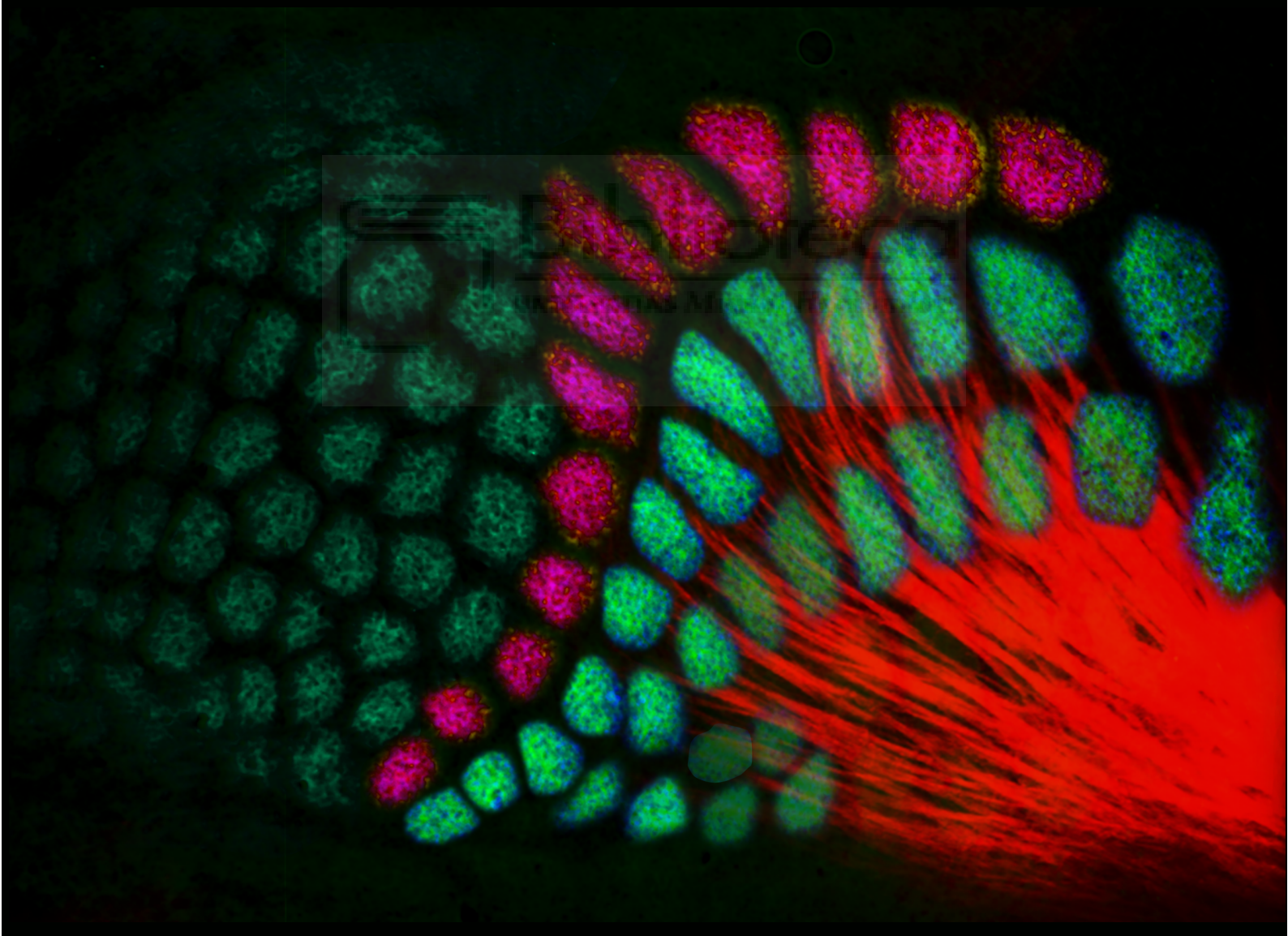
Role of thalamic input in the development of sensory cortical maps

PhD Thesis
Noelia Antón Bolaños



CSIC UNIVERSITAS Miguel Hernández
INSTITUTO DE NEUROCIENCIAS
UNIVERSITAS Miguel Hernández

Instituto de Neurociencias (CSIC-UMH)

Programa de Doctorado Neurociencias

Universidad Miguel Hernández, Alicante (España)

Role of thalamocortical input in the development of sensory cortical maps

Doctoral thesis

Presented by

Noelia Antón Bolaños

Director:

Guillermina López Bendito, CSIC

San Juan de Alicante, 2019

DOCTORAL THESIS BY COMPENDIUM OF PUBLICATIONS

To whom it may concern:

The doctoral thesis developed by me, Noelia Antón Bolaños, with title: "Role of thalamocortical input in sensory cortical maps development" is a compendium of publications and includes the following publications, in which I am the first author:

Antón-Bolaños N., Espinosa A., López-Bendito G. (2018). Developmental interactions between thalamus and cortex: a true love reciprocal story. Curr. Opin. Neurobiol. 2018 Oct; 52:33-41. DOI: 10.1016/j.conb.2018.04.018.
Impact factor 6.541 (2018 Q1 Multidisciplinary Sciences).

Antón-Bolaños N.*, Sempere-Ferrández A.*, Guillamón-Vivancos T., Martini F.J., Pérez- Saiz L., Gezelius H., Filipchuk A., Valdeolmillos M., López-Bendito G. (2019). Prenatal activity from thalamic neurons governs the emergence of functional cortical maps in mice. Science. 2019 02 May. DOI: 10.1126/science.aav7617.
Impact factor 41.037 (2018 Q1 Multidisciplinary Sciences).

I declare that these publications will not be used in any other thesis.

Yours sincerely,

San Juan de Alicante, 4th of June 2019

Noelia Antón Bolaños

DOCTORAL THESIS BY COMPENDIUM OF PUBLICATIONS

To whom it may concern:

The doctoral thesis developed by Noelia Antón Bolaños, with title: "Role of thalamocortical input in sensory cortical maps development", includes the following publications that correspond to the main topic of the dissertation:

Antón-Bolaños N., Espinosa A., López-Bendito G. (2018). Developmental interactions between thalamus and cortex: a true love reciprocal story. Curr. Opin. Neurobiol. 2018 Oct; 52:33-41. DOI: 10.1016/j.conb.2018.04.018.
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Impact factor 41.037 (2018 Q1 Multidisciplinary Sciences).

As the director of this PhD. Thesis and the corresponding author of the articles, I declare that Ms. Noelia Antón Bolaños is a first author of both articles and is a major contributor of the work presented in this publication. I declare that this publication will not be used in any other thesis.

Yours sincerely,

San Juan de Alicante, 4th of June 2019
Dra. Guillermina López-Bendito

A QUIEN CORRESPONDA:

Prof. Miguel Valdeolmillos López, Coordinador del Programa de Doctorado en Neurociencias del Instituto de Neurociencias, Centro Mixto de la Universidad Miguel Hernández-UMH y la Agencia Estatal Consejo Superior de Investigaciones Científicas-CSIC,

CERTIFICA:

Que la Tesis Doctoral presentada por compendio de publicaciones "*Role of thalamocortical input in sensory cortical maps development*" ha sido realizada por Dña. Noelia Antón Bolaños (DNI: 74364678C) bajo la dirección de la Dra. Guillermina López Bendito y da su conformidad para que sea presentada a la Comisión de Doctorado de la Universidad Miguel Hernández.

Para que así conste a los efectos oportunos, firma el presente certificado en San Juan de Alicante a 04 de junio de 2019.

Dr. Miguel Valdeolmillos López

“As long as our brain is a mystery, the universe, the reflection of the structure of the brain will also be a mystery.”

Santiago Ramón y Cajal

Me gustaría aprovechar este momento para agradecer, primero de forma general, a todas aquellas personas que han hecho posible que alcance este momento, el cual muchas veces a lo largo de este proceso parecía imposible. Ya sea científica o anímicamente, gracias a todos aquellos que me dedicaron parte de su tiempo, gracias por las sonrisas por los pasillos del instituto, por escucharme, entenderme y sobretodo por aconsejarme.

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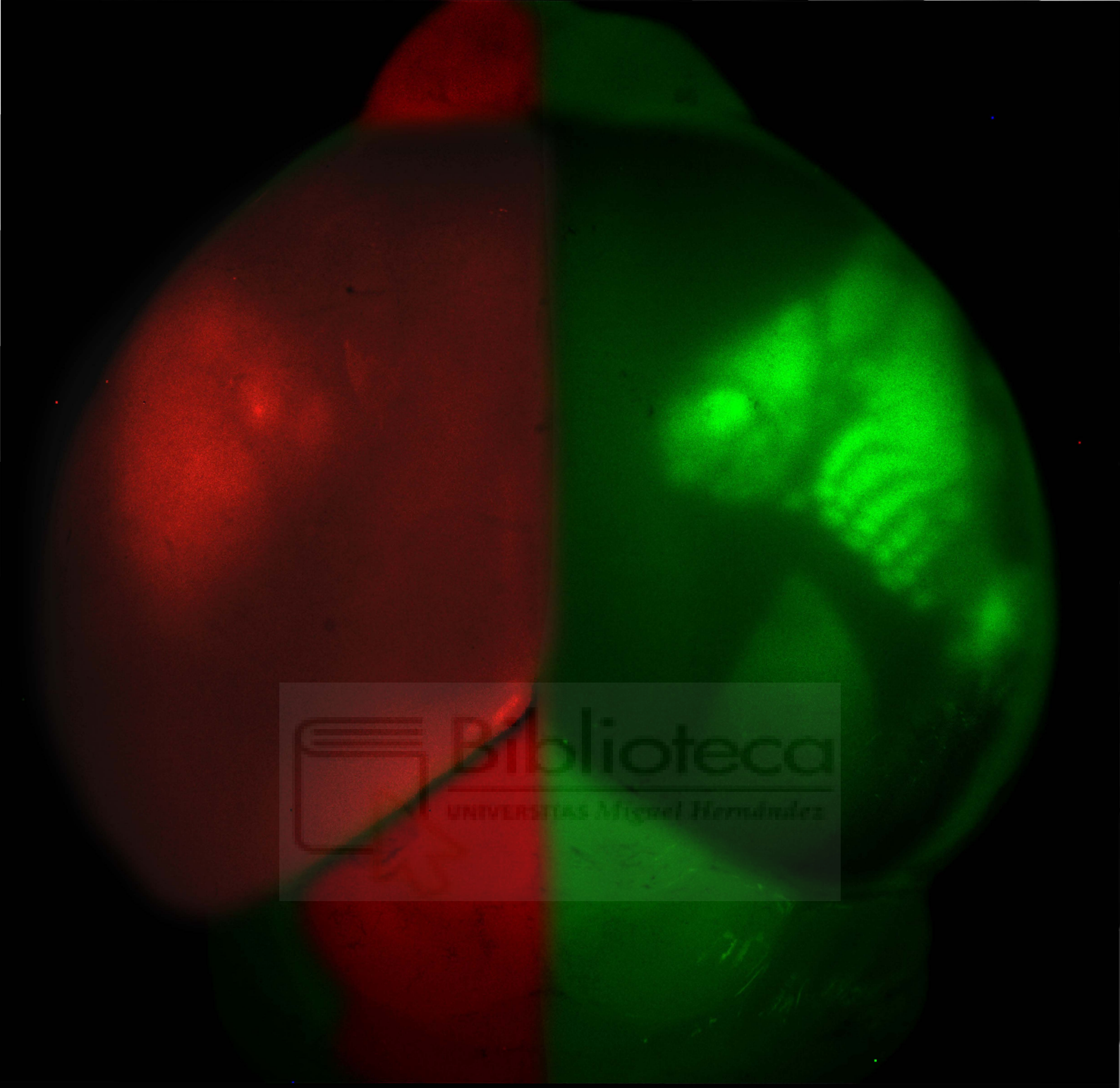

Whole brain images of a P7 TCA-GFP mice. View of the distict sensory cortical areas. The control animal is represented in green and the *Th*^{Kir} mice is represented in red.

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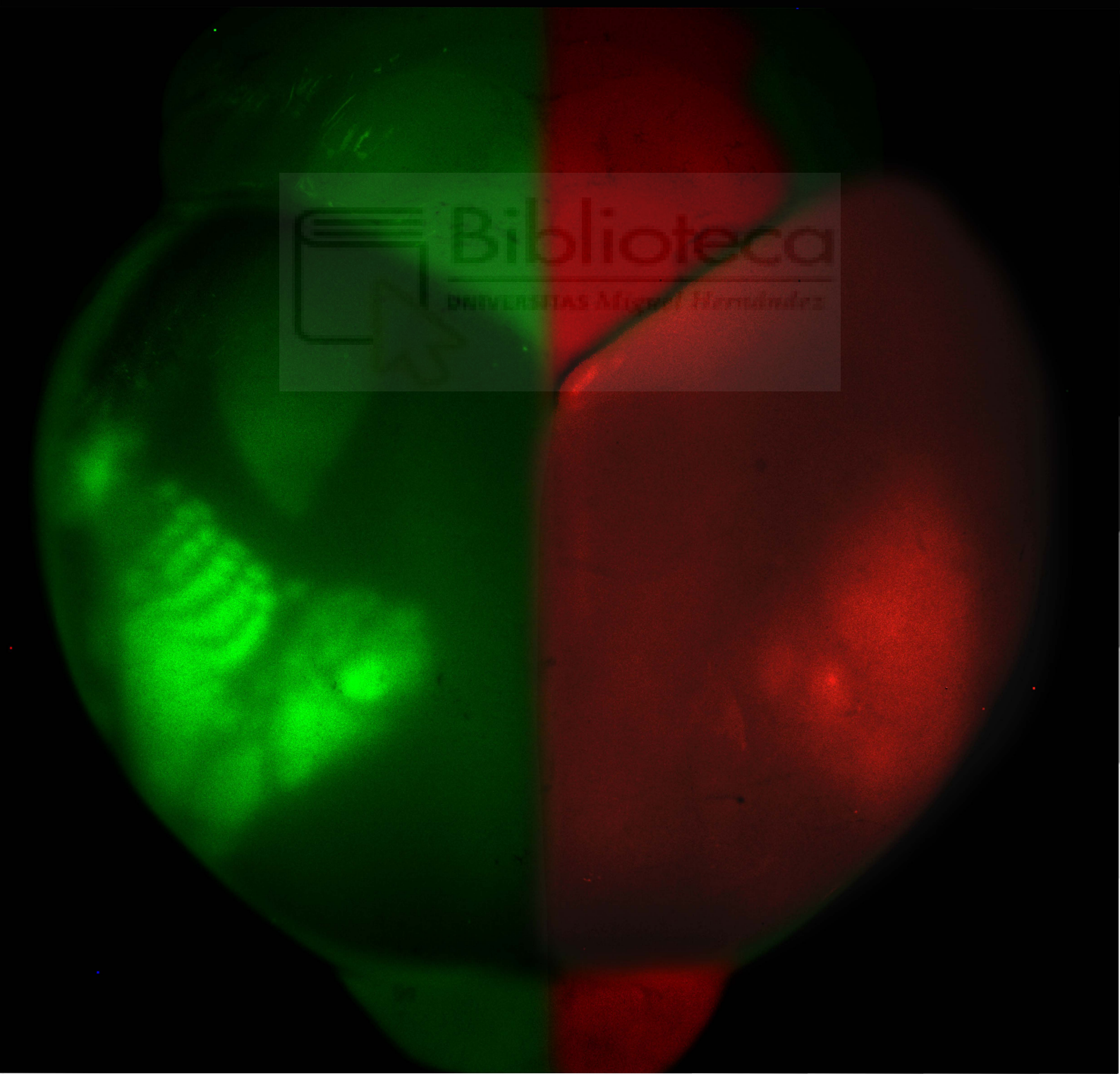

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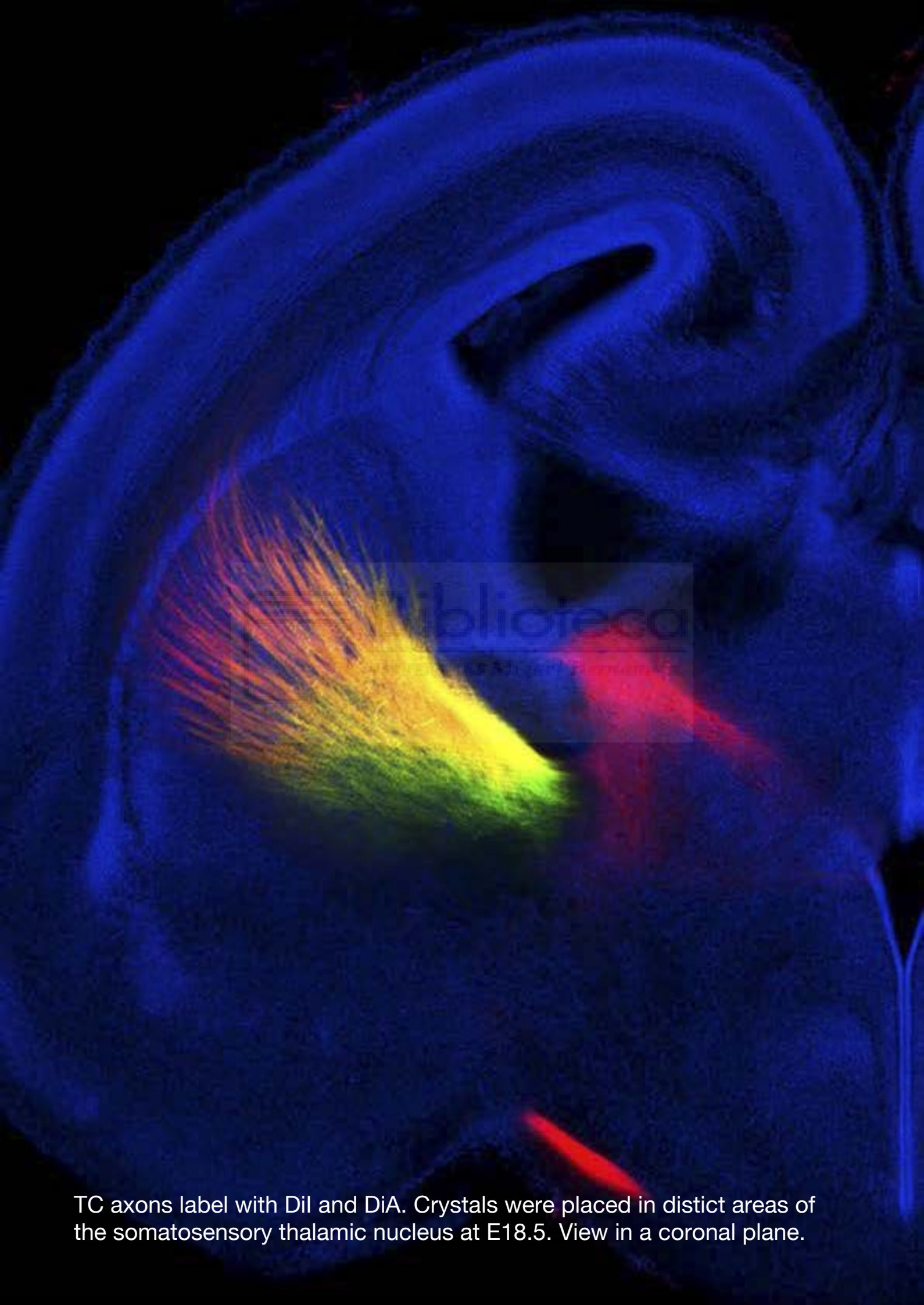
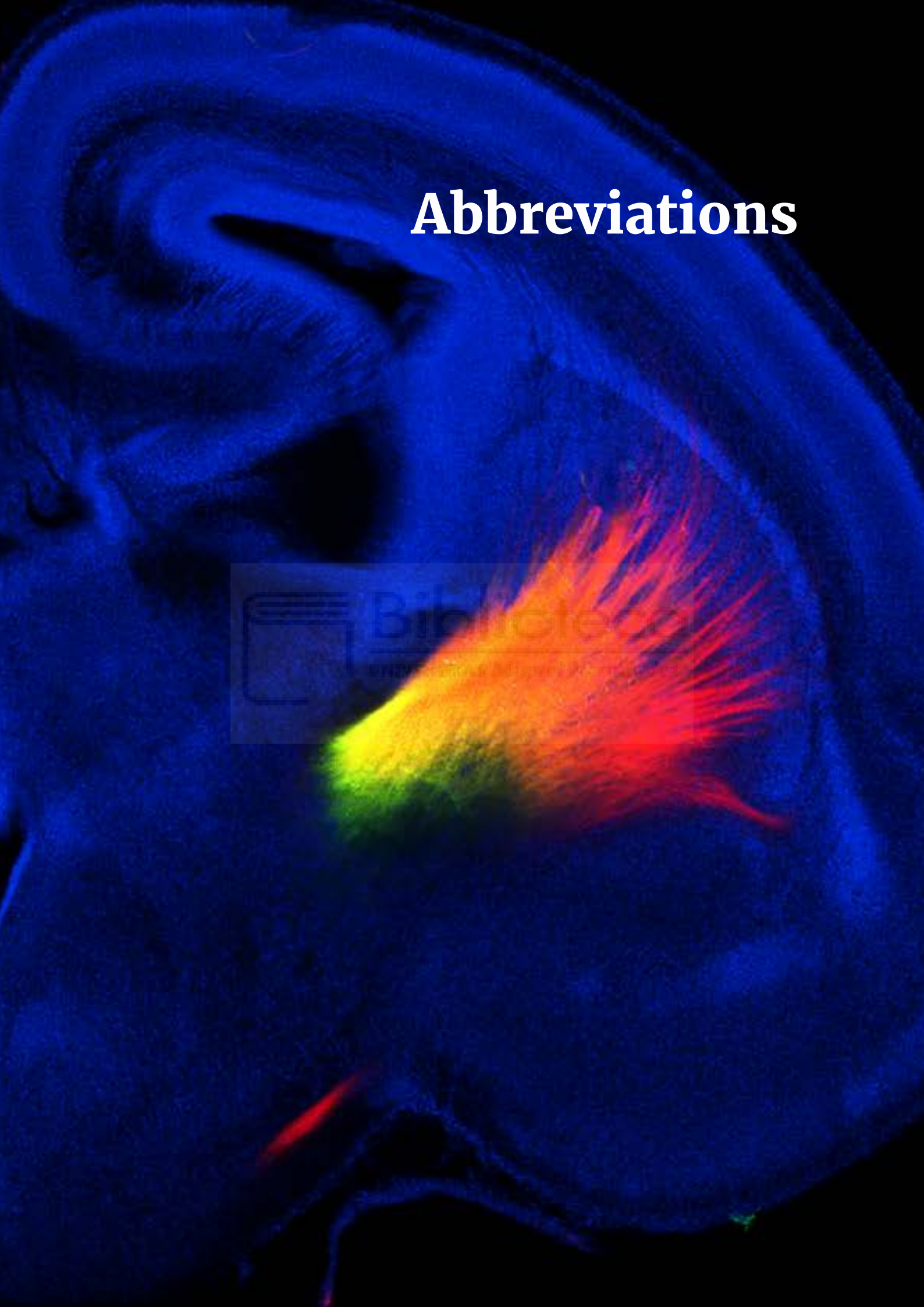


TC axons label with DiI and DiA. Crystals were placed in distict areas of the somatosensory thalamic nucleus at E18.5. View in a coronal plane.

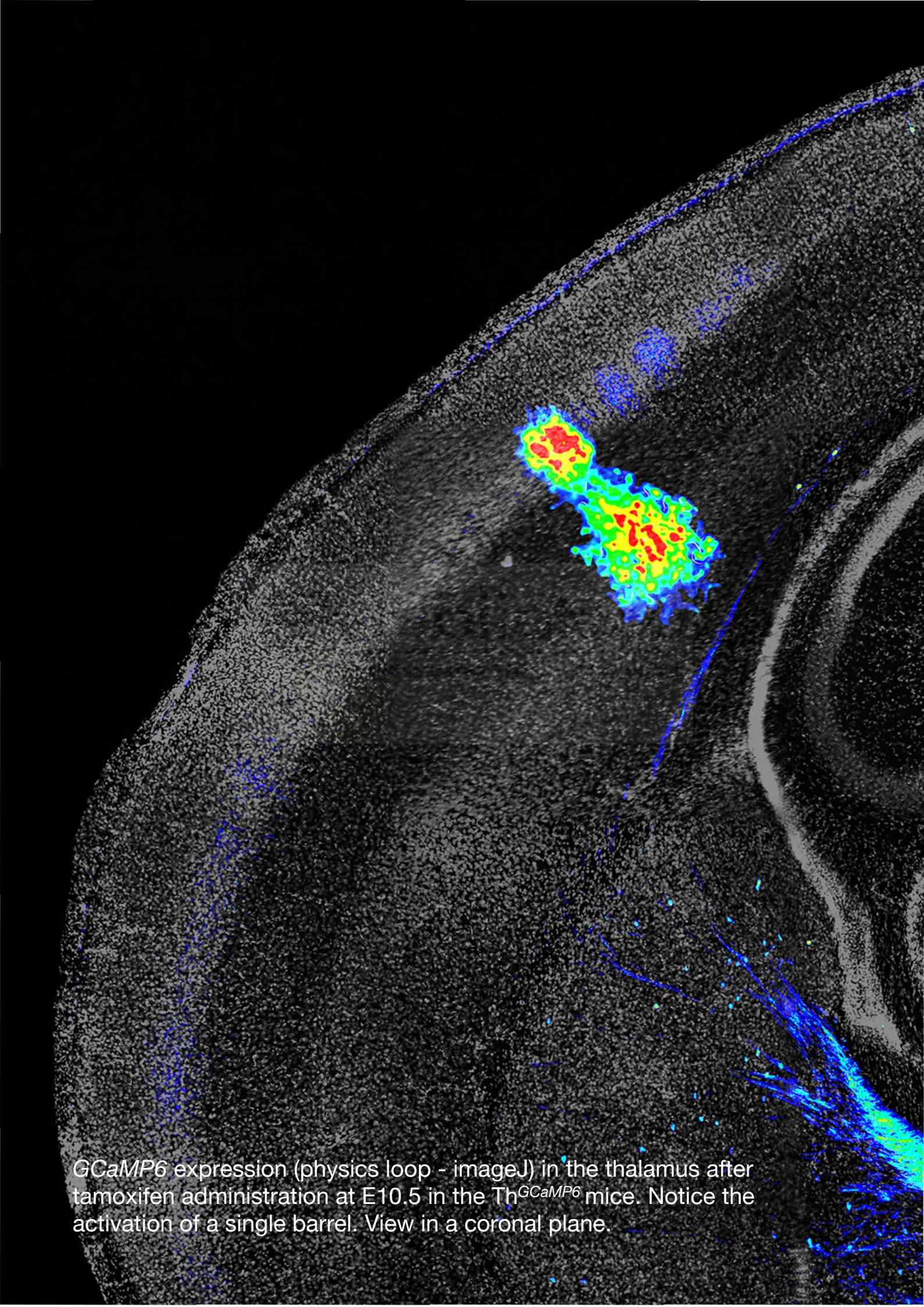
Abbreviations



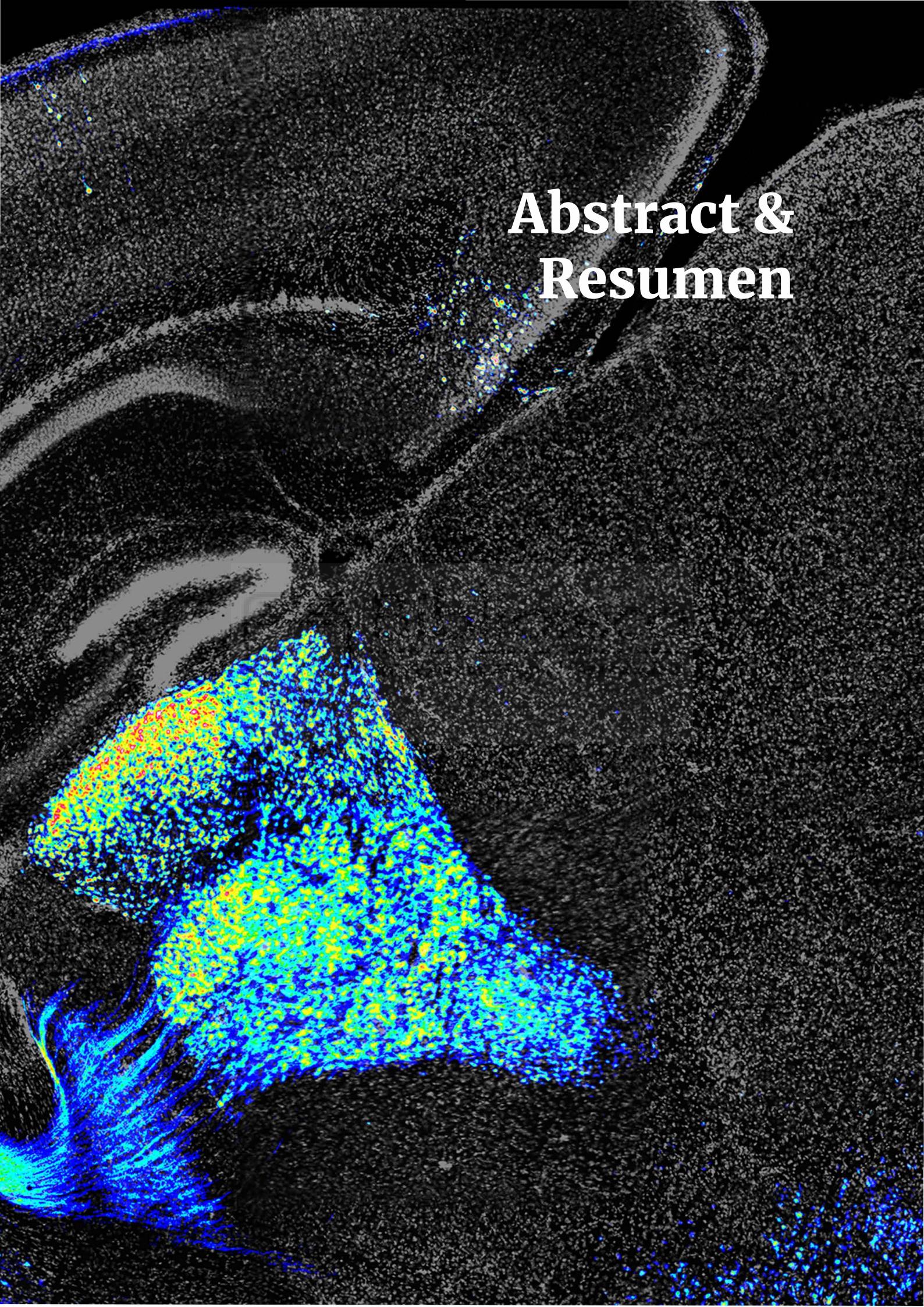
ABREVIATIONS

A1	Primary auditory cortex
AC1	Adenylyl cyclase 1 (AC1)
ANR	Anterior neural ridge
BMP	Bone morphogenetic protein
cENOS	Cortical network oscillations
CFAs	Corticofugal axons
CN	Cochlear nucleus
CoP	Commissural plate
Cp	Cerebral peduncle
CTAs	Corticothalamic axons
dLGN	Dorsolateral geniculate nucleus
DMO	Mid-diencephalic organizer
dPrV	Dorsal portion of the PrV
DTB	Diencephalon–telencephalon boundary
E	Embryonic day
EGF	Epidermal growth factor
EGOs	Early gamma oscillations
FGF	Fibroblast growth factors
FO	First-order
HO	High-order
IC	inferior colliculus
ICp	internal capsule Sonic
IGL	Intergeniculate leaflet
IHC	Inner hair cells
ION	Infraorbital nerve
IZ	Intermediate zone (IZ)
L	Layer
LP	Lateral posterior nucleus
MGB	Medial geniculate body
MGd	Dorsal division of the medial geniculate body
mGlu5	Metabotropic glutamate Receptor 5
MGv	Ventral division of the medial geniculate body
NMDAR1	NMDA receptor1
OHC	Outer hair cells
PKARIIβ	Type IIβ regulatory subunit of the protein kinase (PKARIIβ)
POm	Posteriomedial complex
PrV	Principal trigeminal nucleus
PSPB	Pallial-subpallial boundary
PV	Parvalbumin
RGCs	Retinal ganglion cells
RT	Reticular nucleus
S1	Primary somatosensory cortex
S2	Secondary somatosensory cortex
SC	Superior colliculus

Th-R	Rostral domain of the thalamic complex
SGNs	Spiral ganglion neurons
Shh	Sonic hedgehog
SOC	Superior olivary complex
sp	Star pyramidal
SPAs	Synchronous plateau assemblies
SpVi	Caudal region of the interpolar spinal trigeminal nucleus
SpVo	Rostral pole of the interpolar spinal trigeminal nucleus
Ss	Spiny stellate
SST	Somatostatin
SuPN	Subplate neurons
TC	Thalamocortical
TF	Transcription factor
TG	Trigeminal ganglion
Th-C	Caudal domain of the thalamic complex
TTX	Tetrodotoxin
V1	Primary visual cortex
VL	Ventrolateral
vLGN	Ventrolateral geniculate nucleus
VPM	Ventral posteromedial nucleus
VPmd	Dorso-medial portion of the VPM thalamic nucleus
VPMl	Ventro-lateral portion of the VPM thalamic nucleus
vPrV	Ventral portion of the PrV
VZ	Ventriculkar zone
WNT	Wint/wingless family
ZI	Zona incerta



GCaMP6 expression (physics loop - imageJ) in the thalamus after tamoxifen administration at E10.5 in the Th^{GCaMP6} mice. Notice the activation of a single barrel. View in a coronal plane.



Abstract & Resumen

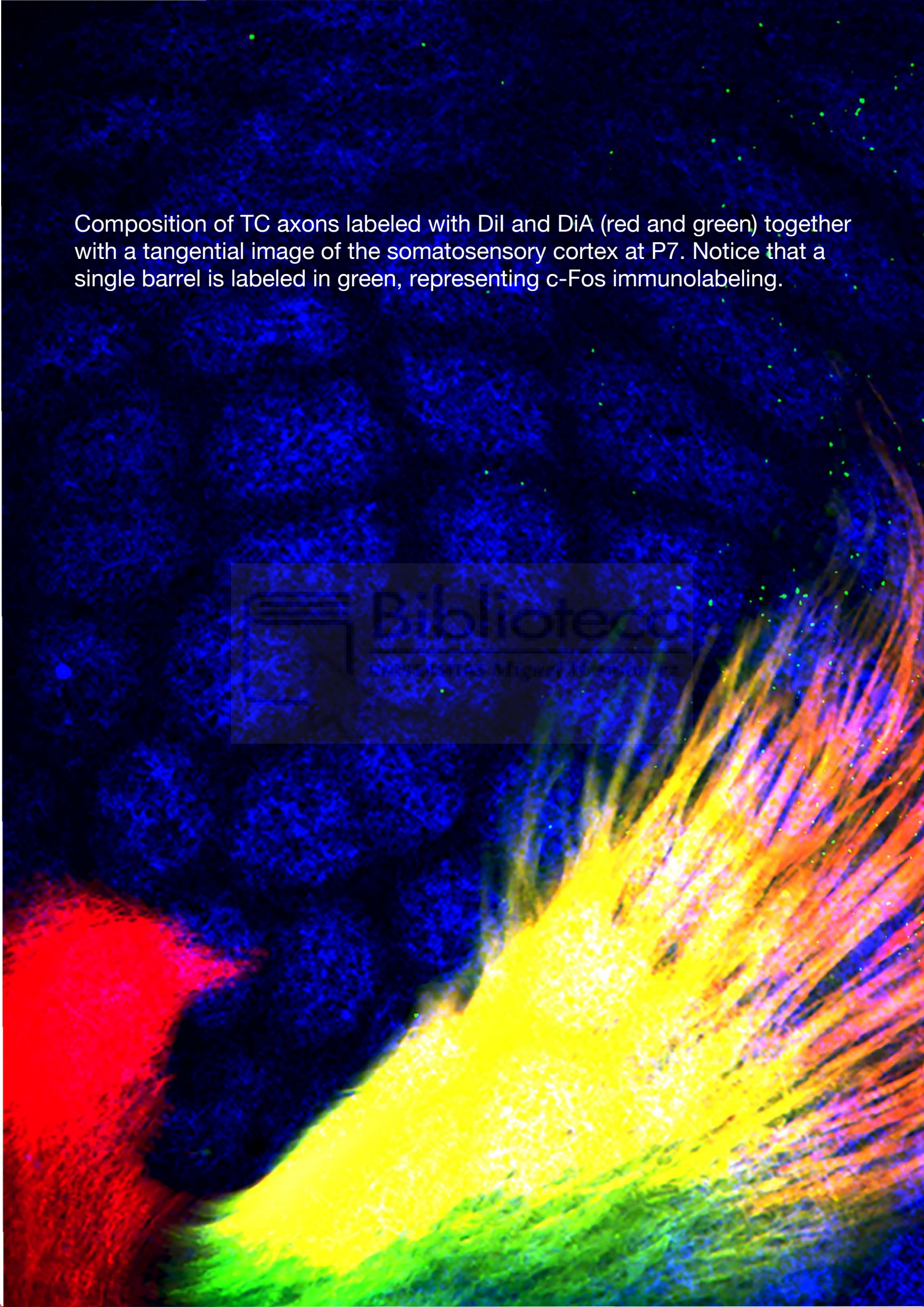
Abstract

Sensory systems are represented in the primary sensory areas of the cerebral cortex by anatomical and functional maps, as the whisker pad representation in the barrel field of S1 in rodents. The compartmentalization of the cerebral cortex into specialized sensory-modality areas is primarily defined by intrinsic molecular determinants. Many intrinsic and extrinsic factors have been proposed to shape sensory maps during early development. Neuronal activity from the sensory periphery is assumed to drive topographic organization of sensory cortex, but thalamocortical columns, a key organizational feature of the cortex, emerge even without peripheral sensory input. Thalamocortical axons (TCA) form a precise topographical projection that conveys the majority of sensory and motor information to the cerebral cortex. This connectivity is formed prenatally and is becoming increasingly clearer that TCA influence several aspects of cortical development, such as cortical areal specification or sensory maps tuning. Previously, we reported that embryonic thalamic activity regulates the size of sensory cortical areas in mice. Now, this thesis demonstrates that thalamic waves are not only essential in the context of plasticity after sensory input loss but we provide the first causal link between intrinsic thalamic activity in the embryo and cortical map formation. In this work, we identify that the fundamental columnar organization of the thalamocortical somatotopic map already exists in the mouse embryo. Blocking thalamic calcium waves results in hyperexcitability of cortical circuits, columnar organization fails, barrels never emerge, and the somatosensory map loses its point-to-point spatial and functional organization. Our results reveal that a self-organized, intrinsic-protomap in the embryonic thalamus drives functional assembly of thalamocortical sensory circuits.

