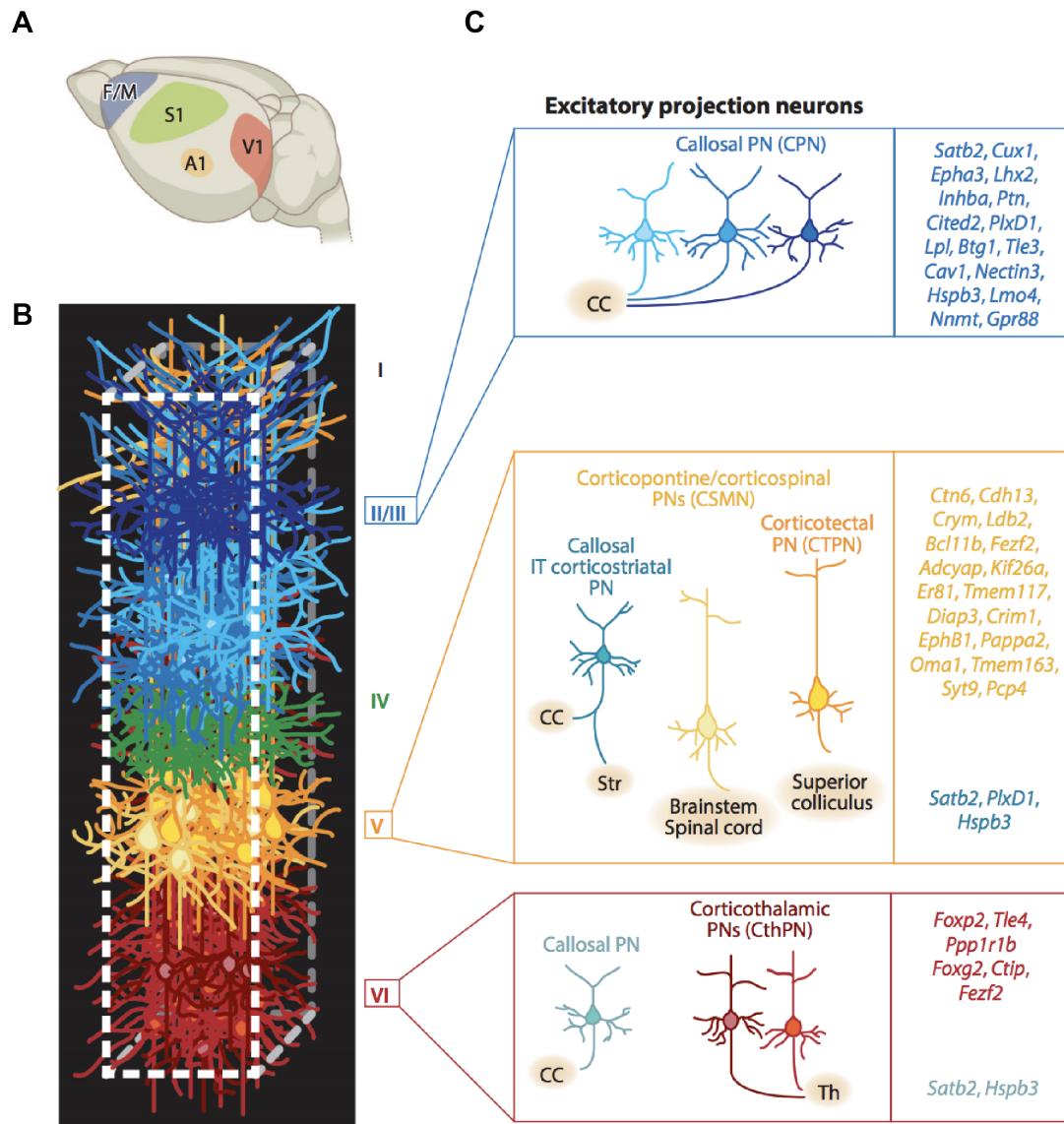


Figure 1 | Organization of the neocortex: tangential in layers and radial in columns

(A) Schematic representation of the primary cortical areas, involved in motricity (M1) and sensory processing (S1, A1, V1). (B) Schematic representation of the cortical organization, tangentially in 6 layers and radially in columns, with pyramidal neurons present in each layer. Note that L1 is devoid of pyramidal neurons but contains dense apical dendrites from the pyramidal neurons in the underlying layers. (C) Subclasses of Layer 2/3 commissural neurons, with their specific transcription factors discriminating of their identity. Layer 5 contains pyramidal neurons, often projecting to the different subcerebral targets like the brainstem, spinal cord, and superior colliculus. Layer 6 has different classes of pyramidal neurons, connecting thalamic nuclei or that connect through the Corpus Callosum. Cortical pyramidal neurons subtypes express unique gene signatures that in specific combinations identify each class (listed on right).

A1, auditory cortex; CC, corpus callosum; CPN, callosal projection neuron; CTPN, corticotectal projection neuron; CThPN, corticothalamic; F/M, frontal/motor cortex; IN, interneurons; IT, intratelencephalic; PN, projection neuron; S1, somatosensory cortex; Th, thalamus; V1, visual cortex. From Lodato & Arlotta, 2014.

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Radially, the neocortex is organized in cortical columns (Fig. 1B), which are repetitive modules of neurons responsive to the same stimulation. Mountcastle was the first to propose that cortical columns constitute the minimal processing units of the neocortex (Mountcastle, 1957). The functionality of cortical columns has mainly been established in the visual cortex, where ocular dominance columns have been shown by Hubel and Wiesel to be the core structure underlying function. However, the notion that cortical columns form functional repetitive units underlying neocortical processing has been largely debated since then (Horton & Adams, 2005; Rakic, 2008).

This dichotomy of neocortical organization along the laminar and radial plane is also found at the level of individual pyramidal neurons. The pyramidal neurons are the essential building blocks of the neocortex for the sensory information. Pyramidal neurons located in L2/3 and L5, display a similar morphological organization: a pyramid-shaped cell body, basal dendrites that grow in the laminar plane and an apical dendrite spanning the thickness of the cortex extending into L1, where they form an apical tuft (Fig. 1C) (Marín-Padilla, 1992). Both apical and basal dendrites receive either local or long-range inputs, and together participate to the integration of the information in the neocortex.

1.2.2 Bottom-up information processing

The six layers of the neocortex have a hierarchical configuration enabling a link between structure and function (Feldmeyer, 2012; Larkum et al., 2018). The classical dogma for sensory cortical processing is the following: sensory information from the periphery is conveyed through primary thalamic nuclei and reaches L4 excitatory neurons. L4 excitatory neurons project to upper L2/3 pyramidal neurons, which in turn, target the contralateral neocortex, other cortical regions, and L5 and L6 pyramidal neurons (Bartolini, Ciceri, & Marín, 2013; Harris & Shepherd, 2015). Finally, L5 receive these inputs from upper cortical layers and, together with L6, output onto subcortical regions (Bartolini et al., 2013; Harris & Shepherd, 2015). This is also characterized as the bottom-up information stream, from sensory organs to the neocortex. For example, in the somatosensory system, information relayed through the whisker pad induces,

through the InfraOrbital Nerve (ION), the activation of the rostral principal nucleus in the brainstem (PrV). The trigeminothalamic axons from the PrV project to the Ventral Posteromedial Nucleus (VPM) of the thalamus, which will in turn, projects to L4 in the barrel cortex, from where information is relayed into the cortical column as previously mentioned. The whisker-to-barrel pathway has remarkable topography preserving the spatial relation between adjacent neurons and axonal projections: each whisker has an individual representation in the barrelets of the PrV, the barreloids of the VPM and in the barrels in the L4 of the S1 cortical area, thereby enabling the somatotopic map to be conveyed from the whisker pad onto each synaptic relay up to the neocortex (Gaspar & Erzurumlu, 2013; Lokmane & Garel, 2014).

1.2.3 Top-down information processing

In addition to bottom-up connectivity, driving the information from the sensory organ directly to the neocortex, there is also a top-down flow of information, providing for example context, attention, arousal and internal predictions (Cauller, 1995; L. Fan et al., 2019; Ibrahim et al., 2020; Matthew E. Larkum et al., 2018; Manita, Miyakawa, Kitamura, & Murayama, 2017; Rubio-Garrido, Pérez-De-Manzo, Porrero, Galazo, & Clascá, 2009). Top-down information is mainly relayed through neocortical L1, where different axonal inputs directly reach cortical neurons and modulate connectivity of underlying networks. This modulation is particularly important because it generally reaches apical dendrites of pyramidal neurons located in different neocortical layers, enabling an activation of entire columns of the underlying cortex. The integration of top-down and bottom-up information in L1 is proposed to be key for active perception – notably a model in the somatosensory cortex underlying conscious behavior and active touch discrimination (Cauller, 1995) but also for predictive coding through discrimination between internal and externally generated sensory representation and predictions (Keller & Mrsic-Flogel, 2018).

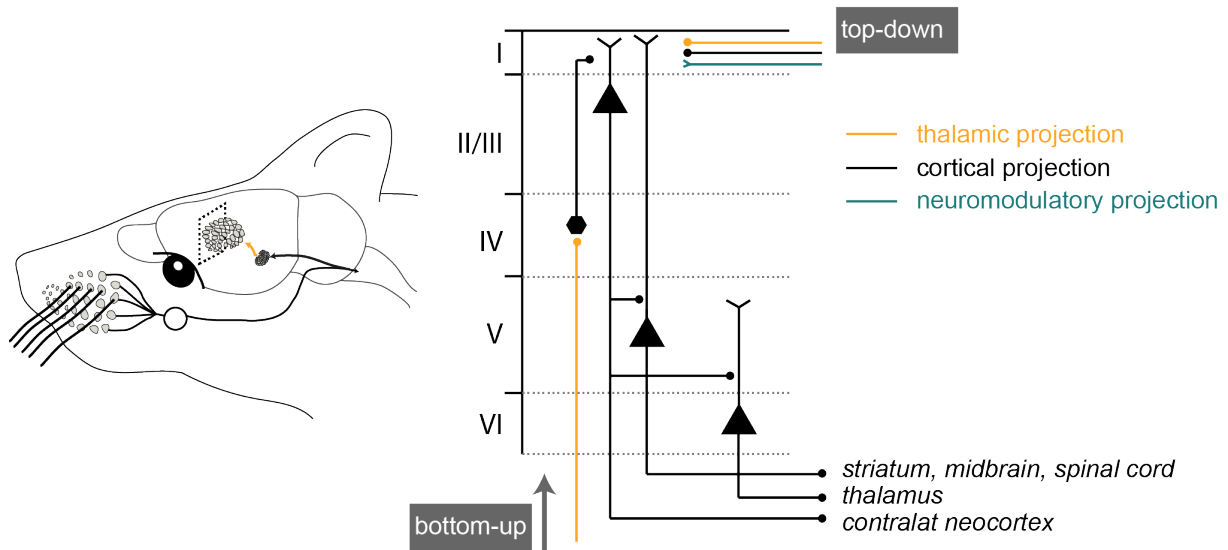


Figure 2 | Connectivity in the somatosensory cortex

Schematic representation of the somatosensory pathway from whiskers to the somatosensory cortex (left). Schematic representation of the cortical connectivity in a coronal plane of the somatosensory cortex (right).

1.3. Layer 1: an atypical layer of the neocortex

1.3.1 Anatomy of neocortical layer 1 in adults

1.3.1.a Cellular composition

L1 is unique since, unlike other cortical layers, it displays a very low cell density, comprising only sparse inhibitory interneurons and glial cells. Most of L1 neurons are GABAergic interneurons, which overall comprise several large classes and shape activity in cortical circuits. Generally, in the entire neocortex, the majority of GABAergic interneurons are either: i) fast spiking Basket and Chandelier cells, which express Parvalbumin (PV^+) and respectively target mainly the perisomatic compartment and the axonal initiation segment, regulating the output of the targeted pyramidal neurons (Bartolini et al., 2013; Huang & Paul, 2019; Lim, Mi, Llorca, & Marín, 2018a; Tremblay et al., 2016; Wamsley & Fishell, 2017); ii) mainly bursting spiking neurons expressing Somatostatin (SST^+), including Martinotti cells that send axons to L1 and target apical dendrites, thereby regulating the entries in L1 (Bartolini et al., 2013; Huang & Paul, 2019; Lim, Mi, Llorca, & Marín, 2018b; Tremblay et al., 2016; Wamsley & Fishell, 2017); iii) a third broad class of neurons expressing Serotonin Receptor 3A ($5HT3R$) that

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include Neurogliaform cells (NGFc) and Single Bouquet cells (SBc) expressing Reelin (RELN), Neuron Derived Neurotrophic Factor (NDNF) and Neuropeptide Y (NPY), Canopy cells (NDNF⁺ NPY⁻), α 7-subunit of Nicotinic Receptors-positive cells, Bipolar cells which express Vasoactive Intestinal peptide (VIP) with specific firing properties and provide either volume inhibition, lateral inhibition or disinhibition by targeting the two first classes of interneurons (Fig. 3A) (Armstrong, Krook-Magnuson, & Soltesz, 2012; Bartolini et al., 2013; L. Z. Fan et al., 2020; Huang & Paul, 2019; Ibrahim et al., 2020; Lim, Mi, et al., 2018b; Niquille et al., 2018; Schuman et al., 2019; Tremblay et al., 2016; Wonders & Anderson, 2006).

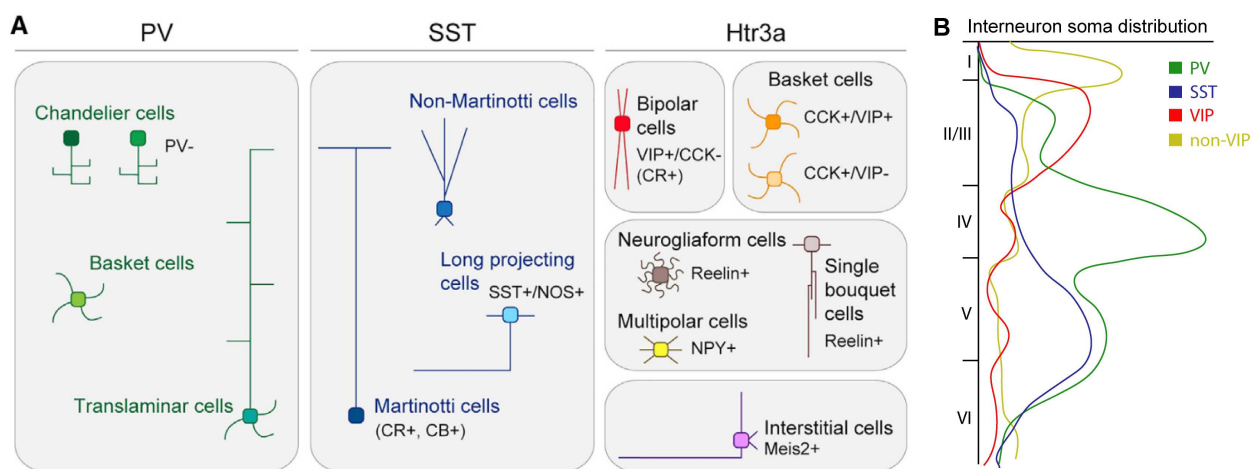


Figure 3 | Interneuron subtypes across cortical layers

(A) Gross classification of the three major classes of interneurons in the neocortex marked by their expression of parvalbumin (PV), somatostatin (SST) and Serotonin Receptor 3A (5HT3R) From Lim, Mi, et al., 2018b. (B) Schematic representation of interneuron soma distribution across the cortical layers in the somatosensory cortex. Adapted from Tremblay et al., 2016. CB, Calbindin; CCK, neuropeptide cholecystikinin; CR, Calretinin; NOS, nitric oxide synthase; NPY, neuropeptide Y; PV, parvalbumin; VIP, Vasointestinal peptide.

Importantly, L1 only comprises sparse neurons of this third class (Ibrahim et al., 2020; Schuman et al., 2019) which can be identified using a combination of unique combination of markers (Frazer et al., 2017; Niquille et al., 2018; Tasic et al., 2016) (Fig. 3B). Using a more specific sub classification, L1 interneurons can be subdivided in four groups based on their molecular profile (Ibrahim et al., 2020; Schuman et al., 2019): NGFc which are NDNF⁺ and NPY⁺, Canopy cells which are NDNF⁺ and NPY⁻, α 7-subunit of Nicotinic Receptors-positive cells and Bipolar VIP⁺ cells (Fig. 4). These L1 interneurons present distinct morphologies and electrophysiological properties. They target mostly apical dendrites located within L1

regulating the top-down entries, sending collaterals deeper in the cortex, or contacting other L1, with implications for disinhibition of cortical circuits (Fig. 4) (L. Z. Fan et al., 2020; Schuman et al., 2019; Takesian, Bogart, Lichtman, & Hensch, 2018).

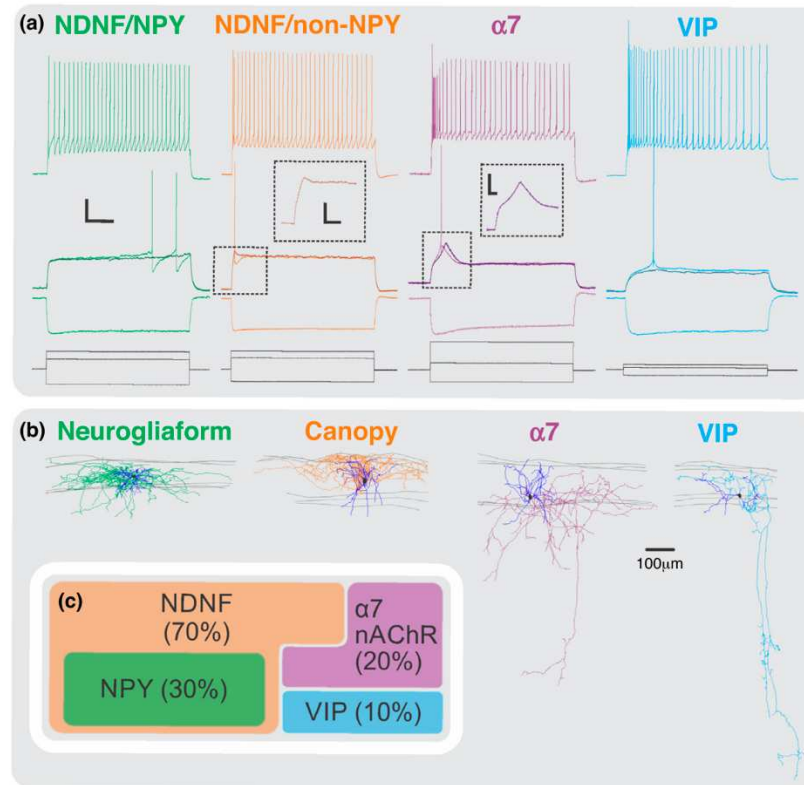


Figure 4 | Interneuron subtypes in adult cortical layer 1

(A) The electrophysiological firing patterns of the four different L1 subtypes, NGF^c, Canopy cells, $\alpha 7^+$ cells and Bipolar cells. (B) Morphological reconstructions of the four subtypes of interneurons in L1. Soma and dendrites are shown in blue and axons in specific colors. Note the axonal arborization of the NDNF⁺ cells mostly restricted to L1, whereas the NDNF⁻ cells present a descending axon. (C) Relative proportions of the four interneuron subtypes in L1.

$\alpha 7$ nAChR, $\alpha 7$ -subunit of Nicotinic Receptors; NDNF, Neuron Derived Neurotrophic Factor; NPY, neuropeptide Y; VIP, Vasointestinal peptide. From Ibrahim et al., 2020.

Besides interneurons, L1 also contains glial cells, astrocytes, microglia and oligodendrocytes. Astrocytes are the only ones to have been studied so far in this structure, and they were shown to have particular Ca^{2+} dynamics and morphology compared to astrocytes in other cortical layers. This suggests they could form a particular subpopulation of pial astrocytes with potentially specific roles (Clavreul et al., 2019; Lanjakornsiripan et al., 2018; Takata & Hirase, 2008).

1.3.1.b Axonal and dendritic composition

Another hallmark of L1 is that it forms a major site of input integration, comprising numerous and heterogeneous populations of axons connecting onto the apical dendrites of upper and deep layers pyramidal neurons as well as onto interneurons (Fig. 5) (Garcia-Munoz & Arbuthnott, 2015).

Long-range entries in L1 are : i) glutamatergic from higher order thalamic nuclei, POM in the somatosensory cortex (Anastasiades, Collins, & Carter, 2020; Audette, Urban-Ciecko, Matsushita, & Barth, 2017; Cruikshank et al., 2012; Wimmer, Bruno, De Kock, Kuner, & Sakmann, 2010), or from other cortical regions (Bonifazi et al., 2009; Ibrahim et al., 2016; Lee, Kruglikov, Huang, Fishell, & Rudy, 2013); ii) neuromodulatory originating from several subcortical structures such as the locus coeruleus, the raphe nucleus or midbrain dopaminergic neurons (Björklund & Dunnett, 2007; Chameau & Van Hooft, 2006; Lewis, Campbell, Foote, Goldstein, & Morrison, 1987; Narboux-Nême, Pavone, Avallone, Zhuang, & Gaspar, 2008; Poorthuis et al., 2018; Rho, Kim, & Lee, 2018; Vitalis, Ansorge, & Dayer, 2013; Vitalis & Verney, 2016); iii) long-range GABAergic inputs generated by the zona incerta (ZI) or long-range projecting cortical GABAergic neurons (Caputi, Melzer, Michael, & Monyer, 2013). The spatial distribution of excitatory inputs on apical dendrites in L1 shows a stereotyped pattern. Thalamic axons mainly observed in the superficial part of L1 – defined as L1a – whereas cortical inputs, neuromodulatory inputs like cholinergic fibers, serotonergic fibers or noradrenergic fibers are present in the whole thickness of the L1 (both L1a and L1b) (Fig. 5). These projections contact apical dendrites of upper and deep layers pyramidal neurons. The apical dendrites of pyramidal neurons are also stereotyped in L1, as the upper layers pyramidal neurons branch in the whole thickness of L1 whereas L5 pyramidal neurons form a denser dendritic plexus in the superficial L1a (Tjia, Yu, Jammu, Lu, & Zuo, 2017). Thus collectively, long-range entries display an ordered spatial organization, suggesting an importance for their dendritic integration.

In addition, L1 contains several GABAergic inhibitory axons that contribute to top-down information and modulate excitatory inputs received in L1 by pyramidal neurons (Ibrahim et

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al., 2020; Schuman et al., 2019). These inhibitory inputs originate from interneurons either located in L1 (Schuman et al., 2019), in other neocortical layers or, as mentioned above, from distally located neurons (Caputi et al., 2013; Chen & Kriegstein, 2015). In particular, Martinotti cells (SST⁺) located in deep neocortical regions and subcortical SST⁺ neurons from Zona Incerta send axons to the superficial L1a where they target the apical dendrites of pyramidal neurons (Chen & Kriegstein, 2015; Favuzzi et al., 2019; Tremblay et al., 2016). Concerning interneurons located in L1, the topography of their local connections remains to be fully established, but it has been already shown that the NDNF⁺ interneurons (NGF_c and Canopy cells) project locally in L1 (Ibrahim et al., 2020; Schuman et al., 2019). The axons of NGF_c are sprouting in the whole L1 thickness, while the axons of Canopy cells are more restricted to L1a (Fig. 4B) (Ibrahim et al., 2020; Schuman et al., 2019). Nonetheless, the overall organization of inhibitory synapses onto pyramidal neurons apical dendrite in L1 seems to be highly specific to the postsynaptic partner (Karimi et al. 2020): i) upper pyramidal neurons receive a strong inhibition at the L1-L2 border, and these inhibitory synapses density drops towards the tip of the apical tuft in L1a; ii) L5 pyramidal neurons present the opposite profile, with a low density of inhibitory synapses at the L1-L2 border but a high density of inhibitory synapses in L1a. This is of potential high importance because the spatial distribution of synapses on apical tufts of pyramidal neurons driven by the spatial organization and segregation of inputs in L1, has significant effects on the computational properties of pyramidal neurons (Favuzzi et al., 2019; Galloni, Laffere, & Rancz, 2020; M. E. Larkum, Kaiser, & Sakmann, 1999; Matthew E. Larkum, 2013).

Altogether this reveals that in adults, L1 is a major site of input integration (Fig. 5).

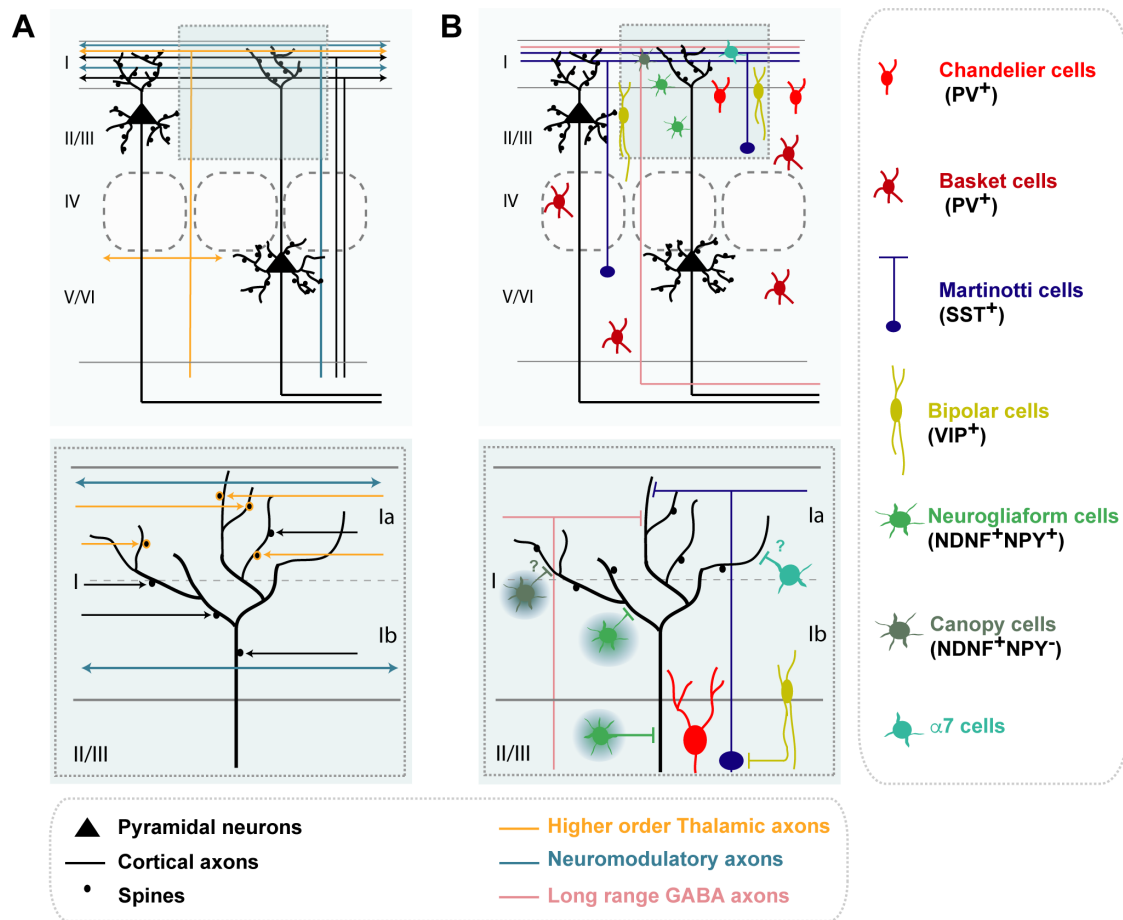


Figure 5 | Layer 1 is a major site of input integration

(A) Schematic representation of excitatory axonal projections (arrows) in the adult somatosensory barrel cortex (up) with a close-up on L1 (bottom) highlighting the spatial organization of inputs on apical dendrites of pyramidal neurons across L1a and L1b (bottom). Dotted lines delineate barrels. (B) Similar representations as in A showing the distribution of GABAergic interneuron subsets, classified by their developmental origins and molecular markers.

la, layer 1a; lb, layer 1b; CGE, Caudal Ganglionic Eminence; MGE, Medial Ganglionic Eminence; NDNF, Neuron Derived Neurotrophic Factor; NPY, Neuropeptide Y; POA, Preoptic Area; PV, Parvalbumin; SST, Somatostatin; VIP, Vasoactive Intestinal Peptide.

1.3.2 Layer 1 in adult neocortical functions

Whereas many studies have focused on deciphering the structure/function relationship in cortical circuits, the most superficial L1 has remained for a long time “a crowning mystery” (Hubel, 1982).

What are the functions of these highly structured circuits in L1? First, the activity in the apical dendrites of L5 pyramidal neurons reflects sensory perception, such activity being perturbed by anesthesia (Lacefield, Pnevmatikakis, Paninski, & Bruno, 2019; Suzuki &

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Larkum, 2020; Takahashi, Oertner, Hegemann, & Larkum, 2016). Inputs regulating apical dendrite activity are thus important for the overall shaping of cortical responses. The excitatory drive on apical dendrites is mainly mediated by higher order thalamic inputs providing a modulation of pyramidal neurons, while the neuromodulatory inputs or GABAergic inputs are either enhancing or suppressing these excitatory entries (Fan et al., 2020; Zhang & Bruno, 2019). In addition, thalamic inputs in somatosensory and other cortices were shown to constitute an important drive not only for pyramidal neurons but also for L1 interneurons (Abs et al., 2018; Audette et al., 2017; Takesian et al., 2018). L1 interneurons can, in turn, either inhibit directly apical dendrites providing feedforward inhibition, or induce disinhibition by targeting other classes of interneurons, especially Basket cells (PV⁺) or Martinotti cells (SST⁺) (Fig. 6) (Abs et al., 2018; Audette et al., 2017; Jiang et al., 2015; Lee et al., 2013; Letzkus et al., 2011; Takesian et al., 2018). A correlated activation of both apical dendrites and interneurons in L1 with feedforward or disinhibition network motifs through the same input, provides a powerful mechanism for rapid sensory plasticity and learning (Abs et al., 2018; Audette, Bernhard, Ray, Stewart, & Barth, 2019; Doron et al., 2019; Williams & Holtmaat, 2018). Finally, direct inputs onto apical dendrites from other cortical or subcortical regions provide top-down information regarding the context of sensory experience, in part through cross-modal communication (Ibrahim et al., 2016; Mesik, Huang, Zhang, & Tao, 2019; Roth et al., 2016; Takesian et al., 2018; Y. H. Tanaka et al., 2018). Collectively, the specific topologic organization of both excitatory and inhibitory inputs onto apical tufts seems to enable an orchestrated connectivity of these entries, leading to the diversity of firing patterns of pyramidal neurons associated to a variety of functions. The intricacy of structure and function in L1 raises the intriguing question of how such spatially organized architecture is built up during development.

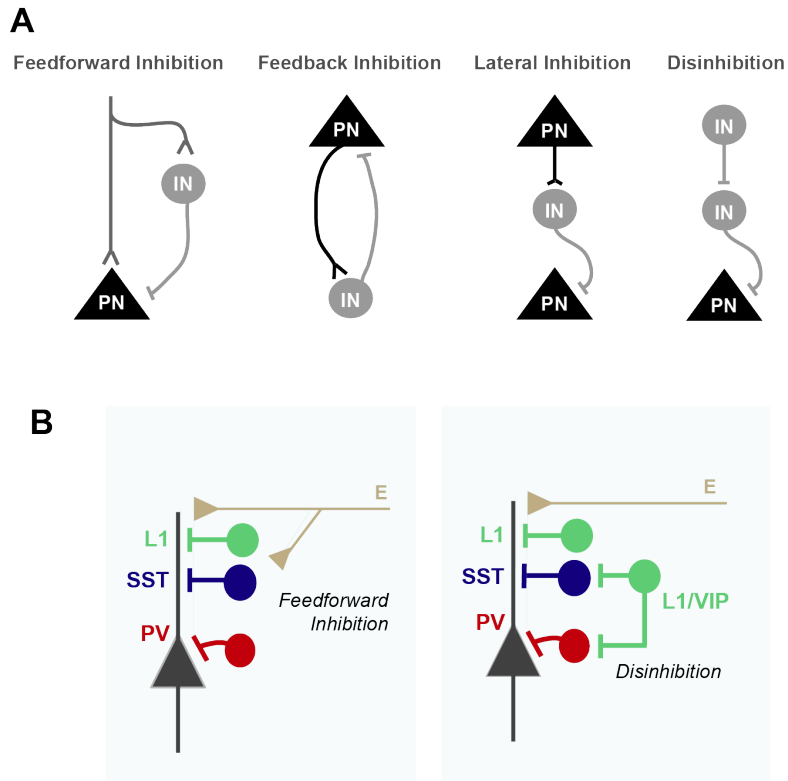


Figure 6 | Connectivity motifs in cortical networks

(A) Schematic representation of different types of connectivity motifs in the brain. In black are represented the pyramidal neurons and in grey are represented the interneurons. Adapted from Tremblay et al., 2016. (B) Schematic representation of types of connectivity motifs in L1, on apical dendrites of pyramidal neurons.

E, excitatory drive; IN, interneurons; L1, L1 interneurons; PN, pyramidal neurons; PV, Parvalbumin interneurons; SST, Somatostatin interneurons.

2. Developmental programs in the neocortex and layer 1 formation

Due to key roles of L1 in brain functions, understanding how this structure assembles from the earliest steps of development is of great importance. The development of L1 is presented in the context of neocortical formation.

2.1. General overview of telencephalic development

The nervous system is derived from the neuroectoderm, which will give rise to its almost entire cellular heterogeneity (Kandel et al., 2012; Rubenstein & Beachy, 1998). During neurulation, the neural ectoderm differentiates into neural plate, which further on bends dorsally and folds to form the neural tube (Figure. 7A). The neural tube is composed by three primary vesicles in the antero-posterior (AP) axis: the prosencephalon – which will give rise to the forebrain telencephalic vesicles and the diencephalon, the mesencephalon which will give rise to the midbrain structures, and the rhombencephalon which will give rise to hindbrain structures (Kandel et al., 2012). In line with the prosomeric model (Puelles & Rubenstein, 2003), the forebrain is subdivided into six prosomeres and the telencephalon arises from prosomeres 1-4 (Fig. 7B). The telencephalon gives rise to the pallium (or cerebral cortex) and the subpallium, which will mainly generate the basal ganglia and migrating neurons. In parallel, the neural tube is patterned along the AP and dorsoventral (DV) axis through signaling centers called organizers (Rubenstein & Beachy, 1998).

Neural tube patterning is mediated through the action of secreted gradients of morphogens, which induce the expression of different transcription factors that are essential to specify neuronal and glial cell identity (Rallu, Corbin, & Fishell, 2002). Amongst morphogens, Sonic Hedgehog (Shh), Wnt (Wingless orthologs), fibroblast growth factors (FGFs), retinoic acid (RA) and Bone Morphogenetic Proteins (BMPs) play particularly important roles in the patterning of the pallium. The morphogens are important to outline different regions of the CNS (dorsal versus ventral) as well as different subdomains like cortical

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areas. FGFs are secreted in the rostral patterning center, close to the septum, Wnt and BMPs are secreted in the caudodorsal midline centers, Shh is secreted in the ventral center, altogether being essential to pattern the pallium (Campbell, 2003; Garel, Huffman, & Rubenstein, 2003; Hoch, Rubenstein, & Pleasure, 2009; Rallu et al., 2002) (Fig. 7C). In particular, they drive the expression of a combination of transcription factors that control the generation of glutamatergic neurons in the pallium and of GABAergic and cholinergic neurons in the subpallium.

A combinatory of transcription factors drives also cortical area identity in the developing pallium. Paired box gene 6 (Pax6), empty spiracle homeobox 2 (Emx2), Sp8, and CoupTF1 are the most studied. Pax6 interacts with Nkx2.1 and Ghs2 transcription factors to define the pallial-subpallial boundary (PSB) (Campbell, 2003). Emx2, Sp8 and CoupTF1 are important for cortical arealization. In the pallium, Pax6 and Emx2 are expressed in complementary gradients of gene expression: Pax6 is expressed in a rostrocaudal and ventro-dorsal high to low gradient while Emx2 presents an increased expression more caudally. Accordingly, Pax6 and Emx2 are involved in frontal/motor and caudal sensory/visual areas, respectively (Bishop, Goudreau, & O'Leary, 2000; Muzio et al., 2002). Sp8 also is involved in the specification of rostral cortical areas (O'Leary et al., 2007; Sahara, Kawakami, Belmonte, & O'Leary, 2007; Zembrzycki, Griesel, Stoykova, & Mansouri, 2007), while CoupTF1 regulates the specification of caudal cortical areas (Armentano et al., 2007). Thus, these transcription factors are expressed in gradients through specific areas of the cortex. How these gradients lead to the formation of discrete domains that reflect area sharp boundaries and to what extent extrinsic mechanisms participate also to this process, is still under debate (O'Leary et al., 2007).

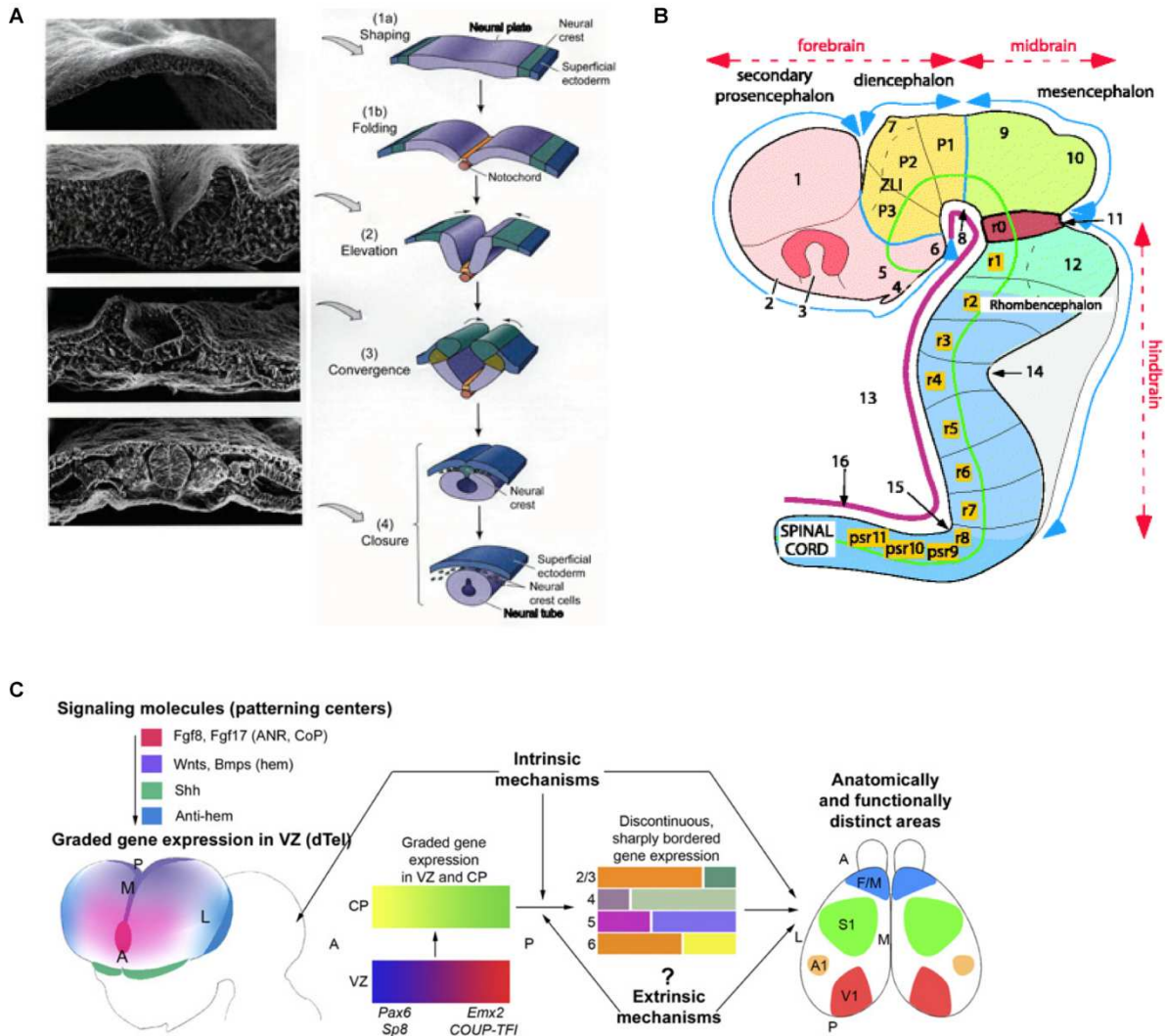


Figure 7 | Development of pallium and acquisition of cortical areal identity

(A) Electron microscopy pictures of the formation of the neural tube in the chick embryo (left) and schematic representation of folding of the neuroectoderm and formation of the neural tube (right) (From Gilbert, 2000). (B) Schematic representation of the prosomeric model of brain development (From Salvador Martínez, Eduardo Puelles and Diego Echevarria, 2013) (C) Schematic representation of the main signaling centers inducing the patterning of the pallium. The gradient of morphogens induce the differential expression of transcription factors, which, together with extrinsic signals participate to the emergence of cortical area identity (From O’Leary et al., 2007).

2.2. Generation and migration of cortical neurons

The building of networks that endow neocortical functions relies on a multistep process starting by neuronal generation, specification, migration to their final position and circuit integration. In this chapter, I will mainly focus on generation and migration, with a specific emphasis on the rodents, when not specifically mentioned.

Specific neuronal populations of the rodent neocortex are generated in overlapping time-windows within distinct progenitor domains of the telencephalon, with a major difference being that glutamatergic neurons are generated in the pallium and GABAergic interneurons in the subpallium (Anderson, Eisenstat, Shi, & Rubenstein, 1997; Oscar Marín & Müller, 2014; Tamamaki, Fujimori, & Takauji, 1997). All these subpopulations follow specific migratory routes from their generation sites to the cortical plate, using distinct modes of migration that can be tangential, radial or a combination of the two. These remarkable migratory processes have been the focus of multiple studies (Bartolini et al., 2017; López-Bendito et al., 2008; O Marín & Rubenstein, 2001; Oscar Marín & Müller, 2014; Miyoshi & Fishell, 2011; Sultan, Brown, Shi, Dicristo, & Studer, 2013; Tamamaki et al., 1997; D. H. Tanaka & Nakajima, 2012). Importantly, the earliest postmitotic neurons form the preplate, which is the first neocortical layer found superficial to the neuroepithelium (Molnár et al., 2006; Valverde, De Carlos, & López-Mascaraque, 1995). Through radial migration of newly generated cells, the preplate splits into superficial Marginal Zone (MZ) and deeper Subplate (SP), both being strategic structures for neuronal migration and axonal development and containing early generated transient neurons with central roles for cortical wiring (Marín-Padilla, 1998; Meng, Yu, Kao, & Kanold, 2020; Montiel et al., 2011)

2.2.1. Generation and migration of Cajal-Retzius cells

Cajal-Retzius cells (CRc) are a transient heterogeneous population of glutamatergic neurons found in the MZ -or developing L1- of the neocortex, hippocampus and olfactory cortex. In mice, CRc are generated early in development, between E (embryonic day) 9.5 and E11 in 4 different cortical domains lining the neocortex: the cortical hem, septum (SE), thalamic

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eminence (ET) and the PSB (Bielle et al., 2005; S. Kirischuk, Luhmann, & Kilb, 2014; Ruiz-Reig et al., 2017; Fadel Tissir et al., 2009; Yoshida, Assimacopoulos, Jones, & Grove, 2006). These cells have been first identified by anatomists as a homogenous population of bipolar elongated cells in the cortical L1 which are a main source of Reelin (RELN) in the developing brain (S. Kirischuk et al., 2014; Marín-Padilla, 1998), but now several genetic tools are available to trace the specific subpopulations of CRc depending on their origins: *Wnt3a^{cre/+}* targets hem-derived CRc (Yoshida et al., 2006), *Dbx1^{cre/+}* enables genetic targeting of SE, ET and PSB-derived CRc (Bielle et al., 2005; Griveau et al., 2010), while altogether CRc generated in the SE, hem and ET can be traced using the *ΔNp73^{cre/+}* line (Fadel Tissir et al., 2009).

Identification Method	Subpopulations of CRc labelled
Embryo: Reelin⁺	hem + septum + thalamic eminence + pallial subpallial boundary
Postnatal: Reelin⁺/Prox1⁻	hem + septum + thalamic eminence + pallial subpallial boundary
<i>ΔNp73^{cre/+}</i>	hem + septum + thalamic eminence
<i>Dbx1^{cre/+}</i>	septum + thalamic eminence + pallial subpallial boundary
<i>Wnt3a^{cre/+}</i>	hem

Table 1 | Several methods to identify and/or genetically manipulate subpopulations of Cajal-Retzius cells

From their origins, CRc migrate tangentially in the MZ in close contact with the meninges (Borrell & Marín, 2006) and tile homogenously the cortical surface. The migration of at least subsets of CRc has been shown to be regulated by Eph/ephrin-dependent contact repulsion (Villar-Cerviño et al., 2013) as well as by Sema3a/PlexinD1 signaling (Bribián et al., 2014). The migration speed of CRc from their origin to the cortical surface is shaped in a cell-autonomous manner by vesicle-associated membrane protein 3 (VAMP3) (Barber et al., 2015).

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While most of CRc homogenously cover the neocortical surface, subsets of CRc (SE and ET-derived) accumulate in a reservoir abutting the lateral olfactory tract (LOT) from which they will reenter tangential migration starting with E15.5, thereby maintaining a proper density while the cortex grows during development (de Frutos et al., 2016). CRc originating from different sources display a specific spatial organization on the early cortical sheet: hem-derived CRc colonize mainly the medio-caudal part of the telencephalic vesicle, SE-derived CRc the medio-rostral part, PSB-derived CRc are mainly found laterally (Barber et al., 2015; Barber & Pierani, 2016; Griveau et al., 2010) whereas it is not yet clear what is the spatial distribution of ET-derived CRc. Moreover, the genetic ablation of SE-derived CRc induces a reorganization of hem-derived and PSB-derived CRc which now populate the medio-rostral ablated cortical area (Barber & Pierani, 2016; Griveau et al., 2010). Thus CRc, while forming a single neuronal population, originate in different sources and position through migration raising the question of whether different CRc have specific roles. Once positioned in the MZ, CRc collectively participate to the migration of both pyramidal neurons and interneurons, cortical arealization, the morphogenesis of pyramidal neuron apical dendrites, and wiring of layer 1 circuits (see chapter 5.3), before being eliminated by cell death (see chapter 4.1.1) (Barber et al., 2015; de Frutos et al., 2016; S. Kirischuk et al., 2014).

2.2.2. Generation and migration of Subplate neurons

Subplate neurons (SPn) constitute a transient heterogenous population of both excitatory and inhibitory neurons generated between E10 - E13 (Price, Aslam, Tasker, & Gillies, 1997) and located deep to the cortical plate and superficially to the intermediate zone (Bruguier et al., 2020; Hoerder-Suabedissen & Molnár, 2013; Montiel et al., 2011). SPn are generated from progenitors located in the cortical ventricular zone and in the rostro-medial telencephalic wall (Pedraza, Hoerder-Suabedissen, Albert-Maestro, Molnár, & De Carlos, 2014) and then they migrate radially or tangentially respectively to reach the preplate. Concomitant with the generation of pyramidal neurons, the preplate splits and the SPn remain restricted to the SP. SPn have been shown to be very important early on, for the guidance of thalamocortical axons

towards the cortical plate, the emergence of electrical activity in the cortex, and later on, essential for the maturation of thalamocortical circuits bridging the thalamus to the cortical layer 4 (Hanganu, Kilb, & Luhmann, 2002; Kanold & Luhmann, 2010; Luhmann, Kirischuk, & Kilb, 2018). The fate of SPn is not completely elucidated, but evidence is showing that some SPn undergo cell death while other subpopulation translocate into Layer 6b cortical neurons (Friedlander & Torres-Reveron, 2009; Kanold & Luhmann, 2010; Luhmann et al., 2018; Valverde & Facal-Valverde, 1988).

2.2.3. Generation and migration of Pyramidal neurons

2.2.3.a Subtypes of pyramidal neurons and their progenitors

Pyramidal Glutamatergic neurons represent approximately 80% of the neuronal population, display a remarkably homogeneous morphology with a pyramidal cell body, basal dendrites around the extending axon, and a tuft of apical dendrites in L1 (Figure 1C). However, bulk and single-cell transcriptomic studies have been instrumental in unraveling the hidden molecular heterogeneity of pyramidal neurons (Arlotta et al., 2005; Fame, Macdonald, Dunwoodie, Takahashi, & Macklis, 2016; Fame, MacDonald, & Macklis, 2011; Greig, Woodworth, Galazo, Padmanabhan, & Macklis, 2013; Matho et al., 2020; Molyneaux et al., 2009; Tasic et al., 2016). Consistently, pyramidal neurons, specified by precise transcriptional programs that control their axonal targets can be classified them into two major classes (Figure 1): i) deep layers mainly corticofugal pyramidal neurons, expressing the transcription factor *Fezf2* and downstream target *Ctip2* (Lodato & Arlotta, 2014; McKenna et al., 2011; Molyneaux et al., 2007), ii) upper layers callosal pyramidal neurons – located in upper cortical layers, which can be identified by the expression of *Cux1* or *Satb2*, which send axonal projections in the contralateral hemisphere that form the corpus callosum (CC) (Alcamo et al., 2008; Lodato & Arlotta, 2014; Molyneaux et al., 2007). Interestingly, although the position of pyramidal neurons is directly correlated with their birthdate, recent findings reveal that similar transcriptomic identity of cortical projecting neurons reflect their axonal target, and not their birth date or laminar position (Klingler et al., 2019).

In mice, pyramidal neurons are generated sequentially between E11 and E16 from pallial progenitors that are all derived from neuroepithelial (NE) cells. First during neurogenesis, NE progenitors divide symmetrically and increase the progenitor pool, before generating Radial Glial Cells (RGc). Schematically, RGc expand in the VZ and they either self-renew or give rise, through asymmetric divisions, to Intermediate progenitors (IP) or to neurons (Govindan & Jabaudon, 2017; Lodato & Arlotta, 2014; Noctor, Martinez-Cerdeño, Ivic, & Kriegstein, 2004; Taverna, Götz, & Huttner, 2014). The IP pool will form the SubVentricular Zone (SVZ) and through a limited number of symmetric divisions, generate the majority of pyramidal neurons (Fig. 8A) (Fietz & Huttner, 2011; Haubensak, Attardo, Denk, & Huttner, 2004; Lodato & Arlotta, 2014; Taverna et al., 2014). Of course, there are a large number of additional progenitor types, that show, for instance, a basal process and contribute to a much larger population of progenitors in mammals with larger or gyrencephalic brains, such as ferrets, monkeys or humans (Fig. 8A) (Borrell & Götz, 2014; Fernández, Llinares-Benadero, & Borrell, 2016; Florio & Huttner, 2014; Hevner, 2019; Pollen et al., 2015)

2.2.3.b Migration and morphological differentiation of pyramidal neurons

Corticogenesis takes place in an “Inside-out” fashion and the laminar position of a pyramidal cell strongly correlates with its birth date (Fig. 8A) (Cooper, 2008; Molyneaux et al., 2007; Parnavelas, 2000). The earliest generated neurons migrate radially and reside in deep cortical layers (L5, L6), whereas later-born neurons migrate pass them and form upper cortical layers (L2-L4) (Cooper, 2008; Molyneaux et al., 2007; Parnavelas, 2000). This radial migration occurs in two steps. During the very early stages of neurogenesis, pyramidal neurons first migrate through soma – translocation (Miyata, Kawaguchi, Okano, & Ogawa, 2001; Nadarajah, Brunstrom, Grutzendler, Wong, & Pearlman, 2001; Nadarajah & Parnavelas, 2002). In this type of migration, the pyramidal neurons extend a radially oriented long process to the MZ, and the cell body will further on translocate apically. As aforementioned, the migration of the first pyramidal neurons splits the prelate into the upper MZ and the inner SP and generates the formation of the Cortical plate (CP). At later developmental stages, this migration is mostly

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replaced by migration along the RGc, which serve as physical scaffold (Nadarajah & Parnavelas, 2002). Time-lapse videomicroscopy studies reveal that the switch between soma translocation and RG-guided migration takes place once the apical process of pyramidal neurons reaches the MZ (Nadarajah et al., 2001; Nadarajah & Parnavelas, 2002). Newly generated pyramidal neurons migrate along the RGc, pass by the earlier generated neurons and last detach from the RGc. They present the apical leading process towards the MZ and their axon towards the VZ. This polarity is in part regulated by a Semaphorin 3A (Sema 3A) gradient, with a highest concentration in the MZ and then in a decreasing concentration towards the ventricular zone. Remarkably, Sema 3A via its interaction with Neuropilin 1 is chemorepulsive for cortical axons (Polleux, Giger, Ginty, Kolodkin, & Ghosh, 1998) and chemoattractant for the apical dendrites (Polleux, Morrow, & Ghosh, 2000), this dual role being explained by a differential expression of cGMP in dendrites and in axons (Polleux et al., 2000; Whitford, Dijkhuizen, Polleux, & Ghosh, 2002).

RELN is very important at multiple steps in this process. The reeler mutant, which presents a spontaneous mutation in the *Reelin* gene, is one of the most studied genetic models with impaired neuronal migration. In the reeler mice, the cortex is inverted notably with pyramidal neurons migrating in an “outside-in” manner (Fig. 8B) (D’Arcangelo et al., 1995; Nadarajah & Parnavelas, 2002; Ogawa & Miyata, 1995; Fadel Tissir & Goffinet, 2003). Consistently, double mutations in the genes coding for the RELN receptors VLDLR and ApoER2 induce the same reeler-like phenotype (Trommsdorff et al., 1999). Analysis of the reeler and similar mutants reveal that RELN is important since early steps in neuronal migration. RELN is modulating the preplate splitting (Caviness et al., 1982), controls soma translocation by inducing the stabilization of actin and Cadherins, control the radial migration along the RGc as well the terminal translocation (Chai et al., 2015; Franco, Martinez-Garay, Gil-Sanz, Harkins-Perry, & Müller, 2011; Gil-Sanz et al., 2013; Hirota & Nakajima, 2017; Sekine et al., 2012; Sekine, Kubo, & Nakajima, 2014). Therefore RELN plays a major role in pyramidal neuron migration

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During early development in mice, the heterogeneous population of pallial glutamatergic neurons follow altogether the same program for dendritic development, with the leading process of pyramidal neurons contacting the MZ. With time, the process of apical dendrite maturation undergoes a specific refinement depending on the glutamatergic neurons subtype: for L2/3 and most of L5 pyramidal neurons (Marín-Padilla, 1992), apical processes become ramified apical dendrites into layer 1, while the layer 4 excitatory spiny stellate neurons, retract this apical leading process at early postnatal stages (Callaway & Borrell, 2011; O'Dell, Cameron, Zipfel, & Olson, 2015; Whitford et al., 2002). Dendritic outgrowth is a very important process in development as the number and morphology of apical dendrites in L1 are determinant for the integration of the sensory information. Several molecular cues have been involved in dendritic growth and branching, including Neurotrophins like Neuronal growth factor (NGF), brain-derived neurotrophic factor (BDNF), Neurotrophin 3 (NT-3) and NT-4, as well as Insulin Growth factor 1 (IGF-1), Notch (Whitford et al., 2002) and RELN signaling pathways (Chameau et al., 2009; Kohno et al., 2015; O'Dell et al., 2015; Okugawa et al., 2020).

The final step in dendritic maturation is the formation of spines. Spines are the physical place where most of the excitatory inputs are made and directly linked to synaptic organization, which is a highly dynamic process, but also displays some stereotypic organization. Even if variable between different cortical areas and cortical layers (Tjia et al., 2017), spines can be specifically located in L1a or L1b as previously mentioned (Chapter 1.3.1). They start to form in mice mainly in the second postnatal week and their density continues to increase in the 1st postnatal month followed by a period of elimination or pruning (Berry & Nedivi, 2017; Whitford et al., 2002). Spine formation/elimination is regulated by intrinsic factors, such as SRGAP2 (Charrier et al., 2012; Fossati et al., 2016; Schmidt, Zhao, Hillman, & Polleux, 2019) environmental signals, such as axon guidance molecules or trophic factors (Chapleau, Larimore, Theibert, & Pozzo-Miller, 2009; Horch, Krüttgen, Portbury, & Katz, 1999; Orefice et al., 2013; Shen & Cowan, 2010) and of course, by activity, as detailed in chapter 3. This spine dynamics has revealed to be key in development and in experience-dependent remodeling of

neuronal circuits (Hofer, Mrcsic-Flogel, Bonhoeffer, & Hübener, 2009; Holtmaat & Svoboda, 2009).

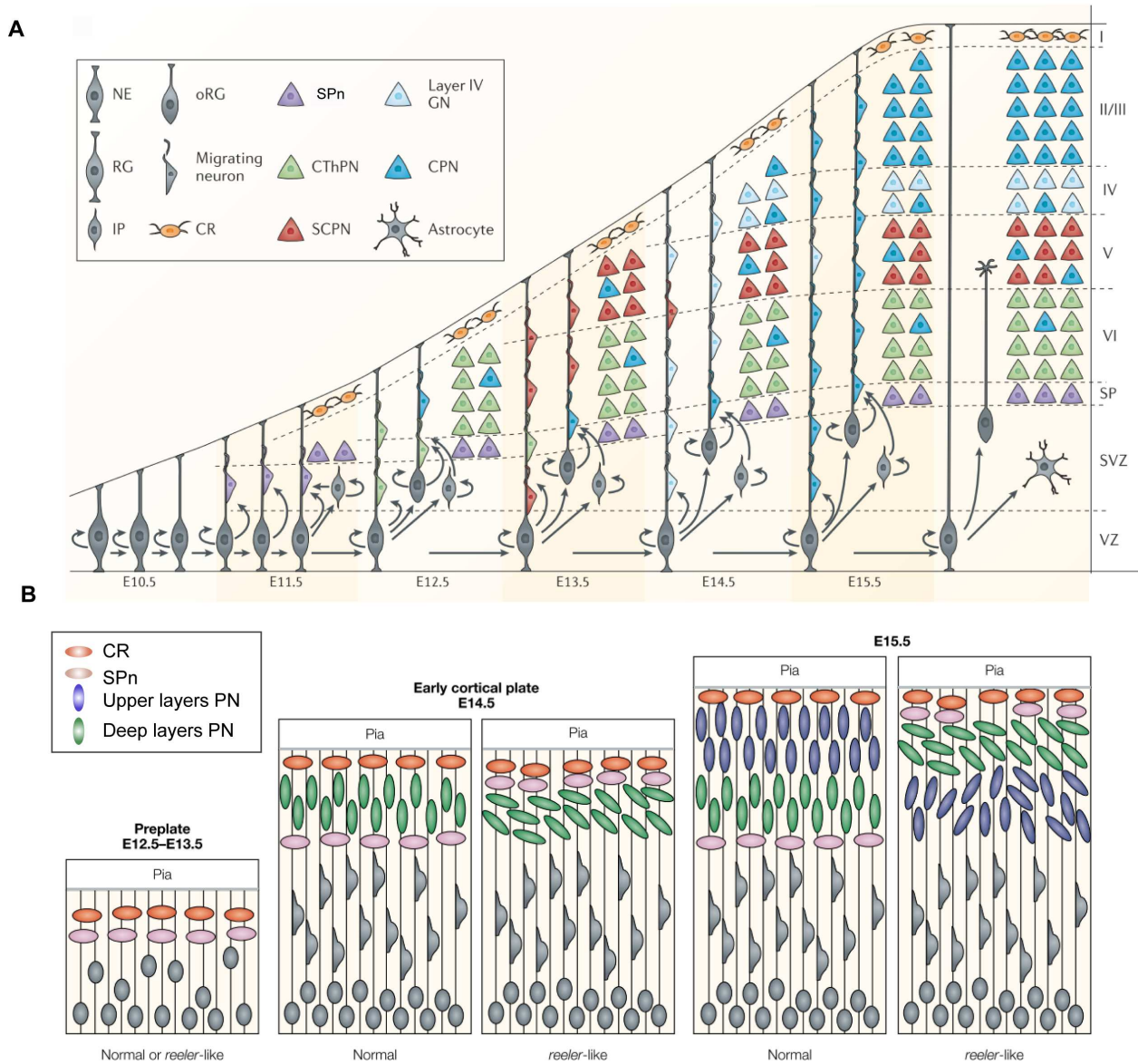


Figure 8 | Generation and distribution of pyramidal neurons

(A) Schematic representation of pyramidal neuron generation and migration in an “inside-out” fashion, with the earliest-born neurons located in deep cortical layers and the late-born neurons located in upper cortical layers. From Greig et al., 2013). (B) Schematic representation of inverted “outside-in” cortical lamination in reeler-like mutants. From Fadel Tissir & Goffinet, 2003.

CR, Cajal-Retzius cell; CThPN, cortico-thalamic projection neurons; CPN, callosal projection neurons; GN, granular layer 4 neurons; IP, intermediate progenitor; NE, neuroepithelial cell; oRG, outer Radial Glia; RG, radial glia; SCPN, subcerebral projection neurons; SPn, subplate neurons

2.2.4. Generation and migration of Interneurons

2.2.4.a Origin of interneurons and link to their diversity

Interneurons originate in the subpallium, meaning that they need to migrate tangentially over long distances before integrating into the cortical plate (Batista-Brito & Fishell, 2009; Lim, Mi, et al., 2018b; Oscar Marín & Rubenstein, 2001; Wamsley & Fishell, 2017). In mice, all interneurons are generated in 3 progenitor domains: the medial ganglionic eminence (MGE), the caudal ganglionic eminence (CGE) and to a lesser extent, the preoptic area (POA) (Bartolini et al., 2013; Lim, Mi, et al., 2018a; Wamsley & Fishell, 2017; Wonders & Anderson, 2006).

The MGE gives rise to the majority of interneurons (approximately 60-70%), which are the earliest born (between E11.5 and E17.5 in mice) (Bartolini et al., 2013). They are generated from progenitors expressing *Nkx2.1* (Xu, Tam, & Anderson, 2008). The MGE gives rise to two main non-overlapping subpopulations: SST⁺ and PV⁺ cells. SST⁺ interneurons include Martinotti (projecting in L1) and non-Martinotti cells (not projecting in L1), PV⁺ include Basket (contacting the somatic region of pyramidal neurons) and Chandelier cells (contacting the axon initial segment - AIS of pyramidal neurons) (Fig. 9). However, single cell transcriptomics and electrophysiological recordings have enabled to identify up to 13 subclasses of these interneurons suggesting their important heterogeneity (Mi et al., 2018; Tasic et al., 2016; Wamsley & Fishell, 2017).

CGE-derived interneurons are generated later from E12.5 until E18.5 with a peak at E16.5 and represent around 30% of the interneuron population. They form a very heterogeneous population of interneurons that can be identified by the combinatorial expression of molecular markers: most of them express the 5HT3R and Prox1, Sp8 or Nr2f2 (CoupTF2) but also some co-express RELN, VIP, Calretinin or NPY (Fig. 9), while the white matter CGE-derived interneurons express also Meis2 (Cai et al., 2013; Frazer et al., 2017; Ma et al., 2012; Miyoshi et al., 2015; Rubin & Kessaris, 2013). The combination of these markers leads to a diverse identity of these cells found also in adult stages, namely SBc, multipolar and bipolar cells (Fig. 9).

POA-derived interneurons only constitute a low percentage of interneurons – approximately 10% of the entire population. POA interneurons can be traced and manipulated using the *Hmx3^{cre/+}* and *Dbx1^{cre/+}* driver lines as well as partially with the *Nkx2.1^{cre/+}* line (D. Gelman et al., 2011; D. M. Gelman et al., 2009; Xu et al., 2008) Fate mapping experiments have shown that the POA generate PV, SST, RELN and NPY cortical neurons (D. Gelman et al., 2011; D. M. Gelman et al., 2009). Importantly, NGF_c – a particular type of interneurons present in upper cortical layers and notably in cortical layer 1 and at the border L1/L2 – is mainly produced in the POA (Niquille et al., 2018) (Fig. 9).

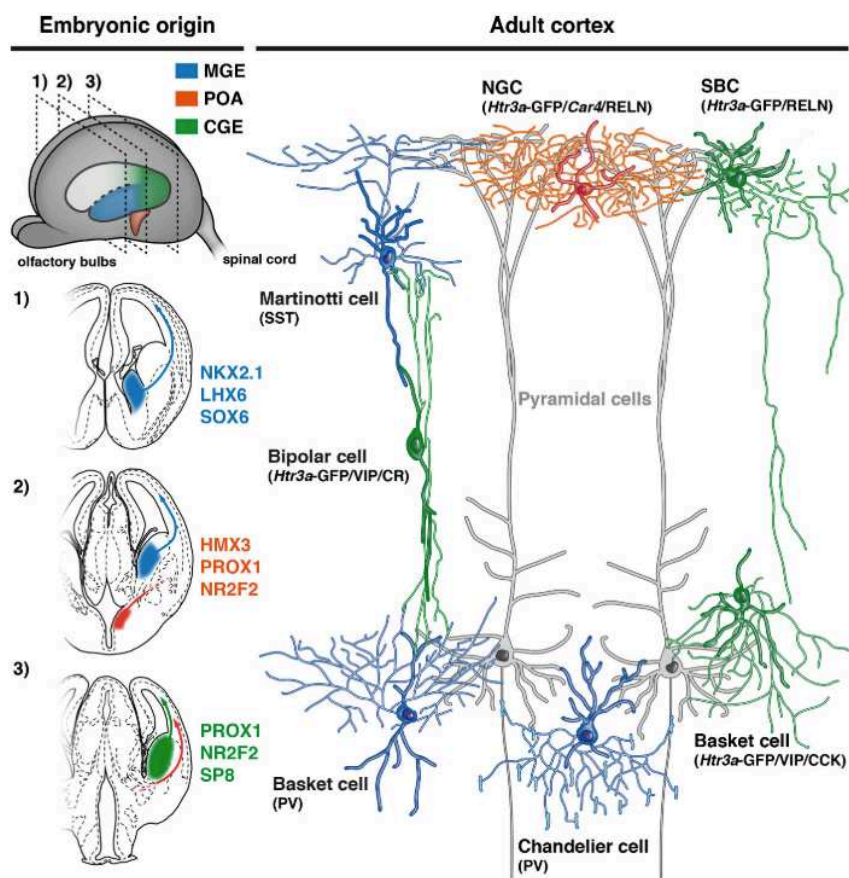


Figure 9 | Origin of main classes of interneurons

Schematic representation of the three origins of interneurons, the MGE, the CGE and the POA (left). Schematic representation of the main subtypes of interneurons depending on their generation site, cortical localization, molecular and connectivity profile (right). From Niquille et al., 2018.

CGE, caudal ganglionic eminence; MGE, medial ganglionic eminence; NGC, Neurogliaform cell; POA, preoptic area; SBC, single-bouquet cell.

2.2.4.b Migration and positioning of interneurons in the neocortex

Cortical interneurons migrate from the subpallium in 3 steps: i) tangential migration through the subpallium towards the pallium either in the SVZ or through the mantle; ii) tangential migration through the cortical MZ and SVZ, and iii) switch to radial migration and integration into the CP (Fig. 10A) (Bartolini et al., 2013; Batista-Brito & Fishell, 2009). Similarly to pyramidal neurons, but in a "looser" way, interneurons colonize the cortex in an inside-out manner, with the first generated interneurons (MGE-derived) mainly localized to deep cortical layers, and the later generated interneurons (CGE-derived) mainly being restricted to upper cortical regions. This organization is likely related to pyramidal neurons as it was shown that L5 can attract their target interneurons when misplaced in the neocortex (Lodato et al., 2011).

The tangential migration across the subpallium is channeled by combinations of chemorepulsive and permissive cues in the basal ganglia and chemoattractant cues in the cortex, including Sema3A/F and Neuregulins (Oscar Marín & Rubenstein, 2001; Moore, West, & Grace, 1999; Nóbrega-Pereira & Marín, 2009; Rudolph, Zimmer, Steinecke, Barchmann, & Bolz, 2010). The choice to pursue cortical migration in the superficial MZ and the SVZ is not random, but does not depend on the generation site of interneurons (Bartolini, 2016; Miyoshi & Fishell, 2011). During this tangential dispersion phase, interneurons are maintained out of the cortical plate through chemoattractant molecules displayed in the migratory streams – such as Cxcl12 - which is recognized by its receptors Cxcr4 and Cxcr7 (López-Bendito et al., 2008). In addition, there is a strong interaction between pallial IP and migrating interneurons, which on the one hand, regulate interneuron migration (Hevner, 2019; Sessa et al., 2008), and on the other hand, influence progenitors (Silva et al., 2018). At later embryonic stages (E16.5), interneurons switch from tangential to radial migration and enter the cortical plate. The mechanisms which regulate the switch between tangential and radial migration are not fully understood, but several hypotheses are in favor of a switch to a lack of responsiveness to Cxcl12 (Li et al., 2008). The final distribution of at least MGE-derived interneurons is driven by Neuregulin 3 (*Nrg3*) expressed in pyramidal neurons and its receptor ErbB4 expressed by

interneurons (Bartolini et al., 2017). Once the interneurons integrate the cortical plate, they form functional cortical circuits. These circuits are later on refined, because postnatally, there is an important phase of physiological death which is further on sculpting cortical circuits (see chapter 4.1.2).

