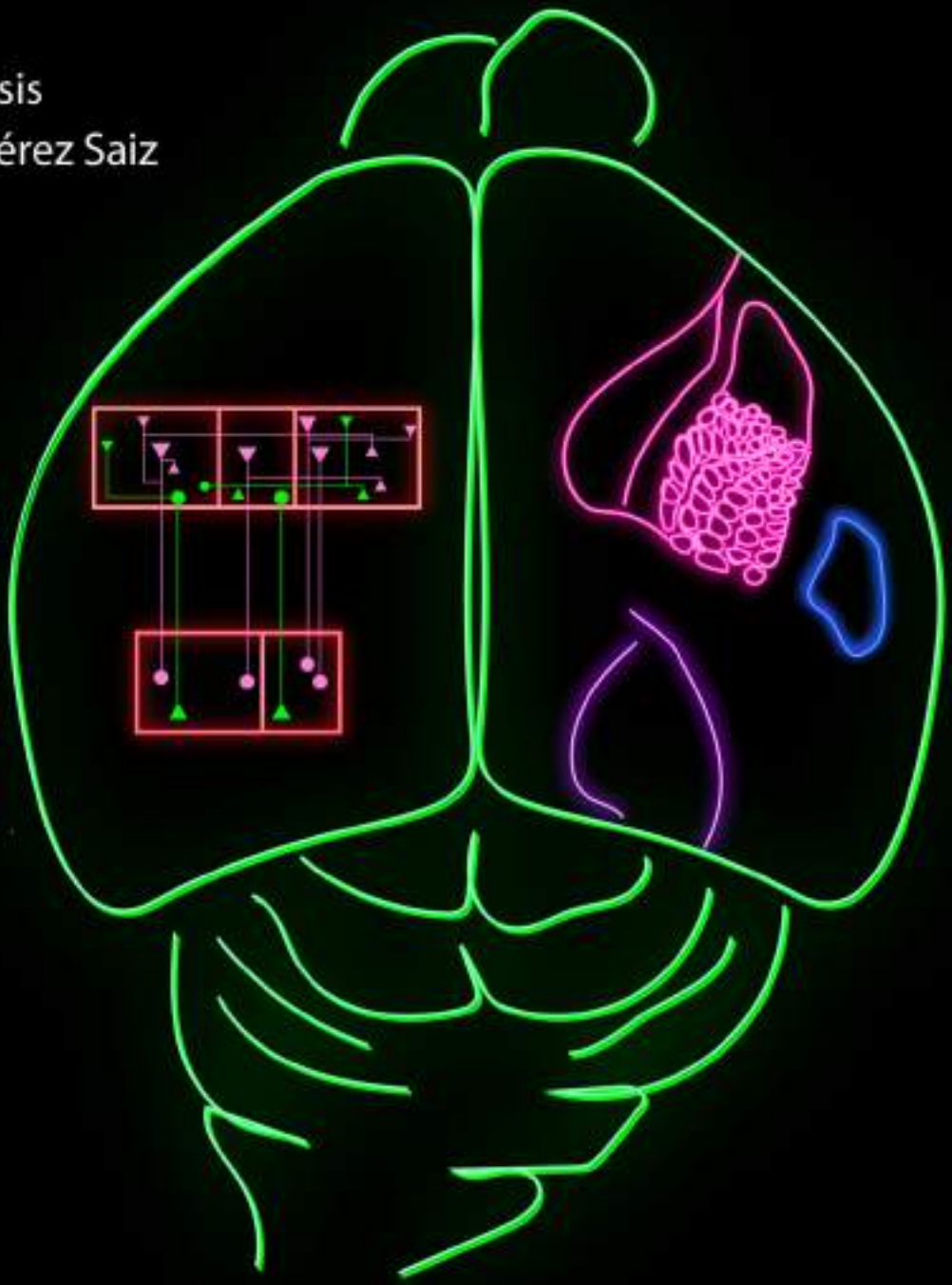
Developmental progression of thalamic and cortical sensory networks in mice

PhD Thesis
Leticia Pérez Saiz



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PhD Programme in Neuroscience
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Developmental progression of functional networks in the sensory thalamus and cortex in mice

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This Doctoral Thesis, entitled **"Developmental progression of functional networks in the sensory thalamus and cortex in mice"** is presented under the conventional thesis form with the following quality indicator(s):

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Sant Joan d'Alacant, __ September 2022

Dra. **Guillermina López-Bendito**, Director, and Dr. **Francisco Martini**, co-director of the doctoral thesis entitled "**Developmental progression of functional networks in the sensory thalamus and cortex in mice"**

CERTIFIES:

That Mrs **Leticia Pérez Saiz** has carried out under our supervision the work entitled "**Developmental progression of functional networks in the sensory thalamus and cortex in mice"** in accordance with the terms and conditions defined in his/her Research Plan and in accordance with the Code of Good Practice of the University Miguel Hernández of Elche, satisfactorily fulfilling the objectives foreseen for its public defense as a doctoral thesis.

We sign for appropriate purposes, at San Juan de Alicante, __ of September of 2022.

Thesis director	Thesis co-director
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Sant Joan d'Alacant, __ September 2022

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INFORMS:

That Mrs **Leticia Pérez Saiz** has carried out under the supervision of our PhD Programme the work entitled "**Developmental progression of functional networks in the sensory thalamus and cortex in mice" in** accordance with the terms and conditions defined in its Research Plan and in accordance with the Code of Good Practice of the University Miguel Hernández de Elche, fulfilling the objectives satisfactorily for its public defense as a doctoral thesis.

Which I sign for the appropriate purposes, in Sant Joan d'Alacant, September 2022.

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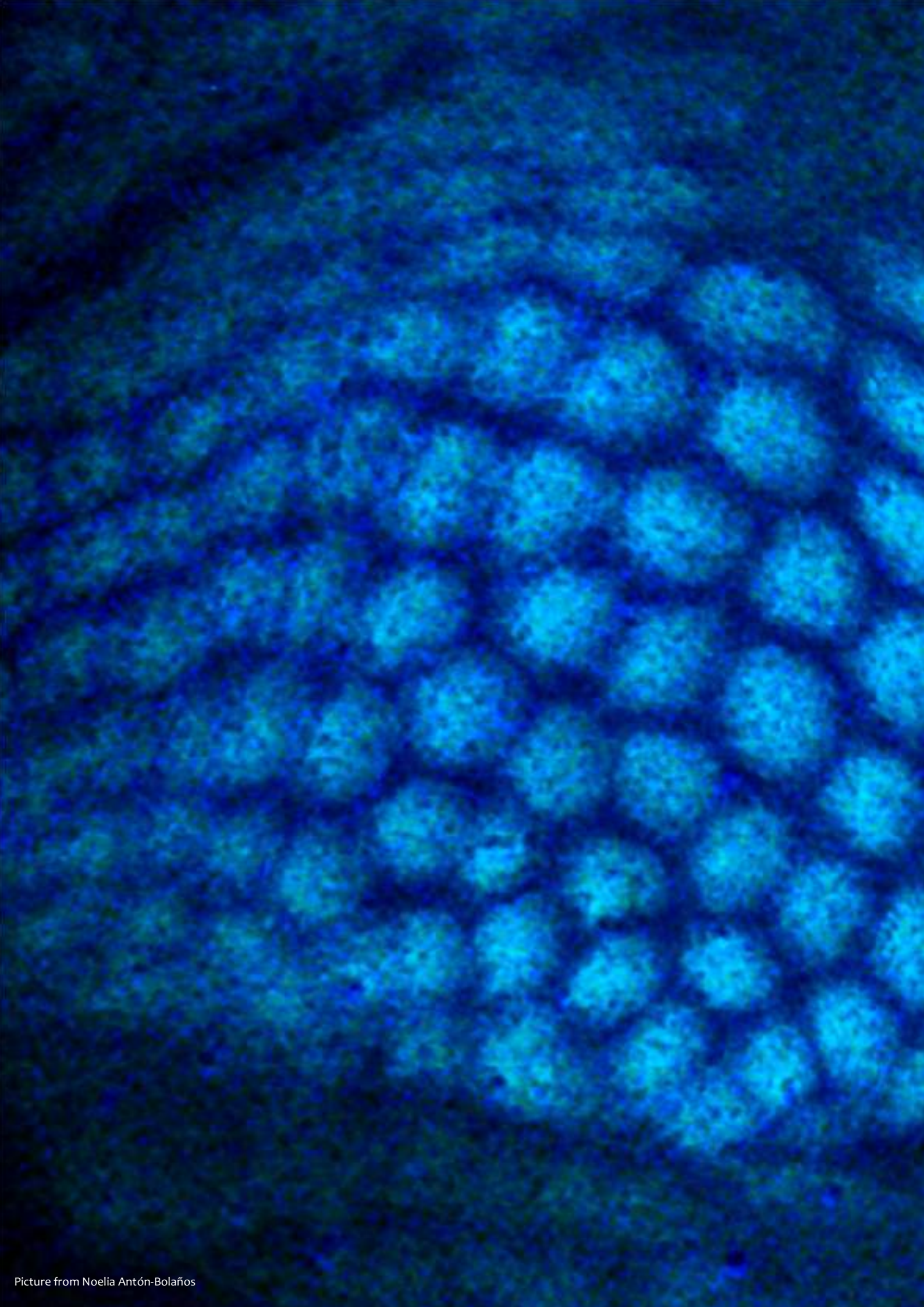

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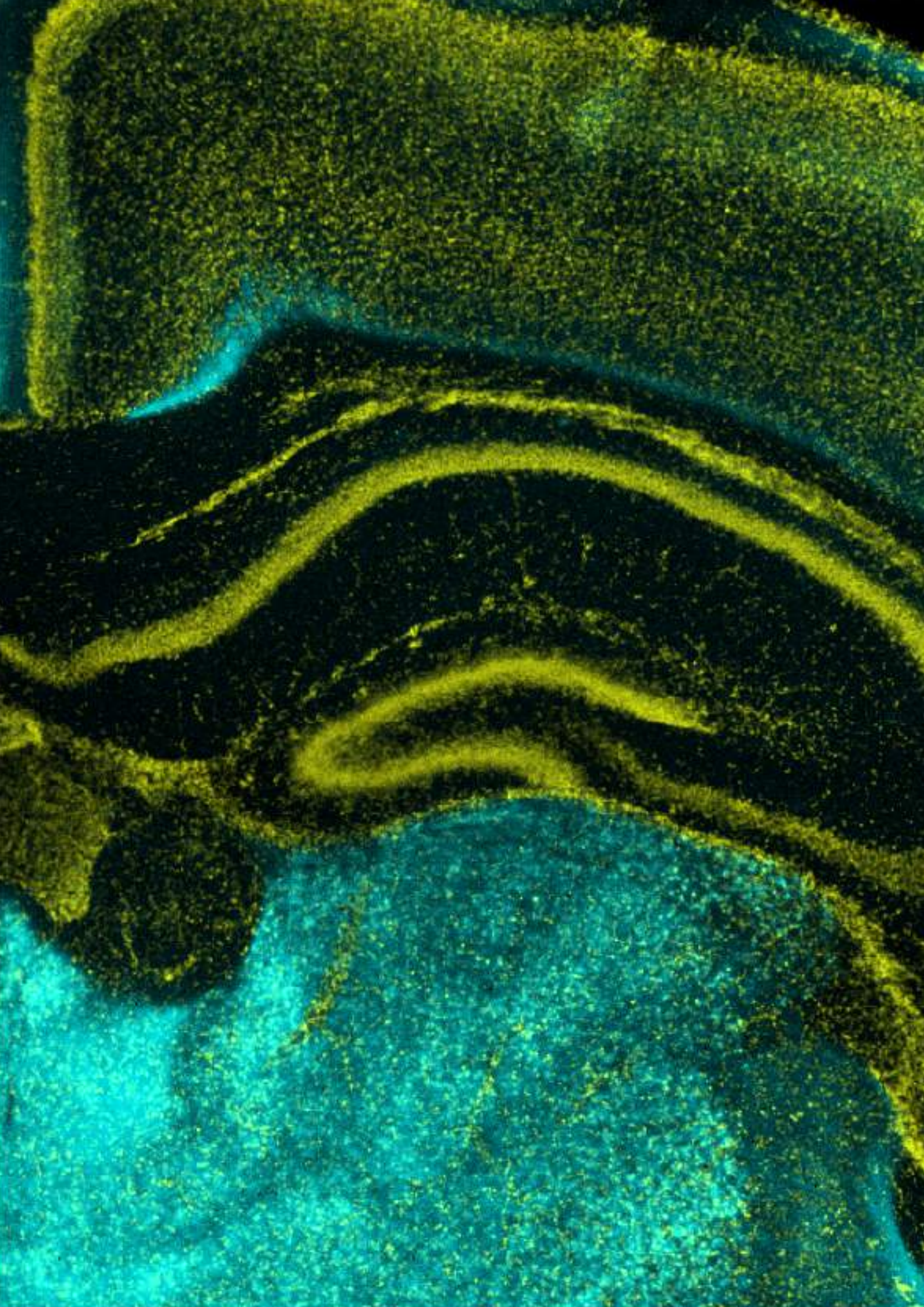
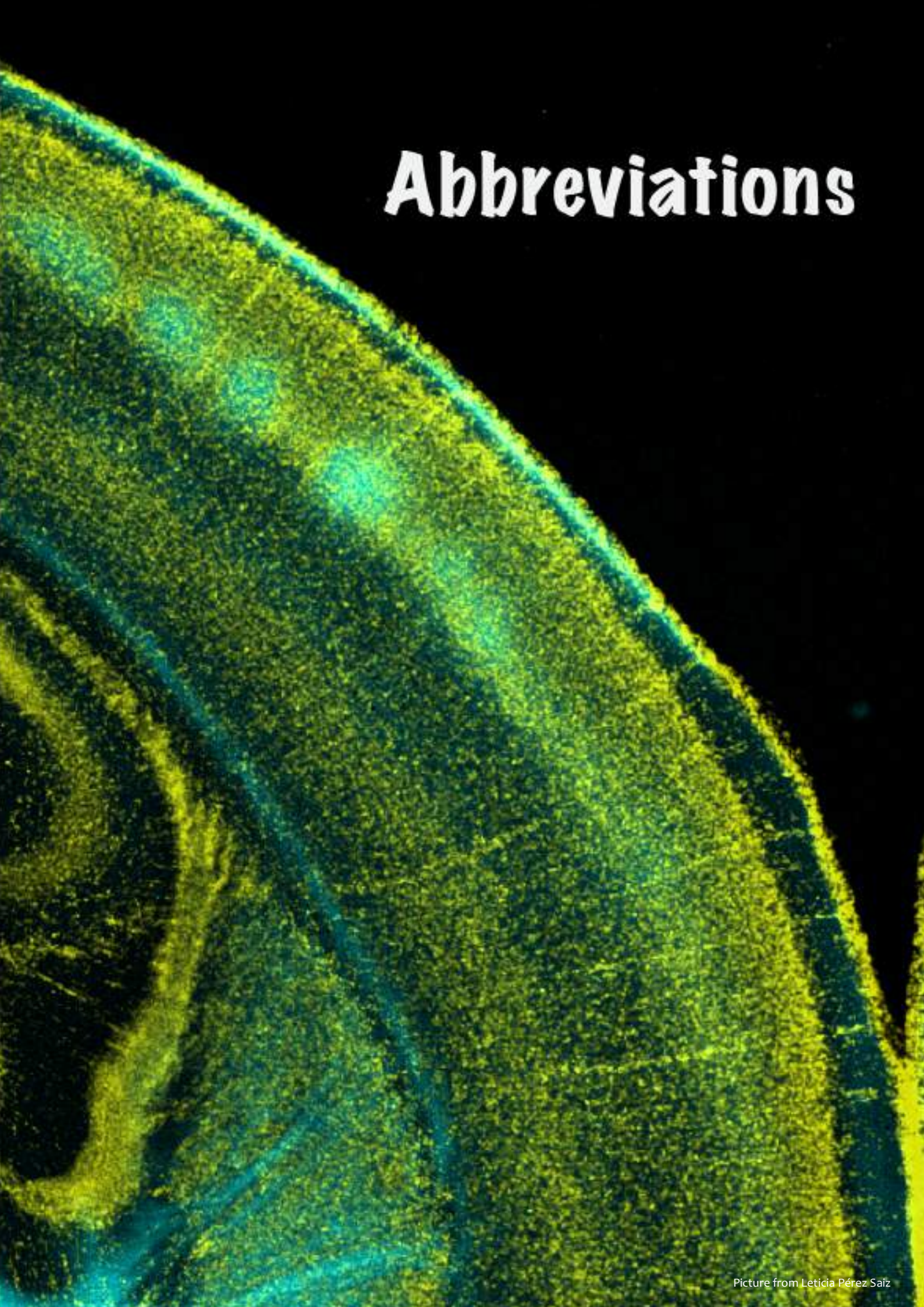


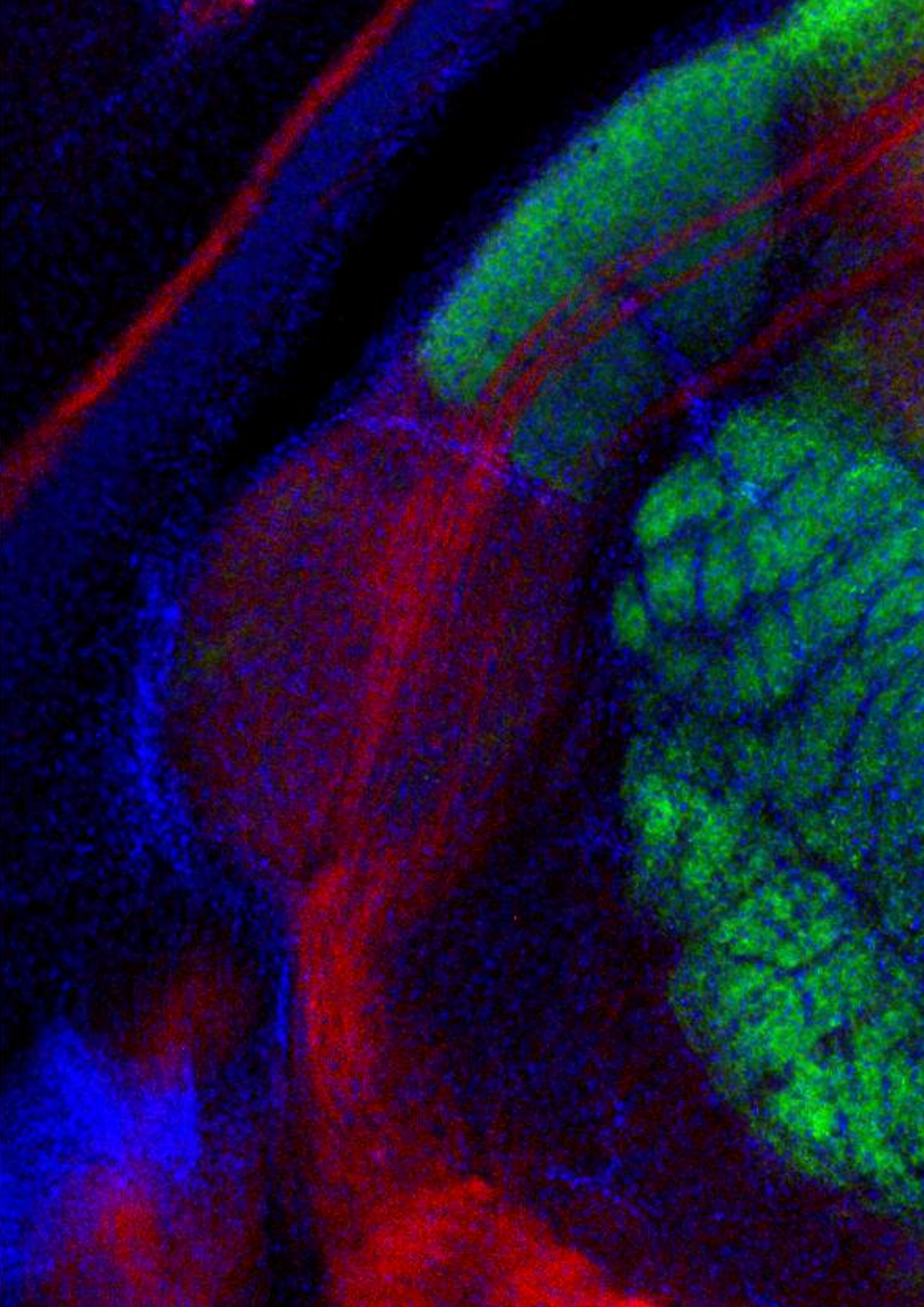
Abbreviations



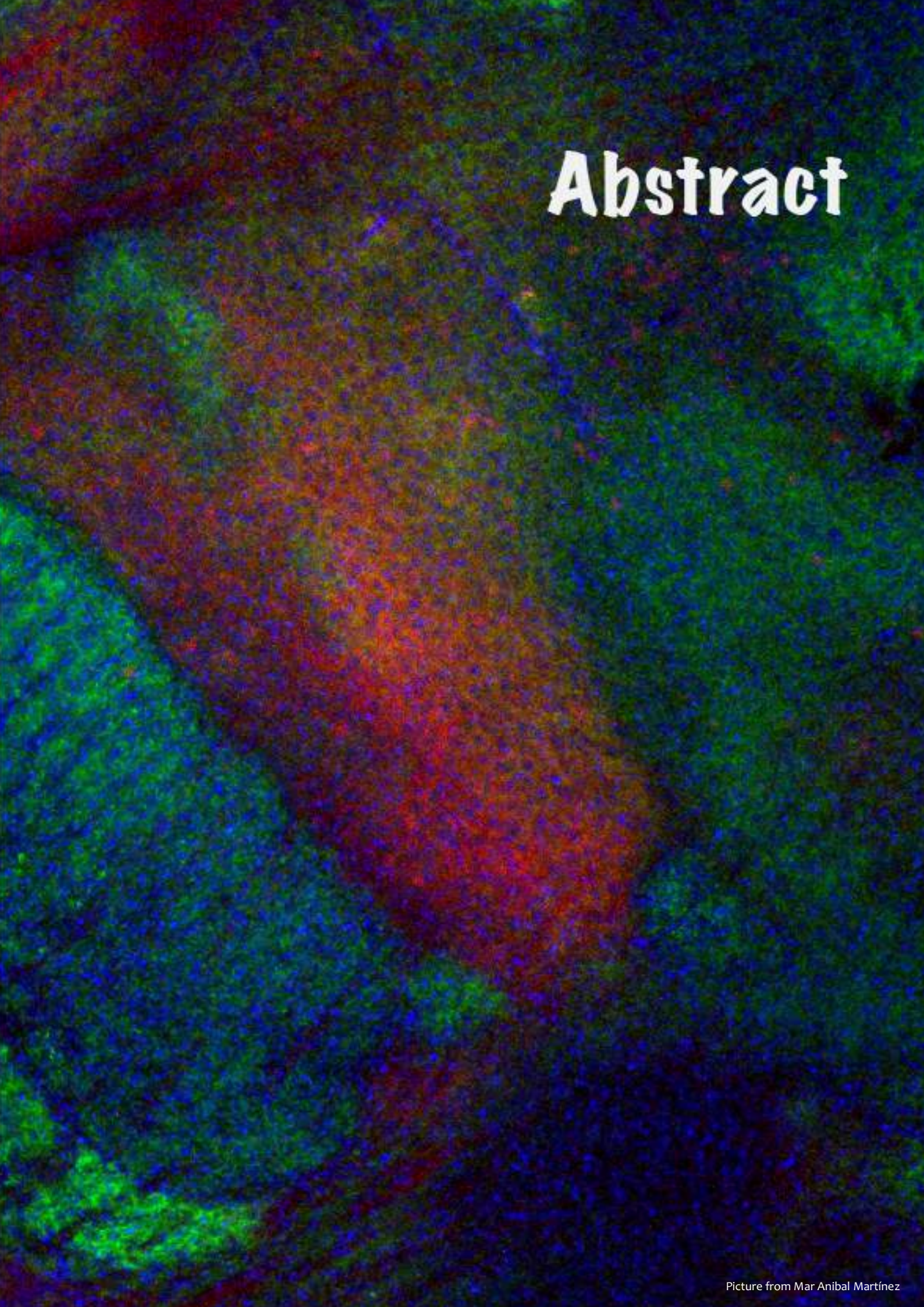
Picture from Leticia Pérez Saiz

A1	Primary auditory cortex	**OC**	Organ of Corti
CN	Cochlear nucleus	**OD**	Ocular dominance
CTAs	Corticothalamic axons	**OS**	Orientation selectivity
DCC	Deleted in colorectal Carcinoma	**OHCs**	Outer hair cells
dLGN	Dorsal lateral geniculate nucleus	**P**	Prosomere
DTB	Diencephalic-telencephalic boundary	**POm**	Posterior medial nucleus
embBE	Embryonic bienucleation	**Prv**	Principal trigeminal nucleus
ER	Electrophysiological recording	**PSPB**	Pallial-subpallial boundary
Fgf	Fibroblast growth factor	**RGCs**	Retinal ganglion cells
FO	First order	**RN**	Reticular nucleus
GABA	Gamma-aminobutyric acid	**S1**	Primary somatosensory cortex
Gbx2	Gastrulation brain homeobox 2	**S2**	Secondary somatosensory cortex
		SC	Superior colliculus
GFP	Green fluorescent protein	**Shh**	Sonic hedgehog
HO	Higher order	**SOC**	Superior olivary complex
IC	Inferior colliculus	**TCAs**	Thalamocortical axons
ICp	Internal capsule	**Th-C**	Caudal domain of the thalamic complex

IHCs	Inner hair cells	**Th-R**	Rostral domain of the thalamic complex
		Th^{kir}	Thalamic Kir
ION	Infraorbital nerve	**TRN**	Thalamic reticular nucleus
L	Layer	**TTX**	Tetrodotoxin
LP	Lateral posterior nucleus	**V1**	Primary visual cortex
L4ss	Layer 4 spiny stellate	**V2**	Secondary visual cortex
L4sp	Layer 4 star pyramidal	**VB**	Ventrobasal complex
		vGlut	Vesicular glutamate transporter
MD	Monocular deprivation	**VPM**	Ventral posterior medial nucleus
MGB	Medial geniculate body		
MGE	Medial ganglionic eminence	**VZ**	Ventricular zone
MGd	Dorsomedial geniculate body	**Wnt**	Wingless-type MMTV integration site family
MGN	Medial geniculate nucleus	**ZLI**	Zona limitans intrathalamica
MGv	Ventromedial geniculate body		
NMDAR	NMDA receptor		