Resumen

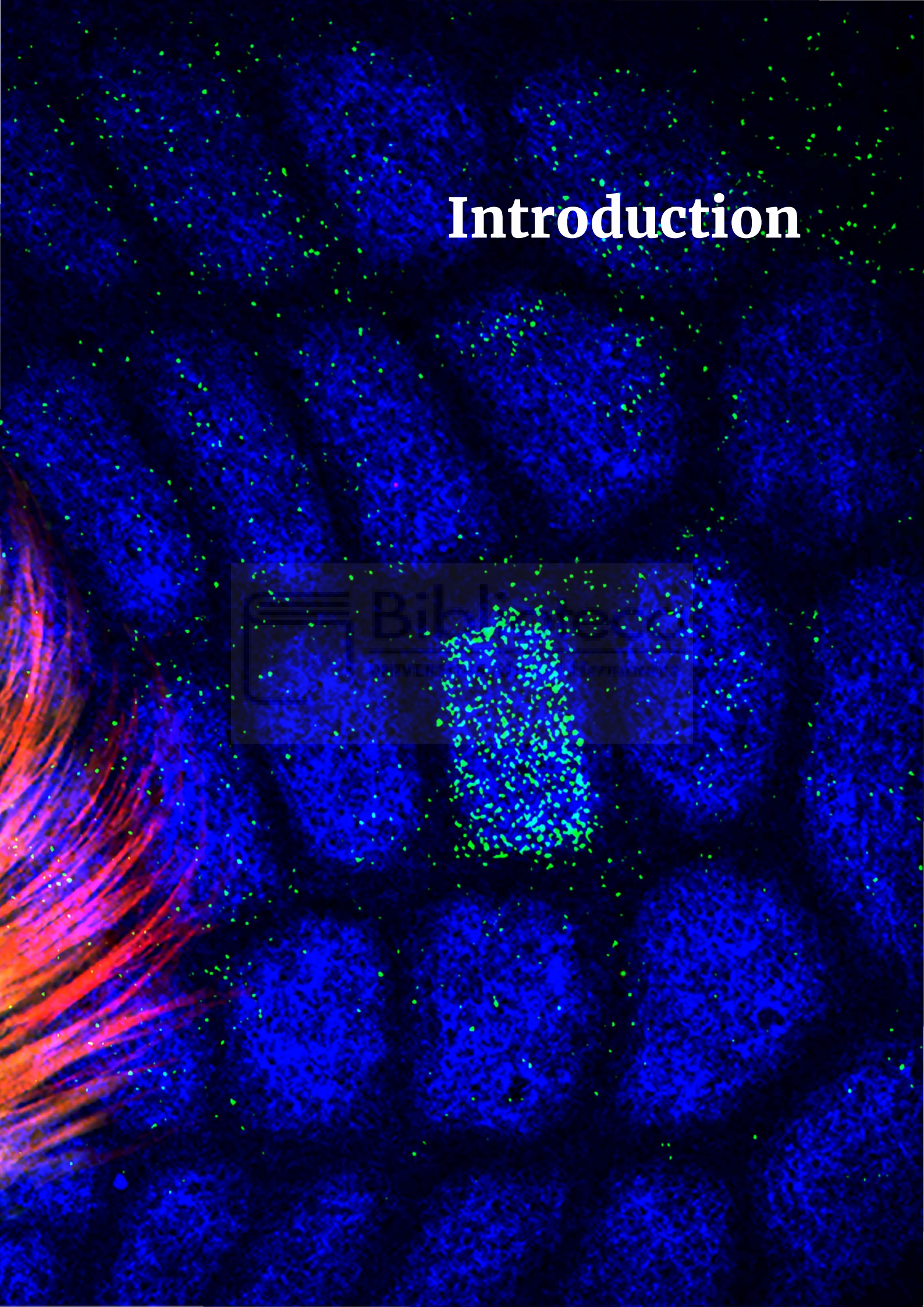
Los sistemas sensoriales se encuentran representados en las cortezas sensoriales primarias del cerebro en forma de mapas anatómicos y funcionales. Tanto factores intrínsecos como extrínsecos han sido propuestos como principales moduladores del desarrollo de las áreas y mapas sensoriales. La actividad neuronal que proviene de los órganos periféricos se considera la principal inductora de la organización topográfica existente en las cortezas sensoriales; sin embargo, las columnas talamocorticales, emergen sin necesidad de input sensorial periférico. Los axones talamocorticales forman una proyección topográfica precisa que transmite la mayor parte de la información sensorial y motora que recibe la corteza. Este circuito se forma prenatalmente y por ello podría estar influyendo diversos aspectos del desarrollo de la corteza cerebral, entre otros la especificación de las áreas corticales y la generación de los mapas sensoriales antes del nacimiento. Previamente, hemos mostrado como la actividad del tálamo prenatal modula el tamaño de las áreas sensoriales de la corteza.

En este trabajo mostramos que el patrón prenatal de la actividad talámica es esencial no sólo como mecanismo homeostático para regular el tamaño de las diferentes cortezas sensoriales, sino también juega una función importante en la formación de los mapas sensoriales. Concretamente, hemos determinado que la organización columnar del mapa somatosensorial existe prenatalmente. Mostramos por primera vez una conexión causal entre la actividad talámica intrínseca en el embrión y la formación del mapa somatosensorial. Cuando modificamos la actividad embrionaria talámica de sincrónica a asincrónica, los circuitos corticales se vuelven hiperexcitables, la organización columnar no se genera, los barriles no se forman y el mapa somatosensorial pierde su organización punto a punto, tanto a nivel anatómico como funcional. Nuestros resultados revelan que el protomapa intrínseco del tálamo controla el ensamblaje anatómico y funcional del circuito talamocortical.



Composition of TC axons labeled with DiI and DiA (red and green) together with a tangential image of the somatosensory cortex at P7. Notice that a single barrel is labeled in green, representing c-Fos immunolabeling.

Introduction



3.1. The thalamus

The thalamus is a complex central brain structure representing a major sensory relay station of the brain. Except olfaction, all sensory inputs from the periphery convey within this remarkable hub and transmit the information further to the cortex (Figure.1). The relationship between these two structures is complex and intimate. A topographically organized reciprocal connectivity (thalamo-cortico-thalamic connections) is believed to be involved in consciousness, which is why the thalamus is a major regulator of sleep and wakefulness. Therefore, the thalamus clearly represents the heart of the pathways that allow us to perceive the world and consequently choose our actions consciously. It is exactly why in a more philosophical meaning the thalamus gained the pseudonym of the "gateway of consciousness" (Crick & Koch 2003).

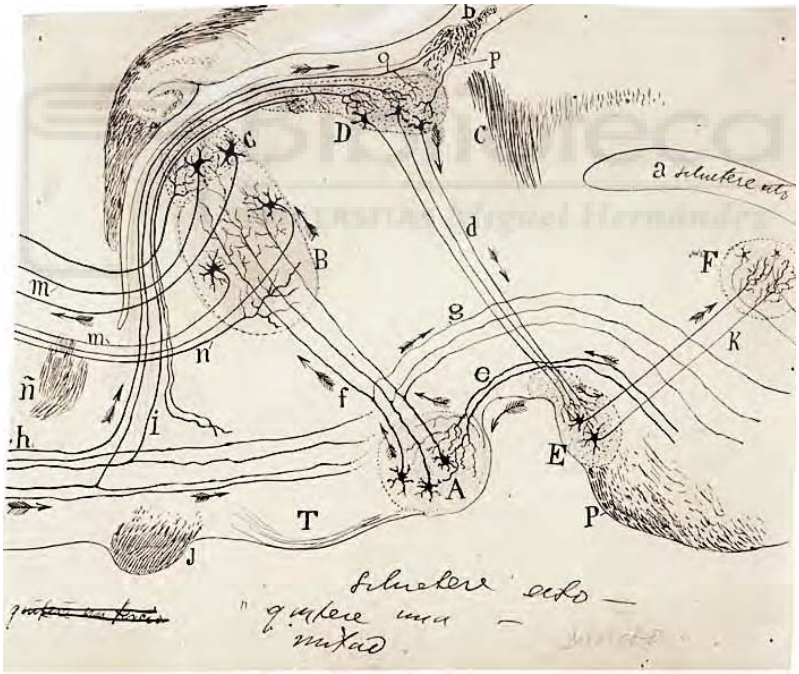


Figure 1. Cajal's drawing of sensory connections, thalamus as a central relay station.

3.1.2. General principles of thalamic development

According to the prosomeric model (Rubenstein et al. 1994; Puelles & Rubenstein 2003), the thalamus develops from the proliferative neuroepithelium of the lateral walls of caudal diencephalon, from the alar domain of the prosomere 2. The thalamic development can be divided into two phases. During the initial proliferative

phase restricted cohorts of polyclonal cell linages differentiate the diencephalon into prosomeres (P1, P2 and P3), mainly established by an acquirement of gene expression patterns. The second phase constitutes the foundations of the future nuclei (E. G. Jones 1985b). As the development advances, the thalamic complex can be subdivided in two domains based in their molecular signature and spatial location: rostral and caudal (Th-R and Th-C). Each domain generates a completely different subpopulation of thalamic neurons. Th-R-derived neurons acquire GABAergic fate (Jeong et al. 2011). They serve as a basis for the intergeniculate leaflet (IGL) territory, also known as the Rim (Kataoka & Shimogori 2008). This is the only thalamic region that is entirely GABAergic, although some interneurons travel from outside thalamic regions to populate the dorso-lateral geniculate nucleus (dLGN) (Jager et al. 2016; Golding et al. 2014). On the other hand, Th-C-derived neurons acquire a glutamatergic fate, giving rise later on to the excitatory projecting neurons (Suzuki-Hirano et al. 2011). The onset of thalamic differentiation is brief, within four days each region develops into a defined pool of thalamic neurons that later migrate and form the final mature nuclei. Based on the classic autoradiography studies performed by Jay B. Angevine in the 60-70's, three consecutive neurogenic gradients define this process (ventro-rostral, caudo-rostral and latero-medial).

The heterogeneous nature of the thalamus is reflected by the diversity of its nuclei, which are arranged by neurons that share similar features. Thus, the thalamic complex is formed by more than 40 different compartments, based on anatomical landmarks delimited by histochemical methods. Thereby, thalamic nuclei are named based on their relative position inside the thalamic complex (E. G. Jones 1985a). Functionally, thalamic nuclei can be classified in sensory, motor and associative. However, based on the origin of their driving inputs they can be also categorized into first-order (FO) and high-order (HO) (Sherman & Guillery 1998). The FO nuclei are defined as those that receive their driving input from peripheral sensory stations, such as the retinal input in the case of the dLGN (visual). On the other hand, HO nuclei receive information principally from the cortex, commonly from layer 5 (L5) neurons, as the amount of ascending subcortical input that they receive is small (Rovó et al. 2012). It has been suggested that thalamic sensory nuclei display a

drive/modulator framework. In this way, FO nuclei act as the driving element, due to their recipient role, and collect their major input from the periphery. Contrarily, HO nuclei work as modulators, as they receive information mainly from the cortex (Sherman & Guillery 2002; Bickford 2015). However, it has been proposed that HO nuclei might be important in transferring information from one cortical area no another as well. Although evidence is lacking *in vivo*, the existence of cortico-thalamic-cortical transmission has been proven in vitro (Theyel et al. 2010). Based on the nature of the sensory driving input that FO and HO relay, sensory thalamic nuclei can be modality-specific categorized in motor, visual, somatosensory and auditory. FO-HO nuclei assembly are formed by ventral posteromedial-Posteromedial nuclei (VPM-PoM), dLGN-Lateral Posterior nuclei (LP) and Ventromedial geniculate body-dorsomedial geniculate body nuceli (MGv-MGd) for somatosensory, visual and auditory inputs respectively. Despite the large diversity between thalamic nuclei in terms of connectivity, molecular determinants, histology and electrophysiological properties among other features are still very well organized and clustered together in the same embryonic landmark, the prosomere 2. Thalamic heterogeneity is based on the specification and segregation of thalamic neuronal precursors at early embryonic stages once the developmental unit is noticeable, as the time of origin is shared between different thalamic areas (Angevine 1970) and thus gives rise to specific thalamic subpopulations later on.

Molecular signature of the developing thalamus. During the last 10 years, much effort has been generated into the molecular characterization of the thalamus and the development of its nuclei. Deciphering the molecular fingerprint of distinct thalamic nuclei appears to be extremely difficult due to the impressive complexity of the thalamus. The fact that at the early developmental stages specific nuclei are hard to define, makes the task even more challenging. Therefore, to fully determine the molecular signature of the thalamus, it is imperative to first understand the source of the genetic heterogeneity of the thalamic primordium.

development (Scholpp & Lumsden 2010). The DMO strongly expresses signalling The ZLI, also known as the mid-diencephalic organizer (MDO) is the local organizer that orchestrates the gene expression patterns during the 9 neural thalamic molecules of the Wnt, Sonic hedgehog (Shh) and fibroblast growth factors (FGF) pathways (Kataoka & Shimogori 2008) (Martinez-Ferre & Martinez 2009). These long-range signalling molecules act as a morphogens and thereby influence cell behaviour by regulating the transcriptional code based on their concentration (Martinez-Ferre & Martinez 2012). Thus, the differential exposure to morphogens will segregate and lead the thalamic neuronal precursors towards specific developmental fates. For instance, Shh signalling has been described as the principal extracellular signalling source that coordinates thalamic development in different species (Blackshaw et al. 2010) (Hagemann & Scholpp 2012a) (Martinez-Ferre & Martinez 2012) (Quinlan et al. 2009; Peukert et al. 2011). High levels of Shh induce the typical pretectum fate, while low levels drive the thalamic genetic signature expressing Gbx2, Dbx1, Olig3 and Lhx2, among others (Scholpp & Lumsden 2010; Hagemann & Scholpp 2012b; Zhang & Alvarez-Bolado 2016). Very recently, an ontogenetic study showed that thalamic progenitors divide generating cohorts of clones fated to specific regions depending on their position, describing for the very first time an early segregation between FO and HO nuclei across distinct modalities. Most probably the precise localization of thalamic progenitors combined with the gradient exposure to signalling molecules regulates linage-fate specification (Shi et al. 2017).

Once thalamic cells become postmitotic and exit the cell cycle they begin to express *Gbx2*, an essential TFs implicated in the segregation of thalamic neurons, and consequently involved in the compartmentalization of the thalamus into distinct nuclei. *Gbx2* is induced very early (E9.5) and exhibits a very dynamic expression, both spatially and temporarily. Hence, different thalamic nuclei will express *Gbx2* at different developmental time-points (Figure 2) and consequently thalamic nuclei can be categorized depending on its expression pattern (Nakagawa & O'Leary 2001; Vue et al. 2007; L. Chen et al. 2009; K. Li et al. 2012; Mallika, Guo & Li 2015a). Several studies, mainly from the group of

James Y. H. Li, have shown that Gbx2 is an imperative determinant for the thalamic acquisition profile. This means that when Gbx2 is absent, the thalamus starts to manifest molecular determinants that define other structures, for instance the habenula (Mallika, Guo & Li 2015b). Lacking *Gbx2* also prevents the acquirement of thalamic activity. While *Gbx2* is missing, thalamocortical axons (TCAs) are misrouted towards the mid/hindbrain instead of following their normal path towards the cortex (Chatterjee et al. 2012). It is also important for the formation of the thalamus itself, as the lack of *Gbx2* affects cell proliferation and produces an abnormal contribution of thalamic neurons to the habenula, resulting in an aberrantly formed thalamus. *Gbx2* also controls thalamic patterning acting over the formation of the early boundaries, specifically the dorsal boundary between the epithalamus and the posterior boundary with the pretectum by influence of cell-adhesion molecules, such as *Cdh6* or *Epha5* (L. Chen et al. 2009).

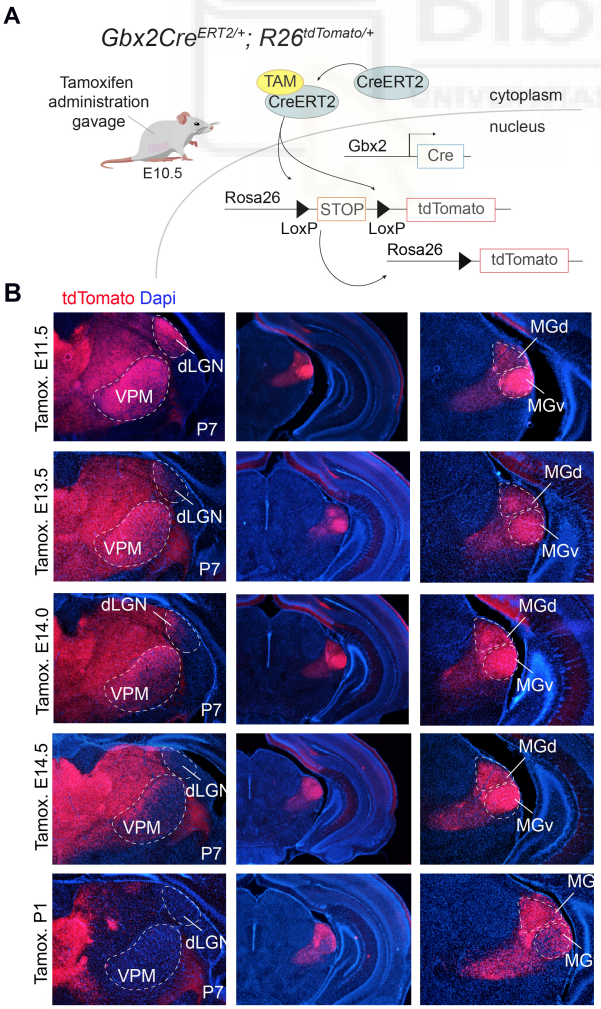


Figure 2. Thalamic Gbx2 expression along development. **A)** Scheme representation of the Cre/flox system used to decipher the temporal expression of *Gbx2* along development. **B)** Coronal view of the thalamic complex at P7. Tamoxifen administration at different time points show the differential expression of *Gbx2* in distinct thalamic nuclei. TdTomato (red) was used as a reporter. VPM, ventro-posterio medial nucleus; dLGN, dorso-latero medial nucleus; MGv ventro-medial geniculate nucleus; MGd dorso-medial geniculate nucleus.

There are other postmitotic molecular determinants of the thalamic complex. One of the most important is the LIM-homeodomain family, which involvement in diencephalic segmentation has been extensively studied. The group of Nakagawa has put much effort in understanding its role in diencephalic segregation. Isl1, Lhx1, Lhx5 are expressed in the prethalamus and Lhx2 and Lhx9 are specific for the thalamic territory. Lhx2 and Lhx9 themselves are differentially expressed in the thalamus, being important for specification and differentiation of thalamic nuclei and cell-types as well (Nakagawa & O'Leary 2001). There is an increasing interest in understanding the transcription factors that specify different thalamic regions and could be used to drive specific genetic manipulations (Price et al. 2012). Recently, our laboratory described novel nuclei-specific genes likely involved in the specification and connectivity of distinct sensory-modality thalamic populations (Gezelius et al. 2017). These novel compartmentalized genes might be implicated in thalamocortical (TC) topographical targeting and organization, hypothesis that needs to be tested. Nowadays, the increase of high-throughput single-cell technology might give the opportunity to understand "on a single cell level" the molecular determinants that characterize each developmental step of the thalamic complex. Importantly, this information would be useful for the generation of novel Cre transgenic mice, essential for opening new avenues for specific-nuclei targeting and manipulations.

3.2. The cortex

3.2.1. General principles of cortical development

The mammalian neocortex is a highly complex but very well-organized structure. It is composed of distinct cortical areas that are responsible for cognitive, sensory and consciousness functions. The neocortex presents a horizontal organization of 6 layers (cortical plate), which accommodates an extensive diversity of neurons and radial glial cells. It also displays a vertical organization arranged in cortical mini-columns (ontogenetic radial columns), representing a chain of neurons that are synaptically and functionally linked across layers. These mini-columns bond together by short-range horizontal connections,

generating cortical columns or modules that share physiological and dynamical properties (Mountcastle 1997). Based on the radial unit hypothesis postulated by Pasko Rakic in the 80's, the foundation of this columnar organization resides in their migration along the radial glial process, which serves as a scaffolding for clonally related neurons (Rakic 1988; Rakic et al. 2009).

In the neocortex we can find two broad classes of neurons: GABAergic interneurons that establish local interactions; and glutamatergic projecting neurons, establishing local and long range connections within intracortical, subcortical and subcerebral targets (Petreanu et al. 2009). The laminar arrangement of the cortical plate follows an inside-out developmental sequence. Excitatory neurons in mice are born consecutively from E10.5 to E18.5, starting from the ones located in the deepest cortical layers (Molyneaux et al. 2007; Jabaudon 2017). A functional consequence of having pools of neurons with distinct maturation stages is that they are differentially influenced by environmental signals, with the superficial (less mature) being the most susceptible to plastic changes (Fox & Wong 2005). Regarding the progenitor specification, a very recent study used single-cell RNA sequencing to determine the existence of early transcriptional waves that offer a dynamic control over neuronal differentiation events. This work has identified specific sets of proliferative, neurogenic and neuronal transcripts, which sequentially instruct and guide newborn neurons to their final fate (Telley et al. 2016). Moreover, cortical neuronal fate specification is also regulated by the bioelectrical membrane status. During corticogenesis, VZ progenitors become more hyperpolarized as sequentially distinct subtypes of neurons are being generated (Vitali et al. 2018). Very interestingly, the Jabaudon's laboratory has recently found that the membrane potential hyperpolarization shifts the progenitor developmental program. Consequently, a premature generation of intermediate progenitors occurs with a switch in the laminar, molecular and morphological features of their neuronal progeny (Vitali et al. 2018). This clearly demonstrates the important role of bioelectrical features in the progenitor state and consequently the transversal organization of the neocortex.

How and when the postmitotic newborn neurons reach their appropriate areal localization led to the proposal of two antagonistic hypotheses. The protocortex theory focuses on how the environment might refine and shape the final synaptic organization (O'Leary 1989). It postulates that the embryonic cortical plate is formed by equipotent cells, that are specified by external, subcortical inputs (mainly thalamic). In contrast, the protomap hypothesis postulates that the laminar areal specification of cortical neurons is pre-specified at the time of their last cell division. This implies that cortical progenitors are born with an intrinsic genetic program, which is independent of external cues (Rakic 1988). Over years, both theories have been developed as independent and contradictory models and supported by different studies. Therefore, it is now widely accepted that during neocortical areal differentiation there is an interplay between intrinsic genetic mechanisms, based on morphogens and early transcriptomic acquirement, and extrinsic mechanisms, governed by sensory input and TC afferents. Intrinsic developmental regulation produces an early rough protomap, whose specification continues in parallel, spatially and temporally, with the development of the TC input. Thus, cortical areas are malleable and exhibit a comprehensive plasticity upon sensory periphery and TC manipulations. The idea of the extrinsic influence originates from classic transplantation studies, in which distinct cortical areas were grafted into others, developing cytoarchitectonic features that characterize the new location (Schlaggar & O'Leary 1991). These grafts were exposed to new TC afferents, that are sensory-modality sorted before reaching the cortical plate, giving an important impulse to TC input as a pivotal extrinsic factor for areal specification. Nowadays, both views are integrated. First, the basic protomap is generated early, however still very plastic and malleable, being susceptible to interactions with prenatal extrinsic inputs, principally thalamic. Recently, Tom Nowakowski and Alex Pollen, using the most recent technology of single cell transcriptomic analysis, have shown that progenitor cells from the prefrontal and visual cortex of the fetal human brain manifest significant gene expression differences that become greater in maturing neurons, showing the importance of the topographical distinctions probably due to extrinsic mechanisms (Nowakowski et al. 2017). Thus, cortical circuits emerge from

dynamic interactions between intrinsic (genetically encoded) and extrinsic (input dependent) mechanisms, which together sculpt mature cortical circuits.

3.3. Thalamocortical system

3.3.1. Thalamocortical projections

Development. The tight reciprocal connectivity that exists between the thalamus and the cortex has been the focus of many studies in the past decades. Not only because it constitutes one of the most eminent connections of the mammalian brain, it also carries sensory and motor information from peripheral centres to the cortex in order to be integrated. The development of the thalamocortical connectivity is a process that starts at the beginning of the second gestational week in mouse (E13.5). It is tightly regulated by different factors, such as gene expression patterns, axon guidance molecules and spontaneous calcium neuronal activity (Molnár et al. 2012; Marcos-Mondéjar et al. 2012; Mire et al. 2012; Leyva-Díaz et al. 2014; Castillo-Paterna et al. 2015; Garel & López-Bendito 2014; Antón-Bolaños et al. 2018; López-Bendito 2018). TCAs start their journey towards their cortical targets, which consist of a very well topographically organized process. In their journey, TCAs need to overcome several boundaries and follow a strictly stereotyped route. First, TCAs are required to cross the boundary that exists between the telencephalon and the diencephalon (Diencefalic-telencefalic boundary, DTB) guided by prethalamic and telencephalic axons and heading towards the internal capsule (IC). At this level, TCAs are already topographically sorted based on morphogens and axon guidance molecules (Molnár et al. 2012). Then, they enter into the subpallium, and slow down at the pallial-subpallial boundary (PSPB). At this point, TCAs wait to meet the pioneer corticofugal axons from SuPNs around E14.0 (McConnell et al. 1989; De Carlos and O'Leary 1992). This intimate encounter between TCAs and corticothalamic axons (CTAs) has been proposed as the "handshake hypothesis" (Blakemore & Molnár 1990), that after several studies showed that TCAs use CTAs as a scaffold. However, nowadays it still remains challenging to test it experimentally. Afterwards,

TCAs head dorsally and finally arrive at the neocortex entering through the intermediate zone at E15.5. At this point, TCAs experience another waiting period in the subplate (SuP). During this waiting period, TCAs established a temporary connection with SuPNs, constituting a key mechanism for early circuit formation. This lasts up till E17.5, when the TCAs start to invade the cortical plate, forming branches (Allendoerfer & Shatz 1994; Del Río et al. 2000; Piñon et al. 2009; Kanold & Luhmann 2010; Viswanathan et al. 2012; Hoerder-Suabedissen & Molnár 2015; Viswanathan et al. 2017).

Remarkably, TCAs start to innervate the cortical primordium at the time when corticogenesis is still ongoing and cortical layers, especially granular layers, are not fully formed yet (Molyneaux et al. 2007). Thus, TCAs are in a very good position (and time) to influence cortical development as an extrinsic subcortical element. The potentially TCA-dependent features could include the proliferation rate of the germinal zone progenitors and, as a consequence, the laminar cytoarchitectural characteristics. From the onset of birth, TCAs are directed towards their principal recipient layer, which is the layer 4 (L4), though they also send collateral connections to L5b. At this particular time, during the first postnatal week, the TCAs refine and form specialized structures, like the barrels in the primary somatosensory cortex (S1) in rodents (López-Bendito & Molnár 2003). Of note, TC development does not only depend on morphogens, TFs expression and axon guidance molecules, but neuronal activity also plays a crucial role during embryonic development (López-Bendito 2018). Nowadays, one of the caveats of the system is to understand the involvement of spontaneous activity during embryonic TC connectivity, and consequently cortical development.

Connectivity. Thalamic FO nuclei receive primary sensory information from peripheral centres and connect with L6, L5b and L4, with the spiny-stellate neurons being their principal recipient cells (Alonso & Swadlow 2017). At the same time, L5a from the primary sensory cortex connects with HO thalamic nuclei, establishing a feedback loop (thalamus-cortex-thalamus). Note that both FO and

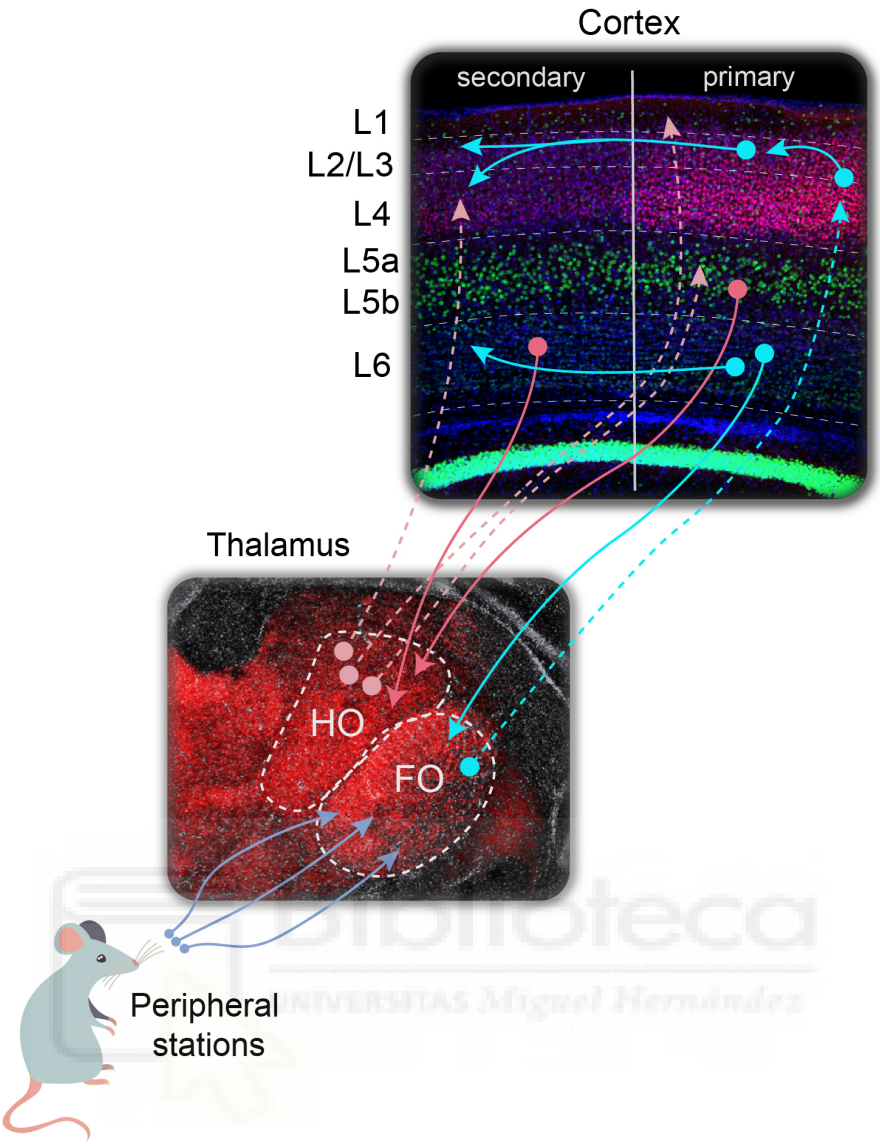


Figure 3. Thalamocortical connectivity. Ascending information from peripheral stations is received by first order (FO) thalamic nuclei. FO thalamic nuclei connect with L4 neurons that connect with L2/L3 neurons. L2/L3 neurons send cortico-cortical projections to secondary areas, specifically to L2/L3 and L4 neurons, closing the thalamo-cortico-thalamic loop as high order (HO) nuclei connect with L4 in secondary areas. HO nuclei receive input mainly from cortical territories, specifically from L5 primary neurons and L6 from secondary cortical areas. HO nuclei also have proyections to L5 and L1 in primary cortical areas.

HO nuclei receive feedback modulatory input from L6 (corticothalamic), but only HO receives feedforward input from L5a (Viaene et al. 2011) (Figure 3). Thereby, peripheral information (driving input) is transmitted from one cortical area to the thalamic recipient cells (L4) of another area through the thalamus (Sherman & Guillery 2002; Reichova & Sherman 2004; Theyel et al. 2010; C. C. Lee & Sherman 2010). This feed-forward connectivity to the thalamus has a principal modulatory role that provides powerful control over thalamocortical transmission. This existent transthalamic cortico-cortical pathway facilitates the cooperation of

cortical areas to carry out various cognitive functions (Seidemann et al. 1998; Fries 2009; Sherman 2016). There is evidence based on optogenetics and calcium imaging, that cortical L5 cells induce waves of activity in other cortical areas (Stroh et al. 203) a process which largely depends on the transthalamic pathway. Following this, we can argue that the thalamus not only relays information, but also provides cortico-cortical communication and contributes to information processing (Sherman 2016). Cortico-cortical communications occur at three different levels: (1) intra-cortical columnar communication between layers (C. C. Lee & Sherman 2009; Thomson 2010; C. C. Lee et al. 2012); (2) inter-cortical horizontal communication between distinct areas by L2/3 and L6 cells; and (3) the additional transthalamic pathways involved in transfer information between cortical areas (Sherman 2016) (described above). Thus, even though cortical areas have a direct connection, they also have a parallel pathway through the thalamus (Sherman 2016). The model of thalamo-cortico-thalamic communication in sensory systems has been simplified in Figure 3.

3.3.2. Corticothalamic projections

All sensory cortical areas receive thalamic input and project reciprocally to distinct thalamic nuclei (Caviness & Frost 1980). CT projections comprise 50% of the synaptic input that thalamic sensory neurons receive. They provide a modulatory input to sensory thalamic neurons, sharpening and shaping thalamic relay information for cortical processing (Sherman & Guillery 1998; Briggs & Usrey 2008; S. R. Olsen et al. 2012). CT projection specificity depends on the laminar identity of the cortical neuron. Hence, three different populations can be distinguished, each within distinct temporal sequence and thalamic targets: SuPNs, L6 and L5 neurons. Curiously, classical studies have assigned the SuPNs as the pioneer CTAs, providing a structural guidance to L5 and L6 corticofugal axons (CFAs). Indeed, their important role in the correct CT axonal pathfinding has been suggested extensively (McConnell et al. 1989; Kim et al. 1991). L5 and L6 CTAs are generated by corticofugal pyramidal neurons that project to FO and HO thalamic nuclei, respectively (Auladell et al. 2000; Price et al. 2006; Molyneaux et al. 2007). They constitute the earliest postmitotic neurons to radially invade

the cortical plate (around E10.5). As they are born, even before they settle in their final localization in the cortical plate, they start to extend the neurites towards their subcortical targets. This establishes a temporal correlation between their navigation timing and their time of birth (Grant et al. 2012; Hoerder-Suabedissen & Molnár 2015).

Along their journey, CT projections expand through the intermediate zone (IZ), pass over the deep cortex and continue towards the IC, at E13.5 experimenting their first waiting period (Jacobs et al. 2007). At E15.5, CTAs cross the PSPB and enter the IC, which also comprises output of subcerebral axons in route towards the cerebral peduncle and pyramidal tract. In the IC the CTs navigate through the corridor cells, which contribute to guide and sort the CT axons (López-Bendito et al. 2006). After crossing the DTB they encounter the prethalamus with the RT and PRN nuclei, experimenting their second waiting period from E16.5 to E17.5 (Garel & Rubenstein 2004; Simpson et al. 2009; Y. Chen et al. 2012; Molnár et al. 2012; Deck et al. 2013; Lokmane et al. 2013; Lokmane & Garel 2014). At this location CFAs are sorted, the vast majority of L5 projections are directed to the cerebral peduncle (Cp) and the remaining L5 and L6 follow their path to their correspondent thalamic targets (Clascá et al. 1995; Molnár & Cordery 1999; Jacobs et al. 2007).

The entrance of CTAs into their thalamic recipient nucleus is a postnatal process that can take several days. This process seems to be correlated with the functional establishment of high sensory processing and performance. Thereby, VPM and ventrolateral (VL, FO sensory and motor thalamic nuclei, respectively) present an early innervation, between E18.5 and P0. On the contrary, MGv and dLGN (FO auditory and visual thalamic nuclei, respectively) are not fully innervated until P8 (Jacobs et al. 2007; Grant et al. 2012). The mechanisms behind the invasion of CT into the thalamic nuclei are still relatively poorly understood. Quite recently, it has been shown that that lack of retinal input provokes a premature entrance of L6 neurons into the dLGN. Moreover, a cross-hierarchical rewiring was observed, with L5 neurons that in normal conditions invade the LP (HO visual nucleus) now aberrantly invaded the

dLGN (FO visual nucleus) (Grant et al. 2016). Actually, it has been observed that the transcriptional identity and the connectivity networks of the FO nuclei is peripheral input-dependent. Input ablation not only drives the induction of HO transcriptional programs into FO nuclei also cause a rewiring of the HO CT input towards FO thalamic nuclei. Thus, changing transcriptional programs and network connectivity from FO to HO by peripheral input ablation, being this fundamental program conserved across sensory modalities (Frangeul et al. 2016). Therefore, neuronal activity, more specifically peripheral sensory activity, might be involved in the development of correct cortico-thalamic connectivity.

3.3.3. Thalamic and cortical interactions during development

Subplate, first interactions. The first interaction between thalamic afferents and cortical neurons occur at the second waiting period of the TCAs journey. A temporary connection is established between TCAs and SuPNs at the time that L4 neurons are being born, corresponding to the first cortical territory that receives synaptic thalamic input (Friauf et al. 1990) . This early synaptic encounter is considered a key developmental process for cortical circuit formation (Luhmann et al. 2018). SuPNs are a transient population settled in the prospective white matter location. They are characterized as a very heterogeneous group that exhibit distinct molecular features, functions, morphology, neurotransmitter identity and even ontogenetic origins (Kanold & Luhmann 2010). First it was thought that SuNPs constitute a passive relay for the entrance of thalamic input to the cortex, however they present a bulk of properties that make them crucial for the correct acquirement of early cortical activity by monitoring, implying and transmitting activity patterns. SuPNs possess a relatively mature state and are already well integrated at very early developmental stages, being electrically coupled by gap-junctions (Dupont et al. 2005). They receive substantial glutamatergic inputs from the thalamus, cortical plate and intra-subplate, including GABAergic interactions (Luhmann et al. 2009). SuPNs send direct synaptic inputs into the developing cortex, branching over all cortical layers tangentially and radially, with extensive arborisations to L4 neurons (the major thalamic recipient layer) (Piñon et al. 2009).

Moreover, they present long-distance axonal projections to the contralateral hemisphere (Del Río et al. 2000) and for a transient developmental period they O'Leary 1992; Viswanathan et al. 2017). Therefore, SuPNs present a wide involvement in cortical functional and anatomical development.

As SuPNs are directly connected with thalamic fibres, it has been shown that this connectivity is imperative for the correct maturation of thalamic synapses and lack of SuPNs induces a low synaptic strength (Kanold et al. 2003).Their involvement in neocortical architecture and maps has also been indicated. Early studies already indicate the role of subplate neurons (SuPNs) in guiding TCAs to their correct target (Ghosh et al. 1990), probably by expressing cues involved in cortical patterning. For instance, an incorrect expression of FGF8 in the SuPNs disrupts the correct TCA targeting (Shimogori 2005), suggesting that SuPNs might contain positional information. However, it remains unclear whether or not the attribute that instructs the putative cortical organization, is rather acquired by the direct contact with TCAs, that are already segregated in a sensory- modality fashion at the time of the "handshake" (Tolner et al. 2012). Their implication in sculpting the neocortex has been also suggested to be due to their possible involvement in the arrangement of radial microcolumns (Mountcastle 1997), as they are electrically coupled by gap junctions with cortical plate cells (Dupont et al. 2005). The role of SNPs in early network activity will be further considered in the Discussion section.

Cortical influence on thalamic development. Thalamic and cortical developmental programs overlap in time and space, influencing each other during brain ontogeny, even though both display an autonomous developmental regulation. This tight relationship has been shown to be fundamental for the correct arrangements of thalamic and cortical territories (Antón-Bolaños et al. 2018). The correct establishment of distinct cortical territories is, a priori, controlled by intrinsic factors based on early genetic programs. However, these intrinsic factors also influence TC targeting to their proper recipient cortical area. For instance, ectopically expressed FGF8 produces duplications and areal shits of sensory cortical areas and thus provides an early positional information

of cortical guidance cues affecting the final pathfinding of TCAs (Shimogori 2005; Assimacopoulos et al. 2012). Similar studies on specific transcription factors for example Emx2, Pax6, Sp8 and COUP-TF1 show an eventual change in the final TC innervation (Hamasaki et al. 2004; Armentano et al. 2007; Manuel et al. 2007; Borello et al. 2014; O'Leary & Sahara 2008). In case of Pax6, a clear top-down plasticity effect can be observed. A miss-expression of Pax6 produces a reduced body map representation in S1, at the same time the equal a miniaturized representation can be observed in the VPM (Zembrzycki et al. 2013). Gene expression patterns are not the only intrinsic cortical mechanisms that affect TC development and connectivity, the cortical neural activity also conform a plausible influence. Early cortical activity patterns emerge during development and influence several developmental features such as layer formation, dendritic and axonal arborisation and navigation (Simi & Studer 2018). Regarding this issue, several studies have been done disrupting specifically cortical activity. NMDA receptor 1 (NMDAR1), adenylyl cyclase 1 (AC1) and metabotropic glutamate receptor 5 (mGlu5) cortical knock-outs (KOs) all show TCAs deficiencies with smaller and blurred barrels in S1. However, these animal models also present a prominent disruption on neuronal organization, lacking the barrel wall formation (Iwasato et al. 2000; Datwani et al. 2002; L.-J. Lee et al. 2005; Iwasato et al. 2008; Ballester-Rosado et al. 2010; Martini et al. 2018; Antón-Bolaños et al. 2018).