2.3. Construction of neocortical layer 1

In contrast to what is observed in adults, the developing L1, or marginal zone (MZ) during embryogenesis, displays several neuronal populations that are transient either because they are either eliminated by cell death and/or because they are only migrating through this structure before reaching their final position in the cortical plate.

2.3.1. Cellular components of the developing neocortical layer 1

As previously mentioned, the first inhabitants of embryonic layer 1 are the CRc, early-born neurons key for cortical wiring which are eliminated by the beginning of the 2nd postnatal week in mice. In addition, the MZ also hosts an important migratory stream for GABAergic cortical interneurons. While interneurons generated in both the MGE and CGE migrate through L1, the MGE-derived interneurons that migrate specifically through this zone and are not present in the adult L1 while subpopulations of CGE-derived interneurons stabilize in L1. In addition, it seems that MGE-derived interneurons that do migrate through L1 give rise to specific populations of interneurons. In particular, part of MGE-derived interneurons that undertake the MZ route reveal a remarkable coupling between migration and axonal target in L1 (Lim, Pakan, et al., 2018). More precisely, recent findings highlight that Martinotti cells travel through the MZ, leave their axon in this structure while their cell body migrates deeper into the CP, leading to their characteristic morphology in adults with a “T-shaped” axonal plexus in L1 (Fig. 9, Fig. 10A) (Lim, Pakan, et al., 2018). Elegant transplantation experiments highlighted that migrating through the MZ is necessary for the SST⁺ Martinotti cells to acquire this axonal morphology (Lim, Pakan, et al., 2018). For CGE-derived interneurons, it remains unclear whether specific

subpopulations preferentially migrate through the MZ- for instance whether the ones that are found in L1 at adult stages have this localization as a consequence of migrating through MZ- or whether migration through the MZ is important for their maturation.

Besides CRc and interneurons, the developing L1 is also hosting glial cells, but their role in the MZ during this timeframe is yet to be discovered.

2.3.2. Axonal components of the developing neocortical layer 1

In addition to its mostly transient cellular components, the MZ displays abundant acellular processes, including basal processes of RGc the leading processes of radially migrating glutamatergic neurons which will give rise to apical dendrites of pyramidal neurons, as well as long-range axonal projections. The L1 receives excitatory inputs transiently from the SP, but also inputs from the thalamus, other cortical regions, subcortical nuclei sending neuromodulatory projections which are present at adult stages (Fig. 10B).

SPn were suggested to be a scaffold for the development of thalamocortical and corticothalamic axons (Barber & Pierani, 2016; Luhmann et al., 2018). However, recently, a subpopulation of glutamatergic SPn, called “pyramidal subplate neurons” was shown to project to L1 somatosensory cortex until at least early postnatal stages (Ghezzi et al., 2020). What are the roles of these L1 projections from the SP, whether they form a scaffold for thalamic projections to L1 and whether they contact CRc or L1 interneurons still remains to be deciphered. Intriguingly a similar population of SPn neurons has been observed in the auditory cortex, during the first postnatal week, with the role of transiently bridging peripheral sensory information relayed by the thalamus up to L1 (Meng et al., 2020).

In addition to transient SPn projections, thalamic, cortical and neuromodulatory axons first reach the SP, then navigate through the CP until they target L1 in the stereotyped manner that characterizes adult L1 circuits (Fenlon, Suárez, & Richards, 2017; Galazo, Martinez-Cerdeño, Porrero, & Clascá, 2008; Rubio-Garrido et al., 2009; Suárez et al., 2014; Vitalis et al., 2013). In addition to this “classical route”, neuromodulatory axons, have an additional second trajectory by reaching directly the MZ (Vitalis et al., 2013). While their trajectory is similar, the

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timing of invasion differs across axonal populations: neuromodulatory axons reach the MZ at embryonic stages, higher order thalamic axons innervate L1 by the beginning of the 1st postnatal week and cortical axons by the beginning of the 2nd postnatal week (Chameau & Van Hooft, 2006; Galazo et al., 2008; Rubio-Garrido et al., 2009; Suárez et al., 2014; Vitalis et al., 2013; Vitalis & Verney, 2016). Since their development, the axons grow in the L1 with a specific topography which is maintained throughout life. The higher order thalamic axons are localized in L1a, enabling thalamic axons and their synapses to be a landmark for this segregation since early stages, while the cortical and neuromodulatory axons sprout in the whole thickness of L1 (Fig. 10B).

Concerning GABAergic inputs, L1 is innervated from birth by long-range inputs from ZI, but also hosts the axonal plexus from L1 NGF_c and Canopy cells, Martinotti cells, Chandelier cells as well as bipolar and multipolar cells located at the border L1/L2 (Che et al., 2018; Chen & Kriegstein, 2015; Dammerman, Flint, Noctor, & Kriegstein, 2000; Lim, Pakan, et al., 2018; Muralidhar et al., 2014; Niquille et al., 2018; Schuman et al., 2019; Silva et al., 2018; Taniguchi, Lu, & Huang, 2013). The topography of at least ZI axons and Martinotti cells axons are also restricted to L1a since development. (Chen & Kriegstein, 2015; Lim et al., 2018).

Whereas the timing and progression of all these axonal tracts has been established, we surprisingly know very little about the cellular or molecular mechanisms controlling axonal navigation in the MZ. Beyond early wiring steps, what do we know about synaptogenesis in L1 and the development of the exquisite synaptic organization observed onto apical dendrites? While most axons reach L1 postnatally, both glutamatergic and GABAergic synaptic markers can be found in L1 from late embryonic stages, likely originating from transient SPn inputs.

While there is still limited information regarding synaptogenesis in L1, evidence suggests that synaptogenesis in L1 is regulated intrinsically through genetic programs and also through environmental signals. Several molecular programs that regulate synapse specificity of L1 projecting interneurons have been recently identified (Favuzzi et al., 2019). For example, *Cbln4* expression in Martinotti cells is sufficient to drive the L1-restricted synapse formation of Martinotti cells on apical dendrites of pyramidal neurons (Favuzzi et al., 2019). Synaptogenic

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factors such as RELN are also present in L1 (Borrell et al., 1999; Bosch et al., 2016). Intriguingly, astrocytes were also shown to play a role in the process by producing the secreted factor Hevin that promotes thalamocortical synapse formation in L1 (Singh et al., 2016).

The developing L1 thus hosts a complex choreography of migrating transient CRc and interneurons, sprouting dendritic and axonal collaterals of both glutamatergic and GABAergic neurons. Proper development of neocortical L1 is crucial for cortical function. Impairments in the wiring of L1 has long lasting consequences on the function of neocortical networks perturbing the perception of the external world (Lim, Mi, et al., 2018a; Takesian et al., 2018).

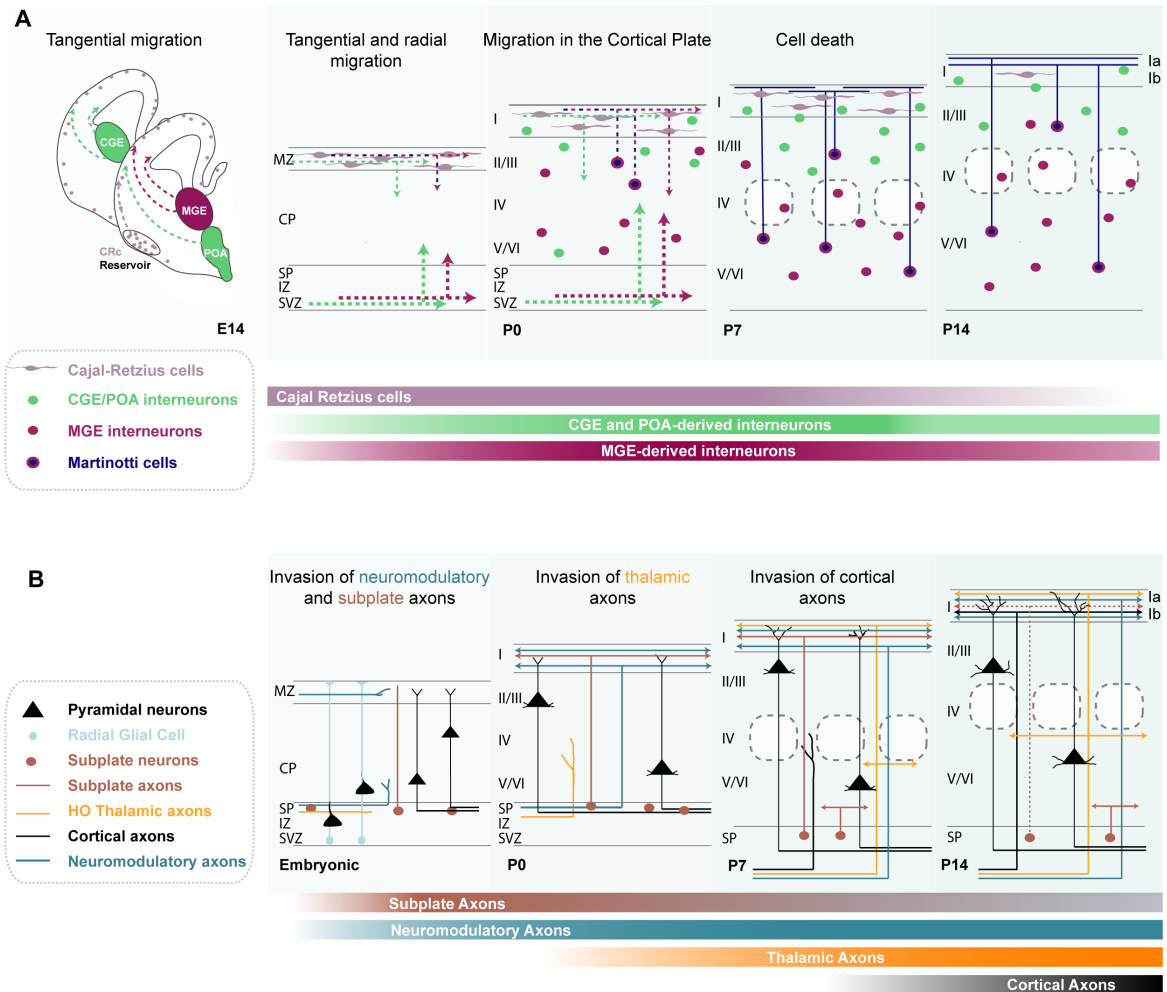


Figure 10 | Timeline of Layer 1 development

(A) Schematic representation of the sequential timing of invasion of Cajal-Retzius cells (CRc) and interneurons in the developing L1, the Marginal Zone (MZ). Panel on the right shows tangential migration of interneurons from their origins and of Cajal-Retzius cells (CRc) from their accumulating reservoir. The dotted lines represent streams of migration. Middle panels depict that Cajal-Retzius cells are localized postnatally in L1 while interneurons switch from tangential migration to radial migration and enter in the cortical plate. Note the migration of Martinotti cells in the MZ, and later on leaving their axon in L1 while they reach their position in the CP. Panels on the right show that CRc start undergoing cell death until their almost complete elimination as well as certain interneurons. (B) Schematic representation of timing of invasion of excitatory axons in L1. Axons which arrive either directly in the MZ (neuromodulatory axons) or through the subplate (SP), cross the CP and reach the L1 at postnatal stages (neuromodulatory, thalamic and cortical axons). Grey dotted lines represent the barrels. MGE: Medial Ganglionic Eminence, CGE: Caudal Ganglionic Eminence, POA: Preoptic Area, CRc: Cajal-Retzius cells, MZ: Marginal Zone, CP: Cortical Plate, SP: Subplate, IZ: Intermediate Zone, SVZ: Subventricular Zone, HO Thalamic Axons: Higher Order Thalamic Axons, FO Thalamic Axons: First Order Thalamic Axons

3. Roles of electrical activity in the development of neocortical circuits

Besides genetic determinants in brain development, electrical activity also emerges as a major regulator of cortical wiring. Electrical activity in development is initially spontaneous in most sensory systems, either locally, in the periphery or in central relays. Then, once the sensory organs fully develop, evoked sensory activity mediated by the thalamus constitutes one of the main drivers for cortical refinement. I will be presenting the roles of electrical activity in neocortical development, at different steps from progenitors, cell-autonomous processes up to the population level and maturation of cortical circuits. Electrical activity is also important to drive neuronal elimination via cell death, but this aspect will be specifically presented in chapter 4.

3.1 Early activity in shaping the formation of neocortical circuits

Electrical activity is present since the earliest stages of neocortical development. Concerning glutamatergic neuronal development, during neurogenesis, the membrane potential of neural progenitors decreases during development and is indicative of the type of division they will undergo (Vitali et al., 2018). However, neural progenitors are not excitable cells and their membrane potential reveals just their biophysical properties. *In vivo* hyperpolarization of neuronal progenitors by overexpression of Kir2.1 inward rectifying channel induces a precocious generation of IP, leading to the generation neuronal progeny with a later status (Vitali et al., 2018). Since E15, some of the first spontaneous Ca^{2+} events appear in RGc in the VZ, and this synchronized propagation shapes the neurogenesis rate (Owens & Kriegstein, 1998; Weissman, Riquelme, Ivic, Flint, & Kriegstein, 2004). Electrical activity is also modulating the radial migration of newly generated pyramidal neurons, affecting cortical lamination (Hurni et al., 2017). Notably, activity of pyramidal neurons has been suggested to be a stop signal for their migration (Hurni et al., 2017) This was suggested to be initially mediated through ambient levels of Glutamate which activates postsynaptic glutamatergic receptors, rather than proper

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synaptic contacts, which are put in place later (Luhmann, Fukuda, & Kilb, 2015). More specifically, a direct role of NMDA receptors in neuronal migration was first shown by Komuro & Rakic, 1993 in the cerebellum and has been also confirmed in the neocortex, where the pharmacological blockade of NMDA signaling results in impairment in the pyramidal radial migration (Behar et al., 1999). In addition, the NMDA signaling has also been suggested to be important for CRc redistribution, as impairment of the GLUN1 subunit of NMDA receptors leads to less CRc in the MZ (de Frutos et al., 2016).

Activity-dependent migration and maturation have also been reported for both MGE and CGE-derived interneurons. First, GABAergic interneurons are also sensitive to ambient GABA and Glutamate which is required for their migration. *In vitro* blockade of AMPAR and NMDAR glutamatergic receptors as well as the expression of KCC2 Cl⁻ transporter arrest interneuron migration (Bortone & Polleux, 2009). The downregulation of NKCC1 Cl⁻ transporter and the upregulation of KCC2 Cl⁻ transporter is responsible for the developmental switch of GABA from depolarizing to hyperpolarizing (Ben-Ari, 2002). Moreover, ambient GABA is acting as a stop signal for radial interneuron migration, since pharmacological blockade of GABA facilitates their radial migration (Furukawa et al., 2014; Luhmann et al., 2015). Consistent with the impact of both glutamatergic and GABAergic signaling, the excitability of interneurons is also modulating their migration. *In vivo* hyperpolarization of CGE-derived interneurons induces impairment in their migration leading to more ectopic cells located in deep cortical layers (De Marco, García, Karayannis, & Fishell, 2011). In conclusion, activity participates on top to core intrinsic genetic programs, to the refinement of cortical circuits in development.

Thalamic spontaneous or evoked activity is also emerging as an important actor for the refinement of circuits. Thalamocortical synapses involve NMDA receptors (NMDAR) (Iwasato et al., 2000). NMDA receptors in the cortex though do not seem to be crucial in the barrel formation as their deletion in the cortex (CxNR1KO) did not completely prevent the formation of TCA patches. However, in absence of NMDA receptors, the spiny stellate excitatory neurons of L4 lose their morphological orientation of dendrites towards the TCA patches (Datwani*, Iwasato†, Itohara‡, & Erzurumlu, 2013; Iwasato et al., 2000; López-Bendito & Molnár, 2003;

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Mizuno et al., 2014). In turn, impairment of the NMDA receptors in sensory thalamus (ThalNR1KO) and as well as hyperpolarization of thalamic axons lead to no barrel formation in the neocortex (Antón-bolaños et al., 2019; Arakawa et al., 2014; Moreno-Juan et al., 2017)

TCAs are also contacting both the MGE and CGE-derived interneurons shaping their distribution, morphology and connectivity. Abolishment of sensory thalamic mediated activity via whisker trimming after the 1st postnatal week induces a decrease in the number of PV⁺ neurons and their synaptic buttons (Jiao, Zhang, Yanagawa, & Sun, 2006). Comparably, perinatal whisker plucking this time during the 1st postnatal week, impairs the morphology of CGE-derived NGF interneurons modulation their integration in the cortical networks, while it does not affect the VIP⁺ interneurons (De Marco García, Priya, Tuncdemir, Fishell, & Karayannis, 2015; De Marco et al., 2011).

Even though electrical activity participate to the refinement of the already genetically programmed circuits, cell intrinsic electrical activity is also inducing the expression transcription factors which shape the identity or connectivity of neocortical neurons, highlighting the link between the external environmental context and intrinsic genetic programs (Wamsley & Fishell, 2017). For example, the activity-dependent expression of Er81 transcription factor in MGE-derived-interneurons is modulating the physiological properties of PV⁺ interneurons (Dehorter et al., 2015). With regards to CGE-derived interneurons, exposing the mice to dark rearing induces an upregulation of Insulin Growth factor 1 (Igf1) which in a cell autonomous manner leads to increased inhibitory inputs onto VIP⁺ neurons (Mardinly et al., 2016). On the other hand, this crosstalk between activity and genetic programs is impacting also on the TCAs and L4 spiny stellate neurons. The activity in thalamic axons regulates the expression of ROR β in L4 neurons which is necessary for barrel-like clustering of cortical neurons (Jabaudon, J. Shnider, J. Tischfield, J. Galazo, & MacKlis, 2012). Activity from the TCAs induces also the expression of BTBD3 in spiny stellate neurons which stimulates the orientation of dendritic arbors of spiny stellate neurons towards the active TCAs in the barrel hollow (Matsui et al., 2013).

Altogether, this suggests that the early electrical activity, either spontaneous or evoked, is impacting on the formation of cortical circuits, from early stages until later network formation.

3.2 Patterns of electrical activity in neocortical maturation

Activity patterns contribute also to large scale population dynamics that are characterize the development and maturation of cortical circuits. The emergence of electrical activity in the cerebral cortex is portrayed by the rise of spontaneous activity among large groups of cells, called assemblies, which contain both pyramidal cells and interneurons (C. Allene et al., 2008). In the developing brain, these events are initially synchronous and neocortical maturation relies on the desynchronization of these networks to allow a more efficient integration of information (C. Allene et al., 2008; Golshani et al., 2009; Khazipov & Luhmann, 2006; Wamsley & Fishell, 2017). The main types of synchronized activity recorded *in vitro* are : Synchronous Plateau Assemblies (SPA), cortical Early Network Oscillations (cENO) and Giant Depolarizing Potentials (GDP) (Allene et al., 2008; Allene & Cossart, 2010; Khazipov & Luhmann, 2006; Luhmann et al., 2016).

All these steps are important for the maturation of cortical circuits, and each type of synchronized activity is regulated by particular processes. SPAs are the earliest monitored form of synchronized activity in the developing brain and they are mediated through gap junctions which can be identified from birth (C. Allene et al., 2008). Later on, cortical dynamics becomes synapse driven. cENO constitute patterns of synchronous activity driven by glutamatergic NMDA signaling. GDP are observed after the cENOs and constitute patterns of synchronous activity driven by GABA_A signaling (Fig. 11) (Allene et al., 2008; Allene & Cossart, 2010; Garaschuk, Linn, Eilers, & Konnerth, 2000). These sequences of patterned activity are also observed in developing human cortical circuits (Khazipov & Luhmann, 2006; Tolonen, Palva, Andersson, & Vanhatalo, 2007; Dreyfus-Brisac C & Larocche 1971).

Recent *in vivo* studies confirmed the presence of these patterns of synchrony in the developing mouse neocortex while providing also information regarding the desynchronization of cortical assemblies. The upper layers pyramidal neurons of S1 present a synchronous

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activation within local clusters of neurons in the first postnatal week (Golshani et al., 2009). During the second postnatal week, the cortical activity desynchronizes and this is independent of the thalamic input (Fig. 11) (Golshani et al., 2009). While Both MGE and L1 CGE-derived interneurons also initially present a synchronized activity patterns, thalamic input is regulating these GABA assemblies and participate to their desynchronization during the beginning of the 2nd postnatal week (Che et al., 2018; Modol et al., 2020).

Therefore, the beginning of the 2nd postnatal week emerges as a time window in which major changes in cortical activity occur. The desynchronization of cortical activity overlaps with several changes in development, like the death of several cells including CRc (see chapter 4). Therefore, this time window seems to be major transition in the wiring of networks and is important to understand whether these phenomena are correlated.

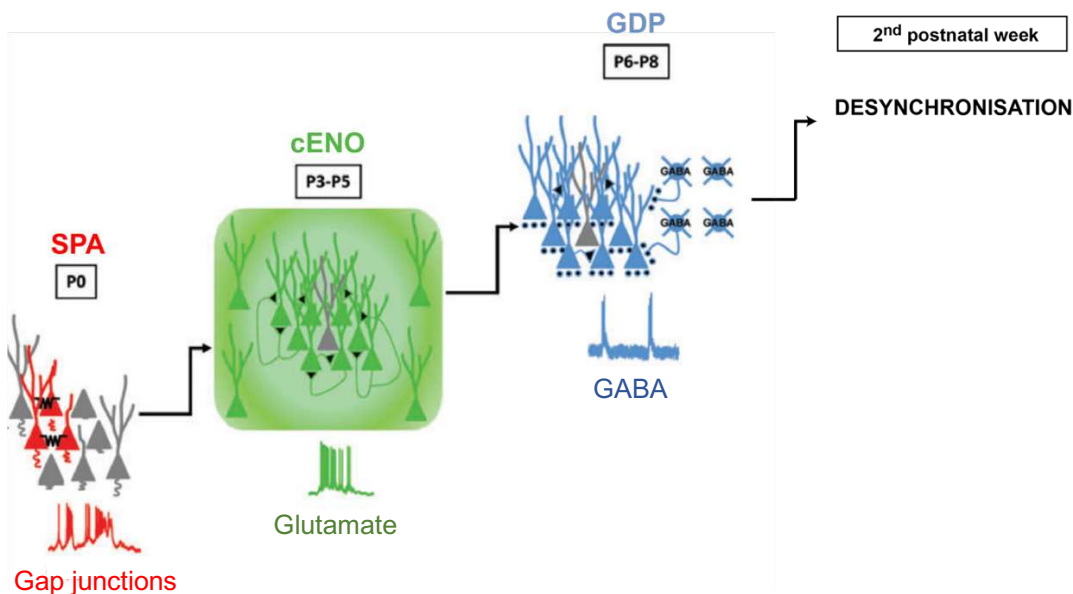


Figure 11 | Emergence of activity in the developing cortex

Schematic representation of the activity patterns in the developing neocortex. At embryonic stages the neocortical activity is decorrelated. At birth, activity becomes correlated and this is mediated via gap-junctions. Later, these patterns evolve to cENO mediated by NMDA receptors, then GDP via GABA receptors, until the desynchronization in the second postnatal week. Adapted from Allene et al., 2008.

4. Programs and electrical activity in neocortical developmental cell death

4.1. Programmed cell death in the developing neocortex

In spite of the energetic cost, neurons are generated in excess during development. While some neuronal subpopulations are scaled down at postnatal stages, some other subpopulations are entirely eliminated (Causeret, Coppola, & Pierani, 2018; Wong & Marín, 2019). What would be the role and through which mechanisms is this elimination taking place? One theory, the 'trophic theory' would be that neurons are competing for limited trophic factors or axonal targets, which leads to the survival of the fittest (Causeret et al., 2018; Fuchs & Steller, 2011; Yamaguchi & Miura, 2015). However, this theory remains to be addressed in the CNS.

The elimination of neurons in development has mostly been characterized through apoptotic pathways and corpse elimination is usually performed by glial cells including professional phagocytes such as microglia and macrophages, or astrocytes (Causeret et al., 2018). Apoptosis is one of the best described mechanism for physiological Programmed Cell Death (PCD), which it is mediated through Bcl signaling which controls the expression of pro-apoptotic genes *Bax* and *Bak*. Their expression leads to the activation of apoptotic proteins like Caspase (Casp) 3 or Casp7, which are responsible for cell death induction (Causeret et al., 2018; Julien & Wells, 2017; Wong & Marín, 2019). However, cell death can also be triggered through non-apoptotic pathways, via different mechanisms including ferroptosis, pyroptosis through inflammation or autophagy (Fricker, Tolkovsky, Borutaite, Coleman, & Brown, 2018; Jung & Chung, 2018).

On the technical detection level, cell death can be assessed or manipulated by several means. The TUNEL method assesses DNA fragmentation associated with most cell death processes, whereas activated Casp3 expression is more specific of apoptosis. In anycase, these methods reveal only a snapshot of dying cells at the moment of the experimentation since death is rapidly followed by corpse elimination (Tawa et al., 2004), thus failing to assess

the whole complexity of cell death if this takes place in a longer time window (Causeret et al., 2018). Other approaches take advantage of genetic models of conditional KO (cKO) of the *Bax/Bak* proapoptotic genes in specific subpopulations of interest to block their apoptotic PCD.

In the neocortex, PCD occurs mainly at postnatal stages leading to the massive or complete elimination of certain populations including CRc and SPn or to the size adjustment of certain populations, for instance pyramidal neurons and interneurons (Wong & Marín, 2019). Irrespective of the neuronal type concerns though, in the neocortex, neuronal PCD peaks between postnatal day 5 (P5) and P7 (Blanquie, Yang, et al., 2017) (Fig. 13).

4.1.1. Substantial elimination of transient neocortical populations

Two main subpopulations that are almost entirely eliminated through PCD are CRc and SPn. In mice, CRc are eliminated starting with the 2nd postnatal week and by P25 almost all CRc have disappeared from the neocortex, while large populations of CRc are maintained in the hippocampus until adulthood. Such elimination in the neocortex is conserved across mammalian species, although recent findings have shown that in humans, there is a small subpopulation of CRc that resists this elimination and are hence called persistent CRc (pCR) (Gundela Meyer & González-Gómez, 2018a). A long-lasting question in the field is to understand the mechanisms underlying the selective disappearance of CRc in the neocortex and not the hippocampus as well as how this feature might change across species. The initial hypothesis proposed either a dilution of CRc resulting from the growth of the neocortex, a trans fate of CRc into RELN⁺ interneurons, or CR elimination through PCD (Causeret et al., 2018; Marín-Padilla, 1990; Parnavelas and Edmundus, 1983). Recent findings demonstrate that subpopulations of CRc, the non-hem derived, are eliminated through PCD via a *Bax*-dependent mechanisms (Ledonne et al., 2016). *Bax/Bak* cKO in CRc using the *DNp73cre* driver which targets 80% of CRc leads to their partial rescue by P21 (Ledonne et al., 2016). However, the same genetic manipulation but this time using the *Wnt3A-cre* driver targeting only hem-derived CRc, does rescue of this subpopulation, suggesting that hem-derived CRc are not eliminated via *Bax*-dependent mechanisms (Ledonne et al., 2016). While the

mechanisms regulating hem-derived CR elimination remain to be characterized, we have so far no information onto why hippocampal CRc, which are also hem-derived, persist until adulthood.

While the roles of SPn in modulating cortical development are becoming increasingly clear (Ghezzi et al., 2020; Hanganu et al., 2002; Luhmann et al., 2018; Marques-Smith et al., 2016), we know less about their elimination and maintenance. The SP, as a structure, has been shown to largely disappear in rodents (Luhmann et al., 2018). While some SPn undergo PCD, some SPn remain and form layer 6B, as supported by the similar morphologies between these two cell types (Marx et al., 2017). Although there is support for some SPn undergoing PCD, the literature contains contradictory information concerning the rate of PCD in this population (Luhmann et al., 2018, Valverde et al., 1989). In humans, the situation is less clear with potentially some neurons maintained in the SP throughout life (Luhmann et al., 2018).

Nonetheless, both CRc and SPn neurons are largely transient, characteristic of developmental stages, where they exert important functions in the wiring of circuits as detailed in the chapters 2.3. and 5.3.

4.1.2. A coordinated downsizing of excitatory and inhibitory neuronal populations

Until recently, the precise timing and rate of PCD in pyramidal neurons was not well established. In the neocortex, PCD occurs mainly during the first postnatal week (Blanquie, Yang, et al., 2017; Wong et al., 2018) and leads to a global reduction in approximately 30% of the pyramidal neuron population (Ferrer, Bernet, Soriano, Del Rio, & Fonseca, 1990; Miller, 1995). More precisely, recent findings revealed that pyramidal neurons PCD peaks between P2 and P5 in the somatosensory cortex and accounts for the elimination of only 13% of pyramidal neurons in this structure (Wong et al., 2018; Wong & Marín, 2019). This death is suggested to be area-dependent, notably with the motor cortex presenting an increased Casp3 signal compared to the somatosensory cortex (Blanquie, Yang, et al., 2017).

Interneurons also undergoes PCD, following pyramidal neurons, leading to an overall reduction of 40% of this neuronal population (Southwell et al., 2012). PCD in cortical

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interneurons peaks around P7, but lasts until the end of the second postnatal week, and relies on apoptotic Bax/Bak signaling. This large elimination of interneurons was shown to be intrinsically determined as it occurs not only *in vivo* but also *in vitro* (Mancia Leon et al., 2020; Southwell et al., 2012). In contrast, recent findings (Wong et al., 2018) show that MGE-derived interneurons die *in vivo* starting at P5 and relies on the death of pyramidal neurons. Indeed, survival of pyramidal neurons through impairment of Bax/Bak apoptotic pathways, rescues interneuron cell death, revealing a remarkable interplay and balance in the numbers of both subpopulations (Wong et al., 2018).

Altogether, the numbers of both pyramidal neurons and interneurons are adjusted in development to ensure a proper cortical function (Fig. 12).

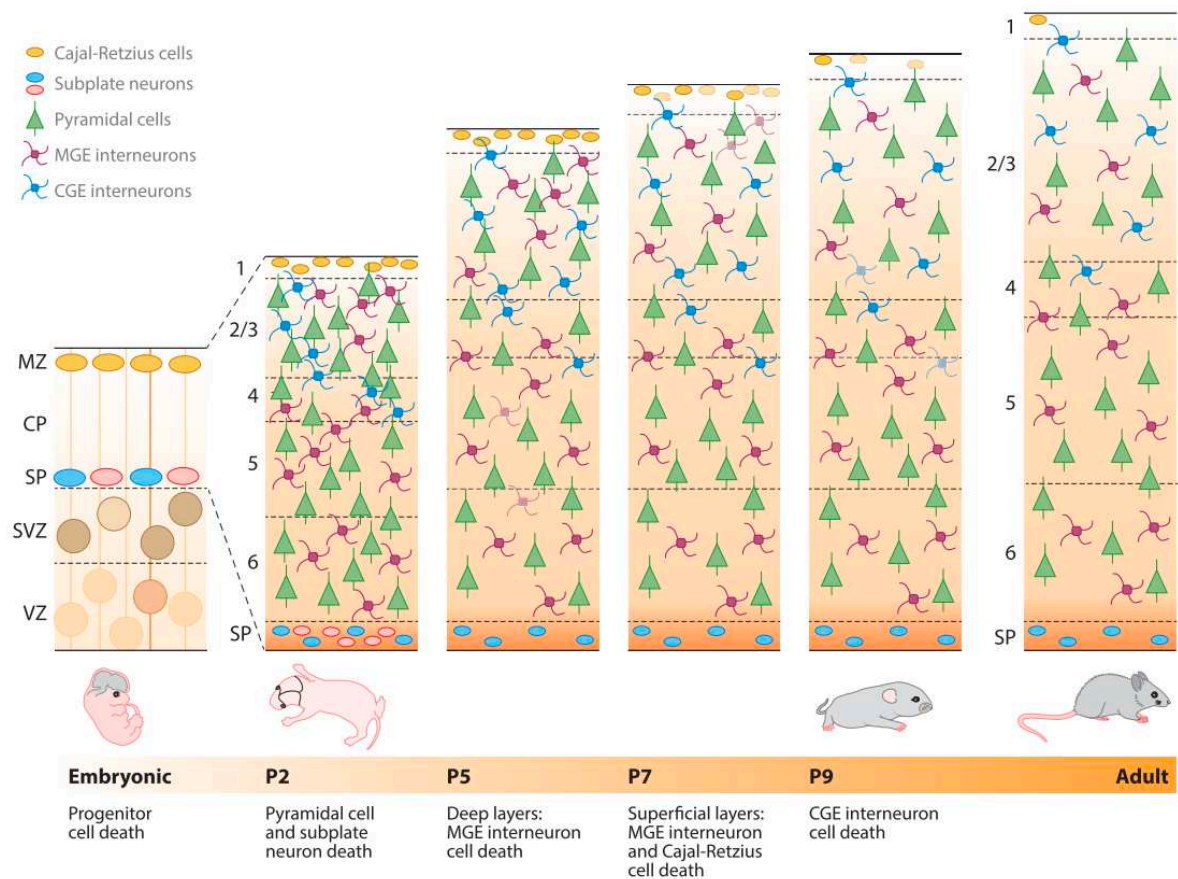


Figure 12 | Developmental timeline of cell death in the neocortex

Schematic representation of the developmental cell death in Cajal-Retzius cells, subplate neurons, pyramidal neurons and interneurons. From Wong & Marin, 2019.

CGE, caudal ganglionic eminence; CP, cortical plate; MGE, medial ganglionic eminence; MZ, marginal zone; SP, subplate; SVZ, subventricular zone; VZ, ventricular zone.

4.2 Electrical activity regulates the death of neocortical neurons

Electrical activity has been proposed for a long time to act as a regulator of PCD in cortical circuits, but has been begun to be recently examined *in vivo*. For cellular populations undergoing down-sizing like pyramidal neurons and interneurons, this has been widely documented with both *in vitro* and *in vivo* approaches. Initial *in vitro* studies have shown that blocking the neuronal activity with the voltage gated Na⁺ channel blocker Tetrodotoxin (TTX) decreases the rate of neuronal survival (Ruijter, Baker, De Jong, & Romijn, 1991) which is consistent with the finding that less active neurons are more prone to elimination (Murase, Owens, & McKay, 2011). Consistently, *in vivo* increased neuronal activity by inducing epileptic seizures decreased the rate of PCD (Blanquie, Yang, et al., 2017). Since pyramidal neuron PCD can vary across different cortices, an intriguing possibility would be that neuronal elimination is dependent on variations in the activity of local circuits (Blanquie, Yang, et al., 2017; Wong & Marín, 2019). Concerning interneurons, their elimination is also mediated through activity-dependent mechanisms, on top of genetic programs (Denaxa et al., 2018; Priya et al., 2018; Southwell et al., 2012; Wong et al., 2018; Wong & Marín, 2019). Using *in vivo* Ca²⁺ imaging, Wong and colleagues showed that less active MGE-derived interneurons are the ones prone to be eliminated through Bax-dependent mechanisms. They furthermore revealed that such activity is driven by pyramidal neurons, which act in a non-cell autonomous manner on the activity of interneurons leading to their elimination or survival (Wong et al., 2018). The elimination of CGE-derived interneurons is also mediated through activity-dependent mechanisms, and the numbers of CGE-derived interneurons is in turn adjusted by the number of MGE-derived interneurons (Denaxa et al., 2018; Priya et al., 2018). Taken together, these studies reveal that the flow of activity through circuits as they assemble is essential to refine neuronal cell numbers, both for excitatory and inhibitory neurons, revealing a remarkable role in shaping mature circuits.

Regarding the CRc and SPNs which are almost completely eliminated, the role of electrical activity in their demise has been debated but remains less established. For SPn, it has been proposed that their elimination might be regulated by activity, however whether they

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undergo apoptotic elimination and/or transmute into L6b pyramidal neurons is yet to be examined (Blanquie, Kilb, Sinning, & Luhmann, 2017; Luhmann et al., 2018). For CRc, both in vitro and in vivo pharmacological experiments support a role for electrical activity in their elimination (Blanquie, Kilb, et al., 2017; Blanquie, Liebmann, et al., 2017; Del Río et al., 1996; S. Kirischuk et al., 2014). Intriguingly, CRc behave in the opposite way compared to pyramidal neurons or interneurons. Briefly, while electrical activity is pro-survival in interneurons, suppression of electrical activity is pro-survival in CRc (Blanquie, Liebmann, et al., 2017; Priya et al., 2018; Wong et al., 2018; Wong & Marín, 2019). *In vitro* experiments on cortical cultures in which electrical activity is completely abolished with TTX application display an increased survival rate of CRc (Blanquie, Kilb, et al., 2017; Blanquie, Liebmann, et al., 2017). This paradoxical situation, which remains to be established in vivo, could potentially be explained by the particular electrophysiological features of CRc (See chapter 5.2) (Blanquie, Liebmann, et al., 2017).

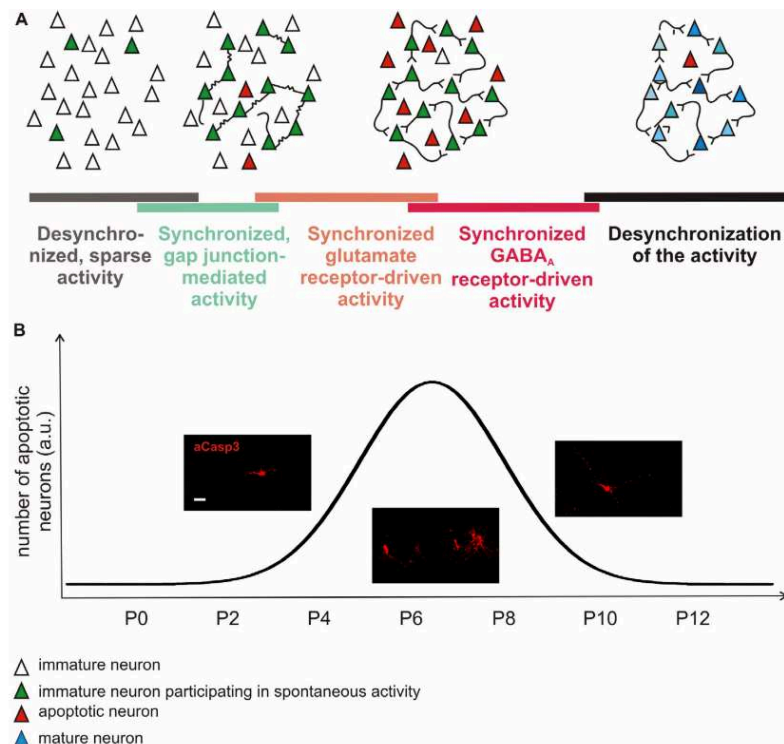


Figure 13 | Interplay between electrical activity and neuronal apoptosis in developing neocortex

(A) Schematic of the sequence of activity-patterns in developing networks. (B) Density of apoptotic neurons in the somatosensory cortex, across the first two postnatal weeks. Note that the pic in the apoptotic neurons coincides with the synaptic-driven activity of cortical assemblies. From Blanquie, Kilb, et al., 2017.

5. Key regulators of cortical layer 1 wiring: Cajal-Retzius cells

5.1. Morphological and molecular characterization of Cajal-Retzius cells

CRc have first been identified by Santiago Ramon y Cajal (1891) and Gustaf Retzius (1893), and were described as superficial cells showing an elongated morphology and covering the surface of the neocortex (Fig. 14) (S. Kirischuk et al., 2014; Marín-Padilla, 1998). CRc are small bipolar neurons located in the neocortical or hippocampal L1. As mentioned before, neocortical CRc are transient and almost completely eliminated by postnatal stages, while the elimination of hippocampal CRc is considerably delayed and some cells persist until adulthood (Anstötz et al., 2016; Anstötz, Quattrocchio, & Maccaferri, 2018). Cardinal features of CRc are their superficial localization and their capacity to express the secreted glycoprotein RELN, which is required for inside-out cortical development (D'Arcangelo et al., 1995; Kwan, Šestan, & Anton, 2012). CRc are also characterized by the fact that they are early-born, glutamatergic neurons and express several additional markers including Tbr1, Calretinin, Δ Np73, Ebf2 and Ebf3, which can vary depending on their site of production, as mentioned before (Chowdhury, 2010; de Frutos et al., 2016; S. Kirischuk et al., 2014; Sun et al., 2019; Fadel Tissir et al., 2009). To identify CRc at early embryonic stages, their pial localization and RELN expression is necessary and sufficient, whereas at postnatal stages, RELN starts to be expressed also in L1 CGE-derived interneurons. Thus a precise identification of CRc requires the expression of RELN and the absence of expression of L1 interneuron markers (for example Prox1 or simply GABA)(D'Arcangelo, Lossi, & Merighi, 2017; S. Kirischuk et al., 2014; Soriano & Del Río, 2005) (see also Table 1, in chapter 2.2.1).

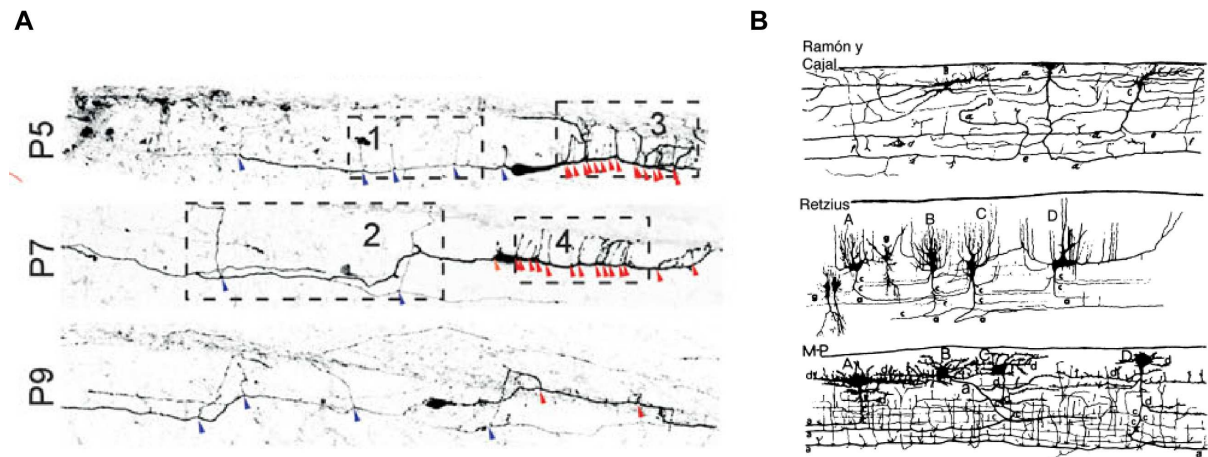


Figure 14 | Morphology of Cajal-Retzius cells in neocortical layer 1

(A) Morphology of mouse CRc in neocortical layer 1 at P5, P7 and P9. Note elongated morphology with projections restricted to cortical L1. From Sun et al., 2019. (B) Drawing from Santiago Ramon y Cajal (1911) of the morphology of CRc from the motor cortex of a two-months old infant (up). Drawing from Gustaf Retzius (1894) of the morphology of CRc from the motor cortex of a twenty-six old fetus (middle). Drawing from Miguel Marín-Padilla (1894) of the morphology of CRc from the motor cortex of a newborn (1892). From Marín-Padilla, 1998 and Martínez-Cerdeño & Noctor, 2014.

5.2. Cajal-Retzius cells are embedded in functional circuits

Neocortical CRc are electrically-active glutamatergic neurons (Jean Marc Mienville, 1998). These cells express the glutamatergic receptors mGluR and NMDAR and postnatally are predominantly innervated by GABAergic inputs (Aguiló et al., 1999; Anstötz et al., 2014; L. A. Cocas et al., 2016; J M Mienville & Pesold, 1999; Riva et al., 2019). Intriguingly though, CRc present throughout their lifetime characteristics of “immature neurons” that never reach the mature state: i) CRc do not present the developmental switch in NMDA receptor subunits ratio NR2B to NR2A (J M Mienville & Pesold, 1999), ii) CRc do not present the developmental switch in the expression of Cl⁻ transporters NKCC1 to KCC2 (Pozas, Paco, Soriano, & Aguado, 2008), and therefore iii) GABAergic inputs to CRc are depolarizing in vitro (Achilles et al., 2007; Jean Marc Mienville, 1998).

CRc are electrically active neurons that are transiently embedded in functional cortical circuits (Laura A Cocas et al., 2016; S. Kirischuk et al., 2014; Sava et al., 2010; Soda et al., 2003). In the L1, CRc receive inputs since early developmental stages in mice (E15), from both serotonergic and noradrenergic projections (Janusonis, 2004; Naqui, Harris, Thomaidou, &

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Parnavelas, 1999) as well as GABAergic inputs from the Subplate (Myakhar, Unichenko, & Kirischuk, 2011). It is not yet clear whether the glutamatergic pyramidal Subplate neurons projecting in L1 are also connecting the CRc or not (Ghezzi et al., 2020). CRc are also innervated by Glutamatergic (Laura A Cocas et al., 2016) and GABAergic inputs, but the origins of these inputs are not fully characterized (Anstötz et al., 2014; S. Kirischuk et al., 2014; Soda et al., 2003; Sun et al., 2019). Martinotti cells projecting to L1 have been shown to connect onto CRc (L. A. Cocas et al., 2016; Cosgrove & MacCaferrri, 2012), GABAergic fibers from the ZI project into L1 but a clear evidence for their connections with CRc is not established (Chen & Kriegstein, 2015). As for L1 CGE-derived interneurons, precise data concerning their connectivity is also missing. In addition to the little information that we have on upstream connectivity of CRc, we know also very little on their downstream targets. CRc have been suggested to output onto L1 apical dendrites (Marín-Padilla, 1998), but their post-synaptic targets still remain largely unknown and constitute an active line of research.

CRc are also embedded in development in transient circuits of the olfactory cortex (de Frutos et al., 2016), and also in adults in hippocampal circuits (Anstötz et al., 2014, 2018; Quattrocolo & Maccaferri, 2014). Notably, in the hippocampus where their connectivity has been the most described, CRc are innervated by NGF_c and from Oriens-Lacunosum/Moleculare interneurons cells in the Molecular Layer and, in turn, they output on on pyramidal neurons and on NGF_c (Fig. 15) (Anstötz et al., 2018; Quattrocolo & Maccaferri, 2014). Whether a similar circuit involving CRc is present also in L1, remains to be elucidated.

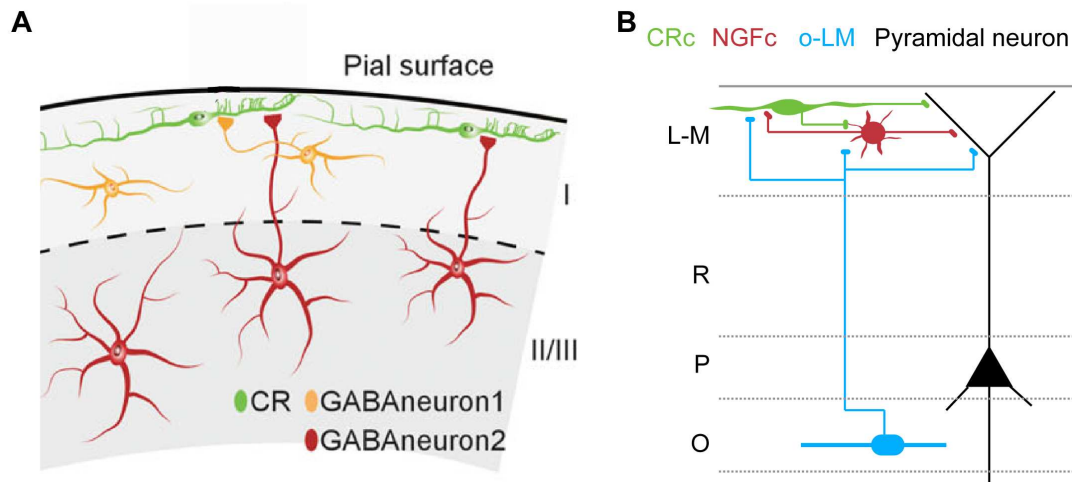


Figure 15 | Input/output connectivity of Cajal-Retzius cells in the neocortical layer 1 and hippocampus

(A) Schematic representation of Cajal-Retzius cells (CRc) in the circuits of neocortical L1. From Sun et al., 2019. **(B)** Schematic representation of input and output connectivity of CRc in the hippocampus. Adapted from (Anstötz et al., 2018; Quattrocolo & Maccaferri, 2014). NGFc, neurogliaform cells; L-M, Stratum lacunosum moleculare; O, Stratum oriens; O-LM, Oriens lacunosum moleculare interneurons; P, Stratum pyramidale; R, Stratum radiatum.

5.3. Functions of Cajal-Retzius cells in the wiring of upper cortical layers

Throughout their lifetime, due to their secreted factors and to their strategical superficial position, neocortical CRc have been proposed to guide the development of the cortex, shaping many important steps in the process. Most of their roles have been associated with RELN secretion, since the CRc are the main source of RELN in the embryonic and early postnatal brain (D'Arcangelo et al., 1995).

First, CRc have been shown to regulate pyramidal neuron radial migration through the expression of RELN binding to the $\alpha3\beta1$ integrin (Michael Frotscher, 1997, 1998; S. Kirischuk et al., 2014) and of Nectin1 adhesion molecule which is binding to Nectin 3, altogether modulating radial migration (Gil-Sanz et al., 2013). This is consistent with the fact that mutants for RELN receptors VLDLR and ApoER2 (Hirota et al., 2015), show impairments in cortical lamination (Hirota, Kubo, Fujino, Yamamoto, & Nakajima, 2018; Hirota & Nakajima, 2020), as well as the reeler mutant, which presents a spontaneous mutation in the *Reelin* gene, presents an inverted outside-in cortex (D'Arcangelo et al., 1995; Michael Frotscher, 1998; Kwan et al.,

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2012; Ogawa & Miyata, 1995). Besides the role on pyramidal neuron migration, CRc are also modulating the radial glia apical endfeet in L1: drastic elimination of CRc through application of domoic acid on the cortical surface of newborn mice, leads to a decrease in the density of radial glia endfeet and an increase in the number of astrocytes in upper cortical layers (Super, 2000). Not only CRc could, through RELN secretion modulate radial migration, but actually the specific distribution of CRc subtypes across the cortical sheet, modulates cortical areal patterning as shown by the impact on the size of higher order cortical areas (Barber et al., 2015; Griveau et al., 2010). Besides these evidence relating CRc and RELN with the migration of pyramidal neurons and cortical identity, Yoshida et al., 2006 show that ablation of hem-derived CRc (which represent a majority of CRc) does not affect the lamination of the cortex. Non-exclusive explanations of this fact could be either that i) several homeostatic mechanisms could induce a compensation by the other subpopulations of CRc (Griveau et al., 2010), ii) the remaining CRc upregulate their level of RELN secretion or that iii) indeed physiologically, low RELN levels are sufficient to induce proper pyramidal neuron migration and lamination of the neocortex.

Second, the density of CRc at embryonic and postnatal stages seems more important for the regulation of interneuron densities, axonal development in L1 as well as morphology of the apical dendrites of upper layer cortical neurons. Since embryonic stages, only a 30% decrease in CRc density is correlated with increased numbers of CGE-derived interneurons in the MZ, and decreased axonal projections in L1, while an increase in the density of CRc in *Ebf3*^{-/-} mutants has the opposite effects (de Frutos et al., 2016). Importantly, a decreased density in CRc during their lifetime has long lasting consequences on the wiring of neocortical circuits, even after the almost complete disappearance of CRc: upper layers pyramidal neurons present an impaired E/I ratio driven by a decreased excitation and a small increase in the inhibitory current received, correlated by a decreased spine density on the apical dendrites of these neurons (de Frutos et al., 2016). However, it still remains to be deciphered whether defects in CRc density have long-lasting impacts on interneurons and apical dendrites of deep pyramidal neurons. Or, in other words, whether CRc modulate L1 inputs on apical tufts

of all pyramidal layers or selective subpopulations. In addition, it will be important to determine what is the nature of the excitatory inputs on apical dendrites which are regulated by CRc – thalamic, cortical or both - helping providing key information the specificity of in L1.

Altogether, the CRc have key roles in the development of cortical circuits, as impairments in CRc density shapes both the excitatory and inhibitory components of the developing brain. It is thus essential to understand how the CRc density is maintained and how these “surplate neurons”, orchestrate the intricate wiring of cortical layer 1.

5.4. Cajal-Retzius cells in human neocortical development

Using RELN expression and a superficial localization in the pallium, CRc were observed in many species from lizards (Goffinet et al., 1999), turtles (B. Bernier et al., 1999), crocodiles (F. Tissir, De Rouvroit, Sire, Meyer, & Goffinet, 2003), chick (Béatrice Bernier, Bar, D’Arcangelo, Curran, & Goffinet, 2000) as well as in rodents, mammals (Ábrahám, Tóth, Bari, Domoki, & Seress, 2005) non-human and human primates (Gundela Meyer & González-Gómez, 2018a). Whereas in mammals they are located superficially in the entire pallium, in chick embryos CRc are restricted to medial pallial regions and olfactory pallial regions, potentially related to their lack of migratory capacities (García-Moreno et al., 2018).

In humans, subsets of CRc are not entirely eliminated and subpopulations persist in the neocortex (Gundela Meyer & González-Gómez, 2018a), revealing an increased heterogeneity and complexity of CRc with evolution (Fig. 14). In humans, CRc have been observed from gestational week (GW) 5, just after the formation of the telencephalic vesicles, being amongst the first neuronal populations generated. Initially most of the CRc are generated in the septum and PSB, and by the GW8 CRc start being generated also from the cortical hem which is increasingly developed in humans, while the thalamic eminence is a less abundant source of CRc in humans (González-Gómez & Meyer, 2014; Gundela Meyer, 2010; Gundela Meyer & González-Gómez, 2018a). Interestingly, the dynamics of life and death of CRc is very different in humans compared to rodents. In humans, most of CRc generated at early developmental stages undergo complete elimination through PCD by GW22-24, hence called

transient CRc (tCRc). At this developmental stage, pyramidal neurons and interneurons have already been generated and migrated into the CP (Gundela Meyer & González-Gómez, 2018b). This population of tCRc seems to be replaced by a new subpopulation of persistent CRc (pCRc), which can be found at low density in the MZ of postnatal human brains throughout life (Gundela Meyer, 2010; Gundela Meyer & González-Gómez, 2018a, 2018b). pCRc have similar molecular identity as the tCRc, but are smaller cells and present a less complex morphology (G. Meyer, 1999; Meyer & González-Gómez, 2018). These cells could indeed come from the retrobulbar area and hence, show similarity with the CRc remigrating from the mouse LOT. However, one could wonder what might be the evolutionary advantage of generating one population of cells which is entirely eliminated to be replaced by a similar one? Gundela Meyer & González-Gómez, 2018a proposed that this could be related to adaptability to cortical function. In early development, tCRc present complex axonal morphologies, are highly secreting RELN and are homogeneously distributed on the surface of the cortex, thereby coordinating the processes of neuronal migration and network wiring. In a second step, pCRc which have a way less complex morphology could contribute to MZ integrity in the growing human brain, potentially having only local roles in postnatal brain development, like for example spine formation. Reexploring human embryos in details, exploring other species and perform cross-species cell transplants will be essential to provide strong support for hypotheses on the roles of these cells in brain evolution.

5.5. Potential roles of Cajal-Retzius cells and Reelin in neurodevelopmental disorders

CRc have been associated either directly through their density or indirectly through RELN secretion, to the etiology of several neurodevelopmental disorders.

First, the density of pCRc is increased in patients suffering from polymicrogyria (Eriksson et al., 2001) and in patients suffering from epilepsy (Ishii, Kubo, & Nakajima, 2016, Gabarelli et al., 2001). These findings have raised the possibility that abnormal elimination or activity of

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CRC could contribute to neurodevelopmental disorders. The potential impact of CRC density is consistent with the recent studies in mice linking CRC density with consequences on neocortical E/I balance (de Frutos et al., 2016). Therefore, these pathologies confirm a potential long-term role of CRC in maintaining a proper E/I ratio required for normal brain function (Oscar Marín, 2012; Nelson & Valakh, 2015)

Second, RELN levels have been correlated with a multitude of neuropsychiatric disorders including Epilepsy, Schizophrenia, Psychosis, ASD or Bipolar Disorder, but it is difficult to discriminate whether these pathologies are due to CRC, to RELN secreting interneurons or to both (Folsom & Fatemi, 2013; Ishii et al., 2016; Fadel Tissir & Goffinet, 2003). In particular, both males and females patients of ASD present increased plasma levels of RELN, which could originate in a perturbed density of elimination of CRC, impairments in RELN secreting interneurons or even an alternative systemic production of this secreted protein (Cuchillo-Ibáñez, Andreo-Lillo, Pastor-Ferrándiz, Carratalá-Marco, & Sáez-Valero, 2020). Genetic support comes from Single Nucleotide Polymorphism (SNP) in the *Reelin* gene which has been associated with increased risk of developing ASD (Wang et al., 2014). Polymorphic GGC repeats sited in the 5' untranslated region of the RELN were associated with autistic disorder, while specific SNP in *Reelin* gene have been associated with increased risk of developing Bipolar Disorders notably in women (Ishii et al., 2016). Consistently, both heterozygouts (rl/+) and homozygouts (rl/rl) reeler mouse models present a certain degree of cognitive and behavioral impairments (Tueting et al., 1999). Moreover, mutations in the *Reelin* locus are also associated in human patients with neurodevelopmental defects linked to neuronal migration like lissencephaly (Hong et al., 2000).

Using mice to dissect the relative contribution of CRC to RELN function will thus be of great interest, not only to progress in the understanding of brain wiring and evolution, but also to link the CRC to the etiology of neurodevelopmental disorders

OBJECTIVES

Building of a functional cerebral cortex relies on the assembly of circuits, in which transient cells, transient connections and early activity are emerging as essential intermediate steps. Still, the roles of transient circuits, how they are remodeled during development or how activity contributes to changes in circuitry remain largely to be deciphered. During my thesis, I tackled these issues in the context of Cajal-Retzius cells (CRc). CRc are early-born layer 1 neurons that form transient circuits until their postnatal massive elimination, and that were shown to regulate some aspects of layer 1 wiring, a major site of input integration. While CRc demise and roles were proposed to rely on neuronal activity, the relative interplay between activity, CRc life and functions have remained elusive. To tackle these issues, I took advantage of genetic models and manipulations in mice to address the following specific questions:

1. Does activity of Cajal-Retzius cells regulate their elimination? (Article 1)
2. To what extent the timed elimination of Cajal-Retzius cells is required for proper brain wiring? (Article 1)
3. Does sensory input drive the activity-dependent elimination of CR cells (Article 2)?
4. Do Cajal-Retzius cells and early sensory activity impact onto the wiring of excitatory entries and inhibitory circuits in cortical layer 1? (Article 2)
5. What is the long-term impact of a reduction in CRc density on the wiring of upper cortical layers? (Article 2)

By addressing these questions, we have found that there is a remarkable interplay between activity and CRc. First, the elimination of Cajal-Retzius cells is activity dependent and impairments in their elimination lead to perturbations of upper layers cortical circuits. Second, during their lifetime, the Cajal-Retzius cells, together with sensory activity, shapes the wiring of cortical layer 1. These findings are instrumental in understanding how transient cells and activity shape the formation of an essential but understudied layer of the neocortex.

RESULTS

RESULTS PRESENTATION

The results of my thesis are presented as two articles, the first one published and the second one in preparation. The last part of this section is a review on cortical layer 1 wiring.

The first article is in close collaboration with the teams of Alessandra Pierani and Maria-Cecilia Angulo (Riva, Genescu, Habermacher et al., 2019). As it has been previously shown that subtypes of CRc die through *Bax*-dependent apoptosis (Ledonne et al, 2016), we questioned what are the mechanisms driving CRc death and what are the consequences of impaired death of CRc on cortical wiring. We have found that the subtypes of CRc that die through *Bax*-mediated apoptosis, die also through activity-dependent mechanisms. Intriguingly, impaired elimination of CRc induces an increase in the excitatory entries on upper layers pyramidal neurons, also in an activity-dependent manner. These results show that the elimination of transient CRc is required for maintaining a proper brain function. In this project, I have performed the experiments on the rescued hyperpolarized CRc (Kir2.1 model), analyzing their rate of survival, the morphology of rescued CRc, as well as 3D reconstructions and morphology analysis in of the upper layers pyramidal neurons in this model.

In the second article (Genescu et al., *in preparation*), studied the contribution of CRc and early sensory activity to the wiring of L1. The early-born CRc are colonizing the surface of the neocortex and were shown to be key in cortical development. With a combination of genetic tools, I show that CRc density together with early sensory activity shape the formation of L1 at early postnatal stages. Intriguingly, the two are not directly related, as early sensory activity, is not impacting on the CRc density. While early sensory activity has a long lasting impact only on the interneuron numbers in L1, CRc density is shaping at long term, both the interneuron numbers and the excitatory entries in L1. These results suggest a key role of CRc and early sensory activity in the formation of an essential but understudied layer of the neocortex. In this project, I performed all the experiments together with the help of Caroline Mailhes-Hamon for viral injections (not present in this manuscript), under the supervision of Sonia Garel.

Article 1

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Activity-dependent death of transient Cajal-Retzius neurons is required for functional cortical wiring

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Abstract Programmed cell death and early activity contribute to the emergence of functional cortical circuits. While most neuronal populations are scaled-down by death, some subpopulations are entirely eliminated, raising the question of the importance of such demise for cortical wiring. Here, we addressed this issue by focusing on Cajal-Retzius neurons (CRs), key players in cortical development that are eliminated in postnatal mice in part via Bax-dependent apoptosis. Using Bax-conditional mutants and CR hyperpolarization, we show that the survival of electrically active subsets of CRs triggers an increase in both dendrite complexity and spine density of upper layer pyramidal neurons, leading to an excitation/inhibition imbalance. The survival of these CRs is induced by hyperpolarization, highlighting an interplay between early activity and neuronal elimination. Taken together, our study reveals a novel activity-dependent programmed cell death process required for the removal of transient immature neurons and the proper wiring of functional cortical circuits.

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Introduction

An emerging player in the assembly of neuronal networks is programmed cell death (PCD). In the nervous system, programmed cell death (PCD) fine-tunes the density of neuronal populations by eliminating 20–40% of overproduced neurons (*Fuchs and Steller, 2011; Causeret et al., 2018; Wong and Marín, 2019*). Only few populations of the mouse cerebral cortex almost completely disappear during the first two postnatal weeks. Amongst these, Cajal-Retzius cells (CRs), the first-born cortical neurons lying in the superficial Layer I (LI), undergo extensive cell death in the mouse during the second postnatal week (*Ledonne et al., 2016*). The persistence of CRs during postnatal life is increased in malformations of cortical development (MCDs) and epilepsies thereby opening the intriguing possibility that the maintenance of CRs contributes to the dysfunction of cortical circuits (for review see *Luhmann, 2013*).

CR play pivotal roles at multiple steps of early cortical development, in addition to their best-known role in the control of radial migration (*Ishii et al., 2016*). They comprise three molecularly distinct subtypes which migrate from different sources that surround the cortical primordium: (i) septum

and eminentia thalami-derived CRs of the Δ Np73/Dbx1 lineage (SE-CRs); (ii) hem-derived CRs of the Δ Np73/Wnt3a lineage (hem-CRs); (iii) pallial-subpallial boundary-derived CRs of the Dbx1 lineage (PSB-CRs) (Bielle et al., 2005; Yoshida et al., 2006; Tissir et al., 2009). Our previous work revealed that their specific embryonic distribution in distinct territories plays key functions in wiring of cortical circuits by controlling the size of functional areas, both primary and higher-order, as well as targeting of thalamocortical afferents (Griveau et al., 2010; Barber et al., 2015; Barber and Pierani, 2016). More recently, we showed that subtype-specific differences also exist in their elimination during early postnatal life, with SE- but not hem-derived CRs dying in a Bax-dependent manner, which is a critical player of the apoptotic pathway (Ledonne et al., 2016).

Before their disappearance, CRs express ionotropic glutamatergic and GABAergic receptors and are embedded into immature circuits where they mainly receive GABAergic synaptic inputs, suggesting that these transient cells might have an activity-dependent role in the development of cortical networks (Kirischuk et al., 2014). Consistently, we found that CRs density shapes axonal and dendritic outgrowth in LI and impacts onto the excitation/inhibition (E/I) ratio in upper cortical layers (de Frutos et al., 2016). Conversely, activity was also proposed as one of the drive of CR demise. Studies from Del Río et al. (1996) first highlighted the role played by electrical activity on CR death in vitro. CRs were shown to display electrophysiological features of immature neurons (Kirischuk et al., 2014; Barber and Pierani, 2016), including the persistent depolarizing action of GABA which was suggested to depend on the maintenance of the chloride inward transporter NKCC1 and the absence of the outward transporter KCC2 (Mienville, 1998; Achilles et al., 2007; Pozas et al., 2008). Interestingly, pharmacological inhibition of activity and GABA signaling in vitro and global inactivation of NKCC1 in vivo were shown to reduce the death of CRs (Blanquie et al., 2017a; Blanquie et al., 2017b). However, very little is known on the role of electrical activity in CR subtype-specific death in vivo, as well as its contribution to the construction of functional and dysfunctional cortical circuits.

Here we show that hyperpolarization of CR neurons by Kir2.1-dependent expression prevented cell death of Δ Np73- but not Wnt3a-derived CRs, corresponding to SE-CRs. By comparing two different mutants in which CR death was similarly rescued, we found that abnormal SE-CR survival promotes exuberance of dendrites and spine density in pyramidal neurons in an activity-dependent manner. This results in an E/I imbalance due to an increase in the excitatory drive of upper cortical neurons. Our findings show that neural activity is involved in CR subtype-specific death and argue in favor of an unappreciated role of the disappearance of these transient neurons in controlling the morphology of pyramidal neurons and the functional properties of their cortical excitatory networks at postnatal stages.