Abstract



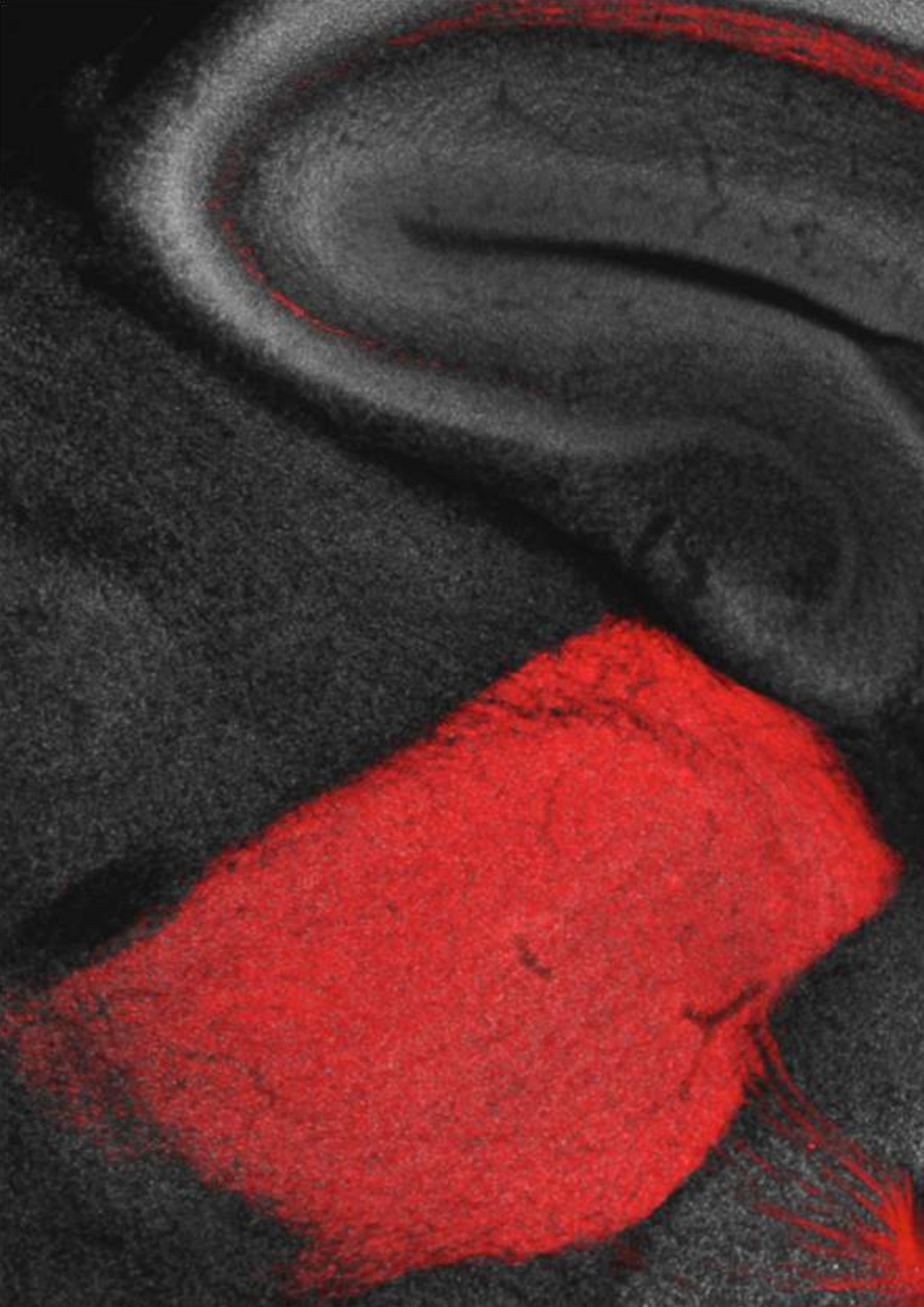
Picture from Mar Anibal Martínez

Abstract

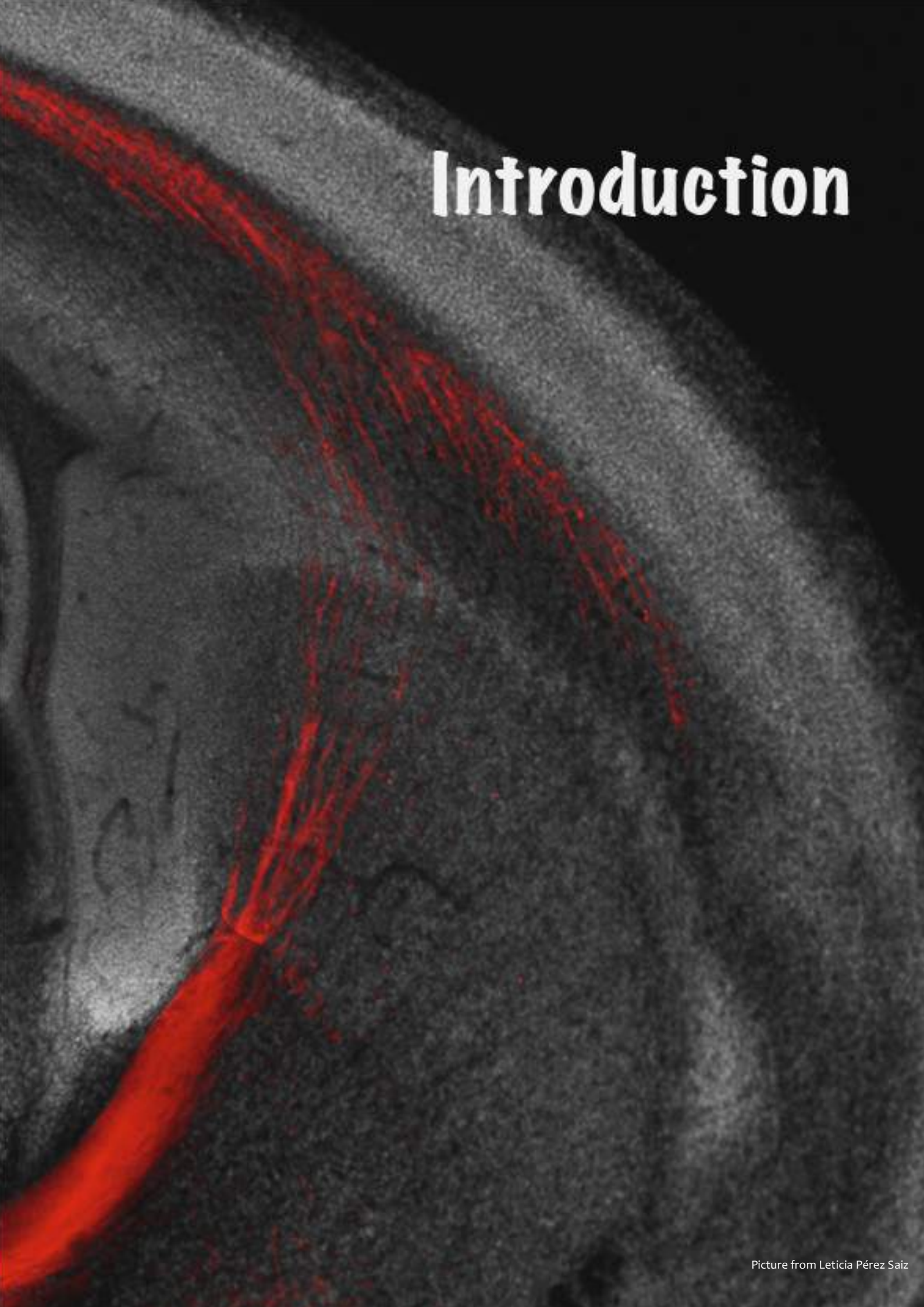
A key question that remains unclear in Developmental Neuroscience is to understand how thalamic spontaneous activity contributes to the emergence and plasticity of cortical sensory maps. Spontaneous neural activity during development plays an important role in the establishment of thalamocortical circuitry in sensory areas before sensory onset. It is thought that the cortical activity at perinatal stages strengthens synaptic connections that will define sensory maps. Spontaneous activity in the developing cortex has been well characterized: it starts as highly correlated patches of active neurons that, after the first postnatal week in mice, switches to a sparser and decorrelated neuronal firing that allows an efficient neuronal coding. By contrast, the profile and function of spontaneous activity in the developing thalamus remains largely unknown. Here, we described the maturation of the spontaneous spiking activity in the thalamic nuclei of mice aged from postnatal day 6 to 14. The overexpression of Kir2.1 in the developing thalamus not only alters this maturation but also affects the electrical activity in the corresponding sensory cortices. We have also observed this link between the maturation of the thalamic and cortical stations in models of early sensory deprivation. When visual input is removed embryonically, the visual and somatosensory thalamus and cortex exhibit abnormal patterns of spontaneous activity at immature stages. In the visually deprived pups, neurons from the primary somatosensory cortex respond faster upon whisker stimulation, a fact that might underlie more efficient information processing in the intact sensory modalities. Our results evidence that normal thalamic activity during development is crucial for the correct organization of cortical circuits and for cross-modal changes both in thalamus and cortex after sensory deprivation.

Resumen

Entender cómo la actividad espontánea del tálamo durante el desarrollo contribuye a la formación y plasticidad de los mapas sensoriales corticales es una pregunta clave en el campo de la neurociencia. Durante el desarrollo del sistema nervioso, los circuitos talamocorticales se activan de manera espontánea con patrones de actividad que van cambiando a medida que los circuitos maduran. Estos cambios de patrones son estereotípicos y se relacionan con un proceso normal de desarrollo del sistema talamocortical. La actividad espontánea en la corteza cerebral ha sido bien caracterizada: comienza siendo sincrónica y en grupos grandes de neuronas y, posteriormente, se desincroniza para ganar eficiencia en la codificación de la información. Por otro lado, el perfil y la función de la actividad talámica durante el desarrollo postnatal todavía no se conocen con claridad. En esta tesis describimos cómo madura el patrón de actividad espontánea en los núcleos talámicos sensoriales de ratones durante las dos primeras semanas postnatales. Cuando alteramos estos patrones mediante la sobreexpresión de Kir2.1 en el tálamo, encontramos que no solo el tálamo presenta defectos sino también las cortezas correspondientes. Esta relación entre los patrones de actividad del tálamo y la corteza se observa también en modelos de deprivación sensorial. Al quitar la entrada sensorial visual, encontramos deficiencias en la maduración de los patrones de actividad espontánea tanto en la vía visual como en la somatosensorial. Además, en estos ratones privados, las neuronas de la corteza somatosensorial primaria responden más rápido a la estimulación de los bigotes. Esta plasticidad entre modalidades sensoriales podría explicar en parte un procesamiento más eficiente de la información somatosensorial ante un déficit visual.



Introduction



Picture from Leticia Pérez Saiz

Introduction

1.The thalamus and the neocortex

1.1 Overview of the neocortex

Brains from all mammals have a neocortex, but its size and complexity vary across the taxa (Herculano-Houzel, 2009). This variability is associated with the different motor skills, sensory specializations or social behavior exhibited by extant mammalian species, from monotremes to primates. Despite these differences, mammalian neocortices share some key features such as functional regionalization and laminar organization. For instance, although the total number of neocortical regions varies, as many as 20 functional regions are shared by all mammalian species, including primary and secondary visual, auditory and somatosensory areas (Kaas, 2011).

In addition, the laminar organization of the mammalian neocortex exhibits a stereotyped connectivity forming vertical modules known as cortical columns or mini-columns (Mountcastle, 1997). Cortical columns are populated by distinct subtypes of neurons: glutamatergic neurons and GABAergic interneurons. While glutamatergic neurons are excitatory and establish local and long-range connections with subcortical and intracortical targets, GABAergic neurons are inhibitory and establish mainly local interactions (Petreanu et al., 2009). The similarities in the neocortical organization among functional regions and species suggest that the cortical columns are common information processors that could deal with inputs of different kinds.

1.2 The thalamus: brief history of a name

In a medical text that dates back more than 2000 years, Galen wrote for the first time the term *thalamus* to name a brain structure located deep in the brain. Actually, the neuroanatomical thalamus is an architectural metaphor. Galen borrowed the

term from the basic layout of the ancient Greek house, where the thalamus was the innermost chamber (Serra et al., 2019). However, it is likely that what Galen called thalamus was actually the third ventricle, a chamber-like structure that resides deep in the brain (Jones, 2007). More than 1000 years later, the German physiologist Karl F. Burdach provided the first accurate description and classification of the thalamus, its subdivisions and connections, setting the basis to understand the relationship between thalamic cytoarchitecture and function (Burdach, 1822).

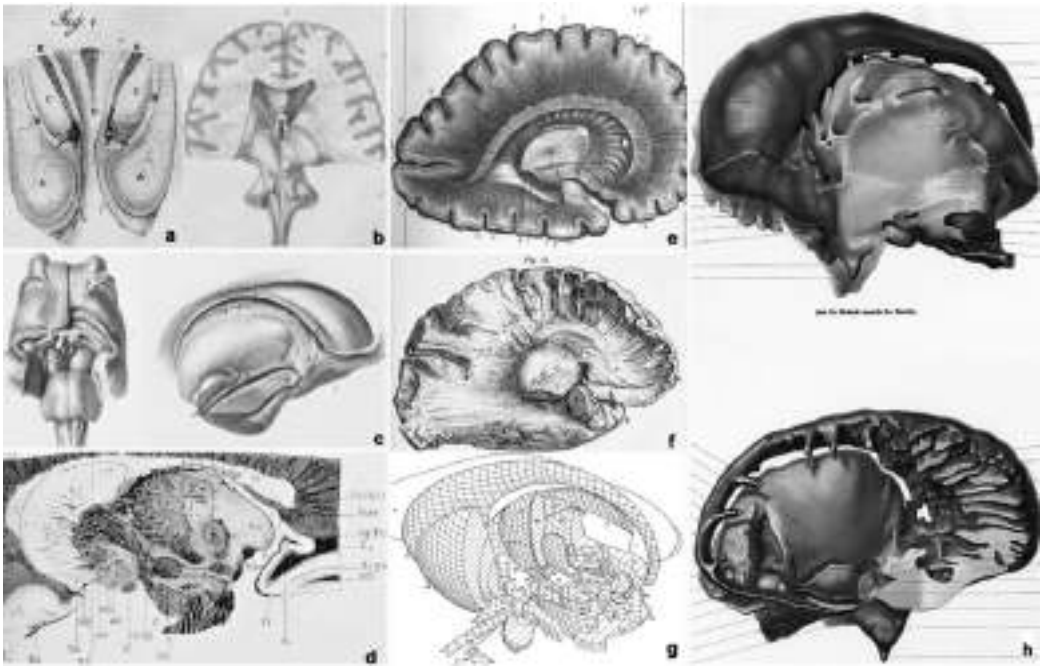


Figure 1. Overview of some of the most renown anatomical illustrations of the thalamus, its macroscopic anatomy and connections (taken from Serra et al., 2019). Illustration from: a) Alexander Monro, b) Friedich Arnold, c) Achile Louise Fovile, d) Jules Dejerine, e) Jules Bernad Luys, f) Theodore Meynert, g) Benno Schlesinger, h) Josef Klinger.

1.3 The organization of the thalamus

The thalamus is not spatially organized in layers as the neocortex. The thalamus is parceled in regions called nuclei, namely, clusters of neurons devoted to similar functions. However, while the cytoarchitecture of thalamus and cortex differ, their functional subdivisions are similar due to the massive interconnection between both

brain structures. Thalamic nuclei can be broadly classified in three groups: sensory, motor and associative. The sensory and motor nuclei are involved in communicating the neocortex with sensory organs and muscles. In contrast, associative nuclei mainly convey and receive information from the cortex. Focusing on the sensory nuclei, they can be further classified in two groups according to the origin of their driving inputs: first-order (FO) and higher-order (HO) (Sherman and Guillery, 1998; Sherman and Guillery, 2002). In the FO nuclei, the driver input derives from ascending pathways; whereas, in the HO nuclei, it comes mainly from layer 5 neurons of the matching sensory areas of the cortex.

FO and HO nuclei receive sensory information segregated by modality, i.e., somatosensory, visual and auditory. Then, there is a pair of FO-HO nuclei in the thalamus for each sensory modality, namely: ventral posteromedial (VPM) and posteromedial (PoM) nuclei for somatosensory information, dorsal lateral geniculate (dLGN) and lateral posterior (LP) nuclei for visual information and ventromedial geniculate body (MGv) and dorsomedial geniculate body (MGd) nuclei for auditory information. Despite these clear-cut functional categories, the genetic analysis of thalamic neurons provides more homologies across hierarchy orders than sensory modalities (Frangeul et al.,2016).

Finally, in a recent review, Halassa and Sherman claim for a classification based on the functionally-relevant connectivity pattern of thalamic cells. They propose to classify each thalamic neuron according to their input and output architecture (Halassa and Sherman, 2019).

2. The development of the connection between thalamus and cortex

2.1 Development of thalamocortical projections

During the second and third weeks of gestation in mice, neurons from the neocortex and thalamus start to extend their axons in order to establish specific and reciprocal

connections. Both thalamocortical and corticothalamic axons travel a long journey through several territories before arriving at their targets. This interconnection is crucial to carry sensory information from the periphery to the cortical corresponding areas and constitute one of the most massive connections of the mammalian brain.

The development of thalamocortical projections is a multi-step process. Around the second gestational week in mice, TCAs exit the thalamus and turn dorsolaterally at the diencephalic-telencephalic boundary in order to reach the internal capsule (López-Bendito and Molnár, 2003; Garel and Rubenstein, 2004). At this point, TCAs are already topographically organized, guided by molecular factors such as Semaphorins/Plexin, ephrins/Eph, or netrins/DCC (Molnár et al., 2012; Garel and López-Bendito, 2014; Braisted et al., 2009). Then, they advance rapidly towards pallial-subpallial boundary where TCAs meet the first corticofugal axons from early born subplate neurons (De Carlos and O'Leary, 1992). Using corridor cells as guide posts (Lopez-Bendito et al., 2006) and CTAs as a scaffold (Blakemore and Molnár 1990), TCAs reach the intermediate zone of the developing cortex by E15.5, before layer 4 neurons are born. Axons wait in the subplate, establishing transient interactions with subplate cells. This waiting period lasts until E17.5, when TCAs start to form branches that invade the cortical plate and form synapses with neurons of the appropriate layers (Allendoerfer and Shatz 1994; Kanold and Luhmann 2010; Viswanathan et al., 2012; Hoerder-Suabedissen and Molnár 2015; Viswanathan et al., 2017). During the first postnatal week, TCA branches undergo a process of pruning that refines their connectivity and prepare the circuits for information processing (López-Bendito and Molnár 2003).

Apart from the intrinsic genetic factors that control thalamocortical connectivity, immature thalamic and cortical circuits exhibit spontaneous activity, another factor that has been implicated in the maturation of the circuit (Molnár et al., 2012; Mire et al., 2012; Leyva-Díaz et al., 2014; Garel and López-Bendito 2014; Antón-Bolaños et al., 2018; López-Bendito 2018). Indeed, one of the main questions of this thesis

is to understand the role of the spontaneous activity during the development of sensory systems.

2.2 Development of corticothalamic projections

In the neocortex, early postmitotic cells form the preplate layer just above the ventricular zone, where they were proliferating (Stewart and Pearlman, 1987). Postmitotic cells migrate in an inside-out gradient within the preplate, splitting this layer into a superficial zone and a deep subplate (Marin-Padilla, 1971; Luskin and Shatz, 1985a,b). The axons of subplate neurons act as pioneers generating the first pathway between these cells and their target. Axonal extension towards subcortical targets starts before the formation of layers 5 and 6 (Grant et al., 2012). They exit the intermediate zone, cross the pallial-subpallial boundary and enter in the internal capsule around E13.5 in mice (Erzurumlu and Jhaveri, 1992; Richars et al., 1997; Jacobs et al., 2007). In the internal capsule, CTAs experience the first waiting period for 24-48 hours (Jacobs et al., 2007; Deck et al., 2013) and, by E16.5, they advance to the prethalamus where they wait until E17.5, possibly in the reticular nucleus (Garel and Rubenstein, 2004; Simpson et al., 2009; Chen et al., 2012; Molnár et al., 2012). At this point, layer 5 and 6 are already formed and their corresponding axons respectively reach FO and HO thalamic nuclei (Cordery and Cordery 1999; Jacobs et al., 2007).

During early postnatal development occurs the final invasion of CTAs into the thalamic nuclei. While motor and somatosensory nuclei are innervated between E18.5 and P0, visual and auditory nuclei complete their innervation by P8 (Jacobs et al., 2007; Grant et al., 2012; Grant et al., 2016). There is still much to decipher regarding to the mechanisms underlying the innervation of thalamic nuclei by CTAs.

2.3 The developmental segregation of sensory systems

Two different hypotheses have been proposed to explain the arealization of the cortex. On the one hand, the protomap hypothesis stands for the idea that cortical

specification is completely independent of external factors. This hypothesis claims that cortical fate of neurons is pre-determined by intrinsic genetic program during neurogenesis (Rakic 1988). On the other hand, the protocortex hypothesis suggests the final cortical fate of neurons is influenced by the external factors such as the input from TCAs (Garel et al., 2002; Vue et al., 2013), which shape the synaptic organization of the cortical circuits (Van der Loos and Woolsey, 1973; O'Leary, 1989). Over last years, both theories have been integrated and it has been accepted that there is a balance between intrinsic (genetic encoded) and extrinsic (input dependent) mechanisms that could affect neocortical differentiation (Grove and Fukuchi-Shimogori, 2003; Arai and Pierani, 2014).

2.4 Thalamocortical connectivity from FO and HO

First-order thalamic nuclei receive, directly or indirectly, information from the periphery. Neurons within these nuclei connect mainly with L4 neurons of their corresponding primary cortical areas. Layer 4 neurons, in turn, connect directly or indirectly with L5b neurons that project back to the thalamus but towards HO nuclei. Then, HO nuclei send projections to L4 neurons of their corresponding secondary cortical areas. This is the general view of the connectivity across sensory system (Figure 2). It is important to note that both FO and HO thalamic nuclei receive modulatory feedback from L6; however, only HO receive feedforward input from L5a (Viaene et al., 2011; Crandall et al.,2015). In this way, the cortico-thalamic-cortical pathway enables the cooperation between different cortical areas (Sherman and Guillery, 2002; Theyel et al., 2010; Lee and Sherman, 2010; Sherman, 2016).

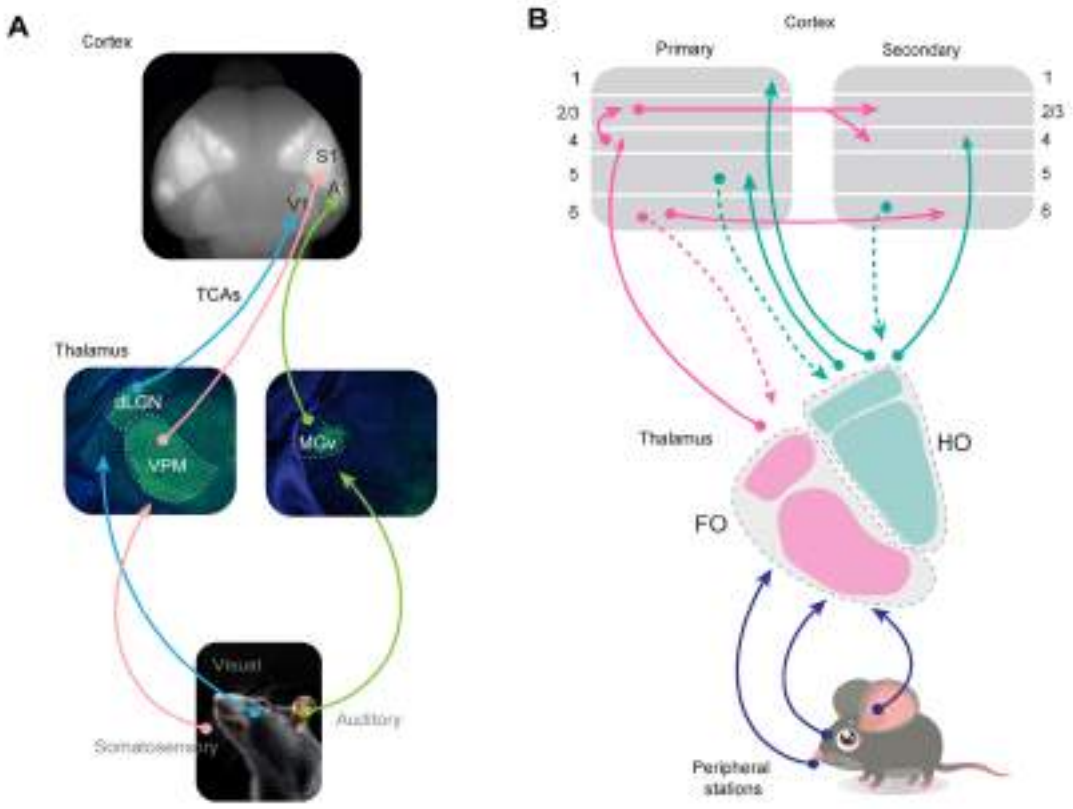


Figure 2. Organization of the primary sensory pathways and main connections. A) Schema representing the organization of the three major peripheral inputs from eyes (blue), whiskers (light orange) and ears (green) towards their corresponding thalamic nuclei: dLGN for visual pathway, VPM for somatosensory and MGv for auditory. From thalamic nuclei, TCAs project to the cortical areas: V1, S1 and A1, respectively. B) Schema showing thalamocortical connectivity. From periphery, ascending information relays in first order thalamic nuclei (FO). That nuclei send information towards L4 in primary sensory cortex and from here, L4 neurons connect with L2/3 neurons. L2/3 neurons spread the information through cortico-cortical connections to L2/3 and L4 neurons of secondary areas. Upper layers in primary and secondary areas connect to L5 neurons that send projections to HO thalamic nuclei. HO project to L4 of secondary cortices and to L5 and L1 of primary cortical areas.

3. Sensory systems

3.1 General principles

Visual, somatosensory and auditory systems exhibit in the ascending sensory stations a topographical representation of the array of receptors in the sensory organ. These topographical representations are: the somatotopic map in the somatosensory system (Woolsey, 1978), the retinotopic map in the visual system (Tusa et al., 1978) and the tonotopic map in the auditory system (Merzenich et al., 1975).

3.2 Visual system

Almost 80% of the information about our surroundings is gained through the visual system. Visual perception is leaded along the neuronal pathway from the retina to the visual cortex in a topographically organized manner and this is possible due to the role of genetic programs and activity dependent mechanisms.

Vision begins within the eye, in the retina. The retina has a laminar organization composed of three main layers of cell bodies (retinal ganglion cell layer, inner nuclear layer and outer nuclear layer) which are separated by two synapse layers. The visual information propagates through these layers as follow. Photoreceptors of the outer cell layer respond to light and send visual information to the inner layer cells, which filter and shape it. From here, visual input arrives at the retinal ganglion cells (RGSs) that form the ganglionic layer. There is a wide diversity of RGCs types and each of them respond to different aspect of the visual field (Danaf and Huberman, 2019). The RGCs send their axons to the central nervous system, forming the optic nerve.

In mice, the RGCs start to extend their axons to the optic chiasm by E12.5. The main target of RGCs are the cells in the thalamic visual nuclei and the superior colliculus (SC). Visual input reaches the thalamus by E15.5 and the SC by E18.5, approximately.

Most of RGC axons cross the midline and project to the contralateral side, only 3-5% of the axons project ipsilaterally (Dräger and Olsen, 1980; Petros et al., 2008). Before P4, ipsilateral and contralateral axons overlap in dLGN and start to occupy their space, with ipsilateral projections forming a patch surrounded by contralateral axons. Both contralateral and ipsilateral projections are kept separated along the visual pathway. This organization is known as eye-specific segregation and allows binocular vision (Godement et al., 1984; Huberman et al., 2008). Underlying eye-specific segregation, two different processes have been described. First, axons reach their relative position and then, they get refined, retracting collaterals and increasing branching in the correct target site. This reorganization occurs mainly during the first postnatal weeks (from P1 up to P10), before eye opening. Apart from eye-specific segregation, central visual pathway shows a spatial representation of the retina, known as retinotopy, that is generated by: i) gradients of molecules (such as Eph/Ephrins) and ii) neuronal activity.

During last years, many studies based on visual manipulations (visual deprivation, dark rearing, visual stimulation) have focused on understanding some particularities of the visual system such as the mechanisms behind the cortical adaptations, the role of the visual experience in the development or how spontaneous activity contributes to the correct formation of visual circuitry. However, the role of the spontaneous activity of the thalamic nuclei has not been addressed. In our lab, it has been demonstrated that binocular deprivation at E14.5, before the retinal axons reach the thalamus, triggers cortical adaptations. Moreover, in these deprived mice, the pattern of activity in the embryonic thalamus is perturbed, giving rise to an increase frequency of spontaneous waves of activity in the dLGN. These findings reveal that embryonic thalamic waves coordinate the pattering of cortical sensory areas (Moreno-Juan et al., 2017).

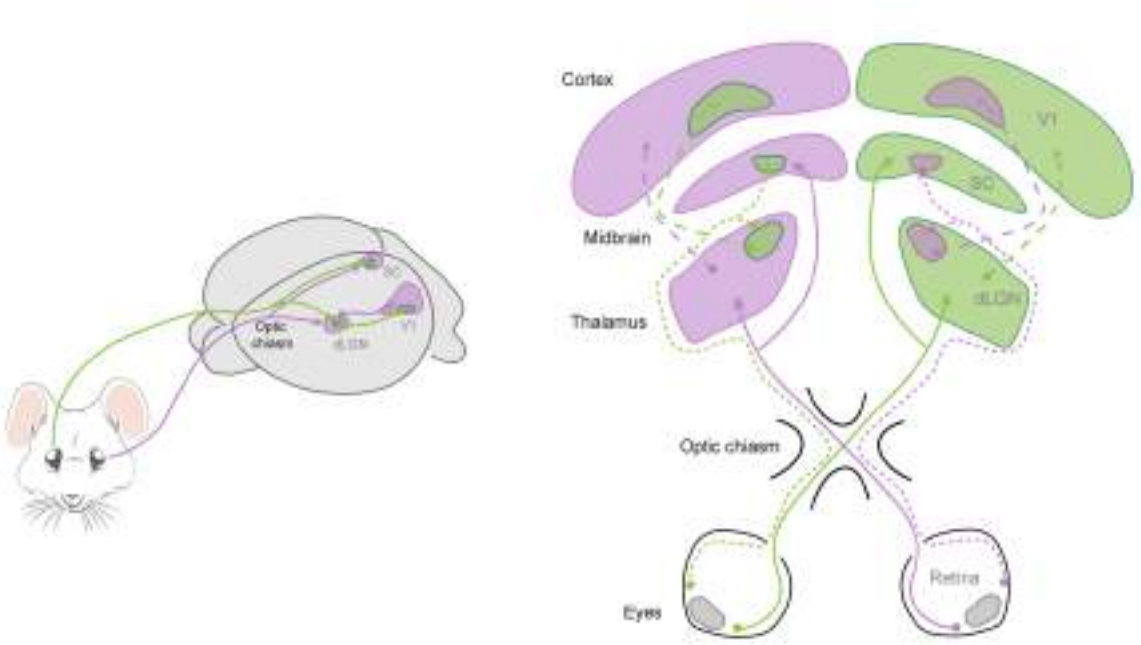


Figure 3. Organization and eye specificity of the visual pathway in mice. These schemes represent the organization of the peripheral axons from the eyes. Axons from both eyes projecting from the temporal or nasal retina are segregated along the visual pathway. Retinal axons advance to the optic chiasm, where most of them cross towards the contralateral hemisphere while some temporal axons stay in their ipsilateral hemisphere. After the chiasm, axons project to the SC and to the thalamus, showing an eye-specificity and retinotopy in their organization. From dLGN, thalamic axons project towards the visual cortex, V1. The scheme on the right is adapted from Seabrook et al., 2017.

3.3 Somatosensory system

The somatosensory system provides information about objects in our surrounding through touch and about the position and movement of our body parts (proprioception) through the activation of muscles. This system also controls the body temperature and detects painful stimuli.

As in the visual system, the topographical representation of the body is retrieved by all sensory stations in the somatosensory pathway. In rodents, the whisker pad is overrepresented respect to other body parts. There are different ascending

pathways in the somatosensory system. These are the lemniscal 1, lemniscal 2, extralemniscal and paralemniscal pathways (Deschenes and Urbain, 2009; Pouchelon et al., 2012). Whereas lemniscal pathways are relayed by VPM and project to S1, the paralemniscal pathway is relayed by the POm and projects to S1 and S2 cortical areas (Pierre et al., 2000; Brech and Sakmann, 2002; Meyer et al., 2010; Oberlaender et al., 2011; Tkusano et al., 2017).

The lemniscal is the most studied pathway. The somatosensory pathway starts in the snout of the mice. Here, whiskers follicles are innervated by trigeminal ganglion cells (TGs) that develop projections towards the hindbrain by E9.5. They enter the principal trigeminal nucleus in the brainstem (PrV) by E14.5, just after its formation (Kitazawa and Rijli, 2018). The maxillary TG axons related with upper jaw and whiskers innervate the ventral part of the PrV whereas the dorsal part is targeted by the mandibular ones, related with the lower jaw (Erzurumlu, 2010; Kitazawa and Rijli, 2018; Iwasato and Erzurumlu, 2018). In the PrV, the axons coming from each single whisker cluster together forming specific spatial representations called barreletes (Ma and Woolsey, 1984). Axons from PrV leave the nuclei and cross the midline around E11.5 in order to reach the contralateral thalamus at E17.5. Here, in the VPM, trigeminal axons arborize and gradually refine, forming the barreloids around P2-P3 (Kivrak and Erzurumlu, 2012). Then, TCAs from VPM extend to the L4 of somatosensory cortex by E18.5 and form the barrels around P3-P4 (Van der Loos and Woolsey, 1973; Woolsey et al., 1975).

Within the barrel cortex, it is established a columnar connectivity. Layer 4 is the principal recipient of thalamic input but it is also implicated in the transmission of intracolumnar information to the remaining layers, generating a columnar circuitry within the barrel cortex (Bruno, 2006). L2/3 neurons situated above the barrel are excited by L4 neurons and, due to their long-range collaterals, they project horizontally towards other cortical domains like other cortical columns of the same S1, towards ipsilateral S2 or even M1 (Yamashita et al., 2018). Additionally, they also target the contralateral S1 through the corpus callosum, integrating the

information of both hemispheres (Petreanu et al., 2007). Layer 5 is the principal output layer and it could be divided in L5a, which receives thalamic input from POm, and L5b, which receives thalamic input from VPM (Jones and Wise, 1977). Layer 6 is also subdivided in L6a, the main source of cortico-thalamic connectivity (receives input from L5b, L6a and L4 excitatory neurons and project to L5 and L6 of many barrels) and L6b, which mainly innervates POm and L1 (Thomson, 2010; Fox and Woolsey, 2009).