Thalamic influence on cortical development. Thalamic input modulates reciprocally cortical development. As mentioned before, TCAs are likely to influence the ontogenic radial arrangement of the neocortex. Past studies demonstrated the extension of thalamic impact in several aspects of cortical development, starting from cortical proliferation, CTA navigation and area specification to interneuron maturation and circuitry assembly. Regarding cortical proliferation, TC afferents have been reported to modulate cell-type specification of cortical neurons (Dehay et al. 1996; Rakic et al. 1991; Zechel et al. 2016). Additionally, thalamic input regulates interneuron identity

and function via activity-dependent mechanisms. Prevention of glutamate release by TCAs, affects the axonal and dendritic development of Reelin-expressing cortical neurons (De Marco García et al. 2015). However, not only Reelin-expressing cortical neurons are modulated by thalamic input, there are studies showing that the absence of TCAs influence also the parvalbumin (PV) and somatostatin (SST) interneurons (Wamsley & Fishell 2017). For instance, during the first postnatal week and before sensory onset, there is a transient innervation of SST cortical interneurons by TCAs. This early circuit seems to be important to modulate thalamic input to L4 pyramidal and PV neurons, clearly contributing to cortical excitatory-inhibitory circuitry assembly (De Marco García et al. 2015; Tuncdemir et al. 2016; Marques-Smith et al. 2016; Takesian et al. 2018; Che et al. 2018).

Pyramidal excitatory neurons are also regulated by the thalamic input, specifically the principal recipient L4 conformed by spiny-stellate neurons. The proper segregation and polarization of these neurons to form the barrel walls fail when thalamic presynaptic release is blocked (Assali et al. 2017; H. Li et al. 2013). Spiny-stellate neurons develop a non-typical apical process, classic feature of non-granular pyramidal neurons (H. Li et al. 2013).

The tight relationship between CTAs and TCAs became clearly evidenced by the Garel's lab. Her group developed a mouse model that lacks TCAs. In this model, the absence of TCAs induced an aberrant pathfinding of CTAs that acquired a cortico-spinal like trajectory, demonstrating that TCAs can modulate the correct topographical navigation of their counterparts (Deck et al. 2013). Moreover, TCAs have an additional role on the specification of primary versus higher-order cortical areas. The genetic restriction required to differentiate the primary areas from the high-order areas is imposed by thalamic input. When TCAs are absent, cortical areas remained in the default mode and engaged in a high-order fate (Chou, Babot, Leingärtner, Studer, Nakagawa & O'Leary 2013a). For instance, in the absence of FO thalamic nucleus the interrelated primary cortical area acquires molecular and functional properties of secondary associative areas (Vue et al. 2013;

Chou, Babot, Leingärtner, Studer, Nakagawa & O'Leary 2013b; Pouchelon et al. 2014). Recently, Jabaudon's group developed animal tools to deeper decipher the mechanism by which TCAs influence the identity and fate determination of cortical cells. The ablation of the VPM pushed presynaptic terminals from the high-order POm to target L4 neurons in S1 aberrantly and, even more surprisingly, they respond to noxious stimuli, a function that is normally carried out by S2 and L4 neurons (Pouchelon et al. 2014). All these observations strongly indicate a primary role of thalamic input over the correct cortical development. TC postsynaptic connectivity is not only crucial for the molecular cortical identity of L4 neurons, it also manifests a clear influence over the final cortical functionality, and consequently the cortical inhibitory and excitatory circuitry assembly.

Starting from very early developmental stages the thalamic and cortical systems are closely interconnected, not only anatomically, but also electrophysiologically. It has been well reported that early spontaneous activity is essential during ontogenic stages (see 3.5, section below). Briefly, neural activity can influence many different physiological processes, such as neurogenesis, neuronal identity acquisition, migration, axonal extension and refinement (Kirischuk et al. 2017; Luhmann & Khazipov 2018; Martini et al. 2018). During the first postnatal week, early gamma oscillations enforce the synchronization between the thalamic and the equivalent cortical territories (for instance, barreloid in VPM and barrel in S1) (Minlebaev et al. 2011), suggesting that early thalamic activity might also influence cortical development. Disruption of different aspects of TC activity, such as the glutamatergic communication and the presynaptic vesicular release, result in defects in the cortical barrel map formation (Narboux-Nême et al. 2012; H. Li et al. 2013; Arakawa, Akkentli, et al. 2014; Suzuki et al. 2015). We previously showed that prenatal thalamic spontaneous activity is crucial to maintain a homeostatic regulation between distinct sensory systems, when a sensorial insult (peripheral or in the CNS) occurs, thalamic inter-nuclear communication potentially orchestrates early events that follow cross-modal

cortical readjustments (Moreno-Juan et al. 2017). Therefore, it is becoming more and more clear that both thalamus and the cortex display a complex synergy supporting the correct development of functional sensory circuitries.

3.4. Development of sensory systems

3.4.1 General overview

Sensory information on its way to the cortex is primarily detected in peripheral stations and passed through multiple sensory centres, including the thalamus. These sensory stations will act as nodes, delivering the information to the next stage, always in a topologically organized manner. During development, neuronal activity acts to strengthen the neighbouring connectivity, thus a point-to-point representation is formed based on the fact that organized stimuli activate adjacent neurons (Bednar & Wilson 2016). Hence, a topographical input mapping is generated and pursues a point-to-point representation from the periphery to the cortical surface. This topographical mapping is a fundamental principle of sensory pathways and it is a common feature shared by all three basic sensory modalities: the retinotopic map in the visual system (Tusa et al. 1978), the somatotopic map in the somatosensory system (Woolsey 1978) and the tonotopic map in the auditory system (Merzenich et al. 1975). This organization is fundamentally shaped by axon guidance molecules, axonal competition and activity regulated mechanisms. Before jumping to the somatosensory system, here I present a reduced overview of the development of other sensory maps, which I personally consider interesting, as all sensory circuitries share common features, helping us to gather a broader picture. The role of neural activity in sensory map formation will be further addressed in the next section 3.5.

3.4.2 Visual map: Retinotopy

The visual system is in charge of receiving, processing, interpreting and building a representation of the visual environment. As the other sensory systems,

it embraces a hierarchically organized topography throughout its course. Thedevelopment of this topography involves precise intrinsic genetic programs and activity mechanisms that go from spontaneous to stimulus-dependent. The visual pathway starts with the retina, which is in charge of translating the light into nerve signals. It is composed by three main layers of cell bodies (retinal ganglion cell layer, inner nuclear layer and outer nuclear layer) intermingled with two synapse layers. The outer cell layer is built of photoreceptors, cones and rods, basically in charge of detecting the changes of light. The inner layer is constituted of bipolar, horizontal and amacrine cells. The horizontal and amacrine cells filter and shape the visual information that arrives to bipolar cells. The bipolar cells receive the filtered input from the photoreceptors pass it to the retinal ganglion cells (RGCs), forming the deepest cell layer (ganglionic layer). Thus, bipolar cells act as an integrator centre of visual information that is pre-processed before reaching the RGCs (Masland 2012). RGCs are very specialized cells, with more than thirty different subtypes that respond to specific stimuli, such as arrangement of light and dark or directions of motions. (Baden et al. 2016; El-Danaf & Huberman 2019) They are the ones in charge of the transmission of visual information to the central nervous system, as their axons assemble into a bundle to exit the eye and form the optic nerve (Erskine & Herrera 2014).

In mice, RGCs axons start their journey to the central brain structures at E12.5. The majority project to the contralateral side crossing the optic chiasm and 2-3% project ipsilaterally (Dräger & J. F. Olsen 1980). RGC axons continue to descend mainly in the direction of the dLGN (thalamus) and the superior colliculus (SC, midbrain) targeting them around E15.5 and E18.5 respectively. Then, both structures project to the visual cortex and receive feed-back cortical projections. Contralateral and ipsilateral projections from each eye are kept segregated throughout the visual pathway, and as a result the dLGN and SC from each hemisphere exhibit a topographical representation of both eyes, feature that allows binocular vision(Huberman et al. 2008). Studies with fluorescent-labelled anterograde tracers manifest

two comprehensive processes during eye-specific segregation, which occurs between P1-P10 (before sensory input): (1) synapse and axon arbor elaboration and (2) synapse and axon elimination. Along the visual pathway, all central brain structures exhibit a representation of the visual map reflecting the visual image that is perceived by the retina. This map is known as retinotopy. The early formation of the retinotopy happens mainly due to genetically encoded programs and axon guidance molecules, such as Ephrins/Eph (Brown et al. 2000). However, neural activity also plays an important parallel role in its formation, which has been the main focus of many investigations during the past decade.

3.4.3 Auditory map: Tonotopy

The auditory system follows a stereotyped pathway from the cochlea, located in the inner ear, to the cortex. Sound waves are transmitted mechanically to the sensory neuroepithelium, the organ of corti, composed of inner (IHC) and outer hair cells (OHC). IHC, constitute the primary receptors and OHC the motor cells that convert the membrane potential to mechanical forces (Mann & Kelley 2011) . Thus, the signal is transmitted through the cochlear nerve fibres to the brainstem nuclei, midbrain (inferior colliculus, IC), thalamus (Medial Geniculate Body, MGB) and auditory cortex, establishing the ascending pathway. The descending pathway goes from the auditory cortex to the MGB, IC, cochlear nucleus (CN) and superior olivary complex (SOC), both located in the brainstem. At the same time, several feedback loops are generated in each station (Malmierca & Ryugo 2012; Terreros & Delano 2015). As all sensory systems, the auditory system develops a topographic map. This map is known as tonotopy and represents the spatial separation of sounds based on their frequency (Russell & Sellick 1977; Mann & Kelley 2011). In the cochlea, neurons are arranged along the longitudinal axis, based on their ability to gradually respond to different frequencies, from high in the basal region to low in the apical region of the cochlear duct. This tonotopic segregation is preserved in the cochlear nucleus and transmitted to the brainstem via topographically ordered axonal pathways. Interestingly, the tonotopic representation along the longitudinal axis of the cochlea is present before

auditory stimuli can drive responses in the auditory nerve. As a matter of fact, in mice hearing onset starts at P12, suggesting that the initial generation of thetonotopic map is independent of peripheral stimulation and that other control mechanisms might take place (Koundakjian et al. 2007; Kandler et al. 2009). It has been suggested that guidance cues, such as tyrosine kinase receptors and Ephrin receptor families are important elements for the tonotopic map ontogeny in the auditory system (Cramer 2005; Huffman & Cramer 2007; Fariñas et al. 2001).

3.4.4 Somatosensory map: Somatotopy

It was in the 70s, when the work of Woolsey and Van der Loos described forcortex by cytoarchitectonic units in L4. These discernible units were ultimately named “barrels” (Woolsey & Van der Loos 1970; Woolsey, C. Welker, et al. 1975; Woolsey, Dierker, et al. 1975). As in the case of the visual and auditory systems, the topographical representation of the whisker pad is not only visible in the cortex. It is in fact formed along all sensory stations of the somatosensory pathway, maintaining a strict somatotopic connectivity.

Whisker follicles located in the rodent’s snout are innervated by neurons from the trigeminal ganglion (TG) which project to different trigeminal nuclei located in the brainstem. This connectivity forms at least four different axonal pathways: lemniscal 1, lemniscal 2, extralemniscal and paralemniscal (Deschenes & Urbain 2009; Feldmeyer 2012; Pouchelon et al. 2012). These axonal pathways differ in their origin in the brainstem, the thalamic region that innervate and their final cortical target (region and layer). For example, the first 3 pathways are relayed by different regions of the VPM and the last by the POm thalamic nucleus. It has been also suggested that all distinct whisker-to-cortex pathways are associated with different somatosensory modalities. This way the lemniscal pathway is involved in the combined whisking-touch signal, the paralemniscal in whisking signal (sensory-motor control) and the extralemniscal the contact signal (C. Yu et al. 2006).

The lemniscal pathway is the most studied pathway, which can be subcategorized in lemniscal (1) and lemniscal (2). Both pathways contain the primary trigeminal station in which whisker information is sent to the principal trigeminal nucleus (PrV) in the brainstem, from where it travels to the dorso-medial part of the VMP thalamic nucleus (VPMdm) (Veinante et al. 2000). Discernible structures named barreletes that topographically connect to the corresponding barrel, can be observed in the PrV. Similarly, one can distinguish a particular shape of the associated barreloid in the contralateral thalamus. The two lemniscal pathways differ in their target region of the VPM barreloid. In the lemniscal 1, PrV targets the core of the VPM barreloids, while in lemniscal 2 targets its head. From the cortical side, the leminiscal 1 axons innervate principally the L4 and L6a and to a lesser extent L3 and L5b neurons of the corresponding barrel in S1 (Meyer et al. 2010; Oberlaender et al. 2011). Of note, each with single-whisker receptive fields. On the other hand, lemniscal (2) axons innervate exclusively the neurons located in L4 barrel septa, with multi-whisker receptive fields (Brecht & Sakmann 2002). The brainstem origin of the paralemniscal pathway are the neurons located in the rostral pole of the interpolar spinal trigeminal nucleus (SpVo) with no apparent barrelete domains (Pierret et al. 2000). Their axons innervate the POm nucleus of the thalamus, which do not show barreloid structures either and project to both, S1 and S2 (secondary somatosensory) cortices. Neurons located in the posterior and anterior part of the POm target respectively L1 and L5a in the S1 cortex (Wimmer et al. 2010). The L4 neurons are also targeted by the POm afferents in S1, but only the ones located in the septa region, although in the S2 POm is targeting L4 neurons (Wimmer et al. 2010; Bosman et al. 2011; Viaene et al. 2011; Pouchelon et al. 2014). The extralemniscal pathway arises from the caudal region of the interpolar spinal trigeminal nucleus (SpVi), in which barrelete-like organization is present. Afferents from SpVi reach the ventro-lateral part of the VPM thalamic nucleus (VPMvl), occupying the tail region of the barreloids, with no presence of discernible barreloid-like structures (Pierret et al. 2000; Bokor et al. 2008). Extralemniscal projections target L3, L4 and L6 neurons of the S1 barrel cortex and more densely neurons located in L4 and L6 of S2 cortex (Pierret et al. 2000).

3.4.5. Development of the somatosensory pathway

To better understand the somatosensory circuitry, this section is focused on the developmental sequence of the pathway, fundamental for the correct assembly and functional maturation of the somatosensory system.

Cutaneous receptors or dermatomes located in the snout of the mice are innervated by TG neurons, which serve as a bridge between these superficial receptors and the hindbrain. The TG neurons in mice develop as early as E9.0 (Erzurumlu et al. 2010) and immediately form three compartments that innervate distinct facial divisions: the ophthalmic, mandibular and maxillary (the whisker-related compartment). As this segregation is established even before the peripheral connections are formed, it has been suggested to be caused by ontogenetic transcriptomic programmes, rather than by the periphery (Hodge et al. 2007; Pouchelon et al. 2012). The TG axons enter the hindbrain at E9.5, well before the innervation of the whisker follicles at E12.5. However they do not extend the collaterals to enter the PrV until E14.5, time at which the PrV is generated (E10.5-E15.5) (Kitazawa & Rijli 2018). Already at this stage, the topographic mapping is apparent and its further whisker-specific refinement takes place at the end of the collateralisation processes at E17.5 (Laumonnerie et al. 2015). The maxillary (upper jaw and whisker-related) TG axon collaterals target the ventral portion of the PrV (vPrV, rombomere3-derived) and the mandibular (lower jaw) the dorsal part (dPrV, rombomere2-derived) (Erzurumlu & Killackey 1983; Erzurumlu 2010; Oury et al. 2006; Kitazawa & Rijli 2018; Iwasato & Erzurumlu 2018). PrV afferents exit the nucleus and around E11.5 cross the midline (before TG axonal collaterals enter the PrV) and reach the contralateral thalamus at E17.5, suggesting a periphery independence at the early steps of this process. The correct VPM-targeting area happens mainly due to intrinsic genetically encoded programs in the different portions of the PrV nucleus, for example driven by the expression of Drg11 and Hoxa2 (Ding et al. 2003; Oury et al. 2006). Lemniscal PrV afferents initially develop diffused arbors in the VPM territory, then gradually refine, first into rows and then into single whisker-

specific patches (barreloids) apparent around P2-P3 (Kivrak & Erzurumlu 2013). As aforementioned, VPM nucleus extend their axons towards the cortex very early,before PrV afferents reach it. Interestingly, these axons are already topographically organized, suggesting a more than probable periphery-independence mechanisms at the very early steps. Which is in fact comparable with the visual and auditory systems, indicating that all three principal sensory systems follow similar rules during map ontogeny assembly.

Now we know that sensory periphery influence the early somatotopic cortical topography after the initial formation, during thalamic fibre ingrowth and arborisation (Martini et al. 2018; Gaspar & Renier 2018). At E18.5 TCAs start to invade the cortical plate tangentially and direct their fibres towards the L4. The segregation takes place gradually in the cortical plate until the TCAs arborize and form the barrels apparent between P3 and P4. There is still a big controversy regarding these last steps of topological refinement. Some seminal studies have indicated the existence of a refinemen processes during barrel formation. Thus TCAs would initially branch profusely and branches that are not correctly located would be eliminated ("pruned"), while those that are correctly located would be stabilized and develop mature TC arbors (Senft & Woolsey 1991b; Senft & Woolsey 1991a; Rebsam et al. 2002). Other studies claim that TCAs are already arranged in columns at the time of growing through the cortical plate. These observations indicate that during the final clusterization of TCAs, when the barrel map formation occurs, initially a poorly-developed branches grow subsequently followed by a substantially and restricted process of arborisation (Agmon et al. 1993; Agmon et al. 1995; Crandall et al. 2017). In fact, it has been demonstrated that disturbing the topographic organization of TCAs along their pathway disrupts the TC clustering, generating an aberrant barrel map (Lokmane et al. 2013). This goes in agreement with the view of a pre-organization of TCAs before reaching the puerile L4.

3.4.6. Intracortical columnar circuitry of the somatosensory cortex

As mentioned, one of the most important features of sensory cortices is their functional organization in radial units that constitutes a hub for afferent, intrinsic and efferent neuronal information. This columnar representation isalready present during early corticogenesis were clonally related neurons (Noctor et al. 2001) migrate from the VZ along the radial glial cells (Fishell &Kriegstein 2003) forming ontogenic columns (Rakic 1988). Thus, this primitive pattern seems to serve as an outline for the future columnar architecture. The next paragraphs are mainly focused on the functional columnar connectivity that is stablished within the barrel cortex in the somatosensory system. Basically, all cortical layers receive thalamic input, either from VPM or from POm nuclei. It is important to further elaborate on the implications of each layer in the thalamo-cortico-thalamic connectivity in order to better understand the functioning of the columnar somatosensory system.

Layer 4 represents the core of the intracolumnar information flow, a part of being the major recipient layer of thalamic input, L4 excitatory neurons are connected with all the remaining layers, distributing the intracortical excitation and generating a columnar circuitry within the barrel (Bruno 2006). There are two types of excitatory neurons, the spiny stellate (ss) and the star pyramidal (sp), both neurons obey to columnar connectivity. Nevertheless, L4ss axon do not project further than the neighbouring barrel and some L4sp neurons exhibit a long-range connectivity over several columns in L4 and infragranular layers. While L4ss act as local signal, L4sp globally integrate horizontal information within a functional column and the neighbouring barrels (Schubert et al. 2003; Egger et al. 2008; Narayanan et al. 2017). The cell body of L4ss form the barrel wall maintaining their dendritic domain polarize to the barrel hollow, where TC arbors are located (Woolsey & van der Loos 2016) and interconnect between them. Actually, the L4-L4 connection is the main intracortical input that L4ss receive (Lefort et al. 2009). L4ss preferentially target other L4ss and pyramidal neurons of L2/3 within the same barrel, however they also innervate L5a, L5b and L6a to a lesser extent (Lefort et al. 2009; Feldmeyer 2012). Differentially, in other sensory

circuits, like the mouse motor cortex, the intracortical circuitry is dominated by L2/3 to L5 connectivity (Weiler et al. 2008), whereas in the barrel cortex L4 dominates de cortical column (Lefort et al. 2009). Excitatory inputs reach the cortex through L4 neurons that are columnar connected with L2/3 neurons (Petersen & Sakmann 2001; Feldmeyer et al. 2004). Focusing in the barrel-septa organization of the somatosensory cortex, several studies have shown that L2 and L3 neurons located above the barrel are highly excited by L4 neurons, however L2 neurons above the septa are more excited by L5a pyramidal neurons, this pathway represents the cortical continuity of the lemniscal (VPM-L4- L2/3 barrel) and paralemniscal (POm-L5a-L2) pathways, being L2 one of the converge points of this two somatosensory pathways (Shepherd 2005; Bureau et al. 2006). L2/3 pyramidal neurons are vertically connected within the column with L5a and L5b (Hooks et al. 2011; Lefort et al. 2009; Shepherd 2005). However, as they are mainly characterised by a long axon that extends down with several long-range collaterals that project horizontally, L2/3 neurons are principally making contacts with surrounding cortical domains, like neighbouring cortical columns within S1 or with the ipsilateral S2 and M1 (Yamashita et al. 2018). Importantly, they also project to the contralateral whisker-related S1 via corpus callosum, thus integrating the activity of the two cortical hemispheres (Petreanu et al. 2007).

Layer 5 is considered the major output layer of the cortex the L5 can be divided in L5a and L5b based on histological (morphology of pyramidal neurons), connectivity (afferent and efferent connections) and functional differences (Wise & E. G. Jones 1977; Larsen & Callaway 2006). Both divisions receive thalamic afferents, from the POm and VPM respectively. In contrast, layer 6 is subdivided in L6a and L6b. L6a is mainly form by pyramidal neurons, L6b is very heterogeneous with highly diverse dendritic domains located in both barrel and no-barrel domains, they mainly innervate the POm and a subset of neurons send their axons to L1. Moreover, L6b neurons are originated by different cortical regions, for instance the subplate and the cortical plate. However, L6a neurons can be

isubdivided in the ones that project intracortically and the ones that project to the thalamus. As aforementioned, L6a has been proposed as the major source of CT connectivity and constitutes a basic element of the feed-back control of thalamic nformation (E. G. Jones & Wise 1977) (Thomson 2010) (Fox & Woolsey 2009). Based on their thalamic target can be divided in: 1) the ones that target only the VPM with columnar collaterals to L4; 2) VPM and POm with collaterals to L4 and L5, to several barrels and no columnar-like distribution; and 3) only to POm located in the lower portion of L6a with some branches to L5b. In the case of L6a pyramidal neurons that project intracortically, mainly innervate L5 and L6 of many barrels, mediating transcolumnar interactions in the infragranular layers of the barrel cortex. L6a neurons receive input form L5b, L6a and also L4 excitatory neurons.

3.5. Spontaneous activity in sensory systems development

3.5.1.OOverview

Neuronal activity can be categorized in two main groups. Spontaneous activity, which is considered to be independent of external input and intrinsically generated in the developing brain structures, and evoked neuronal activity, mainly driven by external peripheral sensory information. Evoked activity has a fundamental role in the final assembly and maturation of sensory maps and circuitry establishment (REFs). Furthermore, intrinsically generated activity patterns mainly play a role in early developmental processes. It is important to bear in mind that both sources are equally important, responsible for specific events of brain developmental and, most probably, highly interrelated. Spontaneous activity recently gained more attention as it is found to play unexpected roles in brain development, for example in sensory systems development.

3.5.2. Spontaneous activity in the visual system

Spontaneous activity in the retina is featured in propagating waves of activity that are defined by highly correlated firing patterns. Spontaneous retinal waves follow a developmental sequence, from late-prenatal stages till the end of the

second postnatal week, just at the time when sensory-experience impact the system. Thus, the basic features of all three stages of retinal waves are as follows: Stage I, has a late-prenatal onset and is characterized by localized bursts of activity mediated by gap junctions that intercommunicate adjacent RGCs (Syed et al. 2004). Stage II is dependent on the cholinergic transmission, present from P1 to P10 matching with retinotopic and eye-specific refinement (Huberman et al. 2008). Lastly, stage III waves, from P10 to P14, are glutamatergic and overcome smaller retina areas of stage II. Since mice open their eyes around P12, the stage III waves overlap with a short period of sensory-experience activity.

Spontaneous retinal activity has been shown to play a pivotal role in eye specific axonal segregation. Many studies have addressed the importance of Stage II retinal waves in this process using different species such as rabbit, ferret, mice and even primates (Huberman et al. 2008; Seabrook et al. 2017). Indeed, blocking cholinergic transmission with an alkaloid epibatidine or using the β2nAChR–/– mouse (only blocks stage II waves) induces retino-thalamic axonal segregation problems in the dLGN, with diffused arbors and larger receptive fields (Torborg & Feller 2005; Blankenship & Feller 2010). However, this effect can be rescued by stage III waves, which are not affected by the lack of β2nAChR neither by epibatidine. Interestingly, blocking the correlated firing of RGCs during stage II period do not induce any segregation impairment, suggesting that spontaneous retinal activity is rather permissive than instructive for eye-specific segregation. The treatment with epibatidine or RGCs-specific drugs severely impairs later developmental processes such as the retinotopic map refinement, ocular dominance and orientation selectivity (Hooks & C. Chen 2006; Huberman 2007; Huberman et al. 2008). Interestingly, spontaneous retinal waves seem to propagate through the entire visual system. This phenomenon has been shown by *in vivo* approaches, where stage II waves dominate the activity in SC, dLGN and V1 (Ackman et al. 2012; Ackman & Crair 2014; Kerschensteiner 2016). Intriguingly, enucleation experiments showed that a subset of stage III spontaneous activity patterns in V1 is dependent on

retina , suggesting that stage III waves are also transmitted along the visual pathway (Siegel et al. 2012). Nevertheless, it remains uncertain whether dLGN or SC intrinsically generate this spontaneous activity (see below) or if it is generated from retina afferents or from cortical feed-back connectivity. Regardless of that, this evidence proves that propagating wavesof spontaneous activity in the visual system play an important role in the formation of retinotopic maps along the pathway. Evoked activity has also a fundamental role in the correct arrangementvisual system initial development is carried out in the absolute absence of the external input.

3.5.3. Spontaneous activity in the auditory system

The role of activity-dependent mechanisms in the auditory tonotopic map refinement have been studied before and after hearing onset. Apparently, both spontaneous and evoked neuronal activity patterns remain important for the final assembly and maturation of this map. Although, the primary generator of the spontaneous neural activity resides in the developing IHC of the cochlea, it remains unclear whether this activity arises intrinsically, or if it is induced by external stimuli. For instance, the closely located inner supporting cells have been shown to periodically release ATP inducing the depolarization of groups of neighbouring IHC, calcium spikes and glutamate release (Tritsch et al. 2010; Wang et al. 2015). At early developmental stages (E16.5-E17.5) IHC can elicit small responses after current injections (Marcotti et al. 2003; Johnson et al. 2005), however it is not until P0 when depolarization and glutamate release from IHC can trigger action potentials of the hindbrain-connected spiral ganglion neurons (SGNs) (Hoffpauir et al. 2009; Tritsch et al. 2010). Thus, the signal transduction in the developing auditory circuits seems to be established well before the onset of input. The spontaneous activity emerges postnatally and is maintained until hearing onset. However, there is no clear consensus about the activity pattern changes that IHC manifests throughout postnatal development before sensory onset.

Spontaneous synchronic patterns of neuronal activity are intrinsically generated along the auditory pathway before the onset of hearing (Tritsch & Bergles 2010; Babola et al. 2018). It is believed that this activity is generated and transmitted from the cochlea. Local application of tetrodotoxin (TTX) to the cochlea or a complete removal of the structure blocks the firing in other structures (Lippe 1994; T. A. Jones et al. 2006; Tritsch & Bergles 2010). Nevertheless, these experiments were performed in chick and further experiments using mammal models will be required to discard the possible generation of spontaneous activity patterns intrinsically along different stations of the auditory pathway. It was recently when the group of Dwight E. Bergles showed for the first time *in vivo* that spontaneous activity originates in the cochlea and is transmitted through all the pathway to the auditory cortex (Babola et al. 2018). These synchronic events show a complex spatio-temporal pattern that is coordinated among auditory stations and resemble an immature tonotopic map. Neurons that will later process similar frequencies are synchronized before the auditory sensory onset. This suggests that spontaneous activity prior to hearing onset serves as a blueprint to the mature tonotopic map, establishing a nascent network prior to the appearance of sensory input (Babola et al. 2018).

Spontaneous activity along the auditory pathway has been implicated in neuronal survival (Zhang-Hooks et al. 2016), synapses maturation (Oleskevich & Walmsley 2002; McKay & Oleskevich 2007), proper target innervation (Franklin et al. 2006; Franklin et al. 2008), refinement and segregation of the tonotopic maps. Indeed, in congenitally deaf animal tonotopy remains normal (Cao et al. 2008; Youssoufian et al. 2008), although SG exhibit broader arbors and more branches connecting with the CN, consequently forming a less defined map (Leake et al. 2006). The role of auditory evoked activity in tonotopic map formation has been studied mostly after hearing onset through the abolishing hearing. In these conditions, the tonotopic maps remain and the overall topographic organization stays normal, however tonotopic resolution and the sharpening of frequency tuning decrease (Friauf & Lohmann 1999).

3.5.4. Spontaneous activity in the somatosensory system

In this section I am going to point out what is known about the role of neuronal activity (spontaneous and evoked), in both the initial establishment of the somatotopic map and in the final arborisation processes that take place to finally develop the barrel columnar representation.The first postnatal week in rodents has been recognized as an important 3critical7 period of plasticity in the developing barrel map. Apparently, the intact periphery is a crucial element for the barrel formation at this stage. This is a so-called "critical period", a time window in which certain conditions are essential, and in their absence irreversible alterations occur. Manipulations after this pivotal time window, however, do not extent significant changes (Erzurumlu 2010; Erzurumlu & Gaspar 2012). Classical studies have shown that sensory deprivation by infraorbital nerve (ION) lesion between P3 and P5 in mice, drive morpho-functional impairments in the barrel map. In such cases, TCAs do not cluster in L4 of the S1 and consequently L4 spiny neurons do not aggregate into barrels, showing the vulnerability of the system to input from the periphery (Belford & Killackey 1980; Durham & Woolsey 1984; Erzurumlu & Gaspar 2012). Nevertheless, denervation of the whisker pad after this period of plasticity, not only blocks peripheral sensory stimulation, it also generates a physical damage of the primary sensory neurons. Neonatal whisker trimming or plucking were also common techniques used to tackle the role of peripheral input in the barrel map formation (Erzurumlu & Gaspar 2012). In this case, sensory activity is not completely blocked, as sensory receptors on the snout can be stimulated by passive contact suggesting that sensory activity could be involved in the correct arrangement of the somatosensory map. However, the essential element they have manipulated in these experiments remained unclear: the peripheral sensory receptors, the peripheral input or the neural activity that flowed through it. Nevertheless, the involvement of peripheral activity remained open as transiently blocking with TTX the ION did not affect the barrel map formation (Henderson et al. 1992).

During this critical period, sensory cortices show sequential stages of spontaneous and evoked activity throughout development, which can be categorized into four stages (Luhmann & Khazipov 2018). The first stage consists exclusively of asynchronous single cell-firing. The second step is a synchronic firing stage, in which synchronous plateau assemblies (SPAs) and beta oscillations are observed. This spontaneous activity is mediated by electric synapses based on gap junctions with a radial component that coupled the column (Dupont et al. 2005; Y.-C. Yu et al. 2012). In the third place, delta-waves are leading cortical neuronal activity early from birth to P7-P8. Usually they incorporate two elements: rapid and oscillatory with early gamma oscillations (EGOs) and spindle bursts. Both are mainly mediated by glutamatergic currents elicited either by TCAs or by SuPNs (Yang et al. 2013) although, spindle bursts require gap junctions from P0 to P3 (Dupont et al. 2005). Both also exhibit local patterns, even though spindle bursts activate larger regions than EGOs, which are usually restricted to a barrel-like column (Luhmann et al. 2016; Luhmann 2017; Khazipov et al. 2013; Yang et al. 2009). Apart from the EGOs and spindle bursts, cortical network oscillations (cENOS) are also present. Their main driver are corticocortical glutamatergic synapses characterized by low-frequency oscillations that involve the entire network (Allène et al. 2008). Last but not least, the fourth stage is the emergence of "mature" activity patterns that are experience-dependent. They are associated with explorative behaviours and active whisking starting around P12 (Landers & Philip Zeigler 2006). Specifically, delta waves (EGOs and spindle bursts) are present in the barrel cortex during a defined developmental time window, and therefore their role in somatosensory map formation has been widely studied (Yang et al. 2013; Minlebaev et al. 2011; Khazipov & Luhmann 2006; Luhmann & Khazipov 2018; Yang et al. 2018).

Taking advantage of *in vivo* approaches by using voltage sensitive dyes and electrophysiological recordings, have been hown that spontaneous activity patterns in the somatosensory cortex were already topographically organized at birth. This demonstrates the existence of a nascent cortical

network that is fairly columnar-restricted even before barrel formation. The elements creating this network were named functional “pre-columns” (Yang et al. 2013; Mitrukhina et al. 2015). Indeed, evoked EGOs/Spindle bursts also exhibit a specific columnar organization after single whisker or barreloid (VPM) stimulation (Yang et al. 2013). Moreover, by using two-photon in vivo calcium imaging, a recent study has shown that L4 excitatory neurons in the barrel cortex exhibit a spontaneous correlated activity within the same barrel, termed as patchwork activity (Mizuno, Nakazawa, et al. 2018). Similarly, to described in Yan et al 2013, this correlated activity is prominent during the first postnatal week, while in the second week L4 neurons tend to fire asynchronously. Despite the fact that it seems to be instructed by periphery, self-generated whisker movements in young pups do not have much effect on this patchwork activity. Thus, stimuli-dependent evoked activity might not be the main source. Notably, the authors also found this spontaneous patchwork activity in TCAs, opening the possibility that the patchwork activity is already patterned in TCAs, and thus the thalamus might play a pivotal role in this cortical patchwork activity and subsequently in the generation of the nascent columnar organization of the somatosensory cortex. Still, it is not clear whether this patterned activity is transmitted from the periphery or it is self-generated in the thalamus, as the ION lesion at P4 did not affect the patchwork activity in the barrel cortex. The spatiotemporal features of the patchwork activity in the somatosensory cortex might indicate a possible role in TC refinement. It would be interesting to record this activity early on, when the anatomical barrels are not present, as it could allow to discriminate whether this activity is instructive for the initiation of map assembly.

The importance of the early thalamic input to the generation of these cortical activity also originates from the fact that thalamic axons interact at early stages with SuPNs. It has been also shown that SuPNs drive local oscillatory columnar networks, probably due to their involvement in map formation. The elimination of the SuPNs disrupts EGOs and prevents the correct columnar structure reflected in the anatomy of the barrel field (Dupont et al. 2005; Tolner et al. 2012). Sensory

peripheral input to the neonatal somatosensory cortex is mainly due to spontaneous whisker movements and passive stimulations by littermates (Akhmetshina et al. 2016). Therefore, even though active whisking starts at P12 in mice, early evoked activity seems to be a key element in driving cortical activity during the development of the somatosensory map.

Cortical oscillatory activity patterns are principally driven by glutamatergic synapses that involve the activation of AMPA and NMDA receptors, most likely from thalamic afferents (Minlebaev et al. 2011; Khazipov et al. 2013). Extensive work on activity-related transgenic animal models have helped to understand the bases of the somatosensory map refinement. For example, while completely eliminating (full KO) either AC1, mGluR5, NMDAR1, type IIβ regulatory subunit of the protein kinase (PKARIIβ) or sodium-dependent 5-HT transporter, the mice exhibit some kind of abnormal barrel map formation (E. Welker et al. 1996; Abdel-Majid et al. 1998; She et al. 2009; Wijetunge et al. 2008; Iwasato et al. 1997; Inan et al. 2006; Persico et al. 2001). What was clear from these studies is that activity itself was important for barrel map formation. However, as these molecules were ablated from the entire organism, it is unclear what somatosensory station was the source of the phenotype. Therefore, engineering conditional transgenic animals was crucial to open new avenues for investigating this issue more profoundly. Indeed, disrupting presynaptic glutamatergic communication between thalamic afferents and cortical territories through a specific gene deletion in either thalamus or cortex produced an aberrant barrel map. For instance, specific thalamic elimination of NMDA receptors, AC1 or a depletion of vGlut2 in a full vGlut1 knock-out background, generates a complete suppression of glutamate release. This manipulation disrupts both TCA afferents and L4 cortical patterning and results in a blurry map (H. Li et al. 2013; Suzuki et al. 2015; Arakawa, Suzuki, et al. 2014). However, manipulation of the activity-dependent presynaptic release in thalamic neurons by elimination of RIM1/RIM2, only lead to structural changes in cortical L4 spiny neurons, affecting their dendritic organization. Noticeable TCAs clustering was rather normal with apparently regular barrel map (Narboux-Nême et al. 2012). There are also several conditional knock outs that

specifically target the cortical territory. For instance, elimination of AC1, mGluR5 and NMDAR1 give rise to major L4 dendritic phenotypes. Under these circumstances TCAs are able to cluster, however, the complexity of their axonal arbors is reduced and the area covered is usually enlarged. Thus the barrel map is partially affected with no clear barrel walls and TC afferents that are not well refined to the barrel (Iwasato 2000; Iwasato et al. 2008; Ballester-Rosado et al. 2010). As expected, the activity related to both, presynaptic and postsynaptic compartments in the TC system, is important to the correct assembly of the barrel map. Interestingly, subcortical structures are also playing a pivotal role, not only having a merely relay function. However, is still unknown what are the rules that the possible commitment of intrinsic thalamic input might follow during the early phases of the functional columnar restrictions in the cortical field.

3.5.5. Spontaneous activity in the developing thalamus

Although it was previously assumed that spontaneous activity was exclusively driven by peripheral stations along sensory pathways, it remained unclear whether sensory stations were able to generate their own spontaneous activity patterns or if the information was simply transmitted from the periphery to cortical territories. We recently discovered that the prenatal thalamus has the potential to generate spontaneous calcium waves independently of the primary peripheral stations (Moreno-Juan et al. 2017). We show that embryonically enucleated mice exhibit thalamic calcium waves in the dLGN even without retina. However, the frequency of these calcium waves was increased in this nucleus, indicating its dependence of peripheral deprivation. Thus, peripheral input might synchronise or restrain the network activity along the pathway in order to instruct the correct development of cortical sensory circuits and maps. Interestingly, the possibility that different stations of a given sensory modality undergo independent spontaneous network events during development remains unexplored.

Additionally, spontaneous thalamic calcium waves have the capability to spread through the TCAs and reach the cortical field when radial migration has not ended yet and areal shape establishment is still highly plastic (Erzurumlu & Gaspar 2012). Therefore, early TC networks are perfectly located in time and space to influence cortical development (Figure 4A). We have previously explored the possible role of thalamic spontaneous activity in cross-modal cortical modifications after sensory deprivation, which have been recently suggested to beindependently driven by sensory experience in young rats (Fetter-Pruneda et al. 2013). Importantly, the thalamus occupies a strategic location in the forebrain, just prior to the cortex and it constitutes a unique design where all sensory modalities convey in the same structure, providing a possible way of communication within sensory systems. To prove our hypothesis, we used distinct animal models to deprive either the visual acquisition (peripherally) or the auditory input by directly ablating (Anton-Bolaños and López-Bendito unpublished) or silencing the auditory thalamus (Moreno-Juan et al. 2017). Spontaneous thalamic calcium waves propagate through gap junctions among sensory-modality thalamic nuclei up to the cortex. This provides a mechanism of communication among sensory systems, revealing the existence of a prenatal sub-cortical mechanism that regulates the cortical areas size in sensory deprived mice. Modifications in the wave pattern of visual or auditory thalamic nuclei promote an increase of frequency in the VPM calcium waves, triggering the upregulation of RORβ that preludes the enlargement of the barrel field. This reveals for the first time that embryonic thalamic calcium waves harmonize cortical sensory areas size prior to sensory processing (Figure 4B) (Moreno-Juan et al. 2017; Martini et al. 2018).

