THE CLOËTTA PRIZE 2017
IS AWARDED TO

PROFESSOR

DENIS JABAUDON

BORN IN 1971 IN VEVEY, SWITZERLAND
DIRECTOR OF THE NEUROCENTER OF THE UNIVERSITY
OF GENEVA

FOR HIS GROUND BREAKING FINDINGS ABOUT GENETIC
MECHANISMS GOVERNING ASSEMBLY AND
FUNCTIONAL INTEGRATION OF CORTICAL AND THALAMIC
NEURONS INTO NEURONAL CIRCUITS

Zürich, 3th November 2017

IN THE NAME OF THE FOUNDATION BOARD:

THE PRESIDENT

THE VICE PRESIDENT

MEMBER

Denis Jabaudon

BIOGRAPHY

Name: Jabaudon, Denis
Date of Birth: 30th January 1971
Place of Birth: Vevey, Switzerland

Education

1989–1995	Medical School	University of Lausanne
1996–1999	MD-PhD Scholar	Brain Research Institute University of Zürich

Positions

1999–2004	Neurology Residency	Lausanne and Geneva University Hospitals
2004–2008	Postdoctoral Fellow	Harvard Medical School Boston, Massachusetts, USA
2008–2009	Junior Research Fellow (Chef de Clinique Scientifique)	Dept. of Basic Neurosciences University of Geneva
2008–	Attending Physician	Clinic of Neurology Geneva University Hospital
2009–2015	SNSF Assistant Professor	Dept. of Basic Neurosciences University of Geneva
2015–	Full Professor	Dept. of Basic Neurosciences University of Geneva

Denis Jabaudon obtained his MD-PhD degree at the Universities of Lausanne and Zurich in Switzerland, where he studied mechanisms controlling synaptic transmission in the laboratory of Prof. Beat Gähwiler. After a neurology residency at Geneva University Hospital, he completed a postdoctoral fellowship at Harvard University, in the laboratory of Prof. J. Macklis, where he began investigating the genetic mechanisms controlling cortical development.

He is currently a professor at the University of Geneva, Switzerland, since 2009, where he has his independent research group, and also practices as a clinical neurologist at Geneva University Hospital.

His work on the genetics of neuronal circuit assembly during cortical development has earned him several prestigious prizes, including the Freedman Prize for Exceptional Basic Research from the Brain and Behavior Research Foundation (NARSAD), the Pfizer Research Prize, and the Bing Prize from the Swiss Academy of Medical Science. Prof. Jabaudon is currently the Director of the Geneva University Neurocenter; he is a member of the FENS Kavli Network of Excellence and his work is funded by the Swiss National Science Foundation (Project and Consolidator Grants) and the Brain and Behavior Research Foundation.

Work in the Jabaudon laboratory is aimed at understanding how genetic and input-dependent mechanisms interact to control neuronal circuit assembly during development. The approaches his team uses to address these questions include the isolation and genetic characterization of single forebrain neurons subtypes, in vivo genetic manipulations during development and optogenetic interrogation of developing circuits.

The long-term aim of his research is to understand how altered environmental conditions and abnormal gene expression interact to lead to circuit miswiring and behavioral changes in neurodevelopmental and psychiatric disorders.

SELECTED PUBLICATIONS

1. Jabaudon D. Fate and freedom in developing neocortical circuits. Nature Communications 2017. doi: 10.1038/ncomms16042. Review.

2. Frangeul* L, Pouchelon* G, Telley* L, Lefort S, Luscher C, Jabaudon D. A cross-modal genetic framework for the development and plasticity of sensory pathways. Nature 2016, 538: 96-98. doi: 10.1038/nature19770. *equal contributors.

3. Telley* L, Govindan* S, Stevant I, Prados J, Dermitzakis E, Nef S, Dayer A, Jabaudon D. Sequential transcriptional waves direct early neuronal differentiation in the mouse neocortex. Science 2016; 351(6280):1443–6. doi: 10.1126/science.aad8361. *equal contributors.

4. Pouchelon G, Golding B, Bellone C, Gambino F, Lüscher C, Holtmaat A, Jabaudon D. Modalityspecific thalamocortical inputs instruct the identity of postsynaptic L4 neurons. Nature 2014, 511(7510): 471–474. doi: 10.1038/nature13390

5. Golding B, Pouchelon G, Bellone C, Di Nardo A, Lüscher C, Shimogori T, Dayer A, Jabaudon D. Retinal activity directs the recruitment of inhibitory interneurons into thalamic visual circuits. Neuron 2014; 81(5): 1057–69. doi: 10.1016/j.neuron.2014.01.032

6. De La Rossa A, Bellone C, Golding B, Moss J, Toni N, Lüscher C, Jabaudon D. In vivo reprogramming of circuit connectivity in postmitotic neocortical neurons. Nature Neuroscience 2013; 16(2): 193–200. doi: 10.1038/nn.3299

FATE AND FREEDOM IN THE DEVELOPING NEOCORTEX

Denis Jabaudon

Summary

The activity of neuronal circuits of the neocortex underlies our ability to perceive the world and interact with our environment. During development, these circuits emerge from dynamic interactions between cell-intrinsic, genetically determined programs, and input/activity-dependent signals, which together shape these circuits into adulthood. Over the past decade or so, technological developments have progressively allowed us to interrogate these nature-nurture interactions with single gene / single input / single cell resolution. In this review, I will discuss some of the genetic and input-dependent mechanisms controlling how individual cortical neurons differentiate into specialized cells to form neuronal circuits and highlight, when appropriate, the contributions we made to this global effort. This monograph is closely adapted from a review I recently published on this topic (Jabaudon 2017).