For half a century now, the somatosensory system and specially the barrel cortex has been an exceptionally useful model to understand the mechanisms underlying the development and plasticity of cortical maps (Erzurumlu and Gaspar, 2020).

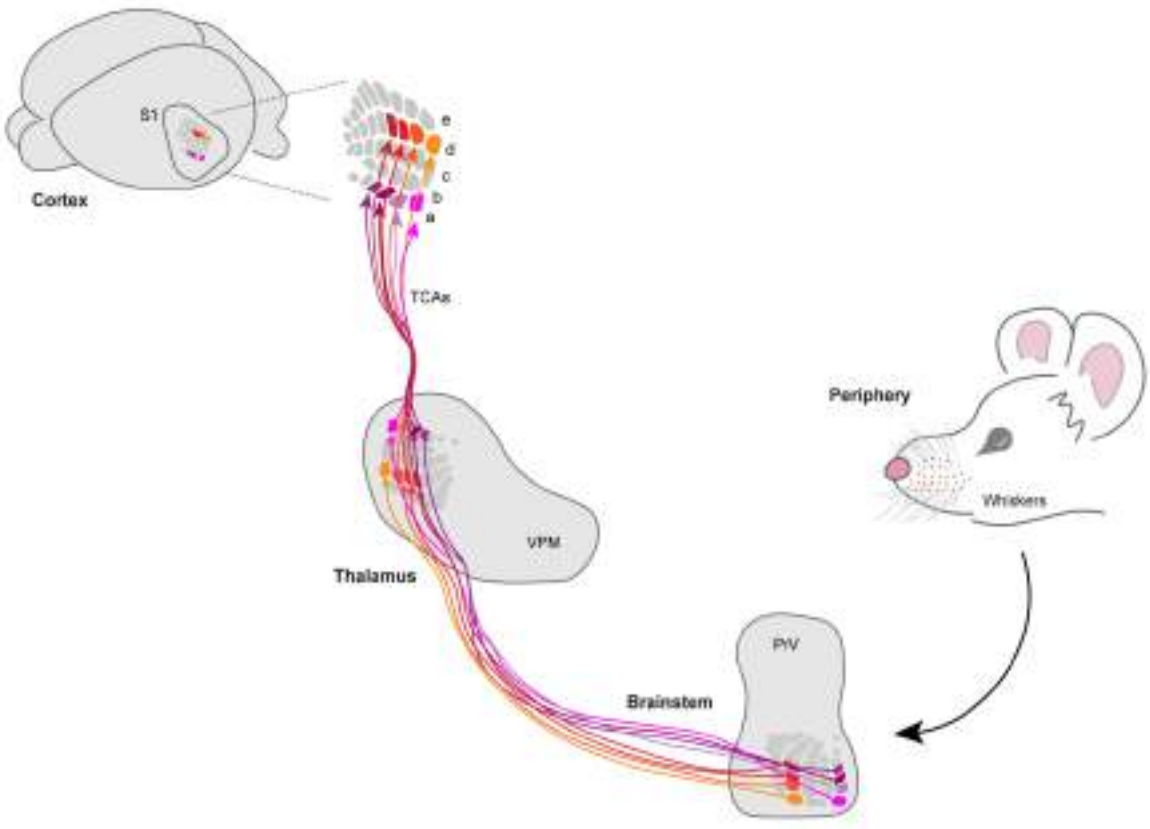


Figure 4. Organization of the somatosensory pathway in mice. Scheme representing the organization of the peripheral axons from the whiskers to the somatosensory cortex, S1. From the whisker pad, where whiskers are organized in five rows, axons are organized in a topographic manner and keep the specific somatotopic connectivity along all sensory stations: PrV in the brainstem, VPM in the thalamus and in the S1.

3.4 Auditory system

The complex circuitry of the auditory system starts in the middle ear, exactly in the auricle, where the sound is collected. Here, the sound generates vibrations that are transmitted to the cochlea, in the inner ear. The cochlea transduces the mechanical energy of sound into electrical signals in the organ of Corti (OC) which is formed by inner hair cells (IHCs) and outer hair cells (OHCs). The cochlea starts to form by E11.5 and at E18.5 IHCs and OHCs are already differentiating. However, at birth, the cochlea is not mature yet and the onset of evoked activity in the auditory pathways is delayed until P12 (Koundakjian et al., 2007). From the cochlea, the auditory information is sent through the cochlear nerve fibers to the cochlear nucleus (CN) in the brainstem, then to the inferior colliculus (IC) in the midbrain, to the Medial Geniculate Body (MGB) in the thalamus and finally to the auditory cortex.

The auditory system exhibits a topographic organization known as tonotopy (Mann and Kelley, 2011). Sounds of different frequencies activate neurons located at different regions of each station of the pathway (Russell and Sellick, 1977; Mann and Kelley, 2011). The first station in which this topographically organization appears is in the cochlea, where cells are organized along the longitudinal axis according to the capability to respond to different frequencies: neurons placed in the basal part respond to higher frequencies and neurons located in the apical part respond to lower frequencies. This tonotopy is also present in the CN and is preserved in the brainstem due to organized axonal pathways. It is thought the establishment of tonotopy is independent of peripheral auditory input (Kandler et al., 2009) and requires gradients of signaling molecules (Fariñas et al., 2001).

4. Spontaneous activity

4.1 Spontaneous activity during development

It has been acknowledged for a long time that spontaneous activity plays a crucial role in brain development. Intrinsically generated activity, which is independent of external input, is involved especially in early developmental processes (Huberman

et al., 2008). The brain of a newborn is able to sense the surrounding environment and interpret it without prior experience. For that, brain circuits have to be correctly connected before birth. This process occurs firstly thanks to molecular factors that guide axons to their correct target (Sanes and Yamagata, 2009) and, in addition, thanks to mechanisms that depend on spontaneous activity (Ackman and Crair, 2014; Blankenship and Feller, 2010).

Spontaneous activity is involved in several processes such as cell differentiation, migration, apoptosis, axon refinement and formation of synapse (Katz and Shatz, 1996; Hanson and Landmesser, 2004; Kilb et al., 2011; Yamamoto et López-Bendito, 2012; Cang and Felheim, 2013). Currently, there are many groups deciphering the mechanisms by which thalamic and cortical neurons communicate during early development. In fact, it has been demonstrated that early gamma oscillations synchronize each thalamic barreloid with its corresponding barrel in the cortex during the first postnatal week (Minlebaev et al., 2011). Spontaneous activity carries topographic information that enables the correct formation of sensory circuits (Erzurumlu and Gaspar., 2012; Mizuno et al., 2018; Arakawa et al., 2014).

4.2 Spontaneous activity in the immature thalamus

The pattern of neuronal activity in the developing thalamus of rodents is modified during embryonic and early postnatal development and it could be subdivided in four different stages. The first one starts around the second embryonic week and is dominated by uncorrelated activity (E12-E15). Then, the pattern becomes more correlated (E16-E18) and, after birth, the third stage is characterized by rhythmic patterns that engage cortical territories. Last, before sensory onset, spontaneous activity changes to a more mature-like pattern.

First stage: calcium spikes and small clusters. From E12 to E15, which correspond with the mid-gestation in mice, spontaneous activity in the thalamus is characterized by calcium transients of asynchronous spontaneous activity in all principal thalamic

nuclei. Spontaneous activity is implicated in the topographical growth of TCAs (Mire et al., 2012; Antón-Bolaños et al., 2018; Martini et al., 2018).

Second stage: thalamic calcium waves. Early uncorrelated activity shifts towards a more correlated pattern dominated by synchronous events known as thalamic calcium waves (Moreno-Juan et al., 2017). They are present in all thalamic principal nuclei from E14.5 and they spread to adjacent nuclei, allowing the communication among them. At the time of birth, thalamic waves are restricted only to dLGN. Thalamic waves also spread from FO to HO thalamic nuclei at perinatal stages but the timeline remains to be studied. Thalamic waves require sodium conductance and gap-junctions because they could be abolished by the application of TTX and carbenoxolone in *ex vivo* thalamic recordings.

Two important papers of the Lopez-Bendito lab have demonstrated the role of thalamic calcium waves in the control of size of cortical areas and in the correct functional organization of cortical circuitry. Regarding to the first role, after bilateral enucleation at E14.5 (before the retinal axons reach the thalamus) there is an increase in the frequency of waves in the VPM, suggesting thalamic waves as a mechanism for intra-thalamic communication and this gives rise to an expansion of S1 and a reduction of V1 (Moreno-Juan et al., 2017). Additionally, experiments in which thalamic waves are disrupted have shown functional defects in the columnar response in the cortex (Antón-Bolaños et al., 2019).

Third stage: spindle bursts and gamma oscillations. After thalamic calcium waves, spontaneous activity patterns gradually change during the first postnatal week. Through extracellular recordings in the thalamic nuclei, it has been demonstrated that thalamic neurons fire action potentials (APs) both spontaneously and after sensory stimulation (Murata and Colonnese, 2016). This mature state consists of early gamma oscillations (30-50 Hz) and spindle bursts, which are fast oscillatory events (8-20 Hz), or a combination of both (Khazipov et al., 2013; Murata and

Colonnese, 2016). These patterns of spontaneous activity could be triggered not only by spontaneous activity but also by external stimulation. In the visual system, the thalamus drives the cortical cells and, when the retina or thalamus are lesioned, most cortical activity disappears (Murata and Colonnese, 2016). Similar results were found for the somatosensory system. After somatosensory stimulation, thalamic responses in the barreloids were synchronized with S1 cortical responses in their corresponding barrels (Minlebaev et al., 2011; Yang et al., 2013). And, the inactivation of the cortical activity affects spontaneous and evoked activity in the thalamus.

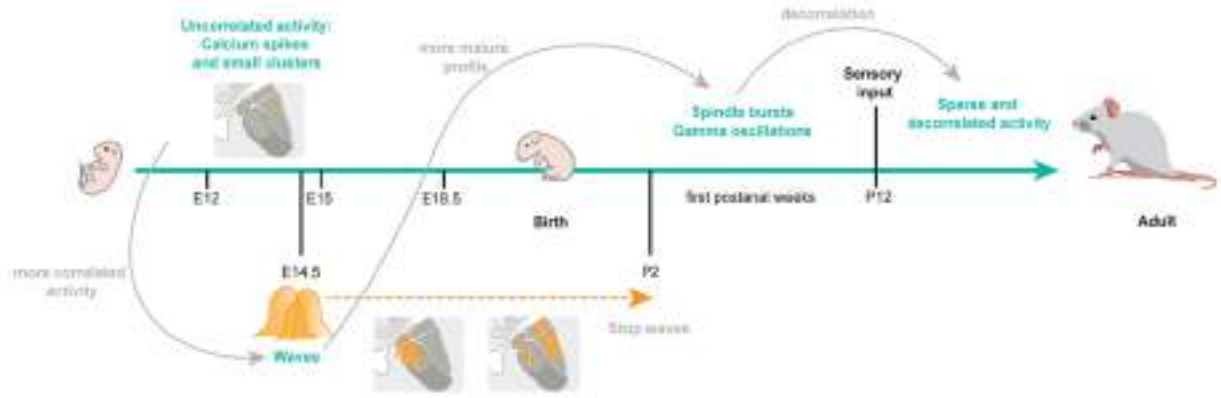


Figure 5. Thalamic spontaneous activity pattern from embryonic stages until first postnatal weeks. Properties of spontaneous activity change from early uncorrelated activity consisting of calcium spikes and small clusters to a more correlated activity (thalamic calcium waves) during late gestation and birth. These waves communicate the three principal thalamic nuclei and at perinatal stages, they spread towards high order nuclei. After birth and during the first postnatal days, spontaneous activity in the thalamus is formed by spindle bursts and gamma oscillations that become sparse and decorrelated activity after sensory onset.

4.3 Spontaneous activity during cortical development

During development, cortical neurons start to establish synaptic connections that allow the generation of organized functional cortical circuits. Genetic factors have a determinant role in the cortical connections (Polleux, 2005), but both spontaneous and sensory-driven activity are also crucial for the correct development of the cortical network (Khazipov and Luhmann, 2006). Electrical activity has been

implicated in different processes as neuronal differentiation, migration, synaptogenesis, specification and synaptic plasticity (Rakic and Komuro, 1995; Fox, 2002; Katz et al., 2002; Feller and Scanziani, 2005; Spitzer et al., 2004). Additionally, sensory-driven mechanisms and spontaneous correlated activity are involved in shaping cortical columns and in the early formation of columnar circuits, respectively (Wiesel and Hubel, 1963; Kanold and Luhman, 2010; Yuste et al., 1992; Dupont et al., 2006; Mizuno et al., 2018; Antón-Bolaños et al., 2019).

Cortical activity during developmental stages is organized in different spatiotemporal patterns.

From uncoordinated to more coordinated pattern. During late embryonic development, cortical neurons experiment the first switch in their spontaneous activity pattern. The early uncoordinated and sparse pattern becomes progressively more coordinated by P0, when neurons start to form connections (Corlew et al., 2004). It has been proposed that this synchronous state of activity strengthen synaptic connections (Kerschensteiner, 2014; Winnubst et al., 2015).

Spindle bursts and gamma oscillations. At early postnatal stages, spontaneous activity in the cortex is dominated by oscillatory patterns known as spindle bursts and gamma oscillations (Luhmann et al., 2016; Yang et al., 2016; Khazipov and Luhmann, 2006; Minlebaev et al., 2007; Hanganu-Opatz, 2010; Luhmann 2017; Khazipov and Milh, 2018). Spindle bursts have a frequency oscillating around 5-25 Hz, last for 1 second and occur with a frequency of approximately 5 events per minute. On the other hand, gamma oscillations have a frequency of 30-40 Hz, last 150-300 milliseconds and occur every 10-30 seconds (Khazipov et al., 2004; Yang et al., 2009; Minlebaev et al., 2011; Gerasimova et al., 2014). While spindle bursts are confined in the space around 200-400 microns and synchronize local neuronal networks, gamma oscillations occupy smaller cortical territories of around 200

microns. In any case, both patterns comprise local events (Khazipov el al., 2004; Hanganu et al., 2006; Yang et al., 2009).

Both spindle bursts and gamma oscillations are triggered by peripheral inputs such as spontaneous movements or retinal waves (Hanganu et al., 2007; Colonnese and Khazipov, 2010). The thalamus is crucial for the generation of both patterns. Silencing the thalamus completely suppresses spontaneous cortical activity (Yang et al., 2013).

Decorrelation of cortical spontaneous activity. There is a critical time point for cortical activity around the second postnatal week in mice, when sensory information starts to reach cortical areas and becomes the main driver for sensory pathways. Therefore, just before sensory onset, cortical circuits are reorganized and activity switches from a correlated pattern to a sparser and more decorrelated neuronal firing (Golshani et al., 2009; Rochefort, 2009; Colonnese et al., 2010; Andre et al., 2010; Colonnese and Phillips, 2018; Mizuno et al., 2018; Nakazawa et al., 2020). This mature state allows a more efficient neuronal coding and primary cortices are able to respond to specific features of stimulation such as the direction of the whisker deflection or the orientation of visual stimulation (Landers and Philip Zeigler, 2006; Hagihara et al., 2015; van der Bourg et al., 2017; Gribizis et al., 2019).

Many efforts have devoted to understand the role of early cortical spontaneous activity in the formation of cortical circuits and cortical columns. Columnar organization in immature circuits has been demonstrated, for example, through extracellular recordings in the neonatal cortex of mice, which have revealed synchronized activity within columns both spontaneously and after sensory stimulation (Dupont et al., 2006; Kummer et al., 2016; Mizuno et al., 2018; Nakazawa et al., 2020). This columnar organization is established before birth, predicting the functional architecture of mature stages (Antón-Bolaños et al., 2019).

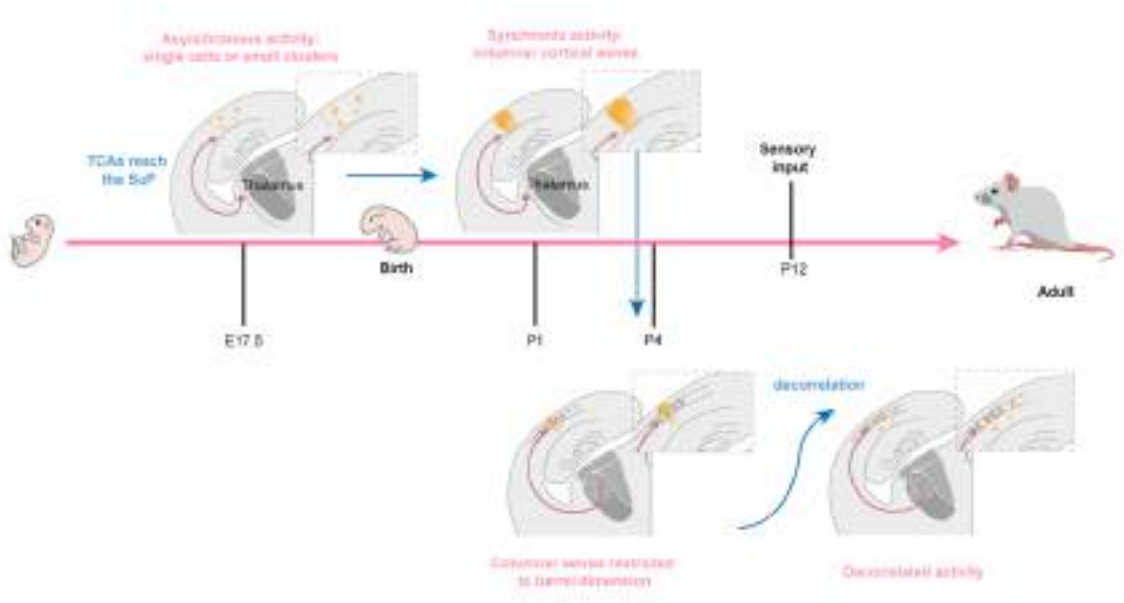


Figure 6. The patterns of cortical spontaneous activity from embryonic stages until the first postnatal weeks. Properties of spontaneous activity change from asynchronous activity during late embryonic development when thalamocortical axons arrive to the subplate to a more synchronous pattern characterized by cortical waves. These waves are more restricted during first postnatal days and at P4, coincide with the dimension of one barrel in the somatosensory cortex. Before the onset of evoked sensory activity, there is a decorrelation of the pattern.

4.4 The role of spontaneous activity in visual system

Spontaneous activity patterns in the immature retina contribute to the generation and refinement of visual circuits. The earliest pattern is featured by highly correlated firing of neighboring RGCs that propagate spatio-temporally, the retinal waves. Spontaneous retinal waves start to appear at late embryonic stages and are present until the second postnatal week, coinciding with eye-opening. These waves have been classified defining three developmental stages: stage I, II and III. Stage I retinal waves occur from around E16 until birth and are defined as bursts of activity mediated by gap junctions that allow the communication among RGCs (Syed et al., 2004; Kahne et al., 2019). Stage II retinal waves are large propagating events which occur from P0 to around P9 and are mediated by cholinergic transmission (Ford et al., 2012). Last, stage III retinal waves are fast and focused events mediated by glutamatergic transmission; they begins around P10 and disappear by eye-opening

(P12-P13) (Firth et al., 2005; Blankeship et al., 2009). Retinal waves are involved in eye-specific segregation, retinotopy and in the organization of receptive fields (Huberman et al., 2008; Ackman et al., 2012; Ackman and Crair, 2014; Arrollo and Feller, 2016; Thomson et al., 2017; Huberman, 2007; Huberman et al., 2008; Seabrook et al., 2017).

Spontaneous retinal waves propagate towards the dLGN, SC and V1 (Colonnese and Khazipov, 2010; Ackman et al., 2012; Siegel et al., 2012). Experiments done during the first postnatal weeks have shown that in these three structures there is wave-like activity driven by retinal waves (Murata and Colonnese, 2016). Moreover, simultaneous *in vivo* calcium imaging recordings of SC and V1 activity have revealed that events in both areas share similar dynamics (Ackman et al., 2012).

In this section we have focused on spontaneous activity; however, evoked activity has also an important role for the correct functional visual circuit. Before eye-opening, light is able to pass through the eyelids and constitute a visual stimulus (Krug et al., 2001). In that way, light could give rise to the generation of stage III retinal waves before the sensory onset and influence the process of eye-specific segregation (Tiriac et al., 2018). Moreover, dark-rearing studies have revealed also an important role of evoked activity in refinement of topography and in some properties such as orientation selectivity (White et al., 2001; Huberman, 2007).

4.5 The role of spontaneous activity in somatosensory system

For the somatosensory system, the electrical messages ascend from the whiskers towards the somatosensory cortex. This transmission is already established at P0 (Iwasato and Erzurumlu, 2018) and it is organized in every station in a topographic manner (Killackey et al., 1995; Sehara and Kawasaki, 2011). The fact that spontaneous activity pattern is topographically organized in all of the

somatosensory stations at birth suggest the presence of an emergent cortical network before the barrel formation. (Golshani et al., 2009; Yang et al., 2013; Mitrukhina et al., 2015). Indeed, when a single whisker or a single barreloid is stimulated in pups, there is a specific columnar response in the cortex (Yang et al., 2013).

However, the mechanisms of propagation of spontaneous correlated activity through these stations remain unclear. On one hand, it has been demonstrated that intact periphery is crucial for the correct formation of the barrel cortex during the first postnatal week (Rice and Van der Loos, 1977). During this period of time, specific conditions are required for the correct assembly of the somatosensory circuit. In fact, whereas the absence of these conditions during this first postnatal week lead to aberrant alterations, later manipulations do not evoke important changes (Van der Loos and Woolsey, 1973; Erzurumlu and Gaspar, 2012). For instance, it has been demonstrated that a lesion in the infraorbital nerve (ION) before the formation of the barrels gives rise to an abnormal organization of TCAs in L4 of the barrel cortex (Weller and Johnson, 1975; Durham and Woolsey, 1984; Erzurumlu and Gaspar, 2012). In addition, experiments of whiskers trimming or plucking revealed that sensory activity is not completely blocked due to the passive contacts that can stimulate the receptors on the snout, suggesting the involvement of sensory activity in the correct arrangement of sensory maps (Erzurumlu and Gaspar, 2012). Therefore, despite active whisking does not start in mice until P12 the, this early evoked activity is a driver of cortical activity during the development of the somatosensory map (Akhmetshina et al., 2016). On the other hand, thalamic input has also demonstrated to be important for the generation of cortical activity. Through *in vivo* simultaneous recordings of VPM and S1, it has been shown that when VPM is lesioned, S1 spontaneous activity is reduced (Yang et al., 2013). The involvement of the thalamic input in the generation of the barrel cortex activity is due to the interaction between thalamic axons with the subplate (Molnár et al., 2003; Kanold and Luhmann, 2010; Hoerder-Suabedissen and Molnar, 2015). When subplate activity is removed, the correct formation of the cortical maps is disrupted

(Dupont et al., 2005; Hanganu et al., 2002; Tolner et al., 2012). Moreover, when receptors in the whisker pad are silenced with lidocaine, spontaneous activity in the somatosensory cortex decreased massively, suggesting that the spontaneous activity in S1 during this period is mainly driven by the periphery (Yang et al., 2013).

Therefore, these results suggest that spontaneous electrical activity acts as a messenger carrying, at least, topographic information. Presynaptic and postsynaptic activity-dependent mechanisms have been involved and they have been thoroughly reviewed in Martini et al., 2018. Peripheral lesions, pharmacological blockade of activity and genetic approaches allowed to find molecules such as NMDAR1 or metabotropic glutamate receptor 5 among others that are crucial for the correct assembly of somatosensory map (Li et al., 1994; Iwasato et al., 1997; Watson, 2006; She et al., 2009; Martini et al., 2017). All of these studies highlight the important role of spontaneous and evoked activity in the correct formation and refinement of the cortical topographic maps.

5. Cross-modal plasticity

Brain plasticity represents an adaptive response of neuronal networks after an insult. Peripheral input deprivation leads to cross-modal plasticity, a phenome by which there are adaptive reorganizations of the intact modalities to compensate to some extent the missing sensory input (reviewed in Martini et al., 2018). In addition, changes in the deprived circuits have also been reported.

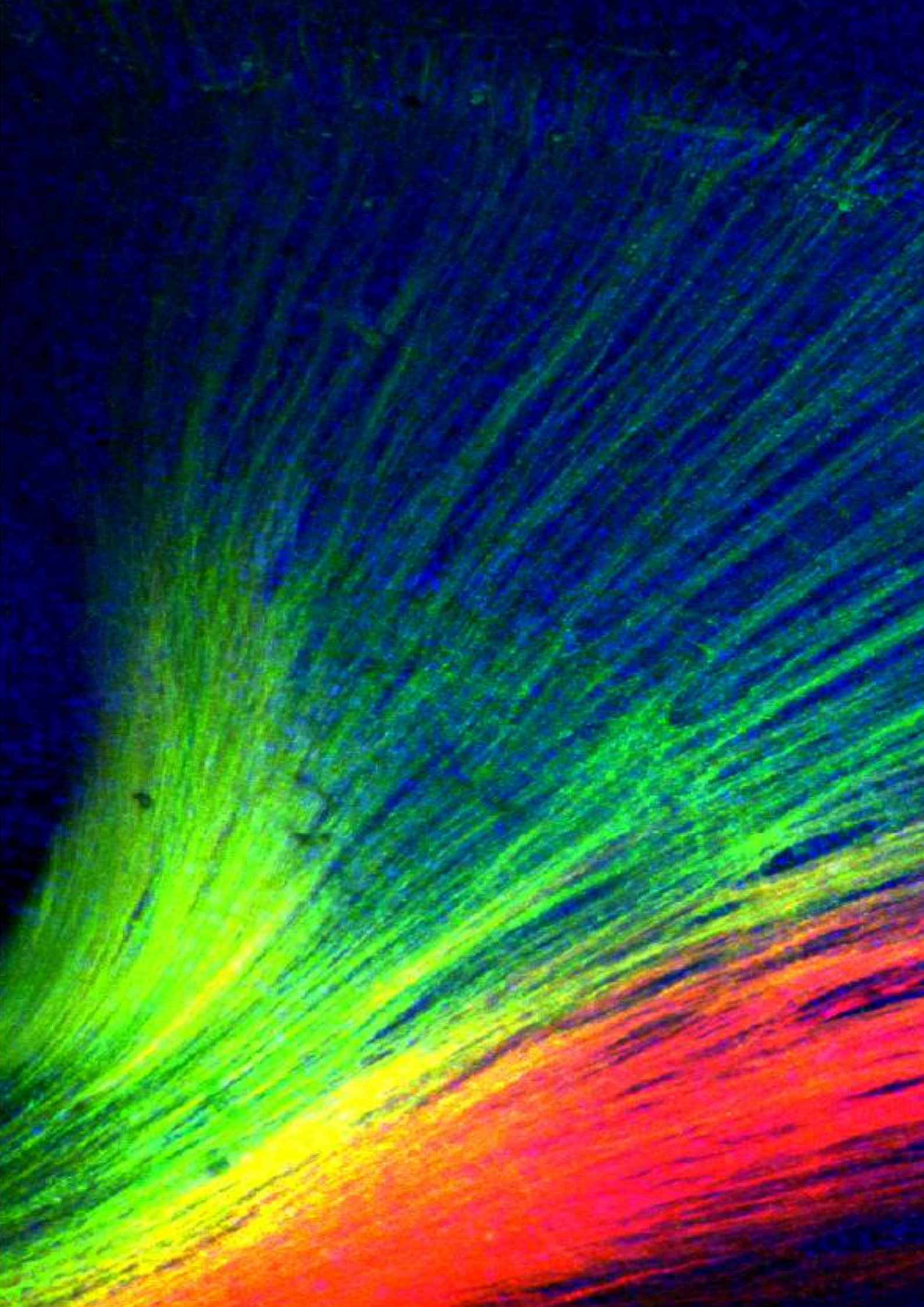
5.1 Mechanisms involved in cross-modal plasticity

For a long time, cross-modal plasticity observed upon sensory loss was attributed to the increase of experience-dependent activity of the intact modalities during the postnatal and adult lifespan. Classical studies of enucleations at P0 in rodents showed an increase in the length of whiskers and an increase in the neural activity driven by experience (Rauschecker et al., 1992; Bronchti et al., 1992; Toldi et al.,

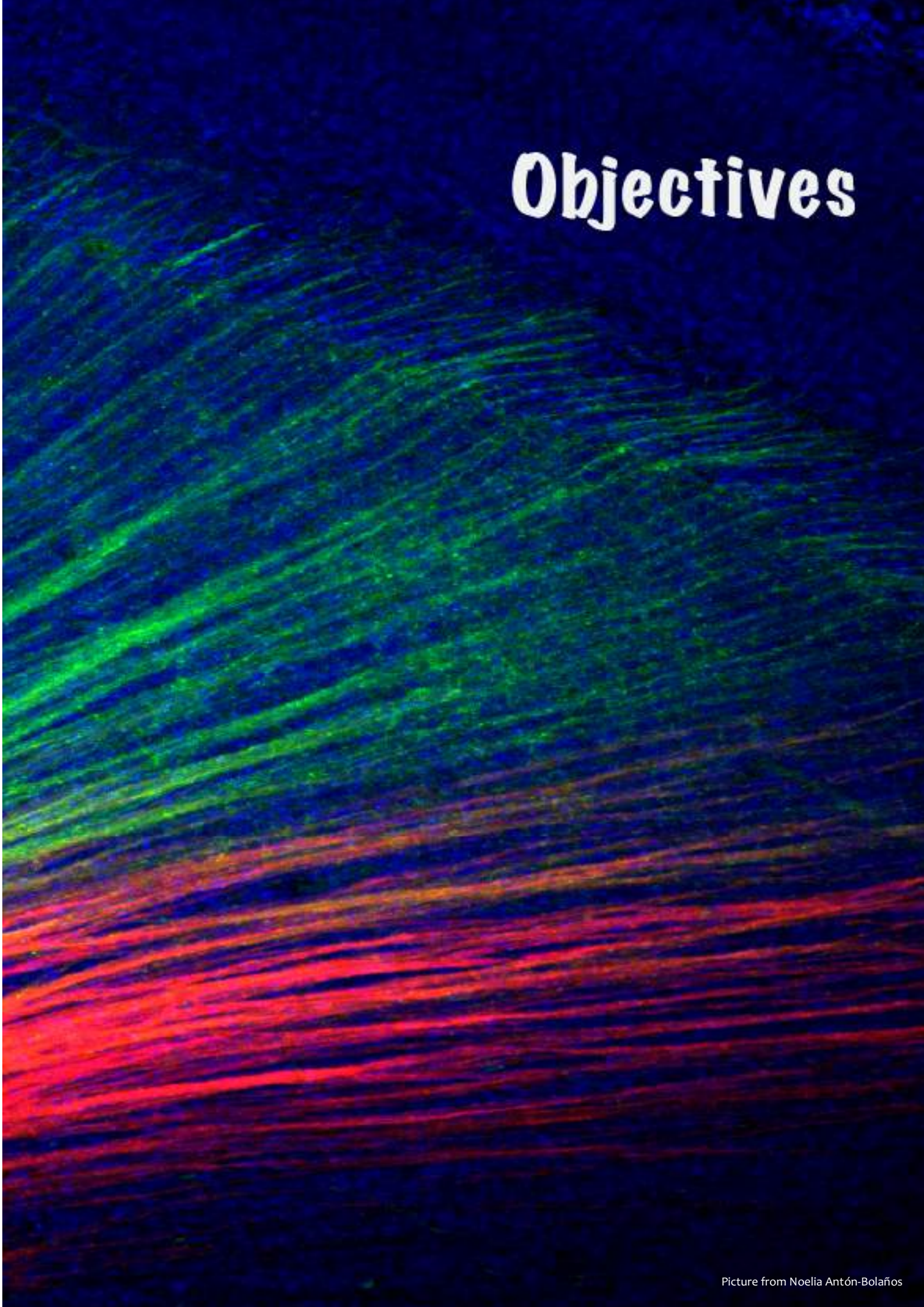
1996). However, these adaptations can be found also at earlier stages, before the onset of sensory experience (Huberman et al., 2006). For instance, enucleations at P0 lead to an expansion in the barrel area in the somatosensory cortex (Fetter-Pruneda et al., 2013). In addition, our group has demonstrated that this increase in the barrel size can be found already at P4 and is related with changes in the expression pattern of thalamic genes and with changes in spontaneous activity, evidencing the influence of the thalamus (Moreno-Juan et al., 2017). So, both peripheral and central stations seem to be involved in the formation of cortical territories before sensory experience.