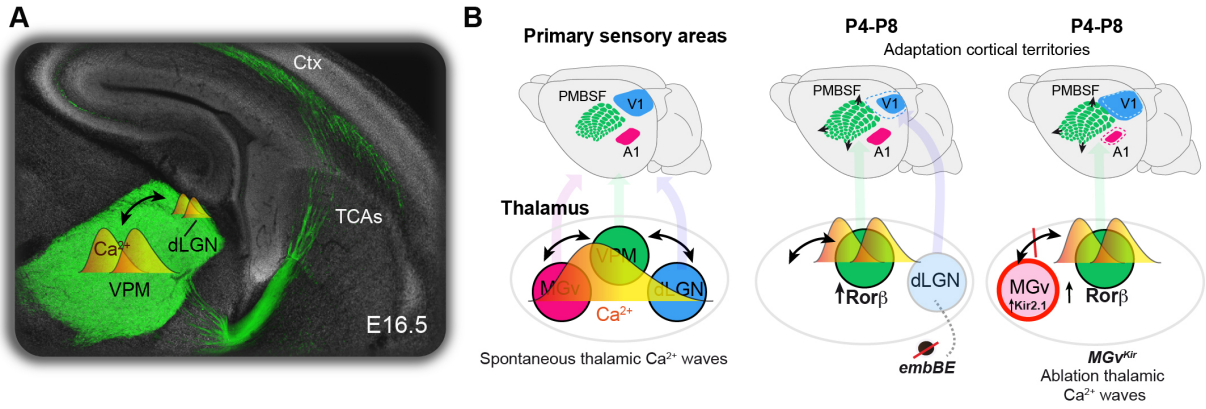
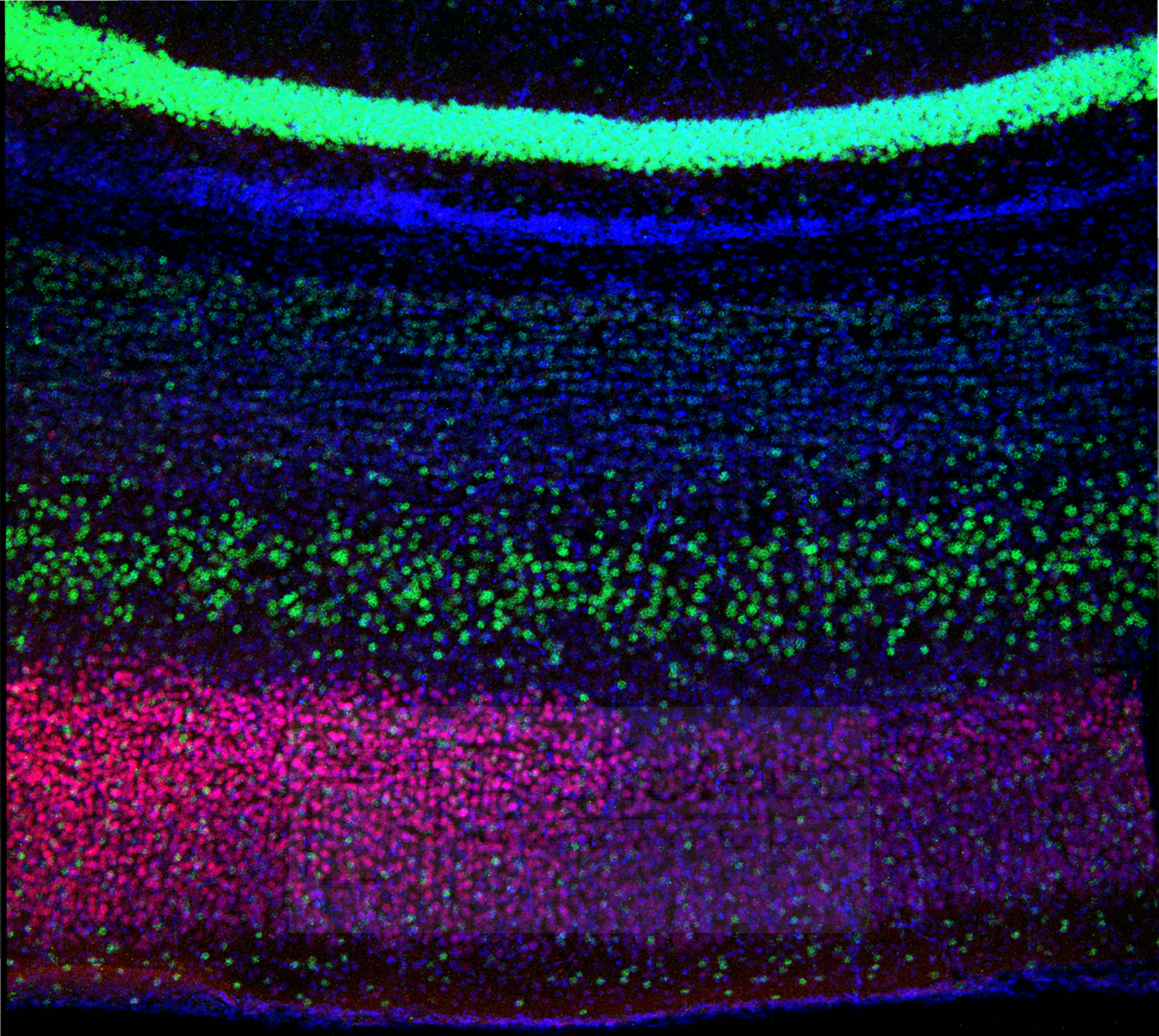
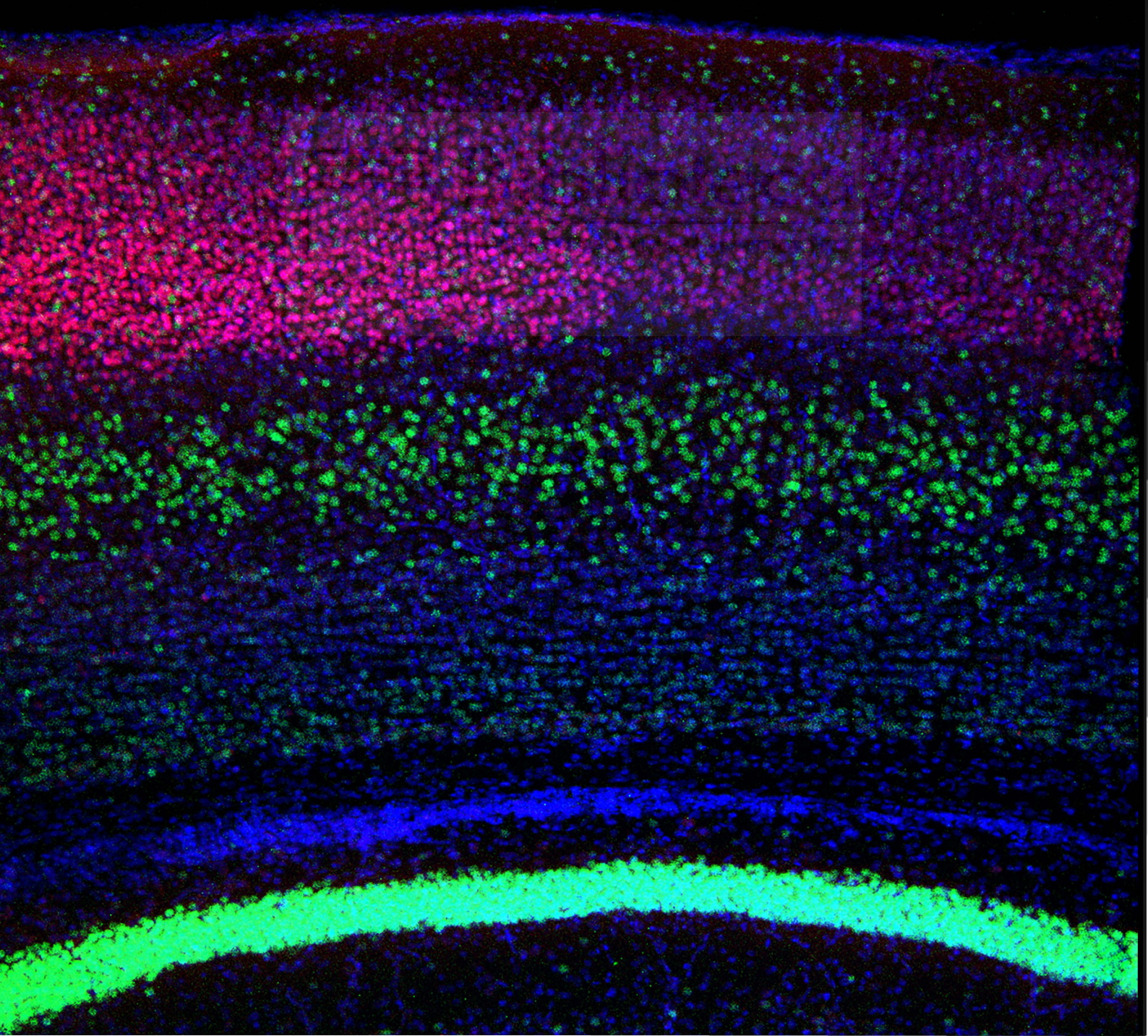


Figure 4. Role of prenatal thalamic spontaneous activity in early cortical adaptations after sensory input deprivation. A) Scheme representation showing the thalamic capability to generate intrinsic spontaneous calcium waves at early developmental stages. Thalamic neurons and projections are label in green, notice that at E16.5 TCAs are present in the cortex. **B)** Scheme representing thalamic calcium waves as a multisensory communication mechanism that regulates cortical area size prior sensory experience. (Adapted from Moreno-Juan et al. 2017)



Mouse cortical plate at P4. Immunohistochemistry of Cux1 in red and Ctip2 in green. View in a coronal plane.

Objectives



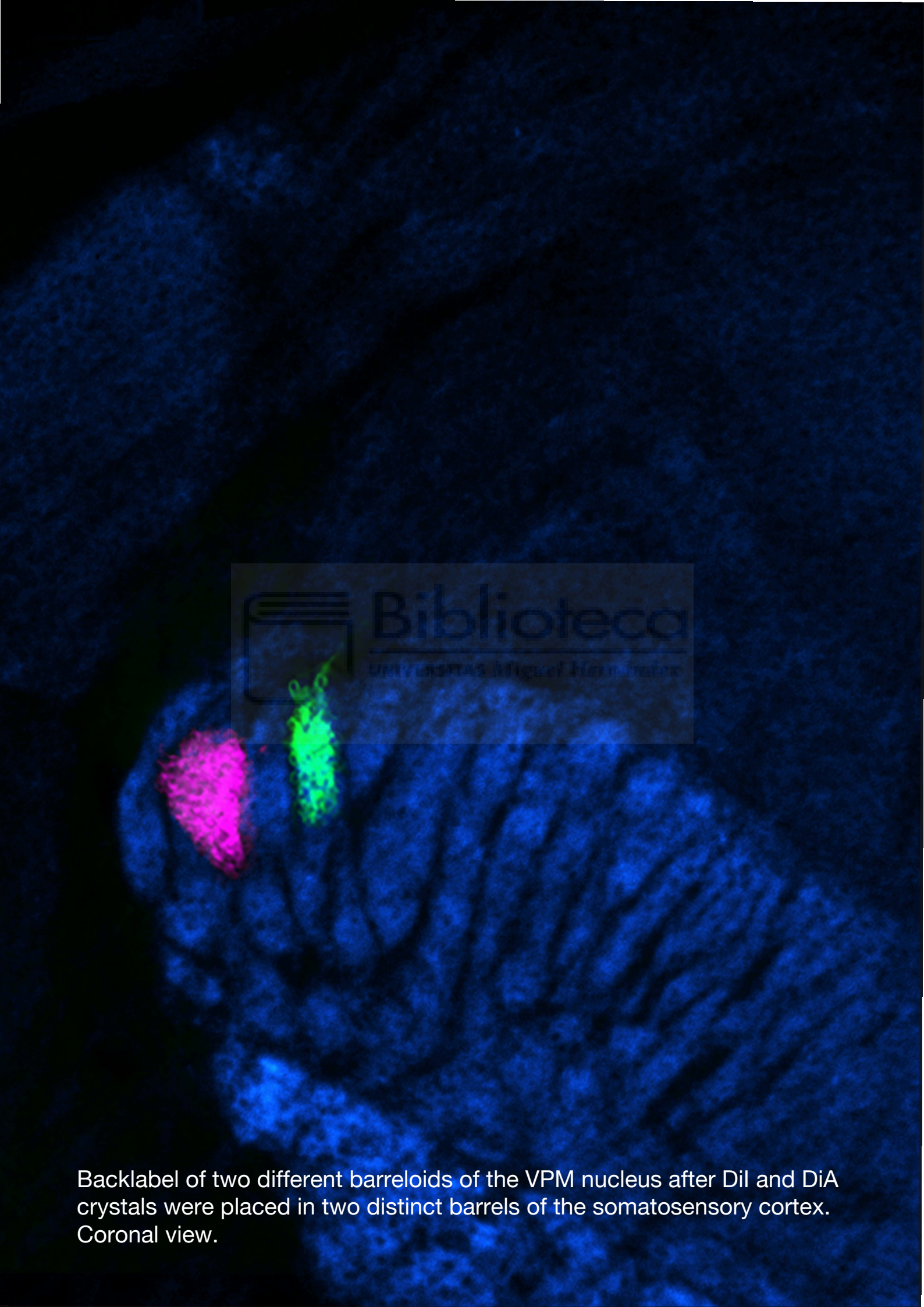
General objectives

1. To understand the rules of the emergence of the somatosensory cortical map.

2. To explore the existence of the functional prenatal columnar sensory map, which might be responsible to pave the cortical territory for the upcoming evoked sensory signals.

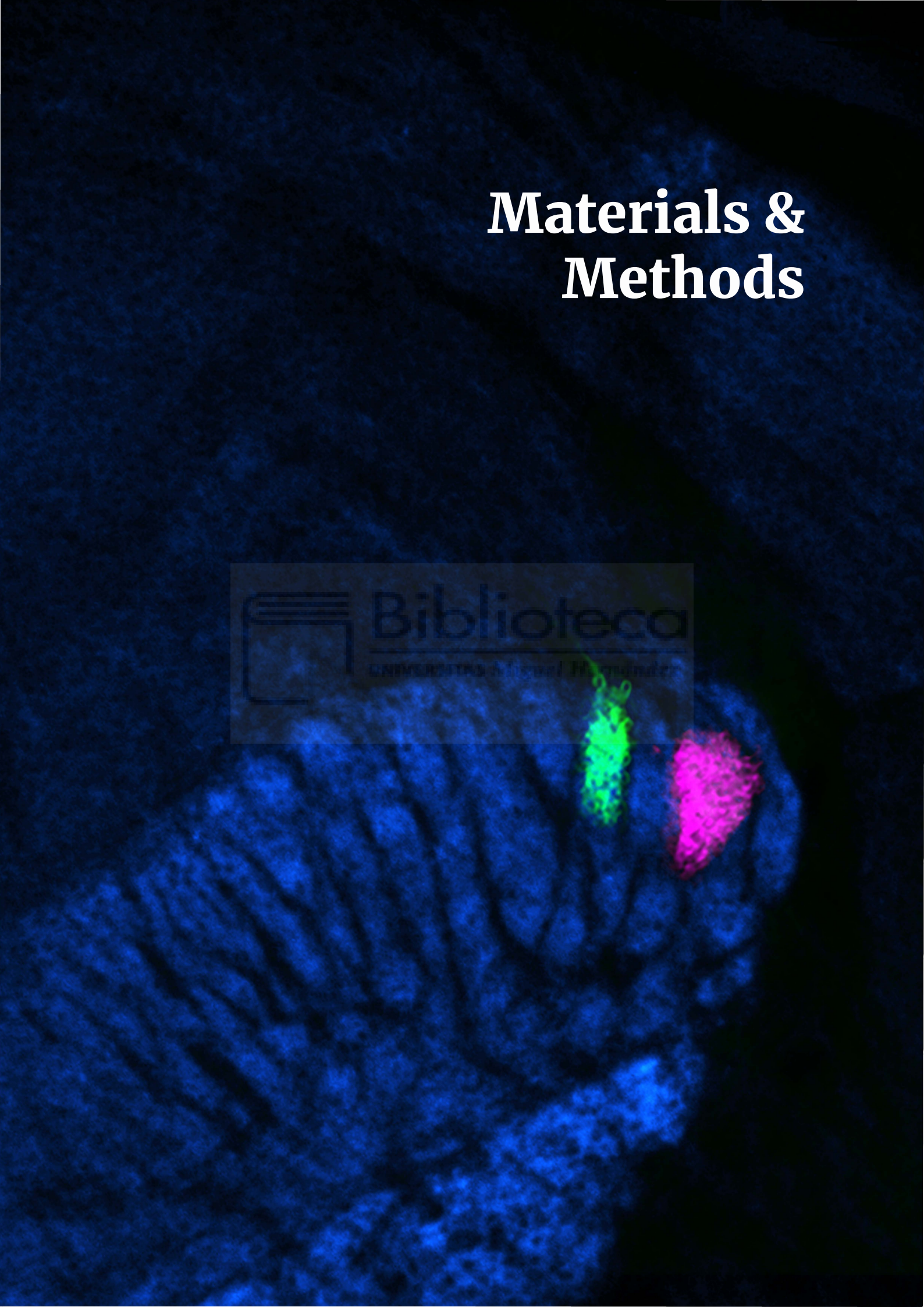
3. To decipher the role of specific patterns of spontaneous prenatal activity in the thalamus in the formation of cortical maps.

4. To explore the possible implication of prenatal thalamic input in the establishment of the functional somatotopic map in the immature cortex.



Backlabel of two different barreloids of the VPM nucleus after DiI and DiA crystals were placed in two distinct barrels of the somatosensory cortex. Coronal view.

Materials & Methods



Materials and Methods

Mouse strains

All transgenic animals used in this study were maintained on an ICR/CD-1 genetic background and genotyped by PCR. The day of the vaginal plug was stipulated as E0.5. The R26tdTomato Cre-dependent mouse line was obtained from Jackson Laboratories (Stock number 007908). The *TCA-GFP Tg,* in which the TCAs are labeled with GFP (19), and the $R26^{Kir2.1\text{-}mCherry}$ (10) mouse lines were previously described. The $R26^{GCaMP6f}$, a Cre-dependent mouse line, was obtained from Jackson Laboratories (Stock number 024105) and crossed with an $Emx1^{Cre/+}$ transgenic mouse (27) to conditionally express the fast calcium indicator GCaMP6f in glutamatergic cortical neurons. The *Thy1-GCaMP6f* mouse line (Jackson Laboratories, 025393) expresses GCaMP6f in excitatory neurons of the brain. In this mouse line, the expression of GCaMP6f in the cortex switches on at perinatal stages and increases its expression to cover the entire primary somatosensory cortex (S1) postnatally. The R26Kir2.1-mCherry, $R26^{tdTomato}$ and $R26^{GCaMP6f}$ floxed mice were crossed with an inducible Cre^{ERT2} mouse line driven by *Gbx2*, an early specific thalamic promoter ($Gbx2^{CreERT2/+}$) (28). Double mutants are referred as Th^{Kir}, $Th^{tdTomato}$ and $Th^{GCaMP6f}$, respectively. We also generated the following triple mutants: *TCA-GFP-*Th^{Kir} and a *Thy1-GCaMP6f-*Th^{Kir}*.* Tamoxifen induction of Cre recombinase in the double/triple mutant embryos was performed by gavage administration of tamoxifen (5 mg dissolved in corn oil, Sigma) at E10.5 to specifically target all primary sensory thalamic nuclei. Tamoxifen administration in pregnant mice produces non-desirable side effects such as delivery problems and decrease survival of newborn pups (29). In order to increase the survival rate of young pups, we administered 125 mg/Kg of progesterone (DEPO-PROGEVERA®) intraperitoneally at E14.5 and implemented C-section procedure at E19.5. Pups were then placed with a foster mother. 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.

Immunohistochemistry and cytochrome oxidase staining

For immunohistochemistry at postnatal stages, mice were perfused with 4% paraformaldehyde (PFA) in PBS (0.01 M), and the brains were dissected and post-fixed overnight. Embryonic brains were directly dissected and fixed in 4% PFA overnight. For cytochrome Oxidase (CytOx) staining, coronal sections were incubated overnight at 37 ºC in a CytOx solution: 0.03% cytochrome c (Sigma), 0.05% 3-3′ diaminobenzidine tetrahydrochloride hydrate (DAB, Sigma #D5637) and 4% sucrose in PBS. For tangential sections, cortical hemispheres were flattened and cryoprotected through steps of 10%, 20% and 30% of sucrose in PBS. Then, a cryotome (MICRON) was used to cut at 80-100 µm tangential sections. Immunohistochemistry was implemented on 60-100 µm vibratome or cryotome brain sections (coronal and tangential) that were first incubated for 1h at room temperature in a blocking solution containing 1% BSA (Sigma) and 0.3% Triton X-100 (Sigma) in PBS. Afterwards, the slices were incubated overnight at 4 °C with the following primary antibodies: guinea pig anti-vGlut2 (1:5000, Synaptic Systems, #135404), chicken anti-GFP (1:3000; Aves Labs, #GFP-1020), rat anti-RFP (1:1000 Chromotek, #5F8), mouse anti-NeuN (1:1000 Merk-Millipore, #MAB377), rabbit anti-Cux1 (1:500, Santa Cruz #13024), rabbit anti-cFos (Synaptic Systems, #226003) and guinea pig anti-cFos (Synaptic Systems, #226004). Sections were then rinsed in PBS and incubated for 2h at room temperature with secondary antibodies: Alexa488 donkey anti-guinea pig (1:500, ThermoFisher, #A11073), Alexa546 donkey anti-guinea pig (1:500, ThermoFisher, #A11040), Alexa488 goat anti-chicken (1:500, ThermoFisher, #A11039), Alexa594 donkey anti-rat (1:500, ThermoFisher, #A21209) and the biotinylated secondary antibody anti-rabbit (1:500, Vector labs #BA-1000). Sections were counterstained with the fluorescent nuclear dye DAPI (Sigma-Aldrich). When signal amplification was required, sections with biotinylated secondary antibodies were incubated with Avidin–Biotin Complex

(ABC 1:500, Vectastain #PK-6100) for 1 h 30 min, washed in PBS-T and Tris-HCl 0.05 M pH 7 and revealed in Tris-HCl 0.05 M with 0.05% DAB and 0.18% H_2O_2.

Slice preparation for calcium measurements

The brains of embryonic or postnatal mice (E16.5-P8) were dissected out rapidly and immersed in an ice-cold slicing solution containing (in mM): 2.5 KCl, 7 $MgSO_4$, 0.5 $CaCl_2$, 1 NaH_2PO_4, 26 Na_2HCO_3, 11 Glucose and 228 Sucrose. Brain hemispheres were separated through the midline, and 350-450 µm thick slices were obtained from each half in a vibratome (VT1200 Leica Microsystems Germany). To preserve thalamocortical connectivity from the ventral postero-medial nucleus (VPM) to S1, a cutting angle of 45º was employed. Slices recovered during 30 min at room temperature in standard ACSF containing (in mM): 119 NaCl, 5 KCl, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 1 NaH_2PO_4, 26 Na_2HCO_3 and 11 glucose. All extracellular solutions were continuously bubbled with a gas mixture of 95% O_2 + 5% CO_2.

Ex vivo calcium imaging

Slices were loaded with the calcium indicator Cal520^{TM} (AAT Bioquest) as previously described (10), transferred to a submersion-type recording chamber and perfused with warmed (32-34 ºC) ACSF at a rate of 2.7-3.5 ml min-1. Images were acquired with a digital CCD camera (Hamamatsu ORCA-R2 C10600-10B) coupled to a Leica DM-LFSA microscope using 5x or 20x water immersion objectives. For recordings of spontaneous calcium activity, frames were acquired with an exposure time of 150 ms, an interframe interval of 300 ms, a frame size of 672x512 pixel and a spatial resolution of 2.5 µm/pixel. With each slice, 1 to 5 epochs of 15 mins (3000 frames) were recorded. For evoked responses, images were acquired with an interframe interval of 100-250 ms and an exposure time of 50-150 ms. For selective blockage of mGlu5 receptors, 2-Methyl-6-(phenylethynyl)pyridine (MPEP, Tocris #1212) was used.

In vivo calcium imaging

At E18.5, embryos were retrieved from the dam's uterus through C-section, kept at 37 ºC and immobilized with soft clay. At P3-P4, mice were anesthetized in ice and their scalp removed. A 3D-printed plastic holder was glued to the skull with cyanoacrylate adhesive and dental cement and attached to a ball-joint holder to immobilize the head. At P3-P4, pups body temperature was kept at around 25 ºC to provide weak anesthesia. Whisker pad stimulation was performed using a 0.16 g von Frey filament (Touch Test®, BIOSEB). To record the calcium responses, we used a 16-bit CMOS camera (Hamamatsu ORCA-Flash 4.0) coupled to a fluorescence stereo microscope (M165FC, Leica). To visualize the whole head, images were acquired at 2.5x zoom and 3.33Hz frequency of acquisition. The frame size was 2048x2048 pixel (6.67x6.67 μm/pixel) for the E18.5 mice and 1024x1024 pixel (12.82x12.82 μm/pixel) for the P3-P4 mice.

Electrical stimulation

Slices were stimulated with a low resistance glass pipette filled with ACSF (0.6-0.8 kΩ). Single, monopolar current pulses (intensity 25-500 μA, duration 0.2 ms) were delivered by a stimulus isolator (Iso-Flex, AMPI) coupled to a computer trough an A/D converter (Digidata 1550-B, Molecular Devices) and controlled by the pulse-generator tool of pClamp 10.6 software (Molecular Devices). The return was trough a low impedance electrode in the bath.

Whole-cell recordings *ex vivo*

Somatic whole-cell recordings from VPM/subplate/cortical neurons were made under visual control using an upright microscope (Leica DM-LFSA) and a water immersion objective (40x). The intracellular solution contained (in mM): 130 K-gluconate, 5 KCl, 5 NaCl, 0.2 EGTA, 10 HEPES, 4 MgATP and 0.4 NaGTP; pH 7.2 adjusted with KOH; 285-295 mOsm. To mimic the expected higher concentration of intracellular Cl- in early stages of development (30), a high-Cl intracellular solution was employed for thalamic embryonic slices (in mM): 90 K-gluconate, 44 KCl, 1 $CaCl_2$, 2 $NaCl_2$, 11 EGTA, 10 HEPES, 2 MgATP and 0.5 NaGTP. Recordings were obtained in current-clamp and/or voltage-clamp mode with a patch-clamp amplifier

(Multiclamp 700A, Molecular Devices). No correction was made for the pipette junction potential, which was estimated at -10 mV using the junction potential calculator from pClamp. Voltage and current signals were filtered at 2-4 kHz and digitized at 20 kHz with a 16-bit resolution A/D converter (Digidata 1550-B, Molecular Devices). The generation and acquisition of pulses were controlled by pClamp. Patch pipettes were made from borosilicate glass (1.5 mm o.d., 0.86 mm i.d., with inner filament) and had a resistance of 4-7 MΩ when filled. In current-clamp experiments, series resistance (Rs) was measured and balanced on-line under visual inspection assisted by the Bridge Balance tool of pClamp software. Rs was monitored at the beginning and at the end of each protocol, and re-balanced if needed. In voltage-clamp experiments, the error in the measured membrane potential (Ve) was computed as Ve = Ihold × Rs and subtracted off-line from the holding potential (Vhold); Ihold stands for the holding current needed to set Vhold. Recordings with Rs >30 MΩ were discarded. Quantification of intrinsic membrane properties and spontaneous neuronal activity was performed on Clampfit 10.7 (Molecular Devices).

In vivo extracellular recordings

P2-P3 mice were anesthetized on ice, 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 barrel field in S1 (0-0.5 mm posterior to bregma and 1.5-2.0 mm from the midline) or in the area of the VPM (1.8 mm anterior to lambda and 2.0 mm from the midline), 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%). Local field potential (LFP, 1-100Hz) was recorded using 4-shank/16-channel silicon probes, with an inter-electrode distance of 50 μm and an inter-shank distance of 200 μm (E16+R-50-S4-L6-200NT, ATLAS). Multi-unit activity (MUA, >300Hz) was recorded with a linear electrode of 16 channels separated by 50 μm (E16+R-50-S1-L6NT, ATLAS). Shank trajectories were stained with DiI (1,1′-dioctadecyl 3,3,3′,3′-

tetramethylindocarbocyanine perchlorate; Invitrogen) diluted in 70% alcohol. Probes were inserted perpendicular into S1, 300-400 μm deep across the cortical layers for S1 and 2800-3000 μm deep for VPM recordings. The electrical signal activity was sampled at 20 kHz by a filter amplifier and visualized using MC_RACK software (Multi Channel Systems). Whisker stimulation was performed by applying a brief (30 ms) air puff (20 repetitions every 4 seconds). 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 and counterstained with the fluorescent nuclear dye DAPI.

Data analysis was made using MatlabTM routines. The LFP was calculated for each channel and 4 sec epochs of activity were extracted using a threshold-based detection (threshold: 3 times baseline standard deviation). For each epoch detected, the electrode with maximum amplitude in each shank was selected and, among them, the electrode of the shank with the strongest signal was picked to calculate cross-correlations against it. Cross-correlations analysis was performed using a temporal window of +/- 2 seconds and then correlation coefficients were averaged in 250 ms bins. In this way, four values were obtained per mouse, one for the auto-correlation (set as distance = 0 μm) and three for correlations with other shanks (up to 600 μm if the strongest response was in a lateral most shank, number 1 or 4). Population averages were calculated from mice averages.

Analysis of fluorescence spontaneous activity

Analysis of spontaneous thalamic calcium activity was performed using custom software written in Matlab™ adapted from the CalciumDX toolbox (available at https://github.com/ackman678/CalciumDX). For each movie, VPM was delineated and the area divided into a grid of 6x6 pixels where each small square is a region of interest (ROI). Events of calcium activity were detected from the average calcium signal of each ROI as a function of time using threshold-based algorithms modified from CalciumDX. To identify significant synchronous activity and discard ROI co-activation that emanates from random temporal coincidence of calcium events, we created surrogated calcium events sequences in Matlab™ for each experiment. The

alternative dataset was built by shuffling randomly the original temporal intervals between calcium transients in every ROI. In this way, the spiking frequency and temporal structure of the calcium activity was preserved. Next, the maximum value of co-activation from shuffled data was calculated. After 1000 iterations, we were able to define a synchronicity threshold as the 95th percentile of the maximum values of co-activation obtained from the shuffled dataset. In each experiment, only activity above the synchronicity threshold was used for calculations and plotting. The beginning of a synchronic event is defined as the frame in which co-activation overpass the threshold and the end is defined as the frame in which co-activation reached 25% of peak synchronicity.

Analysis of fluorescence evoked activity in slices

Data analysis was performed in ImageJ (NIH). Stimulus-locked temporal stacks of ΔF/F0 were computed using a 1.5 s time window prior to stimulus application (F0). In each slice, 5-8 stimulus intensities were tested (2 pulses with inter-stimulus interval of 30 s for each intensity; 5 min inter-stimulus interval within intensities) and an input/output (I/O) curve was generated. The total area activated was quantified for each stimulus intensity. Threshold intensity was defined for each slice as the minimum stimulation current that elicited a measurable calcium response ≥ 0.01 ΔF/F0 at the response origin site (either the subplate or layer 4, depending on the age). The average threshold intensity across ages and genotypes was 127 ± 8 μA. Slices were discarded if threshold intensity ≥ 300 μA. Peri-threshold stimulation intensity was ≤ 50 μA above threshold. The average peri-threshold intensity was 159 ± 9 μA, 32 ± 4 μA above threshold. In Figure 3C and Figure S6D, two stimulus intensities (within the rising phase of the I/O curve and in the plateau of the I/O curve) were sampled for each slice. Response origin was measured as the ΔF/F0 of a 50 μm width column occupying the entire cortical depth and centred in the cortical response initiation site (ΔF/F0,origin). Horizontal spreading of the response within the cortex was determined by computing the distance from response origin at which ΔF/F0 in the column dropped to 50% [distance (0.5*ΔF/F0,origin)]. This was quantified for both sides from the response initiation, referred as + and – sides. In

some experiments, and due to the thickness of the slice, a calcium signal appears beyond the pial surface.

Analysis of fluorescence signals from *in vivo* experiments

Image analysis was performed on ImageJ (NIH). An average of 3-4 images just prior to the stimulation was used as F0. ΔF/F0. Time-series were transformed into 8-bit images and processed with a 2-pixel diameter Gaussian filter. The maximum intensity projection of all frames with activated cortical areas was obtained. The wand tool was used to delineate the ROI with a perimeter of pixels of the same intensity level. In Figure 4D the white lines delimiting ROI # 3 (pink) represent the maximum extension of the activity, covering 6 frames in the control and 8 frames in the Th^{Kir}.

Dye-tracing studies

For axonal tracing, animals were perfused with 4% PFA in PBS, and their brains dissected out and post-fixed overnight. In order to trace VPM thalamocortical axons, a DiD crystal (1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate salt, Invitrogen) was inserted into the VPM at E17.5. In order to trace the corticothalamic pathway small DiI and DiA (4-[4-(dihexadecylamino) styryl]-N-methylpyridinium iodide, Invitrogen) crystals were inserted under a stereo fluorescence microscope (Leica MZ10 F) into distinct barrels: C1 and C4. To reveal the PBMSF, a TCA-GFP transgenic specific animal line was used. To back-label the thalamocortical pathway, fixed brains were embedded in 3% low melting agarose and cut coronally from caudal to rostral until the VPM was exposed. Dye crystals were then placed in the VPM and allowed to diffuse at 37 °C in PFA solution for 1-4 weeks. Vibratome sections (60-100 μm) were obtained and counterstained with the fluorescent nuclear dye DAPI. Image analysis was performed on ImageJ (NIH). A ROI of 250 μm long x 50 μm width was located in the centre of every barrel analysed in control animals. For Th^{Kir} mice the ROI was located in centre of layer 4. In order to quantify the difference in the fluorescence intensity, the 250 μm analysed were divided in 10 bins of 25 μm each.

Fluorescence signal was normalized in every individual by its maximum. The graph in Figure S14 represents the average fluorescence intensity per bin.

Novelty Exposure experiments

Novelty exposure experiments were done in adult mice (~P60). The day before the experiment, mice were initially habituated to the room (light conditions, space and odorants), to the researcher and for 30 min to the open field empty box. Afterwards, the whiskers of the snout were either completely trimmed or the C2 whisker spared. Next day, the mouse was deposited in the open field box with objects. Mice were free to investigate novel objects for 1 hour while their track was recorded. Immediately after, intracardiac perfusion was carried out and brain dissected and post-fixed overnight. For cFos immunostaining, coronal sections of 50-70 μm were cut in a vibratome or tangential flattened sections obtained by a cryotome.

Statistics

Statistical analysis was carried out using GraphPad Prism6TM, Matlab™ and OriginPro 2018™. Statistical comparison between two populations was performed using unpaired two-tailed Student's t-test with Welch correction (equal variance not assumed) or Mann-Whitney U-Test non-parametric two-tailed test when data failed Kolmogorov-Smirnov normality test. For more than two populations and one factor, one-way ANOVA was used combined with Tukey post hoc analysis. In these analyses, P values < 0.05 were considered statistically significant and set as follows $^{*}P < 0.05$; $^{**}P < 0.01$ and $^{***}P < 0.001$. Dependent variables sampled at different age groups and genotypes were independently compared at each age across genotype with a Student's t-test or the Mann-Whitney U-test followed by the false discovery rate (FDR) procedure (31). In this case, thresholds for significance were computed according to the FDR method and are referred in the "Quantifications" section below. For analysis of the horizontal extension of cortical responses after thalamic or intracortical stimulation, both flanks splitting away from the response initiation site where considered as independent variables. However, when the total extension was used as a single variable (including both sides), the statistical result was not affected. No statistical methods were used to predetermine the sample

size, but the number of samples are considered adequate for our experimental designs and consistent with the literature. The mice were not randomized. The investigators were blinded to sample identity except in calcium activity experiments.

Microdissection, purification of total RNA and quantitative real-time PCR

Tissue microdissection, RNA extraction and qPCR protocols were previously described (10). mGlu5 (Gene ID: 2915) gene expression was determined by the primers 5'-GCACGTAGGCAAGTCATC-3' and 5'-GGGTTCTCCTTCTTGTTGATATGG-3'. The housekeeping gene Gapdh was used as a control, (Gene ID: 14433) and determined by the primers 5' CGGTGCTGAGTATGTCGTGGAGT-3' and 5'- CGTGGTTCACACCCATCACAAA-3'.

Quantifications

Main Figures

In Fig. 1A, stimulus intensity 125 μA, stimulus duration 0.2 ms.

In Fig. 2B, mean event amplitude (ΔF/F0): one-way ANOVA test F = 14.48, ***P <0.001; Tukey's multiple comparison test post-hoc analysis **P < 0.01, ***P < 0.001, ns-not significant, P = 0.785). Mean event frequency: one-way ANOVA test: F = 8.606, **P <0.01; Tukey's multiple comparison test post-hoc analysis *P < 0.05, **P < 0.01; ns. P = 0.608). Event duration: one-way ANOVA test: F = 3.44, P = 0.053. Tukey's multiple comparison post-hoc analysis: control vs Th^{Kir} *P < 0.05, control vs $control_{low}$-synch. P = 0.463 and $control_{low}$-synch. vs Th^{Kir} P = 0.437. In Fig. 2D, one-way ANOVA F = 132.0, *** P < 0.001: Tukey's multiple comparison test: control vs Th^{Kir} state1 ns. P = 0.277; control vs Th^{Kir} state2 ***P < 0.001; Th^{Kir} state1 vs Th^{Kir} state2 ***P < 0.001.

In Fig. 3A, Student's t-test. ns. P = 0.16. In Fig. 3C, left panel, quantification of the horizontal spreading. Bars represent the extent of activation from the start of the field of increased calcium to the lateral edges, measured as the point where fluorescence is 50% with respect to the initiation site. Red points lying beyond the

vertical dashed lines correspond to *Th*^{Kir} calcium waves that spread out of the imaged field (± 750 μm from the origin). Student's t-test or Mann-Whitney test p-value for + side spreading: E17-18 <0.001, P0-1 0.006, P2-3 0.007, P4-7 0.052, and for – side spreading: E17-18 0.005, P0-1 0.005, P2-3 <0.001, P4-7 0.034; all these comparisons are significant after FDR correction for multiple comparisons with the exception of + side P4-7. Right panel: Two-way ANOVA test: $F_{genotype}$ = 19.63, **P <0.01. In Fig. 3F, Two-way ANOVA test: $F_{genotype}$ = 8.91, **P <0.01.

In Fig. 4A, control and *Th*^{Kir}, n = 6. In Fig. 4D, area normalized to controls. Mann-Whitney U-test. In Fig. 4E, control and *Th*^{Kir}, n = 5. In Fig. 4F, control and *Th*^{Kir}, n = 3.

Supplementary Figures

In Fig. S1C, one-way ANOVA test: F = 5.808, ***P <0.001. Tukey's multiple comparison test (post-hoc analysis).

In Fig. S2B, n = 6. In Fig. S2C, left: n = 10, right: n = 3. In Fig. S2D, n = 4.

In Fig. S3B, Student's t-test.

In Fig. S4B, Student's t-test corrected with the FDR procedure for multiple comparisons was employed to assess differences across genotypes (Vm rest Pre: ***P < 0.00032, Ba^{2+}: ns. P = 0.093, Post: ***P < 0.00066; rheobase Pre: *P < 0.032, Ba^{2+}: ns. P = 0.723, Post: ns. P = 0.025; Rin Pre: **P < 0.0032, Ba^{2+}: P = 0.891, Post **P < 0.0066).