Introduction

The neuronal circuits of the neocortex underlie our ability to perceive the world and conduct meaningful interactions with our surroundings. Neocortical circuits, through their activity, account for processes such as sensory perception and integration, sensory-motor transformation, motor planning and execution, long-term memory, and attention. These circuits are formed by a diversity of specialized neuronal subtypes, which can be distinguished from each other by anatomical, morphological, physiological, hodological (i.e. relating to connectivity) and genetic features.

Neocortical circuits are both robust and flexible: they reliably carry out complex repetitive tasks, yet are also able to modify the execution of these tasks in response to context and previous experience. To accommodate the seemingly opposing constraints of reliability and plasticity, at least two main driving forces are at play during development: (1) *genetically determined processes,* which act within single cells and allow the generation and differentiation of a core set of specialized neuronal cell types, and (2) non cell-autonomous, *input/activity dependent processes,* which act during critical periods of development to refine these neurons into further subtypes, allowing neural circuit diversification and context-dependent expansion of the behavioral repertoire. Balance between these intrinsic developmental programs and external signals is essential for the proper differentiation and assembly of neurons into circuits, yet the dynamic contribution of these two types of processes to cortical development remains unclear.

Over the past decade or so, my laboratory has been interested in teasing out how genetic and circuit-derived factors bidirectionally interact during development to give rise to the neuronal and circuit diversity found in the adult neocortex. In the current monograph, which is closely transcribed and adapted from a review I recently published on this topic (Jabaudon 2017), I will first provide an introduction on the cerebral cortex and its cellular diversity, then introduce how different types of input modulate cortical neuron differentiation. Throughout this narrative, as per the tradition of the Max Cloëtta Series, I will highlight some contributions of my laboratory to the understanding of these processes.

The neocortex is organized in layers and areas

The neocortex consists in a thick sheet of neurons which covers the surface of both hemispheres in mammals **(Fig. 1).** As introduced above, this structure is the place where sensory inputs converge to generate our conscious perception of the outside world, and where voluntary motor actions are planned and initiated. The neocortex is radially organized into layers, which each are enriched in specialized subtypes of neurons, and tangentially organized into areas, which specialize in diverse sensory, motor, and associative functions **(Fig. 1).** There are six main layers in the neocortex, which have historically been defined by distinct densities of neuronal somas, dendrites and axons. These laminae are not only anatomical landmarks, but actually consist of developmentally and functionally distinct subtypes of glutamatergic neurons **(Fig. 1)** (Jabaudon 2017; Molyneaux et al. 2007; Harris and Shepherd 2015).

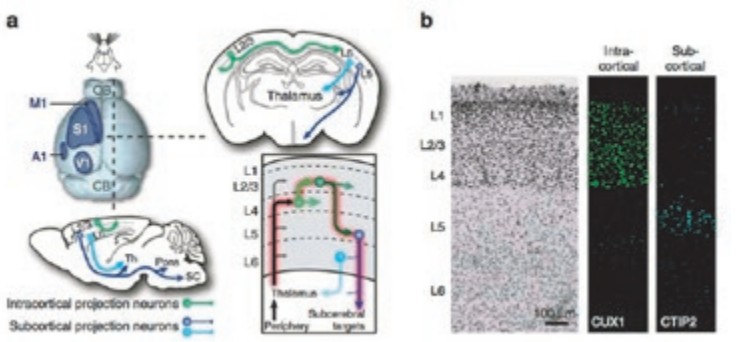

Figure 1: Areal and laminar organization of the neocortex. *(a) Schematic representation of the distinct primary cortical areas in the mouse, and cell-type specific connectivity of cortical projection neurons. A1: primary auditory cortex, CB: Cerebellum, M1 primary motor cortex, S1: primary somatosensory cortex, SC: spinal cord, Th: Thalamus, V1: primary visual cortex. (b) Laminar organization of the neocortex (S1). CUX1 specifically labels intracortical projection neurons while CTIP2 labels corticospinal neurons in layer (L) 5. Taken from Jabaudon, 2017.*

The deepest cortical layers contain neurons whose axons target subcortical structures such as the thalamus (corticothalamic neurons, layer (L) 6) and the tectum, hindbrain and spinal cord ("corticospinal" neurons, L5). In contrast, neurons located more superficially in layers 2 and 3

(L2/3), and 4 (L4) have intracortical axonal targets. Neurons in L4 (also called the "granular layer") differ from the neurons present in other layers in that they are locally-projecting glutamatergic interneurons, i.e. they do not send long-range projections. They are the main targets of neurons in exteroceptive sensory thalamic nuclei (i.e. which receive input from the sense organs) (Petreanu et al. 2009; Erzurumlu, Murakami, and Rijli 2010; Vitali and Jabaudon 2014), and, as such, form the principal sensory gateway to the neocortex. L4 neurons are particularly sensitive to impairments of the sensory organs or their input pathways; as will be discussed in detail later, this is particularly striking in the rodent somatosensory cortex. Within this cortical area, whisker-input receiving L4 neurons are clustered into distinct cellular assemblies called "barrels", which each receive input from a single principal whisker, and are somatotopically distributed such that neighboring barrels receive input from neighboring whiskers (Vitali and Jabaudon 2014; Erzurumlu, Murakami, and Rijli 2010; Pouchelon and Jabaudon 2014). As will be discussed below, in several of my laboratory's research projects, we are taking advantage of the exquisite sensitivity of L4 neurons to input to study the role of activity-dependent signals in neuronal differentiation and circuit formation (see e.g. Pouchelon et al. 2014; Frangeul et al. 2014; Rossa et al. 2013).