The thalamus is the central structure where peripheral sensory information from the different modalities first converge before reaching their cortical targets. Therefore, it seems to be the perfect scenario that facilitates the communication among deprived and intact modalities. Along this line, embryonic thalamic waves communicate visual, somatosensory and auditory thalamic nuclei during normal development. In our lab, it has been shown that, when there is an embryonic visual deprivation or a silencing of the auditory thalamus, the frequency of calcium waves increases in the somatosensory thalamus, a fact that is related to the expansion of barrel-field (Moreno-Juan et al., 2017).

In conclusion, these studies have revealed that both experience-dependent mechanisms and intrinsic mechanisms before sensory experience allow the adaptive changes of cross-modal plasticity that occur after sensory deprivation. Furthermore, within these intrinsic mechanisms, the thalamus emerges as a crucial brain structure in the modulation of early cortical plasticity.



Objectives



Picture from Noelia Antón-Bolaños

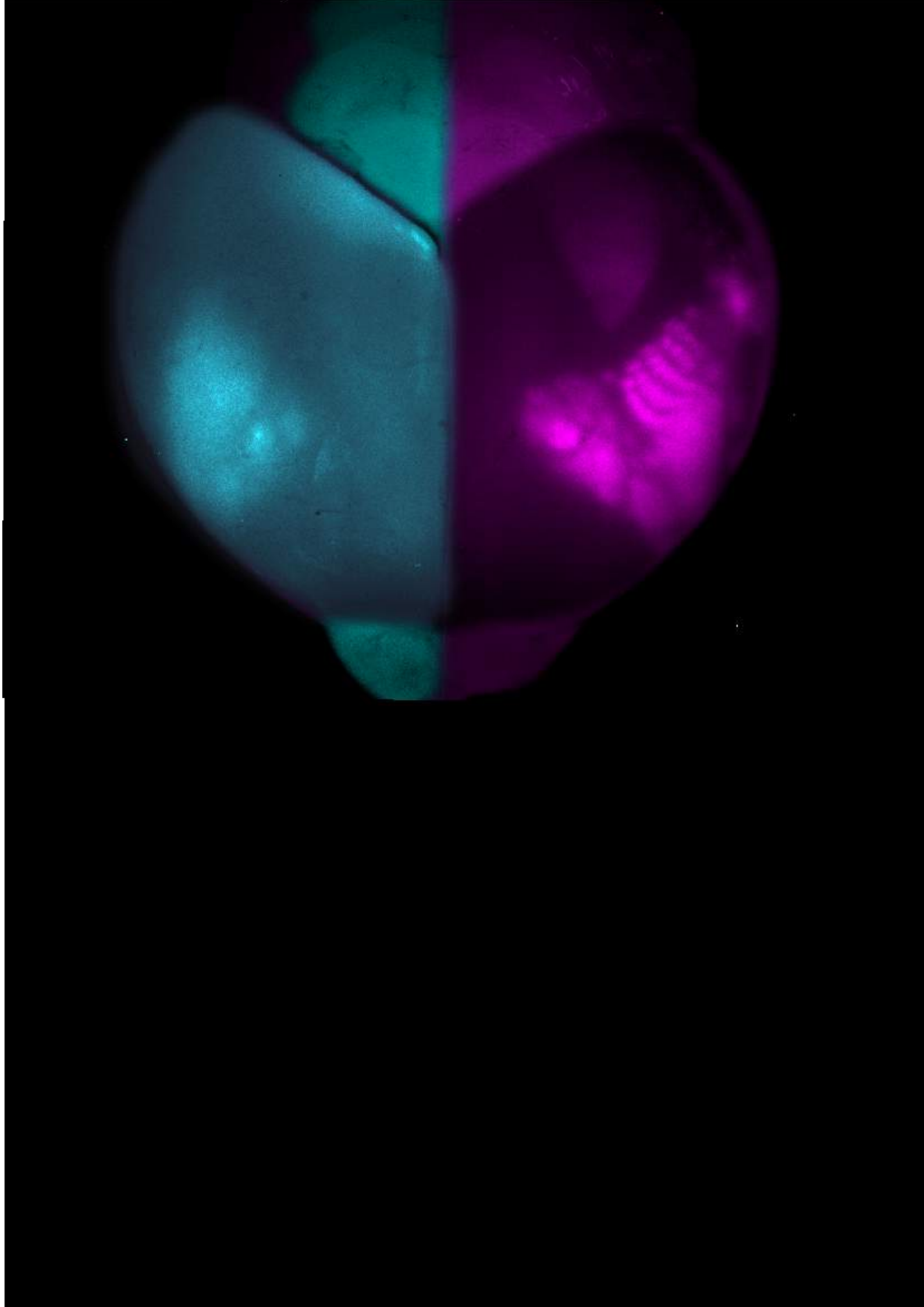
General objectives

The main objective of this PhD Thesis is to investigate the developmental progression of thalamic and cortical spontaneous activity in the sensory pathways of mice. To do so, we pursue the following specific objectives:

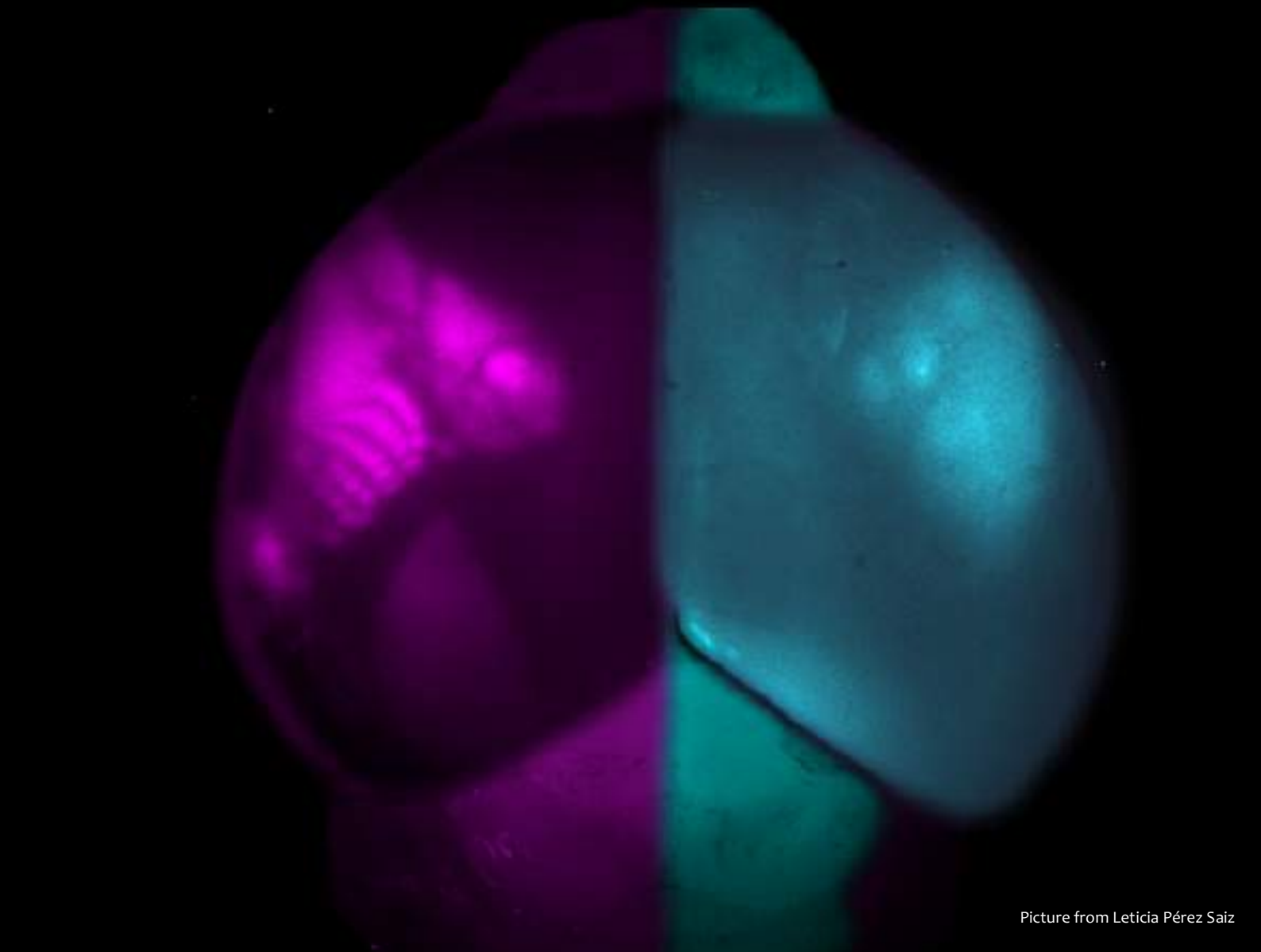
1. To describe the firing properties of thalamic and cortical networks in control mice during early postnatal development by multielectrode recordings.

2. To show how spontaneous activity in the thalamus impacts these firing properties by using models in which thalamic spontaneous avtivity is manipulated.

3. To determine the impact on cortical and thalamic firing properties of early sensory input manipulations.



Material & methods



Picture from Leticia Pérez Saiz

Materials and Methods

Mouse strains

Wild type and transgenic mice used in this study were maintained on an ICR/CD-1 genetic background and all the animals were genotyped by PCR. The day on which the vaginal plug was detected was stipulated as E0.5. These lines have been previously described: the TCA-GFP Tg line, in which the TCAs are labeled with GFP (Mizuno, H. et al., 2014); the R26$^{Kir2.1\text{-}mCherry}$ line (Moreno-Juan, V. et al., 2017), which was generated by inserting a CAG-lox-STOP-loxKir2.1-mCherry-WPRE-pA cassette into the Rosa26 gene locus; and the Rbp4-Cre line, which expresses Cre-recombinase in a subgroup of layer V pyramidal neurons and a small population of VIb neurons (Gong et al., 2007). The R26Kir2.1-mCherry floxed mice were crossed with inducible Cre^{ERT2} mice line driven by the Gbx2 promoter, an early specific thalamic promoter (Gbx2 $^{CreERT2/+}$) (Chen, L. et al., 2009) in order to generate Gbx2$^{CreER/+}$;R26$^{X/+}$ double mutant mice, referred as Th^{Kir}. We also generated the following triple mutant: TCA-GFP-Th^{Kir}.

Tamoxifen induction of Cre recombinase in the double or triple mutant embryos was performed by gavage administration of tamoxifen (5 mg dissolved in corn oil, Sigma) at gestational day 10.5 in order to specifically target all principal sensory thalamic nuclei. The administration of tamoxifen in pregnant mice produces non-desirable side effects such as delivery problems or abortion and decrease survival of newborn pups (Franco, S.J. et al., 2012). In order to prevent these issues, we administered 125 mg/kg of progesterone (DEPO-PROGEVERA®) intraperitoneally at gestational day 14.5. We carried out C-section procedures at gestational day 19.5 and newborns were placed with a foster mother until the day of experiment. In all cases, the Cre^{ERT2}-negative littermates were used as controls of the experimental condition.

The Committee on Animal Research at the University Miguel Hernández approved all the animal procedures, which were carried out in compliance with Spanish and European Union regulations.

In utero enucleation

For in utero enucleation, pregnant females at gestational day 14.5 were deeply anaesthetized with isoflurane (2.5%) to perform the surgery. During the surgery, pregnant mice were kept warm (36-37 °C) by using a heating blanket. Surgical incision was done with a scalpel and 1 ml of ritrodine was applied in order to prevent the uterine contractions during the surgery. When the utero was exposed, both eyes were cauterized in half of the litter. The surgical incision was closed and 1 ml of buprenorphine, was administered subcutaneously to prevent or decrease pain. Embryos were allowed to develop until the age of recording.

In vivo extracellular recordings

P6-P7 and P13-14 mice were deeply anesthetized with isoflurane (2.5%) to perform the surgery. Then, the scalp was removed and the skull cleaned. A 3D-printed holder was glued to the skull by cyanoacrylate adhesive and dental cement, and attached to a stereotaxic apparatus. A 2x2 mm craniotomy was made over the left hemisphere, leaving the dura mater intact. The craniotomy revealed a window above the area of interest, allowing the perpendicular insertion of the multi-channel electrodes. During recordings, mice were kept warm (36-37 °C) by using a heating blanket and lightly anesthetized with isoflurane (0.5%). Recordings were done using 4-shank/16-channel silicon probes, with an inter-electrode pitch of 50 µm and an inter-shank distance of 200 µm (E16+R- 50-S4-L6-200NT, ATLAS) or with a linear electrode of 16 channels pitched 50 µm (E16+R-50-S1-L6NT, ATLAS). Local field potential (LFP, 1-100Hz) and multi-unit activity (MUA, >300Hz) were analyzed separately. Shank trajectories were stained with DiI (1,1′-dioctadecyl 3,3,3′,3′-tetramethylindocarbocyanine perchlorate; Invitrogen) diluted in 70% alcohol. The electrical signal activity was sampled at 20 kHz by a filter amplifier and visualized using MC_RACK software (Multi Channel Systems). Signals were referenced to the reference contact of the probe that laid at the bath level.

Table: Coordinates for the areas of interest (including P2-P3 insertions that were finally discarded)

		Rostro-caudal (from lambda)	Latero-medial (from the midline)	depth
P2-P3	Thalamus	1.8 mm	1.8-2 mm	2-2.6 mm
	S1	1-1.5 mm	1.5-2 mm	0.3-0.6 mm
	V1	0 mm	1-1.5 mm	0.3-0.6 mm
	V2M	0 mm	0.3-0.8 mm	0.3-0.6 mm
P6-P7	Thalamus	2-2.5 mm	1.8-2-5 mm	2.7-3 mm
	S1	3 mm	2-2.2 mm	0.6-1 mm
	V1	0.5-1 mm	1.3-1.8 mm	0.6-1 mm
	V2M	0.5-1 mm	0.3-0.8 mm	0.6-1 mm
P13-P14	Thalamus	2.3-2.8 mm	2-3 mm	3-3.2 mm
	S1	3.2 mm	2-3 mm	1-1.2 mm
	V1	0.8-1.2 mm	1.5-2.5 mm	1-1.2 mm
	V2M	0.8-1.2 mm	0.5-1.5 mm	1-1.2 mm

Whiskers stimulation

Whisker stimulations were performed by applying brief (20-30 ms) air pulses using a syringe place at 2 centimeters from the whiskers. The interval between air pulses was 4 seconds and 30 repetitions were recorded for every insertion.

Histology

After the recording sessions, brains were dissected out and fixed with 4% PFA overnight. In order to determine the position of every channel, 100 µm-thick coronal sections were cut in the vibratome and counterstained with the fluorescent nuclear dye DAPI. As the electrodes were stained with DiI the trajectories of the insertions could be followed.

Data Analysis

The following band-pass filters were applied: 1 Hz to 100 Hz (LFP), 300 Hz to 5 kHz (MUA). We detected spikes using a threshold that corresponds to 4.5 times the standard deviation of the baseline. Analysis was performed using custom scripts written in MATLAB (The MathWorks). Disrupted electrodes were manually discarded. Electrodes were assigned to the thalamic and cortical regions registering them to the histological images. The two most active electrodes were selected from each region and the average frequency was calculated. Correlations were calculated using the spike timing tiling coefficients, a method that discards firing rate as a confounding factor (Cutts and Eglen, 2014).

Immunohistochemistry

For immunohistochemistry, Rbp4-Cre crossed with tdTomato mice were perfused with 4% paraformaldehyde (PFA) in PBS (0.01 M), and the brains were dissected and post-fixed overnight at 4 ºC. Embryonic brains were directly dissected and fixed in 4% PFA overnight. Brains were embedded in 4% agarose and sectioned coronally to 60-70 μm on a vibratome. Brain sections were firstly incubated for 1 hour at room temperature in a blocking solution containing 1% BSA (Sigma) and 0.25% Triton X-100 (Sigma) in PBS 0.01 M. Subsequently, sections were incubated at 4 ºC overnight with the primary antibodies: rat anti-RFP (1:1000, Chromotek, #5F8) and guinea-pig anti vGlut2 (1:5000, Synaptic Systems #135404). After that, sections were rinsed in PBS 0.01 M three times and then, incubated at room temperature for 2 hours with secondary antibodies: Alexa-594 donkey anti-rat (1:500, ThermoFisher, #A21209) and donkey anti-Guinea Pig (1:500, ThermoFisher). Brain sections were rinsed again three times with PBS 0.01 M and finally, were counterstained with the fluorescent nuclear dye DAPI (Sigma-Aldrich).

Statistics

Statistical analysis was carried out using GraphPad Prism6TM and the data are presented as the mean and SEM. Statistical comparison between two populations was performed using unpaired two-tailed Student's t-test or Mann-Whitney U-Test

non-parametric two-tailed test when data failed Kolmogorov-Smirnov or a Shapiro Willk normality tests. A Two-way ANOVA test was performed to compare different thalamic nuclei at different ages, multiple comparisons were corrected using Bonferroni's method. To compare the different cortical areas at different time points between controls and deprived mice, we performed a three-way ANOVA. For evoked activity experiments, Wilcoxon rank-sum test was performed in order to compare the medians of the responses of different populations. P values < 0.05 were considered statistically significant and set as follows *P < 0.05; **P < 0.01 and ***P < 0.001.

No statistical methods were used to predetermine sample sizes, but the number of samples are considered adequate for our experimental designs and consistent with the literature.

Quantifications

Figure 1.

1D, 1E, 1F, 1G. Comparison of firing rate (FR), burst rate (BR), burst duration (BD) and spikes per burst in thalamic nuclei between P6 and P13: two-way ANOVA test, multiple comparison, Bonferroni's method.

1D FR: VPM *P = 0.0219 < 0.05 ; POM ***P < 0.0001; dLGN **P =0.0011 < 0.01; LP ***P < 0.0001

1E BR: VPM P=ns; POM *P= 0.0182 < 0.05; dLGN **P= 0.0030 < 0.01; LP ***P < 0.0001

1F BD: VPM P=ns; POM ***P < 0.0001; dLGN P = ns; LP ***P < 0.0001

1G Spikes per burst: VPM P=ns; POM ***P < 0.0001; dLGN P=ns; LP ***P < 0.0001

1J Correlation amongst pairs of thalamic nuclei from P6 to P13: Two-way anova test, multiple comparison, Bonferroni's method.

VPM-POM *P=0.0206 < 0.05; VPM-dLGN P=ns; VPM-LP P=ns; POM-dLGN ***P=0.0003 < 0.001, POM-LP ***P < 0.0001; dLGN-LP ***P=0.0005 < 0.001

Figure 3.

3B, 3C. Comparison of FR between control and Th^{Kir} for both visual and somatosensory thalamic nuclei. Unpaired two-tailed Student's t-test or Mann-Whitney U-Test non-parametric two-tailed test.

3B: P6-P7, dLGN, ctrl vs Th^{Kir}: *P=0.0406 < 0.05; P13-P14, dLGN, ctrl vs Th^{Kir}: *P=0.0286 < 0.05

P6-P7, LP, ctrl vs Th^{Kir}: P=ns; P13-P14, LP, ctrl vs Th^{Kir}: P=ns

3C: P6-P7, VPM, ctrl vs Th^{Kir}: **P=0.0014 < 0.01; P13-P14, VPM, ctrl vs Th^{Kir}: *P=0.0121 < 0.05

P6-P7, POm, ctrl vs Th^{Kir}: **P =0.0022< 0.01; P13-P14, POm, ctrl vs Th^{Kir}: *P=0.0286 < 0.05

Figure 4.

4B,4C,4D,4E,4F. Burst activity in the developing visual thalamus in control and Th^{Kir} mice. Unpaired two-tailed Student's t-test or Mann-Whitney U-Test non-parametric two-tailed test.

4B BR: P6-P7, dLGN, ctrl vs Th^{Kir}: P=ns; P13-P14, dLGN, ctrl vs Th^{Kir}: *P=0.0286 < 0.05

P6-P7, LP, ctrl vs Th^{Kir}: P=ns; P13-P14, LP, ctrl vs Th^{Kir}: P=ns

4C BD: P6-P7, dLGN, ctrl vs Th^{Kir}: P=ns; P13-P14, dLGN, ctrl vs Th^{Kir}: *P=0.0286 < 0.05

P6-P7, LP, ctrl vs Th^{Kir}: P=ns; P13-P14, LP, ctrl vs Th^{Kir}: P=ns

4D Spikes per burst: P6-P7, dLGN, ctrl vs Th^{Kir}: P=ns; P13-P14, dLGN, ctrl vs Th^{Kir}: *P=0.0501 < 0.05

P6-P7, LP, ctrl vs Th^{Kir}: P=ns; P13-P14, LP, ctrl vs Th^{Kir}: P=ns

4E, 4F ISIs: two-way ANOVA test, multiple comparison, Bonferroni's method.

P6-P7, dLGN, ctrl vs Th^{Kir}: P=ns; P13-P14, dLGN, ctrl vs Th^{Kir}: P=ns

P6-P7, LP, ctrl vs Th^{Kir}: P=ns; P13-P14, LP, ctrl vs Th^{Kir}: P=ns

Figure 5.

5B,5C,5D,5E,5F Burst activity in the developing somatosensory thalamus in control and Th^{Kir} mice. Unpaired two-tailed Student's t-test or Mann-Whitney U-Test non-parametric two-tailed test.

5B BR:P6-P7, VPM, ctrl vs Th^{Kir}: P=ns; P13-P14, VPM, ctrl vs Th^{Kir}: *P=0.0242 < 0.05

P6-P7, POm, ctrl vs Th^{Kir}: P=ns; P13-P14, POm, ctrl vs Th^{Kir}: *P=0.0286 < 0.05

5C BD: P6-P7, VPM, ctrl vs Th^{Kir}: P=ns; P13-P14, VPM, ctrl vs Th^{Kir}: *P=0.0424 < 0.05

P6-P7, POm, ctrl vs Th^{Kir}: P=ns; P13-P14, POm, ctrl vs Th^{Kir}: *P=0.0282 < 0.05

5D Spikes per burst: P6-P7, VPM, ctrl vs Th^{Kir}: P=ns; P13-P14, VPM, ctrl vs Th^{Kir}: *P=0.0424 < 0.05

P6-P7, POm, ctrl vs Th^{Kir}: P=ns; P13-P14, POm, ctrl vs Th^{Kir}: *P=0.0201 < 0.05

5E,5F ISIs: two-way ANOVA test, multiple comparison, Bonferroni's method.

P6-P7, VPM, ctrl vs Th^{Kir}: P=ns; P13-P14, VPM, ctrl vs Th^{Kir}: P=ns P6-P7, POm, ctrl vs Th^{Kir}: P=ns; P13-P14, POm, ctrl vs Th^{Kir}: P=ns

Figure 6.

6C, 6D, 6E, 6F, 6G, 6H. Spontaneous activity in the S1 during first postnatal weeks in control and Th^{Kir} mice. Unpaired two-tailed Student's t-test or Mann-Whitney U-Test non-parametric two-tailed test.

6C FR: P6-P7, S1, ctrl vs Th^{Kir}: *P=0.0107 < 0.05; P13-P14, S1 ctrl vs Th^{Kir}: *P=0.0131 < 0.05

6D BR: P6-P7, S1, ctrl vs Th^{Kir}: *P=0.0164 < 0.05; P13-P14, S1 ctrl vs Th^{Kir}: *P=0.0469 < 0.05

6E BD: P6-P7, S1, ctrl vs Th^{Kir}: P=ns; P13-P14, S1 ctrl vs Th^{Kir}: P=ns

6F Spikes per burst: P6-P7, S1, ctrl vs Th^{Kir}: P=ns; P13-P14, S1 ctrl vs Th^{Kir}: P=ns

6G,6H ISIs two-way ANOVA test, multiple comparison, Bonferroni's method.

P6-P7, S1, ctrl vs Th^{Kir}: P=ns; P13-P14, S1 ctrl vs Th^{Kir}: P=ns

Figure 8.

8D,8E,8F,8G.8H. Comparison of spontaneous activity pattern in visual thalamic nuclei between controls and embBE mice. Unpaired two-tailed Student's t-test or Mann-Whitney U-Test non-parametric two-tailed test.

8D FR P6-P7 dLGN, ctrl vs embBE: ***P=0.0002 < 0.001; LP, ctrl vs embBE: **P=0.0072 < 0.01

P13-P14 dLGN, ctrl vs embBE: P=ns; LP, ctrl vs embBE: P=ns.

8E BR P6-P7 dLGN, ctrl vs embBE: ***P=0.0005 < 0.001; LP, ctrl vs embBE: *P=0.0147 < 0.05

P13-P14 dLGN, ctrl vs embBE: P=ns; LP, ctrl vs embBE: P=ns.

8F BD P6-P7 dLGN, ctrl vs embBE: **P=0.0069 < 0.01; LP, ctrl vs embBE: *P=0.0446 < 0.05

P13-P14 dLGN, ctrl vs embBE: P=ns; LP, ctrl vs embBE: P=ns.

8G Spikes per burst P6-P7 dLGN, ctrl vs embBE: ***P=0.0003 < 0.001; LP, ctrl vs embBE: *P=0.0347 < 0.05

P13-P14 dLGN, ctrl vs embBE: P=ns; LP, ctrl vs embBE: P=ns.

8H ISIs P6-P7 dLGN, ctrl vs embBE: P=ns; LP, ctrl vs embBE: P=ns.

8I ISIs P13-P14 dLGN, ctrl vs embBE: P=ns; LP, ctrl vs embBE: P=ns.

For ISIS: two-way ANOVA test, multiple comparison, Bonferroni's method.

Figure 9.

9C,9D,9E,9F. Correlation within visual thalamic nuclei and among visual and somatosensory thalamic nuclei in control and embBE mice. Two-way ANOVA test, multiple comparison, Bonferroni's method.

9C,9D. P6-P7 dLGN-LP: P=ns; dLGN-VPM: P=ns; dLGN-POM: P=ns, LP-VPM: P=ns; LP-POM: P=ns

9E,9F. P13-P14 dLGN-LP: P=ns; dLGN-VPM: P=ns; dLGN-POM: P=ns, LP-VPM: P=ns; LP-POM: P=ns

Figure 10.

Comparison of spontaneous activity pattern in visual thalamic nuclei between controls and embBE mice. **10C, 10D, 10E, 10F, 10G, 10H**: unpaired two-tailed

Student's t-test or Mann-Whitney U-Test non-parametric two-tailed test. **10I**: two-way ANOVA test, multiple comparison, Bonferroni's method.

10C FR P6-P7 VPM, ctrl vs embBE: ***P=0.0012 < 0.001; POm, ctrl vs embBE: *P=0.0156 < 0.05

P13-P14 VPM, ctrl vs embBE: P=ns; POm, ctrl vs embBE: P=ns

10D BR P6-P7 VPM, ctrl vs embBE: P=ns; POm, ctrl vs embBE: P=ns

P13-P14 VPM, ctrl vs embBE: P=ns; POm, ctrl vs embBE: P=ns

10E BD P6-P7 VPM, ctrl vs embBE: P=ns; POm, ctrl vs embBE: P=ns

P13-P14 VPM, ctrl vs embBE: P=ns; POm, ctrl vs embBE: P=ns

10F Spikes per burst P6-P7 VPM, ctrl vs embBE: *P=0.0342 < 0.05; POm, ctrl vs embBE: P=ns

P13-P14 VPM, ctrl vs embBE: P=ns; POm, ctrl vs embBE: P=ns

10G ISIs P6-P7 dLGN, ctrl vs embBE: P=ns; LP, ctrl vs embBE: P=ns.

10H ISIs P13-P14 dLGN, ctrl vs embBE: P=ns; LP, ctrl vs embBE: P=ns.

For ISIS. two-way ANOVA test, multiple comparison, Bonferroni's method.

10I Correlation VPM-POm: P6-P7 P=ns; P13-P14 P=ns.

Figure 11.

11H,11I Comparison of FR in V2m neurons between control and embBE mice. Unpaired two-tailed Student's t-test or Mann-Whitney U-Test non-parametric two-tailed test.

11H FR of V2m control vs embBE: P6-P7 P=ns; P13-P14 ***P=0.0012 < 0.0001

11I FR of V2m layer IV neurons of control vs embBE: P6-P7 *P=0.0333 < 0.05; P13-P14 *P=0.0148 < 0.05

Figure 12.