In Fig. S5B, control n = 4 and *Th*^{Kir} n = 4. In Fig. S5C, Student's t-test.

In Fig. S6A, Student's t-test or Mann-Whitney U-test corrected with the FDR procedure for multiple comparisons at peri-threshold intensity (Emb: ns. P = 0.141, P0-1: **P < 0.005, P2-3: *P < 0.0375, P4-7: *P < 0.0125). Student's t-test or the

Mann-Whitney U-test corrected with the FDR procedure for multiple comparisons at 500μA (Emb: ns. P = 0.502, P0-1: ns. P = 0.631, P2-3: *P < 0.025, P4-7: *P< 0.0125). In Fig. S6C, Hyperbolic curve fitting using a nonlinear least square regression analysis, fitted parameters from control and *Th*^{Kir} datasets were compared with a global fit using Extra Sum-of-squares F Test method.

In Fig. S6D, Student's t-test or the Mann-Whitney U-test corrected with the FDR procedure for multiple comparisons (+ side Emb: ns. P = 0.192, P0-1: ns. P = 0.452, P2-3: *P < 0.0125, P4-7: *P < 0.025; - side Emb: ns P = 0.173, P0-1: ns. P = 0.180, P2-3: *P < 0.025, P4-7: **P < 0.0025).

In Fig. S7C, stimulation 200 μA. Student's t-test corrected with the FDR procedure for multiple comparisons (Emb: *P < 0.025, P0-1 ns. P = 0.104, P2-3: ns. P = 0.4, P4-5 **P < 0.0025).

In Fig. S8B, Student's t-test. *P < 0.05. In Fig. S8D, Two-way ANOVA test. For cortical area activated P0-2: $F_{genotype}$ = 3.619, ns. P = 0.067; F_{stim} = 13.693, *** P < 0.001; for ΔF/F0 of area activated P0-2: $F_{genotype}$ = 4.277, * P < 0.047; F_{stim} = 5.047, * P < 0.013. For cortical area activated P4-5: $F_{genotype}$ = 37.468, ns. *** P < 0.001; F_{stim} = 2.628, ns. P = 0.089; for ΔF/F0 of area activated P4-5: $F_{genotype}$ = 21.576, *** P < 0.001; F_{stim} = 1.568, ns. P = 0.225; interactions across factors was not significant in any case. In Fig. S8E, Student's t-test corrected with the FDR procedure for multiple comparisons (P0-2 + side: **P < 0.01, P3-5 (+) side: **P < 0.005; P0-2 – side: ns. P = 0.344, P3-5 (–) side: **P < 0.005. In Fig. S8F, n = 3.

In Fig. S9B, Student's t-test. In Fig. S9E, Onset Latency: Two-way ANOVA test and Tuckey's multiple comparisons test; Initial Peak Current, Current Area -70mV and 50mV: Two-way ANOVA test and Sidak's multiple comparisons test for comparing means only between control and *Th*^{Kir}.

In Fig. S10C, Quantification of the horizontal width of the cortical response measured as the length where fluorescence drops 50% with respect to the initiation

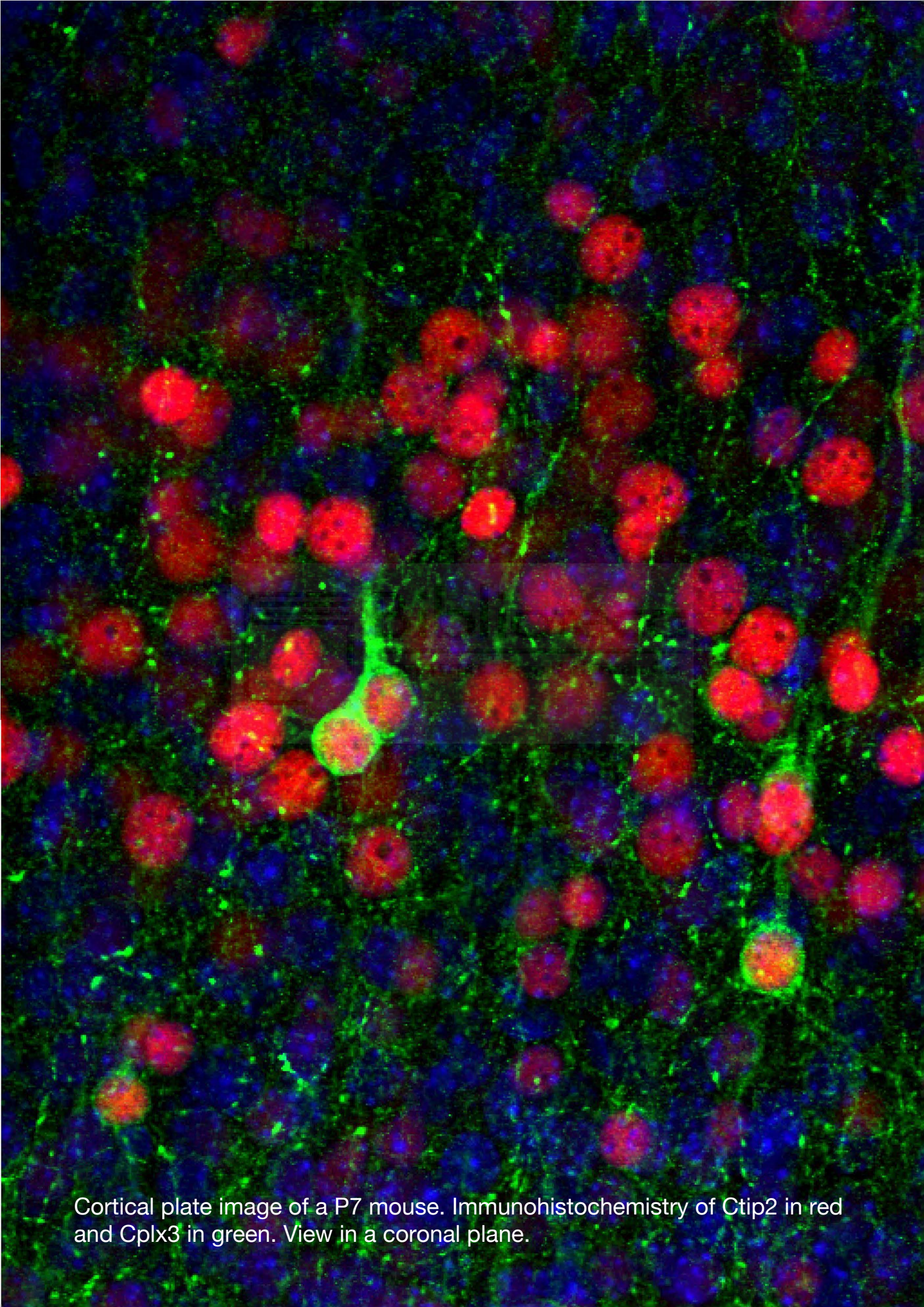
site. Repeated measures two-way ANOVA test and Bonferroni's multiple comparisons test. In Fig. S10D, Student's t-test.

In Fig. S11A, control and Th^{Kir} n = 4. In Fig. S11B, n = 3. In Fig. S11C, control and Th^{Kir} n = 3.

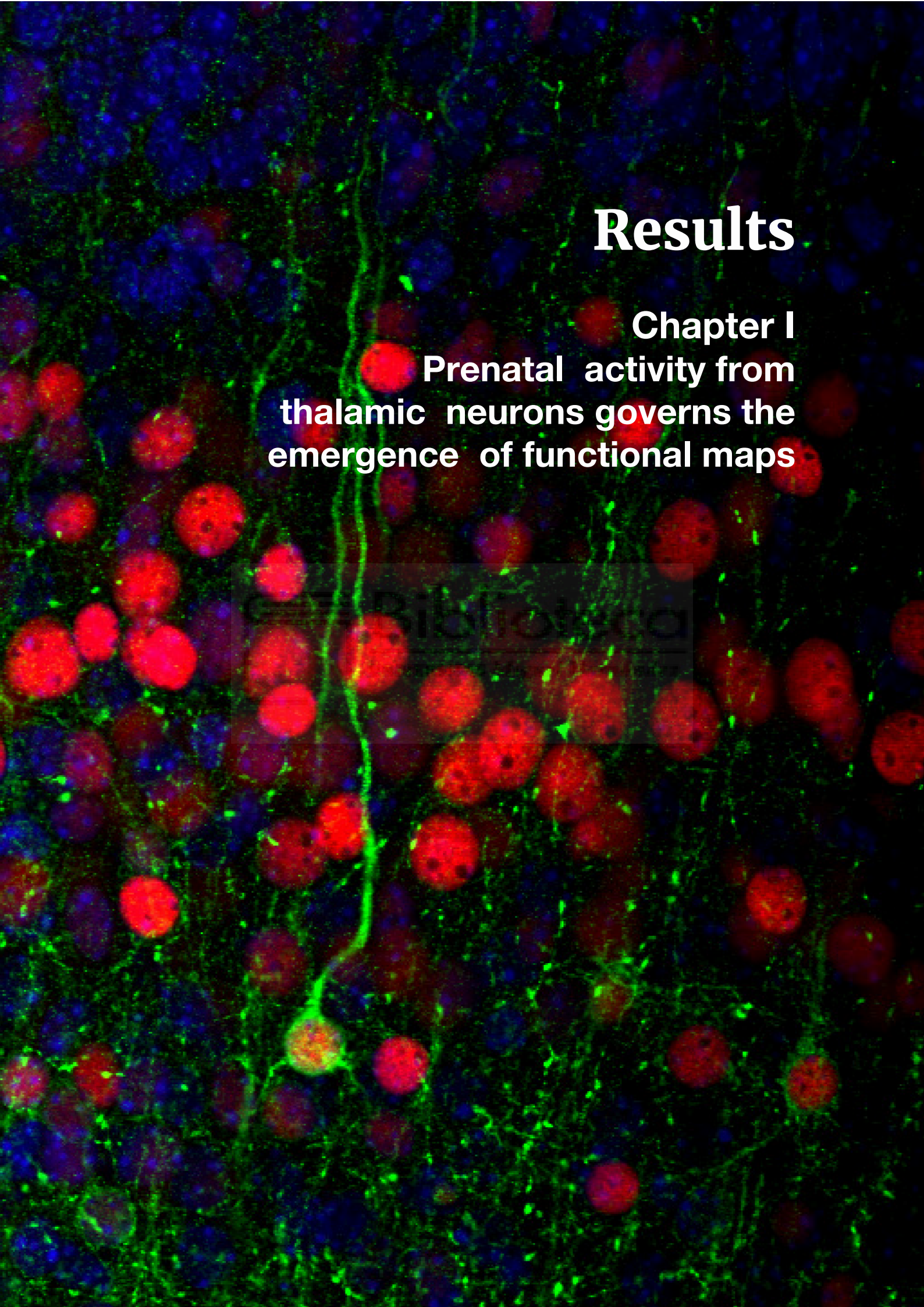
In Fig. S12B, Student's t-test corrected with the FDR procedure for multiple comparisons (Vm P0-1: **P < 0.0075, P2-3: ***P < 0.0003, P6-8: ***P < 0.0005, P15-17: ns P = 0.078; rheobase P0-1: *P < 0.0375, P2-3: ***P < 0.00025, P6-8: *P < 0.05, P15-17: *P < 0.025; Rin P0-1: *P < 0.0375, P2-3: ***P < 0.0003, P6-8: **P < 0.005, P15-17: ns. P = 0.064). In Fig. S12E, maximal responses (Student's t-test, P = 0.494) and latency (Student's t-test, P = 0.4937). In Fig. S12F, Mann-Whitney U-test.

In Fig. S13A, control, n = 8 and Th^{Kir}, n = 6. In Fig. S13C, control, n = 3 and Th^{Kir}, n = 3. In Fig. S13D, control, n = 10 and Th^{Kir}, n = 10.

In Fig. S15A, left: control and Th^{Kir}, n = 5. Right, control and Th^{Kir}, n = 3. In Fig. S15B, control and Th^{Kir}, n = 3. In Fig. S15C, controlC2-whisker and $control_{no\text{-}whiskers}$ n = 3.



Cortical plate image of a P7 mouse. Immunohistochemistry of Ctip2 in red and Cplx3 in green. View in a coronal plane.



Results

Chapter I
Prenatal activity from thalamic neurons governs the emergence of functional maps

Chapter I

Prenatal activity from thalamic neurons governs the emergence of functional cortical maps

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Prenatal activity from thalamic neurons governs the emergence of functional cortical maps in mice

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Abstract

The mammalian brain's somatosensory cortex is a topographic map of the body's sensory experience. In mice, cortical barrels reflect whisker input. We asked whether these cortical structures require sensory input to develop or are driven by intrinsic activity. Indeed, thalamocortical columns, connecting thalamus to cortex, emerge before sensory input and concur with calcium waves in the embryonic thalamus. We show here that the columnar organization of the thalamocortical somatotopic map exists in the mouse embryo before sensory input, thus linking spontaneous embryonic thalamic activity to somatosensory map formation. Without thalamic calcium waves, cortical circuits become hyperexcitable, columnar and barrel organization do not emerge, and the somatosensory map lacks anatomical and functional structure. Thus, a self-organized protomap in the embryonic thalamus drives functional assembly of murine thalamocortical sensory circuits.

The mammalian cerebral cortex is arranged into radial columns that coalesce during development. These columns become functionally organized before adulthood (1-3). Some evidence suggests that genetic factors regulate initial columnar patterning (4); other evidence suggests functional maps arise postnatally as a result of sensory experience (5-9). However, spatially organized patterns of spontaneous activity are evident in the embryonic thalamus, before cortical neurons have completed their radial migration (10). One well-studied functional map is the somatotopic correspondence between whiskers and their associated clusters of layer 4 neurons (called barrels) in the rodent primary somatosensory cortex (S1) (11). Although barrels are apparent anatomically at postnatal day 4 (P4) (12), domains of spontaneously co-activated neurons can be identified at birth in S1 *in vivo* (13-15). We asked whether the emergence of anatomically discernable structures is preceded by organized activity in the mouse embryo. We discovered that structured patterns of neuronal activity in the embryonic thalamus define functional cortical columns and the concomitant functional somatotopic map in the immature cortex.

The functional properties of embryonic thalamocortical connections were assessed by recording the somatosensory cortical calcium responses elicited by the activation of the ventral postero-medial nucleus (VPM) of the thalamus in slices. By embryonic day 17.5 (E17.5), electrical stimulation of the VPM triggered calcium waves that propagated over a large area of the nucleus, resembling previously reported spontaneous activity (10). This thalamic stimulation elicited a cortical calcium response in the S1 (Fig. 1A and B, fig. S1A; movie S1). While activation of thalamocortical axons is confined to the subplate at this stage (fig. S1B), the cortical response spanned the entire thickness of the cortical plate, suggesting that thalamocortical axons activate a radially organized cortical network. From E18.5 onwards, VPM stimulation activated a progressively restricted territory within the nucleus (fig. S1C), allowing us to define the functional topography of the nascent thalamocortical projection. Peri-threshold stimulation of adjacent regions in the VPM activated distinct columnar territories in the cortex (Fig. 1, C and D), indicating the existence of a functional protomap present in these embryonic thalamocortical circuits. This was evaluated *in vivo* by transcranial calcium imaging of glutamatergic

cortical neurons at E18.5. Mechanical stimulation of juxtaposed areas of the whisker pad activated discrete, segregated and spatially consistent cortical territories in the contralateral S1 (Fig. 1, E and F; movie S2), confirming the existence of a cortical somatosensory protomap in the intact embryo.

We then tested whether embryonic thalamic calcium waves influence the emergence of the functional cortical columns that presage the formation of the somatotopic barrel map. To change the normal pattern of spontaneous thalamic activity, we crossed a tamoxifen-dependent *Gbx2*^{CreERT2} mouse with a floxed line expressing the inward rectifier potassium channel 2.1 (Kir) fused to the mCherry reporter (fig. S2) (10). In this model (*Th*^{Kir} hereafter), 78% of the VPM neurons express Kir-mCherry protein upon tamoxifen administration at E10.5 (fig. S2). In control slices, more than half of the spontaneous synchronous events in the VPM corresponded to large amplitude-highly synchronized calcium waves, whereas the remaining activity reflected low amplitude, poorly synchronized events. The highly synchronized waves were not detected in the *Th*^{Kir} mice, in which only small amplitude and mostly asynchronous activity persisted, although at a higher frequency than in controls (Fig. 2, A-C; movies S3 and S4). Collectively, Kir overexpression shifted the pattern of spontaneous activity in the thalamus from synchronized waves to asynchronous activity.

At the cellular level, while control neurons were relatively depolarized at E16.5, *Th*^{Kir} cells displayed a bi-stable pattern of activity with spontaneously alternating periods of hyperpolarized and depolarized membrane potential (Fig. 2D and fig. S3). Action potentials were generated in the depolarized phase in both control and *Th*^{Kir} cells. This change in the electrical properties of the *Th*^{Kir} neurons was sufficient to impede the generation of calcium waves. Indeed, barium, an ion that blocks Kir channels (16), reversed the electrophysiological profile of *Th*^{Kir} neurons, recovering the wave-like activity in *Th*^{Kir} VPM networks (fig. S4 and movie S5). Thus, while there were no propagating calcium waves in the thalamus of *Th*^{Kir} mice, the preservation of its asynchronous activity meant it was not silent.

We analyzed how altering the pattern of spontaneous thalamic activity in our *Th*^{Kir} model affected the functional columnar organization in S1. Peri-threshold VPM stimulation in E17.5-E18.5 slices from control mice triggered a columnar-like cortical response (fig. S5A and movie S6). Conversely, this stimulation in ThKir slices consistently elicited a broader (laterally) cortical calcium wave (fig. S5A; movies S7 and S8). Despite these differences, the subplate was the earliest cortical compartment activated in both control and *Th*^{Kir} mice, followed by the upper cortex (fig. S5, B-D). Next, we tested whether the emergence of the functional topographic map was affected in the *Th*^{Kir} mice. Unlike the controls, stimulation of adjacent regions in the VPM in the *Th*^{Kir} slices activated highly overlapping territories in the cortex (Fig. 3A), indicating that the topographical representation of the thalamocortical circuit does not emerge in the absence of embryonic thalamic waves. Postnatally, the cortical response to VPM stimulation narrowed progressively with time in control slices, coinciding at P4 with the dimensions of the cortical barrel, yet this spatial restriction occurred to a lesser extent in the *Th*^{Kir} mice (Fig. 3, B and C; movies S9 and S10). These differences were observed irrespective of the stimulation strength (fig. S6).

The extended cortical activation in the *Th*^{Kir} mice was not due to more extensive activation of the VPM (fig. S7), yet it was associated with increased levels of intrinsic cortical excitability. This was reflected by the high frequency of spontaneous cortical waves in *Th*^{Kir} slices (fig. S8, A and B) and the widespread cortical response to intracortical stimulation (fig. S8, C-E). Next, we tested whether this change in cortical network excitability occurred in the *Th*^{Kir} mice *in vivo*. Because cortical travelling waves were associated with action potentials bursts (fig. S8F), we recorded extracellular cortical activity with multichannel electrodes. We found extensive spontaneous events of synchronous activity spreading horizontally in the *Th*^{Kir} mice at P2-P3 (Fig. 3, D-F), consistent with the hyperexcitability observed *ex vivo*. Then, we analyzed the possible origin for this excitability and found that the amplitude of the calcium response was the same in the subplate but larger in the upper cortex in the *Th*^{Kir} mice (fig. S9), suggesting a local alteration in the upper

cortical network. As metabotropic glutamate receptors (mGluRs) participate in the propagation of cortical spontaneous activity in newborn rodents (17, 18), we tested whether mGluRs could be involved in the hyperexcitability of cortical networks in Th^{Kir} mice. Bath application of MPEP (100 µM), a mGlu5 specific antagonist, rescued the activation of the thalamocortical-induced cortical network into a column-like domain in Th^{Kir} mice. Albeit MPEP decreased the overall signal intensity in both conditions, it had no effect in the width of the cortical response in controls (fig. S10, A-C). These results are consistent with increased expression of cortical mGlu5 in the Th^{Kir} mice at P0 (fig. S10D). Together, these data reveal that the emergence of functional columns and somatotopic map in the S1 relies on thalamic control of cortical excitability, implicating mGluRs.

To ascertain whether embryonic thalamic activity and functional columns are a pre-requisite to establish the postnatal anatomy of the barrel map, we examined thalamocortical axons clustering in the Th^{Kir} mice in which this projection was labelled by GFP (19). The barrel map was evident at P4 in control mice (12, 20) but no barrels were detected in tangential or coronal sections of Th^{Kir} mice, where thalamocortical axons targeted the layer 4 but did not segregate into discrete clusters (Fig. 4A and fig. S11). Furthermore, there was no arrangement of layer 4 cells into barrel walls in the Th^{Kir} mice. The absence of barrels did not seem to originate from the loss of neurotransmitter release (21, 22), as thalamic neurons in the Th^{Kir} fire action potentials and activate synaptic currents in cortical cells (fig. S4A, S9D and S12, A-C), and respond normally to whisker stimulation *in vivo* (fig. S12, D and E).

The disrupted barrel map in Th^{Kir} mice could reflect altered point-to-point connectivity at several subcortical levels (8, 23, 24). However, the organization of brainstem barrelettes and thalamic barreloids in the Th^{Kir} mice was normal (fig. S13). Since the barrel map ultimately relies on the specific topographic organization of thalamocortical axons (25), we explored whether some spatial segregation was conserved in the Th^{Kir} mice. While dye deposition in barrels C1 and C4 back-labeled

cells in the corresponding barreloids of control mice, the back-labeled territories in *Th*^{Kir} mice were more extensive, including cells located in neighboring barreloids (Fig. 4, B and C). Anterograde tracing from single barreloids also revealed a broader horizontal disposition of thalamocortical axons in the layer 4 of the *Th*^{Kir} mice (fig. S14). Finally, we determined how this aberrant topographic map generated by the lack of thalamic calcium waves affected the relay of sensory stimuli in early postnatal mice *in vivo*. While stimulation of distinct points on the whisker pad at P3-P4 activated discrete barrel-like patches in the control S1, similar stimulations of *Th*^{Kir} mice led to enlarged responses in the barrel-field (Fig. 4D; movies S11 and S12). Together, these data demonstrate that the postnatal anatomic clustering of thalamocortical axons and the somatotopic functional map is disrupted in the absence of embryonic thalamic waves.

As the critical period of thalamocortical plasticity in the S1 closes between P3 and P7 in rodents (6, 20, 26), we assessed whether the loss of columnar organization in the *Th*^{Kir} mice could be overcome by sensory experience. The loss of barrel organization and the lack of a precise functional map persisted in adult *Th*^{Kir} mice, as indicated by vGlut2 staining and the unrestrained cortical activation of cFos (Fig. 4E and fig. S15). The thalamus of *Th*^{Kir} mice retained a normal functional topography when whiskers were stimulated (Fig. 4F). Hence, the natural period of somatosensory-driven plasticity cannot overcome the altered organization that occurs in the embryo.

Our data reveals that embryonic patterns of thalamic activity organize the architecture of the somatosensory map. We show that the development of this map involves the emergence of functional cortical columns in embryos, driven by spontaneous thalamic wave-like activity. These embryonic columns display spatial segregation and somatotopic organization, despite the immature state of the cortical sheet in which they materialize. We propose that patterned activity in pre-cortical relay stations during embryonic stages prepares cortical areas and circuits for upcoming sensory input. As thalamic waves are not exclusive to the

somatosensory nucleus but propagate to other sensory nuclei (e.g., visual or auditory (10)), the principles of cortical map organization described here might be common to other developing sensory systems.

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List of Supplementary Materials

Materials and Methods
Figs. S1 to S15
Movies S1 to S12
References (10, 19, 27-31)

Figure Legends

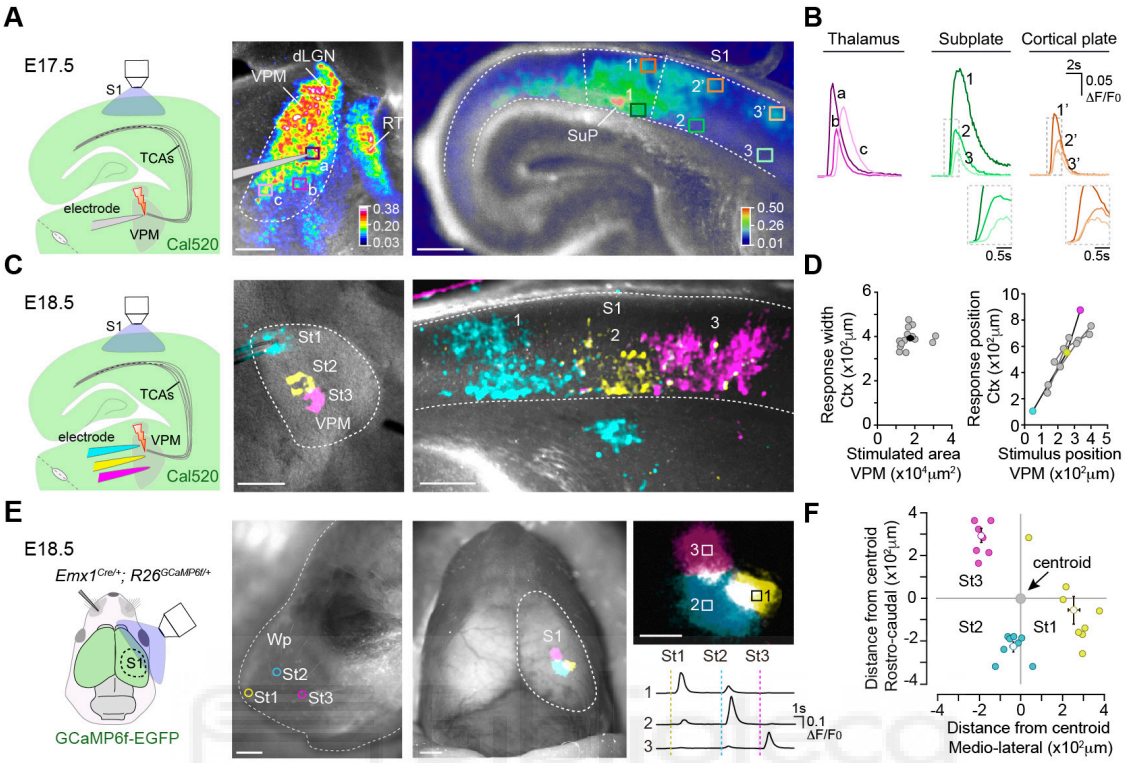

Fig.1. Embryonic thalamocortical stimulation reveals an organized prenatal cortical map. (A) Experimental design. Maximal projection of the calcium responses (ΔF/F0, color coded) in the ventral postero-medial nucleus (VPM) and cortex after VPM stimulation at E17.5. **(B)** Calcium transients from boxes in A. **(C)** Experimental design. Maximal projection of cortical responses after stimulation of three adjacent VPM regions at E18.5. **(D)** Plot of stimulated VPM area versus cortical response width (black dot equals mean value). Right: Plot of the stimulus position in the VPM versus the cortical response location (n = 16). Colored circles represent the data in C. **(E)** Experimental design. Cortical calcium responses elicited by mechanical stimulation of three contralateral whisker pad (Wp) sites (St1-St3) at E18.5. Right: High-magnification and transients recorded in each ROI (boxes 1-3). **(F)** Plot of the position of each cortical response relative to the centroid of the activated area (n = 8). dLGN, dorso-lateral geniculate nucleus; RT, reticular thalamus; SuP, subplate; TCAs, thalamocortical axons. Scale bars, 200 µm in **A**; 1 mm in **E** (left/middle) and 500 µm in **E** (right).

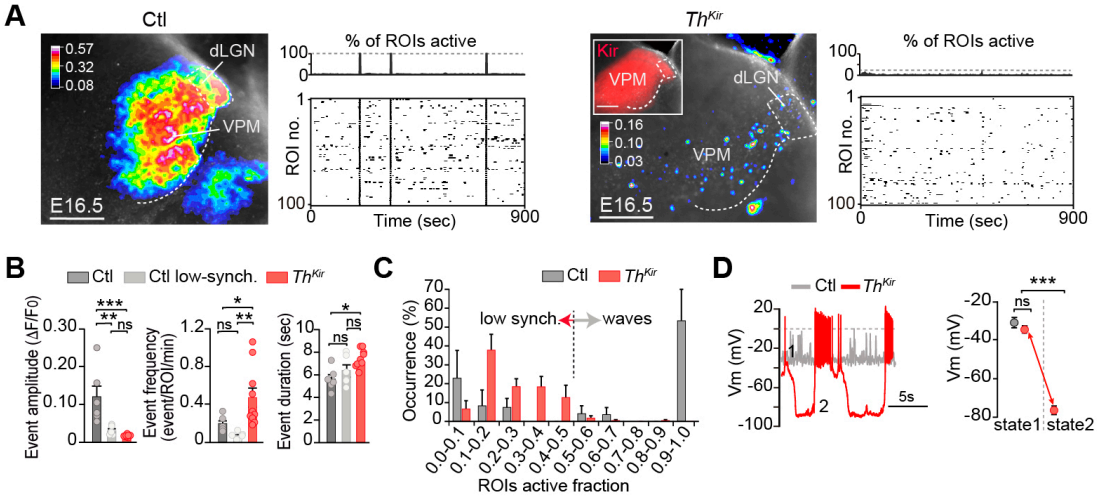

Fig.2. Desynchronizing the embryonic thalamic pattern of activity. (A) Maximal projection of *ex vivo* spontaneous calcium activity in the ventral postero-medial nucleus (VPM) and accompanying raster plots in control and Th^{Kir} slices at E16.5. **(B)** Properties of the VPM calcium events (n = 6 control, n = 10 Th^{Kir}; *P < 0.05, **P < 0.01, ***P < 0.001). **(C)** Percentage distribution of active ROIs. (D) Representative traces and quantification of membrane potential (Vm) in control and Th^{Kir} neurons recorded at E16.5-E18.5 (control n = 7; Th^{Kir} n = 7). ***P < 0.001. dLGN, dorso-lateral geniculate nucleus. Scale bars, 200 µm. Data are means ± SEM.

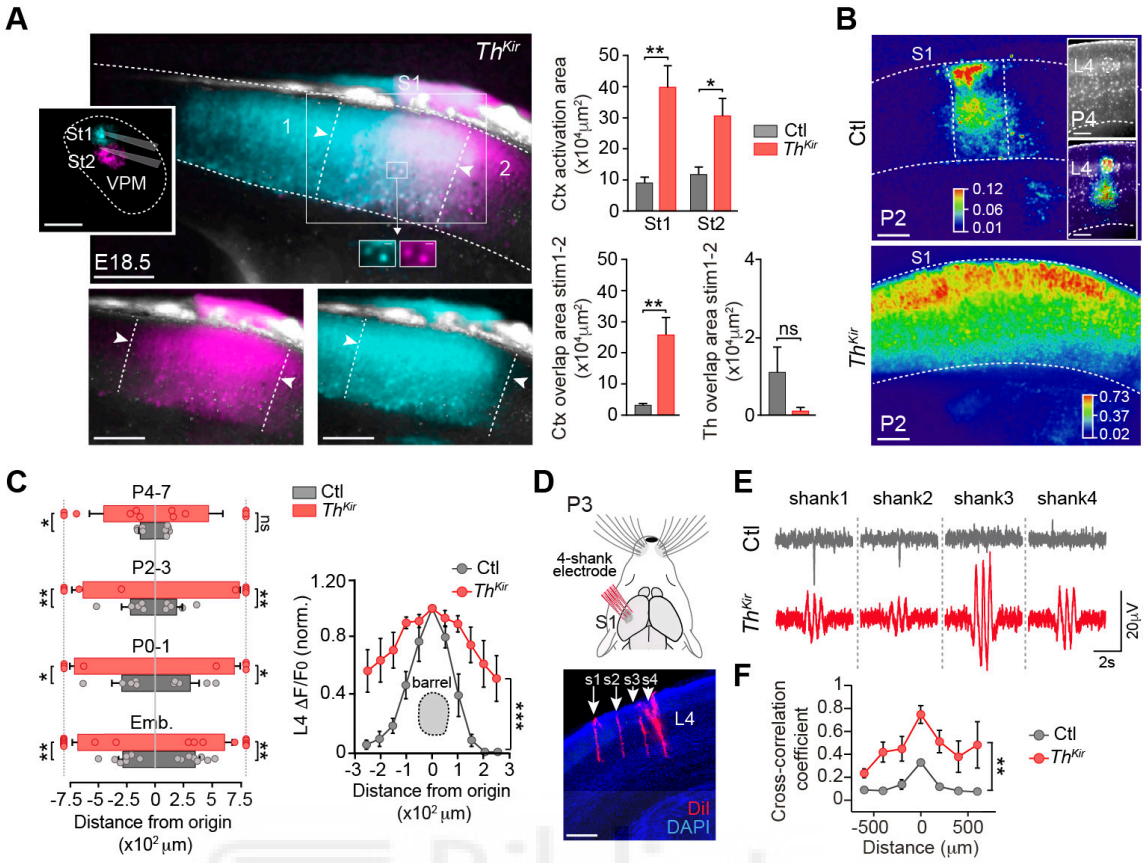

Fig.3. Loss of functional cortical pre-barrel columns in the *Th*^{Kir} mice. **(A)** Maximal projection of cortical responses after stimulation of two adjacent ventral postero-medial (VPM) regions in *Th*^{Kir} slices. Right: Quantifications of the activated area (n = 6 control, n = 6 ThKir; *P < 0.05, **P < 0.01). **(B)** Cortical activation elicited by VPM stimulation at P2 (inset: P4) in control and *Th*^{Kir} slices. **(C)** Quantification of the horizontal spread of the cortical response (E17-18 n = 8 control, n = 9 *Th*^{Kir}; P0-1 n = 5 control, n = 4 *Th*^{Kir}; P2-3 n = 5 control, n = 5 ThKir; P4-7 n = 5 control, n = 6 *Th*^{Kir}). Right: Same in layer 4 at P4-P7 (n = 6 control, n = 6 *Th*^{Kir}; **P < 0.01). **(D)** Experimental design and coronal image showing the 4-shank (s1-s4) electrode insertion in S1 (red). **(E)** Representative *in vivo* recordings of spontaneous cortical network activity. **(F)** Quantification of the cross-correlation coefficient among shanks in control (n = 3) and *Th*^{Kir} mice (n = 6). **P < 0.01. Scale bars, 200 µm. Data are means ± SEM.

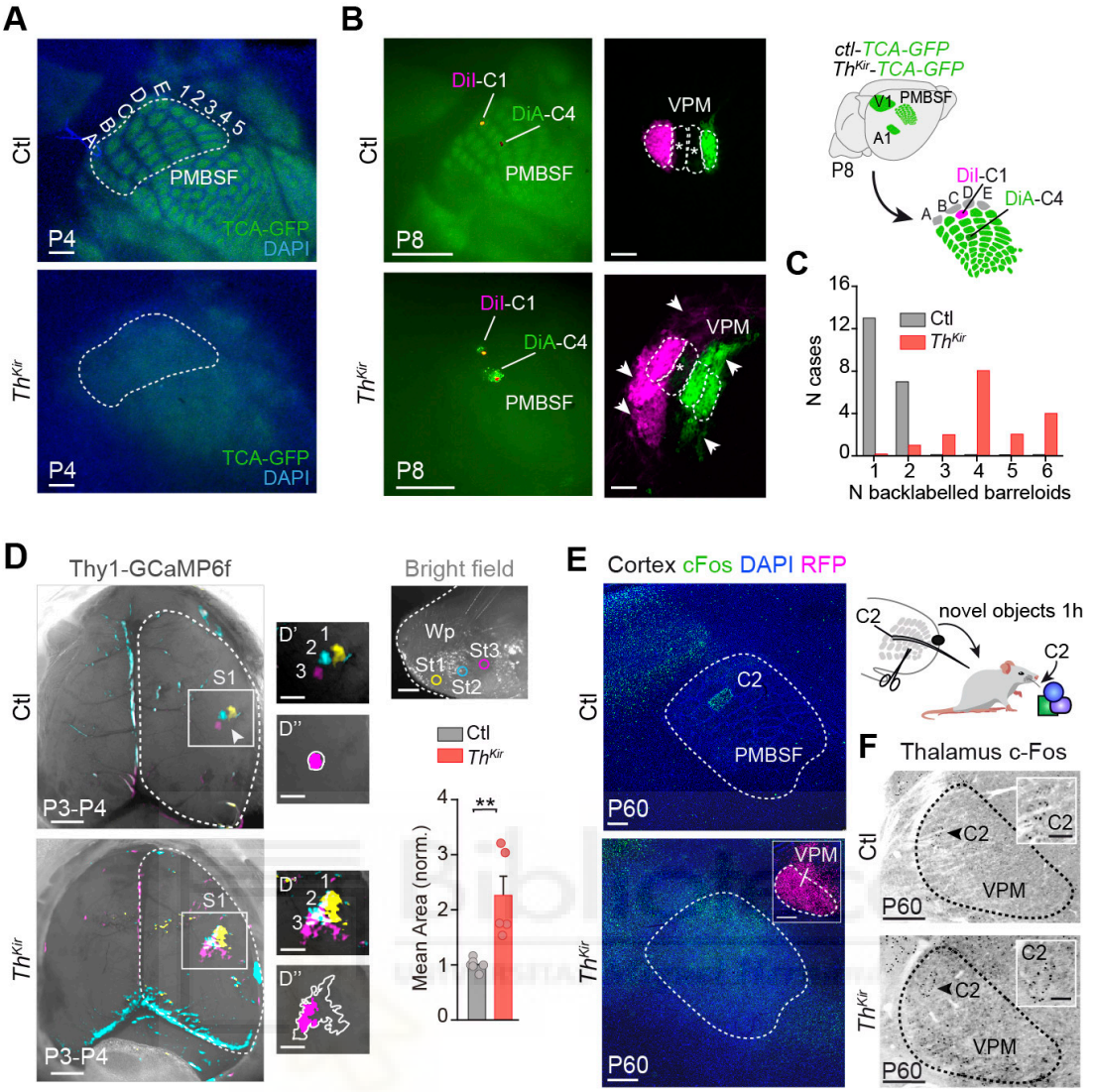

Fig.4. Long-term anatomical and functional changes in S1 of the *Th*^{Kir} mice. (A) Tangential sections showing the postero-medial barrel subfield (PMBSF) in control and *Th*^{Kir} TCA-GFP mice at P4. **(B)** Experimental design and images showing PMBSF injection sites and back-labeled barreloids in the ventral postero-medial nucleus (VPM). **(C)** Quantification of data shown in B (n = 10 control, n = 10 *Th*^{Kir}). **(D)** Maximal projection of the *in vivo* contralateral cortical responses elicited by mechanical stimulation of three whisker pad (Wp) sites at P3-P4 (top right). D': high-power views. D'': drawing of initial (pink) and maximal (outline) extension of representative responses. Bottom-right: Quantification of the data (n = 6 control, n = 5 *Th*^{Kir}; **P < 0.01). **(E)** Experimental design and cortical cFos immunostaining. (F) VPM cFos immunostaining. Scale bars, 300 µm in **A**, **B** right, **E** and **F** (insets 100 µm); 1 mm in **B** left and D (insets 500 µm). Data are means ± SEM.