Results

Subsets of CRs die in an activity-dependent manner

The Δ Np73^{cre/+} mouse line targets approximately 80% of CRs, namely hem-CRs (Wnt3a lineage) and SE-CRs (Tissir et al., 2009; Griveau et al., 2010; Yoshida et al., 2006). Δ Np73-CRs, but not Wnt3a-derived hem-CRs, were shown to be eliminated postnatally via Bax-dependent death, indicating that SE-CRs undergo apoptosis. However, the trigger of such apoptosis or the mechanisms regulating the death of other CR populations are still unknown (Ledonne et al., 2016). To decipher whether activity might regulate the death of specific subsets of CRs in vivo, we first overexpressed the hyperpolarizing channel Kir2.1 (R26^{Kir2.1mcherry/+}) (Moreno-Juan et al., 2017) using the Δ Np73^{cre/+} line. Tracing of CRs at several postnatal stages in control and Kir2.1-expressing mice was performed using DsRed immunostainings to visualize tdTomato and mcherry reporters, respectively. We found that the density of CRs in the somatosensory barrel cortex was unchanged in these animals at postnatal day 7 (P7), that is before CRs undergo massive cell death (Figure 1A and B). In contrast, Kir2.1 channel overexpression in Δ Np73-CRs resulted in an increase of CRs with respect to controls in the somatosensory cortex at P15 and P25 (Figure 1A and B). Importantly, using whole-cell recording, we checked that rescued CRs were, as expected, hyperpolarized and displayed a decrease in the input resistance without exhibiting changes in action potential properties at both P15 and P25 (Figure 1—figure supplement 1A-C). Biocytin-filling and immunostaining further revealed that most morphological properties were preserved in Kir2.1-expressing CRs, apart from a reduced soma at

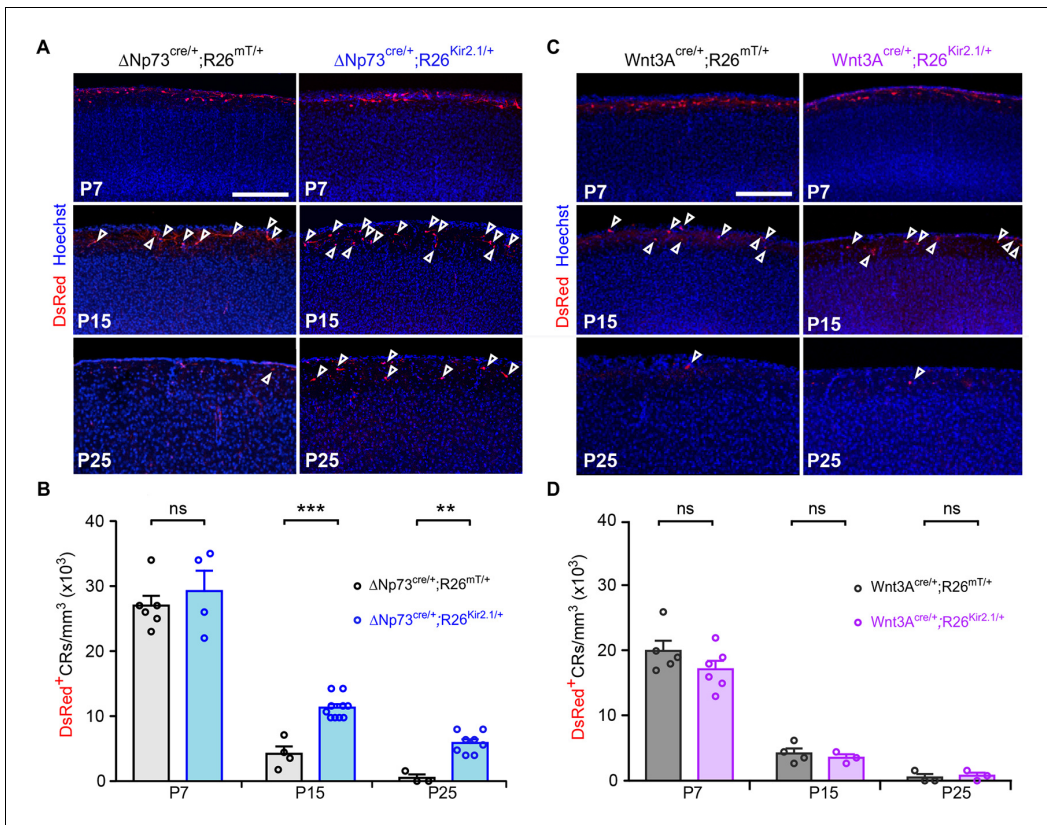


Figure 1. Hyperpolarization induces the survival of SE-CRs. (A) Confocal images of cortical sections from P7, P15 and P25 $\Delta Np73^{cre/+};R26^{mT/+}$ controls (left) and $\Delta Np73^{cre/+};R26^{Kir2.1/+}$ mutants (right) stained for DsRed (red) and Hoechst (blue). Arrowheads indicate DsRed⁺ CRs at P15 and P25. (B) Quantification of CR density (CRs/mm³) at the pial surface in the somatosensory (S1) cortex (P7: n = 6 for controls and n = 4 for mutants, p=0.716; P15: n = 4 for controls and n = 10 for mutants, p=0.001; P25: n = 3 for controls and n = 8 for mutants, p=0.006). (C) Confocal images of cortical sections from P7, P15 and P25 $Wnt3a^{cre/+};R26^{mT/+}$ controls (left) and $Wnt3a^{cre/+};R26^{Kir2.1/+}$ mutants (right) stained for DsRed (red) and Hoechst (blue). Arrowheads indicate DsRed⁺ CRs at P15 and P25. For simplicity as there are too many CRs at P7 arrowheads were not included. (D) Quantification of CR density (CRs/mm³) at the pial surface in the somatosensory (S1) cortex (P7: n = 5 for controls and n = 6 for mutants, p=0.2251; P15: n = 4 for controls and n = 3 for mutants, p=0.771; P25: n = 3 for controls and n = 3 for mutants, p=0.813). Mann-Whitney U Test. Scale bar represents 200 μ m. Data used for quantitative analyses as well as the numerical data that are represented in graphs are available in **Figure 1—figure supplement 1—source data 1**. The online version of this article includes the following source data and figure supplement(s) for figure 1:

Figure supplement 1. Electrophysiological properties and morphology of rescued CRs in $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mice.

Figure supplement 1—source data 1. Density and properties of CRs in the Kir2.1 model.

P25 (**Figure 1—figure supplement 1D**). In particular, rescued CRs displayed similar branching length in LI (**Figure 1—figure supplement 1B and E**) and co-expressed Reelin (Reln) from P7 to P25 (**Figure 1—figure supplement 1F and G**). Taken together, these experiments show that hyperpolarization of $\Delta Np73$ -CRs does not drastically alter cardinal morphological features of CRs but prevents their complete elimination.

A similar proportion of CRs were rescued either by hyperpolarization (**Figure 1A and B**) or by preventing apoptosis (**Ledonne et al., 2016**), raising the intriguing possibility that the same subpopulation of CRs, SE-CRs, might be preserved in both conditions. Since hem-CRs are not eliminated via a Bax-dependent process (**Ledonne et al., 2016**), we investigated whether their survival is sensitive to hyperpolarization. We thus overexpressed Kir2.1 specifically in hem-CRs using the $Wnt3a^{cre/+}$, which corresponds to about 70% of the $\Delta Np73$ -CRs at early postnatal stages (**Figure 1C and D**). We found that hem-CR death was unaffected in this mouse line (**Figure 1C and D**). Moreover, taking into account cortical growth, the proportions of rescued cells in the somatosensory cortex at P25 was evaluated to approximately 30% of the initial pool of $\Delta Np73$ -CRs, which corresponds to the expected number of SE-CRs (**Bielle et al., 2005; Yoshida et al., 2006; Tissir et al., 2009**).

Collectively, these results show that the death of a specific subset of $\Delta Np73^{cre/+}$ SE-CRs is both Bax-dependent and activity-dependent.

CRs rescued by hyperpolarization or blocking Bax-dependent apoptosis are integrated in neuronal circuits

It has been established that CRs are integrated in functional circuits early in the developing postnatal neocortex (Kilb and Luhmann, 2001; Soda et al., 2003; Sava et al., 2010; Cocas et al., 2016). CRs receive GABAergic synaptic inputs and, despite the expression of NMDA receptors (NMDARs) on CR membranes, the presence of NMDAR-mediated synaptic responses is still under debate (Kilb and Luhmann, 2001; Soda et al., 2003; Sava et al., 2010; Schwartz et al., 1998; Mienville and Pesold, 1999; Radnikow et al., 2002; Anstötz et al., 2014). To test whether CRs harbored functional GABAergic and/or glutamatergic synapses during the cell death period and after their rescue, we recorded spontaneous (sPSCs) and evoked postsynaptic currents (ePSCs) of fluorescent CRs with a KCl-based intracellular solution. First, at P9-11 in control $\Delta Np73^{cre/+}; R26^{mt/+}$ mice, sPSCs sensitive to the GABA_A receptor (GABA_AR) antagonist SR95531 (10 μ M) were observed in CRs held at -60 mV, confirming that CRs are innervated mainly by functional GABAergic synaptic inputs (Figure 2—figure supplement 1A–B; data not shown for SR95531 application; $n = 7$). Low-frequency stimulations in LI easily elicited ePSCs that were also completely blocked by SR95531 application (Figure 2—figure supplement 1C–D), even in 0 mM Mg²⁺ condition, which relieves the Mg²⁺ block of NMDA receptors. Taken together, these results indicate that ePSCs were mediated by GABA_ARs and not by AMPA or NMDA receptors. In agreement with previous studies (Sun et al., 2019), the presence of GABAergic synaptic inputs on CRs was confirmed by immunostainings against the presynaptic marker GAD65/67 and the postsynaptic marker Gephyrin (Figure 2—figure supplement 1E). Since the depolarizing action of GABA in CRs was proposed to be partly due to the lack of expression of KCC2, the chloride transporter responsible for maintaining a low intracellular chloride concentration (Mienville, 1998; Achilles et al., 2007; Pozas et al., 2008; Blanquie et al., 2017a; Blanquie et al., 2017b), we also checked for the protein expression of this transporter. In agreement with previous reports (Achilles et al., 2007; Pozas et al., 2008), control GFP⁺ CRs in $\Delta Np73^{cre/+}; Tau^{GFP/+}$ mice expressed very low to undetectable levels of KCC2 (Figure 2—figure supplement 1F). Thus, at the moment of an activity-dependent death, CR cells receive solely GABAergic synaptic inputs.

We further explored whether these inputs are maintained at later stages, when CR cells are not eliminated. To this aim, we performed the same experiments at P23-28 in $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ and $\Delta Np73^{cre/+}; Bax^{lox/lox}; R26^{mT/+}$ mice (Figure 2A–B). First, we found that sPSCs in rescued CRs had similar frequencies, amplitudes and kinetics in both models (Figure 2A–B). Moreover, sPSCs were completely abolished by GABA_AR antagonist SR95531, indicating that rescued CRs remained innervated by functional GABAergic synaptic inputs (data not shown for SR95531 application, $n = 8$ and $n = 5$ for $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ and $\Delta Np73^{cre/+}; Bax^{lox/lox}; R26^{mT/+}$ mice, respectively). Consistently, the complete block of ePSCs by SR95531, even in 0 mM Mg²⁺, revealed that ePSCs were exclusively mediated by GABA_ARs at hyperpolarized holding potentials, as observed in younger mice (Figure 2C–D and Figure 2—figure supplement 1C–D). While these GABAergic inputs were preserved in rescued CRs, we observed a reduced sPSC frequency and ePSC amplitude compared to P9-P11 mice, suggesting a decreased CR connectivity in the more mature neocortex (Figure 2A–B and Figure 2—figure supplement 1A–B).

Altogether, these results indicate that rescued CRs, as previously showed in the early postnatal neocortex (Soda et al., 2003; Sava et al., 2010; Kilb and Luhmann, 2001), receive solely GABAergic synaptic inputs at the time of their death. They further demonstrate that rescued CRs in both mouse models, although displaying a reduced connectivity compared to earlier stages, are kept integrated in functional neuronal circuits.

Survival of electrically-active CRs triggers dendritic exuberance of upper layer pyramidal neurons

In order to test whether CR aberrant survival may alter the function of other neurons in upper cortical layers, we used biocytin-filling and confocal 3D reconstructions to study the morphology of Layer II (LII) and LIII pyramidal neurons in the somatosensory barrel cortex of both $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$

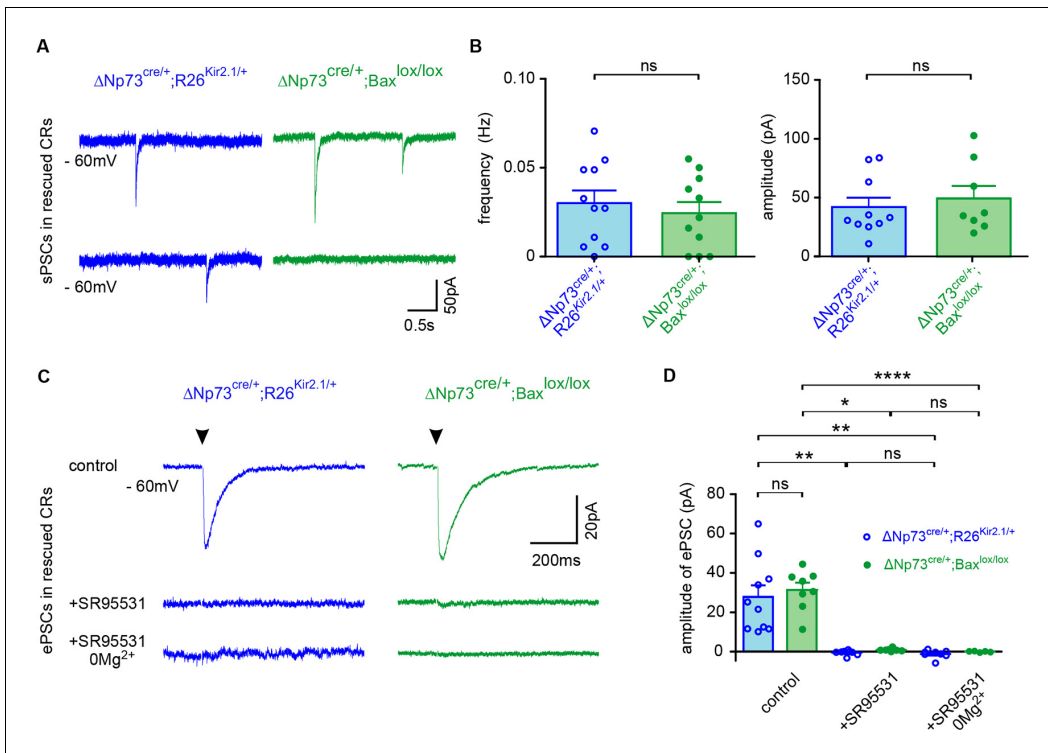


Figure 2. Pure GABAergic sPSCs and ePSCs in rescued CRs. (A) Spontaneous PSCs (sPSCs) recorded in rescued CRs from $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ at P27 (blue) and $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mutants at P26 (green), respectively. (B) Plots of the frequency and amplitude of sPSCs ($n = 11$ for $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ and $n = 11$ for $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mice at P24-29; frequency: $p=0.552$, amplitude: $p=0.580$, Student T Test). Rise time is 2.10 ± 0.42 ms vs 1.02 ± 0.20 ms and decay time 34.26 ± 6.39 ms vs 29.14 ± 3.56 ms for $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ and $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mice, respectively. (C) Mean evoked PSCs (ePSCs) for rescued CRs respectively from a $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mutant at P29 (blue) and a $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mutant at P26 (green) upon stimulation of LI neuronal fibers (stimulation time, arrowhead) in control conditions (top), with SR95531 (middle) and SR95531 in Mg^{2+} -free solution (bottom). Note that ePSCs completely disappeared after bath application of SR95531. (D) Amplitudes of ePSCs in control conditions, with SR95531 and with SR95531 in Mg^{2+} -free solution ($\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mice at P24-29: $n_{control} = 10$, $n_{SR95531} = 8$ and $n_{SR95531/Mg^{2+}free} = 8$; $\Delta Np73^{cre/+}; Bax^{lox/lox}$: $n_{control} = 8$, $n_{SR95531} = 5$ and $n_{SR95531/Mg^{2+}free} = 5$; Kruskal-Wallis test followed by a Bonferroni multiple comparison when comparing the three conditions for each mutant; Student T test for comparison of control ePSCs between $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ and $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mutants, $p=0.638$). To detect CRs in $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mutants the $R26^{mT/+}$ reporter line was used. Data used for quantitative analyses as well as the numerical data that are represented in graphs are available in **Figure 2—figure supplement 1—source data 1**.

The online version of this article includes the following source data and figure supplement(s) for figure 2:

Figure supplement 1. Pure GABAergic sPSCs and ePSCs in control CRs during early postnatal development.

Figure supplement 1—source data 1. Evoked and Spontaneous PSCs in rescued and developing CRs.

and $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mice. While no major changes were observed in the morphology of LII/III pyramidal neurons of $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mice compared to their matched controls (**Figure 3—figure supplement 1**), major defects were observed for these neurons in $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mutants (**Figure 3**). Quantitative analyses revealed an increase in the number of apical and basal dendritic branches at P25 in $\Delta Np73^{cre/+}; Bax^{lox/lox}$ compared to controls (**Figure 3B**). In order to further characterize cell complexity in relation with the distance from the soma, we performed a Sholl analysis. We observed that LII/III pyramidal cells displayed an increased complexity for apical dendrites (between 180 μm and 240 μm from the soma) as well as for basal dendrites (between 60 and 80 μm from the soma) (**Figure 3C**). Interestingly, no statistically different changes in the number of dendritic branches (**Figure 3—figure supplement 1B**) or the complexity of apical and basal dendrites (**Figure 3—figure supplement 1C**) were detected in LII/III pyramidal neurons of the $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ model, in which SE-CRs survive but are hyperpolarized. Thus, the survival of a specific subset of CRs has a general promoting impact onto the dendritic tree of upper layer pyramidal neurons only when they keep their normal intrinsic excitability. Collectively, our analyses show

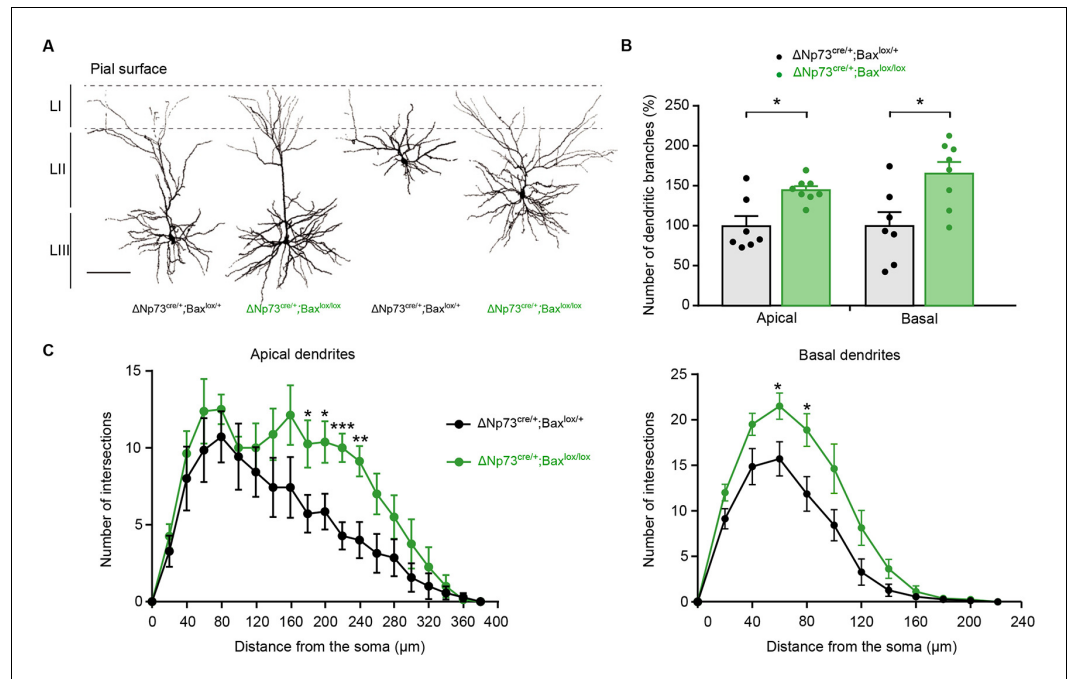


Figure 3. Increased dendritic branches in LII/LIII pyramidal neurons of $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mutants. (A) Representative examples of LII/III pyramidal neurons filled with biocytin in control (P25) and $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mutant (P24) somatosensory cortex. (B) Quantification of the number of dendritic branches in control and $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mutant LII/III pyramidal neurons, expressed as a percentage of dendritic branches relative to the mean of controls ($n = 7$ for controls and $n = 8$ for mutants at P23–28 $p = 0.0182$ for apical dendrites and $p = 0.014$ for basal dendrites; Mann-Whitney U Test). (C) Sholl analysis for the apical and basal dendrites in control and $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mutants showing an increased cell complexity between 180 and 240 μm (p -value = 0.04, 0.027, 0.0007 and 0.005, respectively) and 60 and 80 μm (p value = 0.027 and 0.019, respectively) from the soma, respectively ($n = 7$ for controls and $n = 8$ for mutants). Multiple T-test. Scale bar represents 100 μm . Data used for quantitative analyses as well as the numerical data that are represented in graphs are available in **Figure 3—figure supplement 1—source data 1**.

The online version of this article includes the following source data and figure supplement(s) for figure 3:

Figure supplement 1. Morphological reconstruction of LII/LIII pyramidal neurons in $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mutants.

Figure supplement 1—source data 1. Morphological analyses of layer II/III pyramidal cells in the Bax and Kir2.1 models.

that SE-CRs survival in LI promotes an exuberance of apical and basal dendrites of LII/LIII pyramidal neurons in an activity-dependent manner.

Survival of electrically-active CRs increases excitatory entries in upper layer pyramidal neurons

To examine whether the defects of the dendritic arborization of LII/III pyramidal neurons were related to changes in the synaptic inputs received by these neurons, we first examined spines on both apical and basal dendrites of biocytin-filled pyramidal cells (**Figure 4A–D**). Because excitatory synapses are formed on dendritic spines, the latter can be used as a proxy for the quantification of those synapses. For apical dendrites, we examined terminal ramifications in LI, whereas for basal dendrites, we considered horizontal branches approximately at the same distance from the soma. Spine density on both apical and basal dendrites of pyramidal cells was significantly increased in $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mutants compared to controls (**Figure 4A and B**) whereas no differences were observed in $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mutants (**Figure 4C and D**). These results indicate that the survival of electrically-active CRs not only triggers a dendritic exuberance, but also drives an increase in spine densities.

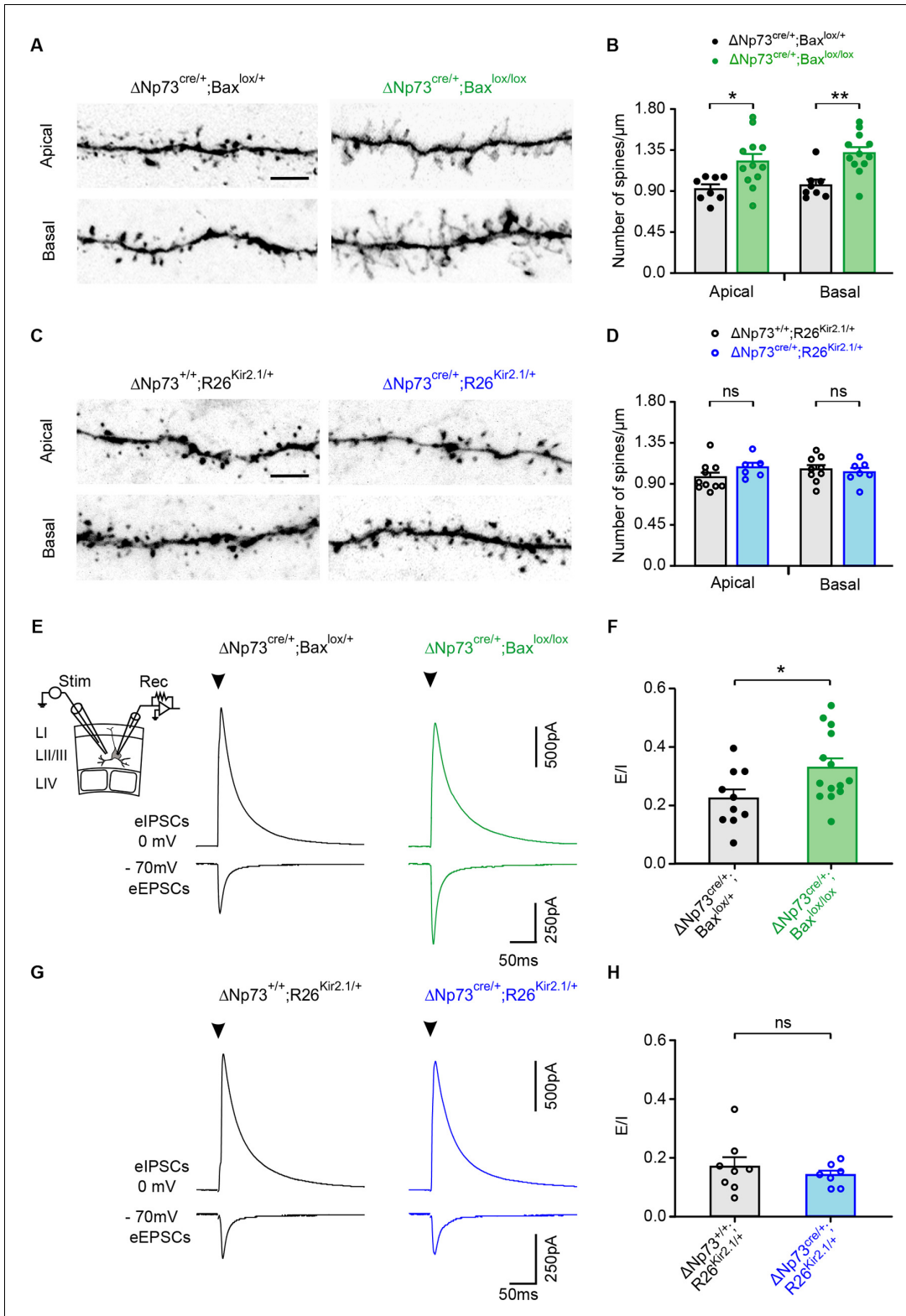


Figure 4. Spine density and evoked synaptic activity recorded in LII/LIII pyramidal neurons in both $\Delta Np73^{cre/+}; Bax^{lox/lox}$ and $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mutants. (A, C) Representative confocal images showing spines in apical and basal dendritic segments of controls (left) and $\Delta Np73^{cre/+}; Bax^{lox/lox}$ (A, right) and $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mutants (C, right) at P24-25. (B, D) Quantification of the spine density (number of spines/ μm) in apical and basal dendrites in LII/LIII pyramidal neurons for both controls and mutants from the same litters (for $\Delta Np73^{cre/+}; Bax^{lox/lox}$ apical and basal dendrites: $n = 8$ for Figure 4 continued on next page

Figure 4 continued

controls and $n = 12$ for mutants at P23-28, $p=0.012$ for apical dendrites and $p=0.0014$ for basal dendrites; for $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ apical dendrites: $n = 10$ for controls and $n = 6$ for mutants at P23-P29, $p=0.166$; basal dendrites: $n = 9$ for controls and $n = 7$ for mutants, $p=0.652$; Mann-Whitney U Test). Scale bar represents $5 \mu m$. (E, G) Pyramidal neurons recorded in voltage-clamp at -70 mV and 0 mV in control at P26 (E, left) and P24 (G, left) and in a $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mutant at P23 (E, right) and a $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mutant at P28 (G, right) during the extracellular stimulation of LII/III fibers as indicated (E, inset). Stimulation artefacts were blanked for visibility. The stimulation time is indicated (arrowheads). (F, H) Plots of E/I ratio calculated from eEPSCs and eIPSCs in controls and $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mutants (F) and $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mutants (H) (for $\Delta Np73^{cre/+}; Bax^{lox/lox}$: $n = 10$ for controls and $n = 14$ for mutants, $p=0.031$, Student T Test; for $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$: $n = 8$ for controls and $n = 7$ for mutants, $p=0.612$; Mann-Whitney U Test). Data used for quantitative analyses as well as the numerical data that are represented in graphs are available in **Figure 4—figure supplement 1—source data 1**.

The online version of this article includes the following source data and figure supplement(s) for figure 4:

Figure supplement 1. Evoked and spontaneous EPSCs and IPSCs of LII/LIII pyramidal neurons in $\Delta Np73^{cre/+}; Bax^{lox/lox}$ and $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mutants.

Figure supplement 1—source data 1. Spine densities, evoked and spontaneous PSCs in LII/III pyramidal neurons in both Bax and Kir2.1 models.

To test whether these morphological modifications in $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mutants is accompanied by modifications in excitatory synaptic inputs, we performed whole-cell recordings of upper layer pyramidal neurons during the extracellular stimulation of LII/III fibers. First, the membrane potential of recorded cells was maintained at -70 mV or 0 mV to respectively record evoked excitatory (eEPSCs) and inhibitory (eIPSCs) postsynaptic currents. Pyramidal cells in $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mutants showed a significant increase in the mean amplitude of eEPSCs, while that of eIPSCs remained unchanged compared to controls (**Figure 4E**, **Figure 4—figure supplement 1A**). This modification is highlighted by a significant increase in the E/I ratio (**Figure 4F**). Together with the increased spine density, these data strongly suggest that pyramidal neurons have enhanced excitatory synaptic inputs. To corroborate this possibility, we then analyzed the spontaneous EPSCs (sEPSCs) of recorded pyramidal neurons. As expected for an increased number of inputs, the sEPSC frequency was significantly higher in $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mutants with respect to controls while the mean sEPSC amplitude remained unchanged (**Figure 4—figure supplement 1C-D**). When the same experiments were performed in $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mutants, no differences were observed either in the amplitudes of both eEPSCs and eIPSCs or in the E/I ratio (**Figure 4G-H**, **Figure 4—figure supplement 1B**). In line with this, changes were neither observed in the frequency of sEPSCs of these mutants (**Figure 4—figure supplement 1E**), showing that defects in the synaptic activity of pyramidal neurons is dependent on the intrinsic activity of rescued CRs. To test whether the effect on pyramidal cells could be due to a direct action of CR activity on excitatory circuits, we produced $\Delta Np73^{cre/+}; Bax^{lox/lox}; ChR2^{lox/+}$ mutant mice to photoactivate rescued CRs while recording neuronal network activity. After defining an efficient photostimulation protocol for reliably eliciting action potentials on recorded CRs (**Figure 4—figure supplement 1F**), we combined photostimulation with of Layer I interneuron recordings in whole-cell configuration and/or local field potentials (LFPs) in different layers (**Figure 4—figure supplement 1G**). We could never detect light-evoked responses during patch-clamp or extracellular recordings, even in the presence of 0 mM Mg^{2+} , 3 mM Ca^{2+} and $50 \mu M$ of the potassium channel blocker 4AP, a treatment that renders all neurons more excitable (**Figure 4—figure supplement 1G**). Although these results do not rule out that rescued CRs contact other neurons through *bona fide* glutamatergic synapses, the low proportion of these cells compared to pyramidal neurons and the lack of electrical extracellular responses during their sustained light stimulation suggest that direct synaptic inputs from CRs cannot account for the robust morphological and functional changes induced in pyramidal cells by the aberrant CR survival.

Overall, these experiments show that the survival of electrically-active SE-CRs increases the excitatory inputs to upper pyramidal neurons, possibly through a non-glutamatergic mechanism, thereby generating a E/I imbalance and functional changes in circuit wiring.

Discussion

Our results show that the elimination of specific subsets of CRs, SE-CRs, is activity-dependent and that this process is essential for proper cortical wiring. Indeed, the persistence of SE-CRs beyond their normal phase of elimination triggered major deficits in LII/LIII somatosensory pyramidal

neurons. Not only the analyzed neurons displayed an increased dendritic arborization and spine density, but they also consistently showed enhanced excitatory inputs leading to a functional E/I imbalance. Remarkably, these anatomical and electrophysiological deficits all relied on the fact that persistent CRs were electrically-active. Our study thus demonstrates that activity is required to eliminate SE-CRs, whose survival would otherwise perturb cortical wiring in an activity-dependent manner. Taken together, it reveals an elegant interplay between transient CRs and neuronal activity in the construction of functional cortical excitatory circuits.

CR subtype-specific pathways in programmed cell death

Activity was reported to promote survival of both glutamatergic and GABAergic neurons in the neocortex and in general in the nervous system (for reviews see [Blanquie et al., 2017a](#); [Causeret et al., 2018](#); [Wong and Marín, 2019](#)), providing a mean to integrate around 70% of neurons into functional circuits. CRs, which completely undergo programmed cell death in the cerebral cortex ([Ledonne et al., 2016](#); [Causeret et al., 2018](#)), have been previously proposed to behave differently. These neurons display 'immature' features such as a depolarized resting potential and a very high input resistance ([Kirischuk et al., 2014](#)). Especially, GABA is depolarizing in these cells due to their elevated intracellular chloride concentration resulting from the activity of the chloride inward transporter NKCC1 in the absence of expression of chloride outward transporter KCC2 ([Mienville, 1998](#); [Achilles et al., 2007](#); [Pozas et al., 2008](#)). Interestingly, the pharmacological blockade of NKCC1 in cell cultures or the genetic ablation of this transporter in vivo promotes the survival of a CR population, probably by preventing GABA_A receptor-mediated depolarization ([Blanquie et al., 2017b](#)). It must be considered, however, that the depolarizing effect of GABA will depend most probably on the levels of neuronal activity since high activity levels attenuate the GABA_A receptor-mediated excitatory drive in CRs ([Kolbaev et al., 2011](#)). Here, we confirmed that CRs receive exclusively functional GABAergic synaptic inputs and express low levels of KCC2 in the second postnatal week, that is during the period of massive cell death. Our findings raise the question of the identity of possible GABAergic neurons that regulate CR subtype elimination. CRs receive GABAergic synaptic inputs from different sources, including local interneurons of Layer I, the underlying layers of the neocortex and the subplate, a transient cortical structure absent in the fourth postnatal week, as well as the zona incerta ([Kirmse et al., 2007](#); [Myakhar et al., 2011](#); [Kirischuk et al., 2014](#); [Chen and Kriegstein, 2015](#); [Sun et al., 2019](#)). A related issue is how GABAergic inputs to CRs might change overtime, since we found that rescued CRs receive less inputs than at earlier stages. One interesting possibility is that rescued CRs in Layer I might lose GABAergic innervation from transient or distant populations (i.e. subplate and zona incerta) during maturation of neuronal networks, restricting their connectivity with more local neocortical inputs. Further investigation is needed to determine the different interneuron subtypes impinging on CRs in immature cortical circuits and after their aberrant survival in adults.

Using conditional Kir2.1 expression in a large subpopulation of CRs, we unequivocally show that only a specific subset of CRs, SE-CRs, dies in an activity-dependent manner in vivo. Since CRs are highly hyperpolarized in this mouse model, a plausible explanation is that GABAergic inputs cannot exert their depolarizing effect as in normal conditions, thereby preventing cell death. In this context, it is also tempting to hypothesize that, as shown in other systems, neuronal activity via intracellular calcium signals could trigger apoptosis, as reported during excitotoxicity ([Blanquie et al., 2017a](#)). The timing of SE-CRs death, namely the second postnatal week, corresponds to a major switch in cortical activity ([Luhmann and Khazipov, 2018](#)) and in GABAergic circuits ([Cossart, 2011](#)), raising the possibility that the elimination of CRs is part of a more global activity-dependent remodeling of cortical circuits. Irrespective of the underlying mechanism, surviving Kir2.1-expressing CRs displayed a relatively normal morphology, expressed Reln and received GABAergic synaptic inputs. Remarkably, SE-CRs is also the subpopulation undergoing a Bax-dependent apoptosis. Quantification of CRs that persist in both models suggests that a vast majority of SE-CRs survives. Indeed, in both deletion of *Bax* ([Ledonne et al., 2016](#)) or over-expression of Kir2.1 (this manuscript) models, a five-fold increase in CR numbers, corresponding to approximately 30% of the initial pool at P7, is detected when using the $\Delta Np73^{Cre}$ line, in contrast to none when using the hem-specific $Wnt3a^{Cre}$ line. Since hem-CRs constitute about 70% of the population targeted by the $\Delta Np73^{Cre}$ line ([Bielle et al., 2005](#); [Yoshida et al., 2006](#); [Tissir et al., 2009](#)), our findings support that the 30% of rescued CRs in $\Delta Np73^{Cre}$ corresponds to a large fraction, if not all, of the SE-CR population.

Together, these results demonstrate that hem-derived CRs die in a *Bax*- and activity-independent manner, in contrast to SE-CRs that survive in *Bax* and *Kir2.1* mutants. Hence, our work reveals that subpopulations of CRs are eliminated by very distinct mechanisms. Interestingly, hippocampal CRs, which mostly derive from the cortical hem (Louvi et al., 2007) display a delayed death which seems to occur independently of the apoptotic-specific Caspase-3 activity (Anstötz et al., 2016; Anstötz et al., 2018). Together, these data support the notion that CR subtypes are intrinsically different in the mechanism determining their demise and argues in favor of complex yet unappreciated subtype-specific pathways leading ultimately to cell death.

CRs aberrant survival perturbs the morphology and connectivity of upper layer neurons in an activity-dependent manner

In this work, we have demonstrated that SE-CRs persistence in mice has a strong effect on LII/III pyramidal neuron morphology and excitatory circuits. Notably, in the $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mutants, we observed an impact on both apical and basal dendrites. While the effect on apical dendrites could be direct via local surviving SE-CRs, the impact on basal ones might be circuit-mediated since excitatory entries in apical dendritic tufts were shown to modify basal dendrite synaptic plasticity (Williams and Holtmaat, 2019). Moreover, SE-CRs survival leads in $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mutants to increased synaptic density with major functional consequences on the E/I ratio. Conversely, recent work showed that aberrant reduction of CRs during development triggers decreased apical dendritic tufts and dendritic spine density of LII/III pyramidal neurons accompanied by a reduction in the E/I ratio (de Frutos et al., 2016). Taken together our findings reveal that the proper balance of CRs constitutes an essential, yet underappreciated, regulator of LII/III pyramidal neuron morphology and wiring.

Importantly, we found in both *Bax* and *Kir* models that surviving CRs are similarly kept embedded into functional networks. Interestingly, rescued CRs are also solely innervated by GABA_A receptor-mediated synapses like their younger control counterparts. Although GABAergic synaptic connectivity often increases during postnatal development (Pangratz-Fuehrer and Hestrin, 2011), some cells may display transient connections that disappear after the second postnatal week, thereby accounting for the reduced connectivity observed in the two models. In contrast, since the effects on pyramidal neurons and cortical excitability are only found in *Bax* mutants, our study reveals that the inappropriate survival of SE-CRs drives an abnormal cortical wiring via an activity-dependent mechanism. However, CRs appear to act on upper layer pyramidal neurons via partially distinct mechanisms at different time points during development. Indeed, *Kir2.1* expressing mice appear largely similar to controls, suggesting that the impact of reducing CR density on LII/III apical dendrites (de Frutos et al., 2016) does not rely exclusively on the intrinsic excitability of CRs. Nevertheless, the mechanism by which rescued CRs induce morphological and functional changes on pyramidal neurons remains unresolved. The lack of response observed during our optogenetic experiments suggest that these changes may not depend on a direct CR excitatory synaptic input onto principal neurons. This is in line with recent data obtained with experiments performed in the hippocampus using ChR2 activation and paired-recordings in the third postnatal week, where CRs are still present in high density (Quattrocchio and Maccaferri, 2014; Anstötz et al., 2016). Indeed, only very few pyramidal cells could be detected as an output of CR cells. Further experiments will be required to determine the relative roles of CR-secreted factors versus circuit-mediated effects onto apical and basal dendrites. Regardless the mechanism and since excitatory entries onto apical dendrites are emerging as major actors in sensory gating, cortical integration and reward (Keller and Mrsic-Flogel, 2018; Khan and Hofer, 2018; Lacefield et al., 2019; Williams and Holtmaat, 2019; Zhang and Bruno, 2019), our findings highlight the importance of a transient cell population in the emergence of functional circuits as well as the deleterious effects of their abnormal demise.

Our study thus shows that the elimination of SE-CRs in the somatosensory cortex is required for proper morphology and wiring of LII/III pyramidal neurons and reveals a remarkable interplay between activity, the elimination of transient cells and cortical wiring. Indeed, activity, likely driven by cortical maturation, regulates the elimination of transient CRs that would otherwise perturb upper layer wiring. Notably, CRs persistence has been described in human pathological conditions, often associated with epilepsy. Our work thus not only provides novel insights onto normal wiring of upper layers, but also addresses the functional consequences of incomplete CR removal with major

relevance for neurodevelopmental diseases, such as autism spectrum disorder, schizophrenia or epilepsy.

Materials and methods

Key resources table

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Strain <i>Mus musculus</i> (males and female)	C57BL6J	Janvier		
<i>Mus musculus</i> (males and female)	$\Delta Np73^{Cre/REGFP}$	Tissir et al., 2009	$\Delta Np73^{Cre}$	
<i>Mus musculus</i> (males and female)	$Wnt3a^{Cre}$	Yoshida et al., 2006	$Wnt3a^{Cre}$	
<i>Mus musculus</i> (males and female)	$Tau^{loxP-stop-loxP-MARCKSeGFP-IRES-nlslacZ}$	Hippenmeyer et al., 2005	Tau^{GFP}	
<i>Mus musculus</i> (males and female)	Bax^{tm2Sjk} , $Bak1^{tm1Thsn/J}$	Takeuchi et al., 2005	$Bax^{lox/lox}$	
<i>Mus musculus</i> (males and female)	$ROSA26^{loxP-stop-loxP-Tomato}$	Madisen et al., 2010	$R26^{mT}$	
<i>Mus musculus</i> (males and female)	$ROSA26^{loxP-stop-loxP-Kcnj2-cherry/+}$	Moreno-Juan et al., 2017	$R26^{Kir2.1/+}$	
<i>Mus musculus</i> (males and female)	Ai32(RCL-ChR2 (H134R)/EYFP)	https://www.jax.org/strain/012569	$ChR2^{lox}$	
Antibody	rabbit polyclonal anti-DsRed	Takara	RRID:AB_10013483	IF(1:500)
Antibody	mouse monoclonal anti-Reelin	Merck Millipore	RRID:AB_565117	IF(1:300)
Antibody	mouse monoclonal anti-Gephyrin	Synaptic systems	RRID:AB_2619837	IF(1:250)
Antibody	rabbit polyclonal anti-GAD65/67	Merck	RRID: AB_2278725	IF(1:250)
Antibody	guinea-pig anti-KCC2	D Ng and S Morton TM Jessell's lab		IF(1:4000)
Antibody	donkey anti-mouse Alexa-488	Jackson Immuno Research Laboratories	RRID:AB_2340846	IF(1:800)
Antibody	donkey anti-rabbit Cy3	Jackson ImmunoResearch Laboratories	RRID:AB_2307443	IF(1:800)
Antibody	donkey anti-rabbit Alexa-647	Molecular Probes	RRID:AB_2536183	IF(1:500)
Antibody	donkey anti-chick Alexa-488	Jackson ImmunoResearch Laboratories	RRID:AB_2340375	IF(1:1000)
Antibody	donkey anti-mouse Alexa-555	Molecular Probes	RRID:AB_2536180	IF(1:1000)
Antibody	goat anti-guinea pig Alexa-555	Molecular Probes	RRID:AB_2535856	IF(1:1000)
Antibody	DAPI (4', 6-diamidino-2-phenylindole)	Invitrogen Molecular Probes	RRID:AB_2629482	IF(1:2000)
Antibody	DyLight 488 streptavidin	Vector Labs	SP-4488	
Sequence-based reagent	CRE genotyping 188 f 167 r	This paper	PCR primers	188 f: TGA TGG ACA TGT TCA GGG ATC 167 r: GAA ATC AGT GCG TTC GAA CGC TAG A

Continued on next page

Continued

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Sequence-based reagent	R26 ^{Kir2.1/+} genotyping AAY101 AAY103 SD297		PCR primers	AAY101: AAAGTCGCTCTGAGTTGTTAT (Rosa26 forward WT) AAY103: GGGAGCGGGAGAAATGGATATG (Rosa26 reverse WT) SD297: GGCCATTACCGTAAGTTATG (CAG promoter reverse)
Chemical compound, drug	Paraformaldehyde	Sigma-Aldrich	CAT:P6148	
Chemical compound, drug	Triton 100X	Eurobio	CAT:GAUTTR00-07	
Chemical compound, drug	SR95531	Abcam	Ab120042	
Chemical compound, drug	4-AP	Sigma Aldrich	A-0152	
Software, algorithm	IMARIS software 8.4.	IMARIS	RRID:SCR_007370	
Software, algorithm	GraphPad Prism 7.0	GraphPad Software	RRID:SCR_000306	
Software, algorithm	ImageJ/FIJI	NIH	RRID:SCR_002285	
Software, algorithm	Adobe Photoshop CS6	Adobe Systems	RRID:SCR_014199	
Software, algorithm	pClamp10.1	Molecular Devices	RRID:SCR_011323	
Software, algorithm	IGOR Pro 6.0	Wavemetrics	RRID:SCR_000325	
Software, algorithm	NeuroMatic	Wavemetrics	RRID:SCR_004186	

Animals

Δ Np73^{Cre}RESGFP (Δ Np73^{Cre}) (Tissir *et al.*, 2009), Wnt3a^{Cre} (Yoshida *et al.*, 2006), ROSA26^{loxP-stop-loxP-Tomato}(R26^{mT}) (Madisen *et al.*, 2010), Tau^{loxP-stop-loxP-MARCKS-eGFP-IRES-nlslacZ} (Tau^{GFP}) (Hippenmeyer *et al.*, 2005) and ChR2^{lox} (Ai32(RCL-ChR2(H134R)/EYFP) (<https://www.jax.org/strain/012569>)) transgenic mice were kept in a C57BL/6J background. The Bax^{tm2Sjk};Bak1^{tm1Thsn}/J line (Takeuchi *et al.*, 2005) harboring the floxed Bax and the Bak knock-out alleles was purchased from the Jackson laboratory as mixed B6;129. Δ Np73^{Cre} and Wnt3a^{Cre} lines were crossed with the R26^{mT} or Tau^{GFP} reporter lines to permanently label CR subtypes. Δ Np73^{Cre} line was crossed to the Bax^{tm2Sjk};Bak1^{tm1Thsn}/J line (Bax^{lox/lox}) to inactivate Bax function in specific CR subtypes. The conditional knock-out also harbored a reporter allele R26^{mT} in order to trace the neurons in which recombination had occurred. Δ Np73^{Cre} and Wnt3a^{Cre} lines were crossed to ROSA26^{loxP-stop-loxP-Kcnj2-cherry/+} (R26^{Kir2.1/+}) (Moreno-Juan *et al.*, 2017) to overexpress the Kcnj2 gene, which encodes Kir2.1, in specific CR subpopulations. Controls used were littermates heterozygous Bax for the Bax model and Δ Np73^{+/+};R26^{Kir2.1/+} for the Kir2.1 model. Animals were genotyped by PCR using primers specific for the different alleles. All animals were handled in strict accordance with good animal practice as defined by the national animal welfare bodies, and all mouse work was approved by the Veterinary Services of Paris (Authorization number: 75–1454) and by the Animal Experimentation Ethical Committee Buffon (CEEA-40) (Reference: CEB-34–2012) and by the Animal Experimentation Ethical Committee Darwin (Reference: 02224.02).

Tissue preparation and immunohistochemistry

For staging of animals, the birth date was considered as postnatal day 0 (P0). Animals were anesthetized with Isoflurane and intracardially perfused with 4% paraformaldehyde (PFA) in 0.1 M PBS, pH 7.4 and post-fixed overnight in 4% PFA at 4°C. Brain were embedded in 3.5% agarose and sectioned in 70 μ m free-floating slices at all stages in **Figure 1** and **Figure 1—figure supplement 1**. For

Figure 2—figure supplement 1E, brains were cryoprotected and sectioned in 50 μm free-floating slices. Immunostaining was performed as previously described (Bielle et al., 2005; Griveau et al., 2010; de Frutos et al., 2016). Primary antibodies used for immunohistochemistry were: mouse anti-Reelin (MAB5364, Millipore 1:300), rabbit anti-DsRed (Takara 632496, 1:500), mouse anti-Gephyrin (147 011, Synaptic Systems, 1:250), Rabbit anti-GAD65/67 (AB1511, Merck, 1:250), guinea pig anti-KCC2 (Gift of S.Morton and D.Ng, 1:4000). Secondary antibodies used against primary antibodies were: donkey anti-mouse Alexa-488 (Jackson ImmunoResearch Laboratories, 1:800), donkey anti-rabbit Cy3 (Jackson ImmunoResearch Laboratories, 1:800), donkey anti-chick Alexa-488 (Jackson ImmunoResearch Laboratories, 1:1000), donkey anti-mouse Alexa-555 (A-31570, Molecular Probes, 1:1000), donkey anti-rabbit Alexa-647 (A31573, Molecular Probes, 1:500), goat anti-guinea pig Alexa-555 (A-21435, Molecular Probes, 1:1000). Hoechst (Sigma-Aldrich 33342, 1:1000) and DAPI (D1306, ThermoFisher Scientific, 1:2000) were used for fluorescent nuclear counterstaining the tissue and mounting was done in Vectashield (Vector Labs).

Image acquisition and cell countings

Immunofluorescence images were acquired using a confocal microscope (Leica TCS SP5), except for anti-KCC2, Gephyrin and GAD65/67 (Figure 2—figure supplement 1E, f) that were acquired on a LEICA SP8 confocal microscope with 93X objective and 2.5 digital zoom (a single optical plane or around 100 z-stacks of 0.07 μm respectively). DsRed⁺ neurons, detected by immunofluorescence, were counted using the ImageJ software, in the somatosensory barrel cortex (S1) for each age and genotype. For each section, the density of CRs (DsRed⁺ CRs/ mm^3) was calculated taking into account the thickness of the section and the surface of Layer I, measured using ImageJ software.

Acute slice preparation, electrophysiology and photostimulation

Acute coronal slices (300 μm) of the neocortex were obtained from $\Delta\text{Np73}^{\text{cre/+}};\text{Bax}^{\text{lox/lox}}$ and $\Delta\text{Np73}^{\text{cre/+}};\text{R26}^{\text{Kir2.1/+}}$ mutants. Excitation light to visualize the tdTomato or Cherry fluorescent proteins was provided by a green Optoled Light Source (Cairn Research, UK) and images were collected with an iXon+ 14-bit digital camera (Andor Technology, UK), as previously described (Ordaz et al., 2015). Patch-clamp recordings were performed at RT using an extracellular solution containing (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4 , 26 NaHCO_3 , 20 glucose, five pyruvate, 2 CaCl_2 and 1 MgCl_2 (95% O_2 , 5% CO_2). Fluorescent CRs were recorded at P13-17 and P24-29 with different intracellular solutions according to the experiment and containing (in mM): either 130 K-Gluconate (K-Glu) or 130 KCl, 0.1 EGTA, 0.5 CaCl_2 , 2 MgCl_2 , 10 HEPES, 2 $\text{Na}_2\text{-ATP}$, 0.2 Na-GTP and 10 $\text{Na}_2\text{-phosphocreatine}$ and 5.4 mM biocytin (pH \approx 7.3). When using a K-Glu-based intracellular solution in whole-cell configuration, potentials were corrected for a junction potential of -10 mV. Recordings were made without series resistance (R_s) compensation; R_s was monitored during recordings and cells showing a change of more than 20% in R_s were discarded. To evaluate the effect of SR95351 (10 μM ; Abcam, Cambridge, UK), drug perfusion reached a steady state at 3 min in the recording chamber. This time was respected before quantification of either spontaneous or evoked PSCs. To test for the presence of eEPSCs mediated by NMDARs receptors at hyperpolarized potentials, the extracellular concentration of MgCl_2 was replaced by CaCl_2 in order to relieve the Mg^{2+} block of these receptors.

Photostimulation of fluorescent ChR2-expressing rescued CRs recorded in whole-cell configuration was obtained by triggering light trains with a blue LED (470 nm, 1 ms pulses; Optoled Light Source, Cairn Research, UK). Light trains of 2, 5, 10 and 20 Hz during 10 s or 30 s were applied to define the optimal frequency inducing an effective activation of recorded ChR2-expressing rescued CRs. For each frequency, we calculated the number of spikes (Ns) with respect to the number of light pulses (NLP) and determined the percentage of success as $[\text{Ns}/\text{NLP}] \times 100$. To test the effect of rescued CRs activation, we performed extracellular recordings and patch-clamp recordings of Layer I interneurons while stimulating with light trains (5 Hz, 10 s or 30 s). Extracellular recordings were recorded with a patch pipette filled with extracellular solution. In a set of experiments, the extracellular solution contained 0 mM Mg^{2+} , 3 mM Ca^{2+} and 4AP for more than 5 min at least.

Recordings were obtained using Multiclamp 700B and pClamp10.1 (Molecular Devices), filtered at 4 kHz and digitized at 20 kHz. Digitized data were analyzed off-line using Neuromatic within IGOR Pro 6.0 environment (Wavemetrics, USA) (Rothman and Silver, 2018). Extracellular

stimulations were performed using a monopolar electrode (glass pipette) placed in Layer I for CRs and Layer II/III for pyramidal neurons (20–99 V, 100 μ s stimulations each 8–12 s; Iso-Stim 01D, npi electronic GmbH, Tamm, Germany). Spontaneous postsynaptic currents were detected with a threshold of 2 times the noise standard deviation during a time window of 3 min for CRs and 1.5 min for pyramidal cells. The V_m was estimated in current-clamp mode as soon as the whole-cell configuration was established. The analysis of R_{in} , action potential amplitudes and duration was performed during pulses of 800 ms in current-clamp configuration from -80 mV during increasing steps of 5 pA as previously described (Ledonne et al., 2016).

Morphological analyses

For morphological analysis, CRs and layer II/III pyramidal cells were loaded with biocytin through patch pipette during whole-cell recordings. The slices were fixed 2 hr in 4% paraformaldehyde at 4°C, rinsed three times in PBS for 10 min, and incubated with 1% triton X-100% and 2% BSA during 1 hr. Then, they were washed three times in PBS and incubated in DyLight 488 streptavidin (Vector Labs, Burlingame, USA) for 2 hr. Successfully labeled CRs and Layer II/III pyramidal cells were visualized either using a LEICA SP5 or SP8 confocal microscope with a 40X objective and a 1.3 digital zoom. Around 250 optical sections of 0.6 μ m were necessary to image the whole dendritic tree of each neuron. For dendritic spines, images of apical dendrites in Layer I and basal dendrites in Layer II/III were acquired with a 63X objective (100 optical sections of 0.25 μ m each). For apical dendrites, a terminal ramification in Layer I was acquired, while for basal dendrites a horizontal dendrite in Layer II/III was acquired approximately at 60 μ m distance from the soma after the first ramification. 3D reconstruction was performed using the IMARIS software 8.4. Statistical analyses were performed based on the data given by IMARIS in apical and basal dendrites separately. Counting of spines was performed manually using ImageJ software on black and white maximum projections on two different segments approximately 50 μ m long for each image. For morphological analysis of CRs, statistical analyses were performed based on the values calculated by image analyses using the IMARIS software (soma diameter and filament length).

Statistical analysis

All data were expressed as mean \pm SEM. A P-value less than 0.05 was considered significant. For statistical groups larger than 7, we performed a D'Agostino-Pearson normality test. According to the data structure, two-group comparisons were performed using two-tailed unpaired Student T test or Mann-Whitney U Test. Bonferroni multiple comparisons were used as post-hoc test following one-way or two-way ANOVA or the non-parametric Kruskal-Wallis Tests. For small statistical groups (less than 7), we systematically performed non-parametric tests (Mann-Whitney U Test or Kruskal-Wallis Tests with Dunn's correction). A reconstructed pyramidal neuron displaying an exceptional basal dendrite projecting to Layer I was submitted to Grubbs test and excluded as an extreme outlier. Statistics and plotting were performed using GraphPad Prism 7.00 (GraphPad Software Inc, USA). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

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Martina Riva, Conceptualization, Data curation, Formal analysis, Supervision, Funding acquisition, Validation, Investigation, Visualization, Methodology, Writing—original draft, Project administration, Writing—review and editing; Ioana Genescu, Chloé Habermacher, David Orduz, Conceptualization, Data curation, Formal analysis, Validation, Visualization, Methodology, Writing—original draft, Writing—review and editing; Fanny Ledonne, Data curation, Formal analysis, Supervision, Validation, Visualization, Methodology; Filippo M Rijli, Guille López-Bendito, Resources; Eva Coppola, Conceptualization, Resources, Formal analysis, Supervision, Validation, Investigation, Methodology, Writing—original draft, Writing—review and editing; Sonia Garel, Conceptualization, Resources, Formal analysis, Supervision, Funding acquisition, Validation, Investigation, Visualization, Methodology, Writing—original draft, Project administration, Writing—review and editing; Maria Cecilia Angulo, Alessandra Pierani, Conceptualization, Formal analysis, Supervision, Funding acquisition, Validation, Investigation, Visualization, Methodology, Writing—original draft, Project administration, Writing—review and editing

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Ethics

Animal experimentation: All animals were handled in strict accordance with good animal practice as defined by the national animal welfare bodies, and all mouse work was approved by the Veterinary Services of Paris (Authorization number: 75-1454) and by the Animal Experimentation Ethical Committee Buffon (CEEA-40) (Reference: CEB-34-2012) and by the Animal Experimentation Ethical Committee Darwin (Reference: 02224.02).

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Additional files

Supplementary files

- Transparent reporting form

Data availability

All data generated or analysed during this study are included in the manuscript and supporting files. The Source data file contains all the data presented in the figures (1 sheet per Figure).

References

- Achilles K, Okabe A, Ikeda M, Shimizu-Okabe C, Yamada J, Fukuda A, Luhmann HJ, Kilb W. 2007. Kinetic properties of cl uptake mediated by na⁺-dependent K⁺-2Cl cotransport in immature rat neocortical neurons. *Journal of Neuroscience* **27**:8616–8627. DOI: <https://doi.org/10.1523/JNEUROSCI.5041-06.2007>, PMID: 17687039
- Anstötz M, Cosgrove KE, Hack I, Mugnaini E, Maccaferri G, Lübke JH. 2014. Morphology, input-output relations and synaptic connectivity of Cajal-Retzius cells in layer 1 of the developing neocortex of CXCR4-EGFP mice. *Brain Structure and Function* **219**:2119–2139. DOI: <https://doi.org/10.1007/s00429-013-0627-2>, PMID: 24026287
- Anstötz M, Huang H, Marchionni I, Haumann I, Maccaferri G, Lübke JH. 2016. Developmental profile, morphology, and synaptic connectivity of Cajal-Retzius cells in the postnatal mouse Hippocampus. *Cerebral Cortex* **26**:855–872. DOI: <https://doi.org/10.1093/cercor/bhv271>, PMID: 26582498
- Anstötz M, Lee SK, Neblett TI, Rune GM, Maccaferri G. 2018. Experience-Dependent regulation of Cajal-Retzius cell networks in the developing and adult mouse Hippocampus. *Cerebral Cortex* **28**:672–687. DOI: <https://doi.org/10.1093/cercor/bhx153>, PMID: 28637318
- Barber M, Arai Y, Morishita Y, Vigier L, Causeret F, Borello U, Ledonne F, Coppola E, Contremoulins V, Pfrieger FW, Tissir F, Govindan S, Jabaudon D, Proux-Gillardeaux V, Galli T, Pierani A. 2015. Migration speed of Cajal-Retzius cells modulated by vesicular trafficking controls the size of Higher-Order cortical Areas. *Current Biology* **25**:2466–2478. DOI: <https://doi.org/10.1016/j.cub.2015.08.028>, PMID: 26387718
- Barber M, Pierani A. 2016. Tangential migration of glutamatergic neurons and cortical patterning during development: lessons from Cajal-Retzius cells. *Developmental Neurobiology* **76**:847–881. DOI: <https://doi.org/10.1002/dneu.22363>
- Bielle F, Griveau A, Narboux-Nême N, Vigneau S, Sigrist M, Arber S, Wassef M, Pierani A. 2005. Multiple origins of Cajal-Retzius cells at the borders of the developing pallium. *Nature Neuroscience* **8**:1002–1012. DOI: <https://doi.org/10.1038/nn1511>, PMID: 16041369
- Blanquie O, Kilb W, Sinning A, Luhmann HJ. 2017a. Homeostatic interplay between electrical activity and neuronal apoptosis in the developing neocortex. *Neuroscience* **358**:190–200. DOI: <https://doi.org/10.1016/j.neuroscience.2017.06.030>, PMID: 28663094
- Blanquie O, Liebmann L, Hübner CA, Luhmann HJ, Sinning A. 2017b. NKCC1-Mediated GABAergic signaling promotes postnatal cell death in neocortical Cajal-Retzius cells. *Cerebral Cortex* **27**:1644–1659. DOI: <https://doi.org/10.1093/cercor/bhw004>, PMID: 26819276
- Causeret F, Coppola E, Pierani A. 2018. Cortical developmental death: selected to survive or fated to die. *Current Opinion in Neurobiology* **53**:35–42. DOI: <https://doi.org/10.1016/j.conb.2018.04.022>, PMID: 29738999
- Chen J, Kriegstein AR. 2015. A GABAergic projection from the zona incerta to cortex promotes cortical neuron development. *Science* **350**:554–558. DOI: <https://doi.org/10.1126/science.aac6472>, PMID: 26429884
- Cocas LA, Fernandez G, Barch M, Doll J, Zamora Diaz I, Pleasure SJ. 2016. Cell Type-Specific circuit mapping reveals the presynaptic connectivity of developing cortical circuits. *Journal of Neuroscience* **36**:3378–3390. DOI: <https://doi.org/10.1523/JNEUROSCI.0375-15.2016>, PMID: 26985044

- Cossart R. 2011. The maturation of cortical interneuron diversity: how multiple developmental journeys shape the emergence of proper network function. *Current Opinion in Neurobiology* **21**:160–168. DOI: <https://doi.org/10.1016/j.conb.2010.10.003>, PMID: 21074988
- de Frutos CA, Bouvier G, Arai Y, Thion MS, Lokmane L, Keita M, Garcia-Dominguez M, Charnay P, Hirata T, Riethmacher D, Grove EA, Tissir F, Casado M, Pierani A, Garel S. 2016. Reallocation of olfactory Cajal-Retzius cells shapes neocortex architecture. *Neuron* **92**:435–448. DOI: <https://doi.org/10.1016/j.neuron.2016.09.020>, PMID: 27693257
- Del Río JA, Heimrich B, Supèr H, Borrell Víctor, Frotscher M, Soriano E. 1996. Differential survival of Cajal-Retzius Cells in Organotypic Cultures of Hippocampus and Neocortex. *The Journal of Neuroscience* **16**:6896–6907. DOI: <https://doi.org/10.1523/JNEUROSCI.16-21-06896.1996>
- Fuchs Y, Steller H. 2011. Programmed cell death in animal development and disease. *Cell* **147**:742–758. DOI: <https://doi.org/10.1016/j.cell.2011.10.033>, PMID: 22078876
- Griveau A, Borello U, Causeret F, Tissir F, Boggetto N, Karaz S, Pierani A. 2010. A novel role for Dbx1-derived Cajal-Retzius cells in early regionalization of the cerebral cortical neuroepithelium. *PLOS Biology* **8**:e1000440. DOI: <https://doi.org/10.1371/journal.pbio.1000440>, PMID: 20668538
- Hippenmeyer S, Vrieseling E, Sigrist M, Portmann T, Laengle C, Ladle DR, Arber S. 2005. A developmental switch in the response of DRG neurons to ETS transcription factor signaling. *PLOS Biology* **3**:e159. DOI: <https://doi.org/10.1371/journal.pbio.0030159>, PMID: 15836427
- Ishii K, Kubo KI, Nakajima K. 2016. Reelin and neuropsychiatric disorders. *Frontiers in Cellular Neuroscience* **10**:229. DOI: <https://doi.org/10.3389/fncel.2016.00229>, PMID: 27803648
- Keller GB, Mrcic-Flogel TD. 2018. Predictive processing: a canonical cortical computation. *Neuron* **100**:424–435. DOI: <https://doi.org/10.1016/j.neuron.2018.10.003>, PMID: 30359606
- Khan AG, Hofer SB. 2018. Contextual signals in visual cortex. *Current Opinion in Neurobiology* **52**:131–138. DOI: <https://doi.org/10.1016/j.conb.2018.05.003>, PMID: 29883940
- Kilb W, Luhmann HJ. 2001. Spontaneous GABAergic postsynaptic currents in Cajal-Retzius cells in neonatal rat cerebral cortex. *European Journal of Neuroscience* **13**:1387–1390. DOI: <https://doi.org/10.1046/j.0953-816x.2001.01514.x>, PMID: 11298799
- Kirischuk S, Luhmann HJ, Kilb W. 2014. Cajal-Retzius cells: update on structural and functional properties of these mystic neurons that bridged the 20th century. *Neuroscience* **275**:33–46. DOI: <https://doi.org/10.1016/j.neuroscience.2014.06.009>, PMID: 24931764
- Kirmse K, Dvorzhak A, Henneberger C, Grantyn R, Kirischuk S. 2007. Cajal retzius cells in the mouse neocortex receive two types of pre- and postsynaptically distinct GABAergic inputs. *The Journal of Physiology* **585**:881–895. DOI: <https://doi.org/10.1113/jphysiol.2007.145003>, PMID: 17962325
- Kolbaev SN, Achilles K, Luhmann HJ, Kilb W. 2011. Effect of depolarizing GABA(A)-mediated membrane responses on excitability of Cajal-Retzius cells in the immature rat neocortex. *Journal of Neurophysiology* **106**:2034–2044. DOI: <https://doi.org/10.1152/jn.00699.2010>, PMID: 21775719
- Lacefield CO, Pnevmatikakis EA, Paninski L, Bruno RM. 2019. Reinforcement learning recruits somata and apical dendrites across layers of primary sensory cortex. *Cell Reports* **26**:2000–2008. DOI: <https://doi.org/10.1016/j.celrep.2019.01.093>
- Ledonne F, Orduz D, Mercier J, Vigier L, Grove EA, Tissir F, Angulo MC, Pierani A, Coppola E. 2016. Targeted inactivation of bax reveals a Subtype-Specific mechanism of Cajal-Retzius neuron death in the postnatal cerebral cortex. *Cell Reports* **17**:3133–3141. DOI: <https://doi.org/10.1016/j.celrep.2016.11.074>, PMID: 28009284
- Louvi A, Yoshida M, Grove EA. 2007. The derivatives of the Wnt3a lineage in the central nervous system. *The Journal of Comparative Neurology* **504**:550–569. DOI: <https://doi.org/10.1002/cne.21461>, PMID: 17701978
- Luhmann HJ. 2013. Cajal-Retzius and Subplate cells: Transient Cortical neurons and Circuits. In: Rubenstein J. L. R, Rakic P (Eds). *Cellular Migration and Formation of Neuronal Connections: Comprehensive Developmental Neuroscience*. Academic Press. p. 843–856. DOI: <https://doi.org/10.1016/B978-0-12-397266-8.00009-0>
- Luhmann HJ, Khazipov R. 2018. Neuronal activity patterns in the developing barrel cortex. *Neuroscience* **368**:256–267. DOI: <https://doi.org/10.1016/j.neuroscience.2017.05.025>, PMID: 28528963
- Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, Ng LL, Palmiter RD, Hawrylycz MJ, Jones AR, Lein ES, Zeng H. 2010. A robust and high-throughput cre reporting and characterization system for the whole mouse brain. *Nature Neuroscience* **13**:133–140. DOI: <https://doi.org/10.1038/nn.2467>, PMID: 20023653
- Mienville JM. 1998. Persistent depolarizing action of GABA in rat Cajal-Retzius cells. *The Journal of Physiology* **512**:809–817. DOI: <https://doi.org/10.1111/j.1469-7793.1998.809bd.x>, PMID: 9769423
- Mienville JM, Pesold C. 1999. Low resting potential and postnatal upregulation of NMDA receptors may cause Cajal-Retzius cell death. *The Journal of Neuroscience* **19**:1636–1646. DOI: <https://doi.org/10.1523/JNEUROSCI.19-05-01636.1999>, PMID: 10024350
- Moreno-Juan V, Filipchuk A, Antón-Bolaños N, Mezzera C, Gezelius H, Andrés B, Rodríguez-Malmierca L, Susín R, Schaad O, Iwasato T, Schüle R, Rutlin M, Nelson S, Ducret S, Valdeolmillos M, Rijli FM, López-Bendito G. 2017. Prenatal thalamic waves regulate cortical area size prior to sensory processing. *Nature Communications* **8**:14172. DOI: <https://doi.org/10.1038/ncomms14172>, PMID: 28155854
- Myakhar O, Unichenko P, Kirischuk S. 2011. GABAergic projections from the subplate to Cajal-Retzius cells in the neocortex. *NeuroReport* **22**:525–529. DOI: <https://doi.org/10.1097/WNR.0b013e32834888a4>, PMID: 21666518

- Orduz D**, Maldonado PP, Balia M, Vélez-Fort M, de Sars V, Yanagawa Y, Emiliani V, Angulo MC. 2015. Interneurons and oligodendrocyte progenitors form a structured synaptic network in the developing neocortex. *eLife* **4**:e06953. DOI: <https://doi.org/10.7554/eLife.06953>
- Pangratz-Fuehrer S**, Hestrin S. 2011. Synaptogenesis of electrical and GABAergic synapses of fast-spiking inhibitory neurons in the neocortex. *Journal of Neuroscience* **31**:10767–10775. DOI: <https://doi.org/10.1523/JNEUROSCI.6655-10.2011>, PMID: 21795529
- Pozas E**, Paco S, Soriano E, Aguado F. 2008. Cajal-Retzius cells fail to trigger the developmental expression of the cl- extruding co-transporter KCC2. *Brain Research* **1239**:85–91. DOI: <https://doi.org/10.1016/j.brainres.2008.08.058>, PMID: 18789906
- Quattrocio G**, Maccaferri G. 2014. Optogenetic activation of cajal-retzius cells reveals their glutamatergic output and a novel feedforward circuit in the developing mouse Hippocampus. *Journal of Neuroscience* **34**:13018–13032. DOI: <https://doi.org/10.1523/JNEUROSCI.1407-14.2014>, PMID: 25253849
- Radnikow G**, Feldmeyer D, Lübke J. 2002. Axonal projection, input and output synapses, and synaptic physiology of Cajal–Retzius Cells in the Developing Rat Neocortex. *The Journal of Neuroscience* **22**:6908–6919. DOI: <https://doi.org/10.1523/JNEUROSCI.22-16-06908.2002>
- Rothman JS**, Silver RA. 2018. NeuroMatic: an integrated Open-Source software toolkit for acquisition, analysis and simulation of electrophysiological data. *Frontiers in Neuroinformatics* **12**:14. DOI: <https://doi.org/10.3389/fninf.2018.00014>, PMID: 29670519
- Sava BA**, Dávid CS, Teissier A, Pierani A, Staiger JF, Luhmann HJ, Kilb W. 2010. Electrophysiological and morphological properties of Cajal–Retzius cells with different ontogenetic origins. *Neuroscience* **167**:724–734. DOI: <https://doi.org/10.1016/j.neuroscience.2010.02.043>
- Schwartz TH**, Rabinowitz D, Unni V, Kumar VS, Smetters DK, Tsiola A, Yuste R. 1998. Networks of coactive neurons in developing layer 1. *Neuron* **20**:541–552. DOI: [https://doi.org/10.1016/S0896-6273\(00\)80993-9](https://doi.org/10.1016/S0896-6273(00)80993-9)
- Soda T**, Nakashima R, Watanabe D, Nakajima K, Pastan I, Nakanishi S. 2003. Segregation and coactivation of developing neocortical layer 1 neurons. *The Journal of Neuroscience* **23**:6272–6279. DOI: <https://doi.org/10.1523/JNEUROSCI.23-15-06272.2003>, PMID: 12867512
- Sun L**, Chen R, Bai Y, Li J, Wu Q, Shen Q, Wang X. 2019. Morphological and physiological characteristics of Ebf2-EGFP-Expressing Cajal-Retzius cells in developing mouse neocortex. *Cerebral Cortex* **29**:3864–3878. DOI: <https://doi.org/10.1093/cercor/bhy265>, PMID: 30307495
- Takeuchi O**, Fisher J, Suh H, Harada H, Malynn BA, Korsmeyer SJ. 2005. Essential role of BAX, BAK in B cell homeostasis and prevention of autoimmune disease. *PNAS* **102**:11272–11277. DOI: <https://doi.org/10.1073/pnas.0504783102>
- Tissir F**, Ravni A, Achouri Y, Riethmacher D, Meyer G, Goffinet AM. 2009. DeltaNp73 regulates neuronal survival in vivo. *PNAS* **106**:16871–16876. DOI: <https://doi.org/10.1073/pnas.0903191106>, PMID: 19805388
- Williams LE**, Holtmaat A. 2019. Higher-Order thalamocortical inputs gate synaptic Long-Term potentiation via disinhibition. *Neuron* **101**:91–102. DOI: <https://doi.org/10.1016/j.neuron.2018.10.049>
- Wong FK**, Marín O. 2019. Developmental cell death in the cerebral cortex. *Annual Review of Cell and Developmental Biology* **35**:523–542. DOI: <https://doi.org/10.1146/annurev-cellbio-100818-125204>, PMID: 31283379
- Yoshida M**, Assimacopoulos S, Jones KR, Grove EA. 2006. Massive loss of Cajal-Retzius cells does not disrupt neocortical layer order. *Development* **133**:537–545. DOI: <https://doi.org/10.1242/dev.02209>, PMID: 16410414
- Zhang W**, Bruno RM. 2019. High-order thalamic inputs to primary somatosensory cortex are stronger and longer lasting than cortical inputs. *eLife* **8**:e44158. DOI: <https://doi.org/10.7554/eLife.44158>, PMID: 30741160



Figures and figure supplements

Activity-dependent death of transient Cajal-Retzius neurons is required for functional cortical wiring

Martina Riva *et al*

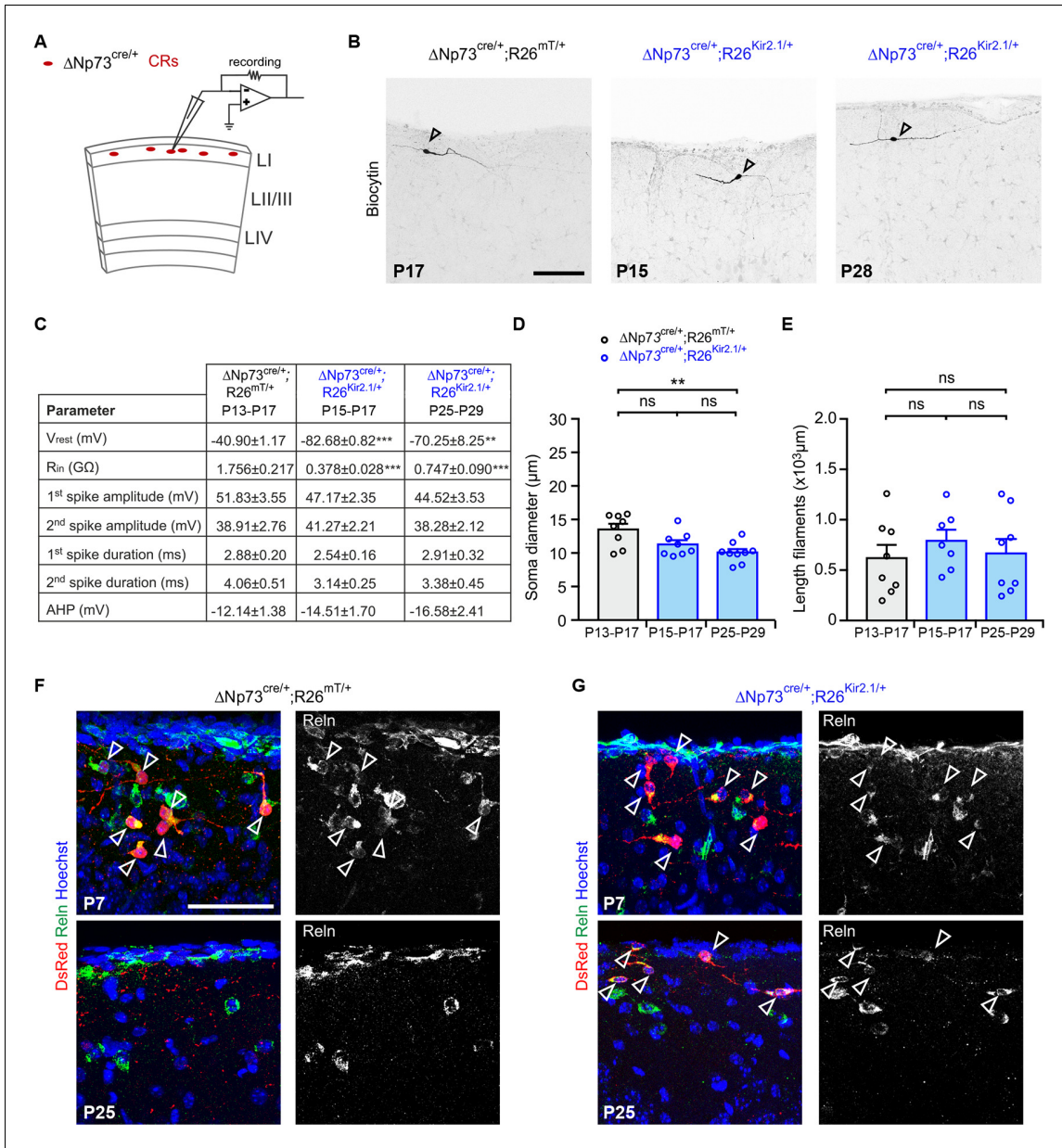


Figure 1—figure supplement 1. Electrophysiological properties and morphology of rescued CRs in $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mice. (A) Schematic representation of the electrophysiological recordings from $\Delta Np73^{cre/+}$ CRs. (B) Biocytin-filled CRs in $\Delta Np73^{cre/+}; R26^{mT/+}$ at P17 and $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ at P15 and P28 displayed a similar characteristic morphology of CRs. (C) Electrophysiological properties of control and rescued CRs expressing Kir2.1. Note that rescued CRs expressing Kir2.1 are hyperpolarized (resting potential, V_{rest}) at both P15-P17 ($n = 16$ for controls and $n = 17$ for mutants, $p=0.0001$) and P25-29 ($n = 11$ for mutants, $p=0.002$; Kruskal-Wallis test followed by a Bonferroni multiple comparison). Moreover, Kir2.1 expression modifies input resistance (R_{in}), without showing modifications of the amplitudes and durations of the first and second spikes or the afterhyperpolarization (AHP) ($n = 12$ for controls and $n = 15$ for mutants at P15-P17 and $n = 7-8$ for mutants at P25-P29; $p<0.0001$ for both ages; ANOVA test followed by a Bonferroni multiple comparison). (D) Quantifications of soma diameter of rescued $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ CRs at P15-P17 and P25-P29 compared to control $\Delta Np73^{cre/+}; R26^{mT/+}$ CRs at P13-P17 ($n = 8$ for controls and $n = 8$ for mutants at P15-P17, $p=0.075$; $n = 9$ for mutants at P25-P29, $p=0.004$, compared to P13-P17 controls; one-way ANOVA test followed by a Bonferroni multiple comparison). (E) Quantifications of filament length of $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ CRs at P15-P17 and P25-P29 compared to control $\Delta Np73^{cre/+}; R26^{mT/+}$ CRs at P13-P17 ($n = 8$ for controls and $n = 7$ for mutants at P15-P17, $p=0.636$; $n = 8$ for mutants at P25-P29, $p=0.964$, compared to P13-P17 controls). (F) Merged and single channel confocal images of P7 and P25 mutant and control brains stained for DsRed (red), Reelin (ReIn, green) and Hoechst (blue), showing that DsRed positive cells express ReIn. Scale bars represent 100 μm . Data used for quantitative analyses as well as the numerical data that are represented in graphs are available in **Figure 1—figure supplement 1—source data 1**.