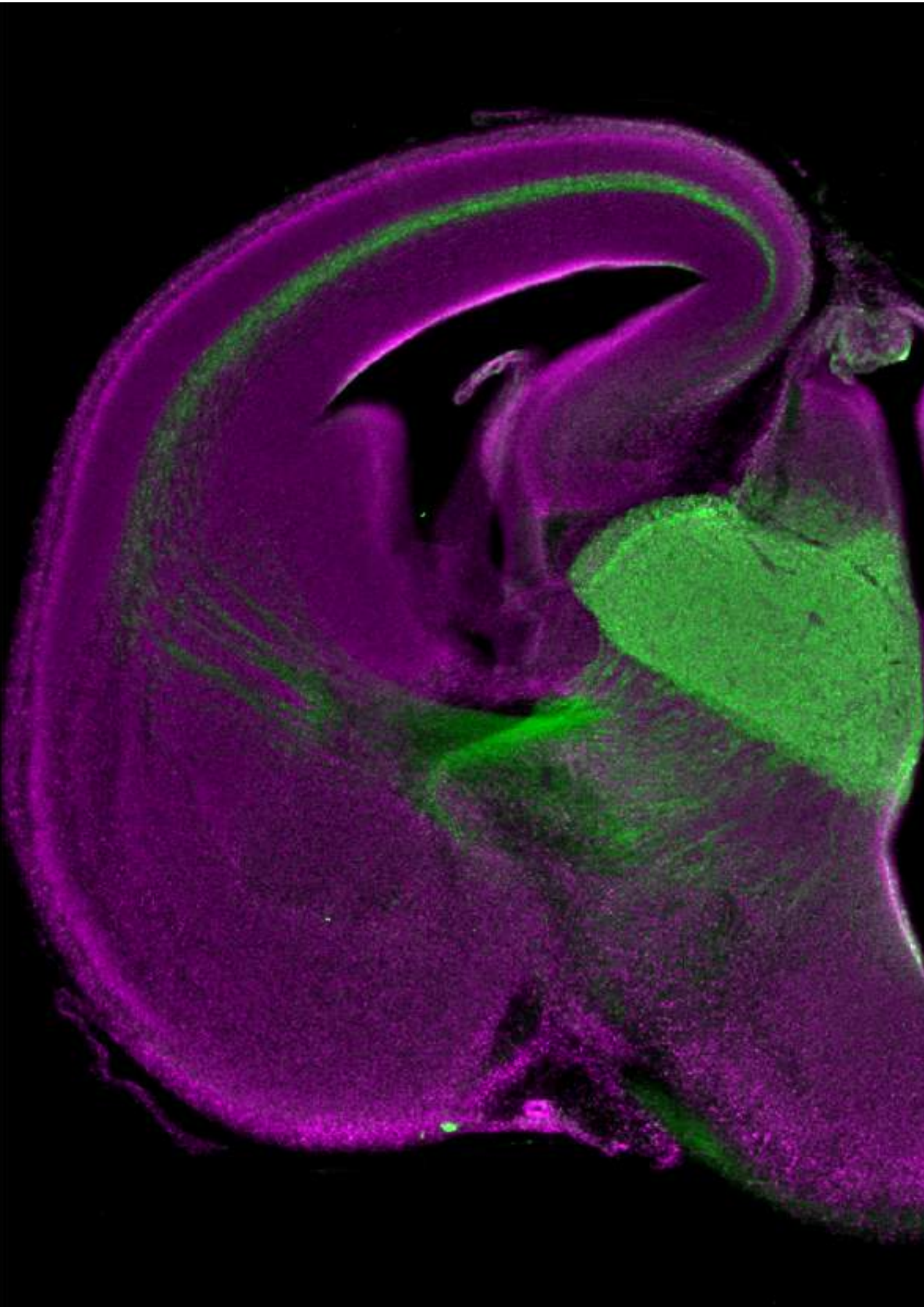
12C FR of S1 control vs embBE: P6-P7 P=ns; P13-P14 **P=0.0178 < 0.005

Figure 13.

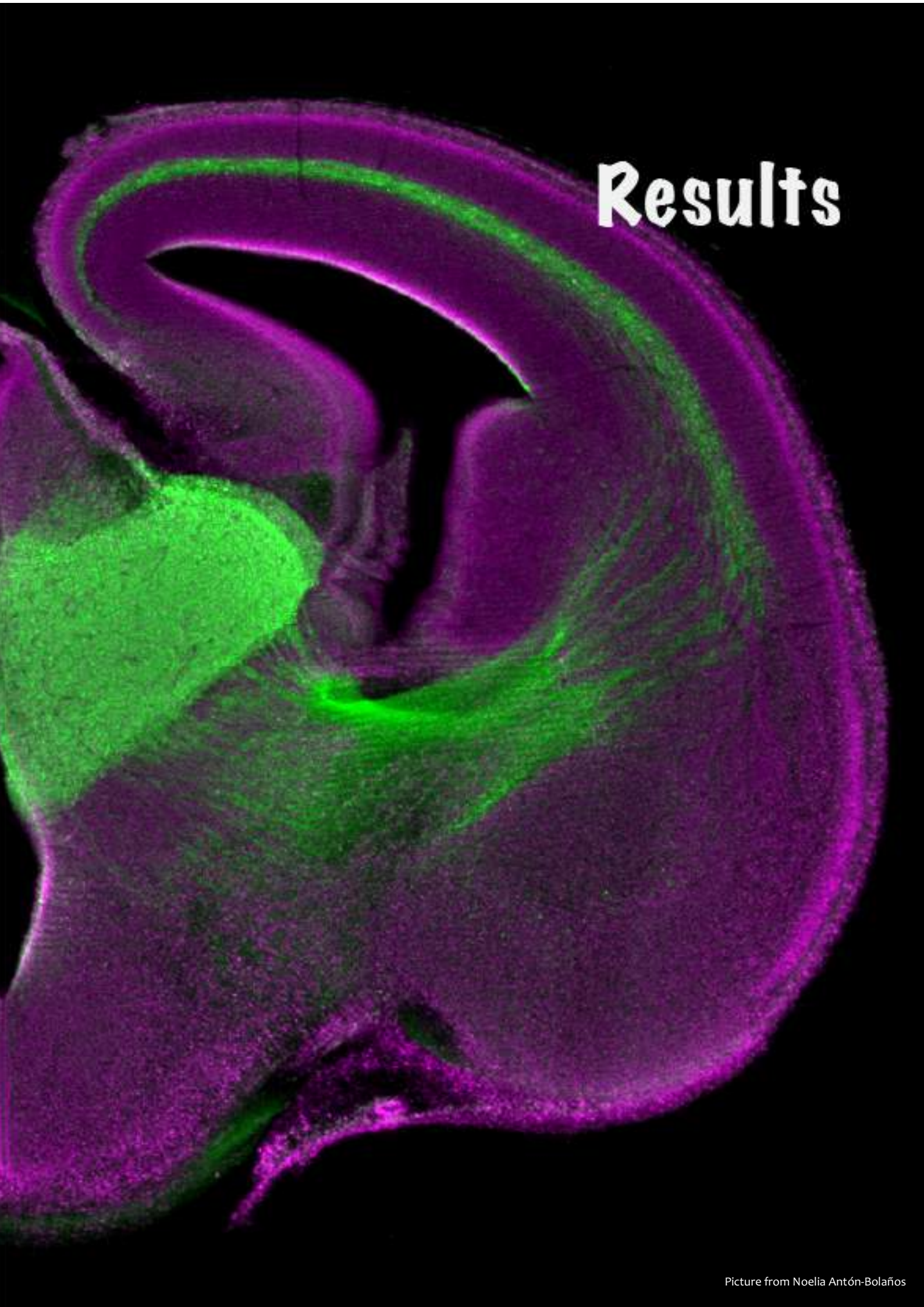
13C. Quantification of the response of S1 neurons to whisker's stimulation. *P=0.02857 < 0.05

13F. Quantification of the response of S1 neurons to whisker's stimulation. *P=0.04714 < 0.05

Wilcoxon rank-sum test.



Results



Picture from Noelia Antón-Bolaños

Results

The role of spontaneous activity on circuit maturation has been extensively studied in the developing sensory cortices (Erzurumlu and Gaspar, 2012; Espinosa and Stryker, 2012; Hanganu-Opatz, 2010; Luhmann et al., 2016). During the first postnatal week, spontaneous activity in the cortex appears as sporadic correlated events that synchronize local and large-scale ensembles of neurons. By the end of the second postnatal week, before the onset of active sensory experience, spontaneous activity becomes continuous and decorrelated resembling the pattern observed in mature networks (Golshani et al., 2009; Rochefort et al., 2009; Colonnese et al., 2010).

The patterns of spontaneous activity sculpt developing circuits. The relevance of these patterns in the assembly of sensory cortices poses an important question about the origin of these patterns. It has been shown that spontaneous activity in the developing sensory cortex is partly driven by peripheral organs such as the retina, the cochlea and muscle spindles (Martini et al., 2021). However, between peripheral sensory organs and cortical sensory areas, the thalamus emerges as a pivotal structure where the ascending pathways from different modalities converge for the first time. Thus, if we are to understand how spontaneous activity sculpts peripheral-to-central pathways, we need to how spontaneous activity develops in the thalamus and to what extent relies on peripheral input.

Chapter I. The firing patterns of spontaneous activity in the thalamus of mice during development

1. The properties of spontaneous firing in the thalamus switch during the second postnatal week

It has been shown that spontaneous activity in the thalamus of rodents emerges at embryonic stages. The embryonic sensory thalamus exhibits spontaneous calcium waves that contribute to the correct development of the cortical circuits (Antón-Bolaños et al., 2018). During the first two postnatal weeks, spontaneous activity in the primary somatosensory and visual thalamus is characterized by bouts of spindle and gamma bursts (Khazipov et al., 2013; Murata and Colonnese, 2016; Yang et al., 2013). By the end of the second postnatal week, the visual thalamus switches towards a mature-like activity pattern (Murata and Colonnese, 2016). Our aim here was to simultaneously record the spontaneous activity in first (FO) and higher-order (HO) nuclei of the visual and somatosensory thalamus and compare their developmental progression.

We performed in vivo extracellular recordings in the first and higher-order nuclei of the thalamus of mice at P6-P7 and P13-P14 (Fig 1A). We used silicon probes with 16 electrodes distributed in 1 or 4 shanks. Similar experiments were performed in P2-P3 mice but these recordings were not reliable and were discarded. To visualize the sensory nuclei and localize the probe after each experiment, we used a TCA-GGP mouse line (Mizuno et al., 2014) in which thalamocortical cells express GFP (Fig 1B).

We characterized first the developmental progression of the firing rate. We found an increase in multi-unit activity (MUA) from P6 to P14 in visual and somatosensory nuclei of the thalamus (Fig 1C and 1D). By P14, a significant proportion of the spiking activity fired in bursts, especially in the dLGN and LP (Fig 1E); but, the burst duration and number of spikes per burst only increased in HO nuclei (LP and POm) (Fig 1F and 1G). In addition, we determined to what extent activity was correlated

among sensory nuclei (Fig 1H and 1I). To avoid changes in correlation owing to the increasing levels of firing rate, we estimated the correlation using the spike time tiling coefficient (STTC) parameter (Cutts and Eglen, 2014). In contrast to what has been previously observed in the somatosensory and visual cortices (Golshani et al., 2009; Rochefort et al., 2009), we found that correlations among sensory nuclei were low at P6-P7 and increased significantly by the end of the second postnatal week (Fig 1J).

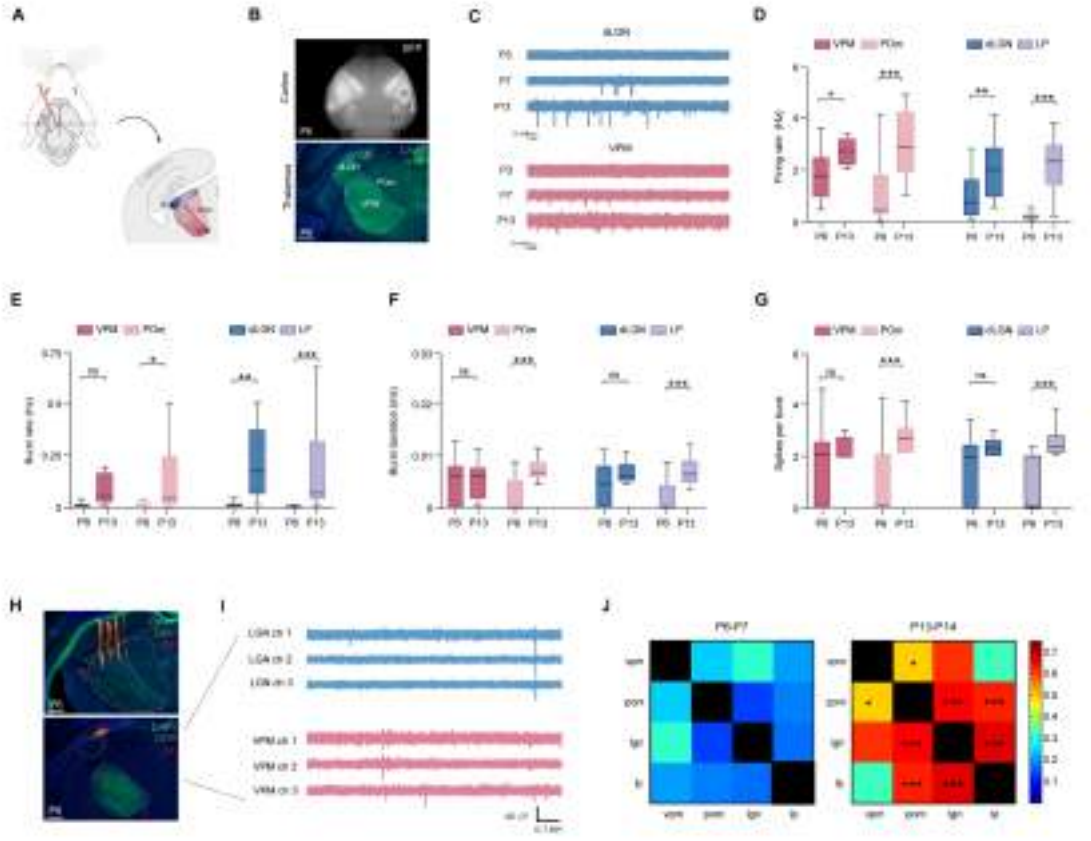


Figure 1. Spontaneous activity in the visual and somatosensory nuclei of the thalamus during the first two postnatal weeks. (A) Experimental design of extracellular recordings in the thalamus. (B) Top, zenithal view of a P6 TCA-GFP mouse brain in which the fluorescent thalamocortical projections depict sensory areas in the cortex. Bottom, coronal section of the same animal showing the expression of GFP in the principal nuclei of the thalamus. Scale bar: 250 μm. (C) Examples of MUA activity in the VPM and dLGN at different postnatal ages. (D) Progression of firing rate from P6 to P13 in somatosensory and visual thalamic nuclei. (E) Progression of burst rate from P6 to P13 in somatosensory and visual thalamic nuclei. (F) Quantification of the duration of bursts from P6 to P13 in somatosensory and visual thalamic nuclei. (G) Quantification of the number of spikes per burst from P6 to P13 in somatosensory and visual thalamic nuclei. (H) Images of brains where two thalamic nuclei have been recorded simultaneously in vivo. Scale bar: 250 μm. (I) Examples of traces recorded simultaneously from the dLGN (blue) and VPM (pink) at P13; only three channels

in each nucleus are shown. (J) The STTC coefficient among thalamic nuclei at P6-P7 and P13-P14. Asterisks indicate significant pair-wise correlations. Number of experiments for firing rate, burst rate, burst frequency and number of spikes per burst: P6-P7: dLGN n=22, LP n=25, VPM n=18, POm n=18. P13-P14: dLGN n=13, LP n=18, VPM n=10, POm n=22. The graphs represent the mean ± SEM. Number of experiments for correlation measurments: P6-P7: VPM-POm n=11, VPM-dLGN n=11, VPM-LP n=5, POm-dLGN n=10, POm-LP n=15, dLGN-LP n=8. P13-P14: VPM-POm n=8, VPM-dLGN n=4, VPM-LP n=6, POm-dLGN n=5, POm-LP n=10, dLGN-LP n=5. *P<0.05, **P<0.01, ***P<0.001.

Chapter II. The spontaneous activity in the developing somatosensory thalamus influences the firing properties of S1 neurons

There is evidence that suggests an impact of thalamic spontaneous activity on cortical development. At perinatal stages in mice, the thalamus exhibits a wave-like pattern of spontaneous activity that traverses the nuclei of the different sensory modalities. These waves control the size and the formation of functional maps in the sensory areas of the cortex during the first postnatal weeks (Moreno-Juan et al., 2017; Antón-Bolaños et al., 2019). Recent studies have demonstrated that the acute inhibition of electrical activity in the developing visual thalamus reduces the firing rate of neurons in V1 (Murata and Colonnese, 2018). However, we ignore to what extent cortical firing properties are affected when thalamic spontaneous activity has been perturbed from embryonic stages onwards.

1.The overexpression of Kir2. modifies the spontaneous firing of thalamic neurons

In order to modify the thalamic activity from embryonic stages in vivo, we overexpressed the inward rectifier potassium channel Kir2.1 in the developing sensory nuclei of the thalamus. To this purpose, we combined the Cre driver mouse line $Gbx2^{CreER}$ with the $R26^{Kir2.1}$ floxed mouse line ($Gbx2^{CreER}$::$R26^{Kir2.1}$, referred herein as Th^{Kir}). The transcription factor Gbx2 is a determinant for the acquisition of a thalamic fate. It is crucial for the segregation of thalamic neurons and for the assembly of sensory nuclei (Chen et al., 2009). It has a temporal dynamic expression in the thalamus and also in other brain structures at different developmental time-points. Thus, to assure a selective expression in the developing sensory nucleus of the thalamus, we implement a tamoxifen-dependent strategy

whereby tamoxifen was administrated to pregnant dams at E10.5 (Fig 2A). At this stage, Gbx2 is expressed almost exclusively in the sensory nuclei of the thalamus (Vue et al., 2007; Chen et al., 2009; Li et al., 2012; Nakagawa and O'Leary, 2001). We confirmed the localization and the level of overexpression of Kir2.1 by immunofluorescence assays against mCherry, a reporter protein fused to Kir2.1 (Fig 2B).

In order to characterize the spontaneous activity pattern of the thalamus in the Th^{kir} mice, we performed in vivo extracellular recordings from the FO and HO visual and somatosensory nuclei of the thalamus at P6-P7 and at P13-P14, just after the onset of active sensory experience (Fig 2C and 2D).

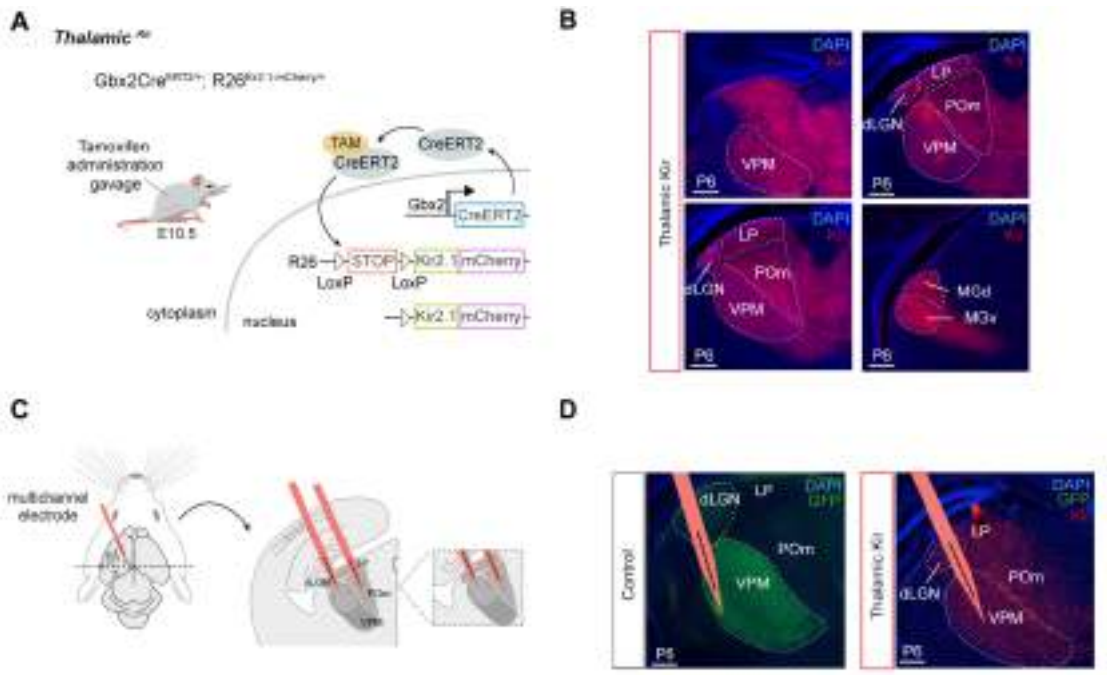


Figure 2. Experimental design of extracellular recordings in mice in which the thalamic activity is modified. (A) Schema that represents the Cre/flox system used to overexpress Kir2.1 in the Th^{Kir} mice by administrating tamoxifen at E10.5. Kir2.1 is fused to the mCherry reporter protein. (B) Coronal slices of the thalamus of Th^{Kir} mice at P6. The recombination is restricted to the FO and HO sensory nuclei and to some medial nuclei. (C) Schema that represents the experimental design used to acquire in vivo extracellular recordings from the developing thalamus. (D) Examples of coronal slices depicting the insertions of 16-channel probes in control and in Th^{Kir} mice. Scale bars, 250 µm.

In the visual system, the average firing rate in the dLGN was similar to that of the

LP at P6-P7. At P13-P14, the activity in the dLGN seems to increase but it is not significantly higher than LP activity (Fig 3B, gray box plots, statistical comparisons not shown in the figure). In the somatosensory system, the POm seems to fire more than the VPm, however, differences do no reach statistical significance (Fig 3C, gray box plots, statistical comparisons not shown in the figure). Comparing the developmental progression within each nucleus, we found that there is a substantial increase in the firing rate with age in control mice (Fig 3B and 3C, statistical comparisons not shown in the figure). This is the general trend except for the POm where the robustness of the data was not enough to determine if there are statistical differences.

The overexpression of Kir2.1 in the thalamic sensory neurons should hyperpolarize the resting membrane potential and reduce the spiking activity in Th^{Kir} mice. Previous studies have demonstrated that the overexpression of Kir2.1 in thalamic neurons switches their pattern of activity. While embryonic thalamic neurons normally exhibit highly synchronous activity in the form of propagating waves, overexpressing Kir2.1 leads to an asynchronous firing without wave-like activity (Moreno-Juan et al., 2017; Antón-Bolaños et al., 2019). Large thalamic waves normally disappeared shortly after birth when they are replaced by a non-propagating synchronous pattern of activity that engages neurons locally and gives rise to small patches of activity (data not shown). We assessed here which is the impact of overexpressing Kir2.1 on this pattern of early postnatal activity in the thalamus. In Th^{Kir} mice, we found that the firing rate in all sensory nuclei but the LP was diminished compared with controls at P6-P7 and P13-P14. In the control LP, the firing rate is rather low at these stages (Fig 3A-3C).

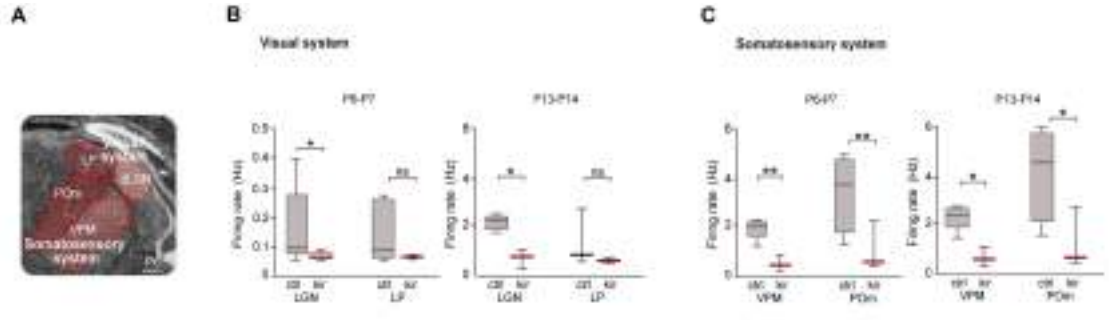


Figure 3. Spontaneous firing rate in the thalamus at P6-P7 and P13-P14 in controls and Th^{Kir} mice. (A) Image of a coronal view of a P6 thalamus showing the visual and somatosensory sensory nuclei. (B) The MUA firing rate at different developmental stages in the FO and HO nuclei of the visual thalamus. (C) The MUA firing rate at different developmental stages in the FO and HO nuclei of the somatosensory system.
Quantifications for firing rate: P6-P7: dLGN control n=5, Th^{kir} n=6; LP control n=6, Th^{kir} n=5. P13-P14: dLGN control n=4, Th^{kir} n=5; LP control n=5, Th^{kir} n=4.
P6-P7: VPM control n=13, Th^{kir} n=15; POm control n=4, Th^{kir} n=10. P13-P14: VPM control n=7, Th^{kir} n=4; POm control n=4, Th^{kir} n=4. The graphs represent the mean ± SEM. *P<0.05, **P<0.01. Scale bar, 250 μm.

We have also characterized to what extent spiking arises as bursts of action potentials. There were almost no bursts in the control visual thalamus at P6-P7 and the same occurred in the Th^{Kir} mice. Activity in burst appeared only at P13-P14 in the control dLGN with bursts that contained 2 or 3 action potentials (Fig 4A-4D). The distribution of the inter-spike intervals (ISIs) were similar in control and Th^{Kir} visual thalamus, suggesting that despite the differences in firing rate the temporal structure of the spiking activity is not affected by overexpressing Kir2.1 (Fig 4E-4F). As in the visual thalamus, the somatosensory nuclei exhibited very low burst activity by P6-P7 in control and Th^{Kir} mice. During the second postnatal week, burst activity significantly increased in control mice but remained low in Th^{Kir} mice (Fig 5A-5B), where bursts tend to have shorter durations and less spikes (Fig 5C-5D). Compared with the visual system, the distribution of ISIs in the control somatosensory thalamus showed a higher proportion of short intervals even at P6-P7 (Fig 5E), suggesting a premature development of the somatosensory circuits respect to the visual ones. In the Th^{Kir} neurons, ISIs were evenly distributed at this stage but, by P13-P14, shorter ISIs became overrepresented and the distribution skewed to the left as in control mice (Fig 5E and 5F)

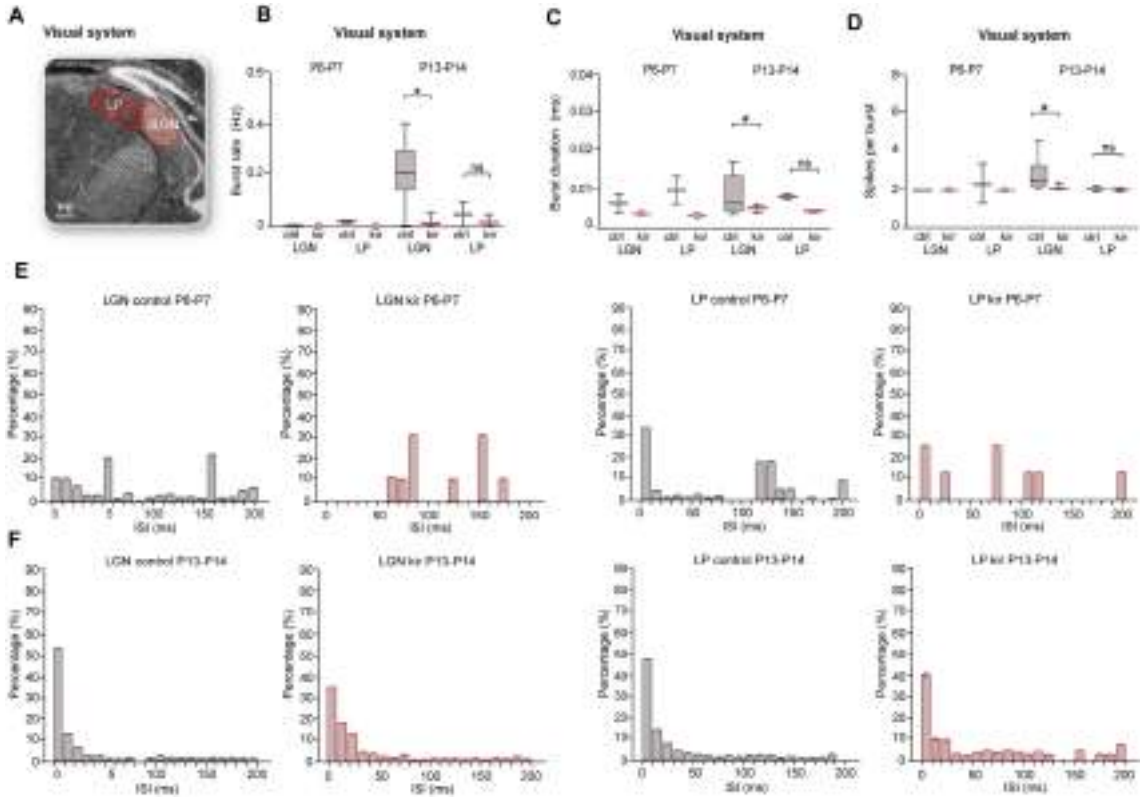


Figure 4. Burst activity in the developing visual thalamus in control and Th^{Kir} mice. (A) Coronal section in which visual thalamic nuclei are outlined in red. (B) Burst rate in the visual thalamic nuclei at P6-P7 and P13-P14. (C) Duration of bursts in the visual thalamic nuclei at P6-P7 and P13-P14. (D) Number of spikes per burst in the visual nuclei at P6-P7 and P13-P14. (E) Distribution of inter-spike intervals (ISIs) in the dLGN and LP at P6-P7. (F) Distribution of ISIs in the dLGN and LP at P13-P14. Quantifications for burst rate, burst frequency, and number of spikes per burst: P6-P7: dLGN control n=5, Th^{kir} n=6; LP control n=6, Th^{kir} n=5. P13-P14: dLGN control n=4, Th^{kir} n=5; LP control n=5, Th^{kir} n=4. Quantification of ISIs: P6-P7: dLGN control n=3, Th^{kir} n=1; LP control n=5, Th^{kir} n=3. P13-P14: dLGN control n=4, Th^{kir} n=5; LP control n=5, Th^{kir} n=3. The graphs represent the mean ± SEM. *P<0.05, **P<0.01. Scale bars, 250 μm.