In addition to excitatory glutamatergic neurons, the neocortex contains another population of neurons, which are inhibitory, use another transmitter, GABA, and are born from distinct germinal zones (i.e. proliferative regions where progenitor are located) than excitatory neurons (see below). The diverse populations of GABAergic interneurons play a pivotal role in the gating and spread of cortical signals through processes such as feedforward inhibition, dis-inhibition and feedback inhibition.

Cortical areas

Layers are not homogenous across the rostro-caudal and latero-medial extent of the neocortex. Instead, local cytoarchitecture varies in an often discontinuous way across the tangential surface of the cortex, defining distinct cortical areas. Histological discontinuities are particularly striking in species with large cortices, and form the basis of the classical Brodmann classification of cortical areas (Zilles and Amunts 2010).

Each cortical area is reciprocally connected with a defined subset of inputs from the thalamus, a structure which relays inputs from the different sense organs (e.g. skin mechanoreceptors, retinal photoreceptors, auditory cells in the cochlea, Fig. 2a). Frontally-located cortical areas are connected with frontally-located thalamic nuclei, including those involved in motor planning and execution, while parieto-occipital and temporal cortical areas are interconnected with more posterior thalamic nuclei, and are involved in sensory perception and integration (López-Bendito and Molnár 2003; Clascá, Rubio-Garrido, and Jabaudon 2012). Delineations between individual cortical areas are particularly sharp in areas receiving input from exteroceptive thalamic nuclei, such as the primary somatosensory, visual, and auditory cortices. Because of their characteristic cytoarchitectural features, these primary sensory areas, and particularly the primary somatosensory (S1) and visual (V1) areas have been extensively used as model systems to study the role of input on cortical differentiation. As a consequence, our understanding of thalamocortical organization and information flow is largely based on the connectivity of primary sensory areas, and particularly somatosensory and visual areas, (Frangeul et al. 2016; Kral 2013). However, these areas only represent a small fraction of the total cortical surface, and different connectivities and information flow exist in other cortical areas and thalamic nuclei, as will be detailed in the next section. A significant part of the work in my laboratory has focused on trying to better characterize the relationship between primary exteroceptive regions and secondary, associative regions, and on trying to understand how input acts to shape secondary regions into primary ones, as discussed later in the text (see e.g. Pouchelon et al. 2014; Frangeul et al. 2016).

Cortical information flow

Sensation starts with detection of stimulus through activation of peripheral receptors, such as skin mechanoreceptors or retinal photoreceptors. Input from these receptors reaches neurons located in exteroceptive, "first order" thalamic nuclei such as the ventrobasalis nucleus (VB, for tactile stimuli), the dorsolateral geniculate nucleus (LG, for visual stimuli), and the ventral medial geniculate nucleus (vMG, for auditory stimuli). Sensory information then reaches primary sensory areas of the neocortex,

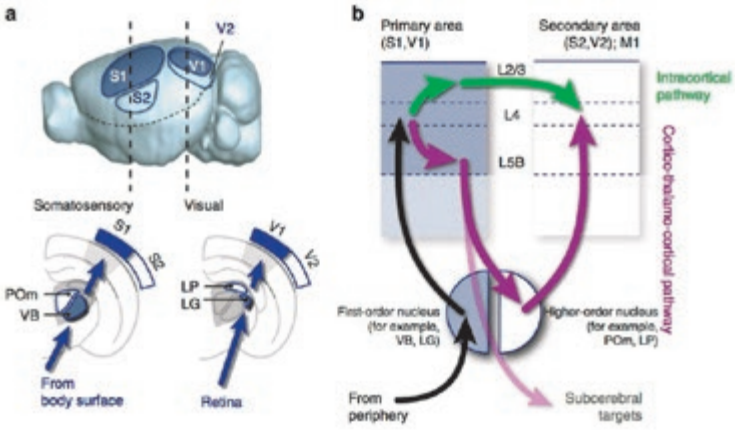

Figure 2: Cortical information flow. (a) *Exteroceptive, first-order thalamic nuclei VB, LG (filled in blue) project to primary cortical areas (S1, V1). Higher order thalamic nuclei and secondary cortical areas are outlined in blue. POm: posteromedial thalamic nucleus; LG: dorsolateral geniculate nucleus; LP: lateroposterior nucleus; VB: ventrobasalis nucleus. (b) Two distinct pathways allow inter-areal communications: an intracortical pathway (green) and a cortico-thalamo-cortical pathway (purple), originating from L5B corticospinal neurons and which transits through higher-order thalamic nuclei. Taken from Jabaudon, 2017.*

where core stimulus properties are perceived, and is then forwarded to secondary sensory (e.g. S2 and V2) and associational areas where stimulus features are dynamically and multi-modally processed **(Fig. 2a)**.

Within primary sensory areas, first order nuclei project particularly strongly onto L4 neurons, which act as the main entry point of extracortical input (Vitali and Jabaudon 2014). From L4 neurons, information is then split into two parallel streams **(Fig. 2b)**: a "classical", intracortical stream, and a more recently characterized extracortical stream. In the intracortical stream, signals are sent to distinct subtypes of L2/3 intracortical neurons, which project to specific sets of cortical areas, including S2, V2, and M1; in the other, cortico-thalamo-cortical stream, L4 signals are sent to infragranular L5B neurons, which send top-down projections to non-exteroceptive, "higher-order" thalamic nuclei (posteromedial nucleus (POm), from S1; lateroposterior nucleus or pulvinar (LP) from V1), and dorsal medial geniculate nucleus (dMG), from A1). Higher-order

thalamic nuclei in turn project to L4 neurons of secondary sensory (i.e. S2, V2) areas, thus closing a cortico-thalamo-cortical loop (Theyel, Llano, and Sherman 2010; Guillery and Sherman 2002). Higher-order thalamic nuclei do not project exclusively to secondary sensory areas but instead have diffuse connections across many cortical areas (Clascá, Rubio-Garrido, and Jabaudon 2012). As such, they may be involved in the coordination of activity across motor and somatosensory cortices during active sensing, as occurs when mice sweep their whiskers back and forth to generate a tactile representation of their environment.