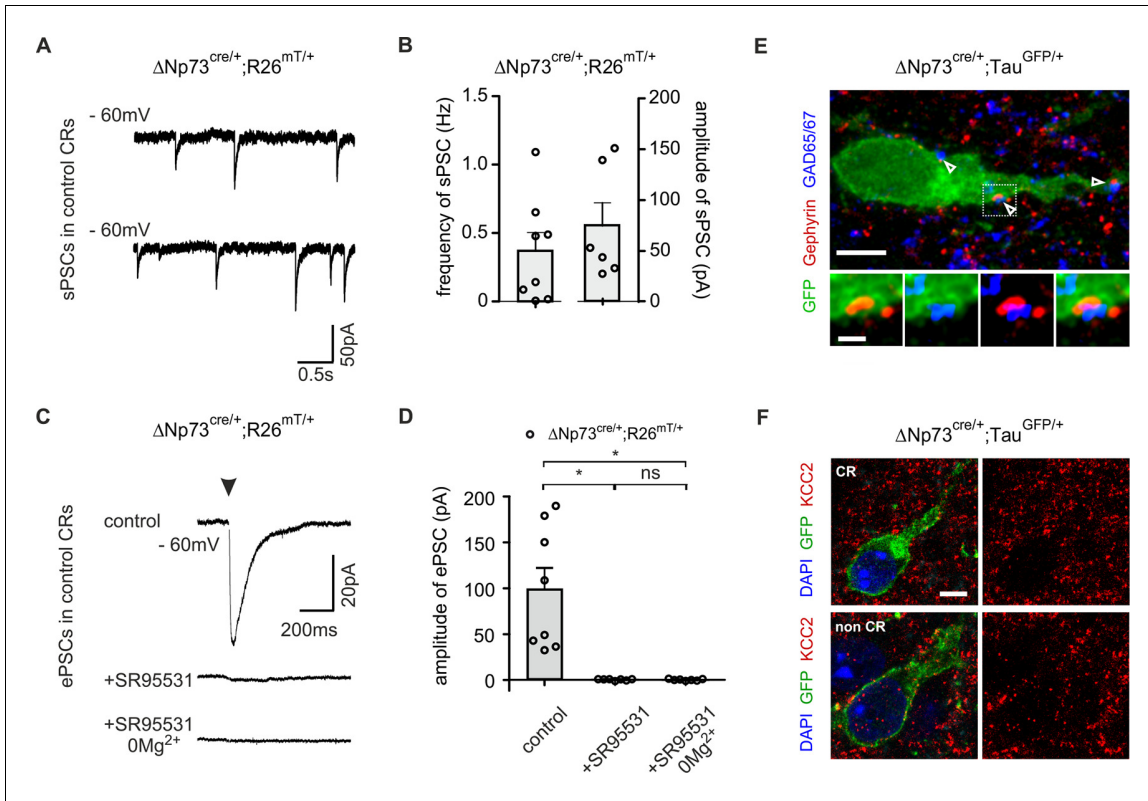


Figure 2—figure supplement 1. Pure GABAergic sPSCs and ePSCs in control CRs during early postnatal development. (A) Spontaneous PSCs (sPSCs) recorded in a control CR from $\Delta Np73^{cre/+};R26^{mT/+}$ at P10. (B) Plots of the frequency and amplitude of sPSCs ($n = 8$) ($p=0.0025$ and $p=0.0022$ for the frequency of sPSCs of controls compared to rescued CRs of both $\Delta Np73^{cre/+};R26^{Kir2.1/+}$ and $\Delta Np73^{cre/+};Bax^{lox/lox};R26^{mT/+}$ mice (Figure 2A); one-way ANOVA followed by a Bonferroni post hoc test). (C) Mean evoked PSCs (ePSCs) for a control CR at P9-11 upon stimulation of LI neuronal fibers in control conditions (top), with SR95531 (middle) and SR95531 in Mg^{2+} -free solution (bottom). Note that ePSCs completely disappeared after bath application of SR95531. Stimulation artefacts were blanked for visibility. The stimulation time is indicated (arrowheads). (D) Amplitudes of ePSCs in control conditions ($p=0.0027$ and $p=0.0067$ for the amplitude of ePSCs in controls compared to rescued CRs of $\Delta Np73^{cre/+};R26^{Kir2.1/+}$ and $\Delta Np73^{cre/+};Bax^{lox/lox};R26^{mT/+}$ mice, (Figure 2D); one-way ANOVA followed by a Bonferroni post hoc test), in the presence of SR95531 and with SR95531 in Mg^{2+} -free solution ($n_{control} = 8$, $n_{SR95531} = 7$ and $n_{SR95531/Mg^{2+}free} = 7$; Kruskal-Wallis test followed by a Bonferroni multiple comparison). (E) Confocal images of a control CR at P10 in $\Delta Np73^{cre/+};Tau^{GFP/+}$ mice expressing GFP (green), Gephyrin (red) and contacted by GABAergic GAD65/67-positive presynaptic terminals (blue; objective 93 \times ; stack of 109 Z sections, each 0.07 μm , $n = 11$). Arrowheads show GABAergic synapses onto the CR. Note the partial co-localization of GAD65/67 and Gephyrin on the GFP⁺ membrane of the CR (Inset). Scale bars: 5 μm and 1 μm (inset). (F) Confocal images of a Layer I CR in the cortex and non-CR in the hypothalamus at P10 stained for DAPI (blue), KCC2 (red) and GFP (green) in $\Delta Np73^{cre/+};Tau^{GFP/+}$ mice (objective 93 \times ; single plane of 0.07 μm , $n = 6$). Note the low level of KCC2 expression in control CR (upper panels) compared to non-CR of $\Delta Np73^{cre/+};Tau^{GFP/+}$ mice (bottom panels). Scale bar represents 5 μm . Data used for quantitative analyses as well as the numerical data that are represented in graphs are available in Figure 2—figure supplement 1—source data 1.

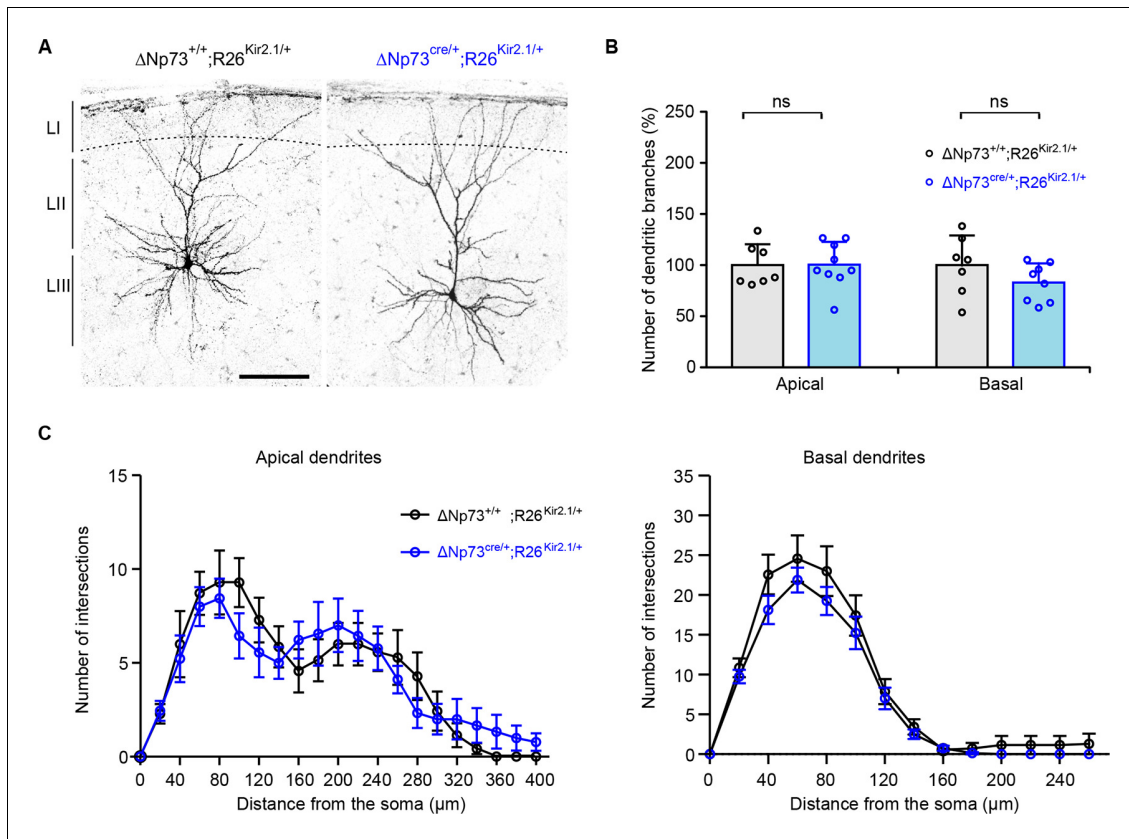


Figure 3—figure supplement 1. Morphological reconstruction of LII/LIII pyramidal neurons in $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mutants. (A) Representative examples of somatosensory cortex LII/III pyramidal neurons filled with biocytin in control (P24) and $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ (P27) mice. (B) Quantification of number of dendritic branches of LII/III pyramidal neurons in controls and $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mice, expressed as a percentage relative to the mean of controls (apical dendrites $n = 7$ for controls and $n = 9$ for mutants at P23-P29, $p = 0.550$; basal dendrites $n = 7$ for controls and $n = 8$ for mutants at P23-P29, $p = 0.199$; Mann-Whitney U Test). (C) Sholl analysis for the apical and basal dendrites in control and $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mutant mice showing no significant change in cell complexity with respect to the distance from the soma, (apical dendrites $n = 7$ for controls and $n = 9$ for mutants; basal dendrites $n = 7$ for controls and $n = 8$ for mutants). Multiple T-test. Scale bar represents 100 μm . Data used for quantitative analyses as well as the numerical data that are represented in graphs are available in **Figure 3—figure supplement 1—source data 1**.

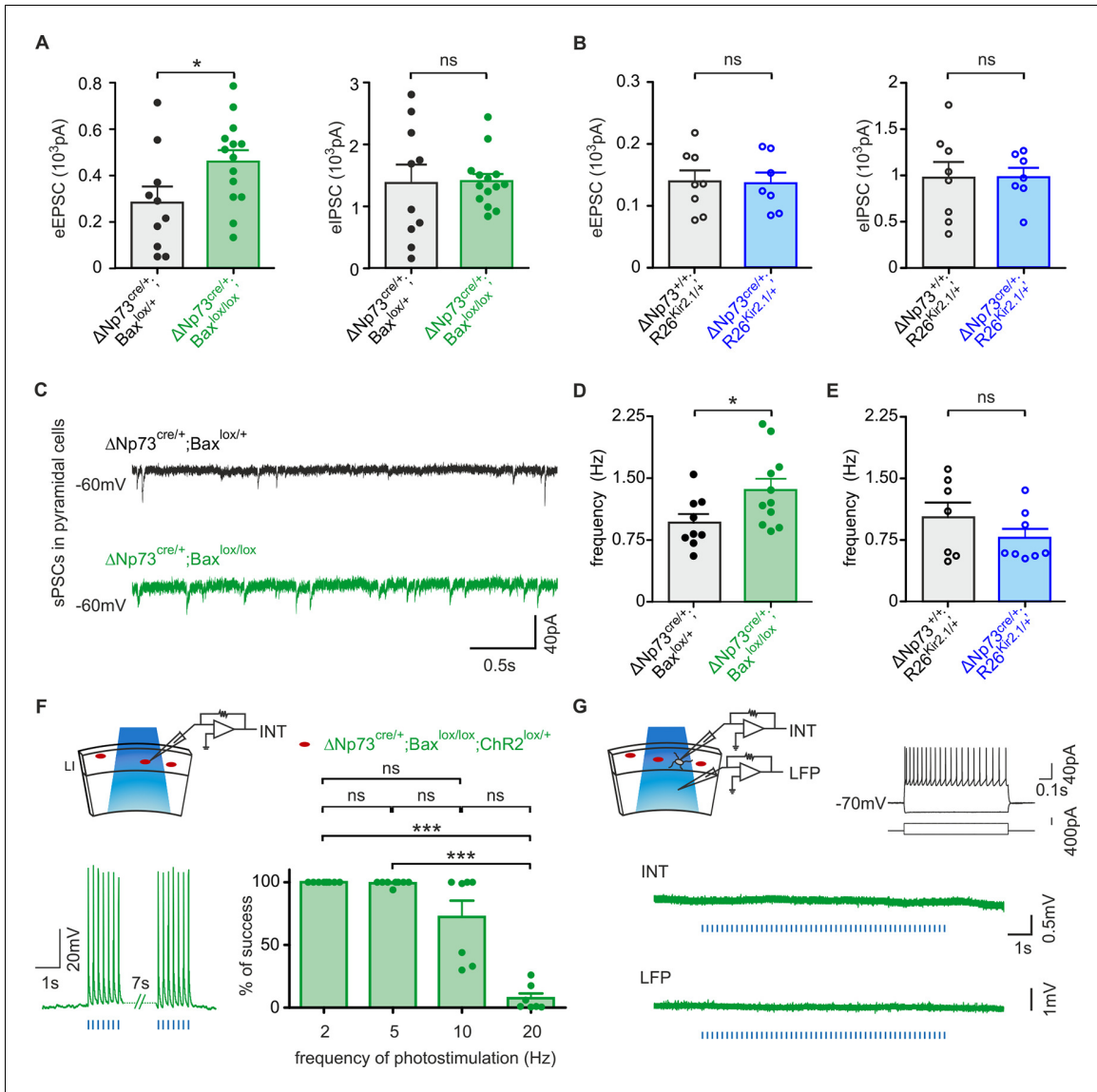


Figure 4—figure supplement 1. Evoked and spontaneous EPSCs and IPSCs of LII/LI/III pyramidal neurons in $\Delta Np73^{cre/+}; Bax^{lox/lox}$ and $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mutants. (A, B) Plots of the mean amplitude of eEPSCs (left) and eIPSCs (right) evoked by extracellular stimulation for the same pyramidal neurons of **Figure 4** held at -70 mV and 0 mV, respectively, in controls and $\Delta Np73^{cre/+}; Bax^{lox/lox}$ (A) and $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ (B) mutants. Note the significant increase in the mean amplitude of eEPSCs for $\Delta Np73^{cre/+}; Bax^{lox/lox}$ ($p=0.042$) but not for $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mutants ($p=0.902$). eIPSCs remained unchanged. (C) sEPSCs recorded in pyramidal neurons held at -70 mV in a control at P26 (black) and a $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mutant at P23 (green). (D, E) Plots of the mean frequencies of sEPSCs in $\Delta Np73^{cre/+}; Bax^{lox/lox}$ (D) and $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ (E) mutants at P23-26 and P23-28, respectively. Note the significant increase in the mean frequency of sEPSCs for $\Delta Np73^{cre/+}; Bax^{lox/lox}$ but not for $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mutants ($n = 9$ for controls and $n = 11$ for $\Delta Np73^{cre/+}; Bax^{lox/lox}$ $p=0.038$, Student T test; $n = 7$ for controls and $n = 8$ for $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mice $p=0.942$, Mann-Whitney U test). For the same cells, mean sEPSC amplitudes: -17.5 ± 2.2 pA for controls vs -19.2 ± 1.4 pA for $\Delta Np73^{cre/+}; Bax^{lox/lox}$ mice ($p=0.501$; Student T test) and -12.20 ± 0.81 pA for controls vs -14.30 ± 1.95 pA for $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ mice ($p=0.9551$; Mann-Whitney U test). (F) Current-clamp recording of a ChR2-expressing rescued CR upon light stimulation in a $\Delta Np73^{cre/+}; Bax^{lox/lox}; ChR2^{lox/+}$ mouse (see diagram). Note that photoactivation (blue pulses, 1 ms) evoked action potentials in response to every light pulse of a 5 Hz-light train of 10 s (bottom left). Similar results were obtained with a 5 Hz-light train of 30 s ($n = 7$) and in the presence of ionotropic receptor antagonists $10 \mu M$ NBQX, $50 \mu M$ AP5 and $10 \mu M$ SR95351 ($n = 2$). Average percentage of success to elicit action potentials with light trains of 10 s delivered from 2 to 20 Hz (right). Note the decreased number of action potentials triggered by photoactivation from 10 Hz ($n = 7$; Kruskal-Wallis test followed by a Bonferroni multiple comparison). (G) Simultaneous Layer II/III extracellular recording and whole-cell recording of a Layer I interneuron localized nearby a ChR2-expressing rescued CR (see diagram, top left). The firing of the interneuron in response to 800 ms depolarizing and hyperpolarizing steps is shown (inset, top right). A 5 Hz-light train of 10 s did not induce Layer II/III LFPs or postsynaptic currents in the recorded interneuron held at -70 mV (bottom left). Similarly, no responses

Figure 4—figure supplement 1 continued on next page

Figure 4—figure supplement 1 continued

were observed during extracellular recordings of Layer I (n = 3), Layer II/III (n = 5) and Layer V (n = 3) or during whole-cell recordings of Layer I interneuron (n = 3) in normal conditions or in the presence of 0 mM Mg^{2+} , 3 mM Ca^{2+} and 4AP (n = 3). Data used for quantitative analyses as well as the numerical data that are represented in graphs are available in **Figure 4—figure supplement 1—source data 1**.

Article 2

Please note that the following article is in progression.

Some experiments are still ongoing to finish the study.

Layer 1 wiring is shaped by Cajal-Retzius cells and early sensory activity

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ABSTRACT

The neocortex, which controls sensory perception, motor behavior and cognitive functions, relies on complex circuits that are organized into 6 layers. The superficial layer 1 (L1) is a major site for input integration as it contains incoming axonal projections, apical dendrites of pyramidal neurons and interneurons, which are essential for the excitation/inhibition (E/I) balance. L1 is thus a convergence point between the bottom-up environmental information and top-down largely internally generated signals. Although there is increasing evidence that L1 plays important roles in sensory integration, there is limited knowledge about its formation. During development and early postnatal stages, L1 is colonized by Cajal-Retzius (CR) cells, a transient population of cortical neurons, which shape underlying cortical circuits. On the other hand, early sensory activity emerges also as a key player in the wiring of cortical circuits in the same timeframe. Here, we report that both CRc density and sensory activity participate to L1 formation. The two though are not directly related since CRc density, which is a parameter tightly regulated during early postnatal life, is unaffected by early sensory deprivation. In contrast, reduced CRc density and impaired early sensory activity both trigger a long-lasting persistent increase in a specific subpopulation of interneurons in L1 as well as a disruption of spine densities of deep layers pyramidal neurons. Overall, this work reveals that transient neurons regulate the E/I balance in upper neocortical layers and display a remarkable interplay with early emerging sensory activity, with important implications for understanding the physiological and pathological brain wiring.

INTRODUCTION

The neocortex, which controls complex functions, via exquisite neuronal networks organized in 6 layers. Cortical layer 1 (L1) is a major site for input integration as it contains incoming axonal projections, apical dendrites of pyramidal neurons and interneurons, which are essential for the excitation/inhibition (E/I) balance (Ibrahim et al. 2020; de Frutos et al. 2016). The E/I balance is essential for circuit function since its deficits have been linked to the etiology of several neurodevelopmental disorders including Autism Spectrum Disorders or Schizophrenia (Marín 2012; Nelson and Valakh 2015). Although there is increasing evidence that L1 plays important roles in sensory integration, cross-modal communication and perception (Ibrahim et al. 2020, 2016; Abs et al. 2018; Poorthuis et al. 2018; Fan et al. 2019; Larkum 2013; Takahashi et al. 2016; Suzuki and Larkum 2020; Manita et al. 2015), little is known about how this structure is put in place. During development and early postnatal stages, L1 is colonized by Cajal-Retzius cells (CRc), a transient population of cortical neurons. CRc are a heterogenous population of first born cortical neurons and play pivotal roles in several processes in brain development (Riva et al. 2019; de Frutos et al. 2016; Barber and Pierani 2016; Kirischuk, Luhmann, and Kilb 2014; Tissir et al. 2009; Griveau et al. 2010; D'Arcangelo et al. 1995; Barber et al. 2015). Recent work suggested that the density of CR cells is maintained prenatally via an activity-dependent process, while the elimination of subsets of CRc is mediated through activity-dependent apoptosis (de Frutos et al. 2016; Riva et al. 2019; Ledonne et al. 2016). Moreover, impaired density of CRc has been shown to modulate in the first postnatal week interneuron densities and axonal outgrowth in L1, thereby regulating the E/I balance in upper layers (de Frutos et al., 2016).

On the other hand, increasing evidence reveals that early sensory activity is very important in shaping cortical circuits. At the global level of population dynamics, since early embryonic development, spontaneous thalamic activity is necessary for regulating sensory cortical areas prior to actual sensory processing (Moreno-Juan et al. 2017; Antón-Bolaños et al. 2019). Later, at the end of the first postnatal week, general pattern of spontaneous activity in the somatosensory cortex undergoes major changes, switching from synchronous activation of assemblies comprising both glutamatergic neurons and interneurons, to decorrelated events. For at least interneurons, these changes are thalamic mediated (Modol et al. 2020). At a more zoomed – in level, early sensory mediated activity shapes postnatally the morphology of subtypes of upper layers interneurons and their integration in cortical circuits, which are indispensable for a proper brain function (De Marco García et al. 2015; Che et al. 2018; De Marco García et al. 2011; Cossart 2011; Kastli et al. 2020). Moreover, early sensory activity is impacting not only on inhibitory circuits, but modulates also spine dynamics of pyramidal neurons shaping the excitatory entries (Tjia et al. 2017). All these evidence point towards a

Results

critical role of early sensory activity in shaping postnatally cortical circuits, notably by modulating both elements of the E/I ratio which is necessary for a proper brain function.

Here, we show that CRc density is an important parameter that is tightly regulated in development and together with early sensory activity participates to the formation of L1. CRc modulates the densities of interneurons via their density and independently of CRc activity. Both impairments in CRc density and early sensory activity have similar consequences on L1 at early postnatal stages – a decreased thickness of L1 and an increased number of interneurons. However, even though the two act similarly on L1 formation, we show that the two are not directly related as early sensory activity is not modulating the CRc density at the cortical surface. In contrast, both CRc density and sensory activity during early postnatal life, have long lasting consequences on cortical wiring.

Our findings show that both early sensory activity and CRc contribute to L1 formation and argue in favor of an unappreciated role of transient changes in electrical activity or transient neurons in controlling the cellular and functional E/I balance of the neocortex. This is instrumental for a better understanding of how both transient CRc and electrical activity sustains neocortex construction and how they could both contribute to miswirings leading to different neurodevelopmental disorders.

RESULTS AND DISCUSSION

Development of cortical layer 1

To investigate the roles of CRc and early sensory activity on L1 wiring, we first characterized the postnatal cellular and neuritic composition of L1 at P7 and P25. Adult L1 is a major point for sensory integration it contains abundant axonal projections which contact the apical dendrites of pyramidal neurons, together with a small number of interneurons which modulate these connections. In contrast, the developing and early postnatal L1 hosts more neurons which are localized in L1 either because they are transient or because they are migrating through this structure. At P7, L1 is hosting transient CRc, the first generated neurons (Kirischuk, Luhmann, and Kilb 2014). Besides CRc, the early postnatal L1 contains also CGE and POA-derived interneurons, while the MGE-derived interneurons are located in deeper cortical layers and only projecting in L1 (**Fig. 1**). Note that the distribution of Martinotti cell axons in L1 is highly organized spatially and restricted to the most superficial part of the L1 (L1a). Both the interneurons located in L1 or projecting in the L1 contact the apical tufts of pyramidal neurons which are instrumental for information processing. By P25, the CRc have almost entirely been eliminated from the S1 (**Fig. 1**) and the L1 contains only few subpopulations of interneurons, together with abundant axonal projections and apical dendrites of pyramidal neurons.

To label the CRc we use a double approach. When possible, we label the CRc with $\Delta Np73^{cre/+}$ mouse line which targets approximately 80% of CRc originating in three out of four origins (hem, septum - SE and thalamic eminence- ET) (Tissir et al. 2009; Bielle et al. 2005; Ruiz-Reig et al. 2017; Griveau et al. 2010) (**Fig. 1**). When not possible to use the $\Delta Np73^{cre/+}$ mouse line, we identify at embryonic stages CRc as Reelin⁺ cells. Postnatally, Reelin starts being expressed also in the interneurons. Therefore to identify the CRc with immunostainings we use Reelin⁺Prox1⁻ marker. As CRc from all origins are expressing Reelin, with this approach we target the vast majority if not all the CRc and no interneurons (**Fig. S1A,B**). To label CGE-derived interneurons, at P7 we use only the Prox1 marker (as CoupTF2 is also expressed in at least subsets of CRc in L1). Postnatally, we label CGE-derived interneurons with a combination of markers which can reveal their identity.

Cajal-Retzius cell density and early sensory activity impact onto layer 1 formation

Both CRc and sensory activity are major regulators of cortical wiring. In this view, we investigated to what extent the two participate to the wiring of cortical L1. To address the role of CRc in the wiring of upper cortical layers, we used a combination of genetic approaches. Because it has been previously suggested that electrical activity in CRc modulates their density

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(de Frutos et al. 2016), we used a comparative approach of different genetic models to investigate whether an impact of CRc density, activity or both, is modulating the wiring of upper cortical layers. We have first used the model $\Delta Np73^{cre/+}; GluN1^{fl/fl}$ in which the GluN1 subunit of NMDA receptors is impaired. In this model, it was already suggested and we have confirmed, a decrease in the number of CRc in L1 (**Fig. S2, Fig. S4**) (de Frutos et al. 2016). Therefore, in this model both the density and electrical activity of the remaining CRc are impaired. In this case, we can notice that at P7 a decreased L1 thickness, no major changes in the upper layers identity and barrel organization, and an increase in the number of CGE-derived Prox1⁺ interneurons in L1 and L2/3 of the somatosensory cortex (**Fig. 2**). In the $\Delta Np73^{cre/+}; R26^{dt-a/+}$ only on the density of CRc is impacted without affecting their activity (**Fig. S2**) (de Frutos et al. 2016). This model presents a decreased L1 thickness and increased number of Prox1⁺ interneurons in upper cortical layers (**Fig. 2**). These results already suggest that the CRc control the thickness of L1 and the interneuron density through their density, independently of their electrical activity. To confirm this hypothesis, we compared these results with the $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ model, in which we overexpress the hyperpolarizing Kir2.1 channel specifically in CRc (Moreno-Juan et al. 2017; Riva et al. 2019). In this model, the density of CRc is not impacted at P7 and the CRc are hyperpolarized (Riva et al. 2019), therefore we impact only the activity of CRc and not with their density. Consistent with our previous findings, in the $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ model, there is no impact on L1 thickness nor on the distribution of CGE-derived interneurons (**Fig. 2**). Altogether, this shows that the CRc are shaping the wiring of upper cortical layers through their density, and not through their activity (**Fig.2**).

Another major regulator of cortical wiring in the early postnatal stages is general, sensory electrical activity. To our surprise, we have noticed that early sensory activity is also important in the formation of L1. By doing Whisker plucking either daily from P1-P3 or at P1 and P5, we have noticed that the L1 is shrunken, similarly with the models of impaired CRc density (L1 thickness at P7 in control $103.8 \pm 16.03 \mu\text{m}$, $\Delta Np73^{cre/+}; GluN1^{fl/fl}$ model $76.72 \pm 9.84 \mu\text{m}$, $\Delta Np73^{cre/+}; R26^{dt-a/+}$ model $69.43 \pm 11.1 \mu\text{m}$, $\Delta Np73^{cre/+}; R26^{Kir2.1/+}$ model $100.1 \pm 17.16 \mu\text{m}$ and Whisker Pluck model $72.24 \pm 8.18 \mu\text{m}$). This model presents also an slight increase in the number of upper layers interneurons but less striking compared to models in which the CRc density is impaired (**Fig.2**).

Altogether, these experiments suggests that both CRc and sensory activity in the L1 participate to the formation.

The density of Cajal-Retzius cells is a parameter that is tightly regulated in development
Impairments in CRc density induce defects in the wiring of circuits, suggesting that CRc density is a parameter that is tightly regulated in space and time, while the brain grows in development.

Results

The CRc density was suggested to be maintained at embryonic stages through a *GluN1*-dependent redistribution of CRc from a reservoir located near the lateral olfactory tract (LOT) (de Frutos et al. 2016). Indeed, we confirm labeling the CRc at embryonic stages with Reelin and postnatally as Reelin⁺Prox1⁻ that not only the density of CRc is indeed perturbed at E15.5, but also that the reduction in CRc density in the somatosensory cortex is maintained by E18.5 and P7 (**Fig. S4**). To confirm that indeed the decrease in CRc density is due to an impaired redistribution from the LOT we have generated the double mutants $\Delta Np73^{cre/+}; GluN1^{fl/fl}; R26^{mT/+}$ which enable genetic tracing of CRc, enabling the visualization of CRc in the LOT which otherwise is delicate. Accordingly, at P7 we noticed a decrease of approximately 30% of CRc in the L1 and a 2.5 fold increase in the density of CRc in the LOT compared to controls (**Fig. 4A**). Therefore, these results indeed confirm that the CRc redistribute from the LOT through a *GluN1*-dependent migration.

Postnatally, the density of CRc is controlled via their elimination. The elimination of CRc mainly occurs in a very narrow temporal window. In the Somatosensory cortex, the density of $\Delta Np73^{cre/+}; R26^{mT/+}$ CRc is quite stable postnatally and drops significantly between P7 (27 ± 3.7 cells/mm) to P9 (15.25 ± 2.8 cells/mm) (**Fig. 3**). The decrease in CRc density can be observed also after P9 but this is likely due to a dilution due to the brain growth that takes place in development, until their almost complete elimination in the Somatosensory cortex by P25 (0.66 ± 1.1 cells/mm). We analyzed this dynamics of CRc density with the $\Delta Np73^{cre/+}; R26^{mT/+}$ model which reveals the behavior of 80% of the CRc. We also checked with Reelin⁺Prox1⁻ method which allows identification of almost all CRc and we found a very similar dynamics of CRc elimination (P7: 31.05 ± 4.9 cells/mm, P9: 17.5 ± 3.6 cells/mm, P25: 1.5 ± 0.5 cells/mm) (**Fig. S3**).

Importantly, the elimination of SE and ET – derived CRc is mediated via activity-dependent apoptosis, unlike the hem-derived CRc which are eliminated via different mechanisms (Ledonne et al. 2016; de Frutos et al. 2016; Riva et al. 2019). We further on questioned whether the activity-dependent death of CRc is mediated, as their redistribution, via NMDA receptors. Using the previously presented $\Delta Np73^{cre/+}; GluN1^{fl/fl}; R26^{mT/+}$, we have noticed that cKO of *GluN1* is not having any impact on the elimination of the CRc (**Fig. 4B**). This is indeed consistent with previous findings suggesting that postnatally, CRc are mainly driven by GABAergic inputs (Riva et al. 2019; Soda et al. 2003; Sun et al. 2019).

Altogether, these results show that the CRc density tightly controlled in time and space is maintained early on via a redistribution from the LOT to the surface of the cortex and postnatally via their elimination. Both these processes are, in part, activity mediated (de Frutos et al. 2016; Riva et al. 2019) but the mechanisms driving these two processes are different: *GluN1*-dependent redistribution and *GluN1*-independent elimination.

General sensory activity is not influencing CRc density

As both CRc density and early sensory activity impact on L1 formation in a similar manner, and as the density of CRc is tightly regulated through activity-dependent mechanisms, we questioned whether the early sensory activity impacts on the density of CRc. To address this question we had a triple approach. First, we investigated whether the evoked activity via the whiskers impact on the CRc density in the somatosensory cortex – by doing bilateral whisker plucking (WP) (Rhoades et al. 1990). Second, we checked whether spontaneous activity independent of the whisker stimulation could impact on CRc density – by doing unilateral ION lesion (as Bechara et al. 2015). Third, we investigated whether the thalamic input and the spontaneous thalamic waves impact on CRc density – by hyperpolarizing the thalamic axons through overexpression of the Kir2.1 channel, using the *Sert^{cre/+}* driver (Moreno-Juan et al. 2017). The WP and the ION experiments were performed in the $\Delta Np73^{cre/+}; R26^{mT/+}$ model in which we genetically trace approximately 80% of the CRc. For the hyperpolarization of the thalamus condition, we identified the CRc with immunostainings as *Reelin⁺Prox1⁻* cells. We performed the analysis at both P7 as a timepoint before the period of cell death, as well as P9 and/or P15 after the peak in CRc death (**Fig. 5**). In this way we could assess whether general early sensory activity is impacting on the density of CRc in the first postnatal week or whether it affects their very timed elimination. To our surprise, we found that all our approaches to impair sensory activity do not impact on the CRc density, neither at P7, neither at later developmental stages (**Fig. 5**).

Collectively, these results show that even though the early sensory activity is key in shaping cortical wiring, early sensory activity is not impacting on the density of CRc.

As sensory activity is not shaping CRc elimination in this timed manner and as CRc are postnatally mainly innervated by GABAergic inputs and not via NMDA signaling (Riva et al. 2019; Sun et al. 2019; Soda et al. 2003), we reasoned that the elimination of SE and ET CRc could be triggered by L1 projecting interneurons. To address this question we overexpressed the HM3Dq DREADD (excitatory) in all *Nkx2.1*-derived interneurons. We hypothesized that if *Nkx2.1*-derived interneurons are killing the SE and ET-derived CRc which are eliminated through activity dependent mechanisms, overactivation of these interneurons would lead to a premature elimination of these CRc. We have overactivated interneurons in a timed manner from P5-P7 and P7-P9 and quantified the density of CRc identified as *Reelin⁺Prox1⁻* cells in L1 of somatosensory cortex. The overactivation of interneurons between these specific time windows does not induce any major perturbations in their density and distribution across the cortical plate (**Fig. S5B**). The overactivation of *Nkx2.1*-derived interneurons induces indeed a decrease in the density of CRc suggesting a premature elimination of CRc at P7 (**Fig. S5A**). Moreover, the number of CRc which are prematurely eliminated in these conditions is

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consistent with the number of CRc which are eliminated through activity-dependent mechanisms (approximately 10 cells/mm in both cases) (Riva et al. 2019).

Overall these results show that CRc density is not modulated by early sensory activity. However, overactivation of L1-projecting interneurons induces a premature elimination of the CRc which die through activity-dependent mechanisms.

CRc density and general sensory activity have long lasting consequences on interneuron densities.

To test whether the defects on cortical wiring induced by CRc density and impaired sensory activity (**Fig 2**) are long lasting, even after CR cells have disappeared, we examined at P25 the L1 thickness and distribution of several subtypes of interneurons.

We found that the L1 thickness is not majorly impacted as it is at P7 either by CRc or early sensory activity modulations (data not shown), but there are long lasting consequences on the distribution of interneurons. Consistently, in the model $\Delta Np73^{cre/+} R26^{dt-a/+}$ which presents a 45% decrease in the number of CRc at P7, there is a statistically significant increase in the number of Reelin⁺, NPY⁺, Calretinin⁺ and CoupTF2⁺ interneurons in upper cortical layers. These markers are overlapping for several subpopulations of interneurons suggesting that CRc are likely modulating the densities of Neurogliaform interneurons (Reelin⁺, NPY⁺, CoupTF2⁺) and Bipolar cells (Calretinin⁺) (**Fig. 6**). Impairment in early sensory activity has a lighter but non negligible effect, inducing an increase in the number of CoupTF2⁺ interneurons, but which are not Reelin⁺, nor NPY⁺ (**Fig. 6**). What is this subpopulation of interneurons still remains to be investigated. These results are consistent with previous data in (de Frutos et al. 2016) showing in the $\Delta Np73^{cre/+} R26^{dt-a/+}$ model a slightly increased inhibitory drive on upper layers pyramidal neurons. The density of CRc and early sensory activity do not modulate only L1 wiring, but has also rather indirect effects on deep cortical layers. Both reduced CRc density during their lifetime as well as impaired early sensory activity lead to a decrease of PV⁺ numbers in L5-6 and no major change in the density and distribution of SST⁺ interneurons (**Fig. S6**).

These results are remarkable because both the Neurogliaform interneurons and Bipolar cells are involved in shaping the top-down inputs arriving in L1 modulating sensory perception, learning and arousal (Williams and Holtmaat 2019; Hochbaum et al. 2014; Lee et al. 2013), revealing a potential role of CRc and early sensory activity in initially regulating these processes.

CRc density modulates spine densities in L1 while early sensory activity shapes spine densities in deeper cortical layers

Decreased CRc density ($\Delta Np73^{cre/+} R26^{dta}$) induces a reduced excitatory input on the apical dendrites of upper layers pyramidal neurons (de Frutos et al. 2016). Nonetheless, little is known about the impact of the CRc on deep layers pyramidal neurons. The two are very different in terms of morphological aspects and physiological properties. For example, while upper layers apical dendrites are branching through the whole thickness of L1, deep layers pyramidal neurons are mainly creating a dendritic plexus in the superficial part of L1 (Tjia et al. 2017). To address this question, we have backcrossed the $\Delta Np73^{cre/+} R26^{dt-a/+}$ model with $Thy1^{gfp/+}$ which labels in a sparse manner the L5b pyramidal neurons (**Fig. 7A**). While the branching of apical dendrites of L5 pyramidal neurons, qualitatively, does not seem to be drastically impaired (**Fig. 7A**), an impaired density of CRc leads to a statistically significant decrease in the spine densities on apical dendrites of L5 pyramidal neurons (**Fig. 7B, C**). Surprisingly, early whisker mediated sensory activity is not impacting the spine densities on the apical dendrites of L5 pyramidal neurons (**Fig. 7B,C**) (control: 0.5 ± 0.11 spines/ μm , $\Delta Np73^{cre/+} R26^{dt-a/+}$: 0.3 ± 0.08 spines/ μm , Whisker Pluck: 0.54 ± 0.11 spines/ μm). To understand whether this is a local effect in L1 or a general impact on spine densities also at long distance, we quantified in the same model the spine densities on the basal dendrites of L5 pyramidal neurons. CRc density has no impact on spine densities on basal dendrites, however, an impaired early sensory activity induces a long lasting decrease in the basal spine densities of L5 pyramidal neurons (**Fig. S7**) (control: 0.74 ± 0.1 spines/ μm , $\Delta Np73^{cre/+} R26^{dt-a/+}$: 0.78 ± 0.17 spines/ μm , Whisker Pluck: 0.62 ± 0.15 spines/ μm).

This suggests that CRc modulate spine densities in L1 in a general manner, shaping the excitatory entries on both upper and deep cortical layers. Early sensory activity is not modulating spine densities in L1 but has a long lasting impact on the spine densities of basal dendrites.

Altogether, these experiments reveal that CRc and early sensory activity converge onto building up the L1. Both modulate the wiring of upper cortical layers in the first postnatal week. But both CRc and early sensory activity have also long lasting consequences on cortical wiring by controlling the numbers of inhibitory interneurons in L1 as well as excitatory entries in L1 and in deeper cortical layers respectively. Therefore, CRc and early sensory activity shape the two elements of the E/I balance, suggesting their important contribution to the cortical function.

MATERIAL AND METHODS

Animals

$\Delta Np73^{Cre/RESGFP}$ ($\Delta Np73^{Cre}$) (Tissir et al. 2009), $Nkx2.1:cre$ (JAX008661 C57BL/6J-TgNkx2-1-cre 2Sand/J), $SST^{cre/+}$ (JAX013044 Ssttm2.1(cre)Zjh/J), $Sert^{cre/+}$ (Narboux-Nême et al. 2008), $GluN1^{flox/flox}$ (Niewoehner et al. 2007), $R26^{Kir2.1-mCherry/+}$ (Moreno-Juan et al. 2017), $Thy1^{gfp/+}$ (Feng et al. 2000) and R26-LSL-hM3Dq-DREADD ($Gq^{DREADD/DREADD}$) (JAX026220 B6N;129-Tg(CAG-CHRM3*, -mCitrine)1Ute/J) mice were kept in a C57BL/6J background. $\Delta Np73^{Cre}$ was crossed with the $R26^{mT}$ reporter line to permanently label CR subtypes, with the $R26^{flox-stop-flox-dt-a}$ (Brockschneider et al. 2007) to induce the partial elimination of CRc, with the $GluN1^{flox/flox}$ to inactivate GluN1 function specifically in CRc and with the $R26^{Kir2.1-mCherry/+}$ to overexpress the Kir2.1 channel in CRc. $Nkx2.1:cre$ mice were crossed with $Gq^{DREADD/DREADD}$ to enable a temporal control of neuronal activity. $Thy1^{gfp/+}$ mice were crossed with $R26^{flox-stop-flox-dt-a}$ to generate in a first time animals $R26^{flox-stop-flox-dt-a}; Thy1^{gfp/+}$. The latter model was crossed with $\Delta Np73^{Cre}$ to enable a modulation of CRc density and a visualization of L5 pyramidal neurons. Controls used were littermates for each cross which were not expressing the *cre*. Animals were genotyped by PCR using primers specific for the different alleles. The day of the vaginal plug was considered E0.5 and the birth date was considered as postnatal day 0 (P0). Animals were handled in accordance with French and European regulations and the local ethics committee.

Drugs

The DREADD receptor is activated through injections of its exogenous ligand CNO. CNO (Tocris 4936) was diluted with 0.9% saline to 0.5 mg/ml. Pups were injected with vehicle (0.9% saline) or CNO (5mg/kg) subcutaneously, twice a day, from P5-P7 or P7-P9.

Sensory deprivation

The whisker plucking was performed bilaterally on pups either at P1 and P5, either daily from P1 to P3. Pups were removed from the mother, anesthetized by hypothermia for 3 min and whiskers were plucked with sterile forceps.

The Infraorbital Nerve Lesion was performed unilaterally only on the right whisker pad at P1, as previously described (Rhoades et al. 1990; Frangeul et al. 2014). In brief, pups were removed from the mother and anesthetized by hypothermia for 3 min. Using a sterile scalpel, an incision was made between the eye and the whisker pad, enabling cutting the InfraOrbital Nerve. Pups were put on a heating pad to recover from the lesion and anesthesia. The efficiency of the manipulation was systematically checked immunostainings for Vglut2 to check for the presence of barrels in S1.

Results

Tissue preparation and immunohistochemistry

Animals were anesthetized with Isoflurane and intracardially perfused with 4% paraformaldehyde (PFA) in 0.1 M PBS, pH 7.4 and post-fixed over-night in 4% PFA at 4°C. Brain were embedded in 3.5% agarose and sectioned in 70 µm free-floating slices at all stages and 300 µm free-floating sections for the *Thy1* mouse model, which allows a better visualization of dendritic arbors. Immunostaining was performed as previously described (de Frutos et al. 2016). Primary antibodies used for immunohistochemistry were: mouse anti-Reelin (MAB5364, Millipore 1:300), rabbit anti-DsRed (Takara 632496, 1:500), goat anti-Prox1(AF2727, R&D Systems, 1:500), rabbit anti-Prox1 (ab28692, Abcam, 1:300), rabbit anti-CoupTF2/Nr2f2 (AB41859, Abcam, 1:300), rabbit anti-NPY (22940, Immunostar, 1:1000), rat anti-SST (MAB354, Millipore, 1:50), chicken anti-gfp (Gfp-1020, Aves, 1:1000), guinea pig anti-VGlut2 (AB2251-I, Millipore 1:1000), rabbit anti-Cux1 (AB-2261231, Santa-Cruz Biotechnology 1:300), rabbit anti-Calretinin (7697, Swant 1:1000), rabbit anti-Parvalbumin (PV27, Swant 1:1000), mouse anti-NeuN (MAB377, Millipore 1:300). Secondary antibodies used against primary antibodies were: donkey anti-mouse Alexa-488 (Jackson ImmunoResearch Laboratories, 1:800), donkey anti-mouse Cy3 (Jackson ImmunoResearch Laboratories, 1:800), donkey anti-chicken Alexa-488 (Jackson ImmunoResearch Laboratories, 1:800), donkey anti-guinea pig Alexa-488 (Jackson ImmunoResearch Laboratories, 1:800), donkey anti-rabbit Cy3 (Jackson ImmunoResearch Laboratories, 1:800), donkey anti-rat Cy3 (Jackson ImmunoResearch Laboratories, 1:800). Hoechst (Sigma-Aldrich 33342, 1:1000) was used for fluorescent nuclear counterstaining the tissue and mounting was done in Vectashield (Vector Labs).

Image acquisition and quantification

Immunofluorescence images were acquired using a confocal microscope (Leica TCS SP5) with objectives of either 10X, 40X and 63X, with or without optical zoom. Cellular and spine quantifications were performed using the ImageJ software, in the somatosensory barrel cortex (S1) or Lateral Olfactory tract (LOT) for the respective ages and genotypes. For each section, the density of CRc was calculated as number of CRc/1mm length of layer 1 on a 10µm thick optical section, measured using ImageJ software.

Statistical Analysis

All data were expressed as mean±SEM. A P-value less than 0.05 was considered significant. According to the data structure, we systematically performed non-parametric tests Mann-Whitney U Test or Kruskal-Wallis Tests with Dunn's correction. Statistics and plotting were performed using GraphPad Prism 7.00 (GraphPad Software Inc., USA). *p < 0.05, **p < 0.01, ***p < 0.001.

REFERENCES

- Abs, Elisabeth, Rogier B. Poorthuis, Daniella Apelblat, Karzan Muhammad, M. Belen Pardi, Leona Enke, Dahlia Kushinsky, et al. 2018. "Learning-Related Plasticity in Dendrite-Targeting Layer 1 Interneurons." *Neuron* 100 (3): 684–699.e6. <https://doi.org/10.1016/j.neuron.2018.09.001>.
- Antón-bolaños, Noelia, Alejandro Sempere-ferrández, Teresa Guillamón-Vivancos, Francisco J Martini, Leticia Pérez-Saiz, Henrik Gezelius, Anton Filipchuk, Miguel Valdeolmillos, and Guillermina López-bendito. 2019. "Functional Cortical Maps in Mice." *Science* 990 (June): 987–90. <https://doi.org/10.1126/science.aav7617>.
- Barber, Melissa, Yoko Arai, Yoshihiro Morishita, Lisa Vigier, Fr??d??ric Causeret, Ugo Borello, Fanny Ledonne, et al. 2015. "Migration Speed of Cajal-Retzius Cells Modulated by Vesicular Trafficking Controls the Size of Higher-Order Cortical Areas." *Current Biology* 25 (19): 2466–78. <https://doi.org/10.1016/j.cub.2015.08.028>.
- Barber, Melissa, and Alessandra Pierani. 2016. "Tangential Migration of Glutamatergic Neurons and Cortical Patterning during Development: Lessons from Cajal-Retzius Cells." *Developmental Neurobiology* 76 (8): 847–81. <https://doi.org/10.1002/dneu.22363>.
- Bechara, Ahmad, Christophe Laumonnerie, Nathalie Vilain, Claudius F. Kratochwil, Vanja Cankovic, Nicola A. Maiorano, Moritz A. Kirschmann, Sebastien Ducret, and Filippo M. Rijli. 2015. "Hoxa2 Selects Barrelette Neuron Identity and Connectivity in the Mouse Somatosensory Brainstem." *Cell Reports* 13 (4): 783–97. <https://doi.org/10.1016/j.celrep.2015.09.031>.
- Bielle, Franck, Amélie Griveau, Nicolas Narboux-Nême, Sébastien Vigneau, Markus Sigrist, Silvia Arber, Marion Wassef, and Alessandra Pierani. 2005. "Multiple Origins of Cajal-Retzius Cells at the Borders of the Developing Pallium." *Nature Neuroscience* 8 (8): 1002–12. <https://doi.org/10.1038/nn1511>.
- Brockschneider, Damian, Yvonne Pechmann, Eva Sonnenberg-Riethmacher, and Dieter Riethmacher. 2007. "An Improved Mouse Line for Cre-Induced Cell Ablation Due to Diphtheria Toxin A, Expressed From the Rosa26 Locus." *Genesis* 45 (2): 76–82. <https://doi.org/10.1002/dvg>.
- Che, Alicia, Rachel Babij, Andrew F. Iannone, Robert N. Fetcho, Monica Ferrer, Conor Liston, Gord Fishell, and Natalia V. De Marco García. 2018. "Layer I Interneurons Sharpen Sensory Maps during Neonatal Development." *Neuron* 99 (1): 98–116.e7. <https://doi.org/10.1016/j.neuron.2018.06.002>.
- Cossart, Rosa. 2011. "The Maturation of Cortical Interneuron Diversity: How Multiple Developmental Journeys Shape the Emergence of Proper Network Function." *Current Opinion in Neurobiology* 21 (1): 160–68. <https://doi.org/10.1016/j.conb.2010.10.003>.
- D'Arcangelo, Gabriella, Graham G Miao, Shu Cheng Chen, Holly D. Scares, James I Morgan, and Tom Curran. 1995. "A Protein Related to Extracellular Matrix Proteins Deleted in the Mouse Mutant Reeler." *Nature* 374 (6524): 719–23. <https://doi.org/10.1038/374719a0>.
- De, Natalia V, Marco García, Theofanis Karayannis, and Gord Fishell. 2011. "Neuronal Activity Is Required for the Development of Specific Cortical Interneuron Subtypes." *Nature* 472. <https://doi.org/10.1038/nature09865>.
- Fan, Linlin, Simon Kheifets, Urs Böhm, Kiryl Piatkevich, Hao Wu, Vicente Parot, Michael Xie, Edward Boyden, Anne Takesian, and Adam Cohen. 2019. "All-Optical Electrophysiology Reveals Excitation, Inhibition, and Neuromodulation in Cortical Layer 1." *BioRxiv*, 614172. <https://doi.org/10.1101/614172>.
- Feng, Guoping, Rebecca H Mellor, Michael Bernstein, Cynthia Keller-Peck, Quyen T Nguyen, Mia Wallace, Jeanne M Nerbonne, Jeff W Lichtman, and Joshua R Sanes. 2000. "Imaging Neuronal Subsets in Transgenic Mice Expressing Multiple Spectral Variants of GFP." *Neuron* 28: 41–51.
- Frangeul, Laura, Cesar Porrero, Maria Garcia-Amado, Benedetta Maimone, Madlyne Maniglier, Francisco Clascá, and Denis Jabaudon. 2014. "Specific Activation of the Paralemniscal Pathway during Nociception." *European Journal of Neuroscience* 39 (9): 1455–64. <https://doi.org/10.1111/ejn.12524>.
- Frutos, Cristina A. de, Guy Bouvier, Yoko Arai, Morgane S. Thion, Ludmilla Lokmane, Maryama Keita, Mario Garcia-Dominguez, et al. 2016. "Reallocation of Olfactory Cajal-Retzius Cells Shapes Neocortex Architecture." *Neuron* 92 (2): 435–48. <https://doi.org/10.1016/j.neuron.2016.09.020>.
- Griveau, Amélie, Ugo Borello, Frédéric Causeret, Fadel Tissir, Nicole Boggetto, Sonia Karaz, and Alessandra Pierani. 2010. "A Novel Role for Dbx1-Derived Cajal-Retzius Cells in Early Regionalization of the Cerebral Cortical Neuroepithelium." *PLoS Biology* 8 (7). <https://doi.org/10.1371/journal.pbio.1000440>.
- Hochbaum, Daniel R, Yongxin Zhao, Samouil L Farhi, Nathan Klapoetke, Christopher a Werley, Vikrant Kapoor, Peng Zou, et al. 2014. "All-Optical Electrophysiology in Mammalian Neurons Using Engineered Microbial Rhodopsins." *Nature Methods* 11 (8). <https://doi.org/10.1038/nmeth.3000>.

Results

- Ibrahim, Leena A., Lukas Mesik, Xu ying Ji, Qi Fang, Hai fu Li, Ya tang Li, Brian Zingg, Li I. Zhang, and Huizhong Whit Tao. 2016. "Cross-Modality Sharpening of Visual Cortical Processing through Layer-1-Mediated Inhibition and Disinhibition." *Neuron* 89 (5): 1031–45. <https://doi.org/10.1016/j.neuron.2016.01.027>.
- Ibrahim, Leena A, Ben Schuman, Rachel Bandler, Bernardo Rudy, and Gord Fishell. 2020. "ScienceDirect Mining the Jewels of the Cortex 's Crowning Mystery." *Current Opinion in Neurobiology* 63: 154–61. <https://doi.org/10.1016/j.conb.2020.04.005>.
- Kastli, Rahel, Rasmus Vighagen, Alexander van der Bourg, Ali Ozgur Argunsah, Asim Iqbal, Fabian F Voigt, Daniel Kirschenbaum, Adriano Aguzzi, and Theofanis Karayannis. 2020. "Developmental Divergence of Sensory Stimulus Representation in Cortical Interneurons." *BioRxiv*, 2020.04.28.065680. <https://doi.org/10.1101/2020.04.28.065680>.
- Kirschuk, S., H. J. Luhmann, and W. Kilb. 2014. "Cajal-Retzius Cells: Update on Structural and Functional Properties of These Mystic Neurons That Bridged the 20th Century." *Neuroscience* 275: 33–46. <https://doi.org/10.1016/j.neuroscience.2014.06.009>.
- Larkum, Matthew E. 2013. "The Yin and Yang of Cortical Layer 1." *Nature Neuroscience* 16 (2): 114–15. <https://doi.org/10.1038/nn.3317>.
- Ledonne, Fanny, David Orduz, Judith Mercier, Lisa Vigier, Elisabeth A. Grove, Fadel Tissir, Maria Cecilia Angulo, Alessandra Pierani, and Eva Coppola. 2016. "Targeted Inactivation of Bax Reveals a Subtype-Specific Mechanism of Cajal-Retzius Neuron Death in the Postnatal Cerebral Cortex." *Cell Reports* 17 (12): 3133–41. <https://doi.org/10.1016/j.celrep.2016.11.074>.
- Lee, Soohyun, Illya Kruglikov, Z. Josh Huang, Gord Fishell, and Bernardo Rudy. 2013. "A Disinhibitory Circuit Mediates Motor Integration in the Somatosensory Cortex." *Nature Neuroscience* 16 (11): 1662–70. <https://doi.org/10.1038/nn.3544>.
- Manita, Satoshi, Takayuki Suzuki, Chihiro Homma, Takashi Matsumoto, Maya Odagawa, Kazuyuki Yamada, Keisuke Ota, et al. 2015. "A Top-Down Cortical Circuit for Accurate Sensory Perception." *Neuron* 86 (5): 1304–16. <https://doi.org/10.1016/j.neuron.2015.05.006>.
- Marco García, Natalia V. De, Rashi Priya, Sebnem N. Tuncdemir, Gord Fishell, and Theofanis Karayannis. 2015. "Sensory Inputs Control the Integration of Neurogliaform Interneurons into Cortical Circuits." *Nature Neuroscience* 18 (3): 393–403. <https://doi.org/10.1038/nn.3946>.
- Marín, Oscar. 2012. "Interneuron Dysfunction in Psychiatric Disorders." *Nature Reviews Neuroscience*. <https://doi.org/10.1038/nrn3155>.
- Modol, Laura, Yannick Bollmann, Thomas Tressard, Agnès Baude, Alicia Che, Zhe Ran S. Duan, Rachel Babij, Natalia V. De Marco García, and Rosa Cossart. 2020. "Assemblies of Perisomatic GABAergic Neurons in the Developing Barrel Cortex." *Neuron* 105 (1): 93–105.e4. <https://doi.org/10.1016/j.neuron.2019.10.007>.
- Moreno-Juan, Verónica, Anton Filipchuk, Noelia Antón-Bolaños, Cecilia Mezzera, Henrik Gezelius, Belen Andrés, Luis Rodríguez-Malmierca, et al. 2017. "Prenatal Thalamic Waves Regulate Cortical Area Size Prior to Sensory Processing." *Nature Communications* 8. <https://doi.org/10.1038/ncomms14172>.
- Narboux-Nême, Nicolas, Luigi Michele Pavone, Luigi Avallone, Xiaoxi Zhuang, and Patricia Gaspar. 2008. "Serotonin Transporter Transgenic (SERT^{cre}) Mouse Line Reveals Developmental Targets of Serotonin Specific Reuptake Inhibitors (SSRIs)." *Neuropharmacology* 55 (6): 994–1005. <https://doi.org/10.1016/j.neuropharm.2008.08.020>.
- Nelson, Sacha B, and Vera Valakh. 2015. "Excitatory/Inhibitory Balance and Circuit Homeostasis in Autism Spectrum Disorders A Theory of Excitatory/Inhibitory Imbalance in Autism HHS Public Access." *Neuron* 87 (4): 684–98. <https://doi.org/10.1016/j.neuron.2015.07.033>.
- Niewoehner, B., F. N. Single, Hvalby, V. Jensen, S. Meyer Zum Alten Borgloh, P. H. Seeburg, J. N.P. Rawlins, R. Sprengel, and D. M. Bannerman. 2007. "Impaired Spatial Working Memory but Spared Spatial Reference Memory Following Functional Loss of NMDA Receptors in the Dentate Gyrus." *European Journal of Neuroscience* 25 (3): 837–46. <https://doi.org/10.1111/j.1460-9568.2007.05312.x>.
- Poorthuis, Rogier B., Karzan Muhammad, Mantian Wang, Matthijs B. Verhoog, Stephan Junek, Anne Wrana, HuiBERT D. Mansvelter, and Johannes J. Letzkus. 2018. "Rapid Neuromodulation of Layer 1 Interneurons in Human Neocortex." *Cell Reports* 23 (4): 951–58. <https://doi.org/10.1016/j.celrep.2018.03.111>.
- Rhoades, Robert W., Carol A. Bennett-Clarke, Nicolas L. Chiaia, Fletcher A. White, Gordon J. Macdonald, John H. Haring, and Mark F. Jacquin. 1990. "Development and Lesion Induced Reorganization of the Cortical Representation of the Rat's Body Surface as Revealed by Immunocytochemistry for Serotonin." *Journal of Comparative Neurology* 293 (2): 190–207. <https://doi.org/10.1002/cne.902930204>.