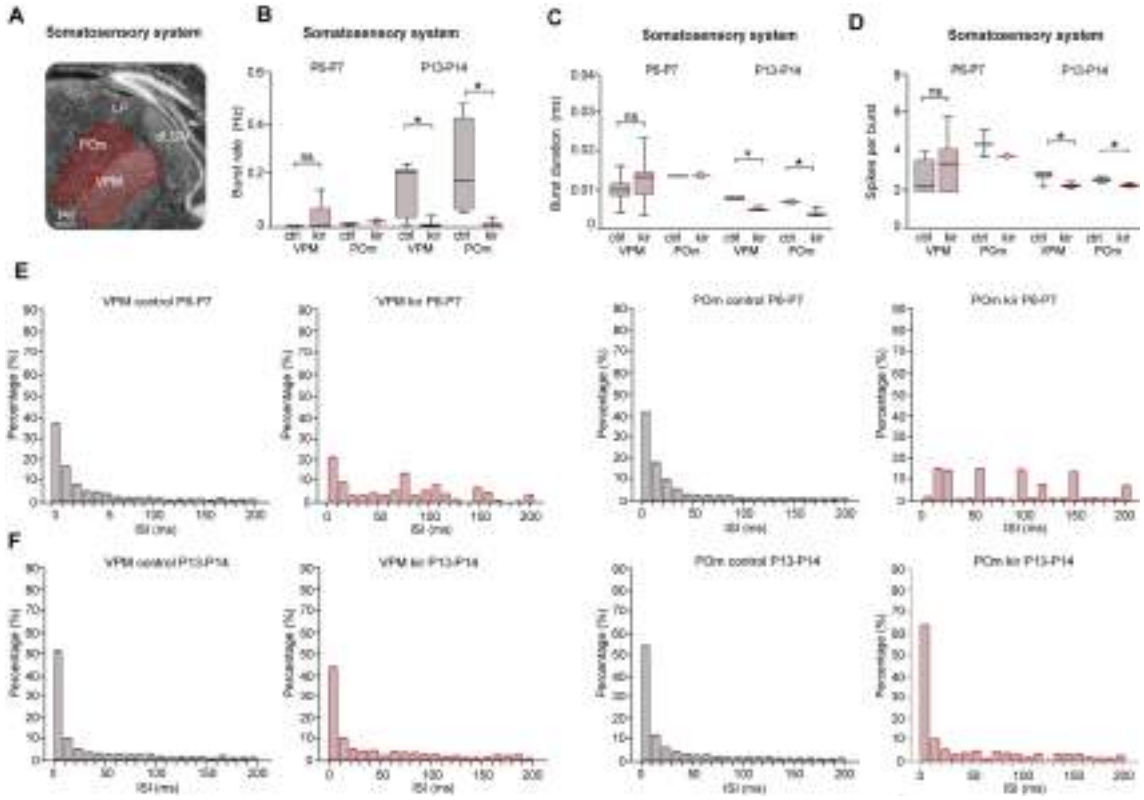


Figure 5. Burst activity in the developing somatosensory thalamus in control and Th^{Kir} mice. (A) Coronal section in which somatosensory thalamic nuclei are outlined in red. (B) Burst rate in the somatosensory thalamic nuclei at P6-P7 and P13-P14. (C) Duration of bursts in the somatosensory thalamic nuclei at P6-P7 and P13-P14. (D) Number of spikes per burst in the somatosensory nuclei at P6-P7 and P13-P14. (E) Distribution of inter-spike ISIs in the somatosensory nuclei at P6-P7. (F) Distribution of ISIs in the somatosensory nuclei at P13-P14. Quantifications for burst rate, burst frequency, and number of spikes per burst: P6-P7: VPM control n=11, Th^{kir} n=16; POm control n= 4, Th^{kir} n=11. P13-P14: VPM control n=7, Th^{kir} n=5; POm control n= 4, Th^{kir} n=3. Quantification of ISIs: P6-P7: VPM control n=11, Th^{kir} n=9; POm control n= 4, Th^{kir} n=7. P13-P14: VPM control n=7, Th^{kir} n=5; POm control n= 4, Th^{kir} n=1. The graphs represent the mean ± SEM. *P<0.05, **P<0.01. Scale bars, 250 μm.

2. The properties of spontaneous firing in S1 neurons of Th^{Kir} mice.

As the sensory thalamus represents the major gate for sensory information into the cortex, our next step was to study to what extent cortical spontaneous and evoked activity are affected in the Th^{kir} mice. In this part, we have focused on the somatosensory system because it is straightforward to activate the pathway stimulating the sensory receptors of the snout. In contrast, in the visual system, the eyelids are closed at P6-P7 precluding the direct stimulation of the retina. Thus, we performed in vivo extracellular recordings in the somatosensory cortex (S1) at P6-P7 and at P14-P14 of control and Th^{Kir} mice (Fig 6A and 6B). We found that

mutants showed a lower frequency of spontaneous activity at P6-P7 and P13-P14 (Fig 6C). This is consistent with the fact that Th^{Kir} exhibited a reduced number of bursts in Th^{Kir} mice (Fig 6D). We did not find significant differences in the duration or in the number of spikes per burst (Fig 6E and 6F). Furthermore, as in the case of thalamic nuclei, the distribution of the ISIs in the cortex showed prevalence of shorter intervals in both control and in Th^{Kir} mice (Fig 6G and 6H). In sum, the firing of S1 neurons is severely affected when thalamic activity is perturbed suggesting that the thalamus is the main driver of cortical activity at these stages.

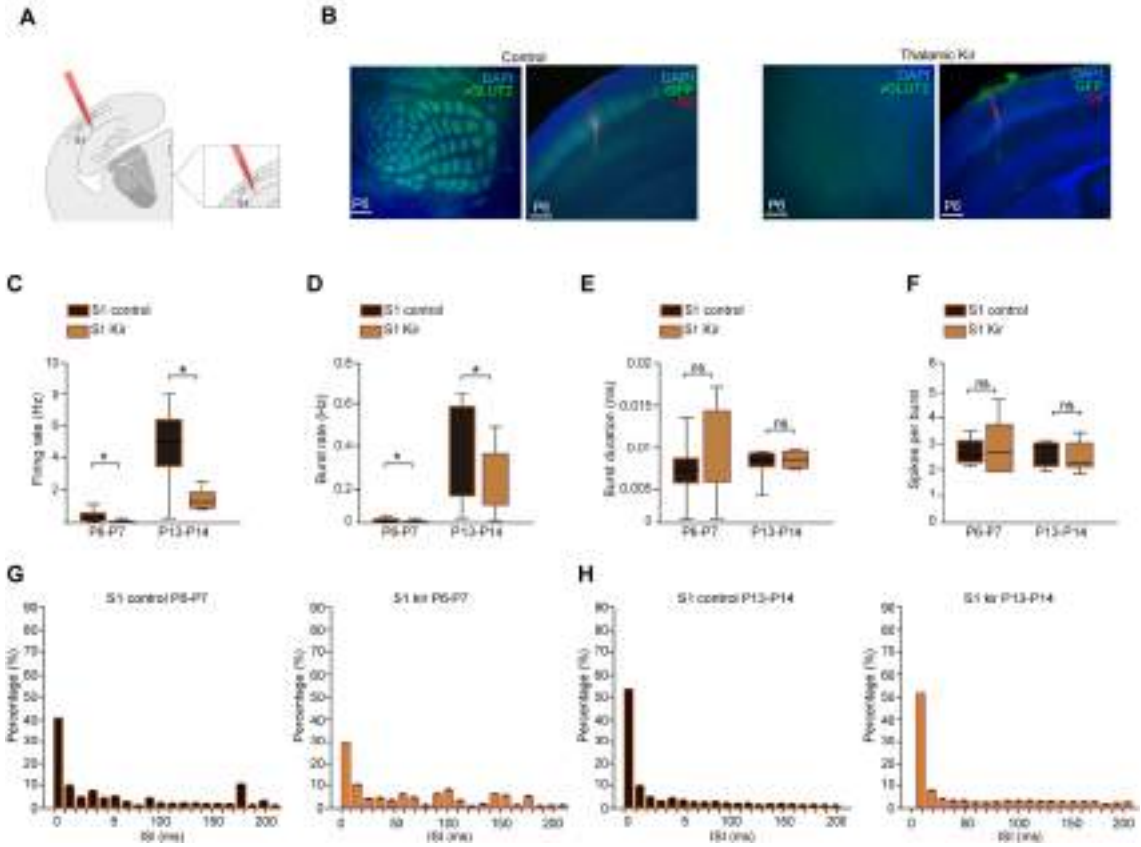


Figure 6. Spontaneous activity in the somatosensory cortex during first postnatal weeks in control and Th^{Kir} mice. (A) Experimental design showing the probe used to record activity in the somatosensory cortex. (B) Horizontal and coronal sections showing the absence of barrels in the somatosensory cortex of the Th^{Kir} mice and the insertion of the recording electrode in S1. (C) Comparison of firing rate between control and Th^{Kir} S1 neurons from at P6-P7 and at P13-P14. (D) Burst rate at P6-P7 and P13-P14. (E) Duration of bursts at P6-P7 and P13-P14. (F) Number of spikes per burst. (G) Distribution of inter-spike intervals (ISIs) in S1 at P6-P7. (H) Distribution of ISIs in S1 at P13-P14.

Quantification of firing rate, burst rate, burst duration, and number of spikes per bursts of S1 in controls and Th^{Kir} animals at during first postnatal weeks. P6-P7: S1 control n=11, Th^{Kir} n=17; P13-P14: S1 control n=8, Th^{Kir} n=7. Quantification of ISIs: P6-P7: S1 control n=10, Th^{Kir} n=12; P13-P14:

S1 control n=8, Th^{Kir} n=7. The graphs represent the mean ± SEM. *P<0.05. Scale bars 250 μm for coronal sections.

3. Evoked responses in the developing somatosensory thalamus and S1 in Th^{Kir} mice.

Knowing that in the Th^{Kir} mice spontaneous activity was significantly decreased both in the thalamus and in the cortex, we wondered whether thalamic and S1 cortical neurons were able to respond to sensory stimuli. To answer this question, we activated the somatosensory pathway by displacing the whiskers with an air puff and recorded in vivo the evoked responses in the VPM using extracellular electrodes. (Fig 7A and 7B). We found that thalamic neurons in the VPM of Th^{Kir} mice were able to respond normally to the peripheral stimulation at the developmental stages considered here (Fig 7C). Thus, it seems that the somatosensory circuits are able to remain functional in spite of the profound defects on the patterns of spontaneous activity during development. To complete this approach, we recorded the activity of the barrel neurons in the somatosensory cortex as well (Fig 7D and 7E). We found that, although the cortical response at P6 seems to be similar both in control and Th^{Kir} mice, the cortical neurons in the barrels of Th^{Kir} mice were not able to follow thalamic activity at P13 and adult stages (Fig 7F).

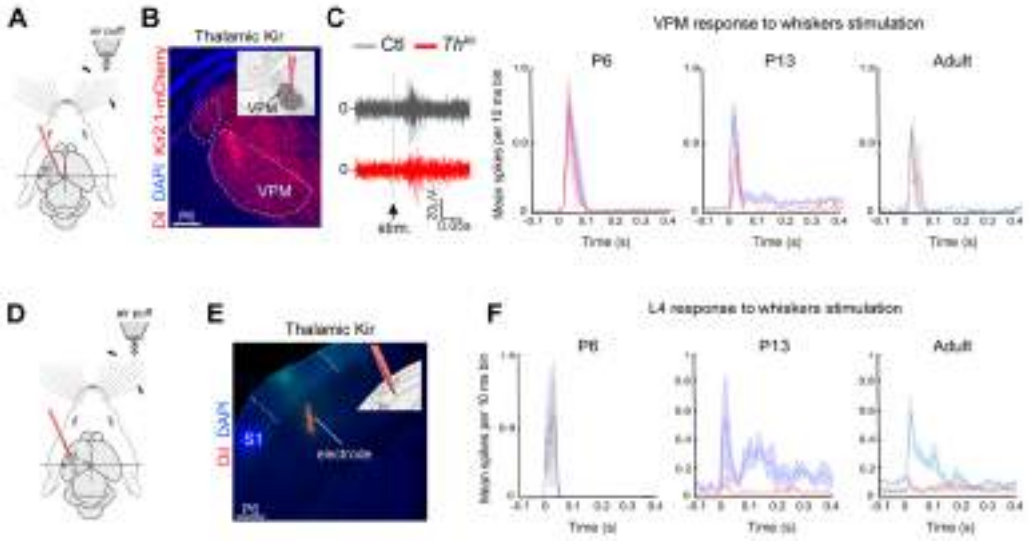


Figure 7. Evoked activity in the thalamus and somatosensory cortex at P6-P7 in control and Th^{Kir} mice. (A) Experimental design to record thalamic activity during whiskers stimulation. (B) Example of

a coronal section of a Th^{Kir} thalamus counterstained with DAPI showing the electrode track labeled with DiI. (C) Left: representative examples of VPM multi-unit activity (MUA) evoked by whisker stimulation in control and Th^{Kir} mice (n=4, n=4, respectively). Right: VPM response to whisker stimulation at P6-P7, P13-P14 and adult mice. Quantification of number of spikes per 10 ms bin at P6-P7 (n=4 control, n=3 Th^{Kir}), P13-P14 (n=6 control, n=2 Th^{Kir}) and adult age (n=2 control, n=2 Th^{Kir}). (D) Experimental design to record S1 activity during whisker stimulation. (E) Example of a coronal section from S1 of a Th^{Kir} mouse counterstained with DAPI showing the electrode track labeled with DiI. (F) S1 response to whiskers stimulation. Quantification of number of spikes per 10 ms bin at P6-P7 (n=4 control, n=4 Th^{Kir}), P13-P14 (n=4 control, n=4 Th^{Kir}) and adult age (n=8 control, n=9 Th^{Kir}). The graphs represent the mean ± SEM. Scale bars, 250 μm.

Chapter III. Spontaneous firing properties in the thalamus during the first postnatal weeks upon visual deprivation.

In the Th^{Kir} model, we have demonstrated that thalamic neurons exhibited abnormal spontaneous activity in the somatosensory and visual nuclei of the thalamus at P6-P7 and at P13-P14. In addition, while the evoked responses in the thalamus were rather normal, cortical responses were profoundly diminished from P13 onwards. In the following experiments, we tackled the same topic but shifting the experimental paradigm. Instead of directly manipulating the thalamus, we studied to what extent the electrical activity in the thalamus is affected when it is deprived of sensory input, and which are the consequences in the corresponding cortical areas.

Among the different kinds of sensory information relayed by the thalamus, the visual modality has been widely chosen to perform peripheral manipulations and assess its impact on the development of the sensory circuits. The advantages of the visual system are mainly practical. In contrast to the somatosensory system, the peripheral organs of the visual and auditory systems are confined in specific regions of the body. Therefore, the manipulation of these organs is straightforward. The auditory system, however, has not a readily accessible primary cortex where to image or record spontaneous activity or evoked responses. Thus, in this chapter we characterized the thalamic and cortical firing properties upon visual deprivation. To do so, we performed bilateral enucleations at embryonic stages (E14.5), this is,

before retinal axons reach the visual thalamus (Fig 8A).

1.Visual deprivation affects the spontaneous firing in the visual thalamus.

Many studies on sensory deprivation have evidenced the presence of genetic and activity changes in the thalamic network. For instance, it has been demonstrated that after embryonic visual deprivation, the spontaneous pattern of waves changes, increasing its frequency in dLGN and in VPM at perinatal stages (Moreno-Juan et al., 2017). Here, we asked how these perinatal changes evolve during the postnatal maturation and to what extent they have an impact on the cortex. To address this question, we first checked the evolution of the pattern of spontaneous activity in the visual thalamic nuclei at P6-P7 and at P13-P14 in control and embBE mice (Fig 8A-8C). In vivo extracellular recordings evidenced strong changes in the spiking activity at P6-P7 in embBE mice. Specifically, the firing rate of dLGN neurons decreased, the firing rate of LP neurons increased (Fig 8D) and the pattern of bursts exhibited nuclei-specific alterations (Fig 8E-8G). Namely, in dLGN neurons, there was a reduction in burst rate, burst duration and number of spikes per burst in embBE mice at P6-P7. Conversely, deprived LP neurons accompanied their increased firing rate with higher frequency of bursts, burst duration and number of spikes per burst. The burst pattern in the deprived visual thalamus at P6-P7 was also revealed in the left-skewed distribution of ISIs (Fig 8H).

Strikingly, the abnormal phenotype observed at P6-P7 in embBE mice was reverted by P13-P14, just after eye-opening (Fig 8D-8G and 8I). It is possible that during postnatal development, compensatory mechanisms get activated in the deprived model to overcome the activity deficit and reach a rather normal level of maturation. Homeostatic plasticity has been described in the visual system at different developmental stages (Riyahi et al.,2021). It seems that the LP plays an important role to promote the maturation of the visual circuits. We have no direct evidences for that but, in previous publications, it has been shown that peripheral deprivation provokes a genetic and physiological reorganization of the hierarchies of FO and HO nuclei of the thalamus (Frangeul et al., 2016).

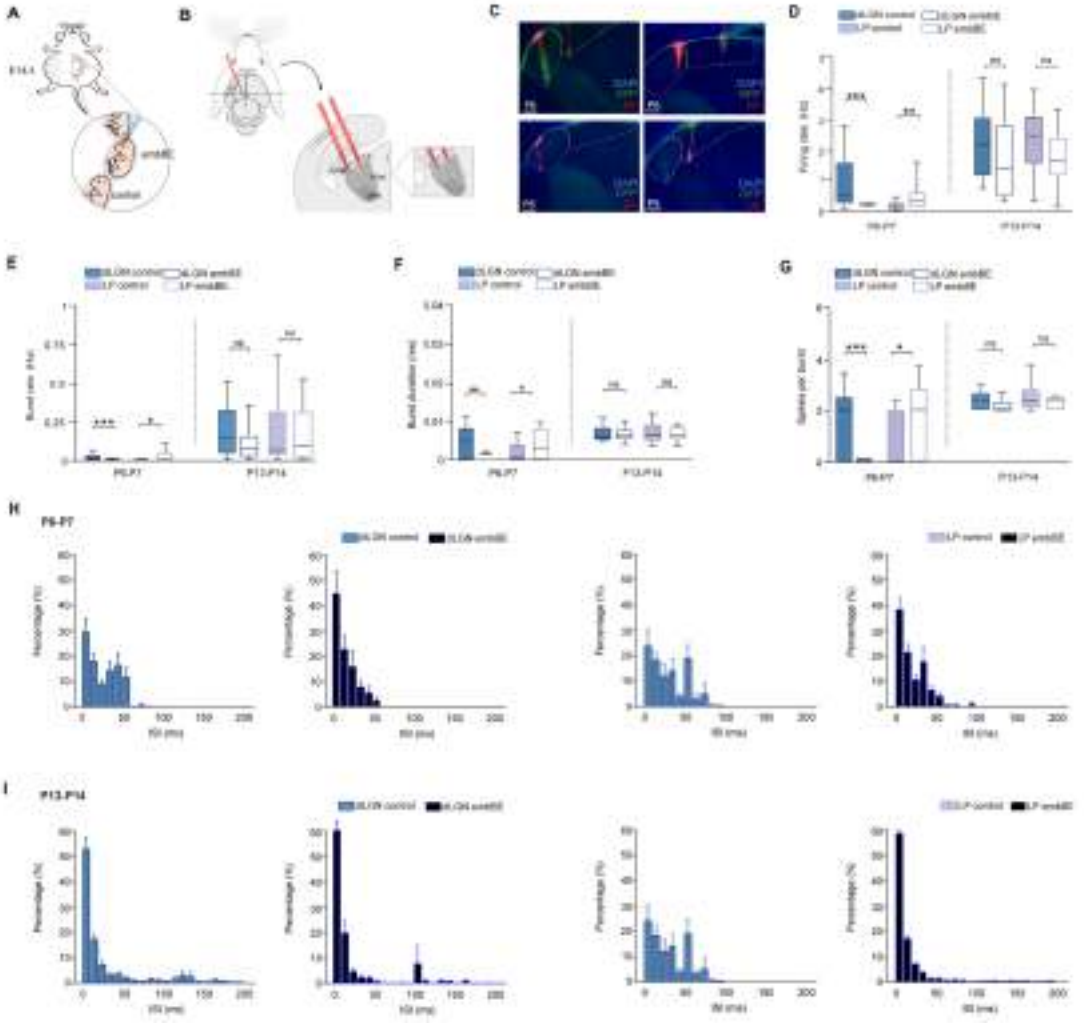


Figure 8. Spontaneous activity pattern during first postnatal weeks after embryonic sensory deprivation in the visual thalamic nuclei: dLGN and LP. (A) Schematic representation of the experimental paradigm used to visually deprived mouse embryos at E14.5. (B) Experimental design implemented to record extracellular activity in the visual thalamus in control and embBE mice. (C) Examples of coronal sections of the dLGN and LP showing the DiI track of the recording probes. (D) Comparison of firing rates between visual thalamic nuclei at P6-P7 and P13-P14 in control and embBE mice. (E) Comparison of burst rate between control and embBE visual thalamic nuclei at P6-P7 and P13-P14. (F) Changes in the duration of these bursts in the visual thalamic nuclei both in control and in embBE mice. (G) Changes in the number of spikes per burst. (H) Distribution of inter-spike intervals at P6-P7 in dLGN and LP in controls and embBE mice. (I) Distribution of inter-spike intervals at P13-P14 in control and embBE mice.

Quantifications for firing rate, burst rate, burst frequency, number of spikes per burst and ISIs: P6-P7: dLGN control n=22, embBE n=16; LP control n=25, embBE n=18. P13-P14: dLGN control n=13, embBE n=13; LP control n=18, embBE n=14. The graphs represent the mean ± SEM. *P<0.05, **P<0.01, ***P<0.001. Scale bars, 250 μm.

2. Visual deprivation might lead to changes in intra-thalamic correlations during early

postnatal development.

As the profile of activity of LP neurons in the deprived model at P6-P7 resembled that of control dLGN, we decided to explore if there was a reconfiguration of connectivity in the visual pathway. Our working hypothesis is that the inputs that normally contact the dLGN are redirected towards the LP in embBE mice. We first studied if the amount of shared input between both visual nuclei increased in the embBE mice. To do so, we recorded simultaneously the activity from the LP and the dLGN with multi-shank electrodes (Fig 9A) and calculated the spike time tiling coefficient (STTC), a parameter that measures the correlation independently of the firing rate (Cutts and Eglen, 2014). We found that the degree of correlation between visual nuclei was low both in embBE and control mice, suggesting that they receive different kind of inputs (Fig 9C). Thus, although we lack evidence to support our hypothesis, it is possible that the rewiring of the LP has occurred earlier in development.

Moreover, as it is known that embryonic visual sensory deprivation also gives rise to some activity changes in the somatosensory thalamic nuclei (Toldi et al., 1996; Fetter-Pruneda et al., 2013; Moreno-Juan et al., 2017), we have also compared the STTC coefficient between visual and somatosensory thalamic nuclei at P6-P7 (Fig 9B). In this case, while primary nuclei exhibited the largest correlations in control mice, the largest correlation were found between the higher order nuclei in the embBE (Fig 9D).

We have also compared the correlation between thalamic nuclei at P13-P14, when the profile of spontaneous activity in embBE resembled that of control mice. In general, the correlations seemed higher than those observed at P6-P7. However, there is general decorrelation trend among all pairs of nuclei in the embBE mice compared to the controls. The only exception are higher order nuclei, the correlation between the firing activity of LP and POm remained high in embBE mice, similar to in control mice (Fig 9E and 9F). Despite the normal pattern of activity observed in embBE thalamic neurons at P13-P14, it seems that higher order nuclei take a

different role in the deprived brain. However, in these experiments and in those conducted at P6-P7, the observed changes in the amount of correlation were not significantly different; they represent a trend that requires more experimental work to obtain conclusive results.

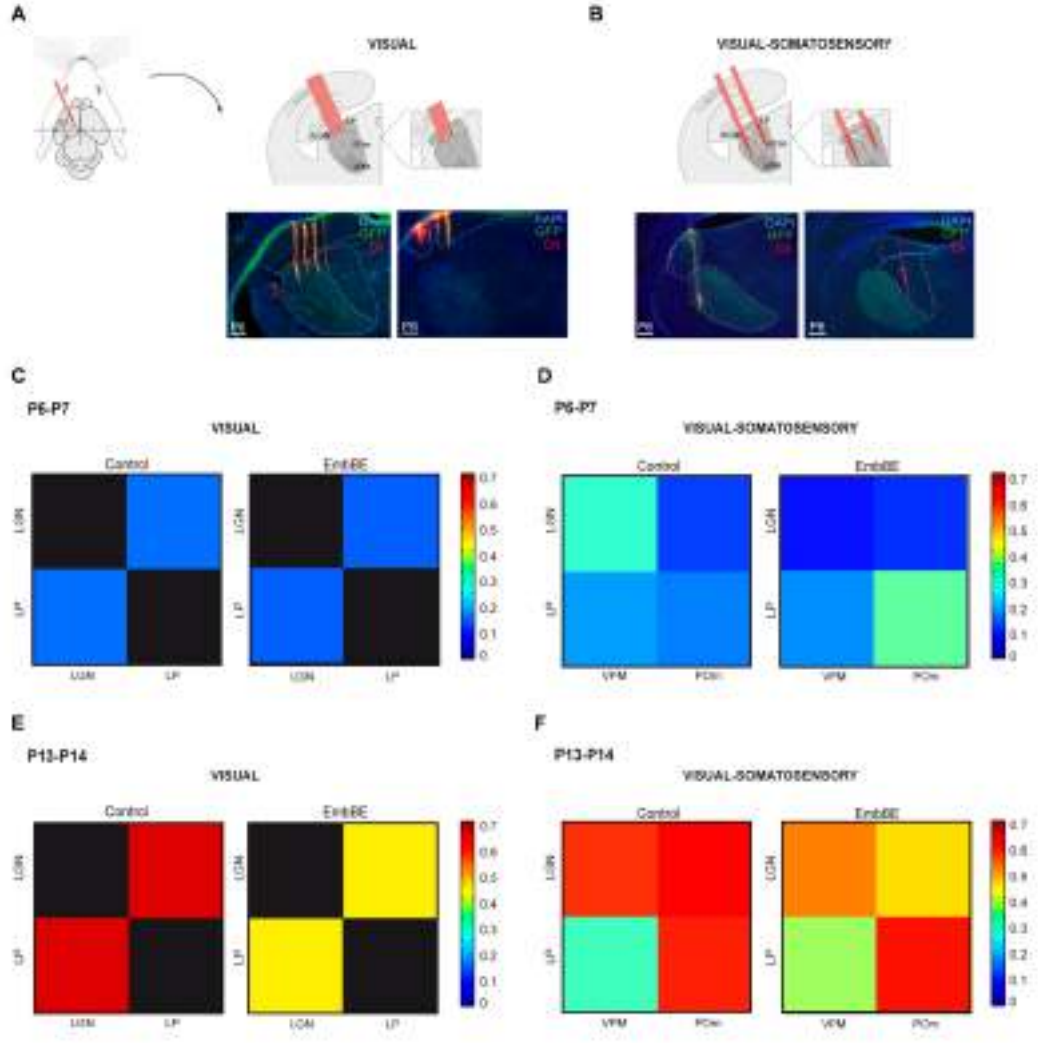


Figure 9. Correlation within visual thalamic nuclei and among visual and somatosensory thalamic nuclei in control and embBE mice. A) Experimental design and coronal sections of extracellular recordings in which visual thalamic nuclei were recorded simultaneously. B) Experimental design and coronal sections of extracellular recordings in which visual and somatosensory thalamic nuclei were recorded simultaneously. C) Comparison of STTC coefficients between control and embBE mice at P6-P7. D) Comparison of STTC coefficients between control and embBE mice at P13-P14. Quantifications for correlation between visual nuclei. P6-P7: dLGN-LP control n=8, dLGN-LP embBE n= 5; P13-P14: dLGN-LP control n=5, dLGN-LP embBE n= 5.
Quantifications for correlation between visual and somatosensory nuclei. P6-P7: dLGN-VPM control n=11, dLGN-VPM embBE n=5; dLGN-POm control n=10 , dLGN-POm embBE n=5; LP-POm control n=15 LP-POm embBE n=8, LP-VPM control n=5 LP-VPM embBE n=5; P13-P14:

dLGN-VPM control n=4, dLGN-VPM embBE n=5; dLGN-POm control n=5, dLGN-POm embBE n=4; LP-POm control n=10 LP-POm embBE n=7, LP-VPM control n=6 LP-VPM embBE n=4. P>0.05, NS.

3.Visual deprivation affects spontaneous firing in the somatosensory thalamus.

Sensory deprivation triggers adaptations not only in the affected modality but also in the spare senses. Cross-modal plasticity has been described in the VPM after visual deprivation (Frangeul et al., 2016) and it is known that bilateral enucleations induce a higher frequency of embryonic waves in mice (Moreno-Juan et al., 2017). From the correlation analysis, it appears that the somatosensory pathway underwent functional changes in the embBE mice at postnatal stages. Thus, we asked how this peripheral manipulation affects the maturation of spontaneous activity during postnatal development in the somatosensory thalamus.

To answer this question, we conducted extracellular recordings in the VPM and POm in control and in embBE mice (Fig 10A and 10B). At P6-P7, we found a decrease in firing rate of both nuclei: VPM and POm. At P13-P4, this effect remains only and to a lesser extent in the POm (Fig 10C). In contrast to the visual nuclei, the burst pattern was not affected neither at P6-P7 nor at P13-P14 (Fig 10D-10F). Consistently, we observed no differences between control and embBE mice in the distribution of ISIs (Fig 10G and 10H). Finally, visual deprivation did not affect the level of correlation between FO and HO somatosensory nuclei (Fig 10I). In sum, we found a decrease in the firing rate of the visually-deprived somatosensory thalamus at P6-P7 that gradually recovers by P13-P14, especially in the VPM.