Interestingly, these two main inter-areal communication pathways have distinct evolutionary histories, since supragranular intracortical projection neurons are a novel acquisition of mammals. In the absence of such intracortical projections, diffuse cortico-thalamo-cortical circuits may have been the main pathway allowing different cortical regions to communicate with one another, as might still be the case in reptiles. Based on results to be discussed below (Pouchelon et al. 2014; Frangeul et al. 2016; Frangeul et al. 2014), we have proposed that by providing a novel pathway to direct information to specific brain areas, supragranular intracortical projection neurons in mammals may have allowed the untethering of cortical function from input-output thalamocortical loops, and emergence of stimulus-dissociated, integrative neocortical functions.

Development of the neocortex

The processes allowing the emergence and functional specialization of cortical circuits start with the genesis of neurons from progenitors and extend into adulthood through experience-dependent developmental processes. Genetic, cell-intrinsic processes, and input/activity-dependent processes thus both play a role in shaping cortical circuits. While input-dependent processes classically occur at later developmental stages, activity and environment are likely to play important roles even early during differentiation, as suggested by recent, unpublished data from our laboratory showing that the bioelectric properties of neuronal progenitors affect their division modes and the type of neurons that they produce. This finding would support earlier findings in which input-dependent controls over progenitor proliferation via thalamocortical afferents oc-

curs, which could in principle contribute to area-specific differences in cytoarchitectures and cell types (Dehay et al. 2001; Rakic, Suñer, and Williams 1991; Zechel, Nakagawa, and Ibáñez 2016).

During embryogenesis, the diverse subtypes of neurons that form cortical circuits are born from a pool of progenitors located deep within the brain, underneath the developing cortex. The neurons that form the distinct layers of the neocortex are sequentially born within two main germinal zones between E10.5 and E18.5 in the mouse: the ventricular zone (VZ) of the dorsal pallium, which gives rise to excitatory glutamatergic neurons (Molyneaux et al. 2007), and a parcellated ventral pallial VZ, including the medial and caudal ganglionic eminences and pre-optic area, which gives rise to distinct subtypes of cortical inhibitory GABAergic interneurons.

Glutamatergic neurons migrate radially into the cortex from the pallial VZ, which they populate in an inside-out manner **(Fig. 3)**. During early corticogenesis (until about E10.5 in mice), VZ progenitors initially self-amplify (at this stage, they are called neuroepithelial cells), and then begin giving rise directly to neurons (at this stage they are referred to as "radial glia"). As corticogenesis proceeds, "direct" neurogenesis decreases; instead, VZ progenitors increasingly generate intermediate progenitors (transit amplifying cells, also called basal intermediate progenitors), which accumulate between the VZ and the developing cortical plate to form an additional germinal zone, the subventricular zone (SVZ), from which most L2/3 neurons are thought to be born (Lui, Hansen, and Kriegstein 2011; Pontious et al. 2008) **(Fig. 3)**.

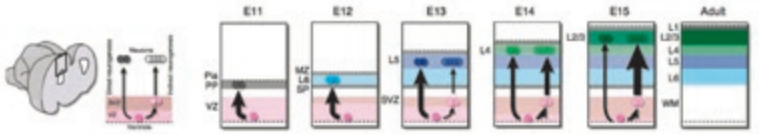

Figure 3: Neurogenic sequence during corticogenesis. *The neocortex is built in an inside-out manner in which neurons born from deeply located germinal zone migrate past earlier-born neurons to reside in more superficial layers. Note that initially, the preplate (PP) is split into a subplate (SP) and superficially located marginal zone (MZ) by incoming L6 neurons, such that early-born neurons are later found in L1. Direct neurogenesis from the ventricular zone (VZ) predominates at early developmental stages, while indirect neurogenesis from the subventricular zone (SVZ) progressively increases during corticogenesis. MZ: marginal zone; PP: preplate; SP: subplate. Taken from Jabaudon, 2017.*

Cortical size depends on the net balance between amplifying divisions, which give rise to new progenitors, and differentiative divisions, which give rise to postmitotic neurons (Florio and Huttner 2014; Dehay and Kennedy 2007). Indirect neurogenesis increases the final number of neurons by amplifying the progenitor pool. This is thought to be a critical step in gyrification, the process through which the neocortex becomes folded in some mammals. This process allows an increase in cortical surface and neuron number within the confined volume of the cranium. The increase in cortical size is particularly striking in supragranular layers (i.e. L2/3), suggesting that cortico-cortical connections increase disproportionately compared to subcortical connections in gyrencephalic species.

An area of intense research is whether fate-restricted VZ progenitors exist (i.e. progenitors which can only give rise to a subset of cortical neurons, as is seen in subpallial proliferative zones), or whether there is a single progenitor type whose competence progresses throughout development (Franco et al. 2012; Eckler et al. 2015). This question has been difficult to investigate because it requires assessing the progeny of single progenitors with clonal resolution in vivo. Progenitors can sequentially give rise to distinct molecularly-defined neuronal cell types in vitro (Gaspard et al. 2008; Shen et al. 2006), and classical transplantation experiments in ferrets support the notion that progenitors can acquire the competence to generate normally later-born, but not earlier-born neurons (McConnell and Kaznowski 1991). Interestingly, this question of the neurogenic plasticity of cortical progenitors has not been reassessed with modern molecular tools and has only been examined in the ferret. The extent to which this principle is generalizable, and the mechanisms at play, remain thus largely unexplored.