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Supplementary Materials for

Prenatal activity from thalamic neurons governs the emergence of functional cortical maps in mice

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This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at science.sciencemag.org/cgi/content/full/science.aav7617/DC1)

Movies S1 to S12

Supplementary Figures and Figures Legends

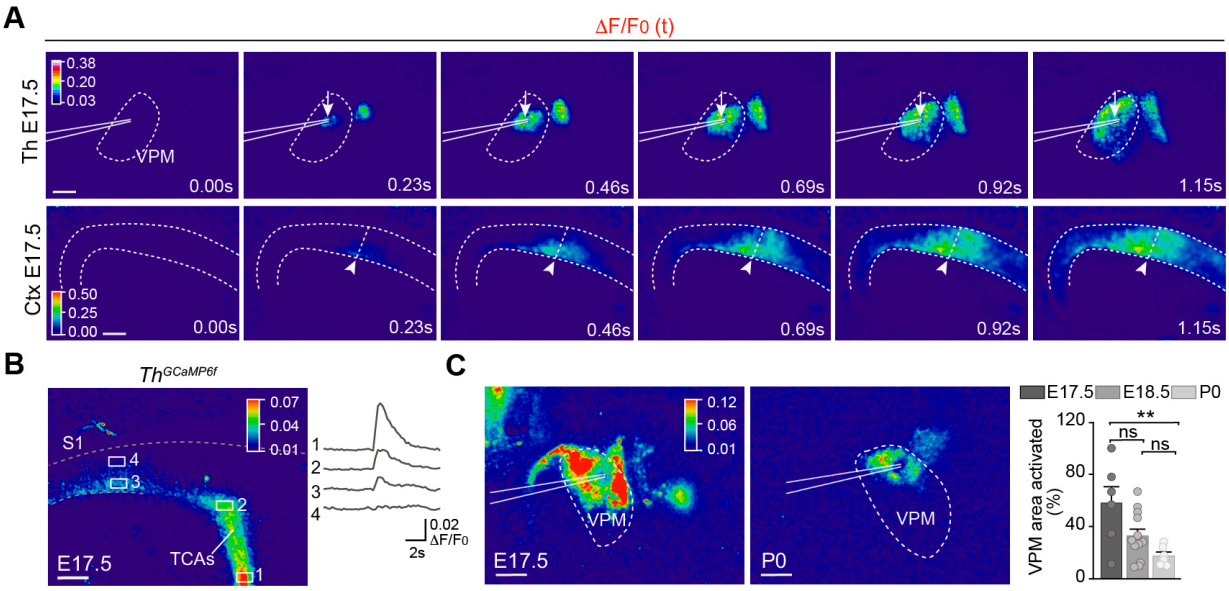


Fig.S1. Time course of thalamic and cortical activation after ventral postero-medial nucleus (VPM) stimulation. **(A)** Propagation of thalamic and cortical calcium responses evoked by a 125 µA pulse in the VPM of a E17.5 slice loaded with Cal520 (same example as in Figure 1A). The response origin is indicated with an arrow in the thalamus and an arrowhead in the cortex. **(B)** Maximal projection of the calcium response in thalamocortical axons after VPM stimulation (300 µA) in a *Th*^{GCaMP6f} mouse at E17.5. **(C)** Thalamic calcium responses elicited after VPM stimulation in an E17.5 and P0 mice. Quantification of the data shown in left panels (E17.5 n = 6 slices from 5 mice; E18.5 n = 12 slices from 10 mice; P0 n = 6 slices from 5 mice. E17.5 vs. E18.5: ns. P = 0.061, E18.5 vs. P0: ns. P = 0.329, E17.5 vs. P0: **P < 0.01). S1, primary somatosensory cortex. Scale bars, 200 µm. Data are means ± SEM.

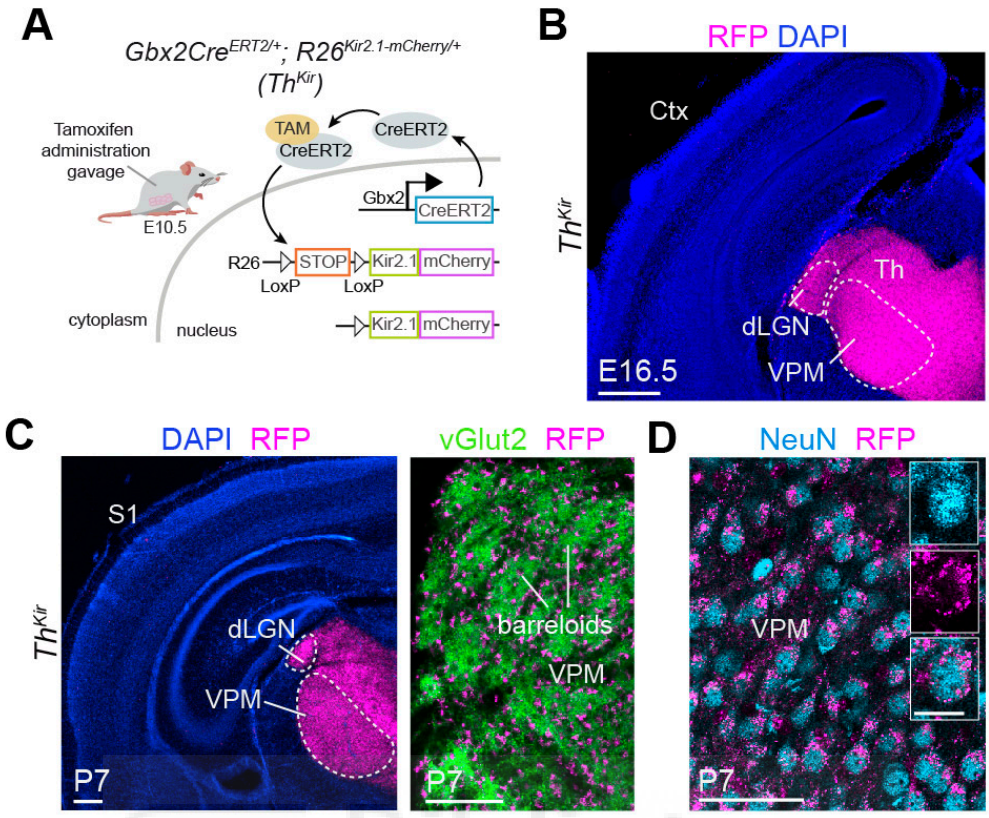


Fig.S2. Selective overexpression of Kir2.1 in the thalamus in the *Th*^{Kir} mouse. (A) Scheme of the genetic conditional overexpression of Kir2.1 in thalamic neurons upon tamoxifen (TAM). Kir2.1 is fused to the mCherry reporter protein. **(B)** Coronal section at E16.5 showing *Kir2.1-mCherry* expression after tamoxifen administration at E10.5. Recombination is restricted to the thalamus (Th) within the forebrain and absent in the cortex (Ctx). **(C)** Coronal sections showing Kir2.1 overexpression (RFP) in the *Th*^{Kir} mice and in barreloids immunostained with vGlut2 at P7. **(D)** RFP-positive neurons immunostained with NeuN in *Th*^{Kir} thalamus at P7. dLGN, dorsal-lateral geniculate nucleus; VPM, ventral postero-medial nucleus. S1, primary somatosensory cortex. Scale bars, 300 µm in **B** and **C** (left); 100 µm in **C** (right); 50 µm in **D** (insets: 12.5 µm).

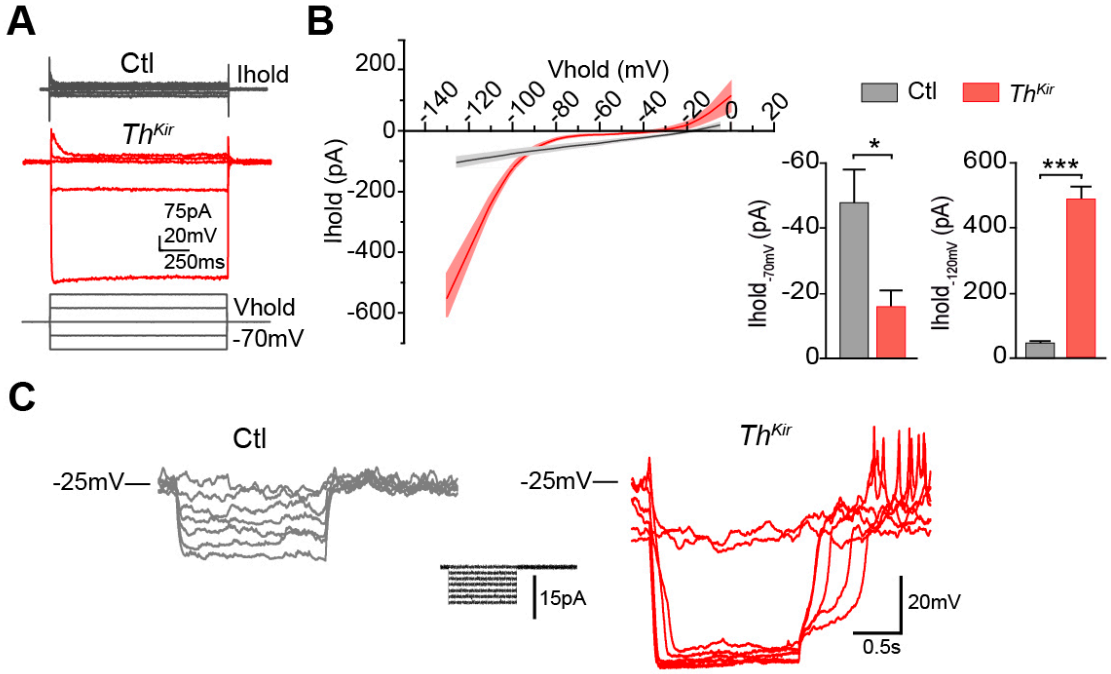


Fig.S3. ***Ex vivo*** **electrophysiological properties of** ***Th^Kir*** **ventral postero-medial (VPM) neurons at E16.5.** **(A)** Responses to a family of voltage steps from a holding potential of -70 mV in a control and a *Th^Kir* neuron. **(B)** Current-voltage relationship (I-V curve) in control and *Th^Kir* thalamic cells. Cells were held at -70 mV and pulses of 1500 ms in 10 mV steps increment were delivered (range -120 to -10 mV). Notice the strong inward rectification in the *Th^Kir* sample, as expected for cells expressing a high Kir2.1 conductance. Right: Quantification of the holding current required to keep Vm at -70 mV and -120 mV in control (n = 5) and *Th^Kir* cells (n = 7). *P < 0.05, ***P < 0.001. **(C)** Injection of low-amplitude (< 20 pA) current pulses induced Vm oscillations in embryonic *Th^Kir* neurons. Data are means ± SEM.

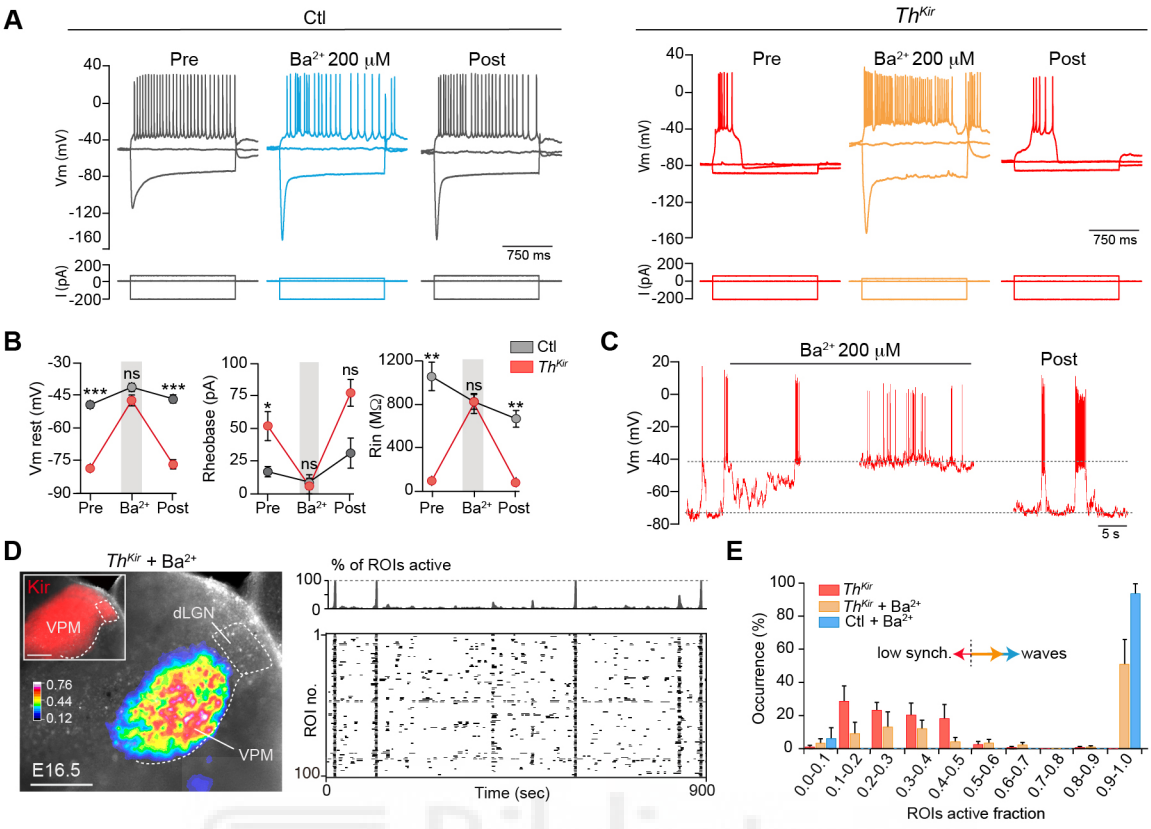


Fig. S4. Blocking Kir2.1 conductance with barium reverts the electrophysiological properties and calcium wave activity in the *Th*^{Kir} mice. **(A)** Representative examples of the membrane voltage (Vm) responses to a family of current steps in P3 control and *Th*^{Kir} neurons perfused with ACSF (pre) and ACSF plus barium Ba^{2+}; 200 µ M), and after washout (post). **(B)** Quantification of the effects of Ba on the resting Vm, rheobase and input resistance (Rin). Control, n = 5 and *Th*^{Kir}, n = 5 neurons from P0-P8; ns. not significant; *P < 0.05, **P < 0.01, ***P < 0.001. **(C)** Representative traces of the effect of Ba^{2+} (200 µM) on the Vm in *Th*^{Kir} neurons. **(D)** Representative example of the effect of Ba2+ (50 µM) on the spontaneous calcium activity in the *Th*^{Kir} mice. Calcium waves reappear when Kir channels are blocked. **(E)** Frequency distribution of the active ROI fraction in the VPM in control + Ba^{2+}, *Th*^{Kir} and *Th*^{Kir}+ Ba^{2+}. Data were obtained from n = 4, n = 7 and n = 5 experiments, respectively. Scale bar, 200 µm. Data are means ± SEM.

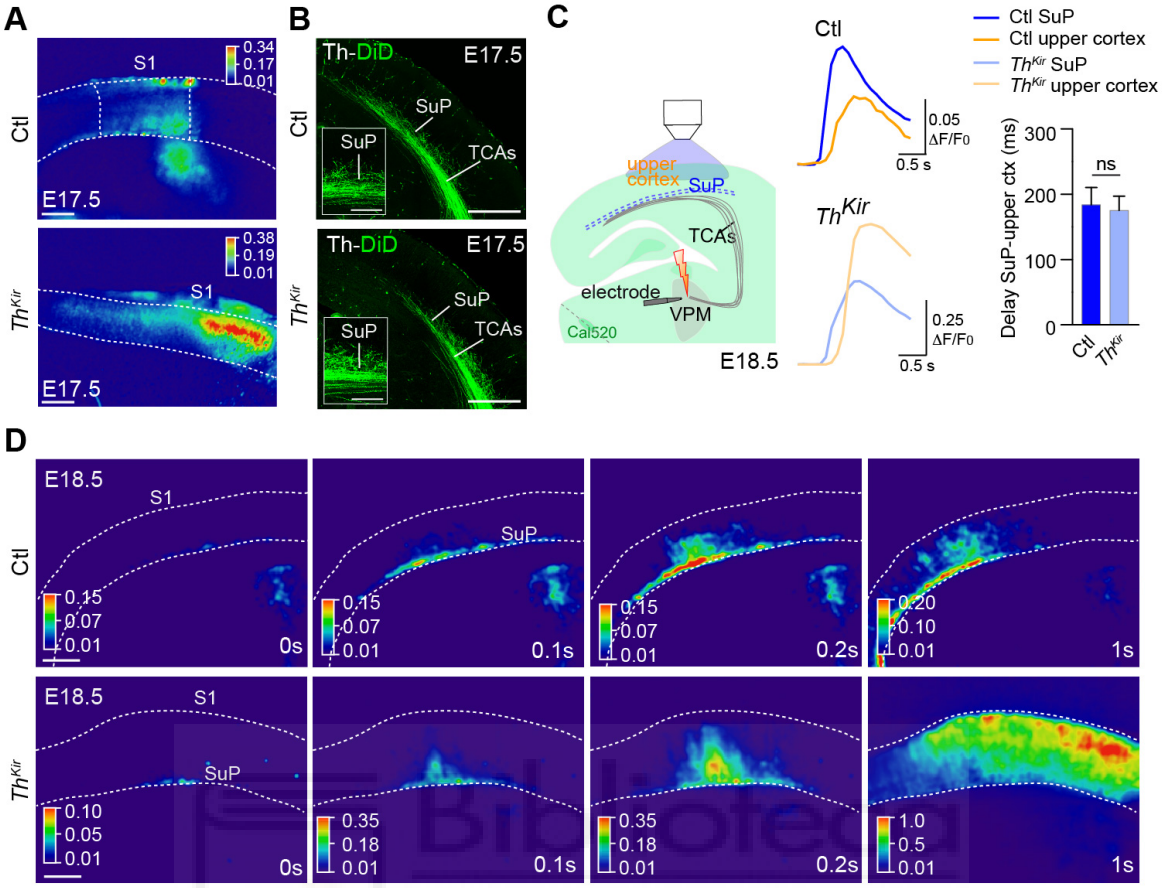


Fig.S5. Widespread embryonic cortical activity and subplate/upper cortex activation delay. **(A)** Maximal projection of the cortical activation elicited by peri-threshold ventral postero-medial nucleus (VPM) stimulation in control and *Th*^{Kir} slices at E17.5 **(B)** Coronal sections showing thalamocortical axons (TCAs) labeled with DiD at E17.5 in control and *Th*^{Kir} mice. **(C)** Experimental design and representative calcium traces. Cortical activation occurs first at the subplate (SuP) level followed by the upper cortex in both control and *Th*^{Kir} mice. Quantification of the delay in both conditions (n = 4 control, n = 7 *Th*^{Kir}, ns. not significant P = 0.806). **(D)** Temporal sequence of cortical activation in the SuP followed by the upper cortex in both control and *Th*^{Kir} mice. S1, primary somatosensory cortex. Scale bars, 200 µm. Data are means ± SEM.

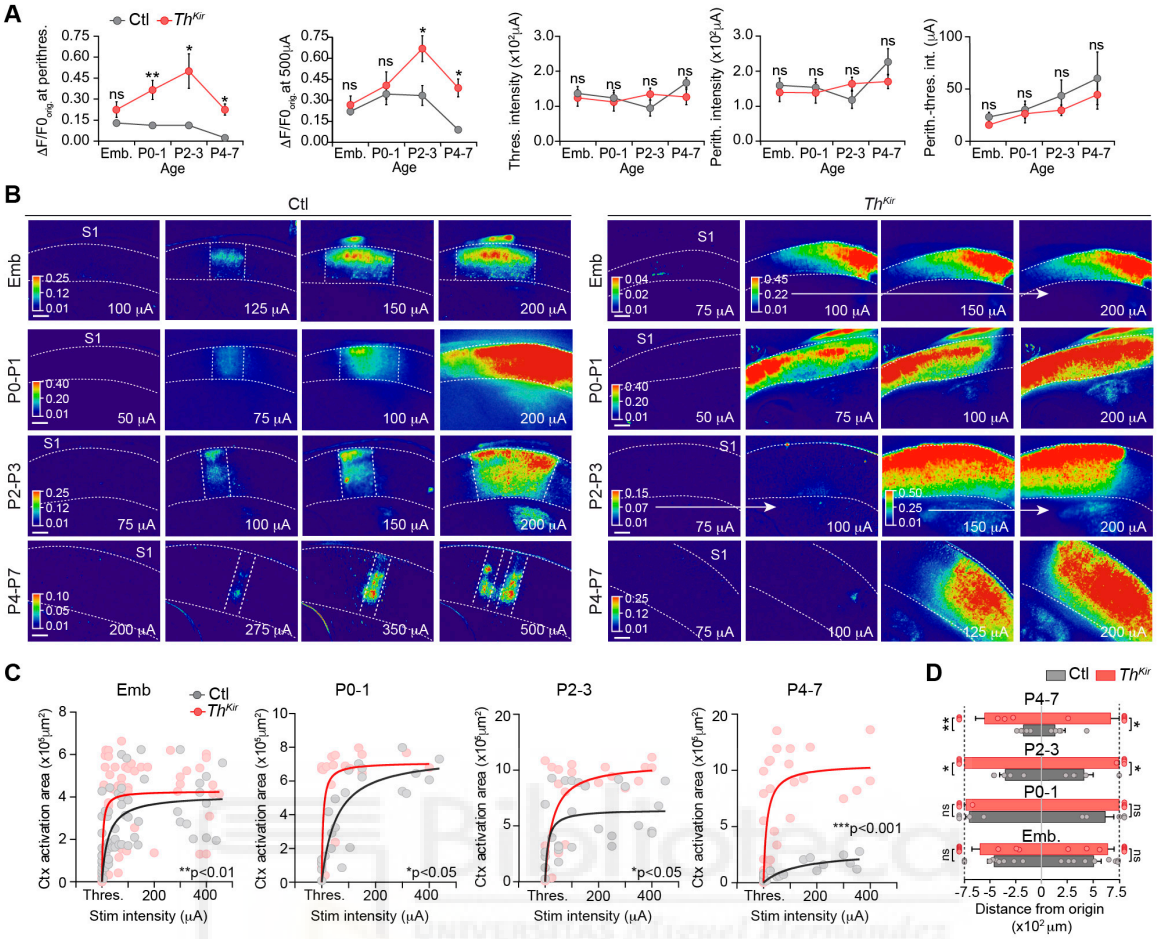


Fig.S6. The development of functional cortical pre-barrel columns is hindered in the ThKir mice. **(A)** Quantification of the developmental profile of cortical calcium signals measured at response origin (ΔF/F0 at origin) after peri-threshold and maximal ventral postero-medial nucleus (VPM) stimulation in slices (E17.5 n = 9 control and n = 9 *Th*^{Kir}; P0-1 n = 5 control and n = 4 *Th*^{Kir}; P2-3 n = 5 control and n = 5 *Th*^{Kir}; P4-7 n = 6 control and n = 7 *Th*^{Kir}). Quantification of the threshold, peri-threshold and peri-thershold minus threshold intensities (see "Quantification" section for p values). **(B)** Maximal projection of the cortical response evoked by VPM stimulation at increasing intensities within the same slice at distinct developmental stages in control and *Th*^{Kir}. White arrows indicate images with the same pseudocolor scale. **(C)** Quantification of the cortical activation area evoked by increasing stimulus intensities at different developmental stages (E17.5 n = 14 control and n = 13 *Th*^{Kir}; P0-1 n = 6 control and n = 5 *Th*^{Kir}; P2-3 n = 5 control and n = 5 *Th*^{Kir}; P4-7 n = 6 control and n = 7 *Th*^{Kir}). **(D)** Quantification of the spreading of the cortical calcium

activation in response to maximal stimulation in control and *Th*^{Kir} slices at different developmental stages. Bars represent the extent of activation, measured as the distance from response initiation to the position where fluorescence decays to half. Red points lying beyond the vertical dashed lines correspond to calcium waves that spread out of the visual field (± 750 µm from the origin). E17.5 n = 9 control and n = 9 *Th*^{Kir}; P0-1 n = 5 control and n = 4 *Th*^{Kir}; P2-3 n = 5 control and n = 5 *Th*^{Kir}; P4-7 n = 6 control and n = 7 *Th*^{Kir}. S1, primary somatosensory cortex. Scale bars, 200 µm. Data are means ± SEM.

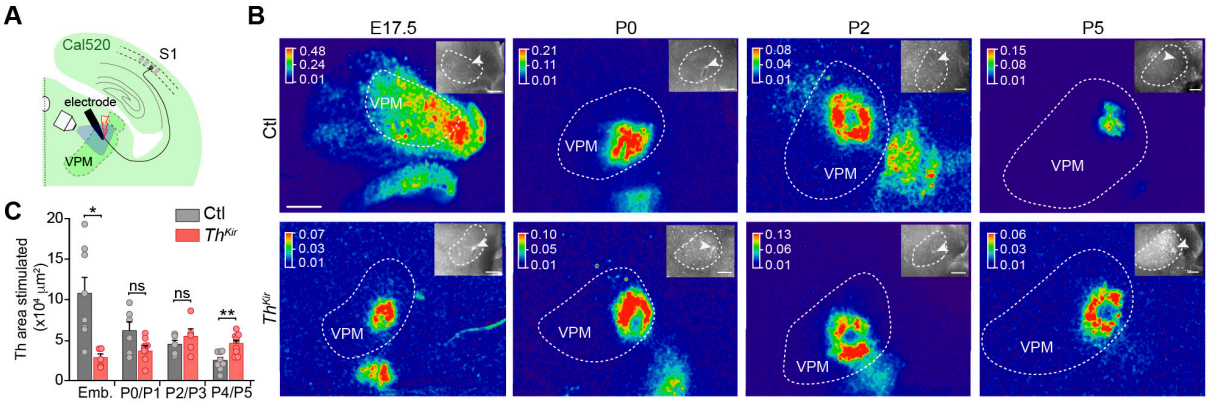


Fig.S7. Extensive cortical activation in *Th*^{Kir} mice is not due to an increment in the area of the ventral postero-medial nucleus (VPM) response recruited by the stimulus. (A) Experimental design. **(B)** Maximum projection of the calcium responses upon VPM stimulation (200 µA) in control and *Th*^{Kir} slices at distinct developmental time points. Arrowheads in insets indicate the position of the electrode. **(C)** Quantification of the thalamic stimulated area at different developmental stages (E17.5: n = 8 control, n = 5 *Th*^{Kir}; P0-1: n = 6 control, n = 9 *Th*^{Kir}; P2-3: n = 5 control, n = 5 *Th*^{Kir}; P4-5: n = 8 control, n = 9 *Th*^{Kir}; *P < 0.05, P0-1 ns. P = 0.104, P2-3: ns. P = 0.4, P4-5 **P < 0.01). Scale bars, 200 µm. Data are means ± SEM.

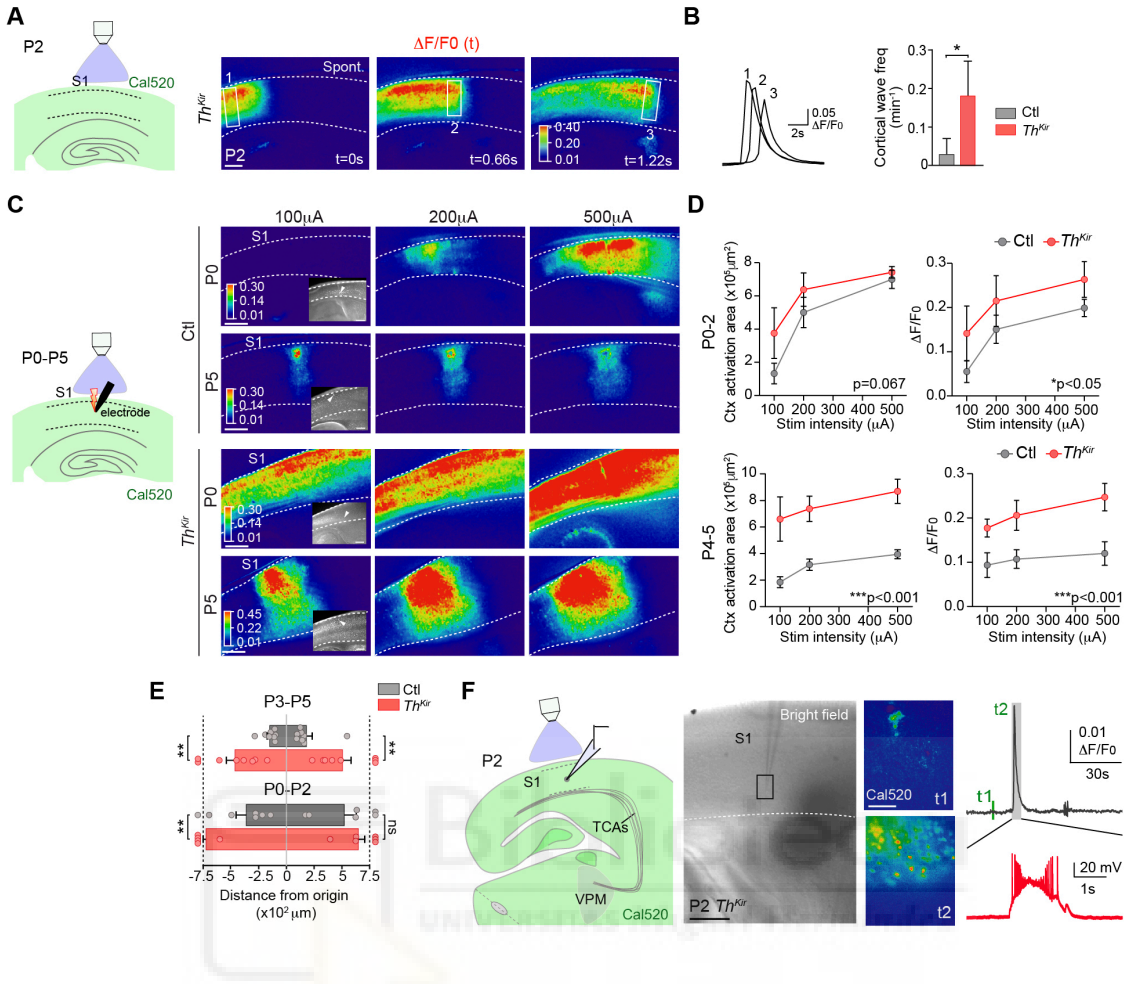


Fig.S8. Prenatal switch of spontaneous thalamic activity modifies intrinsic cortical excitability. (A) Experimental design. Maximum projection of the spread in time of a spontaneous cortical wave in a P2 *Th*^{Kir} slice. **(B)** Time course of the calcium transients in the ROIs and quantification of spontaneous wave frequency in control (n = 6) and *Th*^{Kir} (n = 6). **(C)** Experimental design. Maximum projection of the cortical calcium responses upon cortical stimulation at different intensities at P0 and P5 in control and ThKir mice. **(D)** Quantification of the area and intensity of the cortical response evoked by increasing stimulus amplitudes (control: n = 6 P0-2 and n = 6 P4-5 slices; *Th*^{Kir}: n = 6 P0-2 and n = 6 P4-5 slices). **(E)** Quantification of the horizontal spreading of the response at 200 µA. Bars represent the extent of activation measured as the distance from response initiation site to the position where fluorescence decays to half. Red points lying beyond the vertical dashed lines correspond to calcium waves that spread out of the visual field (± 750 µm from

the origin). P0-2 n = 6 control and Th^{Kir} slices, P3-5 n = 8 control and Th^{Kir} slices. **(F)** Experimental design. Simultaneous acquisition of calcium and current-clamp responses during spontaneous activity in cortical neurons. Spontaneous cortical waves in the Th^{Kir} mice are associated with a transient burst of action potentials in cortical neurons (n = 3). S1, primary somatosensory cortex. Scale bars, 200 μm . Data are means ± SEM.

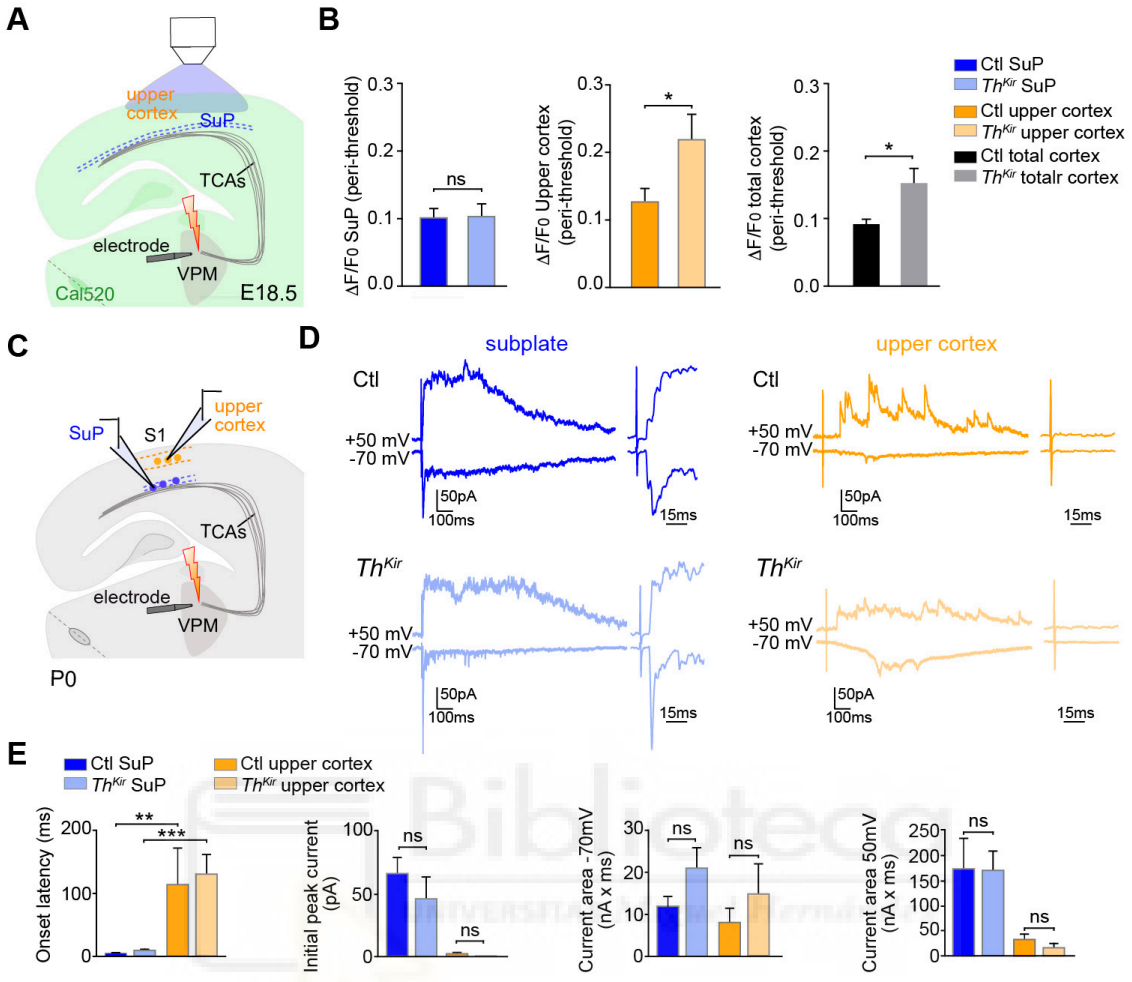


Fig.S9. Calcium dynamics and current properties in subplate (SuP) and upper cortex. (A) Experimental paradigm. **(B)** Quantification of the ΔF/F0 at peri-threshold stimulation in SuP, upper cortex and total cortical wall in control and *Th*^{Kir} mice at E18.5 (control, n = 14; *Th*^{Kir} n = 13; ΔF/F0 Subplate ns. P = 0.934; *P < 0.05). **(C)** Experimental design. **(D)** Traces showing the excitatory postsynaptic currents (EPSCs) triggered in SuP and upper cortical neurons held at -70 mV and 50 mV after ventral postero-medial nucleus (VPM) stimulation. **(E)** Quantification of EPSC properties recorded from SuP and upper cortex neurons in control and *Th*^{Kir} mice at P0 (n = 5-14 control, n = 5-11 *Th*^{Kir}; Onset latency: **P < 0.01, ***P < 0.001; Initial peak current: SuP ns. P = 0.511, Upper Cortex ns. P = 0.999; Current area -70 mV: SuP ns. P = 0.155, Upper cortex ns. P = 0.587; Current area 50 mV: SuP ns. P = 0.999, Upper cortex ns. P = 0.975). S1, primary somatosensory cortex. Data are means ± SEM.

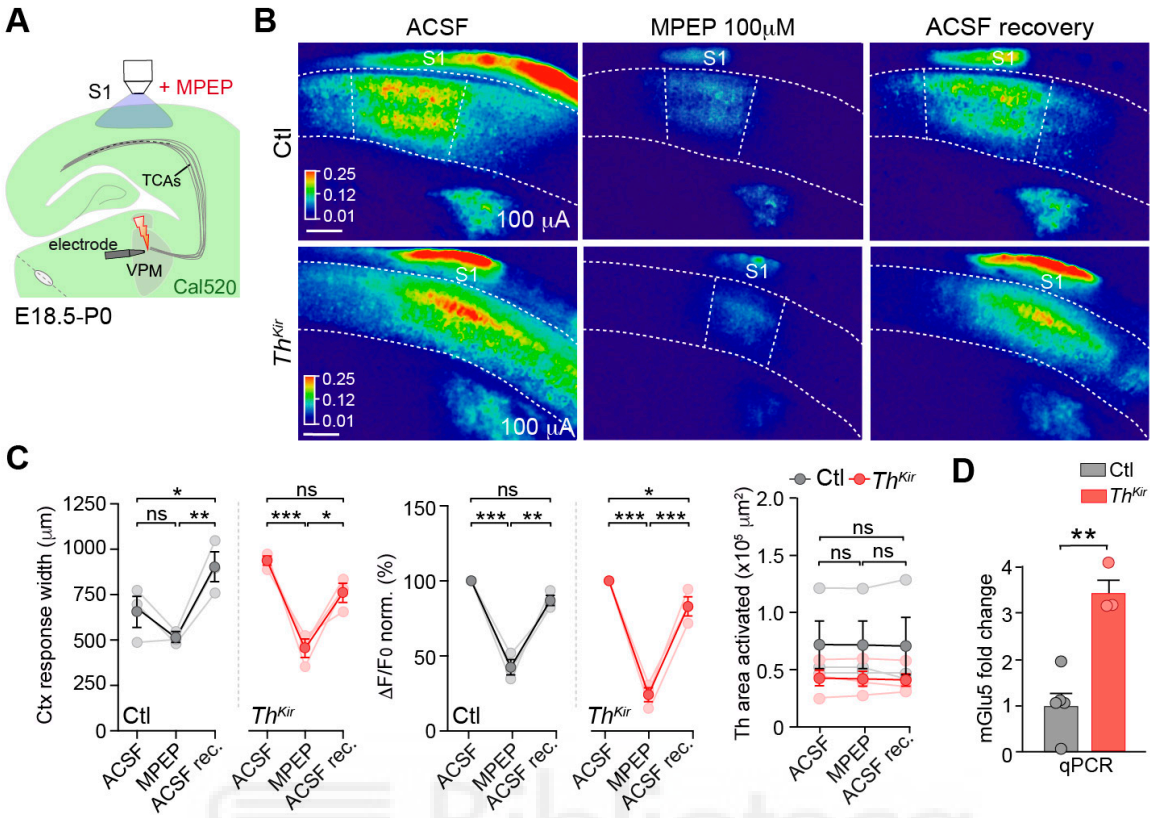


Fig.S10. Effect of mGlu5 antagonist in the thalamocortical-induced cortical response. **(A)** Experimental design. **(B)** Maximum projection of the cortical calcium responses upon ventral postero-medial nucleus (VPM) stimulation before, during and after application of the mGlu5 receptor antagonist MPEP (100 µM) at P0. **(C)** Quantification of the evoked calcium responses shown in **B**. Control (n = 3) and ThKir (n = 3) slices. For cortical response width: Control: ACSF vs. MPEP ns. (not significant) P = 0.226; *Th*^{Kir}: ACSF vs. ACSF recovery ns. P = 0.119; *P < 0.05; **P < 0.01; ***P < 0.001. For ΔF/F0: Control: ACSF vs. ACSF recovery ns. P = 0.134; *P < 0.05; **P < 0.01; ***P < 0.001. For Th area activated: Control: ACSF vs MPEP ns. P > 0.999; MPEP vs Recovery ns. P > 0.999; ACSF vs Recovery ns. P > 0.999; *Th*^{Kir}: ACSF vs MPEP ns. P = 0.998; MPEP vs Recovery ns. P = 0.997; ACSF vs Recovery ns. P = 0.991). Mean values are represented in darker circles. **(D)** Quantitative PCR for mGlu5 levels in the control (n = 5) and *Th*^{Kir} (n = 3) mice at P0, **P < 0.01. Scale bars, 200 µm. S1, primary somatosensory cortex. Data are means ± SEM.