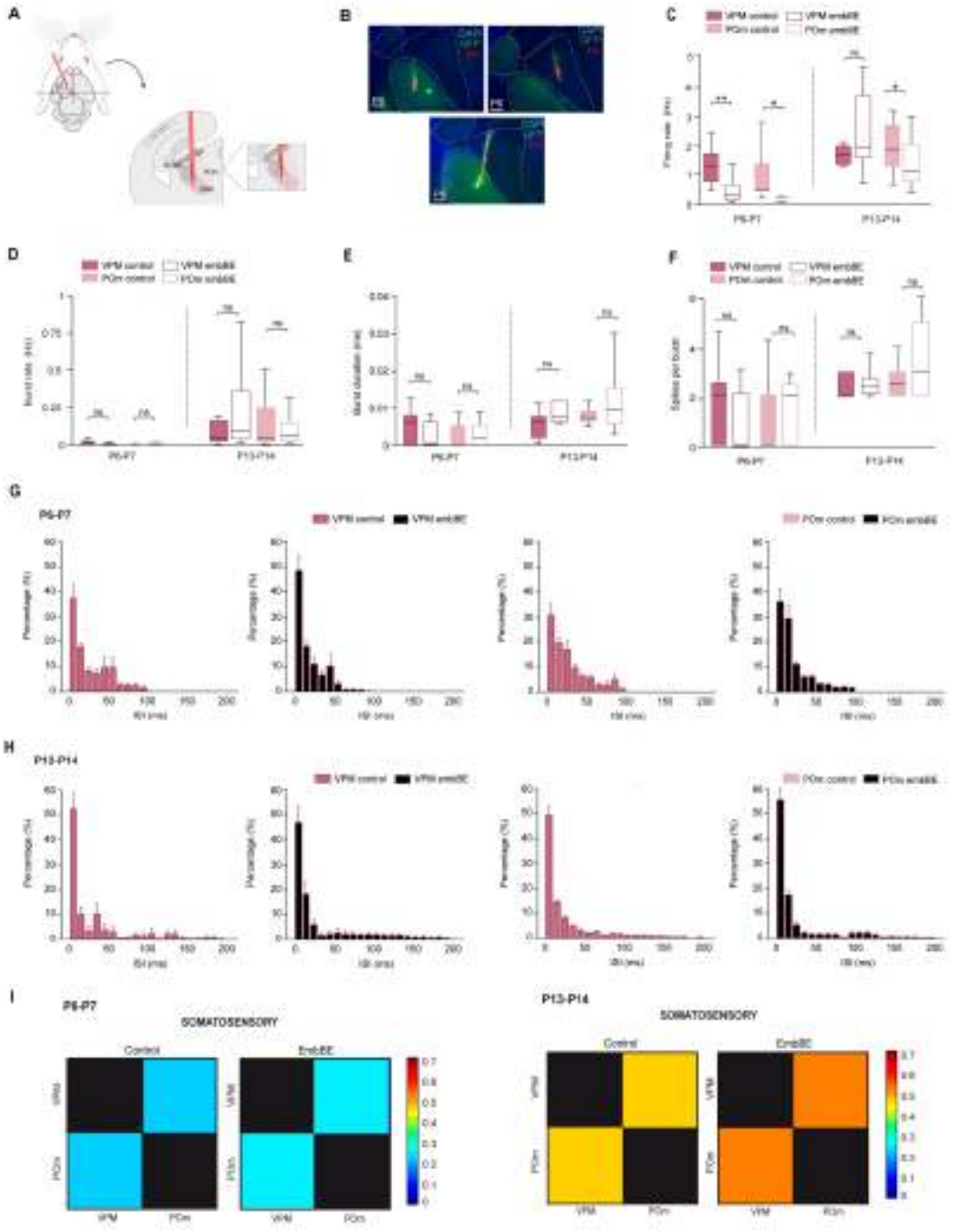


Figure 10. Spontaneous activity pattern during first postnatal weeks after embryonic sensory deprivation in the somatosensory thalamic nuclei: VPM and POm. (A) Experimental design of extracellular recordings in the somatosensory thalamus in control and embBE mice. (B) Examples of coronal sections in which the red track of the electrode indicates de recording sites of the VPM and POm. (C) Spontaneous firing rate in control and embBE somatosensory thalamic nuclei at P6-P7 and P13-P14. (D) Spontaneous burst rate in control and embBE somatosensory thalamic nuclei at P6-P7 and P13-P14. (E) Burst duration in the somatosensory thalamic nuclei both in control and embBE mice. (F) The number of spikes per burst in control and embBE somatosensory thalamic

nuclei at P6-P7 and P13-P14. (G) Distribution of inter-spike intervals at P6-P7 in VPM and POm in control and embBE mice. (H) The same as G but at P13-P14. (I) Firing rate correlation between VPM and POm in control and embBE mice at P6-P7 to P13-P14.
Quantifications for firing rate, burst rate, burst duration, number of spikes per burst and ISIs: P6-P7: VPM control n=18, embBE n=17; POm control n= 18, embBE n=10. P13-P14: VPM control n=10, embBE n=11; POm control n= 22, embBE n=17. The graphs represent the mean ± SEM. *P<0.05, **P<0.01. Scale bars, 250 μm.
Quantifications for correlation between somatosensory nuclei. P6-P7: VPM-POm control n=11, VPM-POm embBE n= 8; P13-P14: VPM-POm control n=8, VPM-POm embBE n= 7. P>0.05, NS.

Chapter IV. The pattern of spontaneous firing in the cortex during the first postnatal weeks upon early visual deprivation.

We have demonstrated that the pattern of spontaneous activity was not normal in the thalamus of mouse pups enucleated at embryonic stages. In the next set of experiments, our aim was to understand to what extent cortical activity was also affected. It has been shown that perinatal visual or somatosensory deprivation leads to an abnormal formation of cortical sensory maps (Espinosa and Stryker, 2012; Huberman et al., 2006; Martini et al., 2021). Consistently, manipulations of peripheral patterns of activity cause defects in the assembly of the sensory pathways. As an example, disrupting the retinal activity before eye-opening impairs the normal development of retinotopy in visual thalamus, superficial layers of the superior colliculus and visual cortex (Arroyo and Feller, 2016). Thus, to study how the maturation of thalamic activity modulates the firing properties of cortical neurons, we performed in vivo electrophysiological recordings in visual and somatosensory cortical areas of control and embBE mice before and just after eye-opening.

1.Visual deprivation at embryonic stages affects spontaneous firing in primary and secondary visual cortical areas.

We performed in vivo extracellular recordings in the primary visual cortex (V1), the main target of dLGN projecting neurons. The experiments were done in control and embBE mice at P6-P7 and at P13-P14 (Fig 11A and 11B). We found that the firing

rate in embBE V1 was lower than that of control mice only after eye-opening, by P13-P14 (Fig 11C). In control mice, dLGN neurons mainly project to L4 of V1 whereas L5 neurons send their axons towards the LP, which was demonstrated to present an increased firing rate after visual deprivation. So, we decided to study whether the firing rate in these layers was differentially affected in embBE mice. While the firing rate of L4 neurons is decreased in embBE mice, the firing rate of L5 neurons does not change significantly, to some extent consistent with the results found in the LP of embBE mice (Fig 11D).

In the same line, as perturbed thalamic spontaneous activity affects the innervation of dLGN by CTAs (Seabrook et al., 2013), we tested whether also the innervation of the LP by L5 CTAs was affected. We identified CTAs arriving to the LP using a transgenic mouse line that labels cortical L5 axons (Rbp4-Cre). We found no differences between control and embBE mice in the timing of the L5 input to the LP, they arrive at P2 in both cases and seem to deploy a normal innervation of the nucleus (Fig 11E). While V1 activity remains low, neurons from V2 exhibited higher firing rates in embBE mice than their control littermates. We found that firing rate of V2 at P13-P14 was higher in embBE than in control mice (Fig 11F-11H) and, indeed, this was significant either for L4, which receives input from LP, and for L5 (Fig 11I).

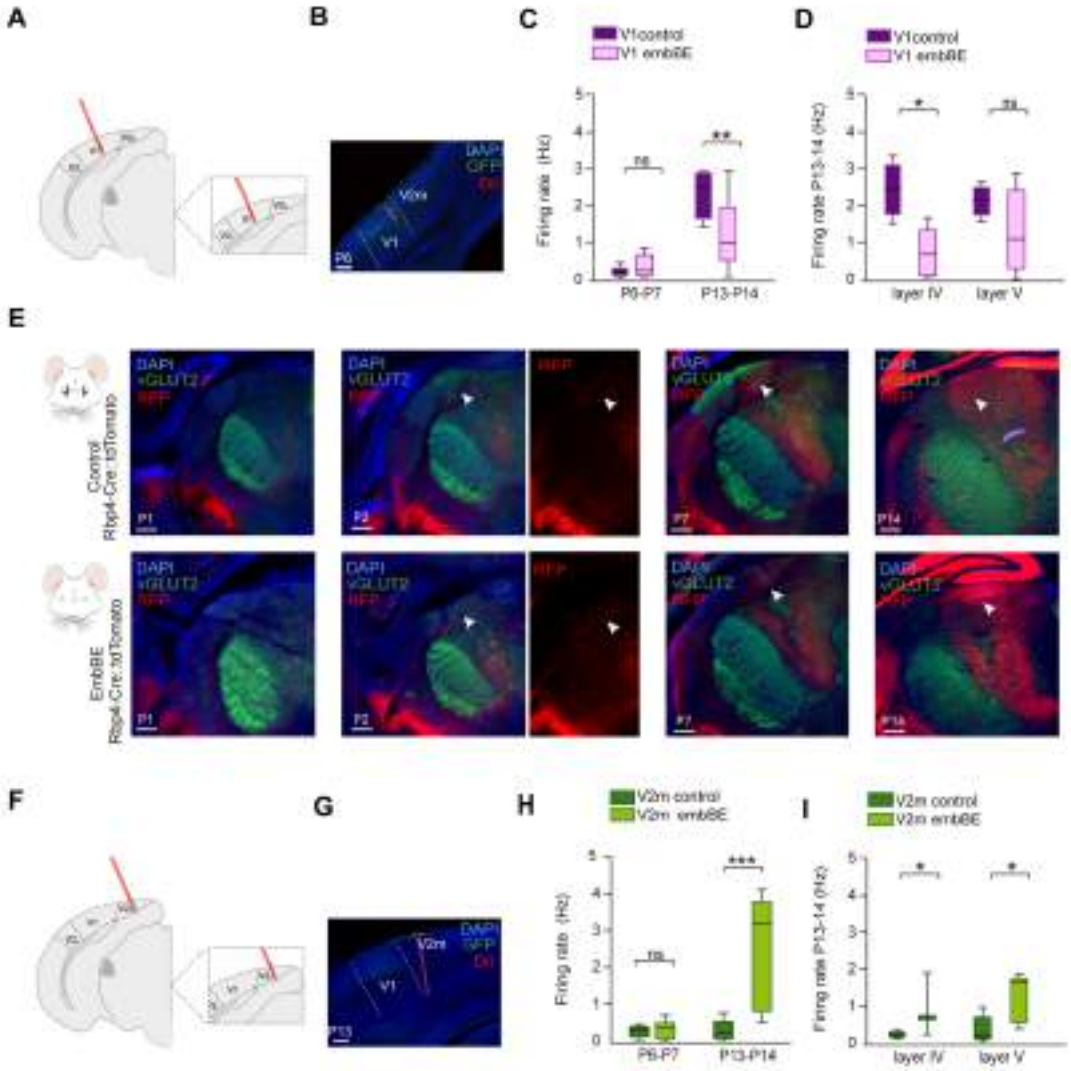


Figure 11. Postnatal consequences of peripheral deprivation in the visual cortical areas. (A) Experimental design used to perform in vivo electrophysiological recordings in the primary visual cortex (V1). (B) Example of a coronal section showing the electrode in V1 (red track). (C) MUA firing rate in V1 of control and embBE mice at P6-P7 (control n=5 and embBE n=4) and P13-P14 (control n=4 and embBE n=5). (D) MUA firing rate in L4 and L5 of V1 in control and embBE mice at P13-P14 (L4 control n=4 and L4 embBE n=5, L5 control n=4 and L5 embBE n=5). (E) Coronal sections of control and embBE Rbp4-Cre::tdTomato mice showing vGLUT2 and RFP immunostaining in FO thalamic nuclei and in CTAs from L5, respectively, at P1, P2, P7 and P14 (control n=5 and embBE n=5, in all ages). Arrows point to CTAs in LP. (F) Experimental design used to perform in vivo electrophysiological recordings in the secondary visual cortex (V2). (G) Example of a coronal section showing the electrode in V2 (red track). (H) MUA firing rate in V2 of control and embBE mice at P6-P7 (control n=12 and embBE n=6) and P13-P14 (control n=9 and embBE n=5). (I) MUA firing rate in L4 and L5 of V2 in control and embBE mice at P13-P14 (L4 control n=9 and L4 embBE n=5, L5 control n=9 and L5 embBE n=5). The graphs represent the mean ± SEM. *P<0.05, **P<0.01, ***P<0.001. Scale bars 250 μm for coronal sections.

2.Visual deprivation at embryonic stages dampens spontaneous firing in the somatosensory cortex.

Previous studies from our lab and others demonstrated that the removal of the visual peripheral input at embryonic stages triggers a reorganization of the deprived and the spare cortical areas (Mezzera and López-Bendito, 2016). As, we have previously shown that embBE mice exhibit a reduced rate of spontaneous activity in the VPM (Fig 10C), the next step was to address what happens in S1. To do so, we performed in vivo extracellular recordings in S1 during the first postnatal weeks in control and embBE mice in order to know whether the effect in the somatosensory thalamus was reflected in the cortex (Fig 12A and 12B). Although the firing rate of VPM neurons in embBE mice was not normal by P6-P7, there is not a significant effect in S1 until P13-P14 (Fig 12C).

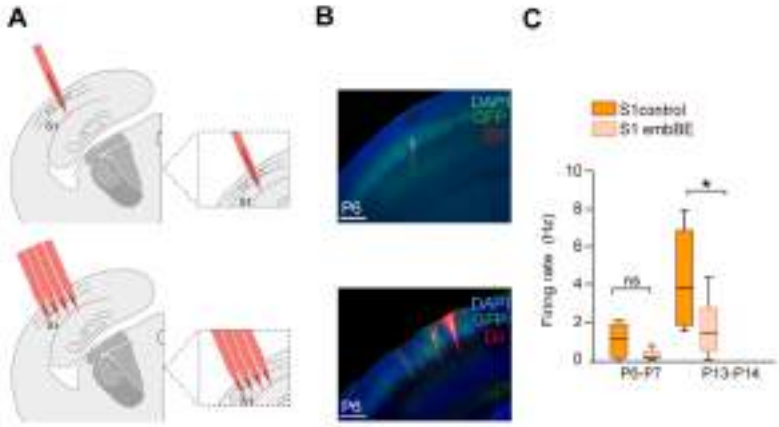


Figure 12. Postnatal consequences of peripheral deprivation in the primary somatosensory cortex (S1). (A) Experimental design used to perform in vivo electrophysiological recordings in the primary somatosensory cortex using multichannel electrodes arranged in 4 shanks (above) or with a linear configuration (below). (B) Two examples of coronal sections showing the electrodes in the barrel field. (C) MUA firing rate in S1 of control and embBE mice at P6-P7 (control n=4 and embBE n=4) and P13-P14 (control n=4 and embBE n=6). The graphs represent the mean ± SEM. *P<0.05. Scale bars 250 μm for coronal sections.

Chapter V. Evoked activity in cortical areas during the first postnatal weeks upon early visual deprivation.

1.Enlarged and faster response of S1 neurons to somatosensory stimulation in visually deprived mice.

Finally, we investigated if cortical function is affected in the somatosensory system of embBE mice. We performed in vivo electrophysiological recording of cortical responses in S1 evoked by whisker stimulation in control and embBE mice at P13-P14 (Fig 13A). We stimulated the whiskers using an air puff and found that deprived cortical neurons presented a faster and larger field response compared to control mice (Fig 13B and 13C). So, although somatosensory thalamic nuclei and cortex suffered a reduction of the rate of spontaneous firing in embBE mice, a somatosensory stimulus not only is able to engage S1 neurons but also provokes responses that surpass those observed in control mice.

2.The presumptive V1 area might be colonized by the somatosensory modality in visually deprived mice.

Many studies in humans and other mammals have reported cross-modal plasticity in sensory deprived individuals whereby a deprived sensory cortex becomes responsive to stimuli from other sensory modalities (Butler and Lomber, 2013; Bavelier and Neville 2002). So, we asked whether the presumptive visual area of the cortex of embBE mice could be engaged by stimuli associated to other modalities. Namely, we have explored to what extent the visual cortex processes somatosensory information in embBE mice. To do so, we performed in vivo electrophysiological recordings of neuronal responses in V1 after whisker stimulation at P13-P14 in both experimental groups (Fig 13D). As expected, we did not find cortical responses in the visual area of control mice evoked by whisker displacements. However, V1 neurons in the embBE model revealed a slow and small field response after whisker stimulation (Fig 13E and 13F). These data suggest that the normally visual area might have been invaded by the somatosensory modality.

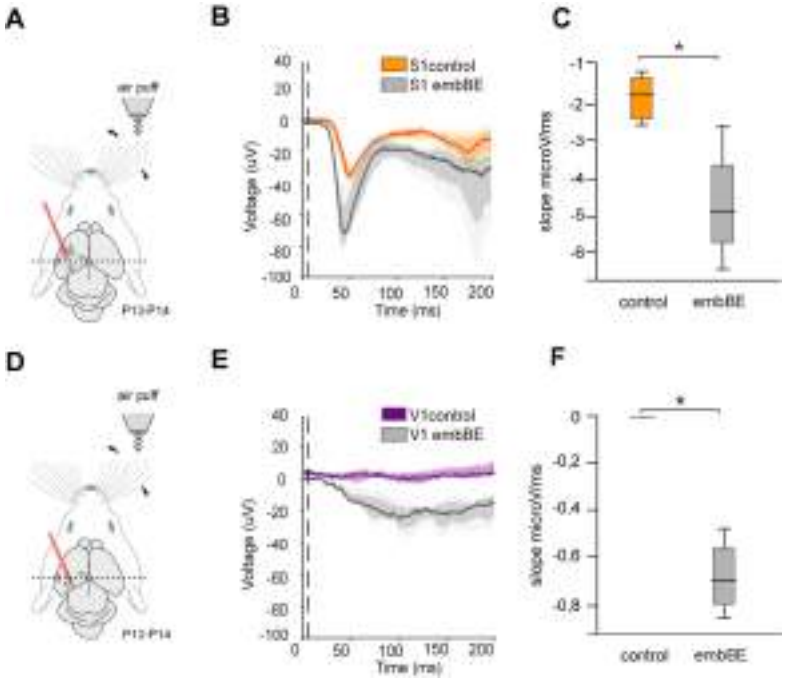
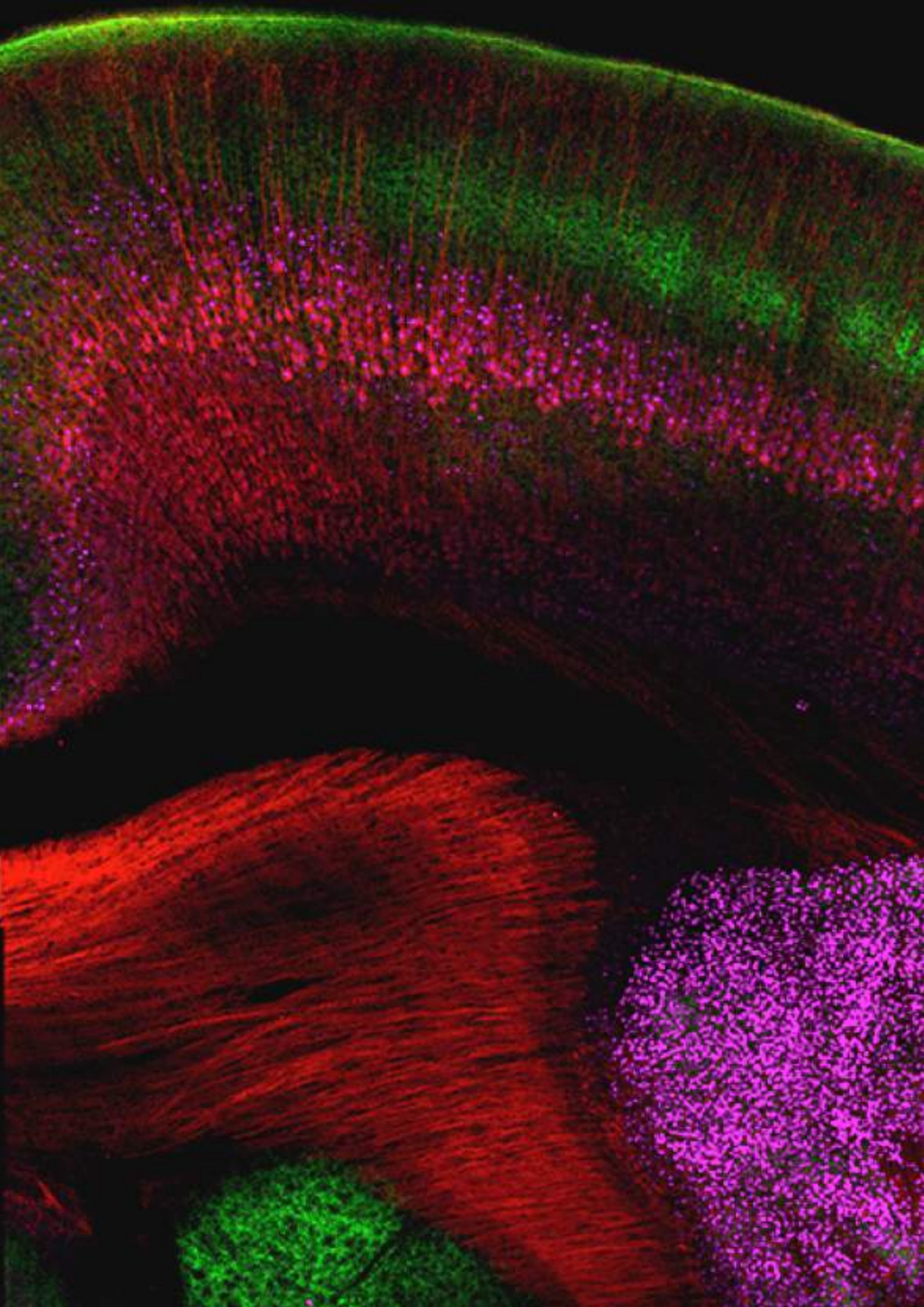


Figure 13. Effects of embryonic visual deprivation in the functional properties of the visual cortex. (A) Experimental design used to record in vivo the response to whisker stimulations in the primary somatosensory cortex at P13-P14. (B) Field responses to whisker stimulation in the somatosensory cortex of control and embBE mice. The thicker line represents the median, the dark shadow represents the interquartile difference and the light shadow represent the extreme values. (C) Quantification of the response slope in B for control and embBE mice at P13-P14. (D) Experimental design used to record in vivo responses to whisker stimulations in V1 at P13-P14. (E) Field responses to whisker stimulation in V1 from control and embBE mice. The thicker line represents the median, the dark shadow represents the interquartile difference and the light shadow represent the extreme values. (F) Quantification of the response slopes in E for control and embBE mice at P13-P14.
Quantifications: S1 control n=4, embBE n=4; V1 control n=4, embBE n=4. *P<0.05. Scale bars, 250 μm



Discussion

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Discussion

The thalamus conveys sensory information from peripheral organs to cortical areas and, as such, it is a potential locus of control for cortical map formation and plasticity. Although spontaneous activity in cortical areas has been largely studied, the influence of thalamic inputs needs further examination. In this dissertation, we have characterized the spiking activity of thalamic nuclei and to what extent it influences the spiking activity in the corresponding sensory areas of the cortex.

Maturation of the patterns of spontaneous activity in the thalamus during the first postnatal weeks.

The specific features related to the formation of the thalamic sensory circuits have not been described in depth. The thalamus is the first station where most sensory pathways converge and, as a result, the segregation of sensory information that occurs during perinatal development is tightly supervised by control mechanisms. In this control, both genetic and activity-driven mechanisms are involved. Genetic mechanisms have been thoroughly described (Gezelius and Bendito, 2017; Nakagawa and Shimogori, 2012, López-Bendito, 2018); however, little is known about thalamic activity. It is described that the pattern of thalamic spontaneous activity is modified during embryonic and early postnatal development in mice. Firstly, from mid-gestation until birth, there is a shift from asynchronous activity characterized by calcium spikes and small clusters of activity to a more synchronized pattern consisting of large calcium waves. Then, shortly after birth, spontaneous activity changes towards a profile encompassing spindle bursts and gamma oscillations (Murata and Colonnese, 2016; Khazipov et al., 2013). However, after these first postnatal days, there are not many evidences about the dynamics of the spontaneous events of activity in the thalamus.

One of the first objectives of this project was to described how the pattern of spontaneous activity evolves during the first two postnatal weeks in different thalamic nuclei. We found that the firing rate in all thalamic nuclei increased along

the postnatal development accompanied by a heightened proportion of firing in bursts. By the end of the second postnatal week, thalamic neurons acquired a rather mature pattern of spontaneous activity that concurs with the onset of active sensory experience. It is possible that HO thalamic nuclei mature at a slower pace and, in fact, bursts in HO nuclei appeared later than in FO where there were many experiments without bursts at P6. Consistently, the duration and the number of spikes per burst increased from P6 to P14 in HO nuclei, while FO retained the same values. Supporting this idea of a delayed maturation of HO, the firing rate of POm and LP is lower than the firing rate of their corresponding FO nuclei. It is known that transcriptional profiles of thalamic nuclei at these stages relate to processing hierarchy better than to sensory modality (Frangeul et al., 2016). In this way, as same order nuclei share similar genetic programs during development, it is likely that they also exhibit a similar developmental progression in the acquisition of their electrophysiological properties. To clarify this, it would be convenient to record spontaneous activity at later stages to check the final progression of the firing patterns.

We have found that correlations between thalamic nuclei at P13-P14 were higher than at P6-P7, an increase that is independent of the firing rate. This data disagrees with results obtained in cortical recordings. It has been largely described that during first postnatal weeks in the cortical areas there is a switch from synchronized patterns of spontaneous activity to a sparser and more decorrelated firing, allowing an efficient neuronal coding (Golshani et al., 2009; Rochefort et al., 2009; Colonnese et al., 2010; Andre et al., 2010). It is possible that thalamic nuclei require higher levels of synchrony but further research is needed to clarify this issue. We have analyzed multi-unit activity, which gives information about neurons adjacent to the electrodes It would be very informative to compare local field signals from different nuclei. Local field potential samples the activity from a larger population of neurons, allowing more comprehensive comparisons.

Thalamic activity influences the firing properties of cortical neurons.

Our lab has recently demonstrated that overexpressing Kir2.1 in the thalamus disrupts spontaneous calcium waves at perinatal stages. Although thalamic spontaneous activity is not completely abolished, there is a shift from synchronic events to a less synchronized mode (Antón-Bolaños et al., 2019). In the present dissertation, we demonstrated that this shift leads to a decreased firing rate and a decreased number of bursts at early postnatal ages. This decreased activity is found in all somatosensory and visual thalamic nuclei except the LP, maybe due to its role in complex cognitive functions such as direct attention and neglect (Kamishina et al., 2008). Nevertheless, our data give more evidence about the consequences of the Kir2.1 overexpression in the thalamic spiking activity. Despite the differences in firing rate, the temporal structure of the spiking activity is not affected since the distribution of ISIs is similar both in control and in Th^{Kir} mice. Furthermore, comparing the distribution of ISIs between visual and somatosensory nuclei in control animals, there is a higher proportion of short intervals in the latter, suggesting a premature development of the somatosensory circuitry.

Decreased spontaneous activity in the thalamus influences the development and consolidation of functional sensory maps.

It has previously been shown that the modification of thalamic activity not only affects spontaneous activity in S1 but also provokes aberrant processing of somatosensory information (Antón-Bolaños et al., 2018). Here, we showed that the spontaneous firing rate of neurons in S1 is diminished in Th^{Kir} compared with control mice from early postnatal development. However, the amplitude of field responses in the somatosensory cortex at P6 seem to be similar in control and Th^{Kir}, demonstrating that thalamic neurons in this nucleus are functional. Only from P6 onwards, we found that S1 neurons of Th^{Kir} mice cannot follow thalamic activity, disrupting completely the transmission of sensory information. In this mouse line, there are defects of information processing from earlier stages. Evoked activity at perinatal stages lacks columnar organization and spontaneous activity spreads throughout larger areas than in control mice (Antón-Bolaños et al., 2018). Together,

our data and previous publications indicate that if spontaneous thalamic activity is abnormal the somatosensory pathway develop rather normally until the thalamic station and defects appear downstream the thalamus in a progressively manner. Last, it would be worthy to check to if abnormal patterns of spontaneous activity are present also in the adulthood in order to check to what extent they reflect the issues on sensory processing shown in mature mice.

Deprivation of visual input at embryonic stages disrupts thalamic spontaneous activity during early postnatal life.

Retinal input provides the major driving input to relay cells in the visual thalamus (Sherman and Guillery, 2002). However, retinal synaptic contacts accounts for less than 10% of the total input in these cells (Bickford et al., 2010). When retinal input is experimentally removed at E14.5 by eye enucleation (embBE), the frequency of calcium waves increases in the dLGN, demonstrating that the thalamus reacts to peripheral damage even at very immature stages of development. We now wander if the maturation of the pattern of spontaneous activity in the thalamus is affected as well at later stages in embBE scenarios. We found that the spontaneous firing rate is diminished in the dLGN at P6-P7. This remarks the relevance of retinal projections as a driver input despite their low relative number of synaptic contacts. It would be interesting to test to what extent the proportion of the other excitatory afferents remains stable, namely, those arriving from the visual cortex, brainstem and superior colliculus. Apart from the synaptic driving, we cannot rule out that retinal input exerts a trophic effect on thalamic relay cells that precludes their normal development in embBE mice. Indeed, the size of the dLGN in embBE mice is reduced suggesting higher levels of programmed cell death in the nucleus (Moreno-Juan et al., 2017).

In contrast to the dLGN, the LP during the first postnatal week increases its level of spontaneous firing activity upon retinal deprivation. The LP receives inputs from different brains structures, some of them characterized by a high frequency of spontaneous firing like the superior colliculus (Yu et al., 2021). The superficial layers

of the superior colliculus send direct projections to LP cells (Naeem et al., 2021), conveying information related to locomotion and saccades (Roth et al., 2016). However, we lack enough evidence on how this pathway develops to hypothesized possible scenarios when retinal input is missing. Alternatively, LP adaptations to sensory deprivation modifications might also involve the cortico-thalamic feedback. Taking in account that L6 CTAs arrive to the dLGN earlier in enucleated than in control mice (Grant et al., 2016), we explored to what extent the major cortical input to the LP, L5 projections, exhibits any adaptation upon retinal deprivation. Using anterograde and retrograde labelling, we found that this projection seems to innervate LP cells normally. We have not found an evident large number of projections or novel projections from non-typical inputs. Nevertheless, it is possible that the corticothalamic projection undergoes adaptations at the synaptic level upon early visual deprivation. In this regard, it would be worthy to explore this issue using patch-clamp recordings from LP cells while stimulating the cortical input.