Results

- Riva, Martina, Ioana Genescu, Chloé Habermacher, David Orduz, Fanny Ledonne, Filippo M. Rijli, Guillermina López-Bendito, et al. 2019. "Activity-Dependent Death of Transient Cajal-Retzius Neurons Is Required for Functional Cortical Wiring." *eLife* 8 (i): 1–18. <https://doi.org/10.7554/eLife.50503>.
- Ruiz-Reig, Nuria, Belén Andrés, Dhananjay Huilgol, Elizabeth A. Grove, Fadel Tissir, Shubha Tole, Thomas Theil, Eloisa Herrera, and Alfonso Fairén. 2017. "Lateral Thalamic Eminence: A Novel Origin for MGluR1/Lot Cells." *Cerebral Cortex* 27 (5): 2841–56. <https://doi.org/10.1093/cercor/bhw126>.
- Soda, Takeshi, Ryo Nakashima, Dai Watanabe, Kazunori Nakajima, Ira Pastan, and Shigetada Nakanishi. 2003. "Segregation and Coactivation of Developing Neocortical Layer 1 Neurons." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 23 (15): 6272–79. <http://www.ncbi.nlm.nih.gov/pubmed/12867512> http://www.ncbi.nlm.nih.gov/pubmed/12867512?ordinalpos=23&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_DefaultReportPanel.Pubmed_RVDocSum.
- Sun, Le, Ruiguo Chen, Ye Bai, Jia Li, Qian Wu, Qin Shen, and Xiaoqun Wang. 2019. "Morphological and Physiological Characteristics of Ebf2-EGFP-Expressing Cajal-Retzius Cells in Developing Mouse Neocortex." *Cerebral Cortex (New York, N.Y.: 1991)* 29 (9): 3864–78. <https://doi.org/10.1093/cercor/bhy265>.
- Suzuki, Mototaka, and Matthew E. Larkum. 2020. "General Anesthesia Decouples Cortical Pyramidal Neurons." *Cell* 180 (4): 666–676.e13. <https://doi.org/10.1016/j.cell.2020.01.024>.
- Takahashi, Naoya, Thomas G Oertner, Peter Hegemann, and Matthew E Larkum. 2016. "Modulate Perception" 354 (6319): 1159–65. <https://doi.org/10.1126/science.aah6066>.
- Tissir, Fadel, Aurélie Ravni, Younès Achouri, Dieter Riethmacher, Gundela Meyer, and Andre M Goffinet. 2009. "DeltaNp73 Regulates Neuronal Survival in Vivo." *Proceedings of the National Academy of Sciences of the United States of America* 106 (39): 16871–76. <https://doi.org/10.1073/pnas.0903191106>.
- Tjia, Michelle, Xinzhu Yu, Lavpreet S. Jammu, Ju Lu, and Yi Zuo. 2017. "Pyramidal Neurons in Different Cortical Layers Exhibit Distinct Dynamics and Plasticity of Apical Dendritic Spines." *Frontiers in Neural Circuits* 11 (June): 1–10. <https://doi.org/10.3389/fncir.2017.00043>.
- Williams, Leena E., and Anthony Holtmaat. 2019. "Higher-Order Thalamocortical Inputs Gate Synaptic Long-Term Potentiation via Disinhibition." *Neuron* 101 (1): 91–102.e4. <https://doi.org/10.1016/j.neuron.2018.10.049>.

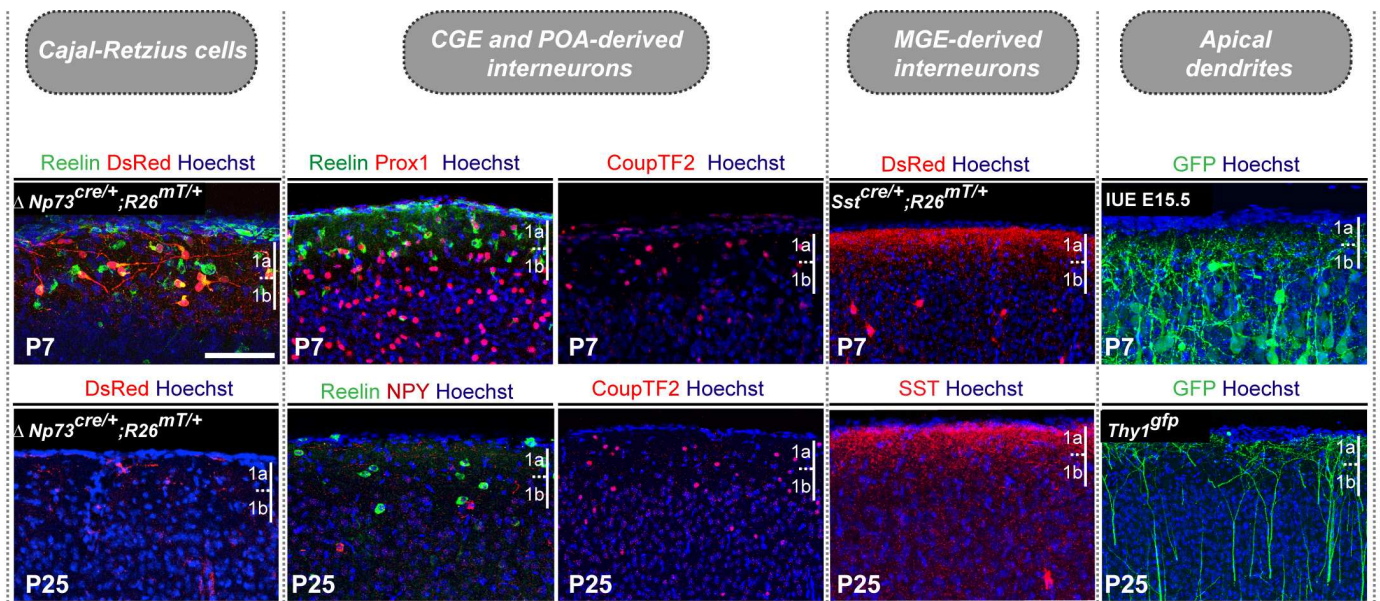


Figure 1 | Postnatal development of cortical layer 1

Confocal images of coronal sections from P7 and P25 brains highlighting the cellular and neuritic composition of L1. At P7, the cortical layer 1 comprises several neuronal populations, like CRc which express Reelin and can be labelled with the $\Delta Np73^{cre/+};R26^{mT/+}$ driver. Besides CRc, L1 hosts CGE and POA-derived interneurons labelled as Reelin⁺, Prox1⁺ or CoupTF2⁺. Despite the cellular components of L1, the landmark of L1 is the abundance of axonal and dendritic projections. For example, SST⁺ axons are projecting in the superficial part of the L1, named L1a. L1 also comprises the apical dendrites of both upper (labelled by IUE at E15.5) and deep (labelled with *Thy1^{gfp}*) layers pyramidal neurons. By P25, CRc have almost completely disappeared in L1 of the somatosensory cortex. At this stage, L1 is formed by sparse populations of CGE and POA-derived interneurons, as well as abundant axonal projections and apical dendrites of both upper and deep cortical layers. Scale bar 100 μ m.

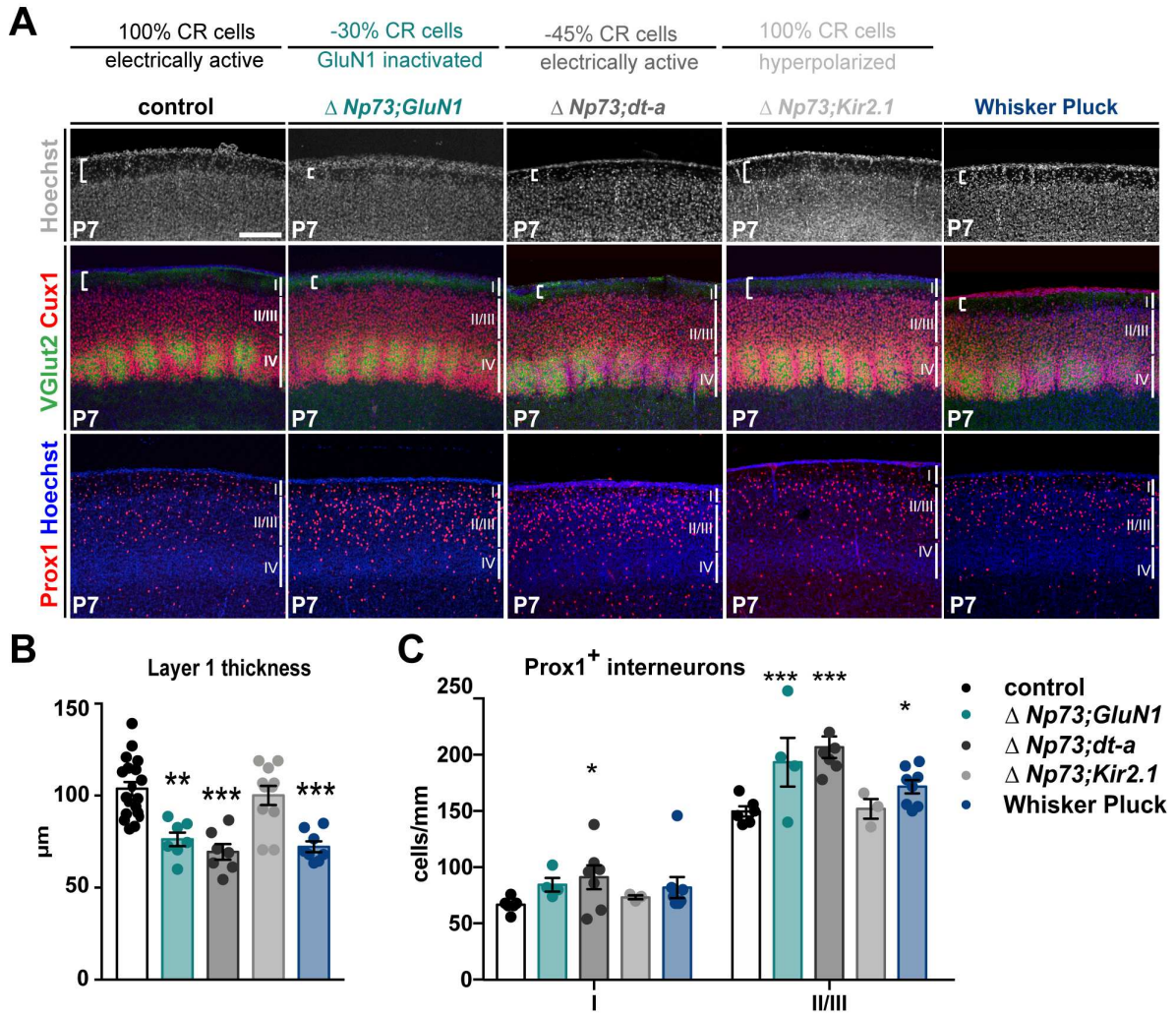


Figure 2 | The density of Cajal-Retzius cells and general sensory activity impact on L1 thickness and interneuron distribution.

(A) Confocal images of coronal sections in the somatosensory cortex of P7 controls, $\Delta Np73^{cre/+};GluN1^{fl/fl}$ mutants, $\Delta Np73^{cre/+};R26^{dt-a/+}$ mutants, $\Delta Np73^{cre/+};R26^{Kir2.1/+}$ mutants and of Whisker plucked animals. The white bracket illustrates the L1 thickness. (B) Quantification of L1 thickness (in μm) (n=19 for control, n=7 for $\Delta Np73^{cre/+};GluN1^{fl/fl}$, n=7 for $\Delta Np73^{cre/+};R26^{dt-a/+}$, n=11 for $\Delta Np73^{cre/+};R26^{Kir2.1/+}$ and n=8 for Whisker Pluck condition). (C) Quantification of Prox1⁺ interneuron densities in L1 and in L2/3 in the control and the four experimental conditions (n=6 for control, n=4 for $\Delta Np73^{cre/+};GluN1^{fl/fl}$, n=7 for $\Delta Np73^{cre/+};R26^{dt-a/+}$, n=11 for $\Delta Np73^{cre/+};R26^{Kir2.1/+}$ and n=8 for Whisker Pluck condition). Mann-Whitney U Test in (B) and 2-ways ANOVA in (C). * p<0.05 ; **p<0.001 ; ***p<0.0001. Scale bar 200 μm .

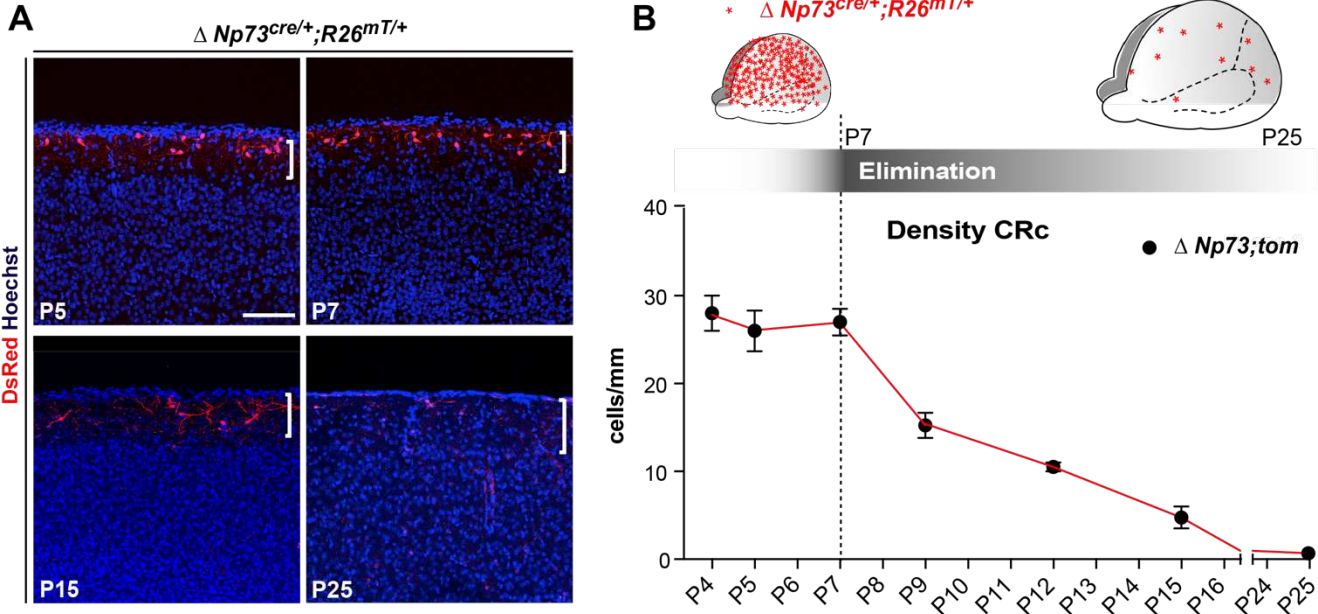


Figure 3 | Time course of postnatal Cajal-Retzius cell elimination

(A) Confocal images of coronal sections in the somatosensory cortex of P5, P7, P15 and P25 control $\Delta Np73^{cre/+};R26^{mT/+}$ brains. The white brackets illustrate the L1 thickness. (B) Schematic representation of the CRc at the surface of the cortex which start undergoing elimination starting with P7 until their almost complete disappearance by P25 (up). Quantification of the densities of CRc (cells/mm length) using the $\Delta Np73^{cre/+};R26^{mT/+}$ model at several postnatal stages (n=2 for P4, n=3 for P5, n=6 for P7, n=4 for P9, n=4 for P12, n=4 for P15, n=3 for P25). Scale bar 200 μ m.

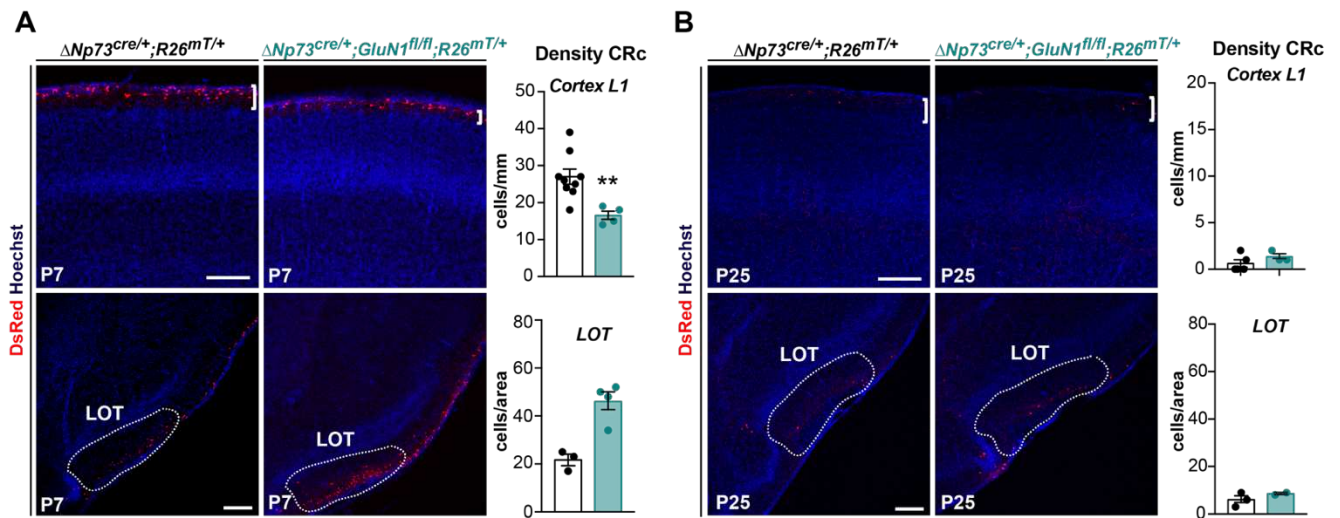


Figure 4 | The density of CRc is maintained through a GluN1 dependent distribution and a non-GluN1 dependent elimination

(A) Confocal images of coronal sections in the somatosensory cortex (up) and lateral olfactory tract (down) of P7 controls and $\Delta Np73^{cre/+}; GluN1^{fl/fl}; R26^{mT/+}$ mutants. The white brackets illustrate the L1 and the dotted line highlights the LOT. At right, quantifications of the densities of CRc in L1 (cells/mm) and in the LOT (cells/area lot). For L1, n=9 controls and n=4 mutants. For the LOT, n=3 controls and n=4 mutants). **(B)** Confocal images of coronal sections in the somatosensory cortex (up) and lateral olfactory tract (down) of P25 controls and $\Delta Np73^{cre/+}; GluN1^{fl/fl}; R26^{mT/+}$ mutants. The white brackets illustrate the L1 and the dotted line highlights the LOT. At right, quantifications of the densities of CRc in L1 (cells/mm) and in the LOT (cells/area lot). For L1, n=4 controls and n=4 mutants. For the LOT, n=3 controls and n=2 mutants). Mann-Whitney U Test, * p<0.05 ; **p<0.001 ; ***p<0.0001. Scale bar 200 μm.

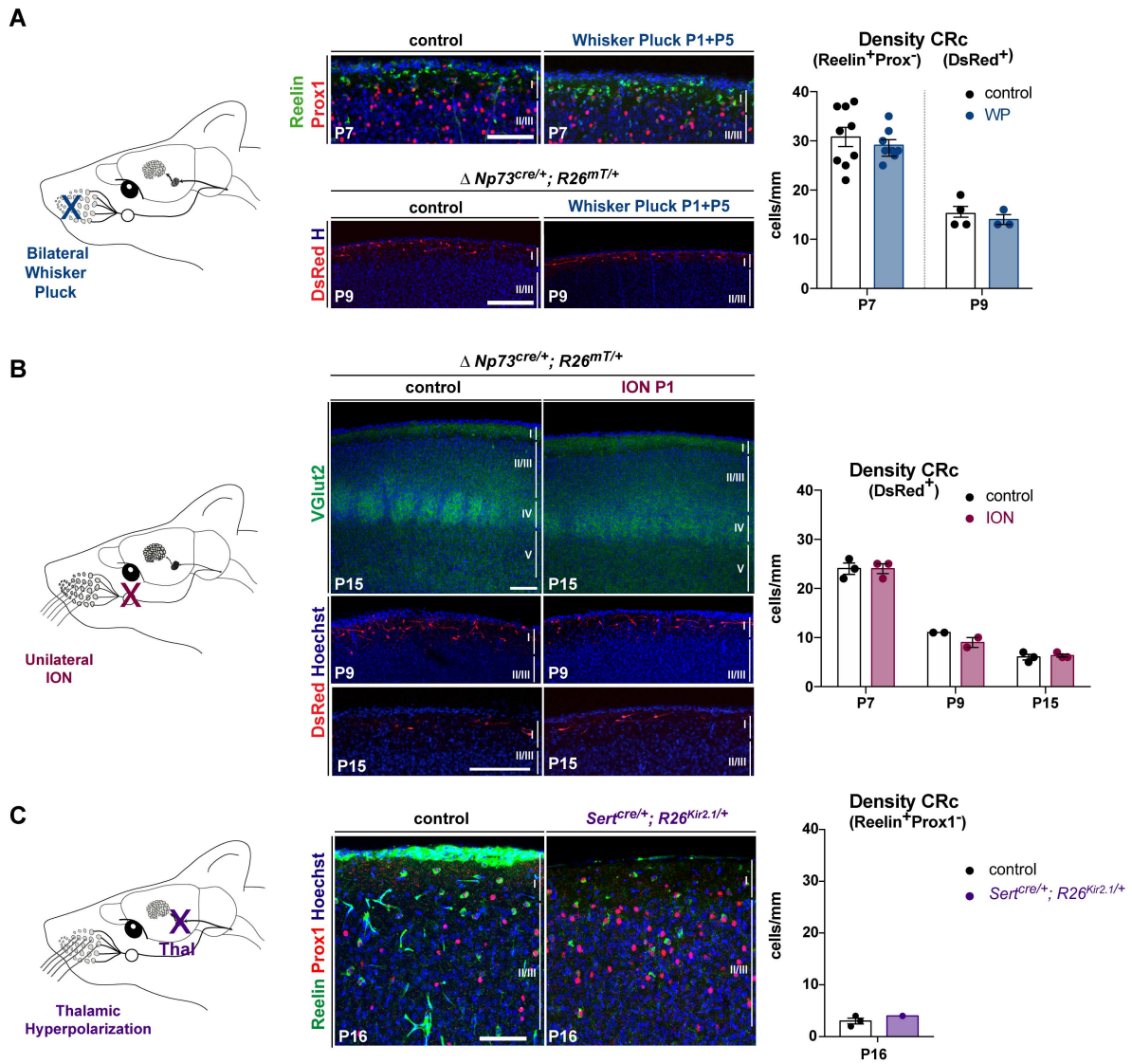


Figure 5 | General sensory activity is not influencing Cajal-Retzius cell density

(A) Schematic representation of Whisker pluck (left). Confocal images of coronal sections in the somatosensory cortex of P7 and P9 controls and Whisker Plucked animals. At P7 the CRc are identified as Reelin⁺/Prox1⁻ cells and at P9 CRc are identified using the $\Delta Np73^{cre/+}; R26^{mT/+}$ reporter line (middle). Quantifications of the density of CRc (cells/mm length) at P7 and P9 (P7: n=9 controls, n=8 Whisker Pluck, P9: n=4 controls and n=3 Whisker Pluck). (B) Schematic representation of unilateral ION (left). Confocal images of coronal sections in the somatosensory cortex of P9 and P15 controls and Whisker Plucked animals. At P15 illustrated the absence of barrels in the ION condition, with Vglut2 staining. At all stages the CRc are identified using the $\Delta Np73^{cre/+}; R26^{mT/+}$ reporter line (middle). Quantifications of the density of CRc (cells/mm length) at P7, P9 and P15 (P7: n=3 controls, n=3 ION, P9: n=2 controls and n=2 ION, P15: n=3 controls and n=3 ION). (C) Schematic representation of Thalamic hyperpolarization in the $Sert^{cre/+}; R26^{Kir2.1/+}$ model (left). Confocal images of coronal sections in the somatosensory cortex of P16 controls and mutants. CRc are identified as Reelin⁺/Prox1⁻ cells (middle). Quantifications of the density of CRc (cells/mm length) at P16 (n=3 controls and n=1 mutant). Mann-Whitney U Test, * p<0.05 ; **p<0.001 ; ***p<0.0001. Scale bar 100 μ m.

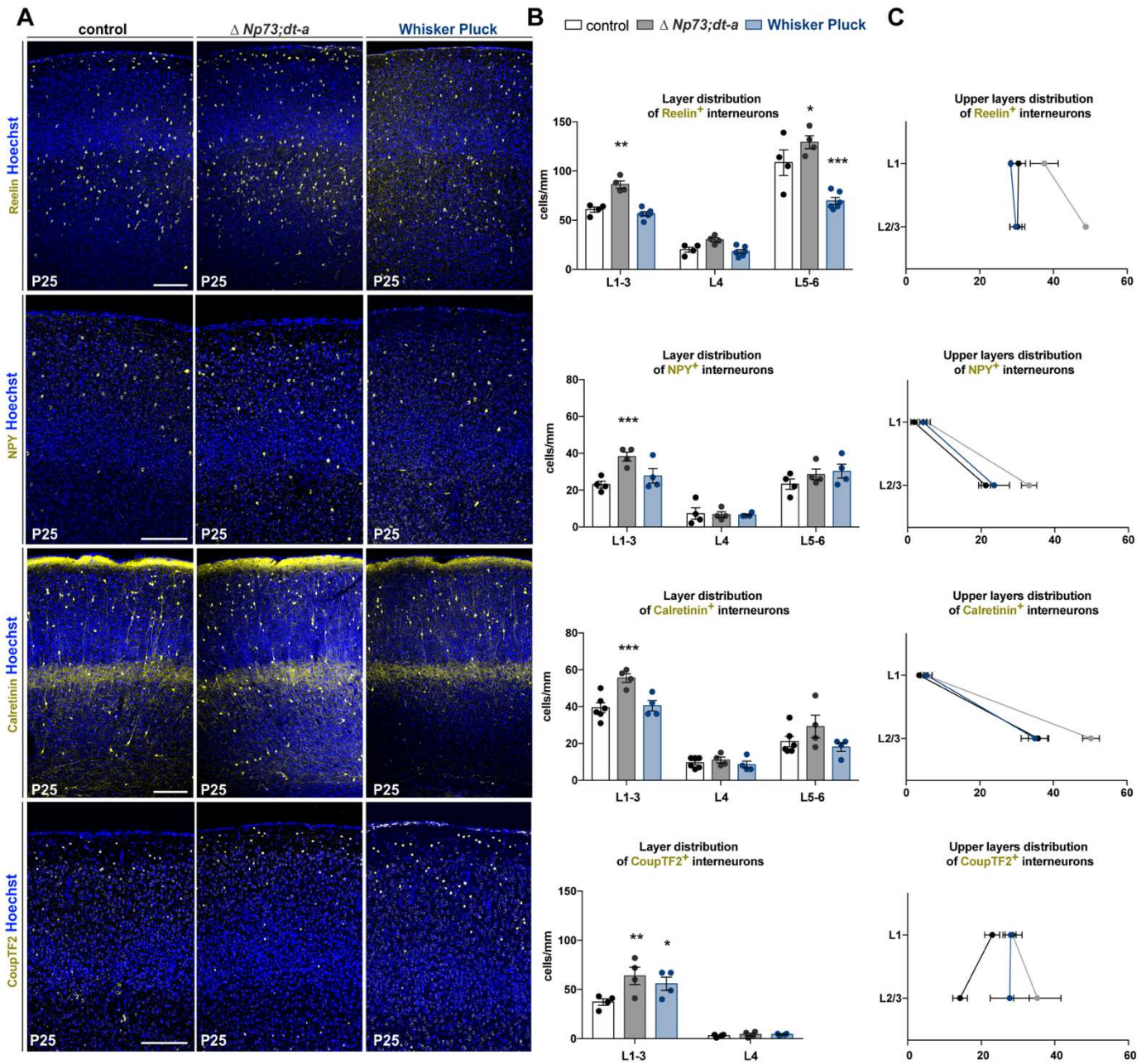


Figure 6 | Impairments of Cajal-Retzius cell density and general sensory activity have long lasting consequences on upper layers interneuron

(A) Confocal images of coronal sections in the somatosensory cortex of P25 controls, $\Delta Np73^{cre/+};R26^{dt-a/+}$ mutants and Whisker Plucked animals. Immunostainings for markers of CGE and POA-derived interneurons. (B) Quantifications of the density of interneurons in upper cortical layers (L1-3), layer 4 and deep cortical layers (L5-6). (C) Quantifications of the density of interneurons in upper cortical layers, L1 vs L2/3. 2-ways ANOVA test with Dunnett's multiple comparison correction, * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$. Scale bar 200 μm .

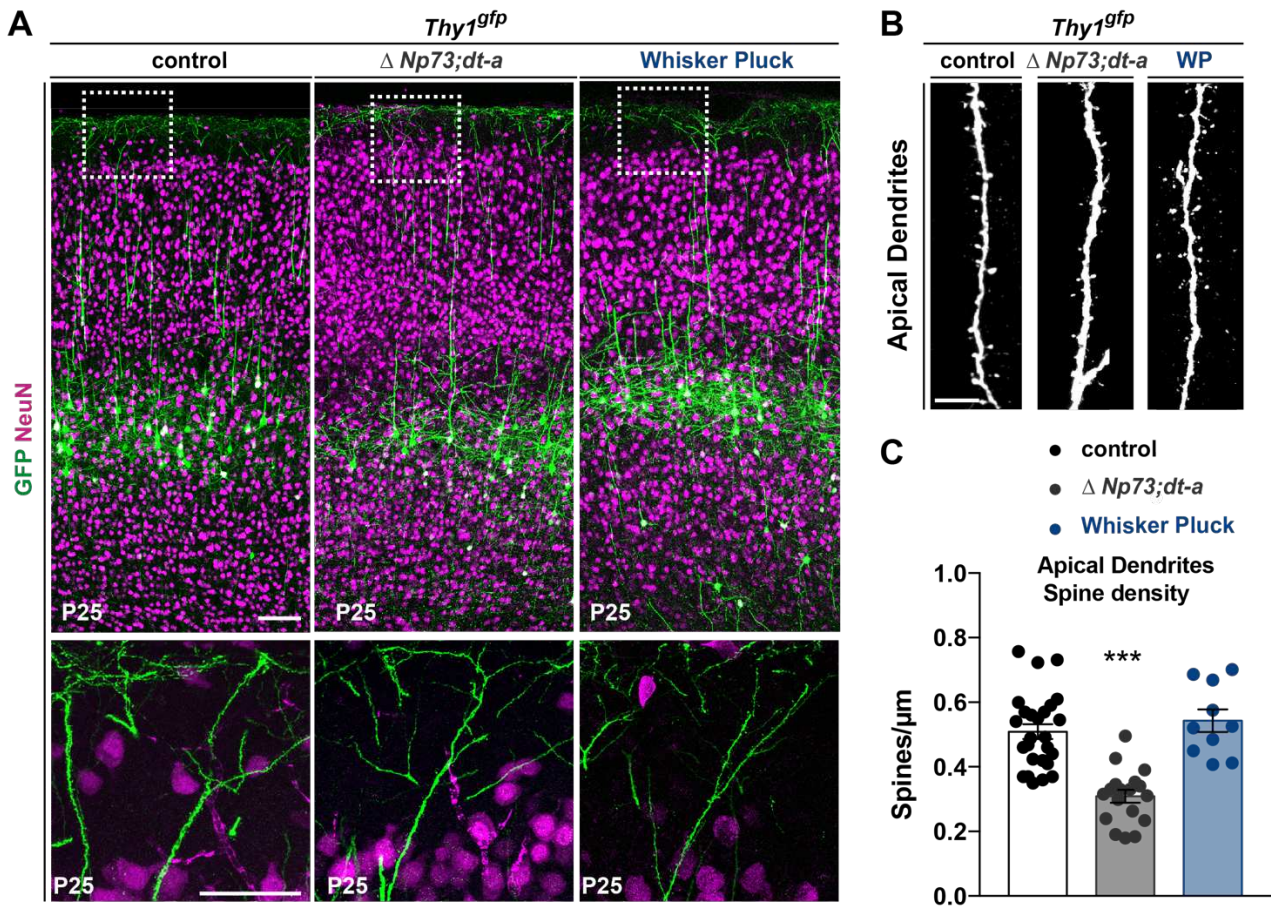


Figure 7 | Long-term effects on the impairment of Cajal-Retzius cell density and general sensory activity on excitatory entries in L1

(A) Confocal images of coronal sections in the somatosensory cortex of P25 controls, $\Delta Np73^{cre/+};R26^{dt-a/+}$ mutants and Whisker Plucked animals all expressing the $Thy1^{gfp}$. Depiction of the whole cortical thickness (up) and a close-up on L1 (down). (B) Confocal images in L1 of spines on the apical dendrites of L5 pyramidal neurons in control, $\Delta Np73^{cre/+};R26^{dt-a/+}$ mutants and Whisker Plucked animals. (C) Quantifications of the spine density in L1 on the apical dendrites of L5 pyramidal neurons Kruskal-Wallis test, * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$. Scale bar 100 μm in A and 5 μm in B.

**Layer 1 wiring is shaped by Cajal-Retzius cells
and early sensory activity**

**Ioana Genescu¹, Caroline Mailhes-Hamon², Maryama Keita¹, Hugues Cartonnet¹,
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SUPPLEMENTAL INFORMATION

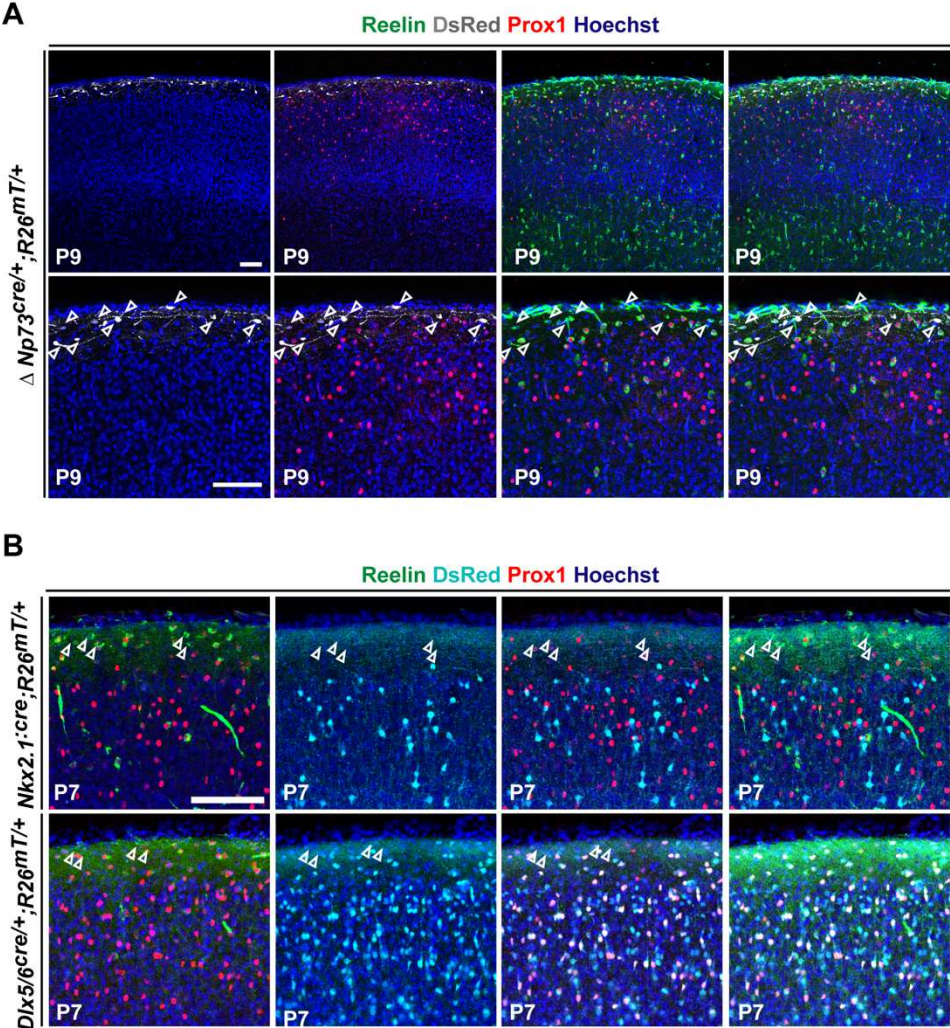


Figure S1 | Methods for identification of Cajal-Retzius cells

(A) Confocal images of coronal sections in the somatosensory cortex of P7 controls $\Delta Np73^{cre/+};R26^{mT/+}$ animals. 80% of the CRc are labelled with the $\Delta Np73^{cre/+};R26^{mT/+}$ line. All the labelled $\Delta Np73^{cre/+};R26^{mT/+}$ CRc are identified as Reelin⁺Prox1⁻ cells. (B) Confocal images of coronal sections in the somatosensory cortex of P7 $Nkx2.1:cre;R26^{mT/+}$ et $Dlx^{cre/+};R26^{mT/+}$ animals which label MGE-derived or respectively all interneurons. The CRc identified as Reelin⁺Prox1⁻ cells are not colocalizing with any interneuron staining. Scale bar 100 μ m.

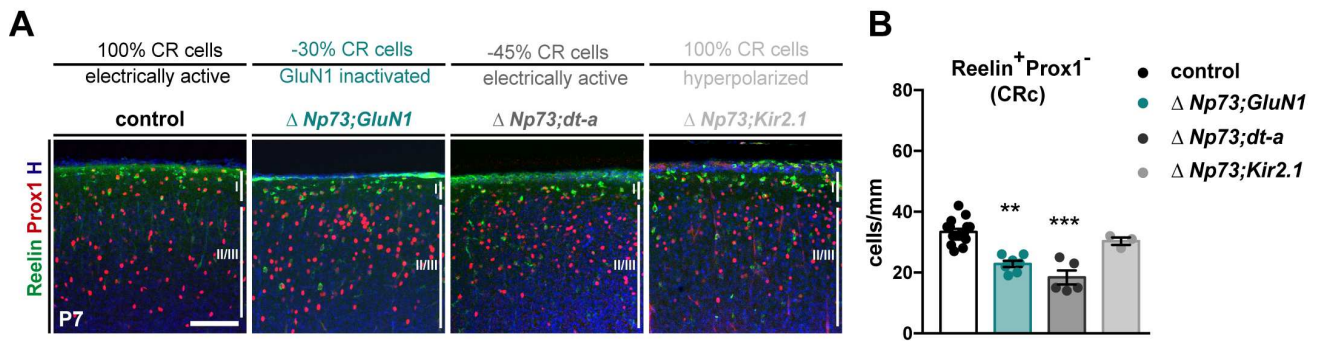


Figure S2 | Genetic models of impaired Cajal-Retzius cells

(A) Confocal images of coronal sections in the somatosensory cortex of P7 controls, $\Delta Np73^{cre/+};GluN1^{fl/fl}$ mutants, $\Delta Np73^{cre/+};R26^{dt-a/+}$ mutants, $\Delta Np73^{cre/+};R26^{Kir2.1/+}$ mutants. CRc are identified as Reelin⁺Prox1⁻ cells. (B) Quantification of the densities of CRc (cells/mm length) identified as Reelin⁺Prox1⁻ cells at P7 in control and the three genetic models (n=11 for controls, n=7 for $\Delta Np73^{cre/+};GluN1^{fl/fl}$, n=5 for $\Delta Np73^{cre/+};R26^{dt-a/+}$ and n=3 for $\Delta Np73^{cre/+};R26^{Kir2.1/+}$. Kruskal-Wallis test, * p<0.05 ; **p<0.001 ; ***p<0.0001. Scale bar 200 μ m.

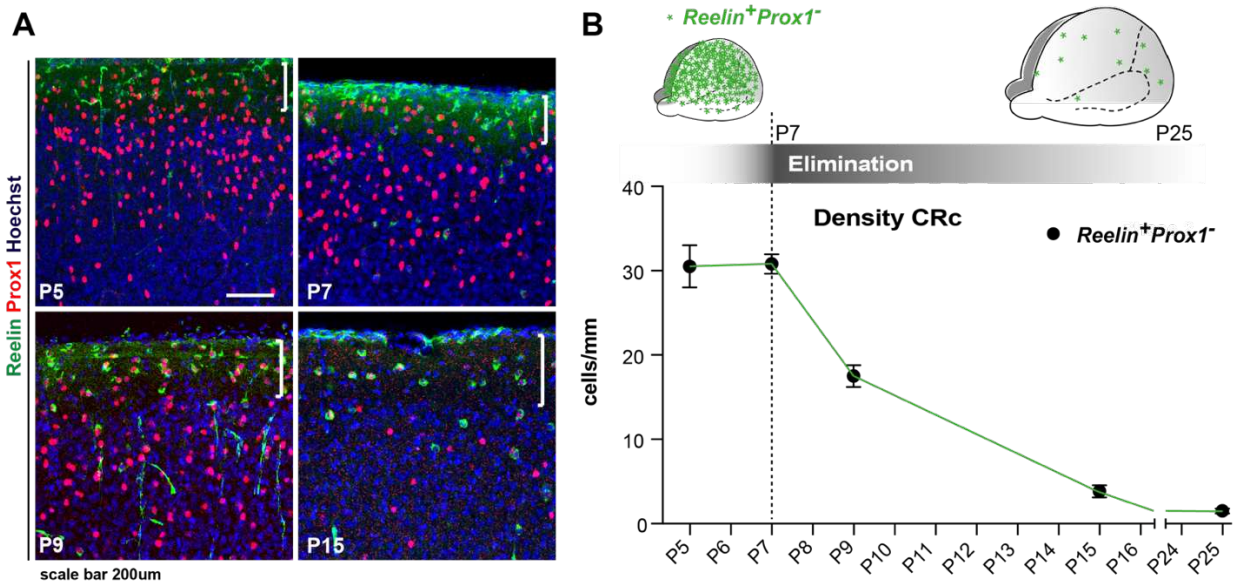


Figure S3 | Time course of postnatal Cajal-Retzius cell elimination using the Reelin/Prox method of identification

(A) Confocal images of coronal sections in the somatosensory cortex of P5, P7, P9 and P15 controls. CRc are identified as Reelin⁺Prox1⁻ cells. The white brackets illustrate the L1 thickness. **(B)** Schematic representation of the CRc at the surface of the cortex which start undergoing elimination starting with P7 until their almost complete disappearance by P25 (up). Quantification of the densities of CRc (cells/mm length) identified as Reelin⁺Prox1⁻ cells at several postnatal stages (n=2 for P5, n=22 for P7, n=8 for P9, n=6 for P15, n=4 for P25). Scale bar 200 μm.

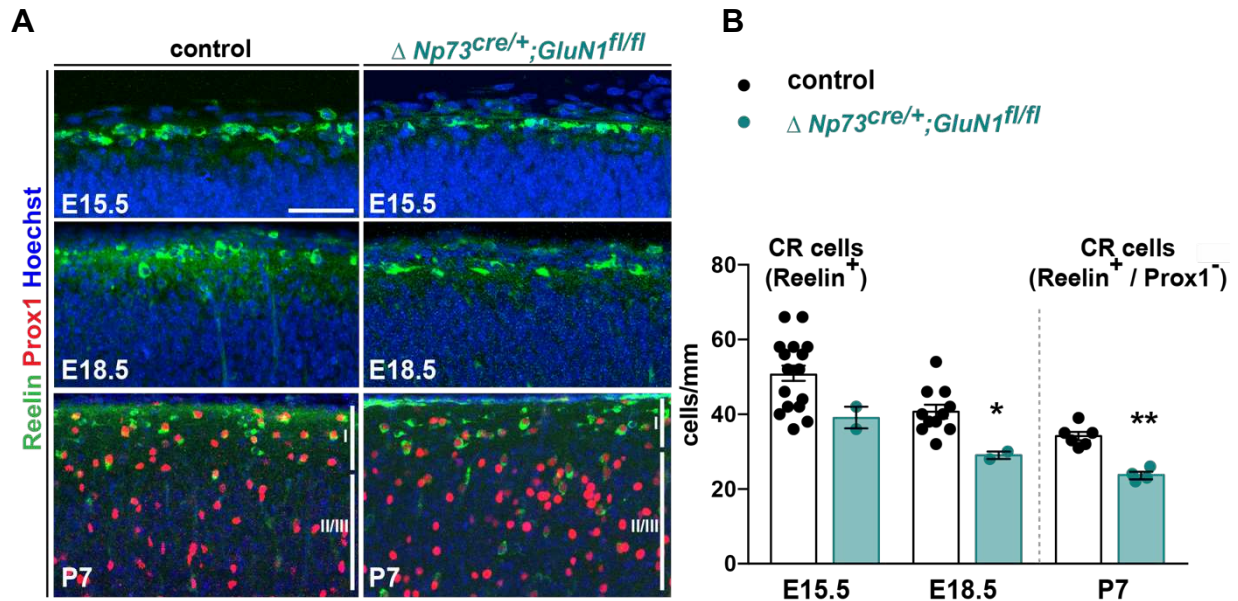


Figure S4 | Early impact of GuN1 on Cajal-Retzius cell density

(A) Confocal images of coronal sections in the somatosensory cortex of E15.5, E18.5 and P7 controls and $\Delta Np73^{cre/+}; GluN1^{fl/fl}$ mutants. CRc are identified at embryonic stages as Reelin⁺ cells while postnatally, they are identified as Reelin⁺/Prox1⁻ cells. (B) Quantification of the densities of CRc (cells/mm length) at E15.5, E18.5 and P7 (E15.5: n=15 for controls and n=2 for mutants, E18.5: n=10 for controls and n=2 for mutants, P7: n=6 for controls and n=4 for mutants). Mann-Whitney U Test, * p<0.05 ; **p<0.001 ; ***p<0.0001. Scale bar 200 μ m.

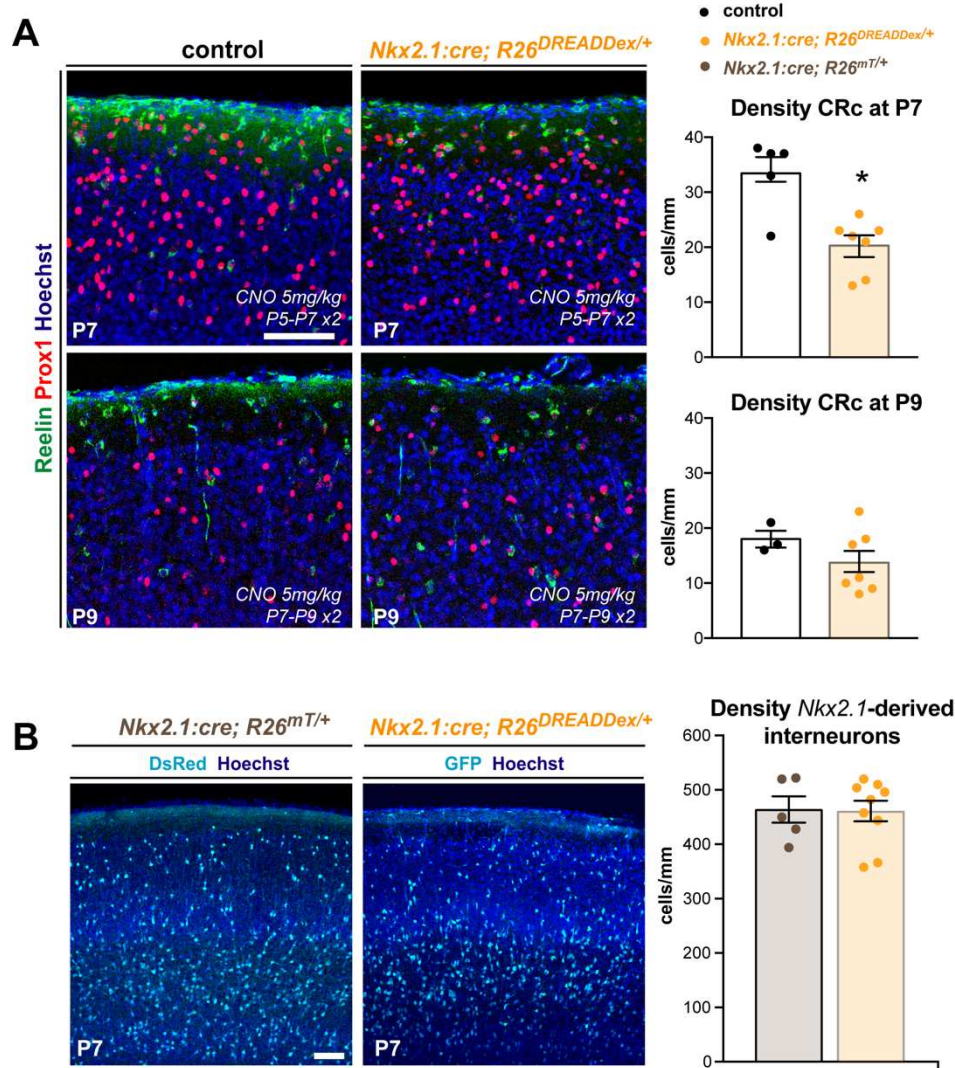


Figure S5 | Stimulation of the interneurons induces a premature elimination of Cajal-Retzius cells

(A) Confocal images of coronal sections in the somatosensory cortex of P7 and P9 controls and *Nkx2.1:cre;R26^{DREADDex/+}* mutants. CRc are identified as Reelin⁺/Prox1⁻ cells (left) Quantification of the densities of CRc (cells/mm length) at P7 and P9 (P7: n=5 for controls and n=7 for mutants, P9: n=3 for controls and n=7 for mutants) (right). (B) Confocal images of coronal sections in the somatosensory cortex of P7 *Nkx2.1:cre;R26^{mT/+}* and *Nkx2.1:cre;R26^{DREADDex/+}* mutants. (left) Quantification of the densities of *Nkx2.1*-derived interneurons (cells/mm length) at P7 (n=5 for *Nkx2.1:cre;R26^{mT/+}* and controls and n=9 for *Nkx2.1:cre;R26^{DREADDex/+}*) (right). Mann-Whitney U Test, * p<0.05 ; **p<0.001 ; ***p<0.0001. Scale bar 100 μm.

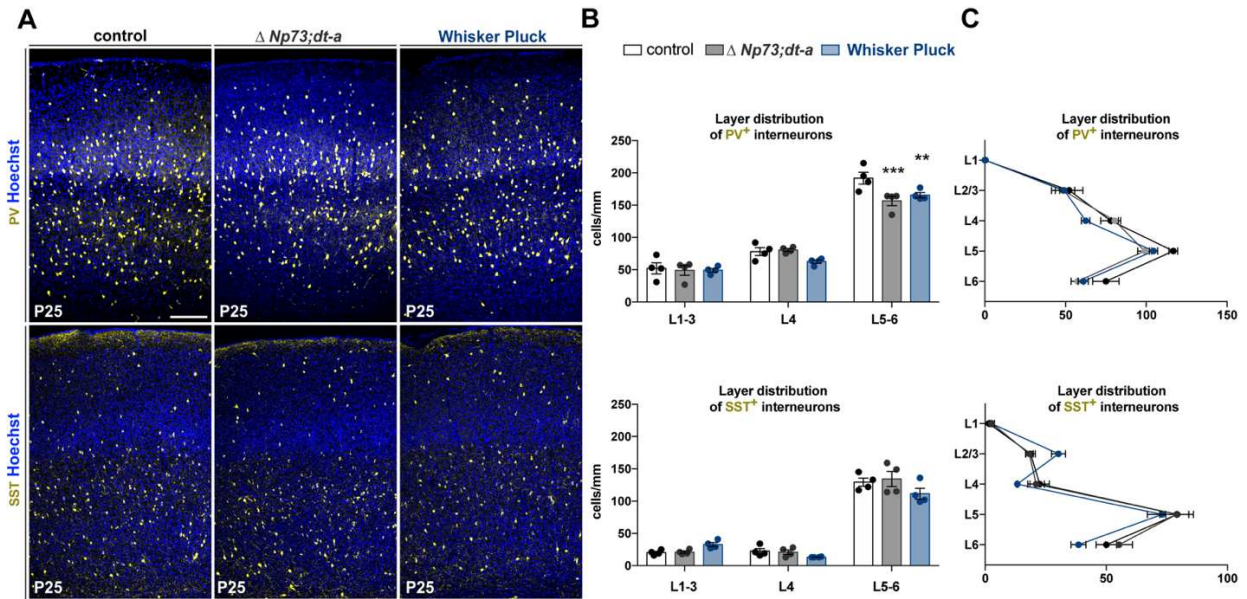


Figure S6 | Impairments of Cajal-Retzius cell density and general sensory activity have long lasting consequences on deep layers interneuron

(A) Confocal images of coronal sections in the somatosensory cortex of P25 controls, $\Delta Np73^{cre/+};R26^{dt-a/+}$ mutants and Whisker Plucked animals. Immunostainings for markers of MGE-derived interneurons.

(B) Quantifications of the density of interneurons in upper cortical layers (L1-3), layer 4 and deep cortical layers (L5-6). **(C)** Quantifications of the density of interneurons in each layer across the cortical thickness. Kruskal-Wallis test, * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$. Scale bar 200 μm .

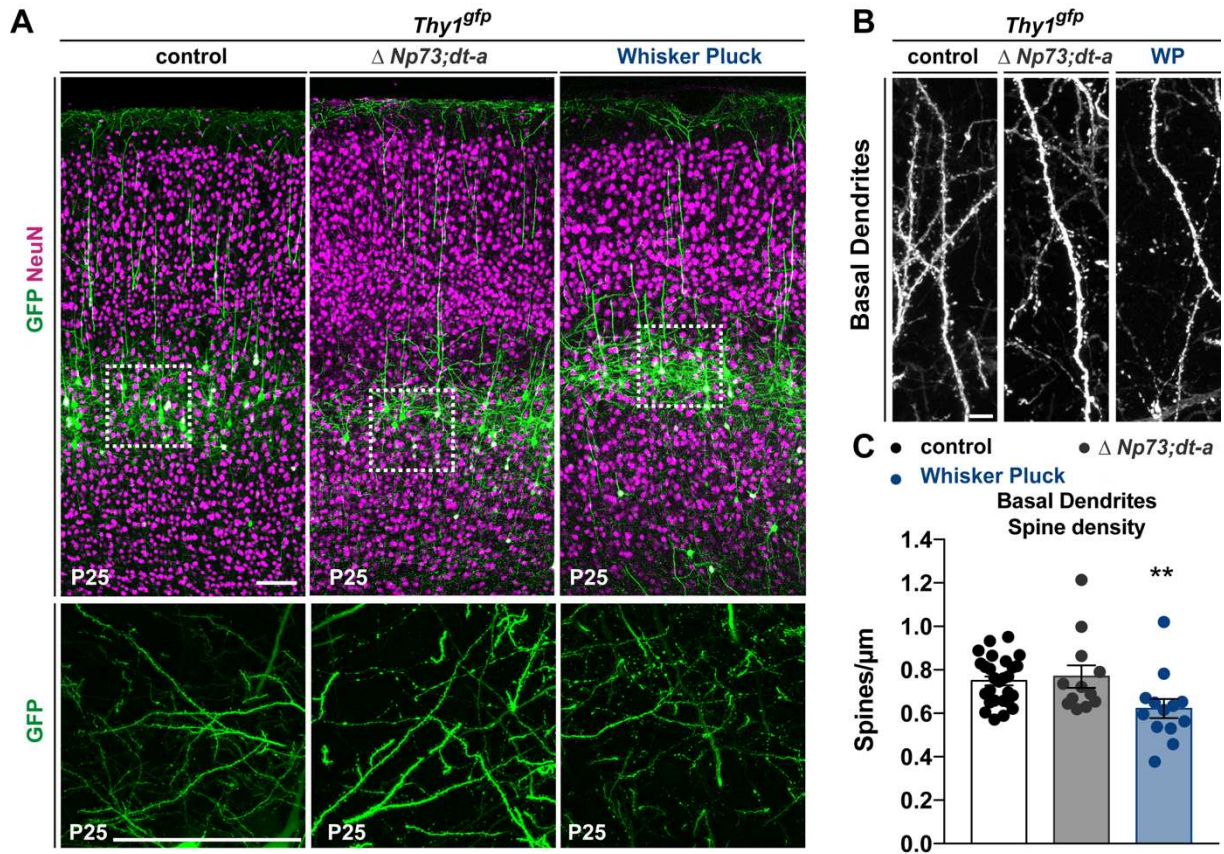


Figure S7 | Long-term effects on the impairment of Cajal-Retzius cell density and general sensory activity on excitatory entries in deep cortical layers

(A) Confocal images of coronal sections in the somatosensory cortex of P25 controls, $\Delta Np73^{cre/+};R26^{dt-a/+}$ mutants and Whisker Plucked animals all expressing the $Thy1^{yfp}$. Depiction of the whole cortical thickness (up) and a close-up on L5 (down). **(B)** Confocal images in L5 of spines on the basal dendrites of L5 pyramidal neurons in control, $\Delta Np73^{cre/+};R26^{dt-a/+}$ mutants and Whisker Plucked animals. **(C)** Quantifications of the spine density in L5 on the basal dendrites of L5 pyramidal neurons Kruskal-Wallis test, * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$. Scale bar 100 μm in A and 5 μm in B.

Review

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Being Superficial: A Developmental Viewpoint on Cortical Layer 1 Wiring

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Abstract

Functioning of the neocortex relies on a complex architecture of circuits, as illustrated by the causal link between neocortical excitation/inhibition imbalance and the etiology of several neurodevelopmental disorders. An important entry point to cortical circuits is located in the superficial layer 1 (L1), which contains mostly local and long-range inputs and sparse inhibitory interneurons that collectively regulate cerebral functions. While increasing evidence indicates that L1 has important physiological roles, our understanding of how it wires up during development remains limited. Here, we provide an integrated overview of L1 anatomy, function and development, with a focus on transient early-born Cajal-Retzius neurons, and highlight open questions key for progressing our understanding of this essential yet understudied layer of the cerebral cortex.

Highlights

- Cortical layer 1 is a major site of integration providing context for sensory information
- Layer 1 inputs exhibit a stereotypical organization generated early in development
- Layer 1 interneurons contribute to disinhibition and hence plasticity and learning
- Developing layer 1 transiently hosts migrating interneurons and Cajal-Retzius cells
- Cajal-Retzius cells regulate layer 1 circuits and upper cortical layers

Short title: Cortical layer 1 in the wiring of functional circuits

Keywords: Neocortex, Development, Cortical layer 1, Cajal-Retzius cells, Interneurons, Apical dendrites, E/I ratio, Top-down inputs.

Introduction

The neocortex controls sensory perception, motor behaviors and cognition [1]. To do so, this brain structure relies on complex circuits that assemble two large classes of neurons: excitatory glutamatergic neurons, including pyramidal neurons that project long distances; and inhibitory GABAergic interneurons, which are morphologically and functionally heterogeneous, and form local circuits. A precise balance between excitation and inhibition is essential to ensure normal cortical activity and function. Indeed, an imbalance in the excitation/inhibition (E/I) ratio is linked to the etiology of several neurodevelopmental disorders, including Autism Spectrum Disorders and Schizophrenia [2]. The constituent microcircuits of the neocortex are further organized into six layers, which collectively ensure an organized flow of information and enable the remarkable computational properties of the neocortex.

Whereas many studies have focused on deciphering the structure/function relationships in cortical circuits, the most superficial layer 1 (L1) has long remained “a crowning mystery” [3]. L1 has an atypical composition compared to other cortical layers, containing axons and dendrites, glial cells but only a limited number of neuronal cell bodies [4]. In particular, L1 comprises long-range excitatory, inhibitory and neuromodulatory inputs that target apical dendrites of pyramidal neurons located in different neocortical layers, as well as sparse inhibitory interneurons (**Figure 1**) [4], [5]. Despite increasing evidence that L1 is important for sensory processing, cross-modal communication, learning and arousal [6]–[11], we still have a fragmented understanding of how it wires up during development. In this review, we collate the current knowledge on L1 structure in adults and how it emerges during development, focusing on the model system of the mouse barrel cortex and highlighting the key regulators of cortical wiring, the transient Cajal-Retzius neurons.

Anatomy and function of the mature cortical layer 1

To grasp how L1 develops, it is essential to first understand its mature architecture and associated functions. A hallmark of L1 is that it hosts apical dendrites of pyramidal cells from distinct layers (**Figures 1A,B**) that integrate “top-down” information providing context, value,

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meaning and congruence to “bottom-up” sensory information, which is mostly relayed through basal dendrites [6], [12].

Another feature of L1 is that it hosts only few cell bodies, namely sparse GABAergic interneurons and glial cells. GABAergic interneurons of the neocortex shape activity patterns and comprise three main classes. First, Parvalbumin (PV⁺) neurons, including Basket and Chandelier cells, respectively target the soma and axonal initiation segment of pyramidal neurons, thereby regulating neuronal output (**Figure 1B,C**) [12], [13]. Second, Somatostatin (SST⁺) neurons, including Martinotti cells which send axons to upper L1 (or L1a) and target apical dendrites of pyramidal neurons, regulate dendritic excitatory entries (**Figure 1B,C**) [12]–[14]. Third, a broad class of 5HT3R⁺ neurons, with various morphologies, elicits either local inhibition or disinhibition by targeting PV⁺ or SST⁺ interneurons (**Figures 1B,C**) [12], [13], [15]–[18]. L1 interneurons all belong to this third class of 5HT3R⁺ neurons and have been recently characterized as four distinct sub-populations characteristic of L1, with particular morphologies, electrophysiological properties and molecular markers: i) Bipolar cells (VIP⁺), which are also largely present in deeper cortical layers; ii) Neurogliaform (NGF) cells, which are also found in deeper cortical layers but specifically co-express Reelin⁺ NDNF⁺ NPY⁺ in L1; iii) Canopy cells (NDNF⁺ NPY⁻); iv) α 7-subunit of Nicotinic Receptors cells and Bipolar cells (VIP⁺). Their axonal projection is either restricted to L1 – for NGF and Canopy cells - or dives deeper into the neocortex – for Bipolar VIP⁺ and α 7-subunit of Nicotinic Receptors cells (**Figure 1B,C**) [4], [16]–[19]. In addition, L1 contains dendritic arborization of several interneurons residing outside of L1, especially Chandelier cells and Bipolar VIP⁺ cells of L2/3 (**Figure 1B,C**). Collectively, such organization highlights inhibitory modules that contribute to L1 circuits as well as to the deeper neocortical layers (**Figure 1B,C**) [4], [16]–[19].

Besides interneurons, L1 also contains glial cells such as astrocytes and microglia (**Figure 1B,C**). L1 astrocytes have been so far the main focus of study and form a particular subpopulation of pial astrocytes, with a characteristic morphology and with potentially specific roles [20]–[22].

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L1 also contains several long-range projections coming from higher order thalamic nuclei, such as the POm or motor thalamus in the barrel cortex [23]–[25], from other cortical regions [26], [27] and from subcortical structures that convey neuromodulatory or GABAergic inputs (**Figure 1A,B**) [28]–[30]. These long-range projections exhibit precise spatial ordering: thalamic axons and GABAergic projections from the Zona Incerta are mainly restricted to the upper L1a, whereas cortical and neuromodulatory inputs are spread across L1, meaning L1a and L1b (**Figure 1A,1B**) [18], [24], [29].

L1 inputs target both apical dendrites, with a remarkably stereotyped organization and ordering [31], as well as interneurons which, in turn, provide another level of regulation [13], [23], [24], [29]. For instance, higher order thalamic inputs constitute a major excitatory drive of apical dendrites and L1 interneurons [19], [23], [32], [33], which either directly inhibit apical dendrites providing feedforward inhibition, or target PV⁺ or SST⁺ Martinotti cells, thereby disinhibiting pyramidal neurons (**Figure 1C**) [8], [10], [19], [23], [26]. The correlated activation of these thalamic inputs can thus sharpen or potentiate the pyramidal neuron responses via interneuron circuits, ultimately forming a powerful mechanism for rapid sensory plasticity and learning [8], [11], [32]–[35]. Direct inputs from other cortical or subcortical regions also provide top-down information regarding the context of sensory experience [9], [19], [36], [37].

Collectively, the precise topology of excitatory and inhibitory synapses onto apical tufts [31] and local interneuron circuits [4] enable the diversity of pyramidal neuron firing patterns associated with their functions. The tight structure-function relationship in L1 raises the intriguing question of how such a spatially organized architecture forms during development.

Layer 1 as a migration hub: more than building up adult circuits

In contrast to what is observed in adults, the embryonic and early postnatal L1, called the marginal zone (MZ), hosts multiple transient neuronal populations, which are later either eliminated by cell death or migrate out of this structure (**Figure 2A**).

The first inhabitants of the MZ are Cajal-Retzius cells (CRc) [38], [39]. These cells are amongst the first cortical glutamatergic neurons to be generated and are the main embryonic

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source of Reelin - a secreted glycoprotein essential for cortical lamination and whose dysfunction is linked to the etiology of several neurodevelopmental disorders [38]–[40]. In mice, CRc are generated between embryonic day (E)9 and E12 from four different sources around the neocortex: the cortical hem, septum (SE), pallial-subpallial boundary (PSB) and thalamic eminence (ET). From their production sites, CRc migrate tangentially in the MZ in close contact with meninges and tile the cortical surface, in part via contact repulsion [38], [41]–[44]. This process leads to cell gradients, for instance with hem-derived CRc more concentrated medio-caudally and more SE-derived CRc medio-rostrally [43], [45], [46]. Such distribution is linked to the migration dynamics since the genetic ablation of SE-derived CRc induces a reorganization of other CRc which now populate the depleted area [43], [45], [46]. During this early migration phase, some SE and ET-derived CRc accumulate in a reservoir located in the olfactory cortex, from which they can re-enter migration, thereby maintaining CRc density in the neocortex during brain developmental growth (**Figure 2A**) [47]. Thus, distinct steps of migration in the MZ are essential to regulate overall CRc density, as well as the local relative proportion of CRc from the different sources. While CRc were shown to contribute to cortical wiring, these cells are almost entirely eliminated in the postnatally neocortex of mice (**Figure 2A**) [39], [47]–[51]. Intriguingly, SE and ET-derived CRc are eliminated by *Bax*-dependent apoptosis whereas the other populations are demised by a process that remains to be characterized [48], [49]. The roles of CRc, their heterogeneity and the mechanisms regulating their density and elimination will be detailed in the next section.

Together with the Subventricular zone (SVZ), the MZ is an important migration stream for GABAergic interneurons (**Figure 2A**), which are produced by the medial ganglionic eminence (MGE) for PV⁺ and SST⁺ interneurons, and by the Caudal Ganglionic Eminence (CGE) and Preoptic Area (POA) for 5HTR3⁺ subtypes (**Figures 1B and 2A**) [12], [15], [51]–[53]. While NGF cells were shown to be preferentially generated by the POA, the exact origins of other 5HTR3⁺ interneurons remain to be characterized in detail [15]. Before E16.5 in mice, cortical interneurons first migrate tangentially through the MZ and SVZ and then radially to position into the cortical plate (CP) where they undergo functional maturation (**Figure 2A**) [12],

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[54]–[56]. Thus a fraction of both MGE and CGE-derived interneurons are only passing through the MZ but for some of them, this migration constitutes an important step in their development. This is the case of SST⁺ Martinotti cells, which preferentially migrate superficially, leaving their axon in the MZ while their cell bodies migrate deeper into the CP, thereby defining the cellular morphology and synaptic targeting of this major player in L1 circuits [14], [57]. The development and migration of the CGE and POA-derived interneurons that populate the mature L1 is less clear [15], [53], [58], [59]; additional genetic tracing studies will be necessary to assess how these subtypes are generated and whether they also preferentially migrate through the MZ. Recent studies have also aimed to address whether the migratory events occurring in the MZ are coordinated. While the migration of both interneurons and CRc relies on the meningeal Cxcl12 chemokine and its cognate CXCR4/CXCR7 receptors [43], [60]–[63], additional experiments suggest that impairing the migration of CRc might impact on the distribution of interneurons [47], [63]–[65]. Here too, further studies will be needed to decipher potential cell-cell interactions that occur in the MZ as well as their functional impacts.

Taken together, the developing L1 appears to be a central hub for the migration of GABAergic neurons, which are key regulators of the postnatal emergence of functional cortical circuits [54], [55], [66]. Are there any developmental roles of L1 interneurons or L1 projecting SST⁺ Martinotti cells? Strikingly, these interneurons regulate circuit wiring in the early postnatal stages via early synchronization of their activity mediated by sensory inputs [67], as well as shape, visual and auditory processing [14], [19]. Thus the MZ is a key hub for the construction of GABAergic circuits and hence for the functional emergence of cortical circuits during development.

Layer 1 wiring: from transient circuits to synaptic organization

In addition to cell bodies, the MZ contains several acellular processes including radial glia end feet (the leading processes of radially-migrating glutamatergic neurons which will give rise to apical dendrites), as well as long-range axonal projections (**Figure 2B**). While the formation of apical dendrites has been the focus of several reviews [68], [69], much less is known about

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the pathfinding of long-range axonal projections. Thalamic, cortical and neuromodulatory axons first reach the subplate (a transient structure located beneath the CP), then navigate through the CP and reach L1 in the stereotyped manner characteristic of adult circuits [30], [70]–[72]. In addition to this “classical route”, neuromodulatory axons can also follow a secondary tangential trajectory that directly reaches the MZ [30]. While axons share similar routes, the timing of invasion differs across populations: neuromodulatory axons reach the MZ at embryonic stages, higher order thalamic axons during the first postnatal week and cortical axons by the beginning of the second postnatal week (**Figure 2B**). For GABAergic inputs, L1 is innervated from birth by long-range inputs from the Zona Incerta that regulate apical dendrite morphogenesis, and by the axonal plexus left by migrating Martinotti cells, as aforementioned (**Figures 1 and 2**) [14], [29], [73].

In addition, the MZ is also innervated by the subplate, a transient structure eliminated postnatally by cell death, with few neurons being integrated in L6b [74], [75] (**Figure 2B**). While the subplate forms transient circuits essential for the development of thalamic inputs [43], [74], recent findings indicate that it also targets L1 interneurons during the first postnatal week, thereby transiently bridging peripheral sensory information relayed by the thalamus up to L1 [76]. This connection is potentially of major importance, since early activity in L1 has been shown to be key for the maturation of interneurons as well as for the building of sensory circuits [19], [51], [67]. Interestingly, early subplate to L1 connections have also been proposed to be observed in human fetal brains, where synapses have been detected in L1 from gestational week (GW)12 [77], suggesting that this feature is evolutionarily conserved. How these transient early L1 circuits contribute to cortical function in rodents as well as in primates will be an important research focus.