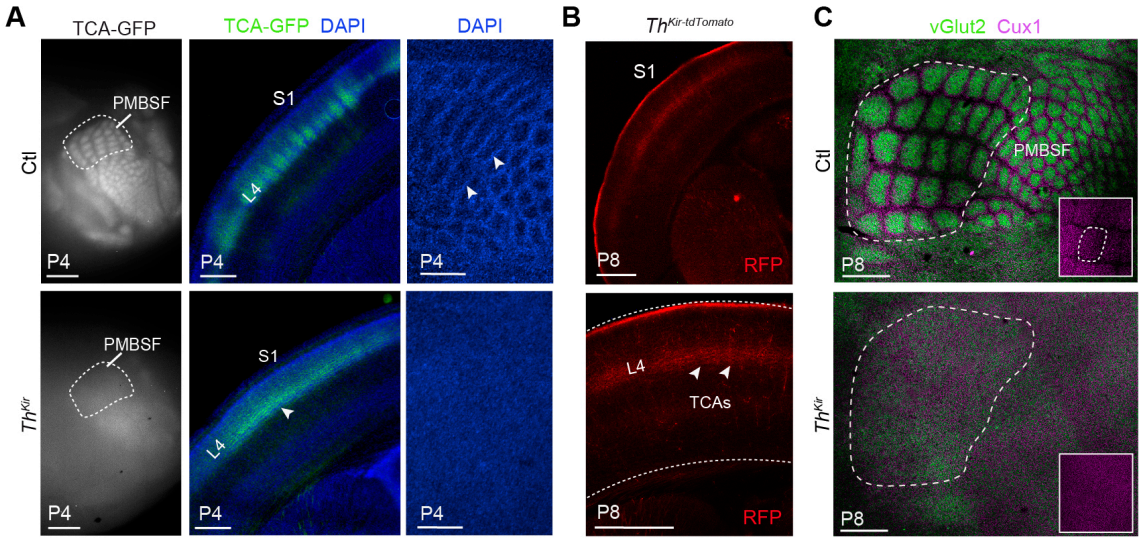


Fig.S11. Barrel map formation is compromised after disruption of thalamic waves. **(A)** In toto (left), coronal (middle) and tangential (right) view of the PMBSF map formation. In the TCA-GFP genetic background, TCAs are labeled in green at P4 in control and *Th*^{Kir} mice. DAPI (blue) shows the disposition of layer 4 (L4) neurons in the PMBSF. **(B)** Coronal section showing thalamocortical axon (TCAs) terminals expressing Kir (tdTomato) in the L4 of *Th*^{Kir} mice at P8. **(C)** Tangential view of the PMBSF sensory map representation at P8. vGlut2 immunostaining of TC terminals and of Cux1 immunostaining of cortical cells. Notice the lack of PMBSF map in *Th*^{Kir} mice. S1, primary somatosensory cortex. Scale bars, 1 mm in **A** (left panels) and 300 μm in **A** (middle/right panels), **B** and **C**.

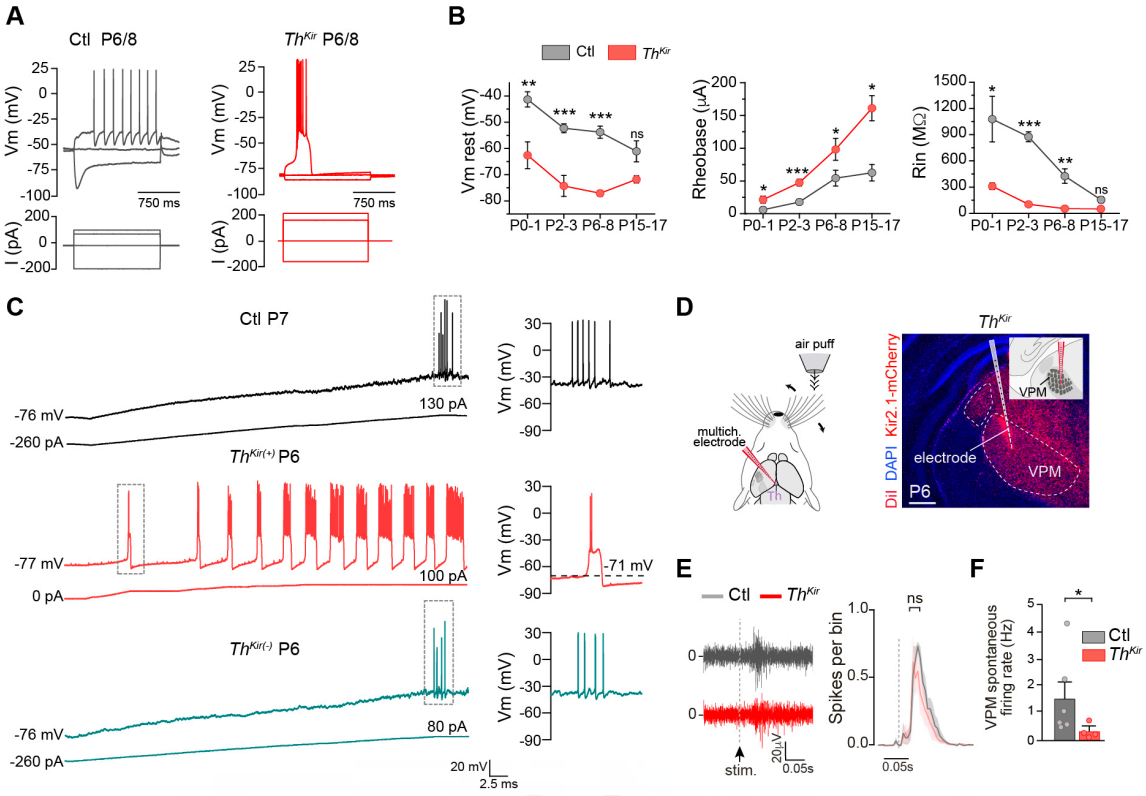


Fig.S12. ***Ex vivo*** **and** ***in vivo*** **electrophysiological properties of ventral postero-medial nucleus (VPM)** Th^{Kir} **neurons during early postnatal development. (A)** Representative examples of the voltage responses to a family of current steps in control and Th^{Kir} VPM cells at P6-P8. **(B)** Quantification of membrane potential (Vm), rheobase and membrane input resistance (Rin). n = 4 to 22 cells for each condition; *P < 0.05, **P < 0.01, ***P < 0.001. **(C)** Voltage changes associated to a current ramp in control, Th^{Kir}-positive and Th^{Kir}-negative neurons. Note that Vm oscillates when reaches -71 mV in the Th^{Kir}. **(D)** Experimental design and coronal section counterstained with DAPI showing the electrode labeled with DiI. **(E)** Representative examples of VPM multi-unit activity (MUA) evoked by whisker stimulation in control (n = 4) and Th^{Kir} (n = 4). Dashed line represents the onset of the stimulus (time = 0). Right: Quantification of number of spikes per 5 ms bin. **(F)** Quantification of the spontaneous firing rate in the VPM of control (n = 6) and Th^{Kir} (n = 4) mice. *P < 0.05. Scale bar, 300 µm = 4) mice. *P < 0.05. Scale bar, 300 µm. Data are means ± SEM.

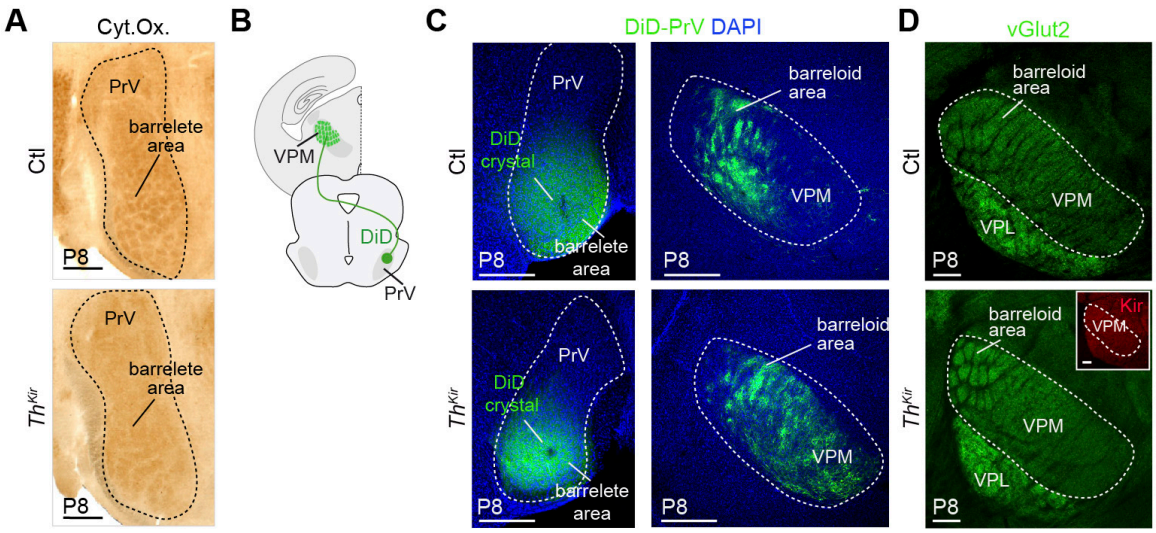


Fig.S13. Subcortical structures are not affected in *Th*^{Kir} mice. **(A)** Representative images of cytocrome Oxidase (Cyt. Ox.) staining showing the barrelettes in the principal sensory trigeminal nucleus (PrV) of the hindbrain in control and *Th*^{Kir} mice at P8. **(B)** Experimental design. **(C)** Representative images of DiD crystal localization in the PrV (left panels) in control and *Th*^{Kir}. Right: Coronal sections showing PrV axons targeting the ventral postero-medial nucleus (VPM) in both control and *Th*^{Kir} mice at P8. **(D)** Coronal view of the VPM labeled with vGlut2 in both control and *Th*^{Kir} mice at P8. Notice the presence of barreloids in *Th*^{Kir}. Scale bars, 300 μm.

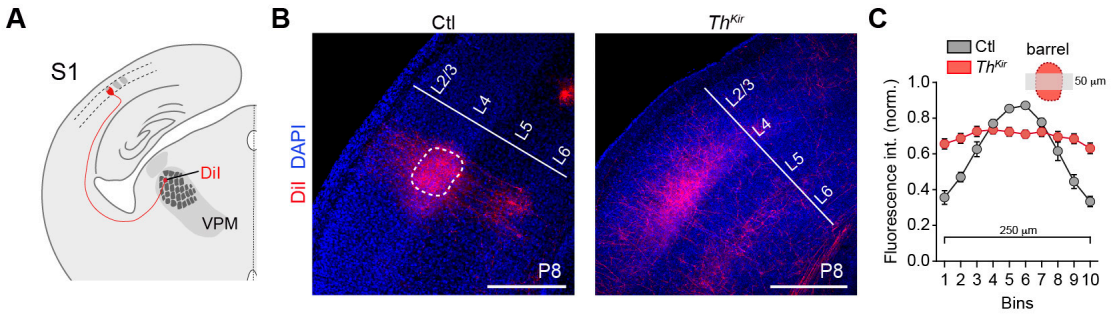


Fig.S14. Thalamocortical axons do not cluster in *Th*^{Kir} mice. (A) Experimental design. A small DiI crystal was placed in the barreloid area of the ventral postero-medial nucleus (VPM). **(B)** Coronal sections showing thalamocortical axons clusters labelled with DiI forming barrels in control mice. TCAs are horizontally oriented at the layer 4 (L4) in *Th*^{Kir} mice. **(C)** Quantification of the fluorescence intensity in L4 (250 x 50 µm ROI) normalized by the maximum intensity of each sample in control (n = 13) and *Th*^{Kir} (n = 21) mice. Each bin represents 25 µm (total: 250 µm). S1, primary somatosensory cortex. Scale bars, 300 µm. Data are means ± SEM.

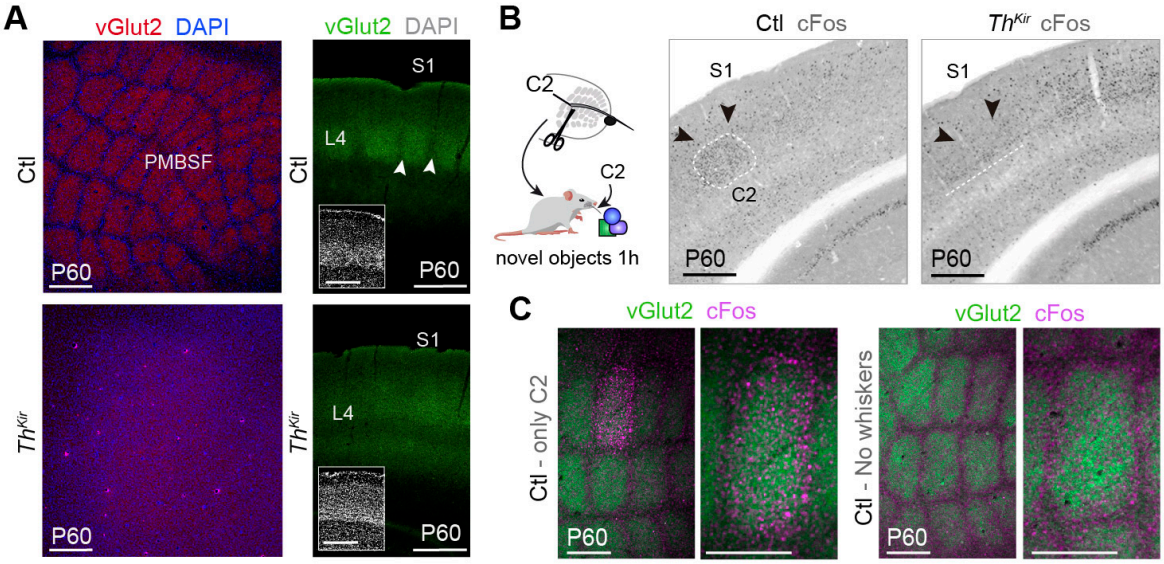


Fig.S15. Functional disruption of the map representation in adult *Th*^{Kir} mice. (A) Flattened tangential sections immunolabeled for vGlut2 in the adult PMBSF control and *Th*^{Kir} mice. Right: Coronal sections showing vGlut2 immunolabeling in the primary somatosensory cortex (S1) of control and *Th*^{Kir} mice at P60. Insets show DAPI staining. **(B)** Experimental design. Animals were kept 1 hour in the open field arena with distinct novel objects. Prior to the novelty exposure test, all whiskers were trimmed except C2. Right: Coronal sections showing cFos immunolabeling in control and *Th*^{Kir} mice. Note C2 activation in the control S1. **(C)** Tangential sections of the PMBSF somatosensory map stained by vGlut2 and cFos immunostaining in control mice. L4, layer 4. Scale bars, 300 μm.

Legends to Supplementary Movies

Movie S1. Example of the cortical response elicited by VPM stimulation (125 μA, 0.2 ms) at E17.5. Calcium concentration is represented in pseudocolor. 1x speed, 110 ms interframe interval.

Movie S2. Representative example of *in vivo* cortical calcium responses elicited after peripheral stimulation in an *Emx1*$^{Cre/+}$;*R26*$^{GCaMP6f/+}$ embryo at E18.5. 1x speed, 300 ms interframe interval.

Movie S3. Representative example of a control spontaneous thalamic activity at E16.5. Calcium concentration is represented in pseudocolor. Real interframe interval: 300 ms.

Movie S4. Representative example of thalamic activity in a *Th*^{Kir} mouse at E16.5. Calcium concentration is represented in pseudocolor. 30x speed, 300 ms interframe interval.

Movie S5. Representative example of the thalamic activity after Ba^{2+} (50 μM) in a *Th*^{Kir} mouse at E16.5. Calcium concentration is represented in pseudocolor. 1x speed, 300 ms interframe interval.

Movie S6. Cortical calcium response elicited in a control E18.5 mouse after VPM stimulation. Calcium concentration is represented in pseudocolor. 1x speed, 300 ms interframe interval.

Movie S7. Cortical calcium response elicited in a *Th*^{Kir} mouse at E17.5 after peri-threshold VPM stimulation. Calcium concentration is represented in pseudocolor. 1x speed, 230 ms interframe interval.

Movie S8. Cortical calcium response elicited in a *Th*^{Kir} mouse at E18.5 after peri-threshold VPM stimulation. Calcium concentration is represented in pseudocolor. 1x speed, 230 ms interframe interval.

Movie S9. Cortical calcium response elicited in a control P2 mouse after peri-threshold VPM stimulation. Calcium concentration is represented in pseudocolor. 1x speed, 230 ms interframe interval.

Movie S10. Cortical calcium response elicited in a *Th*^{Kir} mouse at P2 after peri-threshold VPM stimulation. Calcium concentration is represented in pseudocolor. 1x speed, 230 ms interframe interval.

Movie S11. Representative example of *in vivo* cortical calcium responses elicited after peripheral stimulation in a *Thy1-control* P4 mouse. 1x speed, 300 ms interframe interval.

Movie S12. Representative example of *in vivo* cortical calcium responses elicited after peripheral stimulation in a *Thy1- Th*^{Kir} P4 mouse. 1x speed, 300 ms interframe interval.

TC axons labeled with DiI and DiA. Crystals were placed in distict areas of the somatosensory thalamic nucleus at E18.5. View in a coronal plane.

General discussion

General Discussion

Specialized sensory areas in the neocortex are responsible for processing and integrating sensory modalities information. While intracortical transcriptional programs principally control the basic cytoarchitecture of sensory cortical maps (Rakic et al. 2009), little is known about the possible implications of subcortical structures. The thalamus is perfectly positioned to influence and modulate the correct arrangement of cortical sensory maps and areal size (Moreno-Juan et al. 2017). While cortical layers are still underdeveloped, TCAs have already encountered the SuPNs, establishing the first synaptic interactions between the thalamus and cortex (Luhmann et al. 2018) . At this point the thalamus is already compartmentalized, and thalamic sensory nuclei are already noticeable (Angevine 1970). Formerly, TCAs navigate towards cortical areas and reach the cortical plate topographically segregated, carrying both sensory-modality information and instructing the positional information of a given sensory modality. These events occur before birth and prior to sensory-experience (López-Bendito & Molnár 2003).

Central brain structures inherit their topographical organization from peripheral sensory centres, such as the retina, cochlea or the whisker pad in the snout of the mouse. Peripheral neuronal activity consolidates neighbour connectivity, which is the principal rule for mapping disposition (Hanganu-Opatz 2010; Bednar & Wilson 2016). Hence, many studies have shown that at the period of early cortical plasticity, when sensory cortical areas are still malleable, peripheral input and spontaneous network activity from sensory peripheral neurons are critical to fine-tune the formation of cortical sensory maps (Tritsch & Bergles 2010; Seabrook et al. 2017; Yang et al. 2013; Mitrukhina et al. 2015). However, the role of spontaneous activity in central brain structures in the formation of sensory circuits remains largely unknown. Only recently, it has been shown that retinal and cochlear waves propagate throughout the visual and auditory pathway to central stations (Ackman et al. 2012; Babola et al. 2018). Nevertheless, it is not clear whether the capability to transmit this spontaneous activity works solely as a transfer of information, or if it might synchronize the

intrinsically generated spontaneous activity in each structure. Consequently, if the later is true, intrinsic spontaneous activity generated in every station should not be neglected. Our previous work has pointed out that the thalamus possesses the capability to intrinsically generate spontaneous calcium waves that serve as a cross-modality communication mechanism among sensory systems. This way of communication maintains the homeostatic regulation of areal size upon sensory loss (Moreno-Juan et al. 2017). This has been the first evidence showing that spontaneous thalamic activity might be a key regulatory element during cortical development. Furthermore, it has pointed out that further research is required to define the role of embryonic thalamic networks in sensory maps development, independent of external input.

The functional blueprint of the somatosensory columnar organization is already formedprenatally

While intrinsic genetic factors are considered to regulate the initial patterning, functional maps are considered to arise postnatally by peripheral input (Killackey et al. 1978; Woolsey & Wann 1976; Tiriac et al. 2018; Gaspar & Renier 2018; Hensch 2004). Specifically, the emergence of the somatosensory cortex has been one of the most frequently studied sensory systems in mice, due to the formation of the discernible barrel columns that are already apparent at P4 (Woolsey & Van der Loos 1970). Several groups proposed that prior to anatomical barrel appearance, columnar clusters of cortical neurons are functionally synchronized (Yang et al. 2013; Mitrukhina et al. 2015; Mizuno, Ikezoe, et al. 2018; Dupont et al. 2005; Luhmann & Khazipov 2018). In this work, we present the first *x i* and *i i* evidence of the existence of a functional blueprint that precedes the cytoarchitectural sensory map generation. Although anatomical cortical columns are not formed at embryonic stages (Agmon et al. 1995), functional pre-columnar patterns that pave the cortical field can already be recognized. Prenatal thalamic and peripheral stimulations show the existence of a functional topographic cortical map, indicating that the somatosensory system is able to send information and instruct a topographic organization even before birth. Our data supports previous neonatal observations that indicate that early postnatal

spontaneous and evoked cortical activity is already topographically organized (Yang et al. 2013; Mitrukhina et al. 2015; Mizuno, Ikezoe, et al. 2018). This suggests the presence of a nascent cortical network that is fairly columnar-restricted even before barrel formation and serves as a layout for the putative sensory map. It is known that spontaneous whisker movements and passive stimulations in neonatal pups induce an early evoked sensory cortical response, which are probably involved in the correct somatosensory map arrangement (Mitrukhina et al. 2015; Akhmetshina et al. 2016; Tiriac et al. 2018). However, only 11% of the patchwork activity described in the work of Mizuno *et al.* was correlated with spontaneous body movements, and also persist after ION lesioning. Then, a question arises: from where is this cortical pattern of activity in the somatosensory cortex originating? Different scenarios can be considered: i) this activity might be originated in the trigeminal ganglia under the influence of external input, or ii) it might be generated in central subcortical structures. Definitely, altogether this data seems to point out to the second possibility and that emergent cortical synchronization of neuronal cohorts is peripheral-input independent and thus, other mechanisms should be involved (Mizuno, Ikezoe, et al. 2018). Our data indicates that cortical functional precolumns are instructed embryonically, before early peripheral stimulation starts. Furthermore, in agreement with our findings, patch activity has been found in the TCAs, suggesting that the cortex might be instructed by thalamic input. Supportively, infraorbital nerve lesions did not affect this correlated activity, excluding the peripheral input as an instructive signal (Mizuno, Ikezoe, et al. 2018).

Desynchronization of prenatal thalamic activity networks

We used Kir2.1 overexpression as a tool to disrupt thalamic spontaneous activity at early developmental stages. We modified the default mode of highly synchronic events in a form of waves and shifted the pattern to a non-wave poorly synchronized mode in Th^{Kir} animals. Initially, the fact that Kir2.1 overexpression did not completely abolish thalamic spontaneous activity was rather unexpected, as Kir2.1 overexpression has been widely used as a hyperpolarizing tool (C. R. Yu et al. 2004;

De Marco García et al. 2011). In this animal model 78% of the VPM neurons exhibit an overexpression of the Kir2.1 channel by the Cre/flox system, meaning that this mouse model is a mosaic in which not all VPM neurons are hyperpolarize. This is consistent with the fact that, although VPM neurons are still asynchronous active, they fail to develop calcium waves as a network. Intriguingly, we showed that at the cellular level thalamic relay neurons become bi-stable. Kir2.1 overexpression results in a change of the electrical properties of these neurons, that start to spontaneously oscillate with fluctuating periods of hyperpolarized and depolarized membrane potentials. Action potentials are only generated at the depolarized phase. This effect is most probably caused by a specific thalamic current, named I_H (hyperpolarization-activated cationic current). This current is activated when thalamic neurons experience a membrane hyperpolarization and promotes a rebound of depolarization in thalamic relay neurons (Pape 1996; Santoro et al. 2000; Yue & Huguenard 2001). The oscillations that Th^{Kir} neurons undergo, combined with a dramatic alteration of their intrinsic electrophysiological properties, shape the basis of the activity pattern shifted in the Th^{Kir} mice. Therefore, encountering a large number of Th^{Kir} neurons that are at state1 (depolarized and able to fire), potentially creating a wave, is probabilistically difficult. Consequently, spontaneous waves are not engaged even though individual neuronal activity persist in the embryonic thalamus of these mice. We unequivocally demonstrate that overexpression of Kir2.1 only modifies spontaneous thalamic activity patterns, as we succeeded in recovering the wave-like activity pattern, even though we reversed the electrophysiological profile of Kir2.1 neurons after the addition of barium. Thus, this indicates that thalamic neuronal networks were conserved.

Modification of VPM spontaneous thalamic activity patterns influences the initial establishment of the functional pre-columnar state of the somatosensory system. Our results reveal a causal link between intrinsic thalamic activity and the initial establishment of the somatosensory cortical map. Altering the pattern of spontaneous thalamic networks induce drastic changes in the emergence of the

functional topographic map. Contiguous stimulations of VPM territories induce overlapping responses in the cortical field before birth, rather than the fairly-restricted responses previously observed in controls. To unequivocally show that overlapping responses were not due to an enlarged activation of VPM territories, we measured VPM area responses after stimulation. Interestingly, while VPM-activated areas along embryonic stages are larger in controls with a clear wave pattern, Kir condition manifests restricted patch-like responses. This result reveals that overlapping cortical responses in Th^{Kir} mice are independent of VPM extension responses.

Prenatal switch of spontaneous thalamic activity modifies intrinsic cortical excitability

We show that the shift of the spontaneous thalamic activity from highly synchronic to asynchronic induces hyperexcitability of the neocortex, from embryonic to early postnatal stages. In control mice, thalamic stimulation triggered a columnar cortical response, that became increasingly narrow at postnatal stages, finally matching the dimension of a discernible barrel. Remarkably, in Th^{Kir} mice similar restricted thalamic stimulations induced exuberant intra-cortical calcium waves. These larger responses were correlated with higher levels of intrinsic cortical excitability. Cortical circuitry disruption was triggered prior to barrel formation, as we have observed this phenomenon even at prenatal stages. Our results suggest that the origin of the cortical excitability resides in the upper cortical network, as the amplitude of calcium responses did not exhibit changes in the SuP but were larger in the upper cortex. However, this result does not imply a direct defect over the cortical plate, as SuPNs are highly interconnected with the remaining layers from early stages. Future experiments should be directed to elucidate the possible mechanisms underlying this cortical hyperexcitability and its link with the absence of functional columnar organization. Hypothetically, the lack of a correct pattern of thalamic spontaneous stimulation prevents cortical neurons from maturating correctly (H. Li et al. 2013). Here we demonstrate that the activation of the TCA network is primarily induced in the SuP and then radially extended along the cortical plate. Therefore, thalamic input might instruct the columnar organization via SuPNs, which are considered to be involved in

establishing the neocortex (Luhmann et al. 2009; Kanold 2009; Wess et al. 2017). SuPNs might be important for early cortical microcolumns as they are coupled by gap junctions with the cortical plate neurons (Kanold 2009; Luhmann et al. 2018; Singh et al. 2018), and are probably also involved in the barrel map formation, as its removal disrupts the somatosensory map (Tolner et al. 2012). Considering that the SuP is the first interaction between the thalamus and the cortex, the cortical network properties could change due to SuP alterations (i.e. Gap Junctions, interneurons). Since we have observed a highly excitable cortical state, ionotropic and/or metabotropic receptor might be involved. Indeed, metabotropic glutamate receptors (mGluRs) are largely expressed during cortical development (López-Bendito et al. 2002) and have been reported to be involved in the propagation of early events of cortical spontaneous activity (Calderon et al. 2005; Wagner & Luhmann 2006; Ballester-Rosado et al. 2010; Ballester-Rosado et al. 2016; C. C. Lee & Sherman 2009). Subsequently, we tested the possible implications of mGluRs in the hyperexcitability experienced by cortical networks in ThKir mice. We found higher expression levels of mGlu5 in the cortex at P0. Moreover, the mGlu5 specific antagonist MPEP, rescued the activation cortical networks back to a columar-like response in Th^{Kir} mice. Thus, our data show a direct control of prenatal thalamic networks over the excitability state of cortical circuits.

As previously mentioned, the thalamocortical system has an intimate relationship with SuPNs, providing excitation to the developing circuits from the very early stages (Allendoerfer & Shatz 1994; Luhmann et al. 2009; Kanold & Luhmann 2010; Tolner et al. 2012; Wess et al. 2017). The lack of the proper thalamic input, specifically the absence of highly correlated spontaneous activity, might influence the intrinsic developmental properties of SuPNs. We should keep in mind that the SuPNs are considered to be crucial regulators of cortical development and circuitry (De Carlos & O'Leary 1992; Hanganu et al. 2002; Kanold et al. 2003; Kanold 2009; Yang et al. 2018; Luhmann et al. 2016; Luhmann et al. 2018). It has been shown that the elimination of the SuP results in a cortical hyperexcitability either directly by an increase in GABAergic

activity or by an imbalance in the glutamatergic activity. SuPNs are important for the proper inhibitory neuron maturation, as their early removal prevents the developmental increase of KCC2 expression, crucial for controlling GABA as an inhibitory signal. As a consequence, GABAergic circuits remain depolarized instead of being inhibited (Kanold & Shatz 2006; Kanold 2009). This inhibitory maturation is strongly dependent on the sensory experience, as several studies showed the BDNF involvement in the developmental upregulation of KCC2, resulting in the functional inhibitory maturation of GABA-A (Huang et al. 1999). Sensory deprivations prevent this phenomenon and induce a decrease in the levels of BDNF (Erzurumlu & Gaspar 2012). However, evidence showing the lack of prenatal thalamic input independent of sensory experience being involved in controlling cortical BDNF levels during early developmental stages is still missing.

Connexins might be playing a role in the generation of the hyperexcitable state that the *Th*^{Kir} cortex exhibits. During development, neocortical territories exhibit highly synchronic activity events that are functionally coupled, for instance, synchronic calcium waves, neuronal domains and plateau assemblies (Khazipov & Luhmann 2006). These events allow a formation of a propagating network along the cortical field. This early network has been described to be dependent on the electrical synapses via gap junctions. The gap junctions constitute about 40-70% of the connectivity between cortical neurons during the first postnatal week and are causing a subsequent downregulation of connexins (Montoro & Yuste 2004; Connors et al. 1983). This suggests a decrease in the importance of the electrical synapses and a developmental switch towards the key role of chemical synapses containing NMDA receptors (Dupont et al. 2005). Propagating calcium waves are present from E16.5 to P2 in the neocortical territory, with a peak in P0. In our model this propagating network persists, and is even enhanced, beyond P2. We do not know whether connexins are normally suppressed during these developmental stages, but the impairment in this process could explain the continuous electrical coupling of *Th*^{Kir} cortical neurons.

Many studies have observed that synchronically correlated cortical patterns are, apart from involving gap-juctions function, governed by other factors. Again, metabotropic receptors could induce gap junctions regulation via glutamate, triggering the inositol triphosphate (IP3) pathway and resulting in an increase of intracellular calcium influx (Kandler & Katz 1998). Additionally, NMDA, AMPA and even GABA receptors have been involved in the early cortical synchronic network. A classical view of the cortical compartmentalization that might generate the adult columnar arrangement involves both the maturation of the inhibitory input and the developmental switch from electrical to chemical synapses. This might promote a temporal and spatial organization of the neocortical space (Yuste et al. 1992; Kandler & Katz 1995; Montoro & Yuste 2004).

All in all, we are facing a very complex system that is formed by a highly mature layer of cells that correspond to the SuPNs, receiving early thalamic input. The information is transmitted next to the cortical plate, which in contrast to the SuPNs, presents a less mature state. Probably both structures exhibit distinct communication mechanisms governed by different factors, which challenges the dissection of the various mechanisms conducting the early development of cortical networks.

Lack of the prenatal functional columns result in the absence of the anatomical and functional somatosensory map even in the adulthood.

Our data demonstrate a causative connection between prenatal thalamic activity patterns and the foundation of cortical columns in the somatosensory system. We show that even tough TCAs are correctly located in the L4, they are not segregated into discrete barrels to form the barrel field. We observed this phenomenon from P4 when barrels start to be noticeable in S1. The lack of anatomical barrels is not caused by defects in the peripheral point-to-point organization, which is preserved from in the PrV and VPM. However, we observed the loss of the fine-tuned point-to-point TC connectivity in the barrel cortex of the Th^{Kir} mice, while the gross-topographic organization is maintained. We observed that TCAs are not correctly organized in the L4 and

instead of forming their typical cortical arbors (Agmon et al. 1995; López-Bendito & Molnár 2003), TCAs seem to be horizontally oriented along the cortical plane. As the functional TC topographical map is embryonically perturbed in the ThKir, we conclude that the initial establishment of the barrel map does not emerge. Thus, the absence of the pre-columnar organization preludes the failure of the postnatal barrel formations. Nevertheless, we cannot rule out that an additional TC refinement deficiency during postnatal development may also contribute to the absence of anatomical and functional barrels observed in the ThKirmice. Our results show that the cortical response to a stimulation of a single thalamic area gets refined during postnatal stages, suggesting that a functional refinement process also occurs in Th^{Kir} mice, although to a lesser extent. This data explains the presence of a gross-topographic organization in the Th^{Kir} mice. We believe that in this model TCAs do not cluster properly. Functionally, we show that cortical responses after VPM stimulations are restricted along development but this refinement of the responses occur to a lesser extent. Thus, probably, there is a combination of both: a refinement defect and an abnormal overgrowth of TCAs. In addition, it needs to be considered that the Th^{Kir} mice exhibit a hyperexcitable cortex. When TCAs invade the CP to reach the L4 and segregate to form the columnar clusters, the niche that they encounter is impaired in terms of spontaneous activity. Its electrophysiological properties, neuronal maturation, receptors, expression of activity regulated genes or even neurotrophic factors might have changed as well. Mice lacking mGlu5 and NR1 exhibit TCA arborization defects (Martini et al. 2018; Iwasato et al. 2000; L.-J. Lee et al. 2005; Wijetunge et al. 2008), pointed out that glutamate receptors are guiding TCAs growth into highly branched patterns. In addition, postsynaptic activity dependent messengers that may act presynaptically have been identified in the TCA system and might be involved in the axonal branching and map refinement processes. For instance, endocannabinoids, nitric oxide, neurotransmitters (glutamate), trophic factors (BDNF), ephrin/Eph signaling among others. As an example, mGlu5 signaling modulates the

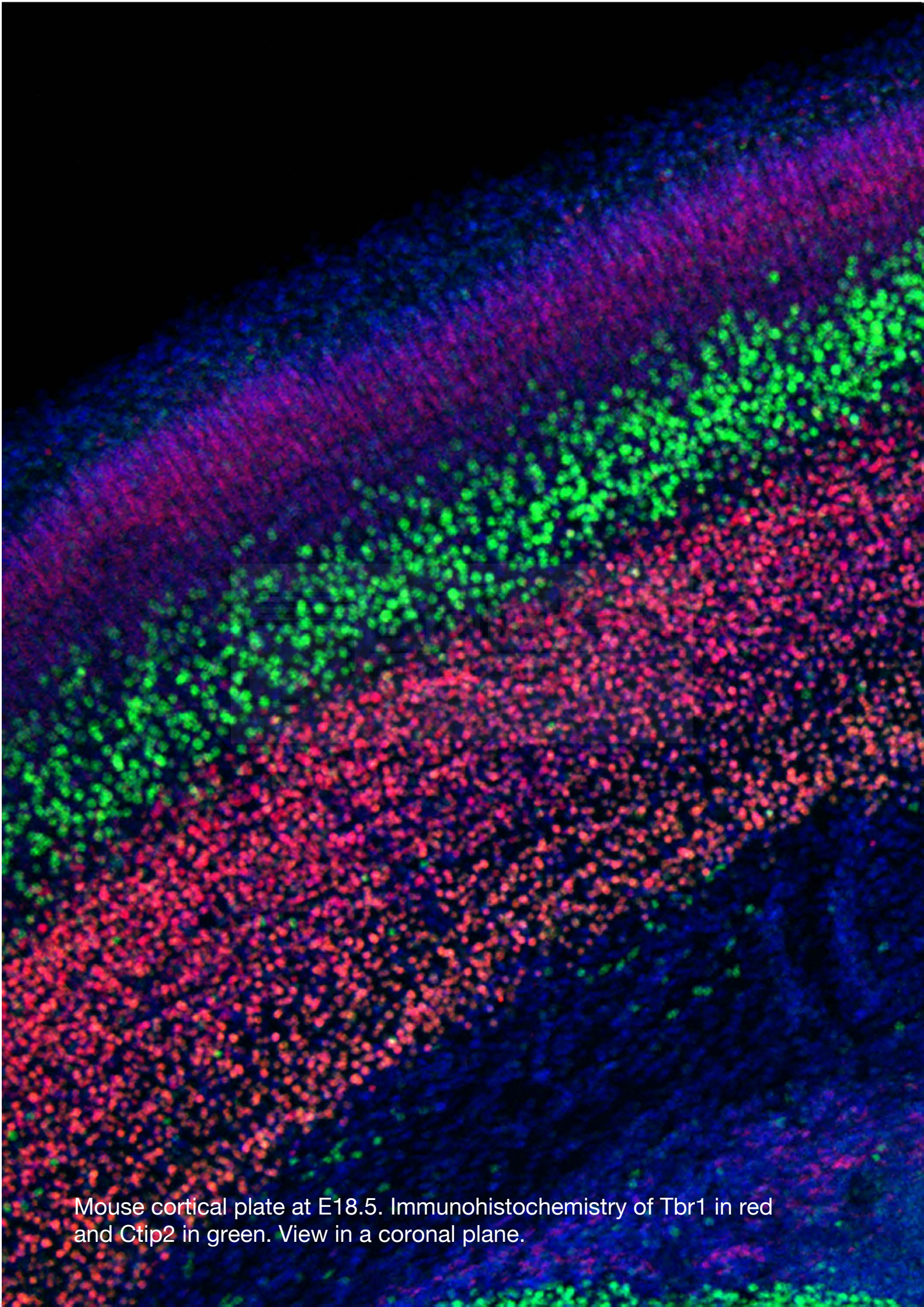
postsynaptic synthesis of endocannabinoids and BDNF, which are expressed in the developing S1. Interestingly, this neurotrophic factor , has been shown to also arrange axon branching in the developing brain (Vicario-Abejón et al. 1998; Cohen-Cory 1999; Marshak et al. 2007). We cannot neglect the multiply studies focused on presynaptic manipulations in this equation. For instance, blocking glutamatergic neurotransmission critically disrupts the clustering of TCAs into the barrel pattern. In contrast to our study, the Kir2.1 is still able to fire and induce the cortical response, even though Kir2.1 overexpression disrupts the electrophysiological properties of thalamic neurons. In the previously mentioned study, glutamatergic neurotransmission was blocked pre- and postsynaptically (H. Li et al. 2013), and therefore it remained unclear whether the defect was ruled out by either manipulations in the cortex, in the thalamus or both. Interestingly, the loss of columnar organization in the Th^{Kir} mouse was not conquered by sensory experience, as the lack of somatosensory barrel organization persisted in adulthood. The period of thalamocortical plasticity in the somatosensory system closes between P3 and P7 (Erzurumlu & Gaspar 2012), whereas sensory driven experience, seen as "whisking" behavior, starts at the end of the second postnatal week. Somatosensory deprivation paradigms have taught us that early manipulations lead to irreversible structural alterations in the barrel map patterning (Woolsey & Wann 1976; Erzurumlu & Gaspar 2012; Gaspar & Renier 2018). In our model, we have identified functional defects to be present at prenatal stages, interrupting the initial establishment of the columnar organization that characterized the somatosensory cortex. In conclusion, once the system develops aberrantly from the beginning, it seems impossible to retrieve the pattern once the critical period of plasticity has already ended.