The presence of DNA mosaicism in postmitotic neurons, likely resulting from DNA rearrangements immediately following mitosis, represents an additional potential source of neuronal functional diversity (Lodato et al. 2015). Such mosaicism may contribute to inter individual differences in cell types, circuits and behavior, and may be relevant to the broad spectrum of psychiatric disorders. If clinically relevant, diagnosis of such conditions will be challenging since causal mutations are only present in affected neurons and would not be detected by classical methods of DNA

collection, such as buccal swabs or blood samples. In a recent study to be described below, we have identified an increase in an enzyme which repairs DNA damage shortly after cell division, providing additional support for a substantial amount of mosaicism in the developing neocortex (Telley et al. 2016).

Neocortical neuron specification and migration

Once neurons are born, they still have to migrate and mature (i.e. develop their characteristic morphological, molecular and synaptic features). Several transcription factors control the differentiation and function of specific neuronal subpopulations of cortical neurons (Molyneaux et al. 2007), some of which I have contributed to characterize during my postdoctoral fellowship (Lai et al. 2008; Jabaudon et al. 2011). These include FEZF2 (Molyneaux et al. 2005; Chen et al. 2005) and CTIP2 (Arlotta et al. 2005) for L5B corticospinal neurons, and SATB2 for intracortical projection neurons (Alcamo et al. 2008; Britanova et al. 2008). While the initial events that control acquisition of neuron-type specific features following mitosis remain poorly characterized, we have recently shown that early neuronal differentiation is directed by a series of transcriptional waves whose sequence is critical for normal progression through development, as will now be detailed (Telley et al. 2016).

The distinct types of neurons that compose the neocortex have different connectivities but also different transcriptional signatures. While all excitatory neurons are born from progenitor cells located in the ventricular zone, below the cortex, the mechanisms that control the generation and differentiation of distinct neuronal cell types from progenitors are overall poorly understood. A main limitation in understanding these processes has been the inability to identify and isolate pure progenitor / early postmitotic cells, since the ventricular zone is a highly heterogeneous and dynamic region. To circumvent these limitations, we have developed a technology we called FlashTag that enables the isolation and visualization of neurons at the very moment they are born. Using this approach, we have identified transcriptional programs controlling neuronal differentiation (Telley et al. 2016). Upon FlashTag injections into the ventricular space, dividing progenitors are tagged with a fluorescent marker that persists in

their progeny, based on the fact that they undergo mitosis in contact with the ventricular wall **(Fig. 4)**. Thus, nascent cohorts of simultaneously-born neurons can be labeled and isolated for transcriptional analysis using single-cell RNA sequencing (Telley et al. 2016) **(Fig. 5)**. Using this approach, we identified and functionally characterized neuron-specific primordial transcriptional programs as they dynamically unfolded **(Fig. 6)**. Our results revealed early transcriptional waves that instruct the sequence and pace of neuronal differentiation events, guiding newborn neurons toward their final fate. Beyond its contribution to the understanding of early neuronal differentiation events, this work potentially provides a genetic road map to be used for the reverse engineering of specific classes of cortical neurons from undifferentiated cells.

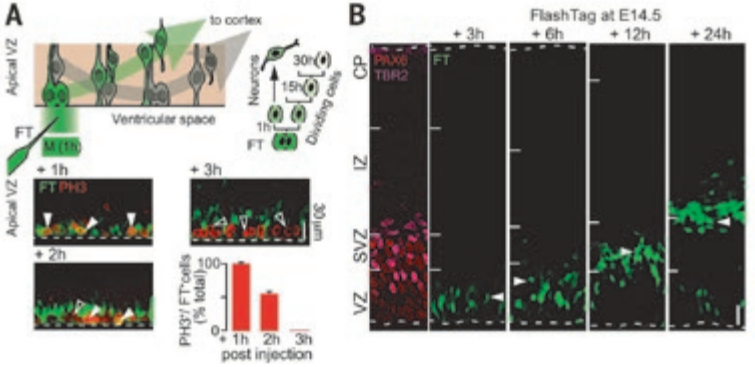

Figure 4: FlashTag (FT) labels time-locked cohorts of newborn cells during corticogenesis. (A) *(Top) Schematic representation of the labeling principle. (Bottom) Pulse-labeling of isochronic mitotic cells using FT at E14.5. PH3, phospho-histone 3, an M-phase marker. (B) Isochronic cohorts of FT positive cells radially migrate from the VZ to the cortex. PAX6 and TBR2 delineate the VZ and SVZ. CP: cortical plate; Cx, cortex; IZ, intermediate zone. Taken from Telley et al., 2016.*

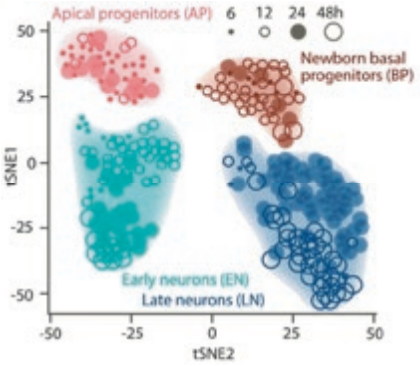

Figure 5: Identification of newborn cortical neurons. *Apical progenitors, daughter basal progenitors, and newborn neurons can be distinguished by unbiased clustering using their transcriptional signatures, obtained through single-cell RNA sequencing. Taken from Telley et al., 2016.*