Beyond the early wiring steps, what do we know about synaptogenesis in L1 and the development of the exquisite synaptic organization observed onto apical dendrites? While there is still limited information, evidence suggests that synaptogenesis in L1 is regulated by both intrinsic programs characteristic of the neuronal populations undergoing synaptogenesis, as well as by local environmental signals. Several molecular programs that regulate synapse

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specificity of L1 projecting interneurons have been recently identified [57]. For example, Cbln4 expression in SST⁺ Martinotti cells is sufficient to drive L1-restricted synapse formation onto apical dendrites of pyramidal neurons [57]. In addition, local L1 signals, produced by either neurons or glial cells have been shown to act as synaptogenic factors. Prime examples are provided by the secreted glycoprotein Reelin [78], [79], which is produced by CRc and some CGE/POA-derived interneurons, and by Hevin, which is produced by astrocytes and promotes thalamocortical synapse formation in L1 [80]. Deciphering the cellular and molecular logic that shapes the exquisite L1 synaptic organization, in particular assessing the roles of transient neuronal circuits and glial cells, will constitute an important milestone.

Cajal-Retzius cells: pioneer neurons involved in layer 1 wiring

One of the major players in L1 wiring are the CRc, which are transient excitatory early-born neurons characterized by an elongated morphology, specific electrophysiological properties and the expression of molecular markers, such as Reelin and Tbr1 [38], [81]. They are classically viewed as a single population of “guidepost cells” important for the migration of pyramidal neurons [82]–[84]. However, several studies over the past years have collectively contributed to change our perspective on these superficial transient cortical neurons.

As mentioned, CRc form a heterogeneous population, being generated in distinct sources [38], [41], [44], [85]–[87], express selective molecular markers, including Calretinin, Δ Np73, Ebf2, Ebf3, depending on their origin [38], and overall comprise transcriptionally distinct subtypes [88]. Remarkably, the elimination of SE-derived cells and their replacement by hem-derived CRc revealed a specific role in cortical arealization [46]. These findings support the intriguing hypothesis that distinct CRc populations might be playing specific roles through their strategic positioning on the neocortical surface [43], [45], [46].

In addition to their signaling activities, CRc are also electrically active neurons embedded in circuits. Irrespective of their origins, CRc are innervated by both serotonergic and noradrenergic axons, express mGluR and NMDA receptors and, postnatally, are predominantly innervated by GABAergic neurons [50], [81], [89]–[94]. In turn, CRc have been

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suggested to output onto L1 apical dendrites [95], but their post-synaptic targets still remain largely unknown. Intriguingly, the mechanisms regulating CRc density, their remigration and their cell death have been shown to be partially activity-dependent [47], [91]. Indeed, the density of SE and ET-derived CRc is regulated through a NMDA-dependent mechanism of redistribution from an olfactory reservoir (**Figure 2A**) [47], while CRc hyperpolarization or general activity blockade impairs the *Bax*-dependent apoptosis of SE and ET-derived CRc (**Figure 3B**) [91], [96]. Intriguingly, the elimination of hem-derived CRc in the neocortex is not dependent on *Bax* and not regulated by activity, highlighting a major difference between CRc subsets [48], [91]. Thus the life and death of CRc subsets is tightly regulated by activity, raising the question of their integration in transient circuits and their roles in the early wiring of the neocortex.

A certain level of understanding of the roles of CRc in cortical wiring has been gained by the use of genetic models that either decrease their density or impair their *Bax*-dependent apoptotic elimination (**Figure 3 A,B**) [47], [48], [91]. The first model, which uses a the $\Delta Np73^{cre}$ driver (80% of CRc, including hem, SE and ET-derived CRc) and a conditional allele expressing the A subunit of the diphtheria toxin, exhibits a 45% reduction in the number of CRc at P7 in the murine barrel cortex [86]. This reduction in CRc density has an early influence on most actors of cortical L1: increased density of CGE/POA-derived interneurons, decreased density of axonal collaterals and decreased complexity of apical dendrite morphology (**Figure 3A**) [47]. Interestingly, an early reduction in CRc density has the opposite effect to an impairment of CRc elimination [47], [91]. Early reduction in CRc density induces decreased apical dendrite branching and spine densities in L1, consistent with a decreased E/I ratio in upper cortical layers (**Figure 3B**) [47]. In contrast, when electrically active CRc are not fully eliminated (via conditional invalidation of *Bax* proapoptotic gene specifically in CRc), the surviving CRc aberrantly integrate into circuits, leading to increased branching of apical dendrites, spine number and E/I ratio in upper cortical layers (**Figure 3B**) [48], [91]. Hence, CRc have key roles in the wiring of upper cortical circuits, in particular the morphogenesis of

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apical dendrites and their associated synaptic inputs, and the complete elimination of CRc is required for proper brain wiring.

There are two main possible non-exclusive explanations for how CRc execute their roles: they might act through activity dependent mechanisms and/or through molecular cues. Impairing the demise of CRc triggers an activity-dependent miswiring, indicating that these defects are mediated by the electrical activity of aberrant surviving SE and ET-derived CRc (**Figure 3B**) [91]; however, hyperpolarization of CRc has no visible consequence for cortical circuits, suggesting that physiological functions of CRc might not rely on their electrical activity but rather on signaling mechanisms that remain to be characterized. One likely candidate would be Reelin since this secreted factor is known to promote synaptogenesis and deletion of its C-terminal portion leads to a morphologically similar phenotype as the ones observed in mutants with impaired CRc density [97]–[99].

CRc are thus emerging as important mediators of the wiring of cortical L1 and of upper cortical layers. Still, we are yet to fully define the inputs driving both the activity-dependent CRc remigration and their elimination, as well as the mechanisms through which these transient neurons shape the wiring of cortical circuits.

Conclusions and perspectives

As functional roles of L1 emerge, it appears that the well stratified synaptic organization of this region is essential for the integration of information across apical dendrites. Linking the synaptic architecture to computation properties and ultimately behavioural responses, as well as deciphering the developmental mechanisms shaping L1 architecture constitute major challenges for the upcoming years. Transient CRc seem to be important regulators of L1 wiring; determining their roles, interplay with interneurons and connectivity will represent important steps forward in the field. A notable challenge is the heterogeneity of the CRc population: these cells derive from four distinct sources and it is yet to be discovered whether different CRc origins confer specific functional properties to these cells.

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To date, most of the studies presented herein have been performed in rodents, raising the question of L1 in primates and humans. As circuits change during evolution, the importance of L1 for cortical function could be amplified because L1 interneurons in humans exhibit additional features, such as an increased complexity of apical dendrites and the axonal plexus [28], [100]. Another key difference is that, unlike rodents, in the human cortex subtypes of CRc are not entirely eliminated [101]. It is thus essential to understand how the intricate processes of L1 construction are coordinated both in space and time, how this varies across species with potential implications for pathologies, and how CRc, these “surplate neurons”, orchestrate such wiring.

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Conflict of interest

The authors declare no conflicts of interest.

FIGURES

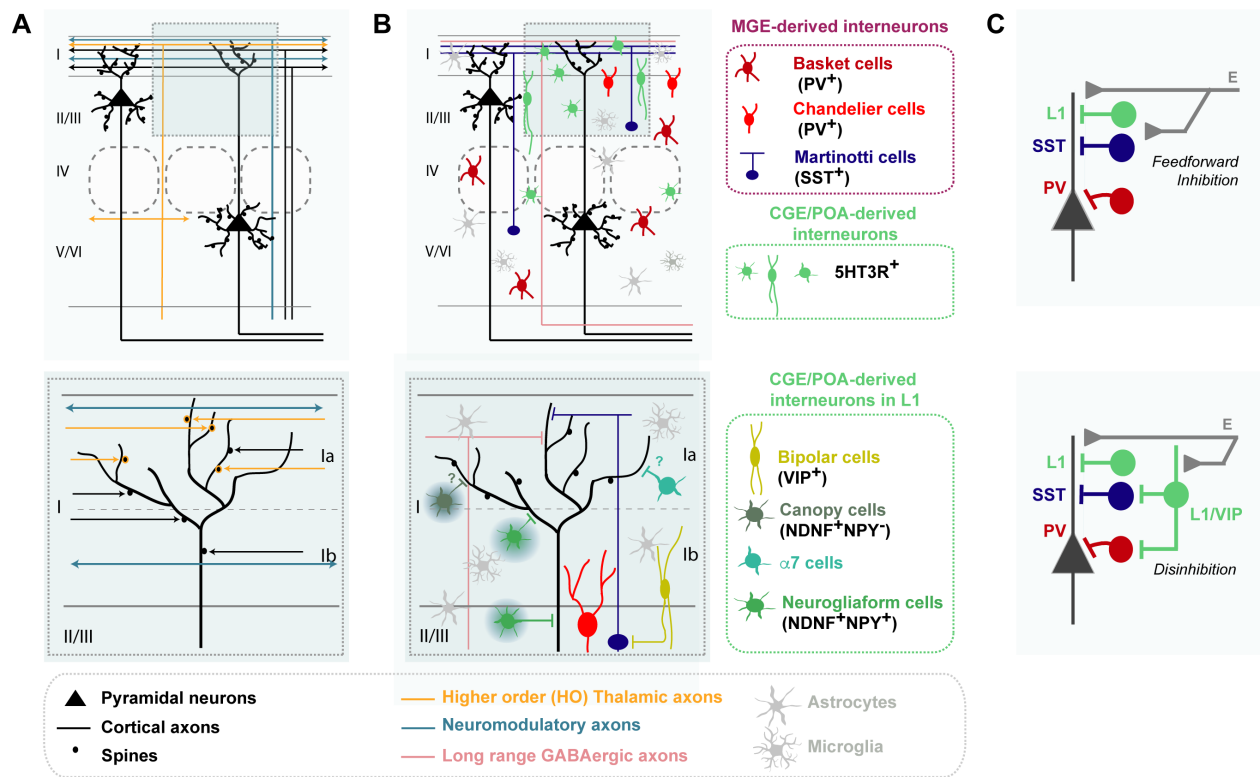


Figure 1. Architecture of circuits in the mature cortical L1

(A) Schematic representation of excitatory axonal projections (arrows) in the adult somatosensory barrel cortex (up) with a close-up on L1 (bottom) highlighting the spatial organization of inputs on apical dendrites of pyramidal neurons across L1a and 1b (bottom). Dotted lines delineate barrels. (B) Similar representations as in A showing the distribution of GABAergic interneuron subsets, classified by their developmental origins and molecular markers. (C) Schematic representations of circuit motifs recruited by excitatory (E) inputs in L1. Ia: layer 1a; Ib: layer 1b; CGE: Caudal Ganglionic Eminence; MGE: Medial Ganglionic Eminence, NDNF: Neuron Derived Neurotrophic Factor, NPY: Neuropeptide Y; POA: Preoptic Area, PV: Parvalbumin, SST: Somatostatin, VIP: Vasoactive Intestinal Peptide.

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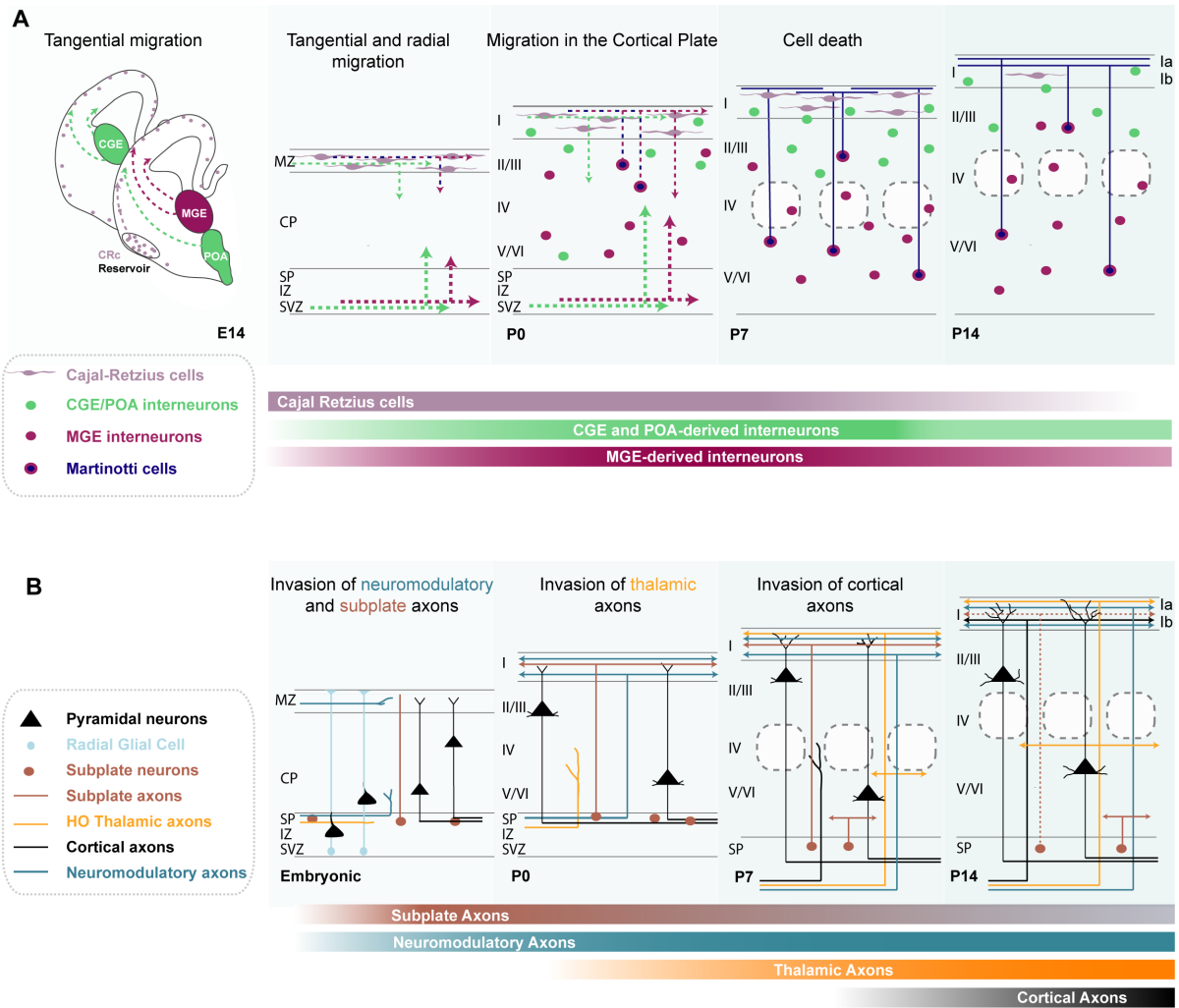


Figure 2. Developmental timeline of L1 wiring

(A) Schematic representation of the sequential migration steps and timing of invasion and death of Cajal-Retzius cells (CRc) and interneurons in the Marginal Zone (MZ), with developmental stages presented below each panel. Dotted lines represent streams of migration. Note the migration of Martinotti cells in the MZ, leaving their axon in L1 while they reach their axon position in the CP. (B) Similar timeline showing the timing of invasion of excitatory axons in L1. Axons arrive either directly in the MZ or via the subplate (SP), cross the CP and reach L1 at postnatal stages. Grey dotted lines represent barrels. CGE: Caudal Ganglionic Eminence, CP: Cortical Plate, CRc: Cajal-Retzius cells, HO Thalamic Axons: Higher Order Thalamic Axons, IZ: Intermediate Zone, MGE: Medial Ganglionic Eminence, MZ: Marginal Zone, POA: Preoptic Area, SP: Subplate: SVZ: Subventricular Zone.

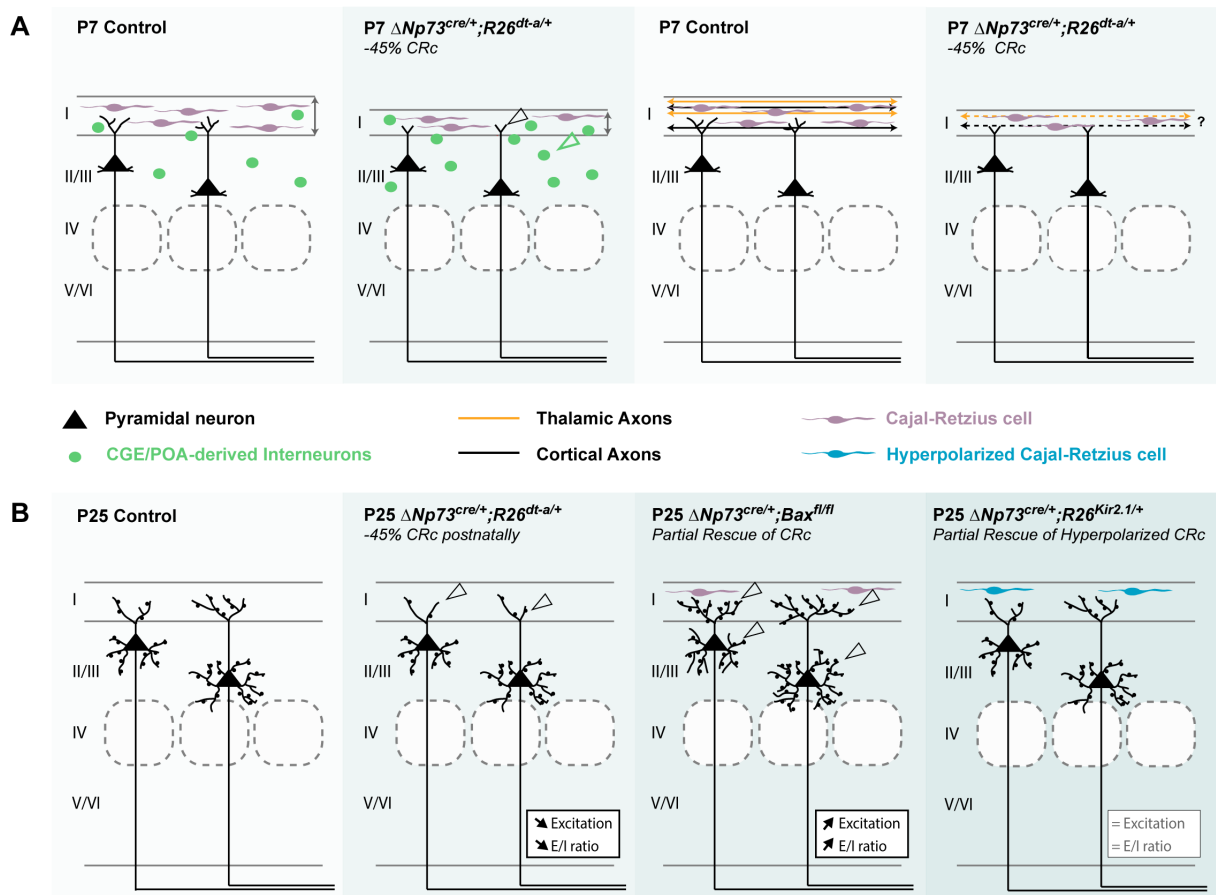


Figure 3. Models of Cajal-Retzius cell alterations and their impact on cortical wiring

(A) Schematic representation of the consequences of reduced density of Cajal-Retzius (CRc) cells ($\Delta Np73^{cre/+}; R26^{dt-a/+}$) on upper layer connectivity at P7, highlighting an impact on L1 thickness, number of interneurons (green arrowheads), complexity of apical dendrites (black arrowheads) and axonal sprouting. (B) Similar representation at P25 highlighting that both impaired CRc density ($\Delta Np73^{cre/+}; R26^{dt-a/+}$) or impaired elimination of electrically-active CRc ($\Delta Np73^{cre/+}; Bax^{fl/fl}$) shape the morphology of apical dendrites and the number of spines (black arrows) and modulate the Excitation/Inhibition (E/I) balance. Note that hyperpolarization of CRc ($\Delta Np73^{cre/+}; R26^{Kir2.1/+}$) impairs their elimination but not the branching, spine density or E/I ratio of the upper layers pyramidal neurons. CGE: Caudal Ganglionic Eminence, E/I: Excitation/Inhibition ratio, POA: Preoptic Area.

References

- [1] K. D. Harris and G. M. G. Shepherd, "The neocortical circuit: Themes and variations," *Nat. Neurosci.*, vol. 18, no. 2, pp. 170–181, 2015.
- [2] O. Marín, "Interneuron dysfunction in psychiatric disorders.," *Nat. Rev. Neurosci.*, vol. 13, no. 2, pp. 107–20, 2012.
- [3] D. H. Hubel, "Cortical Neurobiology," *Annu. Rev. Neurosci.*, vol. 5, pp. 363–370, 1982.
- [4] L. A. Ibrahim, B. Schuman, R. Bandler, B. Rudy, and G. Fishell, "ScienceDirect Mining the jewels of the cortex 's crowning mystery," *Curr. Opin. Neurobiol.*, vol. 63, pp. 154–161, 2020.
- [5] X. Jiang, G. Wang, A. J. Lee, R. L. Stornetta, and J. J. Zhu, "The organization of two new cortical interneuronal circuits," *Nat. Neurosci.*, vol. 16, no. 2, pp. 210–218, 2013.
- [6] L. Caulier, "Layer I of primary sensory neocortex: where top-down converges upon bottom-up," *Behav. Brain Res.*, vol. 71, no. 1–2, pp. 163–170, 1995.
- [7] L. M. Palmer, J. M. Schulz, S. C. Murphy, D. Ledergerber, M. Murayama, and M. E. Larkum, "Interhemispheric Inhibition," *Science (80-.)*, vol. 335, no. February, pp. 989–993, 2012.
- [8] **E. Abs et al., "Learning-Related Plasticity in Dendrite-Targeting Layer 1 Interneurons," *Neuron*, vol. 100, no. 3, pp. 684-699.e6, 2018. (**)**
Using two-photon imaging, viral and genetic approaches, electrophysiology and behavioral tasks, the authors show that L1 interneurons identified at NDNF⁺ directly inhibit the apical dendrites of pyramidal neurons in L1, hence participating in learning-related plasticity.
- [9] L. A. Ibrahim et al., "Cross-Modality Sharpening of Visual Cortical Processing through Layer-1-Mediated Inhibition and Disinhibition," *Neuron*, 2016.
- [10] **Letzkus, Johannes J., Steffen B. E. Wolff, Elisabeth M. M. Meyer, Philip Tovote, Julien Courtin, Cyril Herry, and Andreas Lüthi. 2011. "A Disinhibitory Microcircuit for Associative Fear Learning in the Auditory Cortex." *Nature* 480(7377):331–35. (*)**
Using in vivo functional imaging, electrophysiology and optogenetics, the authors show that, in the auditory cortex, recruitment of disinhibitory circuits is necessary for the acquisition of associative fear memory.
- [11] G. Doron et al., "Perirhinal input to neocortical layer 1 controls learning," *bioRxiv*, p. 713883, 2019.
- [12] G. Bartolini, G. Ciceri, and O. Marín, "Integration of GABAergic Interneurons into Cortical Cell Assemblies: Lessons from Embryos and Adults," *Neuron*, vol. 79, no. 5, pp. 849–864, 2013.
- [13] Z. J. Huang and A. Paul, "The diversity of GABAergic neurons and neural communication elements," *Nat. Rev. Neurosci.*, vol. 20, no. 9, pp. 563–572, 2019.
- [14] L. Lim et al., "Optimization of interneuron function by direct coupling of cell migration and axonal targeting," *Nat. Neurosci.*, vol. 21, no. 7, pp. 920–931, 2018.
- [15] M. Niquille et al., "Neurogliaform cortical interneurons derive from cells in the preoptic area," *Elife*, vol. 7, pp. 1–24, 2018.
- [16] **Schuman, B., Machold, R., Hashikawa, Y., Fuzik, J., Fishell, G., & Rudy, B. (2018). Four unique interneuron populations reside in neocortical layer 1. *The Journal of Neuroscience*, 1613–1618. <https://doi.org/10.1523/JNEUROSCI.1613-18.2018>(**)**
Using several genetic tools, in vitro electrophysiology and morphological reconstruction, the authors characterized and classified the interneurons in layer 1 in the adult somatosensory cortex, into four groups, depending on their molecular profiles.
- [17] **L. Z. Fan et al., "All-Optical Electrophysiology Reveals the Role of Lateral Inhibition in Sensory Processing in Cortical Layer 1," *Cell*, vol. 180, no. 3, pp. 521-535.e18, 2020. (*)**
Using genetically encoded voltage indicators and in vivo functional imaging in L1, the researchers show that interneurons provide important lateral inhibition that is key for timely responses following whisker stimulation, and that neuromodulatory inputs enhance excitatory responses.
- [18] **P. G. Anastasiades, D. P. Collins, and A. G. Carter, "Mediodorsal and ventromedial thalamus engage distinct L1 circuits in the prefrontal cortex," *bioRxiv*, vol. 007308, p. 2020.01.08.898817, 2020. (**)**

Results

Using genetic tracing, electrophysiology and in vivo functional imaging approaches, the authors show that different thalamic nuclei target different subpopulations of L1 interneurons in the prefrontal cortex.

- [19] **Takesian, A. E., Bogart, L. J., Lichtman, J. W., & Hensch, T. K. (2018). Inhibitory circuit gating of auditory critical-period plasticity. *Nature Neuroscience*, 21(2), 218–227. <https://doi.org/10.1038/s41593-017-0064-2> (**)**
Using genetic tools and in vitro electrophysiology, the authors show that in the developing L1 auditory cortex, interneurons are integrating both neuromodulatory and thalamic inputs and drive plasticity during the critical window of auditory cortex development.
- [20] S. Clavreul *et al.*, “Cortical astrocytes develop in a plastic manner at both clonal and cellular levels,” *Nat. Commun.*, vol. 10, no. 1, pp. 1–14, 2019.
- [21] D. Lanjakornsiripan *et al.*, “Layer-specific morphological and molecular differences in neocortical astrocytes and their dependence on neuronal layers,” *Nat. Commun.*, vol. 9, no. 1, 2018.
- [22] N. Takata and H. Hirase, “Cortical layer 1 and layer 2/3 astrocytes exhibit distinct calcium dynamics in vivo,” *PLoS One*, vol. 3, no. 6, 2008.
- [23] N. J. Audette, J. Urban-Ciecko, M. Matsushita, and A. L. Barth, “POm Thalamocortical Input Drives Layer-Specific Microcircuits in Somatosensory Cortex,” *Cereb. Cortex*, no. March 2017, pp. 1–17, 2017.
- [24] S. J. Cruikshank *et al.*, “Thalamic Control of Layer 1 Circuits in Prefrontal Cortex,” *J. Neurosci.*, vol. 32, no. 49, pp. 17813–17823, 2012.
- [25] **Zhang, Wanying and Randy M. Bruno. 2019. “High-Order Thalamic Inputs to Primary Somatosensory Cortex Are Stronger and Longer Lasting than Cortical Inputs.” *ELife* 8:1–22. (*)**
Using optogenetic and in vivo electrophysiology approaches, the authors show that the excitatory drive on apical dendrites of upper layers pyramidal neurons in L1 is mainly mediated by higher-order thalamic input.
- [26] S. Lee, I. Kruglikov, Z. J. Huang, G. Fishell, and B. Rudy, “A disinhibitory circuit mediates motor integration in the somatosensory cortex,” *Nat. Neurosci.*, 2013.
- [27] A. Caputi, S. Melzer, M. Michael, and H. Monyer, “The long and short of GABAergic neurons,” *Curr. Opin. Neurobiol.*, vol. 23, no. 2, pp. 179–186, 2013.
- [28] **Poorthuis, Rogier B., Karzan Muhammad, Mantian Wang, Matthijs B. Verhoog, Stephan Junek, Anne Wrana, Huibert D. Mansvelder, and Johannes J. Letzkus. 2018. “Rapid Neuromodulation of Layer 1 Interneurons in Human Neocortex.” *Cell Reports* 23(4):951–58. (*)**
Here the authors compare the electrophysiological properties of L1 interneurons in mice versus humans. In both species, L1 interneurons receive cholinergic inputs via nicotinic receptors, but human L1 interneurons present specific features.
- [29] J. Chen and A. R. Kriegstein, “A GABAergic projection from the zona incerta to cortex promotes cortical neuron development,” *Science (80-.)*, vol. 350, no. 6260, pp. 554–558, 2015.
- [30] T. Vitalis, M. S. Ansorge, and A. G. Dayer, “Serotonin homeostasis and serotonin receptors as actors of cortical construction: Special attention to the 5-HT3A and 5-HT6 receptor subtypes,” *Front. Cell. Neurosci.*, vol. 7, no. JUNE, pp. 1–20, 2013.
- [31] **A. Karimi, J. Odenthal, F. Drawitsch, K. M. Boergens, and M. Helmstaedter, “Cell-type specific innervation of cortical pyramidal cells at their apical dendrites,” *Elife*, vol. 9, 2020. (*)**
Using 3D-electron microscopy, the authors describe the stereotypic organization of both excitatory and inhibitory synapses on L1 apical dendrites of both upper and deep pyramidal neurons.
- [32] **Williams, L. E., & Holtmaat, A. (2018). Higher-Order Thalamocortical Inputs Gate Synaptic Long-Term Potentiation via Disinhibition. *Neuron*, 1–12. <https://doi.org/10.1016/j.neuron.2018.10.049> (**)**

Using optogenetic and electrophysiology approaches, the authors show that in the barrel cortex, higher order thalamic input on L1 apical dendrites of upper cortical layers coupled with thalamic driven disinhibition through VIP⁺ interneurons is required for cortical long-term potentiation.

- [33] **N. J. Audette, S. M. Bernhard, A. Ray, L. T. Stewart, and A. L. Barth, “Rapid Plasticity of Higher-Order Thalamocortical Inputs during Sensory Learning,” *Neuron*, vol. 103, no. 2, pp. 277-291.e4, 2019. (**)**
Using behavioral approaches, viral manipulations and electrophysiology, the authors show that the behavior of learning by association of multi-whisker stimulation and water reward relies at the circuit level, on a potentiation of higher order thalamic inputs to cortical pyramidal neurons.
- [34] **C. O. Lacefield, E. A. Pnevmatikakis, L. Paninski, and R. M. Bruno, “Reinforcement Learning Recruits Somata and Apical Dendrites across Layers of Primary Sensory Cortex,” *Cell Rep.*, vol. 26, no. 8, pp. 2000-2008.e2, 2019.(*)**
Using in vivo functional imaging, this study shows that in reinforcement learning, reward mediates increased activity in layer 1 apical dendrites of both upper and deep pyramidal neurons of the somatosensory cortex.
- [35] N. Takahashi, T. G. Oertner, P. Hegemann, and M. E. Larkum, “Modulate Perception,” vol. 354, no. 6319, pp. 1159–1165, 2016.
- [36] L. Mesik, J. J. Huang, L. I. Zhang, and H. W. Tao, “Sensory- and Motor-Related Responses of Layer 1 Neurons in the Mouse Visual Cortex,” *J. Neurosci.*, vol. 39, no. 50, pp. 10060–10070, 2019.
- [37] Y. H. Tanaka, Y. R. Tanaka, M. Kondo, S. I. Terada, Y. Kawaguchi, and M. Matsuzaki, “Thalamocortical Axonal Activity in Motor Cortex Exhibits Layer-Specific Dynamics during Motor Learning,” *Neuron*, vol. 100, no. 1, pp. 244-258.e12, 2018.
- [38] S. Kirischuk, H. J. Luhmann, and W. Kilb, “Cajal-Retzius cells: Update on structural and functional properties of these mystic neurons that bridged the 20th century,” *Neuroscience*, vol. 275, pp. 33–46, 2014.
- [39] E. Soriano and J. A. Del Río, “The cells of cajal-retzius: Still a mystery one century after,” *Neuron*, vol. 46, no. 3, pp. 389–394, 2005.
- [40] G. D’Arcangelo, L. Lossi, and A. Merighi, “Editorial: Reelin-related neurological disorders and animal models,” *Front. Cell. Neurosci.*, vol. 10, no. January, pp. 2016–2017, 2017.
- [41] F. Bielle *et al.*, “Multiple origins of Cajal-Retzius cells at the borders of the developing pallium.,” *Nat. Neurosci.*, vol. 8, no. 8, pp. 1002–12, 2005.
- [42] A. Bribián *et al.*, “Sema3E/PlexinD1 regulates the migration of hem-derived Cajal-Retzius cells in developing cerebral cortex,” *Nat. Commun.*, vol. 5, no. December, 2014.
- [43] M. Barber and A. Pierani, “Tangential migration of glutamatergic neurons and cortical patterning during development: Lessons from Cajal-Retzius cells,” *Dev. Neurobiol.*, vol. 76, no. 8, pp. 847–881, 2016.
- [44] N. Ruiz-Reig *et al.*, “Lateral Thalamic Eminence: A Novel Origin for mGluR1/Lot Cells,” *Cereb. Cortex*, vol. 27, no. 5, pp. 2841–2856, 2017.
- [45] **M. Barber *et al.*, “Migration speed of Cajal-Retzius cells modulated by vesicular trafficking controls the size of higher-order cortical areas,” *Curr. Biol.*, vol. 25, no. 19, pp. 2466–2478, 2015. (**)**
Using genetic tools, the authors show that the migration speed of several subtypes of CRc on the cortical surface shapes the size of higher-order areas.
- [46] **A. Griveau *et al.*, “A novel role for Dbx1-derived Cajal-Retzius cells in early regionalization of the cerebral cortical neuroepithelium,” *PLoS Biol.*, vol. 8, no. 7, 2010. (**)**
Using genetic tools, the authors show that distinct subpopulations of CRc colonize specific cortical territories and regulate arealization. Importantly, genetic ablation of SE-derived CRc results in a redistribution of CRc subtypes on the neocortical surface, thereby modifying arealization.
- [47] **C. A. de Frutos *et al.*, “Reallocation of Olfactory Cajal-Retzius Cells Shapes Neocortex Architecture,” *Neuron*, vol. 92, no. 2, pp. 435–448, 2016.(**)**
Using several genetic models, the authors show that CRc accumulate in a reservoir from which they re-enter migration and thereby maintain cell density in L1. Impairments in CRc density has

long-lasting effects on the wiring of circuits, even after the CRc elimination.

- [48] **F. Ledonne *et al.*, “Targeted Inactivation of Bax Reveals a Subtype-Specific Mechanism of Cajal-Retzius Neuron Death in the Postnatal Cerebral Cortex,” *Cell Rep.*, vol. 17, no. 12, pp. 3133–3141, 2016. (**)**
Using genetic models to conditionally impair the Bax proapoptotic gene in CRc, the authors elegantly show that the elimination of specific CRc subtypes is mediated through Bax-dependent mechanisms.
- [49] F. Causeret, E. Coppola, and A. Pierani, “Cortical developmental death: selected to survive or fated to die,” *Current Opinion in Neurobiology*, vol. 53, pp. 35–42, 2018.
- [50] M. Anstötz, K. E. Cosgrove, I. Hack, E. Mugnaini, G. Maccaferri, and J. H. R. Lübke, “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.*, 2014.
- [51] J. Ma, X. H. Yao, Y. Fu, and Y. C. Yu, “Development of layer 1 neurons in the mouse neocortex,” *Cereb. Cortex*, vol. 24, no. 10, pp. 2604–2618, 2014.
- [52] S. Frazer *et al.*, “Transcriptomic and anatomic parcellation of 5-HT3AR expressing cortical interneuron subtypes revealed by single-cell RNA sequencing,” *Nat. Commun.*, vol. 8, p. 14219, 2017.
- [53] D. Gelman *et al.*, “A wide diversity of cortical GABAergic interneurons derives from the embryonic preoptic area,” *J. Neurosci.*, vol. 31, no. 46, pp. 16570–16580, 2011.
- [54] B. Wamsley and G. Fishell, “Genetic and activity-dependent mechanisms underlying interneuron diversity,” *Nat. Rev. Neurosci.*, vol. 18, no. 5, pp. 299–309, 2017.
- [55] R. Batista-Brito and G. Fishell, “Chapter 3 The Developmental Integration of Cortical Interneurons into a Functional Network,” *Curr. Top. Dev. Biol.*, vol. 87, no. 09, pp. 81–118, 2009.
- [56] G. Limoni, M. Niquille, S. Murthy, D. Jabaudon, and A. Dayer, “PlexinA4-Semaphorin3A mediated crosstalk between main cortical interneuron classes is required for superficial interneurons lamination,” *bioRxiv*, p. 2020.06.23.166926, 2020.
- [57] E. Favuzzi *et al.*, “Neurodevelopment: Distinct molecular programs regulate synapse specificity in cortical inhibitory circuits,” *Science (80-.)*, vol. 363, no. 6425, pp. 413–417, 2019.
- [58] G. Miyoshi *et al.*, “Prox1 Regulates the Subtype-Specific Development of Caudal Ganglionic Eminence-Derived GABAergic Cortical Interneurons,” *J. Neurosci.*, vol. 35, no. 37, pp. 12869–89, 2015.
- [59] D. M. Gelman, F. J. Martini, S. Nóbrega-Pereira, A. Pierani, N. Kessar, and O. Marín, “The embryonic preoptic area is a novel source of cortical GABAergic interneurons,” *J. Neurosci.*, vol. 29, no. 29, pp. 9380–9389, 2009.
- [60] V. Borrell and O. Marín, “Meninges control tangential migration of hem-derived Cajal-Retzius cells via CXCL12/CXCR4 signaling,” *Nat. Neurosci.*, vol. 9, no. 10, pp. 1284–1293, 2006.
- [61] G. López-Bendito *et al.*, “Chemokine signaling controls intracortical migration and final distribution of GABAergic interneurons,” *J. Neurosci.*, vol. 28, no. 7, pp. 1613–1624, 2008.
- [62] F. Trousse *et al.*, “CXCR7 receptor controls the maintenance of subpial positioning of cajal-retzius cells,” *Cereb. Cortex*, vol. 25, no. 10, pp. 3446–3457, 2015.
- [63] G. Li *et al.*, “Regional distribution of cortical interneurons and development of inhibitory tone are regulated by Cxcl12/Cxcr4 signaling,” *J. Neurosci.*, vol. 28, no. 5, pp. 1085–98, 2008.
- [64] S. Alcántara, E. Pozas, C. F. Ibañez, and E. Soriano, “BDNF-modulated spatial organization of Cajal-Retzius and GABAergic neurons in the marginal zone plays a role in the development of cortical organization,” *Cereb. Cortex*, vol. 16, no. 4, pp. 487–499, 2006.
- [65] G. Caronia-Brown and E. A. Grove, “Timing of cortical interneuron migration is influenced by the cortical hem,” *Cereb. Cortex*, vol. 21, no. 4, pp. 748–755, 2011.
- [66] R. Cossart, “The maturation of cortical interneuron diversity: How multiple developmental journeys shape the emergence of proper network function,” *Curr. Opin. Neurobiol.*, vol. 21, no. 1, pp. 160–168, 2011.
- [67] **A. Che *et al.*, “Layer I Interneurons Sharpen Sensory Maps during Neonatal Development,” *Neuron*, vol. 99, no. 1, pp. 98–116.e7, 2018. (**)**
Using genetic tools and in vivo functional imaging on pups, the authors show that L1 interneurons are synchronously activated during the first postnatal week, which is dependent on sensory activity and the correlation is lost later on

Results

- [68] K. L. Whitford, P. Dijkhuizen, F. Polleux, and A. Ghosh, "Molecular Control of Cortical Dendrite Development," *Annu. Rev. Neurosci.*, vol. 25, no. 1, pp. 127–149, 2002.
- [69] J. Hakanen, N. Ruiz-Reig, and F. Tissir, "Linking cell polarity to cortical development and malformations," *Front. Cell. Neurosci.*, vol. 13, no. June, pp. 1–22, 2019.
- [70] L. R. Fenlon, R. Suárez, and L. J. Richards, "The anatomy, organisation and development of contralateral callosal projections of the mouse somatosensory cortex," *Brain Neurosci. Adv.*, vol. 1, p. 239821281769488, 2017.
- [71] M. J. Galazo, V. Martínez-Cerdeño, C. Porrero, and F. Clascá, "Embryonic and postnatal development of the layer I-directed ('matrix') thalamocortical system in the rat," *Cereb. Cortex*, vol. 18, no. 2, pp. 344–363, 2008.
- [72] P. Rubio-Garrido, F. Pérez-De-Manzo, C. Porrero, M. J. Galazo, and F. Clascá, "Thalamic input to distal apical dendrites in neocortical layer 1 is massive and highly convergent," *Cereb. Cortex*, vol. 19, no. 10, pp. 2380–2395, 2009.
- [73] H. Taniguchi, J. Lu, and Z. J. Huang, "The spatial and temporal origin of chandelier cells in mouse neocortex," *Science (80-.)*, vol. 339, no. 6115, pp. 70–74, 2013.
- [74] H. J. Luhmann, S. Kirischuk, and W. Kilb, "The superior function of the subplate in early neocortical development," *Front. Neuroanat.*, vol. 12, no. November, pp. 1–14, 2018.
- [75] F. Ghezzi *et al.*, "Non-canonical role for Lpar1-EGFP subplate neurons in early postnatal somatosensory cortex," *bioRxiv*, p. 2020.05.12.088450, 2020.
- [76] **Meng, Xiangying, Yanqing Yu, Joseph Kao, and Patrick O. Kanold. 2020. "Transient Coupling between Subplate and Subgranular Layers to L1 Neurons before and during the Critical Period." *BioRxiv* 009607:2020.05.05.077784 (*)**
Using laser scanning photo-stimulation, the authors describe the development of L1 functional circuits in the auditory cortex, especially highlighting early translaminar excitatory inputs from from the subplate, with a change in L1 functional diversity by the third postnatal week.
- [77] N. Zecevic, "Synaptogenesis in layer I of the human cerebral cortex in the first half of gestation," *Cereb. Cortex*, vol. 8, no. 3, pp. 245–252, 1998.
- [78] V. Borrell *et al.*, "Reelin regulates the development and synaptogenesis of the layer-specific entorhino-hippocampal connections," *J. Neurosci.*, vol. 19, no. 4, pp. 1345–1358, 1999.
- [79] C. Bosch *et al.*, "Reelin Regulates the Maturation of Dendritic Spines, Synaptogenesis and Glial Ensheatment of Newborn Granule Cells," *Cereb. Cortex*, vol. 26, no. 11, pp. 4282–4298, 2016.
- [80] S. K. Singh *et al.*, "Astrocytes Assemble Thalamocortical Synapses by Bridging NRX1 α and NL1 via Hevin," *Cell*, vol. 164, no. 1–2, pp. 183–196, 2016.
- [81] B. A. Sava *et al.*, "Electrophysiological and morphological properties of Cajal-Retzius cells with different ontogenetic origins," *Neuroscience*, vol. 167, no. 3, pp. 724–734, 2010.
- [82] M. Ogawa and T. Miyata, "The reeler Gene-Associated Antigen on Cajal-Retzius Neurons Is a Crucial Molecule for Laminar Organization of Cortical Neurons," vol. 14, pp. 899–912, 1995.
- [83] K. Sekine, K. Kubo, and K. Nakajima, "How does Reelin control neuronal migration and layer formation in the developing mammalian neocortex?," *Neurosci. Res.*, vol. 86, pp. 50–58, 2014.
- [84] O. Yabut, A. Renfro, S. Niu, J. W. Swann, O. Marín, and G. D. Arcangelo, "Abnormal laminar position and dendrite development of interneurons in the reeler forebrain," vol. 40, 2005.
- [85] H. J. Luhmann, A. Fukuda, and W. Kilb, "Control of cortical neuronal migration by glutamate and GABA," *Front. Cell. Neurosci.*, vol. 9, no. JAN, pp. 1–15, 2015.
- [86] F. Tissir, A. Ravni, Y. Achouri, D. Riethmacher, G. Meyer, and A. M. Goffinet, "DeltaNp73 regulates neuronal survival in vivo.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 106, no. 39, pp. 16871–16876, 2009.
- [87] M. Yoshida, S. Assimakopoulos, K. R. Jones, and E. A. Grove, "Massive loss of Cajal-Retzius cells does not disrupt neocortical layer order," *Development*, vol. 133, no. 3, pp. 537–545, 2006.
- [88] J. Li *et al.*, "Integrative genomic analysis of early neurogenesis reveals a temporal genetic program for differentiation and specification of preplate and Cajal-Retzius neurons," p. 331900, 2018.
- [89] S. Janusonis, "Early Serotonergic Projections to Cajal-Retzius Cells: Relevance for Cortical Development," *J. Neurosci.*, vol. 24, no. 7, pp. 1652–1659, 2004.
- [90] S. Z. H. Naqui, B. S. Harris, D. Thomaidou, and J. G. Parnavelas, "The noradrenergic system influences the fate of Cajal-Retzius cells in the developing cerebral cortex," *Dev. Brain Res.*, vol. 113, no. 1–2, pp. 75–82, 1999.

Results

- [91] **Riva, M., Genescu, I., Habermacher, C., Orduz, D., Ledonne, F., Rijli, F. M., Lopez-Bendito, G., Coppola E., Garel, S., Angulo, M.C., Pierani, A. (2019). Activity-dependent death of transient cajal-retzius neurons is required for functional cortical wiring. *ELife*, 8(i), 1–18. <https://doi.org/10.7554/eLife.50503> (**)**
This study shows that SE and ET-derived Cajal-Retzius cells die in an activity-dependent manner, and that their proper elimination is required for the wiring of the upper layer pyramidal neurons.
- [92] L. A. Cocas, G. Fernandez, M. Barch, J. Doll, I. Zamora Diaz, and S. J. Pleasure, “Cell Type-Specific Circuit Mapping Reveals the Presynaptic Connectivity of Developing Cortical Circuits,” *J. Neurosci.*, vol. 36, no. 11, pp. 3378–3390, 2016.
- [93] A. Aguiló *et al.*, “Involvement of cajal-retzius neurons in spontaneous correlated activity of embryonic and postnatal layer 1 from wild-type and reeler mice.,” *J. Neurosci.*, vol. 19, no. 24, pp. 10856–68, Dec. 1999.
- [94] J. M. Mienville and C. Pesold, “Low resting potential and postnatal upregulation of NMDA receptors may cause Cajal-Retzius cell death.,” *J. Neurosci.*, vol. 19, no. 5, pp. 1636–1646, 1999.
- [95] M. Marín-Padilla, “Cajal-Retzius cells and the development of the neocortex,” *Trends Neurosci.*, vol. 21, no. 2, pp. 64–71, 1998.
- [96] **Blanquie, Oriane, Lutz Liebmann, Christian A. Hübner, Heiko J. Luhmann, Anne Sinning, Human Genetics, Friedrich Schiller, and D. Jena. 2017. *Cerebral Cortex* (February):1644–59. (**)**
Using cellular cultures of CRc, the authors show that their elimination in vitro is activity-dependent. Blockade of the NKCC1 transporter in vitro or genetic deletion in vivo induces CRc survival.
- [97] S. Ha, P. P. Tripathi, A. B. Mihalas, R. F. Hevner, and D. R. Beier, “C-Terminal Region Truncation of RELN Disrupts an Interaction with VLDLR, Causing Abnormal Development of the Cerebral Cortex and Hippocampus,” *J. Neurosci.*, vol. 37, no. 4, pp. 960–971, 2017.
- [98] E. Okugawa *et al.*, “Physiological significance of proteolytic processing of Reelin revealed by cleavage-resistant Reelin knock-in mice,” *Sci. Rep.*, vol. 10, no. 1, pp. 1–13, 2020.
- [99] K. Sakai, H. Shoji, T. Kohno, T. Miyakawa, and M. Hattori, “Mice that lack the C-Terminal region of Reelin exhibit behavioral abnormalities related to neuropsychiatric disorders,” *Sci. Rep.*, vol. 6, no. March, pp. 1–13, 2016.
- [100] G. Eyal *et al.*, “Human cortical pyramidal neurons: From spines to spikes via models,” *Front. Cell. Neurosci.*, vol. 12, no. June, pp. 1–24, 2018.
- [101] G. Meyer and M. González-Gómez, “The heterogeneity of human Cajal-Retzius neurons,” *Semin. Cell Dev. Biol.*, vol. 76, pp. 101–111, 2018.

DISCUSSION AND CONCLUSION

Discussion and conclusion

Neocortical function relies on complex circuits organized in six layers. Increasing evidence is pointing out that the cortical layer 1 (L1), the most superficial layer of the neocortex, is a major site of input integration and is essential for higher order functions like sensory processing, learning, arousal and consciousness (Doron et al., 2019; Suzuki & Larkum, 2020; Takahashi et al., 2016; Williams & Holtmaat, 2018). While its anatomy and roles in adult neocortex start being unraveled, there is little knowledge about how this essential structure is put in place in development.

During development, L1 hosts a particular transient population, the Cajal-Retzius cells (CRc), which have been already shown to be very important for key processes in development like neuronal migration, neocortical lamination and arealization. Built on with previous work, in this PhD thesis, we have shown that CRc are major regulators of cortical wiring. Through their density which is tightly regulated in space and time, the CRc are modulating the wiring of upper cortical layers during early postnatal life. We have shown here that CRc density is gracefully maintained by at least two steps, which are regulated in part by activity-dependent mechanisms: a *GluN1*-dependent redistribution and by a partial activity-dependent elimination which is not *GluN1* driven (de Frutos et al., 2016; Article 1: Riva, Genescu, Habermacher et al., 2019; Article 2). Remarkably, perturbations in the density of CRc during their lifetime as well as impaired elimination of CRc have long lasting impacts on both aspects of the E/I balance and induces long term deficits in the functioning of the neocortex (de Frutos et al., 2016; Article 1: Riva, Genescu, Habermacher et al., 2019; Article 2). Therefore, the CRc emerge as major regulator of neocortical development, with implications not only during their lifetime but also long time after they have already been eliminated. These observations raise a number of questions. During this chapter I will discuss the roles of electrical activity in the life and death of CRc (Part 1), the heterogeneity of CRc leading to different behavior during their life and elimination (Part 2) and the roles of CRc in modulating at long term the neocortical E/I ratio which is required for a normal cortical function (Part 3) and finally briefly the roles of CRc in evolution (Part 4).

1. Neuronal activity in life and death of Cajal-Retzius cells

1.1. Cajal-Retzius cell density is shaped by in two different activity-dependent phases

The maintenance of CRc density which is tightly regulated in both space and time is essential for neocortical architecture, as impairments in CRc density have long lasting consequences on cortical wiring (de Frutos et al., 2016; Article 1: Riva, Genescu, Habermacher et al., 2019; Article 2). CRc density is sustained through at least two steps: i) subtypes of CRc accumulate in a reservoir near the LOT during embryogenesis from which they progressively redistribute (de Frutos et al., 2016) and ii) CRc are eliminated during early postnatal life (Ledonne et al., 2016; Article 1: Riva, Genescu, Habermacher et al., 2019). Previous research and our work suggested that both these phases are, at least in part, activity dependent.

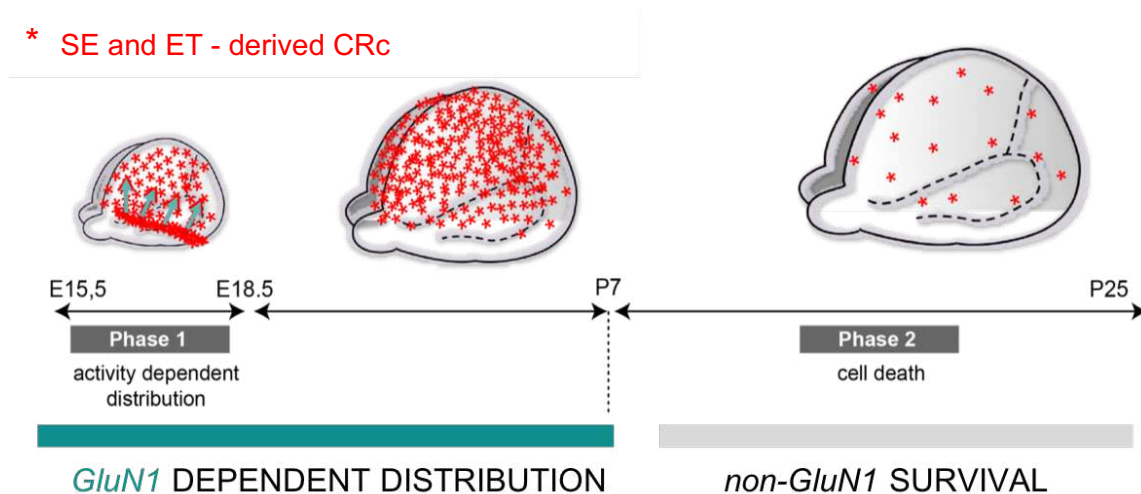


Figure 16 | Cajal-Retzius cell density is in part modulated by activity-dependent mechanisms

Schematic representation of the density of CRc covering the surface of the neocortex. An embryonic *GluN1*-dependent remigration of CRc maintains the density of CRc during their lifetime. A second step in controlling CRc density is via their almost complete elimination starting with the second postnatal week, which is also, in part, activity-dependent but not *GluN1*-mediated. Both these phases are very important for regulating cortical wiring.

CRC density is modulated through a *GluN1* dependent redistribution of subsets of CRC from a reservoir situated near the LOT (de Frutos et al., 2016), as the *GluN1* cKO mutants present less CRC in S1 and accumulations of CRC near the LOT (de Frutos et al., 2016; Article 2). Strikingly however, we have noticed that CRC elimination is not impaired in these *GluN1* cKO mutants (Article 2). Even though not-*GluN1* dependent, CRC elimination is in part activity mediated, as both *in vitro* blocking of neuronal activity or *in vivo* hyperpolarization of CRC is leading to partial rescue of CRC (Blanquie, Liebmann, et al., 2017; Del Río et al., 1996; J M Mienville & Pesold, 1999; Article 1: Riva, Genescu, Habermacher et al., 2019). This suggests that electrical activity shapes the two phases of maintaining a proper density of CRC, but through two different mechanisms: *GluN1*-dependent redistribution and *GluN1*-independent elimination. An important question that remains to be addressed is what triggers these two activity-dependent phases that shape CRC density?

1.2. By which mechanisms activity modulates Cajal-Retzius cell density?

1.2.1. Cajal-Retzius cell redistribution

What could be the inputs (*if any*) triggering these two activity-dependent phases of remigration and elimination, which control CRC density? The *GluN1* dependent migration has already been demonstrated in developing neocortex, as both *in vitro* and *in vivo* blocking or activation of the NMDA receptors leads to slowing down cortical migration (Behar et al., 1999; Hirai et al., 1999; Kihara et al., 2002; Sergei Kirischuk et al., 2017; Reiprich, Kilb, & Luhmann, 2005). The *GluN1* dependent remigration of CRC is likely not a direct synaptic event, as it happens early on during development, between E13.5 and E15.5 and the synaptogenesis has not taken place yet. The activation of the *GluN1* receptor is probably mediated by ambient glutamate, which could originate from the LOT axons. An argument favoring this hypothesis is that CRC near the LOT (CR-lot cells) respond in a synchronous manner to LOT stimulation and their response is completely abolished by application of pharmacological activity blockers like TTX (de Frutos et al., 2016). One way to directly test this hypothesis is by hyperpolarizing the LOT axons to decrease their probability of glutamate release and check whether this impacts on the

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redistribution of the CRc located near the LOT. We did try to test this hypothesis by using the *Er81^{creERT2/+}* model crossed with *R26^{Kir2.1/+}* which, through Tamoxifen injection at E11.5, leads to the expression of hyperpolarizing Kir2.1 channel in the LOT axons. However, the number of CRc near the LOT is difficult to assess only with immunostainings and without using genetic tracings, therefore this experiment is so far inconclusive.

1.2.2 Cajal-Retzius cell elimination

While the GluN1 signaling is key for CRc remigration, the mechanisms driving CRc elimination at the beginning of the second postnatal week are still elusive. One possibility that we cannot disregard is that part of CRc spontaneously activate at the beginning of the second postnatal week. This overactivation could be explained by a release of Ca^{2+} from internal stores, leading to increased intracellular Ca^{2+} concentration which becomes toxic and leads to their cell death. The low resting membrane potential of CRc (J M Mienville & Pesold, 1999; Article 1: Riva, Genescu, Habermacher et al., 2019; Sava et al., 2010; Sun et al., 2019; Wong & Marín, 2019) could indeed facilitate the excitotoxic effect of Ca^{2+} .

A non-exclusive possibility is that the elimination of subsets of CRc is, in part, input driven. The time window of CRc death at the beginning of the 2nd postnatal week, corresponds to a major changes in cortical activity (Luhmann & Khazipov, 2018) and in GABAergic circuits (Cossart, 2011), raising the prospect of CRc elimination as part of a global activity-dependent remodeling of neocortical networks. Our first candidate was general thalamic mediated sensory activity, which is very important in shaping circuits especially in this timeframe of CRc elimination (Erzurumlu & Gaspar, 2012; Leighton & Lohmann, 2016). To address this question, we impacted the activity at several relays in the somatosensory pathway and addressed : i) the role of whisker induced sensory activity by bilateral whisker plucking, ii) whether activity from the periphery independent of sensory experience has an impact on CRc elimination, by infraorbital nerve lesion and iii) the contribution of thalamic waves and activity of thalamic axons innervating the cortex in this process, by expressing the hyperpolarizing Kir2.1 channel in thalamic axons. In contrast with what we would have expected, the sensory activity which is

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a main driver of cortical circuits during this early postnatal life, is not the main driver of CRc demise (Article 2). Or at least, sensory activity is not sufficient for inducing CRc elimination. This suggests it should be a local or long range input leading to the elimination of the CRc in this specific timeframe, independently or at least not driven only by sensory activity.

Reasoning with regards to the axonal projections in L1, the inputs inducing the elimination of subsets of CRc could either originate in the glutamatergic thalamic and cortical projections in L1, or in GABAergic inputs in L1 (from Martinotti cells, L1 interneurons or long range GABAergic SST⁺ inputs from the ZI). Glutamatergic inputs on CRc (J M Mienville & Pesold, 1999) are less likely to be involved in CRc elimination as cKO of *GluN1* is not impacting on CRc demise (Article 2). On the other hand though, GABA has been suggested to be involved in CRc elimination as: i) postnatally, CRc are mainly innervated by GABAergic inputs (Article 1: Riva, Genescu, Habermacher et al., 2019; Sun et al., 2019), ii) in CRc, GABA is excitatory which is consistent with a rescue of CRc through their hyperpolarization (Pozas, Paco, Soriano, & Aguado, 2008; Article 1: Riva, Genescu, Habermacher et al., 2019) iii) full KO of NKCC1 (Cl⁻ transporter) is leading to an increase of Calret⁺ cells in L1 which could be both interneurons and CRc (Blanquie, Liebmann, et al., 2017), and iv) *in vitro* pharmacological blocking of GABA_A receptors is leading to an increase number of CRc (Blanquie, Liebmann, et al., 2017).

Consistent with these arguments which favor GABA as a trigger for CRc elimination, we found that overexcitation of interneurons projecting in L1 during postnatal week, leads to a decrease in the number of CRc. Overactivation of both all *Nkx2.1* - derived interneurons (Article 2) and SST⁺ GABA projections from Martinotti cells and long-range ZI (data not shown), just before the period of CRc elimination, has a similar effect suggesting a premature elimination of CRc in this specific timeframe (Article 2). The hypothesis that Martinotti SST⁺ cells could be triggering the elimination of CRc is also supported by data from Cocas et al., 2016 who demonstrated with rabies virus backlabelling of presynaptic inputs that indeed postnatally CRc are indeed innervated by deep layers Reelin⁺ interneurons which in part are Martinotti cells. However, these experiments only suggest a potentiality of L1 projecting

Discussion and conclusion

interneurons, through overactivation, to induce a premature elimination of CRc. The opposite experiments of temporal silencing of L1 projecting interneurons would be needed, to prove a necessity of interneuron activity inducing CRc elimination. Besides L1 projecting interneurons, other candidates for driving CRc elimination are also the GABAergic CGE-derived interneurons located in L1. Their role in CRc elimination remains to be fully investigated.

Hyperpolarization of CRc leads to the partial rescue of CRc, which is the opposite behavior compared to other systems where the electrical activity promotes survival (Blanquie, Kilb, Sinning, & Luhmann, 2017; Causeret, Coppola, & Pierani, 2018; Denaxa et al., 2018; Heck et al., 2008; Ikonomidou et al., 1999; Priya et al., 2018; Article 1: Riva, Genescu, Habermacher et al., 2019; Wong et al., 2018). The number of both pyramidal neurons and interneurons is dynamically adjusted through activity-dependent cell death mechanisms, which are modulated *Lhx6* and PTEN expression in MGE-derived interneurons (Denaxa et al., 2018; Wong et al., 2018) and Calcineurin levels in CGE-derived non-VIP interneurons (Priya et al., 2018). Why activity plays opposite roles in different cell types remains still to be largely explored. This paradoxical effect could be though explained by the fact that CRc still present characteristics of “immature neurons” previously mentioned, compared to pyramidal neurons or interneurons: CRc present low resting membrane-potential (J M Mienville & Pesold, 1999; Article 1: Riva, Genescu, Habermacher et al., 2019; Sava et al., 2010; Sun et al., 2019) and they receive depolarizing GABAergic inputs (Jean Marc Mienville, 1998). Notably, it would be interesting to know with transcriptomic analysis whether subsets of CRc, unlike pyramidal neurons or interneurons, present changes in the expression of different receptors which could be responsible for their partial activity-dependent demise. Nonetheless, the CRc, alongside with pyramidal neurons and interneurons, are also attesting the homeostatic interplay between neuronal apoptosis and electrical activity in neocortical development.

2. All Cajal-Retzius cells are not born equal in terms of their response to electrical activity

The activity-dependent redistribution and elimination of CRc which regulate their density is concerning only particular subsets of CRc. Using the $\Delta Np73^{cre}$ driver to trace and genetically manipulate the CRc, both previous work and our work showed that only the SE and ET-derived CRc redistribute from the reservoir and are eliminated in a *Bax* and activity-dependent fashion (de Frutos et al., 2016; Ledonne et al., 2016; Article 1: Riva, Genescu, Habermacher et al., 2019). The hem-derived CRc, which are a majority of CRc and can be targeted using the *Wnt3A^{cre}* driver, do not act in this manner: the hem-derived CRc do not accumulate in the reservoir (de Frutos et al., 2016), and while they are indeed eliminated in the neocortex, their death is not *Bax* – dependent and not activity-mediated (Fig. 17B) (Ledonne et al., 2016; Article 1: Riva, Genescu, Habermacher et al., 2019). The behavior of the PSB-derived CRc still remains to be investigated, as these subtypes of CRc are not targeted by the $\Delta Np73^{cre}$ driver (Fadel Tissir et al., 2009).

Indeed, in models of cKO of *Bax* (Ledonne et al., 2016) or overexpression of Kir2.1 in CRc (Article 1: Riva, Genescu, Habermacher et al., 2019), the numbers of rescued CRc are very similar. There is a five-fold increase in CRc numbers using the $\Delta Np73^{cre}$ driver, in contrast to no change in CRc numbers when using the hem-specific *Wnt3a^{cre}* line. Moreover our quantifications suggest that hyperpolarization of the CRc rescues a large proportion if not all SE and ET-derived CRc. At P15, hyperpolarization of CRc using the $\Delta Np73^{cre}$ driver leads to a partial rescue CRc (Fig.17A), while at the same timepoint there is no impact in the hem-derived *Wnt3A^{cre}* subpopulation (Fig. 17B). This already suggests that the rescued CRc are non-hem derived. On top of that, the number of rescued CRc using the $\Delta Np73^{cre}$ driver (Fig.13 A) is highly similar with the number of SE and ET-derived CRc in control conditions, before the period of cell death (Fig. 13 C). Altogether, this suggests that almost all the SE and ET-derived CRc die in an activity-dependent manner.

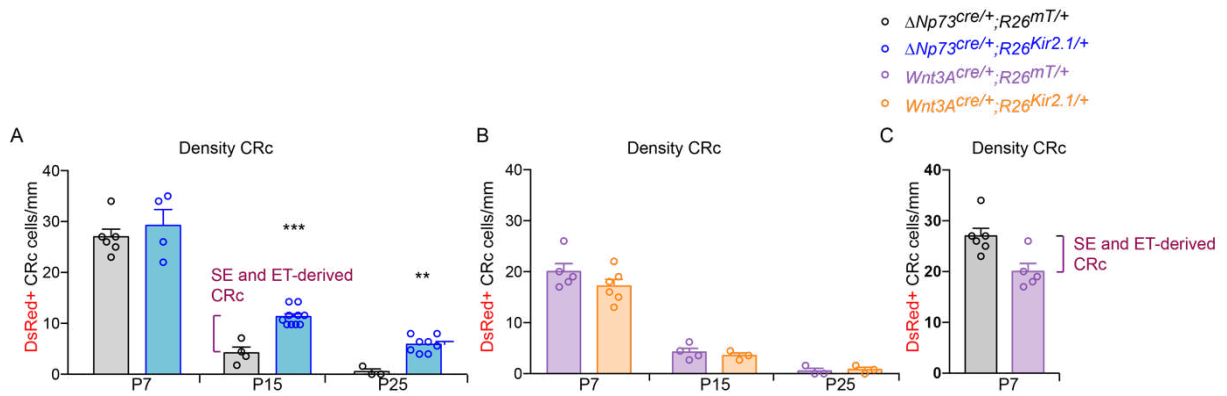


Figure 17 | Most of the SE and ET-derived Cajal-Retzius cells die in an activity-dependent manner

(A) Quantification of CR density at the pial surface in the somatosensory (S1) cortex in both controls ($\Delta Np73^{cre/+};R26^{mT/+}$) and $\Delta Np73^{cre/+};R26^{Kir2.1/+}$ mutants. Note that hyperpolarization of CRc induces their partial survival. (B) Quantification of hem-derived CR density at the pial surface in the somatosensory (S1) cortex in both controls ($Wnt3A^{cre/+};R26^{mT/+}$) and $Wnt3A^{cre/+};R26^{Kir2.1/+}$ mutants. Note that hyperpolarization of hem-derived CRc is not activity mediated. (C) Quantification of CR density at the pial surface in the somatosensory (S1) cortex of SE, ET and Hem-derived CRc ($\Delta Np73^{cre/+};R26^{mT/+}$) and only Hem-derived CRc ($Wnt3A^{cre/+};R26^{mT/+}$). Note that the number of SE and ET-derived CRc is very similar with the number of CRc rescued by hyperpolarization. Adapted from Article 1: Riva, Genescu, Habermacher et al., 2019
 CRc, Cajal-Retzius cells; ET, Thalamic eminence; SE, Septum.

This very different behavior between the hem-derived CRc and the non-hem derived CRc rises several questions.

First, why the subpopulations of CRc behave so differently in response to electrical activity? One of the hypothesis would be that depending on the subpopulations, the CRc are embedded in a specific manner in cortical circuits, receiving differential inputs. This can be questioned *in vivo* using retroviral injections to label the presynaptic partners of subpopulations of CRc, using the $Wnt3A^{cre/+}$ driver for hem-derived CRc and the $Dbx1^{cre/+}$ driver for the non-hem derived CRc. But Sava et al., 2010 have already shown *in vitro* that the electrophysiological and morphological properties of CRc from different subpopulations are very similar, receiving comparable EPSCs. These observations are however made between P1-P7 so before the period of CRc elimination, therefore not excluding the possibility of different inputs target differentially the subpopulations of CRc at the beginning of the 2nd postnatal week. Another

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approach of understanding these differences would be with transcriptomic analysis, to check whether the subpopulations of CRc differentially express subtypes of receptors, which could explain their different behavior towards changes in electrical activity.

Second, the hem-derived CRc not only act differently than the other CRc with different ontogeny, but their fate changes also depending on their environment: the hem-derived CRc in the neocortex are almost completely eliminated in S1 by P25, while in the hippocampus, the hem-derived CRc which represent the majority of hippocampal CRc, are persistent (Anstötz et al., 2016). This suggests that environment underlined by the networks in which these cells are embedded impacts on their fate.

3. Cajal-Retzius cells regulate the excitation/inhibition balance of the neocortex

L1 is a major site of neocortical integration as it contains dendritic tufts of pyramidal neurons which receive both excitatory and inhibitory inputs, altogether participating to cortical function (Harris & Shepherd, 2015). Using a combination of genetic approaches, we showed that changes in the density of CRc during their lifetime or perturbations in the elimination of CRc have long lasting consequences on the cellular and dendritic organization of L1. These impairments, as a consequence, shape the E/I ratio which is required for a normal brain function (de Frutos et al., 2016; Article 1: Riva, Genescu, Habermacher et al., 2019).