Interestingly, the development of both visual nuclei seems normal by P13-P14, at least, by studying the basic firing properties of their cells. This means that some of the fundamental functional features of the thalamocortical system is controlled by homeostatic mechanisms. Similar results were found in other studies where enucleation has been performed at P6. In these cases, there is an acute silencing of the visual system, but two days after deprivation, the firing activity begins to recover (Riyahi et al., 2021). It is likely that this autoregulation could be exerted at the level of the thalamus and cortex (Weliki and Katz, 1999), but further research is needed to answer these questions.

Deprivation of visual input at embryonic stages heightens intra-thalamic communication.

In previous studies, firing rate correlations have been measured within a single nucleus of the thalamus or in the sensory cortex (Colonnese et al., 2017; Weliki and Katz, 1999). However, to the best of our knowledge, this is the first attempt to explore correlations between neurons in different thalamic nuclei. Simultaneous

recordings from two different thalamic nuclei are challenging. In addition to the small size of the nuclei at the developmental stages, their spatial arrangement dictates that, to get neurons from more than one nucleus at the same time, multi-shank probes should be used or the angle of insertion must be controlled with precision. By mastering both techniques, we were able to obtain recordings as reliable as those from a single thalamic nucleus.

Pair-wise correlations between sensory thalamic nuclei are expected to be low since they do not present a considerable amount of shared input and also because direct intra-thalamic communication has not been reported in the adult. We have hypothesized that any kind of relationship between nuclei may account for either cross-modal plasticity or homeostatic control in early visually deprived mice. However, we have not found any relevant direct or indirect communication between nuclei in deprived mice. Actually, all pair-wise correlations seem to be lower in deprived mice than in control mice at the ages studied, except for the LP-POM pair at P6. This pair, indeed, deserves special attention because involves both HO nuclei; however, differences did not reach statistical significance and thus more research is needed to shed light on this issue. Moreover, in control mice, it is remarkable that correlations reach such high values contrasting to previous observations obtained from calcium recordings (Golshani et al., 2009; Rochefort et al., 2009) but in accordance with other studies reporting correlation based on extracellular electrical recordings (Colonnese et al., 2017). Thus, this controversy is telling us that we lack a clear understanding of the meaning of neuronal correlation and its progression during development.

Mild cross-modal changes in the pattern of spontaneous activity of somatosensory thalamus upon early visual deprivation.

It is widely known that sensory deprivation leads to changes not only in the affected modality but also in the other ones due to cross-modal plasticity processes. It has been well described that eye enucleation at embryonically or at early postnatal ages triggers an important reorganization of deprived and non-deprived sensory cortical

areas, probably aimed to increase processing power of the remaining senses (Karlen and Krubitzer, 2009; Moreno-Juan et al., 2017). Although many studies argue that sensory experience accounts for these adaptations (Rauschecker et al., 1992; Bronchti et al., 1992; Toldi et al., 1996), it has been shown that they are evident before the onset of active sensing (Huberman et al., 2006; Fetter-Pruneda et al., 2013; Moreno-Juan et al., 2017). We have then analyzed the patterns of spontaneous activity of the somatosensory nuclei of the thalamus. We performed electrophysiological recordings and found that spontaneous firing was reduced in VPM and POm cells of embBE mice at P6. However, this activity gradually recovers, especially in the VPM, by P13-P14. Since there are clear anatomical adaptations in S1 in embBE mice, such as the enlargement of barrel area (Moreno-Juan et al., 2017), we expected to find functional changes as well. For example, we have hypothesized that the speed of functional maturation may be accelerated or that excitability of the nuclei may be increased. However, the functional changes were mild and mimic those found in the visual pathway. One piece of information that is missing in our work and in most of the related publications is the assessment of extent to which cross-modal adaptations yield relevant improvements in the behavior of the animal.

Consequences of embryonic visual input deprivation on the cortical properties.

The influence of patterned activity on the development of sensory cortical maps is a key question of this dissertation work. There are evidences, for instance, about the crucial role of thalamic waves over cortical development by controlling both the size of cortical areas and the functional organization or cortical columns (Moreno-Juan et al., 2017; Antón-Bolaños et al., 2019). We have seen that enucleations at embryonic stages trigger important changes in thalamic spontaneous activity mainly during the first postnatal week, we wonder now to what extent the sensory cortices are affected.

Functional changes in the cortex were expected in our model of early visual deprivation partly based on anatomical data that reveal an expansion of the

somatosensory area of the cortex in blind animals (Karlen and Krubitzer, 2009; Bronchti et al., 1992; Moreno-Juan et al., 2017). However, the function of S1 cells is expected to follow the behavior of its main driving input, the VPM, which revealed mild changes in embBE mice. Extracellular recordings in S1 revealed that there is not a significant effect in the spontaneous firing of S1 until P13-P14. By P6-P7, it seems that there is a tendency that reveals a less active somatosensory cortex but it is only one week later that the differences are robust enough to indicate an effect. It is remarkable that spontaneous neuronal firing declined in S1 during the second postnatal week since the somatosensory thalamus remains rather normal. We decided to investigate if the decreased spontaneous firing observed in S1 of embBE mice impacts on the evoked activity in the thalamocortical circuit. We explored then the capability of S1 neurons to respond to the stimulation of the whiskers in control and in embBE mice at P13-P14. We found that neurons of S1 in embBE not only are able to respond to the stimulation but also the evoked response was faster and larger than in control mice, suggesting that cortical plasticity in enucleated animals could lead to better processing in the somatosensory modality. Many questions remain open. Why spontaneous activity is reduced in S1 of embBE mice? Which changes at the circuit or synaptic level explain this reduction? Are these changes the same that explain the enhanced evoked responses?

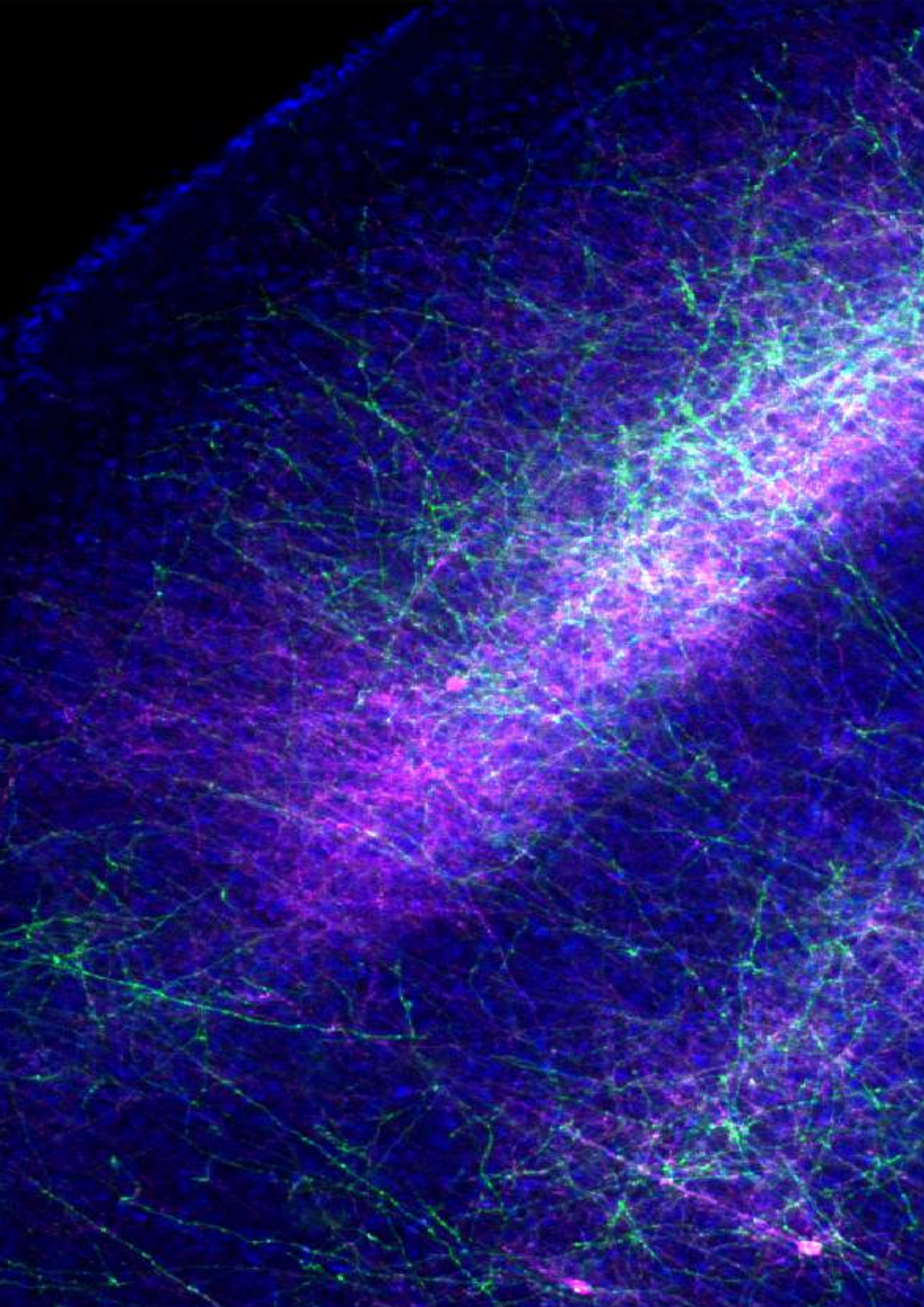
Regarding the visual modality, it is clear that both dLGN and LP underwent important changes in the rate of spontaneous spiking when the visual input is removed at early stages. We wonder then to what extent spontaneous activity in the visual cortex has been affected by early visual deprivation. On the one hand, we found that the cells in the secondary visual cortex of embBE mice fired at a higher frequency compared with control mice, resembling the results found in the LP. On the other hand, the low levels of activity found in the dLGN in embBE mice matched with the decreased in activity recorded from V1 neurons. It is interesting that whereas the decrease of activity was significant for neurons in L4, which receives most of the dLGN projections, L5 neurons did not present significant changes. As layer 5 neurons are one of the main inputs of LP cells, these data indicate that the increased activity observed in LP cells may not have a cortical origin. In this case, it

is possible that the projections from the superior colliculus take over. The abnormal effects were only visible after P13-P14, when circuits are almost mature and begin to actively process visual information. Subtler changes may be uncovered earlier in development using different techniques such as patch-clamp recordings or single-unit analysis.

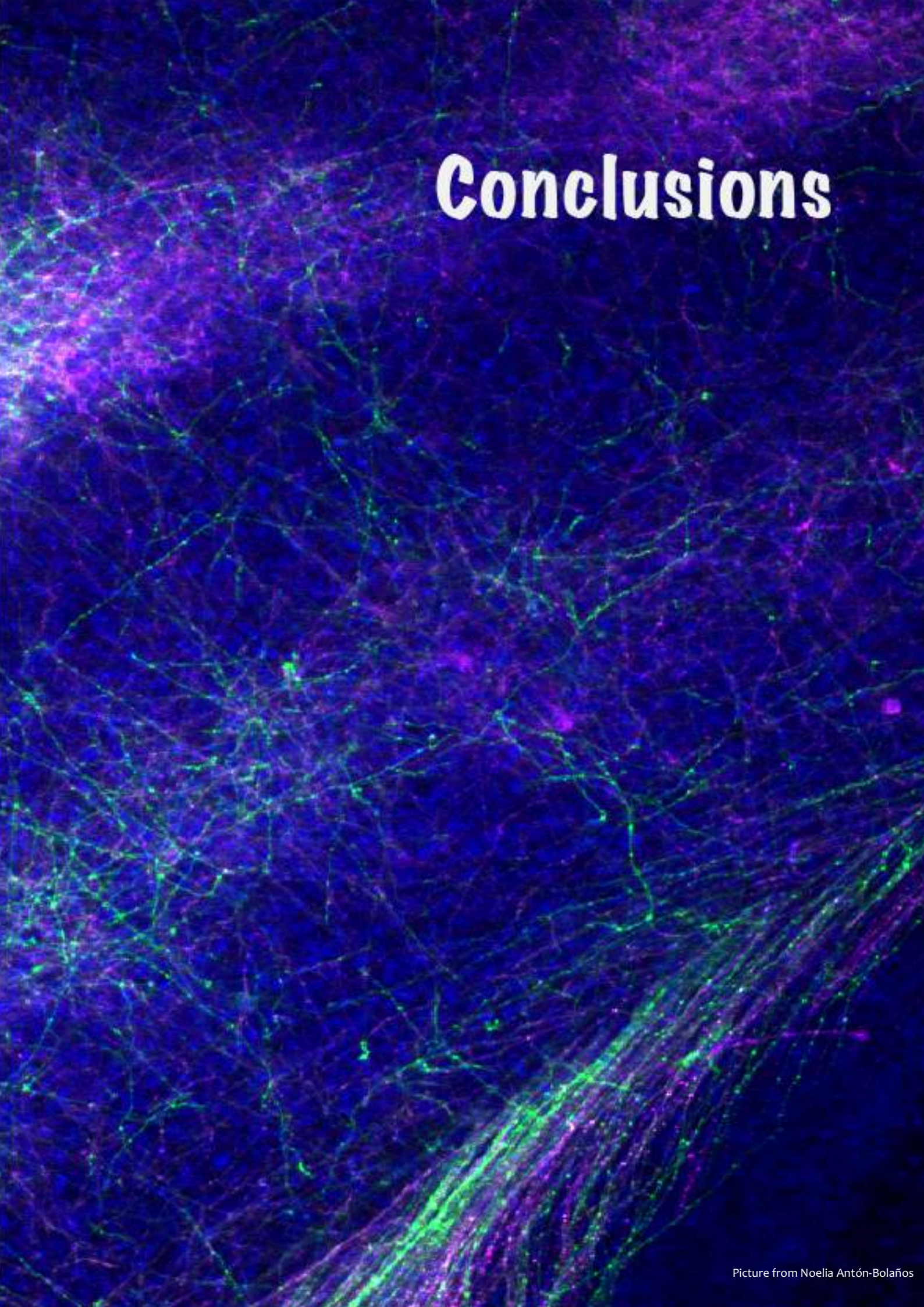
The functional and behavioral meaning of these intra and cross-modal changes is not straightforward. In the literature, the evidence suggests that a fraction of the normally visual neurons in the cortex are overtaken by non-visual modalities after visual deprivation (Ewall et al., 2021). However, we still lack a comprehensive understanding of the exact relevance for the animal of these reconfiguration (Ramamurthy et al., 2021). We thought that our experimental model could be a valid tool to examine this topic. To this, we first need to check if non-visual stimuli are able to drive formerly visual neurons in the deprived visual cortex. We began to tackle this question by recording the field response of V1 neurons to whisker stimulations in controls and in embBE mice. Whereas somatosensory stimulations did not evoke responses in V1 of control mice, there was slow response driven by the whiskers in the putative visual areas of embBE mice. These results suggest that the somatosensory modality might partially invade the otherwise visual cortex in embBE mice. Further functional and behavioral studies in this model may elucidate the role of the deprived territories in processing the information of the spare modalities.

Concluding remarks

Although many efforts have been focused on demonstrating the role of the thalamic activity in the correct development of cortical sensory maps, and although cortical spontaneous activity has been largely described, the maturation of spontaneous activity pattern during postnatal development in the thalamus and how it contributes to the emergence and plasticity of cortical maps has not been explored thoroughly. In this study, we begin to describe the pattern of spontaneous activity in the visual and somatosensory nuclei of the thalamus during the end of the first and second postnatal weeks. Moreover, we modified the spontaneous thalamic activity during development by using embryonic bilateral enucleations and by overexpressing Kir2.1 in the thalamus. We demonstrated that, when the thalamus is manipulated, there are cortical consequences both in spontaneous activity and in the functional properties. In addition, our embBE data provide more evidence regarding the intra- and cross-modal reorganization of sensory modalities, which may lead to a better processing of somatosensory information in visually deprived animals.



Conclusions



Picture from Noelia Antón-Bolaños

Conclusions

1. The firing pattern of spontaneous thalamic activity switches from sparse and decorrelated towards synchronous and bursty during the second postnatal week.

2. Somatosensory circuits seem to present a premature development respect to the visual circuits.

3. The overexpression of Kir2.1 in the thalamic sensory neurons does not suppress the neuronal activity but perturbs the pattern of spontaneous activity in both visual and somatosensory nuclei at postnatal ages.

4. By overexpressing Kir2.1 in the developing sensory nuclei of the thalamus, the firing rate is diminished in all sensory nuclei from P6-P7.

5. Regarding the visual thalamus, the activity pattern in bursts appears only at P13-P14 in the dLGN in control mice. However, the temporal structure of the spiking activity seems to be not affected by the Kir2.1 overexpression.

6. As in the visual thalamus, somatosensory nuclei in Th^{Kir} exhibit lower burst rate at the end of the second postnatal week, with shorter durations, fewer number of spikes and shorter ISIs.

7. However, VPM neurons of Th^{Kir} mice can respond normally to peripheral stimulation. These data suggest that somatosensory circuits remain functional in spite of the severe defects observed in the pattern of spontaneous activity.

8. The firing of spontaneous activity of S1 neurons is severely affected when thalamic activity is perturbed, suggesting that the thalamus is the main driver of cortical activity at postnatal ages.

9. Furthermore, S1 cortical neurons of Th^{Kir} mice are not able to follow thalamic activity from P13 onwards; they show responses weaker than control neurons when whiskers are stimulated.

10. Embryonic visual input deprivation causes adaptations in the pattern of spontaneous activity in visual thalamic nuclei during the first postnatal week. Namely, whereas dLGN neurons undergo a decrease in firing rate, activity in the LP is heightened. The pattern of bursts also exhibits nucleus-specific adaptations. However, these changes vanish after the onset of sensory experience by P13, possibly due to compensatory mechanisms.

11. Although spontaneous activity recovers by the end of the second postnatal week, visual thalamic nuclei show low correlation both in control and embBE mice, suggesting a reconfiguration of connectivity in the visual pathway; visual thalamic nuclei could be receiving afferences from different sources.

12. Visual sensory deprivation diminishes spontaneous activity in somatosensory nuclei during the first postnatal week. Afterwards, there is a gradual recovery by P13-P14, especially in the VPM.

13. Effects of embryonic visual input deprivation on visual thalamic nuclei provoke layer-specific changes in their corresponding cortical areas, V1 and V2 by P13-P14.

14. The decreased spontaneous activity in the somatosensory thalamic nuclei caused by visual deprivation leads to a diminished activity in the primary somatosensory cortex at P13-P14.

15. Although somatosensory thalamic nuclei and cortex undergo a reduction of spontaneous firing in embBE mice, the response of S1 to whisker stimulations is faster and larger than in control mice, suggesting a cross-modal compensation in processing somatosensory information.

16. V1 neurons show mild responses to whisker stimulations after embryonically visual input deprivation, suggesting a partial colonization of the visual cortical area by the somatosensory modality.

Conclusiones

1. En ratones control, la actividad espontánea del tálamo cambia desde un patrón de actividad espontánea con escasos disparos neuronales decorrelacionados hacia un estado caracterizado por un patrón de actividad más sincrónico en forma de ráfagas. Esto ocurre durante la segunda semana postnatal, después de la entrada sensorial.

2. Los circuitos somatosensoriales parecen desarrollarse antes que los visuales.

3. La sobrexpresión de Kir2.1 no suprime la actividad neuronal del tálamo pero genera un cambio en el patrón de actividad espontánea en los núcleos talámicos, tanto en los somatosensoriales como en los visuales, en edades postnatales.

4. Sobreexpresando Kir2.1 en los núcleos sensoriales del tálamo durante el desarrollo, la tasa de disparo disminuye en todos los núcleos sensoriales desde P6-P7.

5. Respecto al tálamo visual, el patrón de actividad en ráfagas solo aparece a P13-P14 en dLGN en ratones control. Sin embargo, la estructura temporal de los disparos no parece estar afectada por la sobreexpresión de Kir2.1.

6. Como sucede en el tálamo visual, los núcleos somatosensoriales en el Th^{Kir} exhiben un menor número de ráfagas al final de la segunda semana postnatal, con duraciones más cortas, menos disparos e intervalos entre disparos más cortos.

7. Sin embargo, las neuronas del VPM del Th^{Kir} son capaces de responder de manera normal a la estimulación periférica. Estos datos sugieren que los circuitos somatosensoriales permanecen funcionales a pesar de los defectos en el patrón de actividad espontánea.

8. La tasa de disparo espontánea de las neuronas en S1 disminuye severamente cuando se perturba actividad del tálamo, lo que sugiere que el tálamo es el principal conductor de la actividad cortical en edades postnatales.

9. Además, las neuronas de S1 del Th^{Kir} no son capaces de seguir la actividad del tálamo desde P13 en adelante; muestran una respuesta mucho menor que los controles cuando estimulamos las vibrisas.

10. La enucleación visual embrionaria causa adaptaciones en el patrón de actividad espontánea en los núcleos talámicos visuales durante la primera semana postnatal; mientras que el dLGN disminuye su actividad espontánea, el núcleo secundario, LP, la incrementa. En concordancia con los cambios en la tasa de disparo, el patrón de disparo en ráfagas también muestra adaptaciones específicas de cada núcleo. Sin embargo, estos cambios desparecen cuando comienza la experiencia sensorial, a P13, posiblemente debido a mecanismos compensatorios.

11. Aunque la actividad espontánea se recupera a finales de la segunda semana postnatal, los núcleos visuales muestran una correlación baja tanto en animales controles como en enucleados, lo que sugiere una reconfiguración de la conectividad en la vía visual; los núcleos visuales podrían estar recibiendo aferencias de diferentes fuentes.

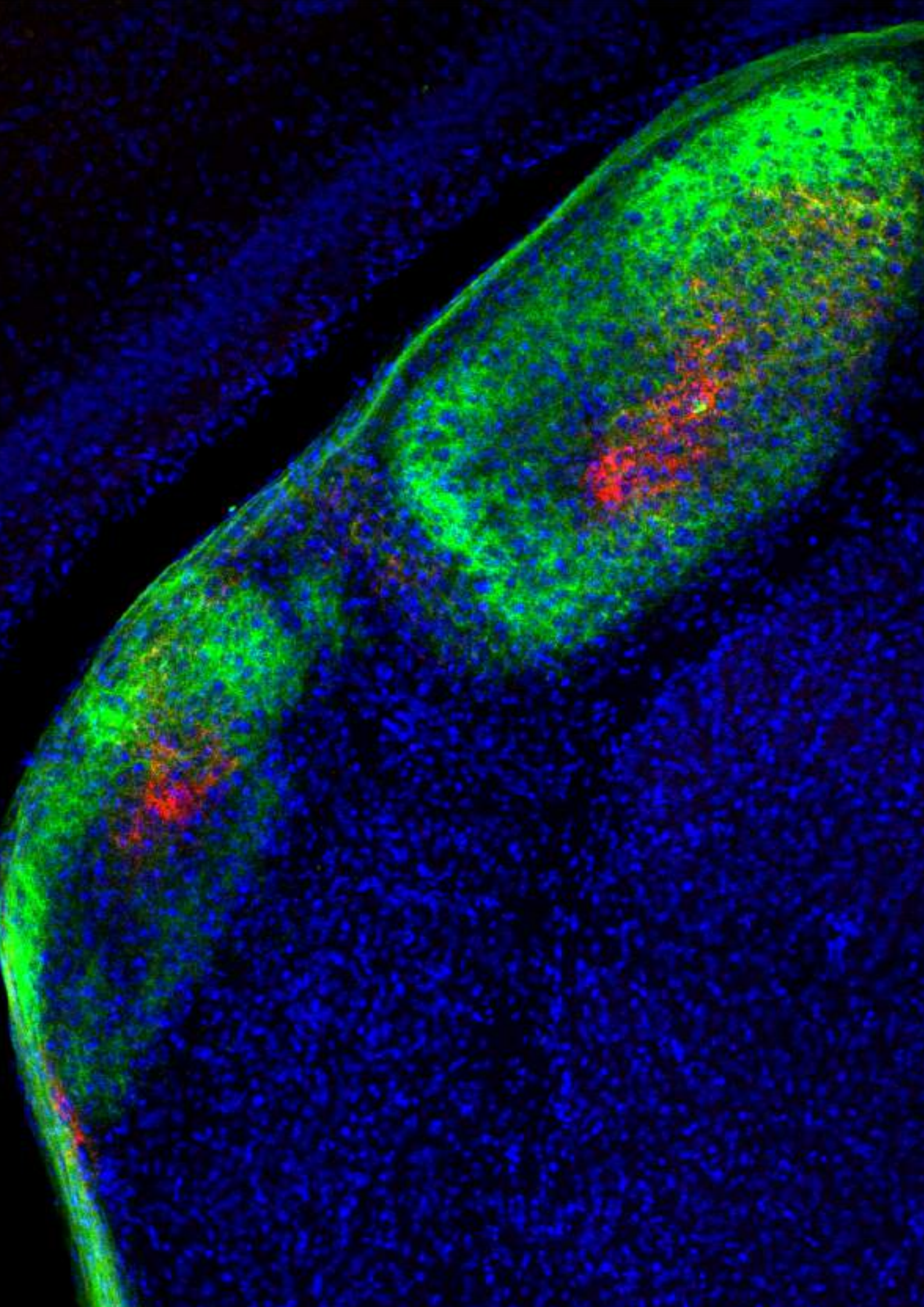
12. La deprivación sensorial disminuye la actividad espontánea en los núcleos somatosensoriales durante la primera semana postnatal. Después hay una recuperación gradual a P13-P14, especialmente en el VPM.

13. Los cambios en la actividad espontánea de los núcleos visuales (dLGN y LP) debidos a la deprivación visual, tienen consecuencias en las capas específicas de sus correspondientes áreas corticales (V1 y V2) a P13-P14.

14. La disminución de actividad espontánea en los núcleos somatosensoriales causados por la deprivación visual conduce a una actividad también reducida en S1 a P13-P14.

15. Aunque los núcleos talámicos y la corteza somatosensorial sufren una reducción de la actividad en animales ciegos, la respuesta cortical evocada por el movimiento de las vibrisas es más rápida y de mayor amplitud que en los controles, sugiriendo una mejora en el procesamiento de la información somatosensorial.

16. Las neuronas de V1 responden aunque de manera relativamente pobre a la estimulación de las vibrisas en ratones que han sido enucleados durante estadios embrionarios, sugiriendo una colonización parcial del área cortical visual por parte de la modalidad somatosensorial.



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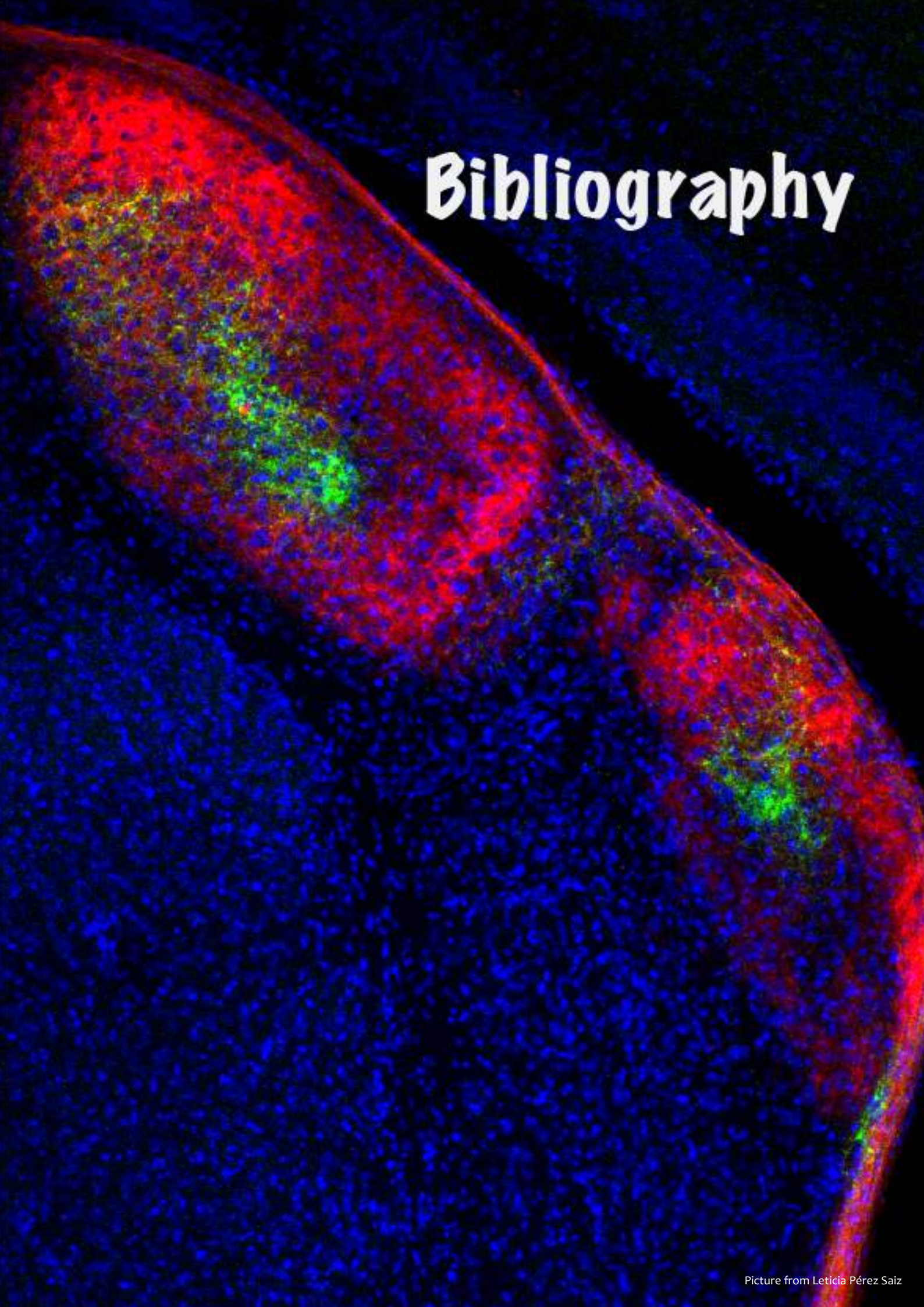

Picture from Leticia Pérez Saiz

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