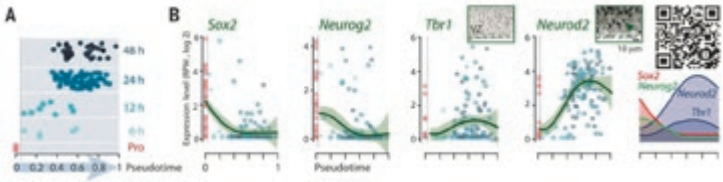

Figure 6: Real-time functional transcriptomics of early postmitotic neurons in vivo. *(A) Genetically-identified neurons are staggered by age along a pseudotime axis, based on their transcriptional similarities. (B) Gene expression dynamics for classical proliferative (Sox2), neurogenic (Neurog2), and neuronal (Tbr1) genes can be identified using this pseudotime alignment. Note for example that Neurod2 is expressed more strongly and earlier than Tbr1, which enables newborn neurons to be identified at earlier time points than previously possible. QR code, http:// genebrowser.unige.ch/science2016, for access to dynamics of all transcripts. Taken from Telley et al., 2016.*

Reprogramming postmitotic neurons

Manipulating gene expression in cortical progenitors can modify their competence to generate specific subtypes of neurons (Molyneaux et al. 2007), but whether neurons can be post-mitotically reprogrammed remained, until recently, unknown. To address this question, in a study published a few years ago (De la Rossa et al. 2013), we sought to reprogram L4 neurons of the mouse neocortex, which receive input connections from the thalamus, into L5B cortical output neurons. For this purpose, we used a gene delivery technique developed in the laboratory (De la Rossa and Jabaudon 2015), which enables rapid expression of select transgenes in postmitotic neurons *in vivo,* to ectopically express a transcription factor specific to layer 5B output neurons, *Fezf2,* into postnatal L4 neurons. *Fezf2* was an ideal candidate to act as an identity switch, because it is both necessary and sufficient to generate L5B neurons during development. Using a combination of *in vivo* and *ex vivo* approaches, including optogenetic interrogation of the reprogrammed circuits, we demonstrated that *Fezf2*-expressing L4 neurons acquire the cardinal molecular, morphological, physiological, and input/output circuit properties of L5B output neurons. A remarkable feature of the reprogrammed cells was that they integrated existing circuits at the correct location (i.e. as expected for normal, naturally *Fezf2*-expressing cells), which we could demonstrate using cell-type specific optogenetic circuit interrogation **(Fig. 7)**. These findings are interesting because they provided a proof-of-principle for the postnatal re-engineering of neuronal circuits *in vivo,* demonstrating for the first time that gene expression dynamically controls the circuit identity of cortical neurons. Furthermore, they revealed a previously unsuspected level of plasticity in postmitotic neurons, which has also been found in other cortical cell types (Rouaux and Arlotta 2013). Thus, following mitosis, neuronal fate becomes progressively restricted rather than irreversibly switched.

The cellular and molecular mechanisms controlling neuron migration from the VZ to the cortex have been well described, in particular with regard to the migration along radial glia processes and the critical role of extracellular Reelin (Tissir and Goffinet 2003). However, the cell-type specific processes controlling final laminar location remain poorly understood. From mid-corticogenesis on, there appears to be a tight rela-

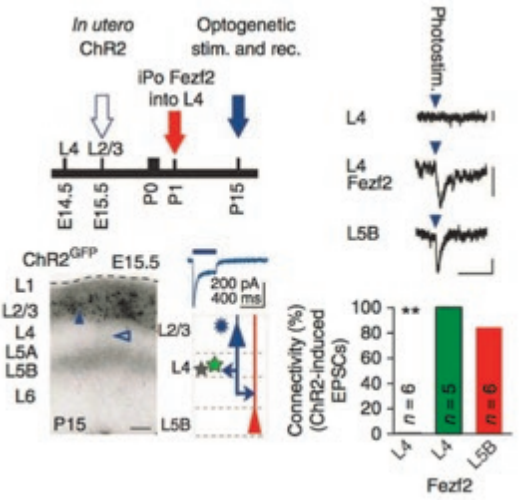

Figure 7: In vivo interrogation of the circuit integration of reprogrammed neurons. *L2/3 neurons are born after L4 neurons, and normally receive unidirectional projections from these cells (i.e. they do not project back to L4 neurons). In contrast, L5B neurons receive input from L2/3 cells. Using optogenetic stimulation of L2/3 cells (through in utero electroporation of channelrhodopsin, ChR2, into L2/3 cells at the time of their birth), the connectivity between L2/3 cells and L4 cells, Fezf2-expressing L4 cells, and L5B cells, can be interrogated. Results show that reprogrammed Fezf2-expressing L4 cells receive L2/3 input, as L5B cells normally do. Taken from De la Rossa et al, 2007.*

tionship between date of birth and laminar position, since VZ-born isochronic neurons align along a narrow sublamina in L4 and L2/3, but at earlier stages, we recently obtained data suggesting that the relationship between date of birth and precise laminar location is less strictly determined (Telley et al. 2016). The mechanisms underlying a more stringent control over laminar location (and perhaps cell type identity) are unknown, and may relate to non-cell autonomous, input-dependent factors, including arrival of thalamocortical afferents, which may act to synchronize progenitor cell behavior (Dehay et al. 2001).