3.1. Cajal-Retzius cells impact on the density of specific populations of interneurons

3.1.1 Early impact of Cajal-Retzius cell density on interneurons

The density of CRc, independently of their activity, regulates the density of CGE/POA-derived interneurons labelled with Prox1 (de Frutos et al., 2016; Rubin & Kessar, 2013). To perturb the density of CRc we have used the $\Delta Np73^{cre/+}; R26^{dt-a/+}$ model, which presents -30% of CRc at E18.5 (de Frutos et al., 2016) and -45% of CRc by P7 (de Frutos et al., 2016, Article 2). In this model, we do not know whether the elimination of CRc is specific to certain subpopulations or it targets cells from all origins. Less CRc induce an increase in the numbers of CGE/POA-derived interneurons in upper cortical layers at both E18.5 and P7 (de Frutos et al., 2016, Article 2), while an increased CRc density embryonically using the $Ebf3^{-/-}$ model induces the opposite effect leading to slightly less CGE/POA-derived interneurons in upper cortical layers (de Frutos et al., 2016) (Fig. 15).

These findings suggest a possible cross-talk between CRc and interneurons. This could indeed be the case at least at embryonic periods, as CRc are located in the MZ which is also

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a stream of migration for interneurons before they enter the cortical plate and reach maturation. Several questions can be raised: i) do CRc control the migration of Prox1⁺ CGE/POA-derived interneuron subpopulations through the MZ? This could be addressed with *ex vivo* or *in vivo* timelapse imaging at embryonic stages in genetic model presenting an decreased density of CRc together with a reporter for CGE-derived interneurons, like for example $\Delta Np73^{cre/+}; R26^{dta}; Gad65^{gfp}$ ii) do CRc modulate not only the numbers of interneurons but also their morphology? This is particularly interesting as cell morphology is indeed determinant for the input that they receive. Whether the morphology of CGE-derived interneurons is affected by CRc density could be assessed in the same genetic model $\Delta Np73^{cre/+}; R26^{dta}; Gad65^{gfp}$ through co-stainings for different subpopulation markers and 3D-morphology analysis. The same question can be asked also for Martinotti cells for which the migration through the MZ is necessary to reach their morphological maturation (Lim, Pakan, et al., 2018). Even though there is no evidence about impacts of CRc density on the morphology of Martinotti cells early on, we have found that less CRc during their lifetime does not have long lasting effects on the axonal plexus of Martinotti cells in L1 (Fig. 18).

What could be the mechanisms through which the CRc density shapes the density of CGE/POA-derived Prox1⁺ interneurons at late embryonic and early postnatal stages? There is yet no evidence suggesting that the CRc could impact on the generation of interneurons. This hypothesis is also unlikely because the progenitor domains of CRc and the CGE and POA for interneurons are segregated. On the other hand, CRc especially through their strategic position in the MZ could be modulating interneuron migration within the cortical plate or from their birthplace to the neocortex. These questions remain to be investigated. Nevertheless, in mouse model of impaired CRc density, while there is an increased number of Prox1⁺ interneurons specifically in upper cortical layers, there are no changes Prox1⁺ interneuron numbers in deep cortical layers in the barrel field S1 (*data not shown*). Therefore, if CRc modulate the migration of interneurons without affecting their generation, one would expect a decrease in the number of interneurons in other brain regions.

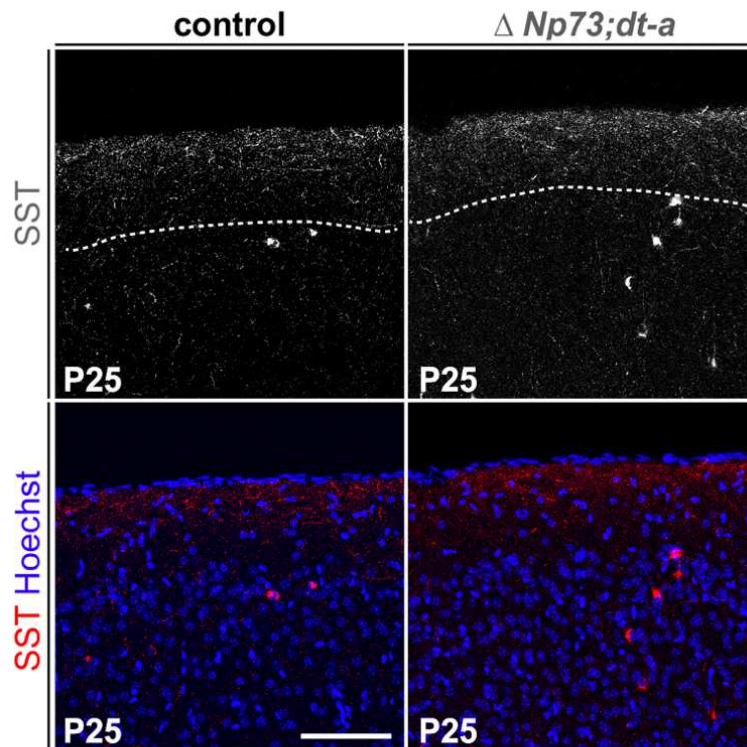


Figure 18 | Decreased Cajal-Retzius cell density is not drastically impacting, on a long term, the axonal plexus of Martinotti cells

Coronal sections of P25 somatosensory cortex immunostained with Somatostatin which labels Martinotti L1 projecting interneurons. Note that the axonal plexus in L1 of Martinotti cells is not drastically perturbed when the density of CRc is impacted at embryonic and postnatal life. Scale bar 100 μ m.

CRc, Cajal-Retzius cells; SST, Somatostatin.

3.1.2 Long-lasting impact on Cajal-Retzius cell density on interneurons

Impaired density of CRc indeed have long lasting consequences on the number of interneurons within the somatosensory cortex. A 45% decrease in the number of CRc at P7 leads also to a long lasting increase in the numbers of upper layers interneurons (Fig. 19). The impact of CRc on interneurons is mainly concerning the POA and CGE-derived interneurons (Fig. 19). According to their molecular signature, these interneurons are likely POA-derived Neurogliaform cells, which are actually the most abundant interneuron subpopulation in neocortical L1 (Hestrin & Armstrong, 1996; Muralidhar et al., 2014; Niquille et al., 2018) as well as subsets of Calretinin⁺ Bipolar or Multipolar cells which are CGE-derived (Caputi, Rozov, Blatow, & Monyer, 2009; Lim, Mi, et al., 2018a; Tremblay et al., 2016). This cellular phenotype

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is consistent with previous electrophysiology results revealing that a decreased density of CRc leads to a slightly increased inhibitory drive on upper layers pyramidal neurons (de Frutos et al., 2016). It is though remarkable the role of CRc in shaping the number of upper layers interneurons, which are highly associated with modulating top-down neocortical inputs, providing contextual information and controlling sensory perception (L. Fan et al., 2019; Ibrahim et al., 2020; Williams & Holtmaat, 2018).

What could be the mechanisms through which perturbed CRc density is leading to increased interneurons densities at later postnatal stages? One hypothesis is that the phenotype observed at P25 is a direct consequence of the effect at early postnatal stages. On top of that, CRc could also modulate interneuron elimination. As CRc undergo elimination mostly before the process of activity-dependent refinement of interneurons, this should be an indirect mechanism that still needs to be unraveled.

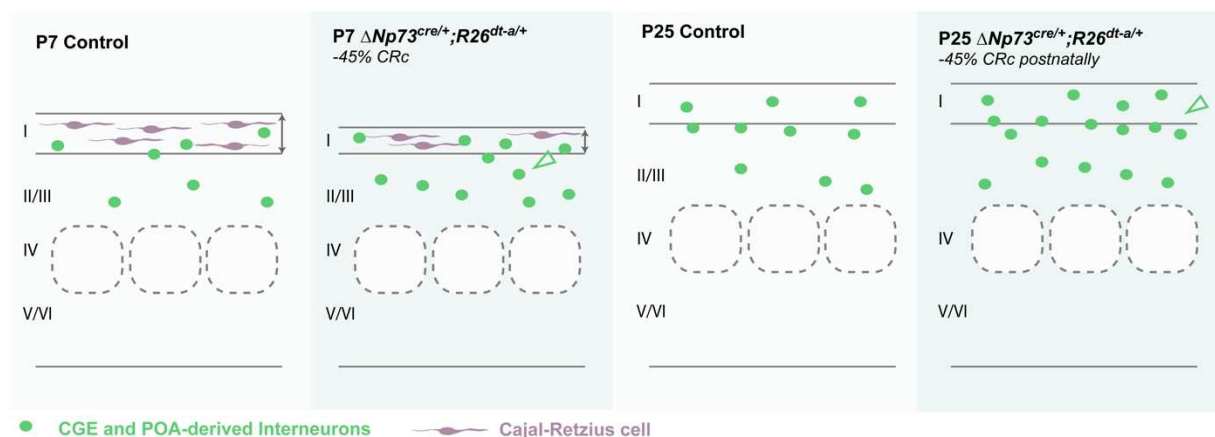


Figure 19 | Impact of Cajal-Retzius cell density on the CGE and POA-derived interneurons

At P7, a 45% decrease in the density of CRc in the somatosensory cortex leads to an increase in the number of CGE and POA-derived interneurons in upper cortical layers. Even after the CRc have already been eliminated at P25, impaired CRc density induces long lasting increase in the number of CGE and POA-derived interneurons.

CGE, Caudal Ganglionic Eminence; CRc, Cajal-Retzius cells; POA, Preoptic Area.

3.2. Cajal-Retzius cells regulate excitatory inputs in Layer 1

3.2.1 Do CRc have a general impact on branching and spine formation in L1?

Recent findings revealed that the CRc density modulates the branching and spine densities of the apical dendrites of upper layers pyramidal neurons (de Frutos et al., 2016; Article 1: Riva, Genescu, Habermacher et al., 2019). Less CRc during their lifetime leads to decreased branching of the upper layers pyramidal neurons, whereas impaired elimination of CRc has the opposite effect (de Frutos et al., 2016). Rescued electrically active CRc lead to a hyper arborization of apical dendrites of upper layers cortical neurons (Fig. 16) (Article 1: Riva, Genescu, Habermacher et al., 2019). The branching of apical dendrites is an important factor in modulating the integration of the information notably through top-down inputs (Karimi et al., 2020; Matthew E. Larkum, 2013). A similar effect is observed also on spines. A decreased CRc density is leading to a long term reduction in the number of spines on apical dendrites, whereas an impaired elimination of subsets of CRc is leading to an increase in the number of spines of apical dendrites of upper layers pyramidal neurons (Fig. 20) (de Frutos et al., 2016; Article 1: Riva, Genescu, Habermacher et al., 2019). Altogether, the apical branching and the spine density both are indicators of the excitatory inputs received by upper layers pyramidal neurons. Reliably, the impaired density of CRc induce a long-lasting decreased excitatory drive on upper layers pyramidal neurons, while partial rescue of CRc is leading to an increase in the excitatory drive on upper layers pyramidal neurons (de Frutos et al., 2016; Article 1: Riva, Genescu, Habermacher et al., 2019).

Do the CRc regulate specifically the excitatory entries on upper layers pyramidal neurons or do they generally promote synaptogenesis? To address these questions we used the *Thy1^{gfp/+}* line backcrossed with genetic model of impaired CRc density to label in a “Golgi-like” manner the L5 pyramidal neurons (Feng et al., 2000). In this context we could not assess the branching of the apical dendrites of deep cortical layers as the density of labelled L5 pyramidal neurons is too high to enable a morphological analysis of a single cell (Feng et al., 2000). We could however analyze the spine densities and found that indeed, impaired density

of CRc during their lifetime induces also a decrease in the spine densities in L1 of deep cortical layers (Article 2). As for the upper cortical layers, this effect is present only in the L1 for the apical dendrites, and not also for the basal dendrites in deeper cortical layers (de Frutos et al., 2016, Article 2), suggesting that the CRc could modulate locally the synaptogenesis.

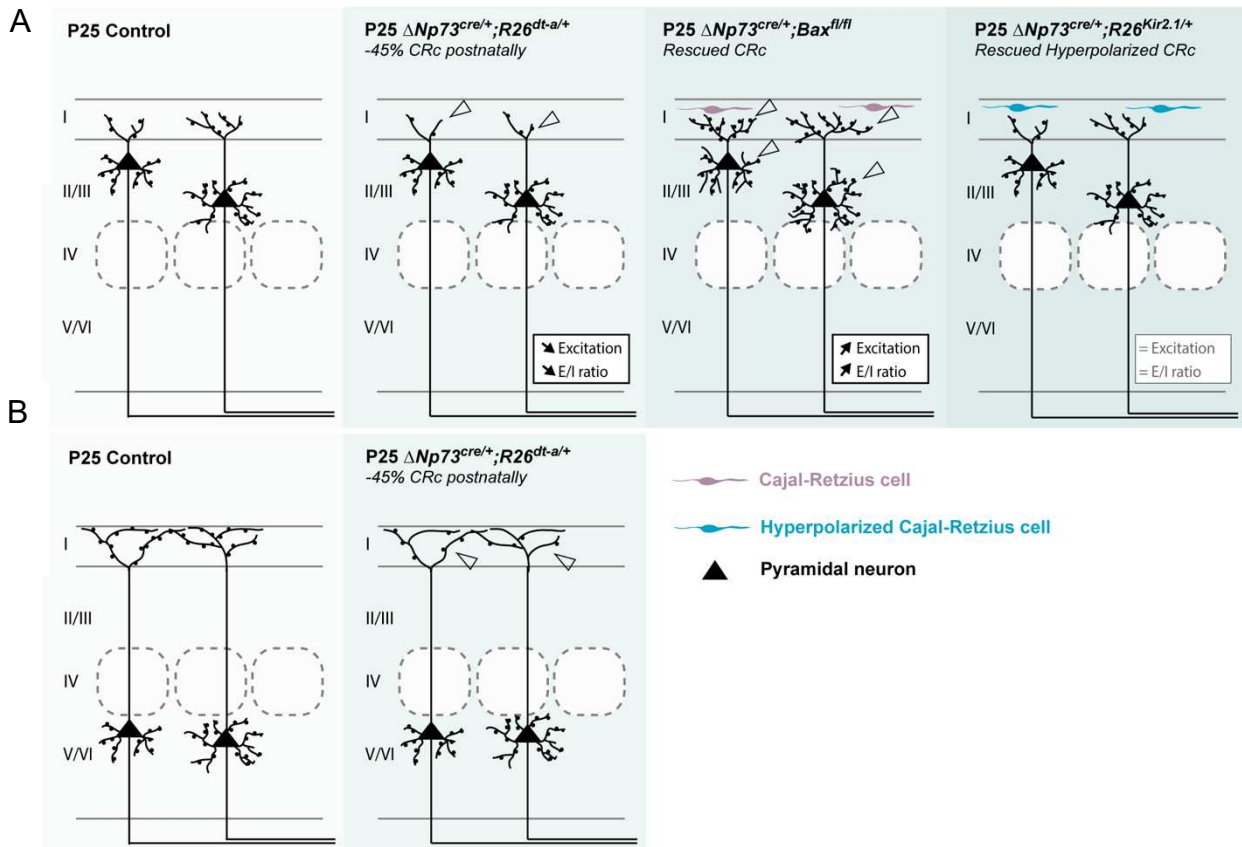


Figure 20 | Impact of Cajal-Retzius cell density on the apical dendrites of pyramidal neurons

(A) Impaired CRc density ($\Delta Np73^{cre/+}; R26^{dt-a/+}$) or impaired elimination of electrically-active CRc ($\Delta Np73^{cre/+}; Bax^{fl/fl}$) shape the morphology of apical dendrites and the number of spines (black arrows) and modulate of the Excitation/Inhibition (E/I) balance off upper layers cortical neurons. Note that hyperpolarization of CRc ($\Delta Np73^{cre/+}; R26^{Kir2.1/+}$) impairs their elimination but not the branching, spine density or E/I ratio of the upper layers pyramidal neurons.

(B) Impaired CRc density ($\Delta Np73^{cre/+}; R26^{dt-a/+}$) modulates the spine densities of apical dendrites of also deep layers cortical neurons.

CGE, Caudal Ganglionic Eminence; CRc, Cajal-Retzius cells; E/I, Excitation/Inhibition ratio; POA: Preoptic Area.

3.2.2 Through which mechanisms could Cajal-Retzius cells act on branching and spine development?

The roles of CRc in branching of apical dendrites of pyramidal neurons and on spines formation is becoming increasingly clear. However, through which mechanisms CRc modulate these parameters which are a readout for excitatory entries in L1, remains still elusive.

Even though the branching of apical dendrites and the spine formation on apical dendrites are both readouts for inputs arriving in L1, the two are actually quite different. While the apical branching is a process which takes place while CRc are still present in L1, the spine formation is a later process which can take place and be refined even after the CRc start being eliminated. Although it remains to be clarified, the mechanisms underlying the arborization and spine formation on apical dendrites could be different.

There are two main possible non-exclusive explanations for how CRc execute their roles in modulating branching and spine densities: they might act through activity dependent mechanisms and/or through molecular cues. In the $\Delta Np73^{cre/+}; R26^{Kir2.1}$ model when the CRc are constitutively hyperpolarized, there is no impact on the branching nor spine densities of upper layers pyramidal neurons, suggesting that the physiological roles of CRc in branching and spine formation is not mediated via the activity-dependent mechanisms in the CRc. However, in the $\Delta Np73^{cre/+}; Bax^{fl/fl}$ model, where CRc are partially rescued, there is an increase in the branching and spines on upper cortical layers (Article 1: Riva, Genescu, Habermacher et al., 2019). These effects, combined with the data in the Kir 2.1 model, suggests that the aberrant branches and spines are generated through activity dependent mechanisms. As hyperpolarization of CRc has no visible consequence for cortical circuits, this implies that physiological functions of CRc might not rely on their electrical activity but rather on signaling mechanisms that remain to be characterized (Article 1: Riva, Genescu, Habermacher et al., 2019).

One likely candidate would be RELN since this secreted factor is known to promote synaptogenesis and deletion of its C-terminal portion leads to a morphologically similar

phenotype as the ones observed in mutants with impaired CRc density: decreased thickness on L1 and stunted apical tufts in L1 (Ha, Tripathi, Mihalas, Hevner, & Beier, 2017; Okugawa et al., 2020; Sakai, Shoji, Kohno, Miyakawa, & Hattori, 2016). A direct way to assess the role of RELN specifically in CRc in the branching and spine formation on apical dendrites would be to use the cKO for *Reelin* specifically in CRc. RELN is one candidate, but of course, it is still an open question regarding additional molecules potentially involved in this process.

Another possibility is that impaired number of spines in L1 in models with impaired CRc density could be a consequence of impaired development of excitatory axons in L1, from late embryonic or early postnatal stages.

3.2.3. Are Cajal-Retzius cells modulating excitatory axonal inputs in neocortical layer1?

As a decreased density of CRc leads to a reduction of spine densities in L1 and an impaired elimination of CRc has the opposite effect of an increase of spine densities in L1, it is tempting to suppose that CRc density is modulating the excitatory entries in L1 (de Frutos et al., 2016; Article 1: Riva, Genescu, Habermacher et al., 2019). A fundamental question to be asked is what is the nature of these excitatory inputs shaped by CRc. Do CRc shape the formation of thalamic synapses, cortical synapses or both? We tried addressing these questions using combinations of immunostainings for thalamic (presynaptic VGlut2/postsynaptic Homer1) and cortical (presynaptic Vglut1/Postsynaptic Homer1) synapses. Unfortunately, due to the high density of synaptic puncta in L1, these experiments were inconclusive. A complementary approach that we are now exploiting is the use of viral injections of AAVSynaptophysinCherry in the higher order thalamic nucleus Po, M1 or S1 that allow selective labelling of the presynaptic inputs of either Po, M1 or S1 in L1. This would enable us to understand whether the CRc have a selective impact on specific inputs in L1.

Is the impairment in spine densities a consequence of an impairment of axonal targeting in L1 from early postnatal stages guided by CRc? Previous work (de Frutos et al.,

2016) together with our data show that in models with perturbed CRc density, there is a qualitative decrease in the forebrain axonal tracts (labelled with L1) and thalamic axonal terminals (labeled with VGlut2) in cortical layer 1 at P2 (Fig. 21) (de Frutos et al., 2016). Whether the CRc are guiding both cortical and thalamic axons in L1 still remains to be understood.

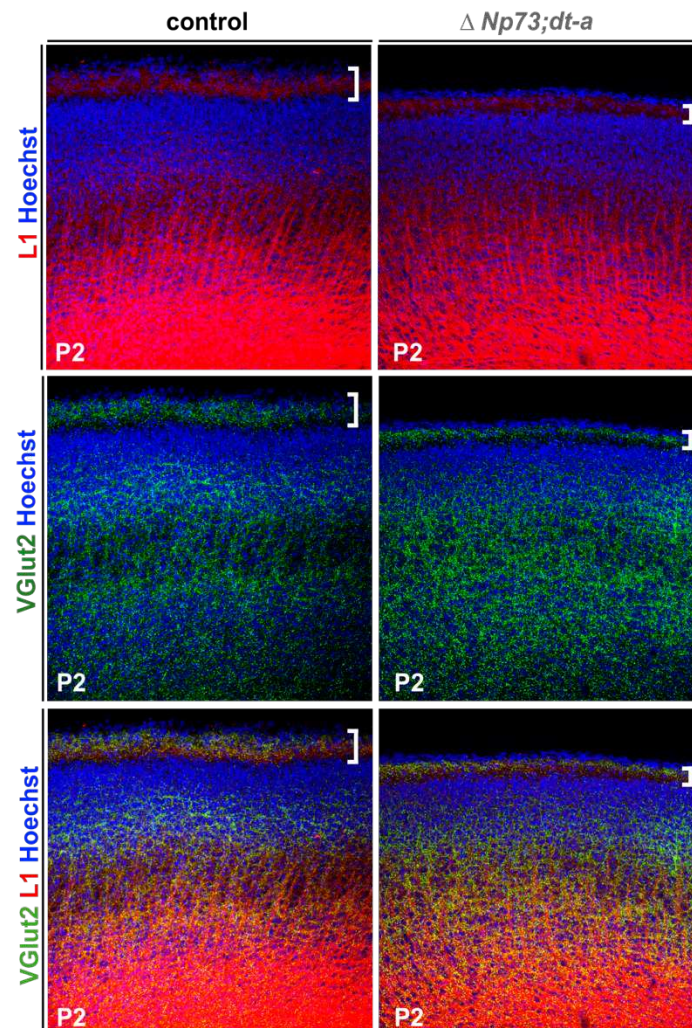


Figure 21 | Cajal-Retzius cell density impacts on axonal plexus in layer 1

Coronal sections of P2 somatosensory cortex of controls and $\Delta Np73^{cre/+};R26^{dt-a/+}$ labelled with general marker of axonal tracts (L1) and thalamic presynaptic terminals (VGlut2). In the $Np73^{cre/+};R26^{dt-a/+}$ model, since P2 the L1 is thinner. L1 and Vglut2 immunostainings show that the mild decrease in the number of CR cells led to a reduction of L1⁺ axons and Vglut2⁺ thalamic terminals in neocortical L1.

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More broadly, in other systems, the CRc were shown to have an important role for guiding axons. In the olfactory cortex, CRc are positioned near the LOT (CR-lot cells) (Dixit et al., 2014). Netrin 1 is a chemoattractant for migrating CR-lot cells towards their ventral position (Kawasaki, Ito, & Hirata, 2006), while Sema 3F and its receptor neuropilin 2 (Nrp-2) maintain the CR-lot cells on the telencephalic surface. The positioning of CR-lot cells occurs before the growth of LOT axons, for which they are a scaffold (Sato, Hirata, Ogawa, & Fujisawa, 1998). CRc have also been shown to be involved in the development of entorhinal projections in the hippocampus. Pioneer entorhinal axons form transient contacts with CRc in the hippocampus and the demise of hippocampal CRc impaired the growth of entorhinal projections, suggesting that indeed, the CRc are placeholders for entorhinal axon growth (Del Río et al., 1996; Förster et al., 1998; Squarzoni, Thion, & Garel, 2015; Supèr, Martínez, Del Río, & Soriano, 1998).

These effects could indeed be mediated by RELN but, in spite of an inverted laminar organization, the reeler mutants do not present major axonal pathfinding defects. However, while the entorhino-hippocampal axons reach their target, their axonal terminations appear to be thinner. Similar results are also observed in co-culture experiments in which CRc are ablated using 6-OHDA (Borrell et al., 1999; Deller et al., 1999; M. Frotscher & Heimrich, 1993; Rio et al., 1997). Therefore, these results suggest a potential role of RELN in axonal development and support that CRc are guideposts for axonal development. Deciphering the molecular cues involved in axonal pathfinding in the olfactory cortex, hippocampus and neocortex is indeed a tremendous open question.

4. Cajal-Retzius cells and evolution

CRc together with L1 itself present a high adaptability in evolution. Subpopulations of CRc are persistent in human adult brain and the L1 in humans presents an increased morphological complexity. The increase in CRc numbers and RELN levels in L1 has been proposed to be instrumental in the progressive enlargement of the pallium in evolution, with the highest RELN in the human neocortex (Gundela Meyer, 2010; Fadel Tissir & Goffinet, 2003). Miguel Marín-Padilla also suggested that, as species evolve, a new pyramidal cell layers is incorporated just beneath the L1, which might serve to discriminate a species from its preceding ones. This theory implies indeed an increased complexity both structural and physiological of cortical layer 1 in evolution, and suggests indeed L1 is a backbone for the increasing complexity of neocortical circuits which takes place during the phlogenetic expansion (Marín-Padilla, 1998).

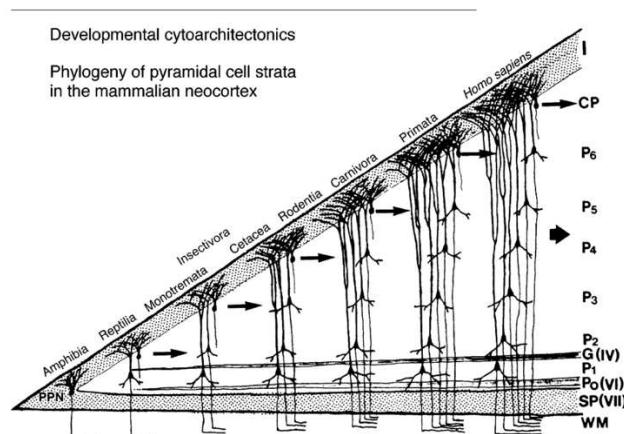


Figure 22 | The theory of neocortical cytoarchitectonics

Schematic representation of the evolution of cortical layers composition supported by the theory of neocortical cytoarchitectonics suggested by Miguel Marín-Padilla.

From Marín-Padilla, 1998

Consistently, in our work we also show that the CRc density and L1 complexity in terms of dendritic arborizations and excitatory entries, are tightly linked. Our findings reveal that CRc density is actually subtly adjusting during the brain growth that is taking place in development and similar mechanisms might also be involved in in evolution to adjust and maintain an increased network complexity.

GENERAL CONCLUSION

In conclusion, this work could participate to a better understanding of the development of layer 1, with a particular highlight on the roles of transient Cajal-Retzius cells in the formation of neocortical wiring. Altogether, these projects show the elegant way electrical activity in CR cells regulate different aspects in their lifetime. First, a remigration of CR cells which maintains a proper density of CR cells and second, an elimination of these cells. Maintaining the density of Cajal-Retzius cells during their lifetime is remarkably important, as perturbations in Cajal-Retzius cell density induce wiring defects in cortical layer 1. Regulating the phase of death is also essential because when Cajal-Retzius cells survive, they induce impairments in the functional connectivity. Therefore the Cajal-Retzius cells are a nice example in development showing that the elimination of a certain cell type is required for a normal brain wiring and function.

Taken together, this work participates at deciphering how transient neuronal populations regulate the wiring of an essential but understudied layer of the neocortex, as well as shed light on how early neuronal activity contributes to this process. All this is instrumental for understanding how electrical activity sustains neocortex construction in physiological conditions, and how this could contribute to miswirings leading to different neurodevelopmental disorders.

ANNEXES

RESUMÉ DE THÈSE

Le néocortex, qui contrôle la perception sensorielle, les comportements moteurs et la cognition, repose sur des circuits complexes contenant à la fois des neurones excitateurs et inhibiteurs. La balance excitation/inhibition (E/I) est essentielle pour le bon fonctionnement des circuits et son déséquilibre est lié à l'étiologie de troubles neuro-développementaux, notamment les troubles du spectre autistique ou la schizophrénie. Une des principales entrées des circuits corticaux est la couche superficielle 1 (L1), qui contient les dendrites apicales des neurones pyramidaux, recevant principalement des entrées excitatrices, ainsi que des interneurons qui régulent l'activité corticale via des circuits spécifiques. En dépit de son rôle de plus en plus reconnu, nous avons encore une compréhension limitée de la façon dont la L1 se met en place au cours du développement. Des études récentes ont mis en évidence les rôles clés d'une population transitoire de neurones précoces, appelés cellules de Cajal-Retzius (CRc) et qui se trouvent dans la L1. Avant leur élimination complète au cours de la deuxième semaine postnatale, il a été démontré que la densité des CRc façonne la morphologie des dendrites apicales des neurones pyramidaux de la couche supérieure, ainsi que leurs entrées excitatrices dans la L1. Cependant, il reste à élucider les mécanismes et la pertinence de l'élimination des CRc et les rôles exacts de cette population neuronale transitoire dans le développement de la L1.

Comme la densité de CRc est importante dans le câblage des circuits de la L1, nous sommes d'abord demandé si leur élimination est également essentielle pour la bonne organisation du cerveau et quels sont les mécanismes régulant cette disparition (collaboration avec A. Pierani et M.C. Angulo). En utilisant plusieurs analyses génétiques *in vivo*, nous avons découvert que des sous-ensembles de CRc meurent de manière dépendante de l'activité, puisque l'hyperpolarisation des CRc conduit à leur survie partielle jusqu'à l'âge adulte. Alors

qu'une réduction précoce de la densité des CRc induisait une réduction de la ramification des dendrites apicales et de la densité des épines dans la L1, correspondant à une diminution de la balance E/I, nous avons constaté que la survie partielle des CRc électriquement actives a un effet inverse : les CRc survivantes s'intègrent de manière aberrante dans les circuits, ce qui entraîne une augmentation de la ramification des dendrites apicales, du nombre d'épines et de la balance E/I dans les couches corticales supérieures. Il est important de noter que nous avons montré que les CRc survivantes induisent ces défauts de façon dépendante de leur activité, ce qui suggère qu'elles sont des acteurs actifs dans les circuits corticaux. Dans l'ensemble, ces travaux révèlent que l'élimination des CRc, en partie régulée par un processus dépendant de l'activité, est nécessaire à une bonne mise en place des circuits neuronaux.

La densité de CRc est donc un paramètre qui doit être étroitement contrôlé au début de la vie postnatale. Comme cela a été suggéré précédemment dans le laboratoire, nous avons confirmé que la densité de CRc est notamment maintenue par une redistribution à partir d'un point d'accumulation, situé près du tract olfactif latéral. Après leur génération, des sous-populations de CRc s'accumulent dans ce réservoir à partir duquel elles migrent au milieu de l'embryogenèse. Nous avons confirmé que cette redistribution des CRc est dépendante de l'activité (notamment par la signalisation des récepteurs NMDA) car les déficiences du GluN1 spécifiquement dans les CRc entraînent une diminution de la densité des CRc couvrant le cortex et une augmentation de la densité des CRc près du tract olfactif latéral. Par ce mécanisme, la densité de CRc est régulée pendant la croissance du cerveau au cours du développement. Il est important de noter qu'en utilisant les mêmes modèles génétiques, nous avons constaté que l'élimination des CRc, même si elle est dépendante de l'activité, n'est pas médiée par la signalisation du GluN1 dans la CRc. Cela suggère que les deux mécanismes qui maintiennent la densité de CRc sont dépendants de l'activité, mais qu'ils sont régis par des mécanismes très différents.

Ainsi, la densité de CRc couvrant le cortex est maintenue par un processus de redistribution précoce et d'élimination postnatale, et ces deux processus sont modulés par

l'activité neuronale. D'autre part, il a déjà été démontré que l'activité sensorielle précoce joue un rôle clé dans la mise en forme et le raffinement postnatals des circuits corticaux. En effet, nous avons montré que l'altération de l'activité sensorielle précoce dans le cortex somatosensoriel induit une diminution de l'épaisseur de la L1 et une altération de la densité des interneurons dans les couches corticales supérieures, phénotype très similaire aux modèles dans lesquels la densité de CRc est diminuée. Par conséquent, nous avons poursuivi en abordant deux questions majeures : i) l'activité sensorielle émergente dans le cortex cérébral régule-t-elle la densité des CRc, participant ainsi au câblage de la L1 ; ii) quelles sont plus largement les conséquences à long terme d'une altération de la densité des CRc postnatale et d'une altération de l'activité sensorielle précoce sur le câblage cortical. De manière surprenante, nous avons constaté que la densité des CRc n'est pas affectée par une activité somatosensorielle précoce altérée, grâce à des approches in vivo complémentaires : l'épilation des vibrisses, la lésion du nerf infra-orbitaire et la réduction de l'activité thalamique spontanée.

En ce qui concerne les conséquences à long terme sur le câblage cortical de l'altération de la densité des CRc ou de l'activité sensorielle, nous avons constaté que : une diminution de la densité des CRc au cours de leur vie déclenche une augmentation persistante et durable dans une sous-population spécifique d'interneurones de la L1, notamment les cellules « Neurogliaform » et les cellules bipolaires. Ces résultats concordent avec les résultats précédents montrant qu'une diminution des CRc entraîne une légère augmentation des entrées inhibitrices dans les couches corticales supérieures. L'altération de l'activité sensorielle a également un impact sur les interneurons de la L1 en induisant une augmentation d'une sous-population spécifique, et a un impact plus fort sur le nombre d'interneurones dans les couches corticales profondes.

En outre, alors qu'il a déjà été démontré que la densité des CRc a des conséquences durables sur la morphologie dendritique et les densités des épines des dendrites apicales dans les couches corticales supérieures, nous avons constaté que les CRc ont aussi une action

similaire sur les projections apicales des neurones corticaux profonds. La ramification des dendrites apicales et les densités d'épines sont toutes les deux des indicateurs des entrées excitatrices. Par conséquent, nos résultats montrent que les CRc contrôlent de manière non spécifique le développement des ramifications apicales des neurones pyramidaux et la densité de leurs épines dans la L1, en régulant les entrées excitatrices dans cette couche. En revanche, l'activité sensorielle précoce ne module pas la densité d'épines dans la L1, mais a un impact à long terme sur la densité d'épines des dendrites basales dans les couches corticales profondes. Dans l'ensemble, ces résultats mettent en évidence les rôles clés des CRc dans la mise en place des réseaux neuronaux même longtemps après leur élimination, et ce parallèlement à l'activité sensorielle.

En conclusion, dans ce travail, nous avons montré que la densité des CRc est un paramètre important à réguler lors de la croissance du cerveau au cours du développement, car des déficiences dans la densité des CRc entraînent des défauts de câblage. Cette densité est déterminée par au moins deux mécanismes : une redistribution des sous-populations de CRc à partir d'un réservoir aux stades embryonnaires et une élimination postnatale des CRc à un moment précis. La redistribution et l'élimination des sous-populations de CRc dépendent toutes deux de l'activité de ces cellules. Les altérations de la densité des CRc au cours de leur vie ainsi que les perturbations de leur élimination ont des conséquences durables sur les réseaux corticaux. Une diminution de la densité des CRc entraîne une augmentation de sous-populations spécifiques d'interneurones dans la L1 et une diminution des entrées excitatrices dans cette couche, tandis qu'une mauvaise élimination des CRc électriquement actives induit une augmentation des entrées excitatrices sur les neurones pyramidaux des couches superficielles.

Dans l'ensemble, mes travaux de doctorat permettent de mieux comprendre les rôles d'une population neuronale transitoire précoce dans la mise en place des circuits corticaux/neuronaux en conditions normales et son rôle potentiel dans l'étiologie des troubles neuro-développementaux.

REFERENCES

-A-

- Ábrahám, H., Tóth, Z., Bari, F., Domoki, F., & Seress, L. (2005). Novel calretinin and reelin expressing neuronal population includes Cajal-Retzius-type cells in the neocortex of adult pigs. *Neuroscience*, *136*(1), 217–230. <https://doi.org/10.1016/j.neuroscience.2005.07.039>
- Abs, E., Poorthuis, R. B., Apelblat, D., Muhammad, K., Pardi, M. B., Enke, L., ... Letzkus, J. J. (2018). Learning-Related Plasticity in Dendrite-Targeting Layer 1 Interneurons. *Neuron*, *100*(3), 684–699.e6. <https://doi.org/10.1016/j.neuron.2018.09.001>
- Achilles, K., Okabe, A., Ikeda, M., Shimizu-Okabe, C., Yamada, J., Fukuda, A., ... Kilb, W. (2007). Kinetic properties of Cl⁻ uptake mediated by Na⁺-dependent K⁺-2Cl⁻ cotransport in immature rat neocortical neurons. *Journal of Neuroscience*, *27*(32), 8616–8627. <https://doi.org/10.1523/JNEUROSCI.5041-06.2007>
- Aguiló, A., Schwartz, T. H., Kumar, V. S., Peterlin, Z. A., Tsiola, A., Soriano, E., & Yuste, R. (1999). Involvement of cajal-retzius neurons in spontaneous correlated activity of embryonic and postnatal layer 1 from wild-type and reeler mice. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *19*(24), 10856–10868. [https://doi.org/10.1016/S0896-6273\(00\)80993-9](https://doi.org/10.1016/S0896-6273(00)80993-9)
- Alcamo, E. A., Chirivella, L., Dautzenberg, M., Dobрева, G., Fariñas, I., Grosschedl, R., & McConnell, S. K. (2008). Satb2 Regulates Callosal Projection Neuron Identity in the Developing Cerebral Cortex. *Neuron*, *57*(3), 364–377. <https://doi.org/10.1016/j.neuron.2007.12.012>
- Allene, C., Cattani, A., Ackman, J. B., Bonifazi, P., Aniksztejn, L., Ben-Ari, Y., & Cossart, R. (2008). Sequential Generation of Two Distinct Synapse-Driven Network Patterns in Developing Neocortex. *Journal of Neuroscience*, *28*(48), 12851–12863. <https://doi.org/10.1523/JNEUROSCI.3733-08.2008>
- Allene, Camille, & Cossart, R. (2010). Early NMDA receptor-driven waves of activity in the developing neocortex: physiological or pathological network oscillations? *The Journal of Physiology*, *588*(1), 83–91. <https://doi.org/10.1113/jphysiol.2009.178798>
- Anastasiades, P. G., Collins, D. P., & Carter, A. G. (2020). Mediodorsal and ventromedial thalamus engage distinct L1 circuits in the prefrontal cortex. *BioRxiv*, 007308, 2020.01.08.898817. <https://doi.org/10.1101/2020.01.08.898817>
- Anderson, S. A., Eisenstat, D. D., Shi, L., & Rubenstein, J. L. R. (1997). Interneuron migration from basal forebrain to neocortex: Dependence on Dlx genes. *Science*, *278*(5337), 474–476. <https://doi.org/10.1126/science.278.5337.474>
- Anstötz, M., Cosgrove, K. E., Hack, I., Mugnaini, E., Maccaferri, G., & 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 Structure and Function*. <https://doi.org/10.1007/s00429-013-0627-2>
- Anstötz, M., Huang, H., Marchionni, I., Haumann, I., MacCaferri, G., & Lübke, J. H. R. (2016). Developmental Profile, Morphology, and Synaptic Connectivity of Cajal-Retzius Cells in the Postnatal Mouse Hippocampus. *Cerebral Cortex*, *26*(2), 855–872. <https://doi.org/10.1093/cercor/bhv271>
- Anstötz, M., Quattrocchio, G., & Maccaferri, G. (2018). Cajal-Retzius cells and GABAergic interneurons of the developing hippocampus: Close electrophysiological encounters of the third kind. *Brain Research*, *1697*(June), 124–133. <https://doi.org/10.1016/j.brainres.2018.07.028>
- Antón-bolaños, N., Sempere-ferrández, A., Guillamón-Vivancos, T., Martini, F. J., Pérez-Saiz, L., Gezelius, H., ... López-bendito, G. (2019). Functional Cortical Maps in Mice. *Science*, *990*(June), 987–990. <https://doi.org/10.1126/science.aav7617>
- Arakawa, H., Suzuki, A., Zhao, S., Tsytsarev, V., Lo, F. S., Hayashi, Y., ... Erzurumlu, R. S. (2014). Thalamic NMDA receptor function is necessary for patterning of the thalamocortical somatosensory map and for sensorimotor behaviors. *Journal of Neuroscience*, *34*(36), 12001–12014. <https://doi.org/10.1523/JNEUROSCI.1663-14.2014>

References

- Arlotta, P., Molyneaux, B. J., Chen, J., Inoue, J., Kominami, R., & MacKlis, J. D. (2005). Neuronal subtype-specific genes that control corticospinal motor neuron development in vivo. *Neuron*, *45*(2), 207–221. <https://doi.org/10.1016/j.neuron.2004.12.036>
- Armentano, M., Chou, S. J., Srubek Tomassy, G., Leingärtner, A., O'Leary, D. D. M., & Studer, M. (2007). COUP-TFI regulates the balance of cortical patterning between frontal/motor and sensory areas. *Nature Neuroscience*, *10*(10), 1277–1286. <https://doi.org/10.1038/nn1958>
- Armstrong, C., Krook-Magnuson, E., & Soltesz, I. (2012). Neurogliaform and Ivy cells: A major family of nNOS expressing GABAergic neurons. *Frontiers in Neural Circuits*, *6*(MAY2012), 1–10. <https://doi.org/10.3389/fncir.2012.00023>
- Audette, N. J., Bernhard, S. M., Ray, A., Stewart, L. T., & Barth, A. L. (2019). Rapid Plasticity of Higher-Order Thalamocortical Inputs during Sensory Learning. *Neuron*, *103*(2), 277–291.e4. <https://doi.org/10.1016/j.neuron.2019.04.037>
- Audette, N. J., Urban-Ciecko, J., Matsushita, M., & Barth, A. L. (2017). POM Thalamocortical Input Drives Layer-Specific Microcircuits in Somatosensory Cortex. *Cerebral Cortex*, (March 2017), 1–17. <https://doi.org/10.1093/cercor/bhx044>

-B-

- Barber, M., Arai, Y., Morishita, Y., Vigier, L., Causeret, F., Borello, U., ... Pierani, A. (2015). Migration speed of Cajal-Retzius cells modulated by vesicular trafficking controls the size of higher-order cortical areas. *Current Biology*, *25*(19), 2466–2478. <https://doi.org/10.1016/j.cub.2015.08.028>
- Barber, M., & Pierani, A. (2016). Tangential migration of glutamatergic neurons and cortical patterning during development: Lessons from Cajal-Retzius cells. *Developmental Neurobiology*, *76*(8), 847–881. <https://doi.org/10.1002/dneu.22363>
- Bartolini, G. (2016). Molecular mechanisms regulating the intracortical migration of interneurons.
- Bartolini, G., Ciceri, G., & Marín, O. (2013). Integration of GABAergic Interneurons into Cortical Cell Assemblies: Lessons from Embryos and Adults. *Neuron*, *79*(5), 849–864. <https://doi.org/10.1016/j.neuron.2013.08.014>
- Bartolini, G., Sánchez-Alcañiz, J. A., Osório, C., Valiente, M., García-Frigola, C., & Marín, O. (2017). Neuregulin 3 Mediates Cortical Plate Invasion and Laminar Allocation of GABAergic Interneurons. *Cell Reports*, *18*(5), 1157–1170. <https://doi.org/10.1016/j.celrep.2016.12.089>
- Batista-Brito, R., & Fishell, G. (2009). Chapter 3 The Developmental Integration of Cortical Interneurons into a Functional Network. *Current Topics in Developmental Biology*, *87*(09), 81–118. [https://doi.org/10.1016/S0070-2153\(09\)01203-4](https://doi.org/10.1016/S0070-2153(09)01203-4)
- Behar, T. N., Scott, C. A., Greene, C. L., Wen, X., Smith, S. V., Maric, D., ... Barker, J. L. (1999). Glutamate acting at NMDA receptors stimulates embryonic cortical neuronal migration. *Journal of Neuroscience*, *19*(11), 4449–4461. <https://doi.org/10.1523/jneurosci.19-11-04449.1999>
- Ben-Ari, Y. (2002). Excitatory actions of GABA during development: The nature of the nurture. *Nature Reviews Neuroscience*, *3*(9), 728–739. <https://doi.org/10.1038/nrn920>
- Bernier, B., Bar, I., Pieau, C., De Rouvroit, C. L., Goffinet, A. M., & Goffinet, A. M. (1999). Reelin mRNA expression during embryonic brain development in the turtle *Emys orbicularis*. *Journal of Comparative Neurology*, *413*(3), 463–479. [https://doi.org/10.1002/\(SICI\)1096-9861\(19991025\)413:3<463::AID-CNE8>3.0.CO;2-F](https://doi.org/10.1002/(SICI)1096-9861(19991025)413:3<463::AID-CNE8>3.0.CO;2-F)
- Bernier, Béatrice, Bar, I., D'Arcangelo, G., Curran, T., & Goffinet, A. M. (2000). Reelin mRNA expression during embryonic brain development in the chick. *Journal of Comparative Neurology*, *422*(3), 448–463. [https://doi.org/10.1002/1096-9861\(20000703\)422:3<448::AID-CNE10>3.0.CO;2-4](https://doi.org/10.1002/1096-9861(20000703)422:3<448::AID-CNE10>3.0.CO;2-4)
- Berry, K. P., & Nedivi, E. (2017). Spine Dynamics: Are They All the Same? *Neuron*, *96*(1), 43–55. <https://doi.org/10.1016/j.neuron.2017.08.008>
- Bielle, F., Griveau, A., Narboux-Nême, N., Vigneau, S., Sigrist, M., Arber, S., ... Pierani, A.

References

- (2005). Multiple origins of Cajal-Retzius cells at the borders of the developing pallium. *Nature Neuroscience*, 8(8), 1002–1012. <https://doi.org/10.1038/nn1511>
- Bishop, K. M., Goudreau, G., & O'Leary, D. D. M. (2000). Regulation of area identity in the mammalian neocortex by *Emx2* and *Pax6*. *Science*, 288(5464), 344–349. <https://doi.org/10.1126/science.288.5464.344>
- Björklund, A., & Dunnett, S. B. (2007). Dopamine neuron systems in the brain: an update. *Trends in Neurosciences*, 30(5), 194–202. <https://doi.org/10.1016/j.tins.2007.03.006>
- Blanquie, O., Kilb, W., Sinning, A., & Luhmann, H. J. (2017). Homeostatic interplay between electrical activity and neuronal apoptosis in the developing neocortex. *Neuroscience*, 358, 190–200. <https://doi.org/10.1016/j.neuroscience.2017.06.030>
- Blanquie, O., Liebmann, L., Hübner, C. A., Luhmann, H. J., Sinning, A., (2017). NKCC1-Mediated GABAergic Signaling Promotes Postnatal Cell Death in Neocortical Cajal-Retzius Cells, *Cerebral Cortex* 1644–1659. <https://doi.org/10.1093/CERCOR>
- Blanquie, O., Yang, J. W., Kilb, W., Sharopov, S., Sinning, A., & Luhmann, H. J. (2017). Electrical activity controls area-specific expression of neuronal apoptosis in the mouse developing cerebral cortex. *ELife*, 6, 1–21. <https://doi.org/10.7554/eLife.27696>
- Bonifazi, P., Goldin, M., Picardo, M. A., Jorquera, I., Cattani, A., Bianconi, G., ... Cossart, R. (2009). GABAergic Hub Neurons Orchestrate Synchrony in Developing Hippocampal Networks. *Science*, 326(5958), 1419–1424. <https://doi.org/10.1126/science.1175509>
- Borrell, V., Del Río, J. A., Alcántara, S., Derer, M., Martínez, A., D'Arcangelo, G., ... Soriano, E. (1999). Reelin regulates the development and synaptogenesis of the layer-specific entorhino-hippocampal connections. *Journal of Neuroscience*, 19(4), 1345–1358. <https://doi.org/10.1523/jneurosci.19-04-01345.1999>
- Borrell, V., & Götz, M. (2014). Role of radial glial cells in cerebral cortex folding. *Current Opinion in Neurobiology*, 27, 39–46. <https://doi.org/10.1016/j.conb.2014.02.007>
- Borrell, V., & Marín, O. (2006). Meninges control tangential migration of hem-derived Cajal-Retzius cells via CXCL12/CXCR4 signaling. *Nature Neuroscience*, 9(10), 1284–1293. <https://doi.org/10.1038/nn1764>
- Bortone, D., & Polleux, F. (2009). KCC2 Expression Promotes the Termination of Cortical Interneuron Migration in a Voltage-Sensitive Calcium-Dependent Manner. *Neuron*, 62(1), 53–71. <https://doi.org/10.1016/j.neuron.2009.01.034>
- Bosch, C., Masachs, N., Exposito-Alonso, D., Martínez, A., Teixeira, C. M., Fernaud, I., ... Soriano, E. (2016). Reelin Regulates the Maturation of Dendritic Spines, Synaptogenesis and Glial Ensheathment of Newborn Granule Cells. *Cerebral Cortex*, 26(11), 4282–4298. <https://doi.org/10.1093/cercor/bhw216>
- Bribián, A., Nocentini, S., Llorens, F., Gil, V., Mire, E., Reginensi, D., ... Del Río, J. A. (2014). Sema3E/PlexinD1 regulates the migration of hem-derived Cajal-Retzius cells in developing cerebral cortex. *Nature Communications*, 5(December). <https://doi.org/10.1038/ncomms5265>
- Bruguier, H., Suarez, R., Manger, P., Hoerder-Suabedissen, A., Shelton, A. M., Oliver, D. K., ... Molnár, Z. (2020). In search of common developmental and evolutionary origin of the claustrum and subplate. *Journal of Comparative Neurology*, (April), 1–22. <https://doi.org/10.1002/cne.24922>
- Butz, A. M. (2017). 乳鼠心肌提取 HHS Public Access. *Physiology & Behavior*, 176(12), 139–148. <https://doi.org/10.1016/j.physbeh.2017.03.040>

-C-

- Cai, Y., Zhang, Q., Wang, C., Zhang, Y., Ma, T., Zhou, X., ... Yang, Z. (2013). Nuclear receptor COUP-TFII-expressing neocortical interneurons are derived from the medial and lateral/caudal ganglionic eminence and define specific subsets of mature interneurons. *Journal of Comparative Neurology*, 521(2), 479–497. <https://doi.org/10.1002/cne.23186>
- Callaway, E. M., & Borrell, V. (2011). Developmental sculpting of dendritic morphology of layer 4 neurons in visual cortex: Influence of retinal input. *Journal of Neuroscience*, 31(20), 7456–7470. <https://doi.org/10.1523/JNEUROSCI.5222-10.2011>

References

- Campbell, K. (2003). Dorsal-ventral patterning in the mammalian telencephalon. *Current Opinion in Neurobiology*, 13(1), 50–56. [https://doi.org/10.1016/S0959-4388\(03\)00009-6](https://doi.org/10.1016/S0959-4388(03)00009-6)
- Caputi, A., Melzer, S., Michael, M., & Monyer, H. (2013). The long and short of GABAergic neurons. *Current Opinion in Neurobiology*, 23(2), 179–186. <https://doi.org/10.1016/j.conb.2013.01.021>
- Caputi, A., Rozov, A., Blatow, M., & Monyer, H. (2009). Two calretinin-positive gabaergic cell types in layer 2/3 of the mouse neocortex provide different forms of inhibition. *Cerebral Cortex*, 19(6), 1345–1359. <https://doi.org/10.1093/cercor/bhn175>
- Cauler, L. (1995). Layer I of primary sensory neocortex: where top-down converges upon bottom-up. *Behavioural Brain Research*, 71(1–2), 163–170. [https://doi.org/10.1016/0166-4328\(95\)00032-1](https://doi.org/10.1016/0166-4328(95)00032-1)
- Causeret, F., Coppola, E., & Pierani, A. (2018). Cortical developmental death: selected to survive or fated to die. *Current Opinion in Neurobiology*. <https://doi.org/10.1016/j.conb.2018.04.022>
- Chai, X., Fan, L., Shao, H., Lu, X., Zhang, W., Li, J., ... Zhao, S. (2015). Reelin induces branching of neurons and radial glial cells during corticogenesis. *Cerebral Cortex*, 25(10), 3640–3653. <https://doi.org/10.1093/cercor/bhu216>
- Chameau, P., Inta, D., Vitalis, T., Monyer, H., Wadman, W. J., & Van Hooft, J. A. (2009). The N-terminal region of reelin regulates postnatal dendritic maturation of cortical pyramidal neurons. *Proceedings of the National Academy of Sciences of the United States of America*, 106(17), 7227–7232. <https://doi.org/10.1073/pnas.0810764106>
- Chameau, P., & Van Hooft, J. A. (2006). Serotonin 5-HT₃ receptors in the central nervous system. *Cell and Tissue Research*, 326(2), 573–581. <https://doi.org/10.1007/s00441-006-0255-8>
- Chapleau, C. A., Larimore, J. L., Theibert, A., & Pozzo-Miller, L. (2009). Modulation of dendritic spine development and plasticity by BDNF and vesicular trafficking: Fundamental roles in neurodevelopmental disorders associated with mental retardation and autism. *Journal of Neurodevelopmental Disorders*, 1(3), 185–196. <https://doi.org/10.1007/s11689-009-9027-6>
- Charrier, C., Joshi, K., Coutinho-Budd, J., Kim, J. E., Lambert, N., De Marchena, J., ... Polleux, F. (2012). Inhibition of SRGAP2 function by its human-specific paralogs induces neoteny during spine maturation. *Cell*, 149(4), 923–935. <https://doi.org/10.1016/j.cell.2012.03.034>
- Che, A., Babij, R., Iannone, A. F., Fetcho, R. N., Ferrer, M., Liston, C., ... De Marco García, N. V. (2018). Layer I Interneurons Sharpen Sensory Maps during Neonatal Development. *Neuron*, 99(1), 98–116.e7. <https://doi.org/10.1016/j.neuron.2018.06.002>
- Chen, J., & Kriegstein, A. R. (2015). A GABAergic projection from the zona incerta to cortex promotes cortical neuron development. *Science*, 350(6260), 554–558. <https://doi.org/10.1126/science.aac6472>
- Chowdhury. (2010). Fate of Cajal-Retzius neurons in the postnatal mouse neocortex. *Frontiers in Neuroanatomy*, 4(March), 1–8. <https://doi.org/10.3389/neuro.05.010.2010>
- Clavreul, S., Abdeladim, L., Hernández-Garzón, E., Niculescu, D., Durand, J., Ieng, S. H., ... Loulier, K. (2019). Cortical astrocytes develop in a plastic manner at both clonal and cellular levels. *Nature Communications*, 10(1), 1–14. <https://doi.org/10.1038/s41467-019-12791-5>
- Cocas, Laura A, Fernandez, G., Barch, M., Doll, J., Diaz, I. Z., & Pleasure, X. J. (2016). Cell Type-Specific Circuit Mapping Reveals the Presynaptic Connectivity of Developing Cortical Circuits, 36(11), 3378–3390. <https://doi.org/10.1523/JNEUROSCI.0375-15.2016>
- Cooper, J. A. (2008). A mechanism for inside-out lamination in the neocortex. *Trends in Neurosciences*, 31(3), 113–119. <https://doi.org/10.1016/j.tins.2007.12.003>
- Cosgrove, K. E., & MacCaferrri, G. (2012). MGlu1 α -dependent recruitment of excitatory GABAergic input to neocortical Cajal-Retzius cells. *Neuropharmacology*, 63(3), 486–493. <https://doi.org/10.1016/j.neuropharm.2012.04.025>
- Cossart, R. (2011). The maturation of cortical interneuron diversity: How multiple developmental journeys shape the emergence of proper network function. *Current*

References

- Opinion in Neurobiology*, 21(1), 160–168. <https://doi.org/10.1016/j.conb.2010.10.003>
- Cruikshank, S. J., Ahmed, O. J., Stevens, T. R., Patrick, S. L., Gonzalez, A. N., Elmaleh, M., & Connors, B. W. (2012). Thalamic Control of Layer 1 Circuits in Prefrontal Cortex. *Journal of Neuroscience*, 32(49), 17813–17823. <https://doi.org/10.1523/JNEUROSCI.3231-12.2012>
- Cuchillo-Ibáñez, I., Andreo-Lillo, P., Pastor-Ferrándiz, L., Carratalá-Marco, F., & Sáez-Valero, J. (2020). Elevated Plasma Reelin Levels in Children With Autism. *Frontiers in Psychiatry*, 11(March), 1–6. <https://doi.org/10.3389/fpsy.2020.00242>

-D-

- D’Arcangelo, G., Lossi, L., & Merighi, A. (2017). Editorial: Reelin-related neurological disorders and animal models. *Frontiers in Cellular Neuroscience*, 10(January), 2016–2017. <https://doi.org/10.3389/fncel.2016.00299>
- D’Arcangelo, G., Miao, G. G., Chen, S. C., Scares, H. D., Morgan, J. I., & Curran, T. (1995). A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. *Nature*, 374(6524), 719–723. <https://doi.org/10.1038/374719a0>
- Dammerman, R. S., Flint, A. C., Noctor, S., & Kriegstein, A. R. (2000). An excitatory GABAergic plexus in developing neocortical layer 1. *Journal of Neurophysiology*, 84(1), 428–434. <https://doi.org/10.1152/jn.2000.84.1.428>
- Datwani*, A., Iwasato†, T., Itoharaf, S., & Erzurumlu, R. S. (2013). NMDA Receptor-Dependent Pattern Transfer from Afferents to Postsynaptic Cells and Dendritic Differentiation in the Barrel Cortex Akash. *Bone*, 23(1), 1–7. <https://doi.org/10.1038/jid.2014.371>
- de Frutos, C. A., Bouvier, G., Arai, Y., Thion, M. S., Lokmane, L., Keita, M., ... Garel, S. (2016). Reallocation of Olfactory Cajal-Retzius Cells Shapes Neocortex Architecture. *Neuron*. <https://doi.org/10.1016/j.neuron.2016.09.020>
- De Marco García, N. V., Priya, R., Tuncdemir, S. N., Fishell, G., & Karayannis, T. (2015). Sensory inputs control the integration of neurogliaform interneurons into cortical circuits. *Nature Neuroscience*, 18(3), 393–403. <https://doi.org/10.1038/nn.3946>
- De, N. V., García, M., Karayannis, T., & Fishell, G. (2011). Neuronal activity is required for the development of specific cortical interneuron subtypes. *Nature*, 472. <https://doi.org/10.1038/nature09865>
- Dehorter, N., Ciceri, G., Bartolini, G., Lim, L., Del Pino, I., & Marín, O. (2015). Tuning of fast-spiking interneuron properties by an activity-dependent transcriptional switch. *Science*, 349(6253), 1216–1220. <https://doi.org/10.1126/science.aab3415>
- Del Río, J. A., Heimrich, B., Supèr, H., Borrell, V., Frotscher, M., & Soriano, E. (1996). Differential survival of Cajal-Retzius cells in organotypic cultures of hippocampus and neocortex. *Journal of Neuroscience*, 16(21), 6896–6907. <https://doi.org/10.1523/jneurosci.16-21-06896.1996>
- Deller, T., Drakew, A., Heimrich, B., Förster, E., Tielsch, A., & Frotscher, M. (1999). The hippocampus of the reeler mutant mouse: Fiber segregation in area CA1 depends on the position of the postsynaptic target cells. *Experimental Neurology*, 156(2), 254–267. <https://doi.org/10.1006/exnr.1999.7021>
- Denaxa, M., Neves, G., Rabinowitz, A., Kemlo, S., Liodis, P., Burrone, J., & Pachnis, V. (2018). Modulation of Apoptosis Controls Inhibitory Interneuron Number in the Cortex. *Cell Reports*, 22(7), 1710–1721. <https://doi.org/10.1016/j.celrep.2018.01.064>
- Dixit, R., Wilkinson, G., Cancino, G. I., Shaker, T., Adnani, L., Li, S., ... Schuurmans, C. (2014). Neurog1 and Neurog2 control two waves of neuronal differentiation in the piriform cortex. *Journal of Neuroscience*, 34(2), 539–553. <https://doi.org/10.1523/JNEUROSCI.0614-13.2014>
- Doron, G., Shin, J. N., Takahashi, N., Bocklisch, C., Skenderi, S., Druke, M., ... Larkum, M. E. (2019). Perirhinal input to neocortical layer 1 controls learning. *BioRxiv*, 713883. <https://doi.org/10.1101/713883>

-E-

- Eriksson, S. H., Thom, M., Heffernan, J., Lin, W. R., Harding, B. N., Squier, M. V., & Sisodiya, S. M. (2001). Persistent reelin-expressing Cajal-Retzius cells in polymicrogyria. *Brain*, *124*(7), 1350–1361. <https://doi.org/10.1093/brain/124.7.1350>
- Erzurumlu, R. S., & Gaspar, P. (2012). Development and critical period plasticity of the barrel cortex. *European Journal of Neuroscience*, *35*(10), 1540–1553. <https://doi.org/10.1111/j.1460-9568.2012.08075.x>

-F-

- Fame, R. M., Macdonald, J. L., Dunwoodie, S. L., Takahashi, E., & Macklis, J. D. (2016). Cited2 regulates neocortical layer II/III generation and somatosensory callosal projection neuron development and connectivity. *Journal of Neuroscience*, *36*(24), 6403–6419. <https://doi.org/10.1523/JNEUROSCI.4067-15.2016>
- Fame, R. M., MacDonald, J. L., & Macklis, J. D. (2011). Development, specification, and diversity of callosal projection neurons. *Trends in Neurosciences*, *34*(1), 41–50. <https://doi.org/10.1016/j.tins.2010.10.002>
- Fan, L. Z., Kheifets, S., Böhm, U. L., Wu, H., Piatkevich, K. D., Xie, M. E., ... Cohen, A. E. (2020). All-Optical Electrophysiology Reveals the Role of Lateral Inhibition in Sensory Processing in Cortical Layer 1. *Cell*, *180*(3), 521–535.e18. <https://doi.org/10.1016/j.cell.2020.01.001>
- Favuzzi, E., Deogracias, R., Marques-Smith, A., Maeso, P., Jezequel, J., Exposito-Alonso, D., ... Rico, B. (2019). Neurodevelopment: Distinct molecular programs regulate synapse specificity in cortical inhibitory circuits. *Science*, *363*(6425), 413–417. <https://doi.org/10.1126/science.aau8977>
- Feldmeyer, D. (2012). Excitatory neuronal connectivity in the barrel cortex. *Frontiers in Neuroanatomy*, *6*(JULY 2012), 1–22. <https://doi.org/10.3389/fnana.2012.00024>
- Feng, G., Mellor, R. H., Bernstein, M., Keller-Peck, C., Nguyen, Q. T., Wallace, M., ... Sanes, J. R. (2000). Imaging Neuronal Subsets in Transgenic Mice Expressing Multiple Spectral Variants of GFP. *Neuron*, *28*, 41–51.
- Fenlon, L. R., Suárez, R., & Richards, L. J. (2017). The anatomy, organisation and development of contralateral callosal projections of the mouse somatosensory cortex. *Brain and Neuroscience Advances*, *1*, 239821281769488. <https://doi.org/10.1177/2398212817694888>
- Fernández, V., Llinares-Benadero, C., & Borrell, V. (2016). Cerebral cortex expansion and folding: what have we learned? *The EMBO Journal*, *35*(10), 1021–1044. <https://doi.org/10.15252/embj.201593701>
- Ferrer, I., Bernet, E., Soriano, E., Del Rio, T., & Fonseca, M. (1990). Naturally occurring cell death in the cerebral cortex of the rat and removal of dead cells by transitory phagocytes. *Neuroscience*, *39*(2), 451–458. [https://doi.org/10.1016/0306-4522\(90\)90281-8](https://doi.org/10.1016/0306-4522(90)90281-8)
- Fietz, S. A., & Huttner, W. B. (2011). Cortical progenitor expansion, self-renewal and neurogenesis—a polarized perspective. *Current Opinion in Neurobiology*, *21*(1), 23–35. <https://doi.org/10.1016/j.conb.2010.10.002>
- Florio, M., & Huttner, W. B. (2014). Neural progenitors, neurogenesis and the evolution of the neocortex. *Development (Cambridge)*, *141*(11), 2182–2194. <https://doi.org/10.1242/dev.090571>
- Folsom, T. D., & Fatemi, S. H. (2013). The involvement of Reelin in neurodevelopmental disorders. *Neuropharmacology*, *68*, 122–135. <https://doi.org/10.1016/j.neuropharm.2012.08.015>
- Förster, E., Kaltschmidt, C., Deng, J., Cremer, H., Deller, T., & Frotscher, M. (1998). Lamina-specific cell adhesion on living slices of hippocampus. *Development*, *125*(17), 3399–3410.
- Fossati, M., Pizzarelli, R., Schmidt, E. R., Kupferman, J. V., Stroebel, D., Polleux, F., & Charrier, C. (2016). SRGAP2 and Its Human-Specific Paralog Co-Regulate the Development of Excitatory and Inhibitory Synapses. *Neuron*, *91*(2), 356–369.