In addition to cell-intrinsic genetic programs and extracellular molecular gradients, activity-dependent processes also control neuronal migration. This has been demonstrated for the tangential migration and differen-

tiation of specific populations of GABAergic interneurons (De Marco García, Karayannis, and Fishell 2011). These cells can be recruited to specific target regions in an input-dependent manner, as shown both in the neocortex (De Marco García et al. 2015) and in the thalamus (Golding et al. 2014), providing an input-dependent mechanism for homeostatic regulation of circuit excitability. In the visual thalamus (i.e. dLGN), we showed that input from the retina is critical for GABAergic interneurons to migrate to their proper location and integrate into circuits (Golding et al. 2014). In this absence of this input, or upon disruption of retinal waves, which are critical for circuit assembly, interneurons mismigrate, resulting in an overall hyperexcitable circuit. This process may represent a homeostatic mechanism to compensate for decreased external inputs. Interestingly, a similar process seems to be at play in the neocortex, where we found that S1 becomes hyperexcitable upon loss of VB input (Pouchelon et al. 2014).

Compared with GABAergic interneurons, early stages of differentiation of glutamatergic excitatory neurons appear to be less dramatically affected by activity. Interestingly, cortical lamination appears to proceed largely normally in the absence of vesicular neurotransmitter release (Washbourne et al. 2002), or in the absence of input/output neocortical connectivity (Zhou et al. 2010), although the morphology and connectivity of neocortical neurons is likely to be affected. Supporting this possibility, chronic hyperpolarization of intracortical projection neurons and thalamic neurons affects axonal elongation and arborization (Mire et al. 2012; Mizuno, Hirano, and Tagawa 2010; Rodríguez-Tornos et al. 2016), and sensory input affects interhemispheric connectivity (Suárez et al. 2014).

Role of input in cortical neuron differentiation

Since the entry point of specific types of thalamic inputs into the cortex coincides with the presence of distinctive cytoarchitectural features (e.g. barrels in S1), a lot of effort has gone into understanding the cellular and molecular mechanisms through which input affects cortical neuron differentiation. This is a topic of particular interest in the laboratory **(Fig. 8),** and this question has been particularly well studied in S1 and

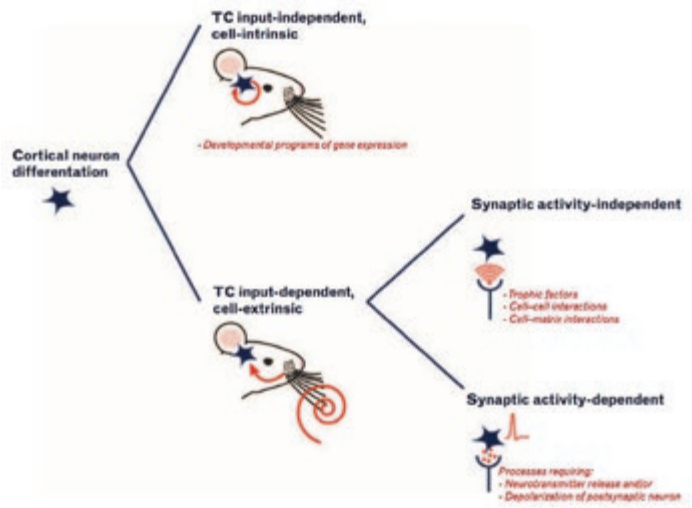

Figure 8: Potential controls over cortical neuron differentiation.

V1. Within these sensory areas, there is a topographical representation of the sensory periphery on the cortical surface, whereby neighbouring neurons respond to activation of neighbouring peripheral receptors, and where densely innervated regions occupy proportionally large regions of the cortical representation. This topographical layout is called somatotopy in S1, retinotopy in V1 and tonotopy in A1.

In S1, the input dependence of these maps was originally studied by lesioning sensory input pathways, such as by section of the infraorbital nerve, which conveys input from the whiskers, or by follicle cautery. These approaches consistently lead to impaired barrel pattering, with shrinkage/disappearance of injured whisker representations and expansion of remaining ones (Fox and Wong 2005; Van der Loos and Woolsey 1973).

These results, however, cannot unambiguously be ascribed to purely developmental mechanisms, since injury-related processes such as axonal sprouting or neuronal death may be at play. To circumvent these limitations, pharmacological attempts have been made to manipulate sensory input, but dose-dependent effects and lack of specificity limit the inter-

pretation of these studies. More recently, manipulations of neuronal activity with cell-subtype specificity together with transcriptional analysis have enabled a better understanding of the molecular and cellular mechanisms that control the assembly of neuronal maps. These studies have shown that synaptic release of glutamate from the thalamocortical axons is required for the assembly of L4 neurons into barrels and dendritic polarisation towards these axons, in particular via activation of NMDA receptors and metabotropic glutamate receptors (López-Bendito and Molnár 2003). In addition, several transcription factors, which we have contributed to identify and include Npas4, Zbtb20 function to polarize L4 dendrites towards incoming VB axons in S1(Pouchelon et al. 2014; Wang et al. 2017; Shetty et al. 2013), and Btbd3 has a similar role in V1-(Matsui et al. 2013).

To study the role of input in cortical neuron differentiation, we developed a genetic mouse model in which VB thalamic neurons, which normally innervate L4 neurons in S1, degenerate shortly after birth, such that the latter cells do not receive their normal thalamic input (Pouchelon et al. 2014) **(Fig. 9)**. Under these circumstances, S1L4 neurons acquired the molecular properties of L4 neurons in associative regions, i.e. in this case S2. A similar process occurs in the visual system (Chou et al. 2013; Vue et al. 2013; Pouchelon et al. 2014). Interestingly, in the case of the somatosensory cortex, deprived S1 circuits acquire S2-like features (i.e. an increase in excitatory/inhibitory ratio), suggesting that thalamic input not only affects L4 neurons but also determines downstream circuit assembly (Pouchelon et al. 2014). As already introduced in a previous chapter, these findings suggest that associative cortical identity is a ground-state feature, and that acquisition of primary cortical area circuit properties is imparted by first-order thalamic input.