References

- <https://doi.org/10.1016/j.neuron.2016.06.013>
- Franco, S. J., Martinez-Garay, I., Gil-Sanz, C., Harkins-Perry, S. R., & Müller, U. (2011). Reelin Regulates Cadherin Function via Dab1/Rap1 to Control Neuronal Migration and Lamination in the Neocortex. *Neuron*, 69(3), 482–497. <https://doi.org/10.1016/j.neuron.2011.01.003>
- Frangeul, L., Pouchelon, G., Telley, L., Lefort, S., Luscher, C., & Jabaudon, D. (2016). A cross-modal genetic framework for the development and plasticity of sensory pathways. *Nature*, 538(7623), 96–98. <https://doi.org/10.1038/nature19770>
- Frazer, S., Prados, J., Niquille, M., Cadilhac, C., Markopoulos, F., Gomez, L., ... Dayer, A. (2017). Transcriptomic and anatomic parcellation of 5-HT3A R expressing cortical interneuron subtypes revealed by single-cell RNA sequencing. *Nature Communications*, 8, 1–12. <https://doi.org/10.1038/ncomms14219>
- Fricker, M., Tolkovsky, A. M., Borutaite, V., Coleman, M., & Brown, G. C. (2018). Neuronal cell death. *Physiological Reviews*, 98(2), 813–880. <https://doi.org/10.1152/physrev.00011.2017>
- Friedlander, M. J., & Torres-Reveron, J. (2009). The changing roles of neurons in the cortical subplate. *Frontiers in Neuroanatomy*, 3(AUG), 1–8. <https://doi.org/10.3389/neuro.05.015.2009>
- Frotscher, M., & Heimrich, B. (1993). Formation of layer-specific fiber projections to the hippocampus in vitro. *Proceedings of the National Academy of Sciences of the United States of America*, 90(21), 10400–10403. <https://doi.org/10.1073/pnas.90.21.10400>
- Frotscher, Michael. (1997). Dual role of Cajal-Retzius cells and reelin in cortical development. *Cell and Tissue Research*, 290(2), 315–322. <https://doi.org/10.1007/s004410050936>
- Frotscher, Michael. (1998). Cajal-Retzius cells, Reelin, and the formation of layers. *Current Opinion in Neurobiology*, 8(5), 570–575. [https://doi.org/10.1016/S0959-4388\(98\)80082-2](https://doi.org/10.1016/S0959-4388(98)80082-2)
- Fuchs, Y., & Steller, H. (2011). Programmed cell death in animal development and disease. *Cell*, 147(4), 742–758. <https://doi.org/10.1016/j.cell.2011.10.033>
- Furukawa, T., Yamada, J., Akita, T., Matsushima, Y., Yanagawa, Y., & Fukuda, A. (2014). Roles of taurine-mediated tonic GABAA receptor activation in the radial migration of neurons in the fetal mouse cerebral cortex. *Frontiers in Cellular Neuroscience*, 8(MAR), 1–18. <https://doi.org/10.3389/fncel.2014.00088>

-G-

- Galazo, M. J., Martinez-Cerdeño, V., Porrero, C., & Clascá, F. (2008). Embryonic and postnatal development of the layer I-directed (“matrix”) thalamocortical system in the rat. *Cerebral Cortex*, 18(2), 344–363. <https://doi.org/10.1093/cercor/bhm059>
- Galloni, A. R., Laffere, A., & Rancz, E. (2020). Apical length governs computational diversity of layer 5 pyramidal neurons. *eLife*, 9, 1–40. <https://doi.org/10.7554/eLife.55761>
- Garaschuk, O., Linn, J., Eilers, J., & Konnerth, A. (2000). Large-scale oscillatory calcium waves in the immature cortex. *Nature Neuroscience*, 3(5), 452–459. <https://doi.org/10.1038/74823>
- García-Moreno, F., Anderton, E., Jankowska, M., Begbie, J., Encinas, J. M., Irimia, M., & Molnár, Z. (2018). Absence of Tangentially Migrating Glutamatergic Neurons in the Developing Avian Brain. *Cell Reports*, 22(1), 96–109. <https://doi.org/10.1016/j.celrep.2017.12.032>
- García-Munoz, M., & Arbuthnott, G. W. (2015). Basal ganglia—thalamus and the “crowning enigma.” *Frontiers in Neural Circuits*, 9(November), 1–10. <https://doi.org/10.3389/fncir.2015.00071>
- Garel, S., Huffman, K. J., & Rubenstein, J. L. R. (2003). Molecular regionalization of the neocortex is disrupted in Fgf8 hypomorphic mutants. *Development*, 130(9), 1903–1914. <https://doi.org/10.1242/dev.00416>
- Gaspar, P., & Erzurumlu, R. (2013). The Birth of the Barrels. *Developmental Cell*, 27(1), 3–4. <https://doi.org/10.1016/j.devcel.2013.09.028>
- Gelman, D., Griveau, A., Dehorter, N., Teissier, A., Varela, C., Pla, R., ... Marín, O. (2011). A

References

- wide diversity of cortical GABAergic interneurons derives from the embryonic preoptic area. *Journal of Neuroscience*, 31(46), 16570–16580. <https://doi.org/10.1523/JNEUROSCI.4068-11.2011>
- Gelman, D. M., Martini, F. J., Nóbrega-Pereira, S., Pierani, A., Kessar, N., & Marin, O. (2009). The embryonic preoptic area is a novel source of cortical GABAergic interneurons. *Journal of Neuroscience*, 29(29), 9380–9389. <https://doi.org/10.1523/JNEUROSCI.0604-09.2009>
- Ghezzi, F., Marques-Smith, A., Anastasiades, P. G., Lyngholm, D., Vagnoni, C., Rowett, A., ... Butt, S. J. B. (2020). Non-canonical role for Lpar1-EGFP subplate neurons in early postnatal somatosensory cortex. *BioRxiv*, 2020.05.12.088450. <https://doi.org/10.1101/2020.05.12.088450>
- Gil-Sanz, C., Franco, S. J., Martinez-Garay, I., Espinosa, A., Harkins-Perry, S., & Müller, U. (2013). Cajal-Retzius cells instruct neuronal migration by coincidence signaling between secreted and contact-dependent guidance cues. *Neuron*, 79(3), 461–477. <https://doi.org/10.1016/j.neuron.2013.06.040>
- Gilbert S.F. (2000). *Developmental Biology* 6th edition, Sunderland (MA): Sinauer Associates. ISBN-10: 0-87893-243-7.
- Goffinet, A. M., Bar, I., Bernier, B., Trujillo, C., Raynaud, A., & Meyer, G. (1999). Reelin expression during embryonic brain development in lacertilian lizards. *Journal of Comparative Neurology*, 414(4), 533–550. [https://doi.org/10.1002/\(SICI\)1096-9861\(19991129\)414:4<533::AID-CNE8>3.0.CO;2-V](https://doi.org/10.1002/(SICI)1096-9861(19991129)414:4<533::AID-CNE8>3.0.CO;2-V)
- Golshani, P., Goncalves, J. T., Khoshkhoo, S., Mostany, R., Smirnakis, S., & Portera-Cailliau, C. (2009). Internally Mediated Developmental Desynchronization of Neocortical Network Activity. *Journal of Neuroscience*, 29(35), 10890–10899. <https://doi.org/10.1523/JNEUROSCI.2012-09.2009>
- González-Gómez, M., & Meyer, G. (2014). Dynamic expression of calretinin in embryonic and early fetal human cortex. *Frontiers in Neuroanatomy*, 8(JUN), 1–12. <https://doi.org/10.3389/fnana.2014.00041>
- Govindan, S., & Jabaudon, D. (2017). Coupling progenitor and neuronal diversity in the developing neocortex. *FEBS Letters*, 591(24), 3960–3977. <https://doi.org/10.1002/1873-3468.12846>
- Greig, L. C., Woodworth, M. B., Galazo, M. J., Padmanabhan, H., & Macklis, J. D. (2013). Molecular logic of neocortical projection neuron specification, development and diversity. *Nature Reviews Neuroscience*, 14(11), 755–769. <https://doi.org/10.1038/nrn3586>
- Griveau, A., Borello, U., Causeret, F., Tissir, F., Boggetto, N., Karaz, S., & Pierani, A. (2010). A novel role for Dbx1-derived Cajal-Retzius cells in early regionalization of the cerebral cortical neuroepithelium. *PLoS Biology*, 8(7). <https://doi.org/10.1371/journal.pbio.1000440>

-H-

- Ha, S., Tripathi, P. P., Mihalas, A. B., Hevner, R. F., & Beier, D. R. (2017). C-Terminal Region Truncation of RELN Disrupts an Interaction with VLDLR, Causing Abnormal Development of the Cerebral Cortex and Hippocampus. *The Journal of Neuroscience*, 37(4), 960–971. <https://doi.org/10.1523/JNEUROSCI.1826-16.2016>
- Hanganu, I. L., Kilb, W., & Luhmann, H. J. (2002). Functional synaptic projections onto subplate neurons in neonatal rat somatosensory cortex. *Journal of Neuroscience*, 22(16), 7165–7176. <https://doi.org/10.1523/jneurosci.22-16-07165.2002>
- Harris, K. D., & Shepherd, G. M. G. (2015). The neocortical circuit: Themes and variations. *Nature Neuroscience*, 18(2), 170–181. <https://doi.org/10.1038/nn.3917>
- Haubensak, W., Attardo, A., Denk, W., & Huttner, W. B. (2004). Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: A major site of neurogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, 101(9), 3196–3201. <https://doi.org/10.1073/pnas.0308600100>

References

- Heck, N., Golbs, A., Riedemann, T., Sun, J. J., Lessmann, V., & Luhmann, H. J. (2008). Activity-dependent regulation of neuronal apoptosis in neonatal mouse cerebral cortex. *Cerebral Cortex*, *18*(6), 1335–1349. <https://doi.org/10.1093/cercor/bhm165>
- Hestrin, S., & Armstrong, W. E. (1996). Morphology and physiology of cortical neurons in layer I. *Journal of Neuroscience*, *16*(17), 5290–5300. <https://doi.org/10.1523/jneurosci.16-17-05290.1996>
- Hevner, R. F. (2019). Intermediate progenitors and Tbr2 in cortical development. *Journal of Anatomy*, *235*(3), 616–625. <https://doi.org/10.1111/joa.12939>
- Hirai, K., Yoshioka, H., Kihara, M., Hasegawa, K., Sakamoto, T., Sawada, T., & Fushiki, S. (1999). Inhibiting neuronal migration by blocking NMDA receptors in the embryonic rat cerebral cortex: A tissue culture study. *Developmental Brain Research*, *114*(1), 63–67. [https://doi.org/10.1016/S0165-3806\(99\)00019-X](https://doi.org/10.1016/S0165-3806(99)00019-X)
- Hirota, Y., Kubo, K. ichiro, Fujino, T., Yamamoto, T. T., & Nakajima, K. (2018). ApoER2 Controls not only neuronal migration in the intermediate zone but also termination of migration in the developing cerebral cortex. *Cerebral Cortex*, *28*(1), 223–235. <https://doi.org/10.1093/cercor/bhw369>
- Hirota, Y., Kubo, K. ichiro, Katayama, K. ichi, Honda, T., Fujino, T., Yamamoto, T. T., & Nakajima, K. (2015). Reelin receptors ApoER2 and VLDLR are expressed in distinct spatiotemporal patterns in developing mouse cerebral cortex. *Journal of Comparative Neurology*, *523*(3), 463–478. <https://doi.org/10.1002/cne.23691>
- Hirota, Y., & Nakajima, K. (2017). Control of Neuronal Migration and Aggregation by Reelin Signaling in the Developing Cerebral Cortex. *Frontiers in Cell and Developmental Biology*, *5*(APR), 1–8. <https://doi.org/10.3389/fcell.2017.00040>
- Hirota, Y., & Nakajima, K. (2020). VLDLR is not essential for reelin-induced neuronal aggregation but suppresses neuronal invasion into the marginal zone. *Development (Cambridge, England)*, *147*(12). <https://doi.org/10.1242/dev.189936>
- Hoch, R. V., Rubenstein, J. L. R., & Pleasure, S. (2009). Genes and signaling events that establish regional patterning of the mammalian forebrain. *Seminars in Cell and Developmental Biology*, *20*(4), 378–386. <https://doi.org/10.1016/j.semcd.2009.02.005>
- Hoerder-Suabedissen, A., & Molnár, Z. (2013). Molecular diversity of early-born subplate neurons. *Cerebral Cortex*, *23*(6), 1473–1483. <https://doi.org/10.1093/cercor/bhs137>
- Hofer, S. B., Mrcic-Flogel, T. D., Bonhoeffer, T., & Hübener, M. (2009). Experience leaves a lasting structural trace in cortical circuits. *Nature*, *457*(7227), 313–317. <https://doi.org/10.1038/nature07487>
- Holtmaat, A., & Svoboda, K. (2009). Experience-dependent structural synaptic plasticity in the mammalian brain. *Nature Reviews Neuroscience*, *10*(9), 647–658. <https://doi.org/10.1038/nrn2699>
- Hong, S. E., Shugart, Y. Y., Huang, D. T., Al Shahwan, S., Grant, P. E., Hourihane, J. O. B., ... Walsh, C. A. (2000). Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. *Nature Genetics*, *26*(1), 93–96. <https://doi.org/10.1038/79246>
- Horch, H. W., Krüttgen, A., Portbury, S. D., & Katz, L. C. (1999). Destabilization of cortical dendrites and spines by BDNF. *Neuron*, *23*(2), 353–364. [https://doi.org/10.1016/S0896-6273\(00\)80785-0](https://doi.org/10.1016/S0896-6273(00)80785-0)
- Horton, J. G., & Adams, D. L. (2005). The cortical column: A structure without a function. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1456), 837–862. <https://doi.org/10.1098/rstb.2005.1623>
- Huang, Z. J., & Paul, A. (2019). The diversity of GABAergic neurons and neural communication elements. *Nature Reviews Neuroscience*, *20*(9), 563–572. <https://doi.org/10.1038/s41583-019-0195-4>
- Hubel, D. H. (1982). Cortical Neurobiology. *Annual Review of Neuroscience*, *5*, 363–370.
- Hurni, N., Kolodziejczak, M., Tomasello, U., Badia, J., Jacobshagen, M., Prados, J., & Dayer, A. (2017). Transient Cell-intrinsic Activity Regulates the Migration and Lamina Positioning of Cortical Projection Neurons. *Cerebral Cortex*, *27*(5), 3052–3063.

References

<https://doi.org/10.1093/cercor/bhx059>

-I-

- Ibrahim, L. A., Mesik, L., Ji, X. ying, Fang, Q., Li, H. fu, Li, Y. tang, ... Tao, H. W. (2016). Cross-Modality Sharpening of Visual Cortical Processing through Layer-1-Mediated Inhibition and Disinhibition. *Neuron*. <https://doi.org/10.1016/j.neuron.2016.01.027>
- Ibrahim, L. A., Schuman, B., Bandler, R., Rudy, B., & Fishell, G. (2020). ScienceDirect Mining the jewels of the cortex ' s crowning mystery. *Current Opinion in Neurobiology*, 63, 154–161. <https://doi.org/10.1016/j.conb.2020.04.005>
- Ikonomidou, C., Bosch, F., Miksa, M., Bittigau, P., Vöckler, J., Dikranian, K., ... Olney, J. W. (1999). Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. *Science*, 283(5398), 70–74. <https://doi.org/10.1126/science.283.5398.70>
- Ishii, K., Kubo, K. I., & Nakajima, K. (2016). Reelin and neuropsychiatric disorders. *Frontiers in Cellular Neuroscience*, 10(OCT2016), 1–13. <https://doi.org/10.3389/fncel.2016.00229>
- Iwasato, T., Datwani, A., Wolf, A. M., Nishiyama, H., Taguchi, Y., Tonegawa, S., ... Itohara, S. (2000). Cortex-restricted disruption of NMDAR1 impairs neuronal patterns in the barrel cortex. *Nature*, 406(6797), 726–731. <https://doi.org/10.1038/35021059>

-J-

- Jabaudon, D., J. Shnider, S., J. Tischfield, D., J. Galazo, M., & MacKlis, J. D. (2012). ROR β induces barrel-like neuronal clusters in the developing neocortex. *Cerebral Cortex*, 22(5), 996–1006. <https://doi.org/10.1093/cercor/bhr182>
- Jabaudon, D., & López Bendo, G. (2012). Development and plasticity of thalamocortical systems. *European Journal of Neuroscience*, 35(10), 1522–1523. <https://doi.org/10.1111/j.1460-9568.2012.08117.x>
- Janusonis, S. (2004). Early Serotonergic Projections to Cajal-Retzius Cells: Relevance for Cortical Development. *Journal of Neuroscience*, 24(7), 1652–1659. <https://doi.org/10.1523/JNEUROSCI.4651-03.2004>
- Jiang, X., Shen, S., Cadwell, C. R., Berens, P., Sinz, F., Ecker, A. S., ... Tolias, A. S. (2015). Principles of connectivity among morphologically defined cell types in adult neocortex. *Science*, 350(6264). <https://doi.org/10.1126/science.aac9462>
- Jiao, Y., Zhang, C., Yanagawa, Y., & Sun, Q. Q. (2006). Major effects of sensory experiences on the neocortical inhibitory circuits. *Journal of Neuroscience*, 26(34), 8691–8701. <https://doi.org/10.1523/JNEUROSCI.2478-06.2006>
- Julien, O., & Wells, J. A. (2017). Caspases and their substrates. *Cell Death and Differentiation*, 24(8), 1380–1389. <https://doi.org/10.1038/cdd.2017.44>
- Jung, Y. J., & Chung, W. S. (2018). Phagocytic roles of glial cells in healthy and diseased brains. *Biomolecules and Therapeutics*, 26(4), 350–357. <https://doi.org/10.4062/biomolther.2017.133>

-K-

- Kandel, E. R., Schwartz, J. H., Jessell, T. M., Siegelbaum, S. A., & Hudspeth, A. . (2012). *Principles of Neural Sciences*.
- Kanold, P. O., & Luhmann, H. J. (2010). The subplate and early cortical circuits. *Annual Review of Neuroscience*, 33, 23–48. <https://doi.org/10.1146/annurev-neuro-060909-153244>
- Karimi, A., Odenthal, J., Drawitsch, F., Boergens, K. M., & Helmstaedter, M. (2020). Cell-type specific innervation of cortical pyramidal cells at their apical dendrites. *ELife*, 9. <https://doi.org/10.7554/eLife.46876>
- Kawasaki, T., Ito, K., & Hirata, T. (2006). Netrin 1 regulates ventral tangential migration of guidepost neurons in the lateral olfactory tract. *Development*, 133(5), 845–853. <https://doi.org/10.1242/dev.02257>
- Keller, G. B., & Mrsic-Flogel, T. D. (2018). Predictive Processing: A Canonical Cortical Computation. *Neuron*, 100(2), 424–435. <https://doi.org/10.1016/j.neuron.2018.10.003>
- Khazipov, R., & Luhmann, H. J. (2006). Early patterns of electrical activity in the developing

References

- cerebral cortex of humans and rodents. *Trends in Neurosciences*.
<https://doi.org/10.1016/j.tins.2006.05.007>
- Kihara, M., Yoshioka, H., Hirai, K., Hasegawa, K., Kizaki, Z., & Sawada, T. (2002). Stimulation of N-methyl-D-aspartate (NMDA) receptors inhibits neuronal migration in embryonic cerebral cortex: A tissue culture study. *Developmental Brain Research*, *138*(2), 195–198. [https://doi.org/10.1016/S0165-3806\(02\)00490-X](https://doi.org/10.1016/S0165-3806(02)00490-X)
- Kirischuk, S., Luhmann, H. J., & Kilb, W. (2014). Cajal-Retzius cells: Update on structural and functional properties of these mystic neurons that bridged the 20th century. *Neuroscience*, *275*, 33–46. <https://doi.org/10.1016/j.neuroscience.2014.06.009>
- Kirischuk, Sergei, Sinning, A., Blanquie, O., Yang, J. W., Luhmann, H. J., & Kilb, W. (2017). Modulation of neocortical development by early neuronal activity: Physiology and pathophysiology. *Frontiers in Cellular Neuroscience*, *11*(November), 1–21. <https://doi.org/10.3389/fncel.2017.00379>
- Klingler, E., De la Rossa, A., Fièvre, S., Devaraju, K., Abe, P., & Jabaudon, D. (2019). A Translaminar Genetic Logic for the Circuit Identity of Intracortically Projecting Neurons. *Current Biology*, *29*(2), 332–339.e5. <https://doi.org/10.1016/j.cub.2018.11.071>
- Kohno, T., Honda, T., Kubo, K. -i., Nakano, Y., Tsuchiya, A., Murakami, T., ... Hattori, M. (2015). Importance of Reelin C-Terminal Region in the Development and Maintenance of the Postnatal Cerebral Cortex and Its Regulation by Specific Proteolysis. *Journal of Neuroscience*, *35*(11), 4776–4787. <https://doi.org/10.1016/j.nima.2009.05.173>
- Komuro, H., & Rakic, P. (1993). Modulation of neuronal migration by NMDA receptors. *Science*, *260*(5104), 95–97. <https://doi.org/10.1126/science.8096653>
- Kwan, K. Y., Šestan, N., & Anton, E. S. (2012). Transcriptional co-regulation of neuronal migration and laminar identity in the neocortex. *Development*, *139*(9), 1535–1546. <https://doi.org/10.1242/dev.069963>

-L-

- Lacefield, C. O., Pnevmatikakis, E. A., Paninski, L., & Bruno, R. M. (2019). Reinforcement Learning Recruits Somata and Apical Dendrites across Layers of Primary Sensory Cortex. *Cell Reports*, *26*(8), 2000–2008.e2. <https://doi.org/10.1016/j.celrep.2019.01.093>
- Lanjakomsiripan, D., Pior, B. J., Kawaguchi, D., Furutachi, S., Tahara, T., Katsuyama, Y., ... Gotoh, Y. (2018). Layer-specific morphological and molecular differences in neocortical astrocytes and their dependence on neuronal layers. *Nature Communications*, *9*(1). <https://doi.org/10.1038/s41467-018-03940-3>
- Larkum, M. E., Kaiser, K. M. M., & Sakmann, B. (1999). Calcium electrogenesis in distal apical dendrites of layer 5 pyramidal cells at a critical frequency of back-propagating action potentials. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(25), 14600–14604. <https://doi.org/10.1073/pnas.96.25.14600>
- Larkum, Matthew E. (2013). The yin and yang of cortical layer 1. *Nature Neuroscience*, *16*(2), 114–115. <https://doi.org/10.1038/nn.3317>
- Larkum, Matthew E., Petro, L. S., Sachdev, R. N. S., & Muckli, L. (2018). A Perspective on Cortical Layering and Layer-Spanning Neuronal Elements. *Frontiers in Neuroanatomy*, *12*(July), 1–9. <https://doi.org/10.3389/fnana.2018.00056>
- Ledonne, F., Orduz, D., Mercier, J., Vigier, L., Grove, E. A., Tissir, F., ... Coppola, E. (2016). Targeted Inactivation of Bax Reveals a Subtype-Specific Mechanism of Cajal-Retzius Neuron Death in the Postnatal Cerebral Cortex. *Cell Reports*, *17*(12), 3133–3141. <https://doi.org/10.1016/j.celrep.2016.11.074>
- Lee, S., Kruglikov, I., Huang, Z. J., Fishell, G., & Rudy, B. (2013). A disinhibitory circuit mediates motor integration in the somatosensory cortex. *Nature Neuroscience*. <https://doi.org/10.1038/nn.3544>
- Leighton, A. H., & Lohmann, C. (2016). The wiring of developing sensory circuits—From patterned spontaneous activity to synaptic plasticity mechanisms. *Frontiers in Neural Circuits*, *10*(SEP), 1–13. <https://doi.org/10.3389/fncir.2016.00071>
- Letzkus, J. J., Wolff, S. B. E., Meyer, E. M. M., Tovote, P., Courtin, J., Herry, C., & Lüthi, A.

References

- (2011). A disinhibitory microcircuit for associative fear learning in the auditory cortex. *Nature*, 480(7377), 331–335. <https://doi.org/10.1038/nature10674>
- Lewis, D. A., Campbell, M. J., Foote, S. L., Goldstein, M., & Morrison, J. H. (1987). The distribution of tyrosine hydroxylase-immunoreactive fibers in primate neocortex is widespread but regionally specific. *Journal of Neuroscience*, 7(1), 279–290. <https://doi.org/10.1523/jneurosci.07-01-00279.1987>
- Li, G., Adesnik, H., Li, J., Long, J., Nicoll, R. A., Rubenstein, J. L. R., & Pleasure, S. J. (2008). Regional distribution of cortical interneurons and development of inhibitory tone are regulated by Cxcl12/Cxcr4 signaling. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 28(5), 1085–1098. <https://doi.org/10.1523/JNEUROSCI.4602-07.2008>
- Lim, L., Mi, D., Llorca, A., & Marín, O. (2018a). Development and Functional Diversification of Cortical Interneurons. *Neuron*, 100(2), 294–313. <https://doi.org/10.1016/j.neuron.2018.10.009>
- Lim, L., Pakan, J. M. P., Selten, M. M., Marques-Smith, A., Llorca, A., Bae, S. E., ... Marín, O. (2018). Optimization of interneuron function by direct coupling of cell migration and axonal targeting. *Nature Neuroscience*, 21(7), 920–931. <https://doi.org/10.1038/s41593-018-0162-9>
- Lodato, S., & Arlotta, P. (2014). Generating Neuronal Diversity in the Mammalian Cerebral Cortex. *Annual Review of Cell and Developmental Biology*, 31(1), annurev-cellbio-100814-125353. <https://doi.org/10.1146/annurev-cellbio-100814-125353>
- Lodato, S., Rouaux, C., Quast, K. B., Jantrachotechatchawan, C., Studer, M., Hensch, T. K., & Arlotta, P. (2011). Excitatory Projection Neuron Subtypes Control the Distribution of Local Inhibitory Interneurons in the Cerebral Cortex. *Neuron*, 69(4), 763–779. <https://doi.org/10.1016/j.neuron.2011.01.015>
- Lokmane, L., & Garel, S. (2014). Map transfer from the thalamus to the neocortex: Inputs from the barrel field. *Seminars in Cell and Developmental Biology*, 35, 147–155. <https://doi.org/10.1016/j.semcdb.2014.07.005>
- López-Bendito, G., & Molnár, Z. (2003). Thalamocortical development: How are we going to get there? *Nature Reviews Neuroscience*, 4(4), 276–289. <https://doi.org/10.1038/nrn1075>
- López-Bendito, G., Sánchez-Alcañiz, J. A., Pla, R., Borrell, V., Picó, E., Valdeolillos, M., & Marín, O. (2008). Chemokine signaling controls intracortical migration and final distribution of GABAergic interneurons. *Journal of Neuroscience*, 28(7), 1613–1624. <https://doi.org/10.1523/JNEUROSCI.4651-07.2008>
- Luhmann, H. J., Fukuda, A., & Kilb, W. (2015). Control of cortical neuronal migration by glutamate and GABA. *Frontiers in Cellular Neuroscience*, 9(JAN), 1–15. <https://doi.org/10.3389/fncel.2015.00004>
- Luhmann, H. J., & Khazipov, R. (2018). Neuronal activity patterns in the developing barrel cortex. *Neuroscience*, 368, 256–267. <https://doi.org/10.1016/j.neuroscience.2017.05.025>
- Luhmann, H. J., Kirischuk, S., & Kilb, W. (2018). The superior function of the subplate in early neocortical development. *Frontiers in Neuroanatomy*, 12(November), 1–14. <https://doi.org/10.3389/fnana.2018.00097>
- Luhmann, H. J., Sinning, A., Yang, J. W., Reyes-Puerta, V., Stüttgen, M. C., Kirischuk, S., & Kilb, W. (2016). Spontaneous neuronal activity in developing neocortical networks: From single cells to large-scale interactions. *Frontiers in Neural Circuits*, 10(MAY), 1–14. <https://doi.org/10.3389/fncir.2016.00040>
- M-
- Ma, T., Zhang, Q., Cai, Y., You, Y., Rubenstein, J. L. R., & Yang, Z. (2012). A subpopulation of dorsal lateral/caudal ganglionic eminence-derived neocortical interneurons expresses the transcription factor Sp8. *Cerebral Cortex*, 22(9), 2120–2130. <https://doi.org/10.1093/cercor/bhr296>
- Mancia Leon, W. R., Spatazza, J., Rakela, B., Chatterjee, A., Pande, V., Maniatis, T., ... Alvarez-Buylla, A. (2020). Clustered gamma-protocadherins regulate cortical interneuron

References

- programmed cell death. *ELife*, 9, 1–36. <https://doi.org/10.7554/eLife.55374>
- Manita, S., Miyakawa, H., Kitamura, K., & Murayama, M. (2017). Dendritic Spikes in Sensory Perception. *Frontiers in Cellular Neuroscience*, 11(February), 1–7. <https://doi.org/10.3389/fncel.2017.00029>
- Mardinly, A. R., Spiegel, I., Patrizi, A., Centofante, E., Bazinet, J. E., Tzeng, C. P., ... Greenberg, M. E. (2016). Sensory experience regulates cortical inhibition by inducing IGF1 in VIP neurons. *Nature*, 531(7594), 371–375. <https://doi.org/10.1038/nature17187>
- Marín-Padilla, M. (1998). Cajal-Retzius cells and the development of the neocortex. *Trends in Neurosciences*, 21(2), 64–71. [https://doi.org/10.1016/S0166-2236\(97\)01164-8](https://doi.org/10.1016/S0166-2236(97)01164-8)
- Marín-Padilla, M. (1990). The structural organization of layer I of the adult human acoustic cortex a golgi and electron microscopy study. *The Journal of Comparative Neurology*, 299(3), 89–105. <https://doi.org/10.3109/00016489309135853>
- Marín-Padilla, M. (1992). Ontogenesis of the pyramidal cell of the mammalian neocortex and developmental cytoarchitectonics: A unifying theory. *Journal of Comparative Neurology*, 321(2), 223–240. <https://doi.org/10.1002/cne.903210205>
- Marín, O., & Rubenstein, J. L. (2001). A long, remarkable journey: tangential migration in the telencephalon. *Nature Reviews. Neuroscience*, 2(11), 780–790. <https://doi.org/10.1038/35097509>
- Marín, Oscar. (2012). Interneuron dysfunction in psychiatric disorders. *Nature Reviews. Neuroscience*, 13(2), 107–120. <https://doi.org/10.1038/nrn3155>
- Marín, Oscar, & Müller, U. (2014). Lineage origins of GABAergic versus glutamatergic neurons in the neocortex. *Current Opinion in Neurobiology*, 26(Figure 1), 132–141. <https://doi.org/10.1016/j.conb.2014.01.015>
- Marín, Oscar, & Rubenstein, J. L. R. (2001). A long, remarkable journey: Tangential migration in the telencephalon. *Nature Reviews Neuroscience*, 2(11), 780–790. <https://doi.org/10.1038/35097509>
- Marques-Smith, A., Lyngholm, D., Kaufmann, A. K., Stacey, J. A., Hoerder-Suabedissen, A., Becker, E. B. E., ... Butt, S. J. B. (2016). A Transient Translaminar GABAergic Interneuron Circuit Connects Thalamocortical Recipient Layers in Neonatal Somatosensory Cortex. *Neuron*, 89(3), 536–549. <https://doi.org/10.1016/j.neuron.2016.01.015>
- Martínez-Cerdeño, V., & Noctor, S. C. (2014). Cajal, Retzius, and Cajal-Retzius cells. *Frontiers in Neuroanatomy*, 8(JUN), 1–7. <https://doi.org/10.3389/fnana.2014.00048>
- Marx, M., Qi, G., Hanganu-Opatz, I. L., Kilb, W., Luhmann, H. J., & Feldmeyer, D. (2017). Neocortical Layer 6B as a Remnant of the Subplate - A Morphological Comparison. *Cerebral Cortex (New York, N.Y.: 1991)*, 27(2), 1011–1026. <https://doi.org/10.1093/cercor/bhv279>
- Matho, K. S., Huilgol, D., Galbavy, W., Kim, G., He, M., An, X., ... Huang, Z. J. (2020). Genetic dissection of glutamatergic neuron subpopulations and developmental trajectories in the cerebral cortex, 1–28.
- Matsui, A., Tran, M., Yoshida, A. C., Kikuchi, S. S., Mami, U., Ogawa, M., & Shimogori, T. (2013). BTBD3 Controls Dendrite Orientation Toward Active Axons in Mammalian Neocortex. *Science*, 342(November), 1114–1118.
- McKenna, W. L., Betancourt, J., Larkin, K. A., Abrams, B., Guo, C., Rubenstein, J. L. R., & Chen, B. (2011). Tbr1 and Fezf2 regulate alternate corticofugal neuronal identities during neocortical development. *Journal of Neuroscience*, 31(2), 549–564. <https://doi.org/10.1523/JNEUROSCI.4131-10.2011>
- Meng, X., Yu, Y., Kao, J., & Kanold, P. O. (2020). Transient coupling between subplate and subgranular layers to L1 neurons before and during the critical period. *BioRxiv*, 009607, 2020.05.05.077784. <https://doi.org/10.1101/2020.05.05.077784>
- Mesik, L., Huang, J. J., Zhang, L. I., & Tao, H. W. (2019). Sensory- and Motor-Related Responses of Layer 1 Neurons in the Mouse Visual Cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, 39(50), 10060–10070. <https://doi.org/10.1523/JNEUROSCI.1722-19.2019>

References

- Meyer, G. (1999). Feature Article: What is a Cajal-Retzius cell? A Reassessment of a Classical Cell Type Based on Recent Observations in the Developing Neocortex. *Cerebral Cortex*, 9(8), 765–775. <https://doi.org/10.1093/cercor/9.8.765>
- Meyer, Gundela. (2010). Building a human cortex: The evolutionary differentiation of Cajal-Retzius cells and the cortical hem. *Journal of Anatomy*, 217(4), 334–343. <https://doi.org/10.1111/j.1469-7580.2010.01266.x>
- Meyer, Gundela, & González-Gómez, M. (2018a). The heterogeneity of human Cajal-Retzius neurons. *Seminars in Cell and Developmental Biology*, 76, 101–111. <https://doi.org/10.1016/j.semcdb.2017.08.059>
- Meyer, Gundela, & González-Gómez, M. (2018b). The Subpial Granular Layer and Transient Versus Persisting Cajal-Retzius Neurons of the Fetal Human Cortex. *Cerebral Cortex (New York, N. Y. : 1991)*, 28(6), 2043–2058. <https://doi.org/10.1093/cercor/bhx110>
- Mi, D., Li, Z., Lim, L., Li, M., Moissidis, M., Yang, Y., ... Marín, O. (2018). Early emergence of cortical interneuron diversity in the mouse embryo. *Science*, 360(6384), 81–85. <https://doi.org/10.1126/science.aar6821>
- Mienville, J M, & Pesold, C. (1999). Low resting potential and postnatal upregulation of NMDA receptors may cause Cajal-Retzius cell death. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, 19(5), 1636–1646.
- Mienville, Jean Marc. (1998). Persistent depolarizing action of GABA in rat Cajal-Retzius cells. *Journal of Physiology*, 512(3), 809–817. <https://doi.org/10.1111/j.1469-7793.1998.809bd.x>
- Miller, M. W. (1995). Relationship of the time of origin and death of neurons in rat somatosensory cortex: Barrel versus septal cortex and projection versus local circuit neurons. *Journal of Comparative Neurology*, 355(1), 6–14. <https://doi.org/10.1002/cne.903550104>
- Miyata, T., Kawaguchi, A., Okano, H., & Ogawa, M. (2001). Asymmetric inheritance of radial glial fibers by cortical neurons. *Neuron*, 31(5), 727–741. [https://doi.org/10.1016/S0896-6273\(01\)00420-2](https://doi.org/10.1016/S0896-6273(01)00420-2)
- Miyoshi, G., & Fishell, G. (2011). GABAergic interneuron lineages selectively sort into specific cortical layers during early postnatal development. *Cerebral Cortex*, 21(4), 845–852. <https://doi.org/10.1093/cercor/bhq155>
- Miyoshi, G., Young, A., Petros, T., Karayannis, T., McKenzie Chang, M., Lavado, A., ... Fishell, G. (2015). Prox1 Regulates the Subtype-Specific Development of Caudal Ganglionic Eminence-Derived GABAergic Cortical Interneurons. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, 35(37), 12869–12889. <https://doi.org/10.1523/JNEUROSCI.1164-15.2015>
- Mizuno, H., Luo, W., Tarusawa, E., Saito, Y. M., Sato, T., Yoshimura, Y., ... Iwasato, T. (2014). NMDAR-regulated dynamics of layer 4 neuronal dendrites during thalamocortical reorganization in neonates. *Neuron*, 82(2), 365–379. <https://doi.org/10.1016/j.neuron.2014.02.026>
- Modol, L., Bollmann, Y., Tressard, T., Baude, A., Che, A., Duan, Z. R. S., ... Cossart, R. (2020). Assemblies of Perisomatic GABAergic Neurons in the Developing Barrel Cortex. *Neuron*, 105(1), 93-105.e4. <https://doi.org/10.1016/j.neuron.2019.10.007>
- Molnár, Z., Métin, C., Stoykova, A., Tarabykin, V., Price, D. J., Francis, F., ... Kennedy, H. (2006). Comparative aspects of cerebral cortical development. *European Journal of Neuroscience*, 23(4), 921–934. <https://doi.org/10.1111/j.1460-9568.2006.04611.x>
- Molyneaux, B. J., Arlotta, P., Fame, R. M., MacDonald, J. L., MacQuarrie, K. L., & Macklis, J. D. (2009). Novel subtype-specific genes identify distinct subpopulations of callosal projection neurons. *Journal of Neuroscience*, 29(39), 12343–12354. <https://doi.org/10.1523/JNEUROSCI.6108-08.2009>
- Molyneaux, B. J., Arlotta, P., Menezes, J. R. L., & Macklis, J. D. (2007). Neuronal subtype specification in the cerebral cortex. *Nature Reviews Neuroscience*, 8(6), 427–437. <https://doi.org/10.1038/nrn2151>
- Montiel, J. F., Wang, W. Z., Oeschger, F. M., Hoerder-Suabedissen, A., Tung, W. L., García-

References

- Moreno, F., ... Molnár, Z. (2011). Hypothesis on the dual origin of the mammalian subplate. *Frontiers in Neuroanatomy*, 5(APRIL), 1–10. <https://doi.org/10.3389/fnana.2011.00025>
- Moore, H., West, A. R., & Grace, A. A. (1999). The regulation of forebrain dopamine transmission: Relevance to the pathophysiology and psychopathology of schizophrenia. *Biological Psychiatry*, 46(1), 40–55. [https://doi.org/10.1016/S0006-3223\(99\)00078-5](https://doi.org/10.1016/S0006-3223(99)00078-5)
- Moreno-Juan, V., Filipchuk, A., Antón-Bolaños, N., Mezzera, C., Gezelius, H., Andrés, B., ... López-Bendito, G. (2017). Prenatal thalamic waves regulate cortical area size prior to sensory processing. *Nature Communications*, 8. <https://doi.org/10.1038/ncomms14172>
- Mountcastle, V. B. (1957). Modality and Topographic Properties Neurons of Cat ' S Somatic Sensory. *Journal of Neurophysiology*.
- Muralidhar, S., Wang, Y., & Markram, H. (2014). Synaptic and cellular organization of layer 1 of the developing rat somatosensory cortex. *Frontiers in Neuroanatomy*, 7(January), 1–17. <https://doi.org/10.3389/fnana.2013.00052>
- Murase, S., Owens, D. F., & McKay, R. D. (2011). In the newborn hippocampus, neurotrophin-dependent survival requires spontaneous activity and integrin signaling. *Journal of Neuroscience*, 31(21), 7791–7800. <https://doi.org/10.1523/JNEUROSCI.0202-11.2011>
- Muzio, L., Dibenedetto, B., Stoykova, A., Boncinelli, E., Gruss, P., & Mallamaci, A. (2002). Emx2 and Pax6 control regionalization of the pre-neuronogenic cortical primordium. *Cerebral Cortex*, 12(2), 129–139. <https://doi.org/10.1093/cercor/12.2.129>
- Myakhar, O., Unichenko, P., & Kirischuk, S. (2011). GABAergic projections from the subplate to Cajal-Retzius cells in the neocortex. *NeuroReport*, 22(11), 525–529. <https://doi.org/10.1097/WNR.0b013e32834888a4>

-N-

- Nadarajah, B., Brunstrom, J. E., Grutzendler, J., Wong, R. O. L., & Pearlman, A. L. (2001). Two modes of radial migration in early development of the cerebral cortex. *Nature Neuroscience*, 4(2), 143–150. <https://doi.org/10.1038/83967>
- Nadarajah, B., & Parnavelas, J. G. (2002). Modes of neuronal migration in the developing cerebral cortex. *Nature Reviews Neuroscience*, 3(6), 423–432. <https://doi.org/10.1038/nrn845>
- Naqui, S. Z. H., Harris, B. S., Thomaidou, D., & Parnavelas, J. G. (1999). The noradrenergic system influences the fate of Cajal-Retzius cells in the developing cerebral cortex. *Developmental Brain Research*, 113(1–2), 75–82. [https://doi.org/10.1016/S0165-3806\(99\)00003-6](https://doi.org/10.1016/S0165-3806(99)00003-6)
- Narboux-Nême, N., Pavone, L. M., Avallone, L., Zhuang, X., & Gaspar, P. (2008). Serotonin transporter transgenic (SERT^{cre}) mouse line reveals developmental targets of serotonin specific reuptake inhibitors (SSRIs). *Neuropharmacology*, 55(6), 994–1005. <https://doi.org/10.1016/j.neuropharm.2008.08.020>
- Nelson, S. B., & Valakh, V. (2015). Excitatory/Inhibitory balance and circuit homeostasis in Autism Spectrum Disorders A theory of excitatory/inhibitory imbalance in Autism HHS Public Access. *Neuron*, 87(4), 684–698. <https://doi.org/10.1016/j.neuron.2015.07.033>
- Niquille, M., Limoni, G., Markopoulos, F., Cadilhac, C., Prados, J., Holtmaat, A., & Dayer, A. (2018). Neurogliaform cortical interneurons derive from cells in the preoptic area. *ELife*, 7, 1–24. <https://doi.org/10.7554/eLife.32017>
- Nóbrega-Pereira, S., & Marín, O. (2009). Transcriptional control of neuronal migration in the developing mouse brain. *Cerebral Cortex*, 19(SUPPL. 1), 107–113. <https://doi.org/10.1093/cercor/bhp044>
- Noctor, S. C., Martinez-Cerdeño, V., Ivic, L., & Kriegstein, A. R. (2004). Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nature Neuroscience*, 7(2), 136–144. <https://doi.org/10.1038/nn1172>

-O-

- O'Dell, R. S., Cameron, D. A., Zipfel, W. R., & Olson, E. C. (2015). Reelin prevents apical

References

- neurite retraction during terminal translocation and dendrite initiation. *Journal of Neuroscience*, 35(30), 10659–10674. <https://doi.org/10.1523/JNEUROSCI.1629-15.2015>
- O’Leary, D. D. M., Chou, S. J., & Sahara, S. (2007). Area patterning of the mammalian cortex. *Neuron*, 56(2), 252–269. <https://doi.org/10.1016/j.neuron.2007.10.010>
- Ogawa, M., & Miyata, T. (1995). The reeler Gene-Associated Antigen on Cajal-Retzius Neurons Is a Crucial Molecule for Laminar Organization of Cortical Neurons, 14, 899–912.
- Okugawa, E., Ogino, H., Shigenobu, T., Yamakage, Y., Tsuiji, H., Oishi, H., ... Hattori, M. (2020). Physiological significance of proteolytic processing of Reelin revealed by cleavage-resistant Reelin knock-in mice. *Scientific Reports*, 10(1), 1–13. <https://doi.org/10.1038/s41598-020-61380-w>
- Orefice, L. L., Waterhouse, E. G., Partridge, J. G., Lalchandani, R. R., Vicini, S., & Xu, B. (2013). Distinct roles for somatically and dendritically synthesized brain-derived neurotrophic factor in morphogenesis of dendritic spines. *Journal of Neuroscience*, 33(28), 11618–11632. <https://doi.org/10.1523/JNEUROSCI.0012-13.2013>
- Owens, D. F., & Kriegstein, A. R. (1998). Patterns of intracellular calcium fluctuation in precursor cells of the neocortical ventricular zone. *Journal of Neuroscience*, 18(14), 5374–5388. <https://doi.org/10.1523/jneurosci.18-14-05374.1998>

-P-

- Parnavelas, J. G. (2000). The origin and migration of cortical neurones: New vistas. *Trends in Neurosciences*, 23(3), 126–131. [https://doi.org/10.1016/S0166-2236\(00\)01553-8](https://doi.org/10.1016/S0166-2236(00)01553-8)
- Pedraza, M., Hoerder-Suabedissen, A., Albert-Maestro, M. A., Molnár, Z., & De Carlos, J. A. (2014). Extracortical origin of some murine subplate cell populations. *Proceedings of the National Academy of Sciences of the United States of America*, 111(23), 8613–8618. <https://doi.org/10.1073/pnas.1323816111>
- Pollen, A. A., Nowakowski, T. J., Chen, J., Retallack, H., Sandoval-Espinosa, C., Nicholas, C. R., ... Kriegstein, A. R. (2015). Molecular Identity of Human Outer Radial Glia during Cortical Development. *Cell*, 163(1), 55–67. <https://doi.org/10.1016/j.cell.2015.09.004>
- Polleux, F., Giger, R. J., Ginty, D. D., Kolodkin, A. L., & Ghosh, A. (1998). Patterning of cortical efferent projections by semaphorin-neuropilin interactions. *Science*, 282(5395), 1904–1906. <https://doi.org/10.1126/science.282.5395.1904>
- Polleux, F., Morrow, T., & Ghosh, A. (2000). Semaphorin 3A is a chemoattractant for cortical apical dendrites. *Nature*, 404(6778), 567–573. <https://doi.org/10.1038/35007001>
- Poorthuis, R. B., Muhammad, K., Wang, M., Verhoog, M. B., Junek, S., Wrana, A., ... Letzkus, J. J. (2018). Rapid Neuromodulation of Layer 1 Interneurons in Human Neocortex. *Cell Reports*, 23(4), 951–958. <https://doi.org/10.1016/j.celrep.2018.03.111>
- Pouchelon, G., Gambino, F., Bellone, C., Telley, L., Vitali, I., Lüscher, C., ... Jabaudon, D. (2014). Modality-specific thalamocortical inputs instruct the identity of postsynaptic L4 neurons. *Nature*, 511(7510), 471–474. <https://doi.org/10.1038/nature13390>
- Pozas, E., Paco, S., Soriano, E., & Aguado, F. (2008). Cajal-Retzius cells fail to trigger the developmental expression of the Cl-extruding co-transporter KCC2. *Brain Research*, 1239, 85–91. <https://doi.org/10.1016/j.brainres.2008.08.058>
- Price, D. J., Aslam, S., Tasker, L., & Gillies, K. (1997). Fates of the earliest generated cells in the developing murine neocortex. *Journal of Comparative Neurology*, 377(3), 414–422. [https://doi.org/10.1002/\(SICI\)1096-9861\(19970120\)377:3<414::AID-CNE8>3.0.CO;2-5](https://doi.org/10.1002/(SICI)1096-9861(19970120)377:3<414::AID-CNE8>3.0.CO;2-5)
- Priya, R., Paredes, M. F., Karayannis, T., Yusuf, N., Liu, X., Jaglin, X., ... Fishell, G. (2018). Activity Regulates Cell Death within Cortical Interneurons through a Calcineurin-Dependent Mechanism. *Cell Reports*, 22(7), 1695–1709. <https://doi.org/10.1016/j.celrep.2018.01.007>
- Puelles, L., & Rubenstein, J. L. R. (2003). Forebrain gene expression domains and the evolving prosomeric model. *Trends in Neurosciences*, 26(9), 469–476. [https://doi.org/10.1016/S0166-2236\(03\)00234-0](https://doi.org/10.1016/S0166-2236(03)00234-0)

-Q,R-

- Quattrocolo, G., & Maccaferri, G. (2014). Optogenetic Activation of Cajal-Retzius Cells Reveals Their Glutamatergic Output and a Novel Feedforward Circuit in the Developing Mouse Hippocampus. *Journal of Neuroscience*, 34(39), 13018–13032. <https://doi.org/10.1523/JNEUROSCI.1407-14.2014>
- Rakic, P. (2008). For the article “Confusing cortical columns,” by Pasko Rakic, which appeared in issue 34, August 26, 2008, of, (34), 12099–12100.
- Rallu, M., Corbin, J. G., & Fishell, G. (2002). Parsing the prosencephalon. *Nature Reviews Neuroscience*, 3(12), 943–951. <https://doi.org/10.1038/nrn989>
- Reiprich, P., Kilb, W., & Luhmann, H. J. (2005). Neonatal NMDA receptor blockade disturbs neuronal migration in rat somatosensory cortex in vivo. *Cerebral Cortex*, 15(3), 349–358. <https://doi.org/10.1093/cercor/bhh137>
- Rho, H. J., Kim, J. H., & Lee, S. H. (2018). Function of selective neuromodulatory projections in the mammalian cerebral cortex: Comparison between cholinergic and noradrenergic systems. *Frontiers in Neural Circuits*, 12(June), 1–13. <https://doi.org/10.3389/fncir.2018.00047>
- Rio, J. A. Del, Heimrich, B., Borrell, V., Forstert, E., Drakewt, A., Alcantara, S., ... Soriano, E. (1997). A role for Cajal-Retzius cells and reelin in the development of hippocampal connections, 385(January), 787–790.
- Riva, M., Genescu, I., Habermacher, C., Orduz, D., Ledonne, F., Rijli, F. M., ... Pierani, A. (2019). Activity-dependent death of transient cajal-retzius neurons is required for functional cortical wiring. *ELife*, 8(i), 1–18. <https://doi.org/10.7554/eLife.50503>
- Roth, M. M., Dahmen, J. C., Muir, D. R., Imhof, F., Martini, F. J., & Hofer, S. B. (2016). Thalamic nuclei convey diverse contextual information to layer 1 of visual cortex. *Nature Neuroscience*, 19(2), 299–307. <https://doi.org/10.1038/nn.4197>
- Rubenstein, J. L. R., & Beachy, P. A. (1998). Patterning of the embryonic forebrain John LR Rubenstein* and Philip A Beachyt. *Current Opinion in Neurobiology*, 8, 18–26. Retrieved from <http://biomednet.com/elecref/0959438800800018>
- Rubin, A. N., & Kessar, N. (2013). PROX1: A Lineage Tracer for Cortical Interneurons Originating in the Lateral/Caudal Ganglionic Eminence and Preoptic Area. *PLoS ONE*, 8(10). <https://doi.org/10.1371/journal.pone.0077339>
- Rubio-Garrido, P., Pérez-De-Manzo, F., Porrero, C., Galazo, M. J., & Clascá, F. (2009). Thalamic input to distal apical dendrites in neocortical layer 1 is massive and highly convergent. *Cerebral Cortex*, 19(10), 2380–2395. <https://doi.org/10.1093/cercor/bhn259>
- Rudolph, J., Zimmer, G., Steinecke, A., Barchmann, S., & Bolz, J. (2010). Ephrins guide migrating cortical interneurons in the basal telencephalon. *Cell Adhesion and Migration*, 4(3), 400–408. <https://doi.org/10.4161/cam.4.3.11640>
- Ruijter, J. M., Baker, R. E., De Jong, B. M., & Romijn, H. J. (1991). Chronic blockade of bioelectric activity in neonatal rat cortex grown in vitro: Morphological effects. *International Journal of Developmental Neuroscience*, 9(4). [https://doi.org/10.1016/0736-5748\(91\)90054-P](https://doi.org/10.1016/0736-5748(91)90054-P)
- Ruiz-Reig, N., Andrés, B., Huilgol, D., Grove, E. A., Tissir, F., Tole, S., ... Fairén, A. (2017). Lateral Thalamic Eminence: A Novel Origin for mGluR1/Lot Cells. *Cerebral Cortex*, 27(5), 2841–2856. <https://doi.org/10.1093/cercor/bhw126>

-S-

- Sahara, S., Kawakami, Y., Belmonte, J. C. I., & O’Leary, D. D. M. (2007). Sp8 exhibits reciprocal induction with Fgf8 but has an opposing effect on anterior-posterior cortical area patterning. *Neural Development*, 2(1), 1–22. <https://doi.org/10.1186/1749-8104-2-10>
- Sakai, K., Shoji, H., Kohno, T., Miyakawa, T., & Hattori, M. (2016). Mice that lack the C-Terminal region of Reelin exhibit behavioral abnormalities related to neuropsychiatric disorders. *Scientific Reports*, 6(March), 1–13. <https://doi.org/10.1038/srep28636>

References

- Sato, Y., Hirata, T., Ogawa, M., & Fujisawa, H. (1998). Requirement for early-generated neurons recognized by monoclonal antibody lot1 in the formation of lateral olfactory tract. *Journal of Neuroscience*, *18*(19), 7800–7810. <https://doi.org/10.1523/jneurosci.18-19-07800.1998>
- Sava, B. A., Dávid, C. S., Teissier, A., Pierani, A., Staiger, J. F., Luhmann, H. J., & Kilb, W. (2010). Electrophysiological and morphological properties of Cajal-Retzius cells with different ontogenetic origins. *Neuroscience*, *167*(3), 724–734. <https://doi.org/10.1016/j.neuroscience.2010.02.043>
- Schmidt, E., Zhao, H., Hillman, E., & Polleux, F. (2019). Humanization of SRGAP2C expression increases cortico-cortical connectivity and reliability of sensory-evoked responses in the mouse brain. <https://doi.org/10.1101/852970>
- Schuman, B., Machold, R. P., Hashikawa, Y., Fuzik, J., Fishell, G. J., & Rudy, B. (2019). Four unique interneuron populations reside in neocortical layer 1. *Journal of Neuroscience*, *39*(1), 125–139. <https://doi.org/10.1523/JNEUROSCI.1613-18.2018>
- Sekine, K., Kawachi, T., Kubo, K. I., Honda, T., Herz, J., Hattori, M., ... Nakajima, K. (2012). Reelin Controls Neuronal Positioning by Promoting Cell-Matrix Adhesion via Inside-Out Activation of Integrin $\alpha 5\beta 1$. *Neuron*, *76*(2), 353–369. <https://doi.org/10.1016/j.neuron.2012.07.020>
- Sekine, K., Kubo, K., & Nakajima, K. (2014). How does Reelin control neuronal migration and layer formation in the developing mammalian neocortex? *Neuroscience Research*, *86*, 50–58. <https://doi.org/10.1016/j.neures.2014.06.004>
- Sessa, A., Mao, C. A., Colasante, G., Nini, A., Klein, W. H., & Broccoli, V. (2008). Tbr2-positive intermediate (basal) neuronal progenitors safeguard cerebral cortex expansion by controlling amplification of pallial glutamatergic neurons and attraction of subpallial GABAergic interneurons. *Genes and Development*, *506*(16), 16–29. <https://doi.org/10.1101/gad.575410>
- Shen, K., & Cowan, C. W. (2010). Plasticity, 1–18.
- Silva, C. G., Peyre, E., Adhikari, M. H., Tielens, S., Tanco, S., Van Damme, P., ... Nguyen, L. (2018). Cell-Intrinsic Control of Interneuron Migration Drives Cortical Morphogenesis. *Cell*, *172*(5), 1063–1078.e19. <https://doi.org/10.1016/j.cell.2018.01.031>
- Singh, S. K., Stogsdill, J. A., Pulimood, N. S., Dingsdale, H., Kim, Y. H., Pilaz, L. J., ... Eroglu, C. (2016). Astrocytes Assemble Thalamocortical Synapses by Bridging NRX1 α and NL1 via Hevin. *Cell*, *164*(1–2), 183–196. <https://doi.org/10.1016/j.cell.2015.11.034>
- Soda, T., Nakashima, R., Watanabe, D., Nakajima, K., Pastan, I., & Nakanishi, S. (2003). Segregation and coactivation of developing neocortical layer 1 neurons. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *23*(15), 6272–6279. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12867512> http://www.ncbi.nlm.nih.gov/pubmed/12867512?ordinalpos=23&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_DefaultReportPanel.Pubmed_RVDocSum
- Soriano, E., & Del Río, J. A. (2005). The cells of cajal-retzius: Still a mystery one century after. *Neuron*, *46*(3), 389–394. <https://doi.org/10.1016/j.neuron.2005.04.019>
- Southwell, D. G., Paredes, M. F., Galvao, R. P., Jones, D. L., Froemke, R. C., Sebe, J. Y., ... Alvarez-Buylla, A. (2012). Intrinsically determined cell death of developing cortical interneurons. *Nature*, *491*(7422), 109–113. <https://doi.org/10.1038/nature11523>
- Squarzonni, P., Thion, M. S., & Garel, S. (2015). Neuronal and microglial regulators of cortical wiring: Usual and novel guideposts. *Frontiers in Neuroscience*, *9*(JUN). <https://doi.org/10.3389/fnins.2015.00248>
- Suárez, R., Fenlon, L. R., Marek, R., Avitan, L., Sah, P., Goodhill, G. J., & Richards, L. J. (2014). Balanced interhemispheric cortical activity is required for correct targeting of the corpus callosum. *Neuron*, *82*(6), 1289–1298. <https://doi.org/10.1016/j.neuron.2014.04.040>
- Sultan, K. T., Brown, K. N., Shi, S.-H., Dicristo, G., & Studer, M. (2013). Production and organization of neocortical interneurons, *1119*(21), 38–1.

References

- <https://doi.org/10.3389/fncel.2013.00221>
- Sun, L., Chen, R., Bai, Y., Li, J., Wu, Q., Shen, Q., & Wang, X. (2019). Morphological and Physiological Characteristics of Ebf2-EGFP-Expressing Cajal-Retzius Cells in Developing Mouse Neocortex. *Cerebral Cortex (New York, N.Y. : 1991)*, 29(9), 3864–3878. <https://doi.org/10.1093/cercor/bhy265>
- Super, H. (2000). Disruption of Neuronal Migration and Radial Glia in the Developing Cerebral Cortex Following Ablation of Cajal-Retzius Cells. *Cerebral Cortex*, 10(6), 602–613. <https://doi.org/10.1093/cercor/10.6.602>
- Supèr, H., Martínez, A., Del Río, J. A., & Soriano, E. (1998). Involvement of distinct pioneer neurons in the formation of layer-specific connections in the hippocampus. *Journal of Neuroscience*, 18(12), 4616–4626. <https://doi.org/10.1523/jneurosci.18-12-04616.1998>
- Suzuki, M., & Larkum, M. E. (2020). General Anesthesia Decouples Cortical Pyramidal Neurons. *Cell*, 180(4), 666–676.e13. <https://doi.org/10.1016/j.cell.2020.01.024>
- T-
- Takahashi, N., Oertner, T. G., Hegemann, P., & Larkum, M. E. (2016). Modulate Perception, 354(6319), 1159–1165. <https://doi.org/10.1126/science.aah6066>
- Takata, N., & Hirase, H. (2008). Cortical layer 1 and layer 2/3 astrocytes exhibit distinct calcium dynamics in vivo. *PLoS ONE*, 3(6). <https://doi.org/10.1371/journal.pone.0002525>
- Takesian, A. E., Bogart, L. J., Lichtman, J. W., & Hensch, T. K. (2018). Inhibitory circuit gating of auditory critical-period plasticity. *Nature Neuroscience*, 21(2), 218–227. <https://doi.org/10.1038/s41593-017-0064-2>
- Tamamaki, N., Fujimori, K. E., & Takauji, R. (1997). Origin and route of tangentially migrating neurons in the developing neocortical intermediate zone. *Journal of Neuroscience*, 17(21), 8313–8323. <https://doi.org/10.1523/jneurosci.17-21-08313.1997>
- Tanaka, D. H., & Nakajima, K. (2012). Migratory pathways of GABAergic interneurons when they enter the neocortex. *European Journal of Neuroscience*, 35(11), 1655–1660. <https://doi.org/10.1111/j.1460-9568.2012.08111.x>
- Tanaka, Y. H., Tanaka, Y. R., Kondo, M., Terada, S. I., Kawaguchi, Y., & Matsuzaki, M. (2018). Thalamocortical Axonal Activity in Motor Cortex Exhibits Layer-Specific Dynamics during Motor Learning. *Neuron*, 100(1), 244–258.e12. <https://doi.org/10.1016/j.neuron.2018.08.016>
- Taniguchi, H., Lu, J., & Huang, Z. J. (2013). The spatial and temporal origin of chandelier cells in mouse neocortex. *Science*, 339(6115), 70–74. <https://doi.org/10.1126/science.1227622>
- Tasic, B., Menon, V., Nguyen, T. N., Kim, T. K., Jarsky, T., Yao, Z., ... Zeng, H. (2016). Adult mouse cortical cell taxonomy revealed by single cell transcriptomics. *Nature Neuroscience*, 19(2), 335–346. <https://doi.org/10.1038/nn.4216>
- Taverna, E., Götz, M., & Huttner, W. B. (2014). *The cell biology of neurogenesis: toward an understanding of the development and evolution of the neocortex. Annual review of cell and developmental biology* (Vol. 30). <https://doi.org/10.1146/annurev-cellbio-101011-155801>
- Tawa, P., Hell, K., Giroux, A., Grimm, E., Han, Y., Nicholson, D. W., & Xanthoudakis, S. (2004). Catalytic activity of caspase-3 is required for its degradation: Stabilization of the active complex by synthetic inhibitors. *Cell Death and Differentiation*, 11(4), 439–447. <https://doi.org/10.1038/sj.cdd.4401360>
- Tissir, F., De Rouvroit, C. L., Sire, J. Y., Meyer, G., & Goffinet, A. M. (2003). Reelin expression during embryonic brain development in *Crocodylus niloticus*. *Journal of Comparative Neurology*, 457(3), 250–262. <https://doi.org/10.1002/cne.10573>
- Tissir, F., Fadel, & Goffinet, A. M. (2003). Reelin and brain development. *Nature Reviews Neuroscience*, 4(6), 496–505. <https://doi.org/10.1038/nrn1113>
- Tissir, F., Fadel, Ravni, A., Achouri, Y., Riethmacher, D., Meyer, G., & Goffinet, A. M. (2009). DeltaNp73 regulates neuronal survival in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, 106(39), 16871–16876.

References

- <https://doi.org/10.1073/pnas.0903191106>
- Tjia, M., Yu, X., Jammu, L. S., Lu, J., & Zuo, Y. (2017). Pyramidal neurons in different cortical layers exhibit distinct dynamics and plasticity of apical dendritic spines. *Frontiers in Neural Circuits*, 11(June), 1–10. <https://doi.org/10.3389/fncir.2017.00043>
- Tolonen, M., Palva, J. M., Andersson, S., & Vanhatalo, S. (2007). Development of the spontaneous activity transients and ongoing cortical activity in human preterm babies. *Neuroscience*, 145(3), 997–1006. <https://doi.org/10.1016/j.neuroscience.2006.12.070>
- Tremblay, R., Lee, S., & Rudy, B. (2016). GABAergic Interneurons in the Neocortex: From Cellular Properties to Circuits. *Neuron*, 91(2), 260–292. <https://doi.org/10.1016/j.neuron.2016.06.033>
- Trommsdorff, M., Gotthardt, M., Hiesberger, T., Shelton, J., Stockinger, W., Nimpf, J., ... Herz, J. (1999). Reeler/disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. *Cell*, 97(6), 689–701. [https://doi.org/10.1016/S0092-8674\(00\)80782-5](https://doi.org/10.1016/S0092-8674(00)80782-5)

-V-

- Valverde, F., De Carlos, J. A., & López-Mascaraque, L. (1995). Time of origin and early fate of preplate cells in the cerebral cortex of the rat. *Cerebral Cortex*, 5(6), 483–493. <https://doi.org/10.1093/cercor/5.6.483>
- Valverde, F., & Facal-Valverde, M. V. (1988). Postnatal development of interstitial (subplate) cells in the white matter of the temporal cortex of kittens: A correlated Golgi and electron microscopic study. *Journal of Comparative Neurology*, 269(2), 168–192. <https://doi.org/10.1002/cne.902690203>
- Villar-Cerviño, V., Molano-Mazón, M., Catchpole, T., Valdeolillos, M., Henkemeyer, M., Martínez, L. M., ... Marín, O. (2013). Contact Repulsion Controls the Dispersion and Final Distribution of Cajal-Retzius Cells. *Neuron*, 77(3), 457–471. <https://doi.org/10.1016/j.neuron.2012.11.023>
- Vitali, I., Fièvre, S., Telley, L., Oberst, P., Bariselli, S., Frangeul, L., ... Jabaudon, D. (2018). Progenitor Hyperpolarization Regulates the Sequential Generation of Neuronal Subtypes in the Developing Neocortex. *Cell*, 174(5), 1264–1276.e15. <https://doi.org/10.1016/j.cell.2018.06.036>
- Vitalis, T., Ansorge, M. S., & Dayer, A. G. (2013). Serotonin homeostasis and serotonin receptors as actors of cortical construction: Special attention to the 5-HT3A and 5-HT6 receptor subtypes. *Frontiers in Cellular Neuroscience*, 7(JUNE), 1–20. <https://doi.org/10.3389/fncel.2013.00093>
- Vitalis, T., & Verney, C. (2016). Sculpting Cerebral Cortex with Serotonin in Rodent and Primate Tania. In *Intech* (Vol. i, p. 13). <https://doi.org/http://dx.doi.org/10.5772/57353>

-W, X, Y, Z-

- Wamsley, B., & Fishell, G. (2017). Genetic and activity-dependent mechanisms underlying interneuron diversity. *Nature Reviews Neuroscience*, 18(5), 299–309. <https://doi.org/10.1038/nrn.2017.30>
- Wang, Z., Hong, Y., Zou, L., Zhong, R., Zhu, B., Shen, N., ... Miao, X. (2014). Reelin gene variants and risk of autism spectrum disorders: An integrated meta-analysis. *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics*, 165(2), 192–200. <https://doi.org/10.1002/ajmg.b.32222>
- Weissman, T. A., Riquelme, P. A., Ivic, L., Flint, A. C., & Kriegstein, A. R. (2004). Calcium waves propagate through radial glial cells and modulate proliferation in the developing neocortex. *Neuron*, 43(5), 647–661. <https://doi.org/10.1016/j.neuron.2004.08.015>
- Whitford, K. L., Dijkhuizen, P., Polleux, F., & Ghosh, A. (2002). Molecular Control of Cortical Dendrite Development. *Annual Review of Neuroscience*, 25(1), 127–149. <https://doi.org/10.1146/annurev.neuro.25.1.12701.142932>
- Williams, L. E., & Holtmaat, A. (2018). Higher-Order Thalamocortical Inputs Gate Synaptic Long-Term Potentiation via Disinhibition. *Neuron*, 1–12.

References

- <https://doi.org/10.1016/j.neuron.2018.10.049>
- Wimmer, V. C., Bruno, R. M., De Kock, C. P. J., Kuner, T., & Sakmann, B. (2010). Dimensions of a projection column and architecture of VPM and POm axons in rat vibrissal cortex. *Cerebral Cortex*, 20(10), 2265–2276. <https://doi.org/10.1093/cercor/bhq068>
- Wonders, C. P., & Anderson, S. A. (2006). The origin and specification of cortical interneurons. *Nature Reviews Neuroscience*, 7(9), 687–696. <https://doi.org/10.1038/nrn1954>
- Wong, F. K., Bercsenyi, K., Sreenivasan, V., Portalés, A., Fernández-Otero, M., & Marín, O. (2018). Pyramidal cell regulation of interneuron survival sculpts cortical networks. *Nature*, 557(7707), 668–673. <https://doi.org/10.1038/s41586-018-0139-6>
- Wong, F. K., & Marín, O. (2019). Developmental Cell Death in the Cerebral Cortex. *Annual Review of Cell and Developmental Biology*, 35(1), 523–542. <https://doi.org/10.1146/annurev-cellbio-100818-125204>
- Xu, Q., Tam, M., & Anderson, S. A. (2008). Fate Mapping Nkx2.1-Lineage Cells in the Mouse Telencephalon. *Journal of Comparative Neurology*, 506(October 2007), 16 –29. <https://doi.org/10.1002/cne>
- Yamaguchi, Y., & Miura, M. (2015). Programmed cell death in neurodevelopment. *Developmental Cell*, 32(4), 478–490. <https://doi.org/10.1016/j.devcel.2015.01.019>
- Yoshida, M., Assimacopoulos, S., Jones, K. R., & Grove, E. A. (2006). Massive loss of Cajal-Retzius cells does not disrupt neocortical layer order. *Development*, 133(3), 537–545. <https://doi.org/10.1242/dev.02209>
- Zembrzycki, A., Griesel, G., Stoykova, A., & Mansouri, A. (2007). Genetic interplay between the transcription factors Sp8 and Emx2 in the patterning of the forebrain. *Neural Development*, 2(1), 1–18. <https://doi.org/10.1186/1749-8104-2-8>
- Zhang, W., & Bruno, R. M. (2019). High-order thalamic inputs to primary somatosensory cortex are stronger and longer lasting than cortical inputs. *ELife*, 8, 1–22. <https://doi.org/10.7554/eLife.44158>

RÉSUMÉ

Le cortex cérébral contrôle des fonctions complexes comme la perception sensorielle, le comportement moteur ou la cognition par le biais de circuits très organisés. Ces circuits se développent dans l'embryon et les mauvais câblages sont liés à l'étiologie de troubles neurodéveloppementaux comme l'Autisme ou la Schizophrénie. La couche la plus superficielle du cortex, la couche 1 (L1), joue un rôle central dans le fonctionnement du cerveau. Elle permet l'intégration des informations de la périphérie par des stimuli internes, ce qui façonnent notre perception. Bien qu'il soit de plus en plus évident que la L1 joue un rôle important dans l'intégration sensorielle, les connaissances sur sa formation sont limitées. Le câblage de L1 est modelé par la densité des cellules de Cajal-Retzius (CRc), une population transitoire de neurones corticaux, qui façonnent les circuits corticaux sous-jacents. Cependant, il reste à déchiffrer comment la densité et l'élimination des CRc sont régulées et si les CRc sont essentielles au câblage cortical. Ici, nous avons démontré que i) la densité des CRc est étroitement maintenue pendant le développement et n'est pas affectée par l'activité sensorielle précoce, ii) l'élimination de sous-populations de CRc est activité-dépendante et iii) les perturbations de la densité et la mort des CRc ont des conséquences à long-terme sur le câblage des circuits sous-jacents. Ces travaux permettent de mieux comprendre les rôles d'une population neuronale transitoire dans la régulation du câblage d'une couche essentielle mais encore peu étudiée du néocortex. Cela permet aussi de comprendre comment les CRc soutiennent la construction du néocortex dans des conditions physiologiques, et comment elles pourraient contribuer aux mauvais câblages menant à différents troubles neurodéveloppementaux.

MOTS CLÉS

Cellules de Cajal-Retzius, couche 1 du néocortex, activité neuronale, balance E/I, interneurones, dendrites apicales

ABSTRACT

The cerebral cortex controls complex functions like sensory perception, motor behavior or cognition via highly organized circuits. These circuits develop in the embryo and miswirings are linked to the etiology of neurodevelopmental disorders like Autism Spectrum Disorder or Schizophrenia. The most superficial layer of the cortex, layer 1 (L1), is playing a central role in brain function. It enables the integration of inputs from the periphery with internal stimuli, shaping our perception. Although there is increasing evidence that L1 plays important roles in sensory integration, there is limited knowledge about its formation. L1 wiring is regulated by the density of transient inhabitants, the Cajal-Retzius cells, a population of cortical neurons, which shape underlying cortical circuits. However, how CRc density and elimination are regulated and whether CRc are key for cortical wiring remained to be deciphered. Here, we have shown that i) the density of CRc is tightly maintained during development and is not impacted by early sensory activity, ii) the elimination of subsets of CRc is activity dependent and iii) impairments in both density and death of CRc have long lasting consequences on the wiring of the underlying circuits. This work provides a better understanding of the roles of a transient neuronal population in regulating the wiring of an essential but understudied layer of the neocortex. This is instrumental in understanding how CRc sustain neocortex construction in physiological conditions, and how they could contribute to miswirings leading to different neurodevelopmental disorders.

KEYWORDS

Cajal-Retzius cells, Cortical layer 1, neuronal activity, E/I balance, interneurons, apical dendrites