Taking a step back, while our early work on the reprogramming of post-mitotic neurons described above (De la Rossa et al. 2013), in which we addressed gene-circuit relationships in the gene-to-circuit direction, here instead we manipulated neuronal circuits and assessed how this affects the genetic identity of post-synaptic target neurons. In both cases, the identity of neurons was congruently reassigned on the genetic, morphological and input-output circuit level. The Pouchelon et al. study showed that the identity of cortical neurons and the circuits they form is signi-

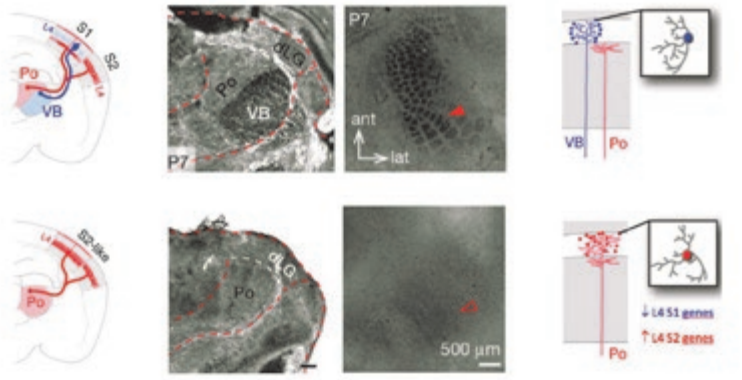

Figure 9: Loss of exteroceptive input leads to a respecification of L4 neurons in S1, which acquire the characteristics of L4 neurons in S2. *A. Under normal conditions, L4 neurons in S1 receive VB input and L4 neurons in S2 receive Po input. Upon genetic ablation of the VB (bottom), Po input is rewired onto S1. (B) Photomicrographs showing loss of VB following genetic ablation (left, compare top and bottom), and loss of barrel patterning in S1 (right). (C) Upon acquisition of Po input, the genetic identity of L4 neurons in S1 ressembles that of L4S2 neurons. Photomicrographs from Pouchelon et al., 2014.*

ficantly determined by sensory input, and identified the molecular mechanisms underlying this effect. Therefore, environmental factors strongly influence neuronal gene expression and circuit formation during development, providing a path through which adverse environmental conditions could lead to abnormal gene expression and secondary circuit miswiring in neurodevelopmental disorders.

More recently, we showed that a similar process is at play within sensory thalamic nuclei, where input ablation experiments support the idea that higher-order genetic identity is a default feature, and that first-order identity is acquired in an input-dependent manner (Frangeul et al. 2016). We and others showed that in the absence of input from the retina, exteroceptive visual nucleus LGN receives input from L5B (Frangeul et al. 2016; Grant, Hoerder-Suabedissen, and Molnar 2016), a normally higher-order nucleus-destined afferent, and develops a corresponding higher-order transcriptional identity (Frangeul et al. 2016). This finding is interesting because it suggests that ascending exteroceptive and descend-

ing corticofugal inputs may thus compete to innervate thalamic nuclei **(Fig. 10).**

From an evolutionary perspective, the findings above support the view that neurons in primary areas and first-order nuclei/areas may have emerged from ancestral secondary/higher-order-type neurons(Slutsky, Manger, and Krubitzer 2000; Sanides 1969). We have thus proposed that first-order neurons may have been co-opted from a ground-state pool of higher-order type neurons based on their ability to convey signals generated by high-resolution body receptors because of specific metabolic, electrophysiological and connectivity features (Frangeul et al. 2016).

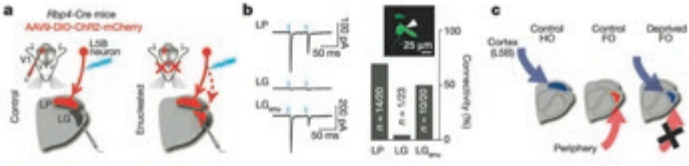

Figure 10: Rewiring of descending cortical input onto de-afferented thalamic nuclei. *(a) Schematic of the experimental setup: Optogenetic stimulation of L5B neurons in the cortex normally activates only LP neurons, while LG neurons are not targeted. In the absence of retinal input, descending inputs now invade the LG. (b) Experimental results (c) Summary of the findings. FO: first order nucleus, HO: higher order nucleus.*

Conclusion and outlook

The level of coordination the assembly of distinct subtypes of neurons into specialized functional circuits across space and time is staggering and raises a number of questions. What is the level of cellular diversity necessary to sustain the functions of the neocortex, and which are the features that delineate these core cell types? How do these features emerge during development and how do they vary across individuals, or in interaction with the environment? To which extent are they involved in the emergence of neurodevelopmental and neuropsychiatric disorders?

I believe that studies involving “non-clonal” model animals might contribute to better define the normal spectrum of variability in cell positioning and circuit assembly, while raising animals in more natural environments could be used to gauge the impact of experience of this process.

While these protocols will introduce natural "noise" in the system, the increase in the resolution of the tools we use to manipulate and assess neurons and circuits, including single-cell RNA sequencing, single-cell optogenetics, and targeted gene editing, will contribute to refine the read-out of these studies and provide a more truthful picture of the degrees of freedom in cortical assembly, and on the limits between normal and abnormal development.

Finally, understanding the number and nature of the independent parameters that define the configuration of the neocortex will be critical in attempts to reverse engineer developmental processes. Understanding these parameters will be important not only to define the relationship between developmental gene expression and mature neuronal function, but also to account for inter-individual variability in brain circuits and behavior in both normal and pathological settings.

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