Concluding remarks

This study reveals how the development of the somatosensory map requires the emergence of cortical functional columns even before birth, a process that is shaped by specific activity patterns of prenatal thalamic networks. These embryonic columns display a remarkable level of spatial segregation and a somatotopic distribution, despite the immature stage of the cortical plate. Our data point out the presence of a nascent cortical network that is fairly columnar-restricted even before barrel formation and serves as a layout for the establishment of the putative sensory map.

We propose that this initial functional map represents the immature blueprint for the forthcoming sensory representation in the mature cortex. We show that altering the pattern of spontaneous thalamic networks from highly synchronic waves to asynchronous individual events induce drastic changes in the emergence of the functional topographic map. Lack of prenatal thalamic waves drive cortical networks into a hyperexcitable mode, which irreversibly perturbs cortical organization of TC axons, as well as the future postnatal establishment of the barrel map. As thalamic waves are not exclusive for the VPM, but propagate to other sensory nuclei, such as visual or auditory centers (Moreno-Juan et al. 2017) the principles of cortical map organization described here might be common to other developing sensory systems. Thus, in the light of our data we would anticipate that the embryonic patterns of thalamic activity may play a significant role in the map formation of other sensory systems.

The conclusion we draw from these studies is that embryonic patterns of thalamic activity are critical to organize the functional architecture of sensory systems as perturbations in the development of thalamic waves alter the cortical columnar organization. We suggest that patterned activity in subcortical relay stations during embryonic stages plays a critical role in preparing cortical areas and circuits for upcoming evoked sensory signals.



Mouse cortical plate at E18.5. Immunohistochemistry of Tbr1 in red and Ctip2 in green. View in a coronal plane.

Conclusions & conclusiones

Conclusions

1. Cortical radial columns are functionally organized and spatially ordered already during the embryonic stages of brain development.

2. Kir2.1 overexpression did not suppress the neuronal activity in the thalamus but shifted the pattern of spontaneous activity from highly synchronous waves to poorly synchronized individual activity.

3. Th^{Kir} cells displayed a bi-stable pattern of activity with spontaneously alternating periods of hyperpolarized and depolarized membrane potentials. This change in the electrical properties of Th^{Kir} cells was sufficient to impair the generation of calcium thalamic waves.

4. Barium, an ion that blocks Kir channels, reverted the electrophysiological profile of the Th^{Kir} neurons and recovered the wave-like activity in the VPM network.

5. The emergence of the functional topographical map organization was disrupted in the Th^{Kir} mice. Prenatal stimulation of adjacent VPM regions activated highly overlapping territories in the cortex, indicating that the point-to-point representation of the TC circuit does not emerge in the absence of embryonic thalamic waves.

6. At postnatal stages, VPM stimulation triggers a fairly restricted columnar activation, becoming increasingly narrow in control mice, coinciding at P4 with the dimensions of the cortical barrel. In contrast, Th^{Kir} mice failed to restrict its spatial domain of activation after VPM stimulation, eliciting intra-cortical calcium waves that spread laterally.

7. The principal source of the extended cortical activation exhibit by Th^{Kir} mice resides in the increment of the intrinsic levels of the cortical excitability. It is reflected by both the widespread cortical response to intracortical stimulation

and the extensive spontaneous events of synchronous activity that spread horizontally.

8. The cortical hyperexcitability origin is located in the upper cortex. While the amplitude of upper cortical responses after thalamic stimulation are larger in the *Th*^{Kir} mice, in subplate territories they remain unchanged

9. The metabotropic glutamate receptor 5 (mGlu5) is involved in induce cortical hyperexcitability in *Th*^{Kir} networks. From the transcriptional point of view, the cortical plate of *Th*^{Kir} mice exhibit increased levels of mGlu5 expression at P0. This result was functionally corroborated by the bath application of its specific antagonist (MPEP), which rescued the cortical default response to a columnar-like shape.

10. *Th*^{Kir} mice displayed both an impaired clusterization of TCAs in the barrels and an incorrect arrangement of the L4 spiny neurons forming the barrel walls. Thus, embryonic thalamic activity patterns and functional columns are a pre-requisite for the establishment of the postnatal anatomy of the barrel map.

11. The trigeminal and thalamic point-to-point somatosensory connectivity is topographically well conserved in the *Th*^{Kir} mice, since the organization of brainstem barrelettes and thalamic barreloids remain unaltered. Meanwhile, the point-to-point TC connectivity was disrupted starting from the embryo.

12. Maintained sensory experience does not prevail the lack of both the anatomical barrel organization and the precise functional map in the *Th*^{Kir} mice. Therefore, the natural period of somatosensory-driven plasticity cannot overcome the prenatal absence of the sensory functional map.

Conclusiones

1. Las columnas corticales radiales se encuentran funcionalmente organizadas y espacialmente ordenadas desde etapas embrionarias del desarrollo cerebral.

2. La sobrexpresión de Kir2.1 no suprime la actividad neuronal del tálamo pero genera un cambio del patrón de su actividad, de actividad altamente sincrónica en forma de ondas a actividad asincrónica.

3. Las neuronas Th^{Kir} presentan un patrón de actividad bi-estable, alternando con periodos espontáneos de potenciales de membrana hyperpolarizantes y despolarizantes. Este cambio en las propiedades eléctricas de las neuronas talamicas del Th^{Kir} es suficiente para bloquear la generación de las ondas de calcio en el tálamo embrionario.

4. La aplicación de bario, ion que bloquea los canales Kir, es capaz de revertir los efectos producidos por la sobreexpresión de Kir2.1 en el tálamo, restaurando tanto las propiedades electrofisiológicas como la actividad sincrónica en forma de ondas.

5. La generación del mapa topográfico funcional no se produce en los animales Th^{Kir}. Las estimulación de regiones adyacentes del VPM genera respuestas corticales con un gran solapamiento, indicando que la representación punto a punto característica de los circuitos talamocorticales no se produce cuando las ondas talámicas son bloqueadas prenatalmente.

6. En estadios postnatales, la estimulación del VPM genera respuestas corticales que son cada vez mas estrechas. A lo largo del desarrollo estas columnas se restringen en los animales controles, coincidiendo con las dimensiones de los barriles a P4. Sin embargo, en lo animales Th^{Kir} no se generan columnas corticales, y aunque hay cierta restricción la respuesta, ésta se extiende

mayoritariamente en el plano horizontal, generando extensas ondas de actividad.

7. La extensa activación cortical en los animales *Th*^{Kir} reside en un aumento de los niveles intrínsecos de excitabilidad de los circuitos corticales.

8. El origen de la hiperexcitabilidad cortical parece localizarse en la parte alta de la corteza cerebral. Mientras que la amplitud de las respuestas es mayor en esta zona cortical, en la subplaca las respuestas a estimulos talamocorticales permanece en niveles normales.

9. El receptor de glutamato mGlu5 está involucrado en la hiperexcitibilidad cortical de los animales *Th*^{Kir}. A nivel transcriptómico, los niveles de mRNA de mGlu5 en la condición *Th*^{Kir} son mas elevados en la corteza somatosensorial. La aplicación de MPEP, un antagonista de mGlu5, rescata la generación de columnas corticales funcionales.

10. Los animales *Th*^{Kir} presentan defectos en la generación del mapa somatosensorial. Los axones talamocorticales no se agrupan en la capa 4 formando los barriles y las neuronas espinosas no se organizan formando las paredes que los conforman. De este modo, la actividad talámica embrionaria en forma de ondas, y en consecuencia las columnas funcionales, son un pre-requisito necesario para establecer la anatomía que conforma el sistema de barriles en roedores.

11. La conectividad topográfica punto a punto se conserva adecuadamente en las estaciones subcorticales en el ratón *Th*^{Kir}, indicando un claro defecto en el desarrollo del sistema talamo-cortical.

12. La experiencia sensorial en estadios postnatales no rescata la ausencia de columnas corticales ni la generación de los barriles anatómicos en el ratón *Th*^{Kir}.

De este modo, el periodo natural de plasticidad somatosensorial no parece estar implicado en este proceso.

Tangential view of the thalamocortical axons labeled in the TCA-GFP mice. Distinct cortical sensory areas has been labeled with distinct colors.

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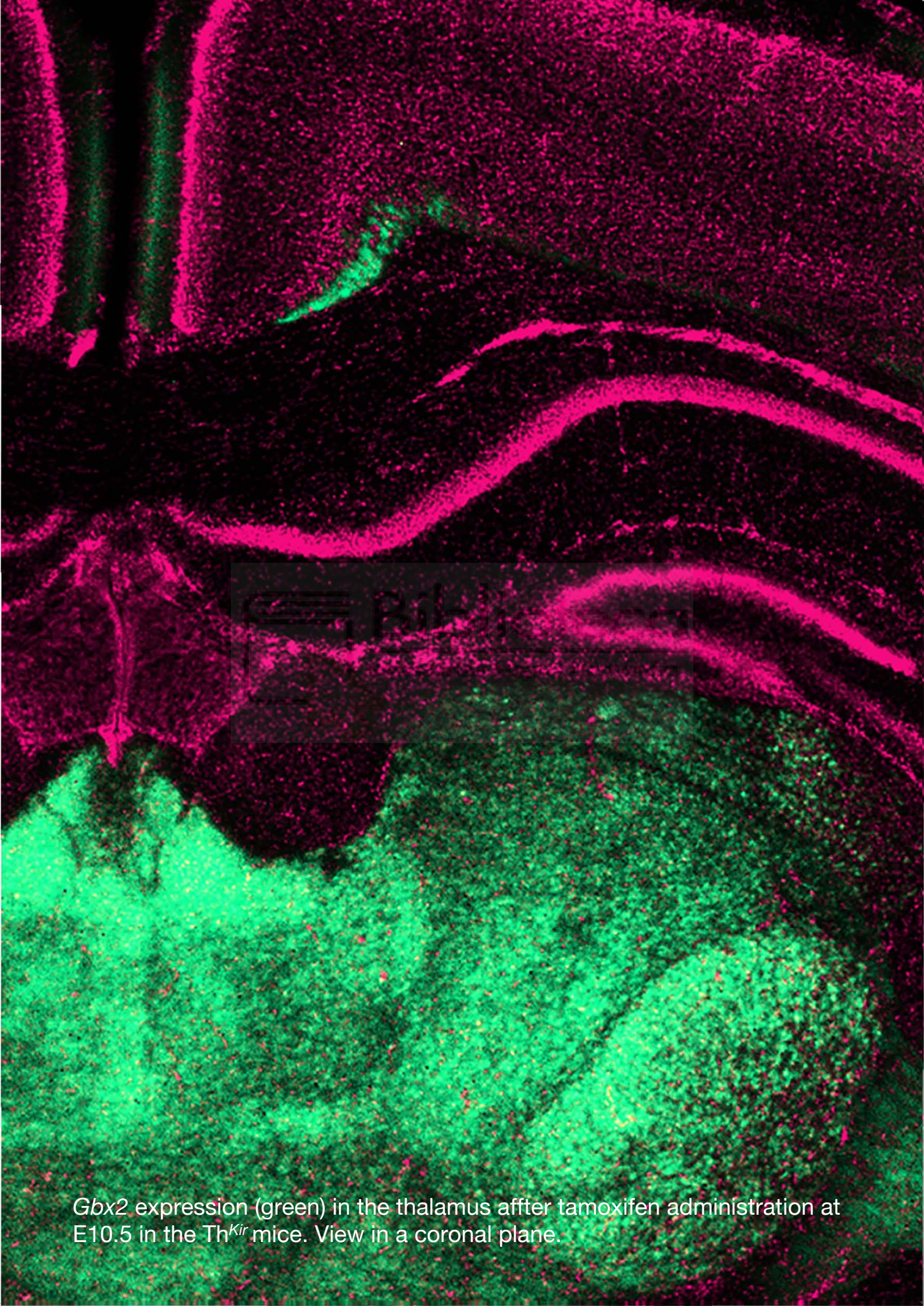
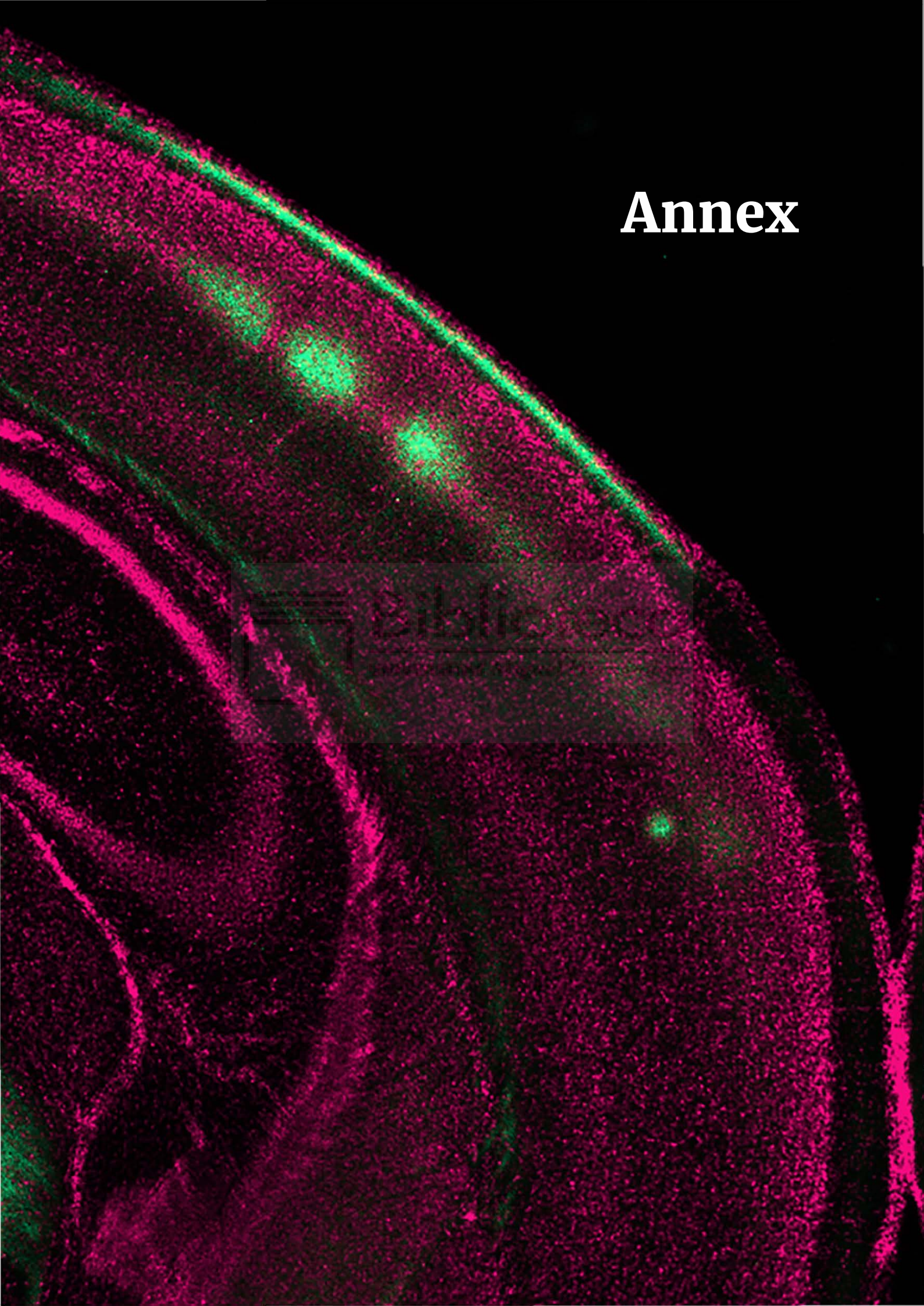


Gbx2 expression (green) in the thalamus affter tamoxifen administration at E10.5 in the Th^{Kir} mice. View in a coronal plane.

Annex



Developmental interactions between thalamus and cortex: a true love reciprocal story

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Abstract

The developmental programs that control the specification of cortical and thalamic territories are maintained largely as independent processes. However, bulk of evidence demonstrates the requirement of the reciprocal interactions between cortical and thalamic neurons as key for the correct development of functional thalamocortical circuits. This reciprocal loop of connections is essential for sensory processing as well as for the execution of complex sensory-motor tasks. Here, we review recent advances in our understanding of how mutual collaborations between both brain regions define area patterning and cell differentiation in the thalamus and cortex.

Introduction

The cerebral cortex contains billions of neurons organized in delimited areas that process particular aspects of sensation, movement and cognition. The mechanisms that allow these neurons to integrate into specific functional circuits during development have received much attention in the last decade. Given that thalamic and cortical developmental programs concur in time and overlap in space (Figure 1), both structures influence each other during brain ontogeny. Hence, the incipient and long-lasting relationship between the cortex and the thalamus can be understood as a love story.

In sensory systems, the thalamus receives information from peripheral organs which is reliably processed and transmitted to cortical territories. Corticothalamic feedback from layer 6 is sent back to the corresponding first-order thalamic sensory nuclei. This thalamo-cortico-thalamic loop is built at late prenatal-early postnatal stages, and has been demonstrated to be fundamental to regulate developmental processes, for instance, the specification of cortical and thalamic territories [1-6].

The interaction between thalamus and cortex has deserved much attention in the last four decades. It was in the late 80's when two influential and opposing views emerged on how cortical areas are developmentally specified; called the Protomap and the Protocortex theories. The Protomap hypothesis postulated that cortical cells are already "pre-specified" at birth, being their area of generation key for the acquisition of identity features [7]. Indeed, neuronal precursors from different cortical areas show variations in cell-cycle kinetics, division mechanisms and cell fate specification [8-10]. Hence, areal differences are set by intrinsic developmental programs that unfold before thalamic fibers arrival. In accordance with this hypothesis, early cortical expression of guidance molecules and transcription factors guide thalamic axons to their final destination [11,12]. However, thalamic axons arrive to the cortex while corticogenesis is still ongoing, influencing the rate of proliferation across the germinal zone and, therefore, determining cytoarchitectural features [13].

On the opposite hand, the Protocortex hypothesis proposes that the cortex is originated as a tabula rasa where all neurons are born equal and multipotent. In this case, afferent axons (mostly thalamic input) impose cortical areal identity through activity-dependent mechanisms [13]. Various studies in primate visual system supported the Protocortex hypothesis [14,15]. Following this line, prenatal enucleation experiments in macaque showed that the depletion of thalamocortical axons (TCAs) during early corticogenesis changes the specification and area size of the visual cortex [16,17]. For several years, even decades, the Protomap and Protocortex hypotheses developed as independent and contradictory models, both supported by a separated amount of studies [18-20]. In the last few years, a similar discussion is applied to the thalamus, where the influence of intrinsic versus extrinsic programs (cortical and peripheral) is being disassembled. The astonishing advances in molecular biology, genetics, imaging, and electrophysiology have led to new evidences and opened novel avenues to understand cortical and thalamic development as processes where intrinsic and extrinsic mechanisms cooperate. The aim of this review is not to give an extensive recapitulation of what is known about the origin and principles of thalamocortical organization; instead, we stress out the present understanding of how thalamus and cortex influence each other's development and function.

The first interactions: Development of the thalamocortical connectivity

Thalamocortical and corticothalamic axons (CTAs) must pursue a long journey passing through several territories before reaching their main targets, layer 4 cortical neurons and neurons of the thalamic nuclei, respectively (Figure 1). Every step of this trip needs to be tightly regulated to ensure a functional precise circuit. Thalamic neurons are generated from the alar plate of prosomere 2, between embryonic day (E)10.5 and E16.5 in mice [21,22]. Subsequently, thalamic axons are extended towards the prethalamus guided by prethalamic and ventral telencephalic axons [23-26]. Slit-Robo mediated repulsions avoid the entrance of TCAs into the hypothalamus, and force them to turn into the internal capsule, headed to the diencephalic-telencephalic border [27-30]. Once in the ventral telencephalon, corridor cells pave the way to traverse the mantle of the medial ganglionic eminence,

based on ErbB4-Neuregulin1 interactions [31]. Also in this region, distinct TCAs get topographically sorted based on molecular interactions [32-37] regulated by spontaneous calcium activity [38,39] and other factors. TCAs must then cross the pallium-subpallium boundary to reach the cortex.

Cortical neurons located in the pallial ventricular zone generate progressively between E11.5 and E16.5 (Figure 1). The earliest generated neurons migrate radially to occupy the deepest layers in the cortical plate, while the ones born at later stages migrate through to form upper layers [40,41]. Early-born subplate neurons (SuPNs) send their axons towards the thalamus around E12.5. According to the "handshake hypothesis", subplate axons meet TCAs at the pallial subpallium boundary, serving them as a scaffold. This close interaction has been demonstrated to be crucial for the arrival of TCAs to the cortex [24,42,43]. In contrast, CTAs grow towards the internal capsule and the thalamic reticular nucleus, where they wait for a day before invading the thalamus around E17.5 [44,45]. At the time TCAs arrive to the cortical primordium, around E15.5 in mice, layer 2-4 neurons are not born yet [44,46], and thus accumulate at the subplate. During this waiting period, TCAs engage in activity-dependent interactions with subplate cells, leading to their realignment before entering the cortical plate [47,48]. Shortly before birth, TCAs invade the cortical plate, form branches and synaptic contacts with layer 4 neurons principally. Cortical cells may influence this process by sending "stop" and "branch" signals [49]. Although cortical regionalization is initially created by graded expression of various cortical genes, TCA input can influence the size and identity of specific cortical areas [2-4,50].

Cortical influence over thalamocortical development

As aforementioned, the arrangement of cortical maps is initially controlled by intrinsic factors and particular genetic developmental programs [20,51]. These factors not only influence the positioning of sensory maps, but are also important for guiding TCAs towards their final destination. For instance, the fibroblast growth factor 8 (FGF8), an essential anterior telencephalic morphogene, promotes an early developmental cascade that specifies distinct cortical areas [52]. Duplications and areal shifts of sensory cortical maps are generated when FGF8 is expressed

ectopically [5,53] (Figure 2). Therefore, FGF8 acts as an indirect regulator of TCA innervation by providing early positional information of cortical guidance cues. Similarly, other transcription factors such as Emx2 [54], Pax6 [55], Sp8 [56] and COUP-TF1 [6,56,57] are important to specify positional information in the cortex [20,58]. For example, Pax6 miss-expression in cortical progenitors, which preferentially determines sensorimotor areas, prompts to an aberrant and miniaturized body map representation in the principal somatosensory area (S1) [1]. This significant reduction of cortical territories engages a top-down plasticity process that generates an aberrant body representation also in the ventro-posteromedial nucleus (VPM) of the thalamus (Figure 2) [1]. Postmitotic COUP-TF1 expression is essential to repress frontal/motor area features in parietal and occipital cortex, thus controlling occipital-related genes such as Ror[57]. When COUP-TF1 is eliminated specifically from the cortex, primary sensory areas suffer a caudal compression, leading to a shift of TCA connectivity [6] (Figure 2). All these evidences highlight the influence of cortical genetic patterns to the final position of TCAs.

Additional cortical factors may also influence TCAs development. For instance, early neuronal activity patterns emerge during the development of the cortex, starting from early asynchronous activity to correlated firing, local burst, and ultimately, experience dependent modifications [59]. This neuronal activity regulates key developmental features, such as, axonal navigation and refinement of topographic maps [60,61]. Disruption of cortical neuronal activity provokes defects in the somatosensory map formation. Studies with specific cortical knock-outs for NMDAR1, AC1 and mGluR5 show deficiencies in barrel wall formation, disturbing neuronal reorganization. These cortico-specific animal models also show slight TCA defects, developing smaller and blurred barrels, while barrelloids in the thalamus remain intact [62-64].

Thalamic influence over cortical development: arealization and circuitry

As aforementioned, the cortex modulates distinct aspects of thalamic development and maturation, but it also receives an influence from the thalamus in a reciprocal fashion. Thalamic input impacts on several aspects of cortical development such as

cortical proliferation, CTAs connectivity, cortical area specification, interneuron maturation and circuits assembly. However, bulk of evidence shows that several features of cortical development can proceed with little thalamic influence, at least embryonically. For instance, a shifted topography of thalamic connectivity during embryonic life only weakly affects cortical regionalization [65]. Moreover, mice lacking TCAs and corticofugal axons, and thus not receiving subcortical influence, develops a grossly normal cortex [66], further supporting the existence of intrinsic cortical mechanisms.

Anyhow, the influence of thalamic input into cortical developmental programs is doubtless. Early on, TCAs release a diffusible factor (related to bFGF signaling) that exerts a mitogenic effect on upper layer cortical progenitors, increasing the number of proliferative divisions and influencing the early developmental features of glutamatergic cortical neurons [67]. Therefore, TCAs might influence cortical arealization. In fact, recent studies have demonstrated that TCAs modulate the correct topographical navigation of their counterparts, the CTAs. Total abolishment of TCAs provokes CTAs to take an abnormal path, and that they acquire a corticospinal-like trajectory at the subpallium [68]. Thus, TCAs direct CTAs into the corridor region [31]. Moreover, TCAs are not only essential for guiding CTAs to their final targets, genetic manipulations of thalamic structures confirmed the importance of the thalamic input as an extrinsic factor, imperative for the correct development and establishment of the right genetic profile of the primary [2] versus high-order [3] cortical sensory areas (Figure 3). TCAs from first-order sensory nuclei provide specific information to instruct target cortical region. In the absence of these TCAs cortical areas will remain in the default mode and engage in a high-order fate [2-4].

A correct topographical axonal organization is crucial to generate cortical maps, being a common feature in different sensory modalities and systems [69,70]. This organization is shaped by axon guidance molecules and axonal competition, that drive the correct axon pathfinding along their route. In the thalamocortical system, the precise point-to-point topographic organization of TCAs plays an important role in the appropriate development of cortical maps [12]. Disrupting the correct

organization of TCAs along their pathway leads to a blurry somatosensory map, which lacks TCAs clustering [71]. This study showed for the first time the importance of the fine ordering of TCAs at the subpallium for the correct arrangement of the functional whisker pad representation in S1. Nevertheless, topographical miss-organization of TCAs subcortically could also affect the barrel map formation indirectly, by influencing the development of the thalamic barrelloids or by top-down mechanisms.

Moreover, thalamic input influences diverse aspects of cortical interneuron ontogeny [72]. Recently, several studies have pointed out the influence of thalamic activity-dependent mechanisms over the process of interneurons identity acquisition and function. For instance, thalamocortical input preferentially activates NR2B receptors expressed by Reelin interneuron population. Thus, manipulating thalamic input by the restricted expression of tetanus toxin in the thalamus prevents thalamic glutamate release, and influences the axonal and dendritic development of Reelin expressing cortical interneurons [73]. Moreover, other types of interneurons are also affected by the lack of TCA input, such as PV and SST neurons [74]. It has been shown that thalamic input innervates transiently somatostatin interneurons during the first postnatal week, before sensory onset. This early synaptic connectivity is important to modulate thalamic input to pyramidal and PV neurons in layer 4, contributing to the correct development of cortical circuits [73,75-77] . More than that, TCA input directly influences the correct development of layer 4 spiny stellate neurons. Blockade of thalamic presynaptic release provokes significant morphological changes in layer 4 cells that fail to develop the proper segregation and polarization needed to form the barrel walls around the TCA clusters [78,79]. In addition, the interference of thalamic activity triggers layer 4 spiny stellate neurons to develop an apical process, which is normally present in pyramidal neurons in non-granular layers [78].

Although the neuronal location is a process mainly controlled by intrinsic cortical mechanisms, the postmitotic identity of these cortical cells could be influenced by subcortical inputs. In the absence of a first-order thalamic nucleus, the corresponding primary cortical area acquires properties of associative secondary

areas [2-4] (Figure 3). The ablation of the VPM not only causes layer 4 neurons of S1 to molecularly resemble of layer 4 neurons from secondary somatosensory areas (S2), but also, that presynaptic terminals from the high-order posteromedial thalamic nucleus (POm) aberrantly target layer 4 neurons in S1. This connection (POm-S1 layer 4) acquires the ability to respond to noxious stimuli, a function carried out by S2 layer 4 neurons in wild-type animals [4]. Altogether, these results strongly suggest that thalamocortical inputs exert a control of the molecular identity and function of postsynaptic layer 4 neurons and their upstream circuits.

The thalamus and the cortex are electrophysiologically interconnected from very early developmental stages. This interconnection is based on spatiotemporal synchronization of electrophysiological patterns from both structures. Early studies have shown that during the first postnatal week early gamma oscillations implement thalamocortical synchronization between a single thalamic barreloid and its equivalent cortical barrel [80]. More recently, it has been well demonstrated that this thalamic neuronal activity also influences the formation of sensory maps [81,82] and it has been recorded in vivo in the postnatal cortex [83]. Disruption of the presynaptic glutamatergic communication between somatosensory TCAs and the cortex disturbs, to some extent, barrel map formation. Ablation of NMDAR in the VPM perturbs profoundly the formation of barrelloids and consequently, the patterning of the posteromedial barrel subfield in S1 prevails blurred, meanwhile, barrellettes in the brainstem remain intact [84]. Manipulations of the presynaptic release in the TCA system also reproduce defects in barrel map formation: RIM1/RIM2 thalamic specific knock-out do not present defects in TC clusterization, although layer 4 spiny neurons acquire significant structural changes [85]. More severe phenotypes can be noticed when vesicular release is blocked from TCAs. Specific thalamic depletion of Vglut2 in a total Vglut1 knock-out background, generates a barrel less phenotype that lacks the somatosensory cortical map. Thus, a thalamic presynaptic vesicular release seems to be important for the cortical map formation [78], although the lack of the postsynaptic cortical Vglut1 may also contribute to the phenotype observed. Presynaptic AC1 is also critical for barrel formation, as thalamic knock-out presents disrupted barrels [86]. Nevertheless, it is not clear whether this effect is produced by a dysregulation of neurotransmitter

release or acting over axon guidance molecules and spontaneous activity, as it has been already shown in the retinotectal projection [87].

It is clear that cortex and thalamus influence each other throughout development within the same sensory modality. But, do they also interact for triggering mechanisms of plasticity as those observed after sensory manipulations? Are cross-modal cortical modifications generated and controlled by subcortical input? The expansion of the posteriomedial barrel subfield in S1 has been described after postnatal visual deprivation [88,89], before sensory-experience onset [90]. A recent study locates this experience-independent cross-modal expansion as a phenomenon that is led by the thalamus, where intercommunication among distinct sensory modalities takes place [50]. This intercommunication occurs by means of spontaneous calcium waves, which control thalamic gene expression and the arborisation of TCAs in sensory cortical areas (Figure 3) [50]. Genetic disruption of prenatal thalamic calcium waves leads to changes in the pattern of thalamic activity in the remaining nuclei. This novel mechanism serves to promote plasticity among developing sensory systems and places the thalamus, for the very first time, as a main input regulator of cross-modal cortical readjustments before sensory onset.

Future perspectives

As highlighted in this review, the interaction between cortex and thalamus has always gained much attention, as the thalamocortical circuit is established from early embryonic stages at the time both structures are developing key features. The generation of tools to specifically target and modify single brain structures has been fundamental for advancing in our knowledge of the thalamo-cortical reciprocal modulation. However, further studies will be necessary to determine many aspects of this cortical and thalamic relationship. For instance, to what extent the acquisition of topographical and electrophysiological functional properties of cortical and thalamic neurons depends on their interaction. Moreover, peripheral input must also be included into the equation, as it has been shown that a correct thalamocortical circuit and interaction depends on it. Finally, the recent findings on the involvement of thalamic spontaneous activity in modulating the developmental aspects of cortical features constitute a fascinating topic for the coming years that will contribute in further understanding the role of neural activity in thalamocortical wiring.

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Figures and legends

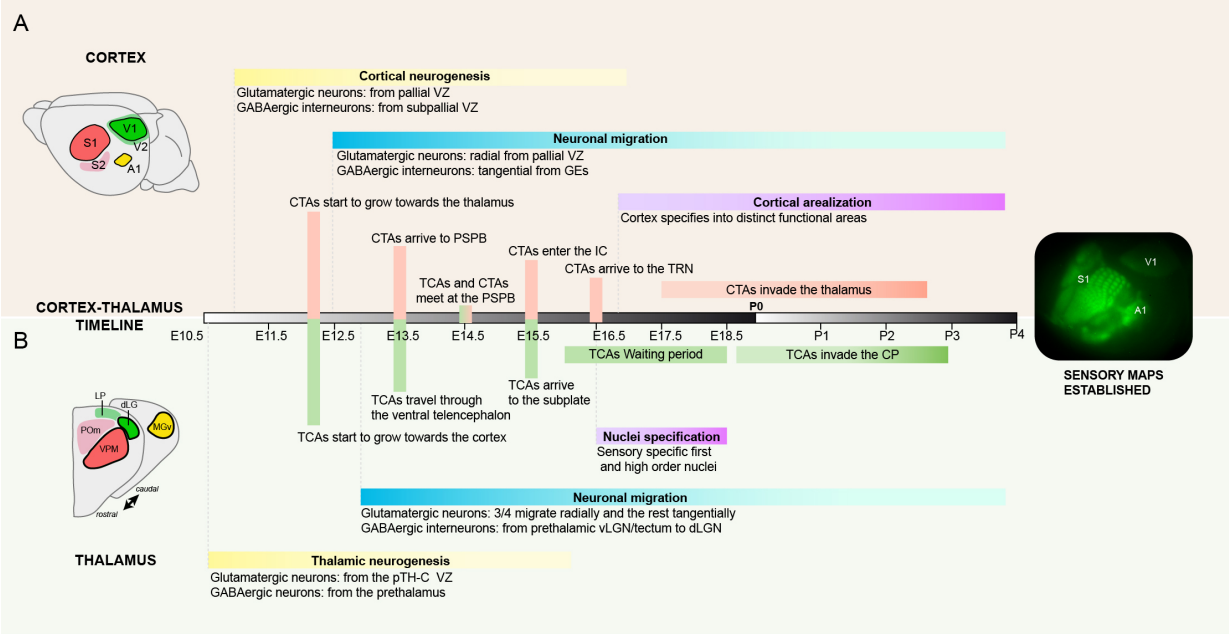

Fig.1. Development of corticothalamic and thalamocortical circuits

Developmental time line of thalamocortical and corticothalamic circuits, notice that the upper part of the scheme represents the cortical development timeline (A) and the lower the thalamic one (B). The central core of the scheme is focused in summarizing the CTAs and TCAs navigation. Key developmental time-points and special features are highlighted in the scheme. (A) Cortical development of excitatory neurons takes place from E11.5 to E16.5, at the same time, GABAergic interneurons invade the cortical plate. (B) Thalamic glutamatergic neurons are born between E10.5 and E16.5 at the ventricular zone of the caudal pre-thalamus. GABAergic thalamic interneurons, which origin remains controversial, invade the thalamus during the first postnatal week.

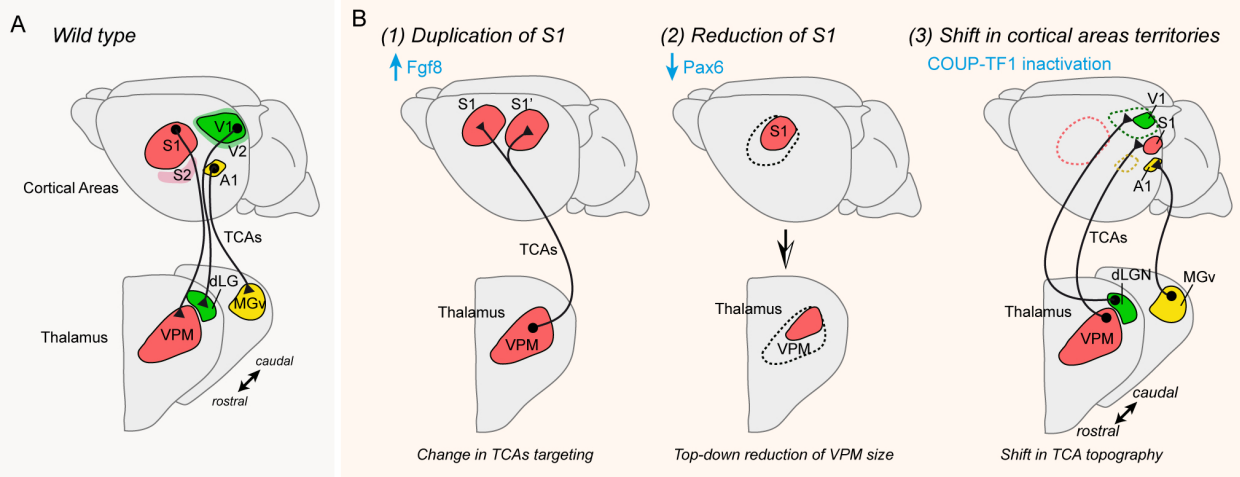

Fig.2. Cortical influence over thalamic development

Schemes representing the interaction between cortex and thalamus at early developmental stages. (A) In the wild type, a sensory representation for each modality (visual, somatosensory and auditory) is represented in both the cortex and thalamus. TCAs are topographically organized in a sensory modality fashion. (B) Modifications of sensory cortical areas promote thalamic rearrangements, either by TCA rewiring or by changing thalamic structures. Ectopic expression of FGF8 at the caudal pole of the cortex generates a duplication of S1, both S1 and S1' are innervated by TCAs from the VPM nucleus. Reduction of Pax6 expression in the cortex generates a miniaturize S1 cortical area, engaging a reduction of the VPM nucleus. Ablation of COUP-TF1 expression in the cortex produces a shift of cortical areas towards de occipital pole of the cortex, TCAs rewire to properly connect with the shifted cortical territories. POm, Posterior Medial nucleus; LP, Lateral Posterior nucleus; dLG, Dorsal Lateral Geniculate nucleus; MGv, ventral division of the Medial Geniculate body; VPM, Ventral Posterolateral nucleus; TCAs, Thalamocortical axons; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; V1, primary visual cortex; V2, secondary visual cortex; A1, primary auditory cortex.

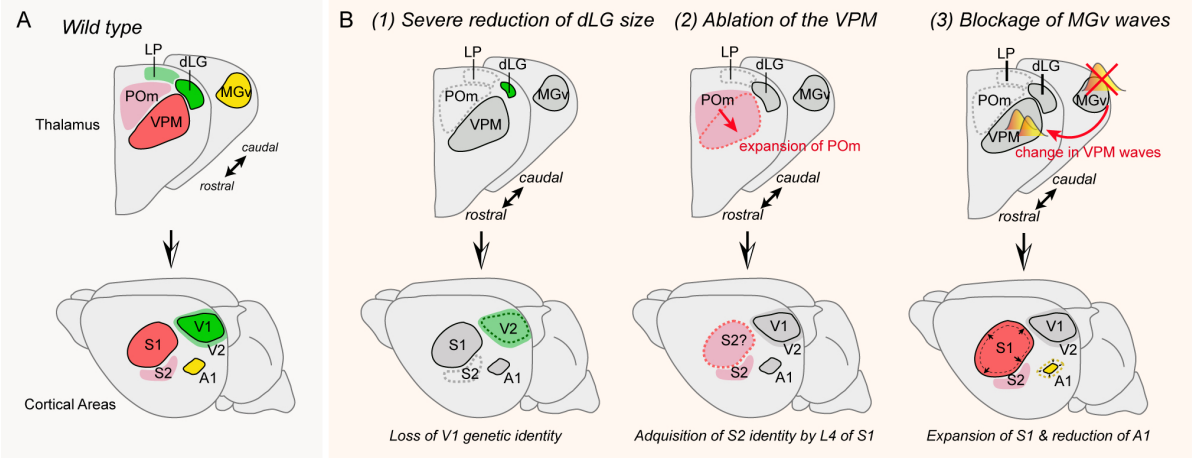

Fig.3. Bottom-up plasticity. Thalamic influence over cortical arealization and circuitry

Schemes representing the interaction between cortex and thalamus at early developmental stages. (A) In the wild type, a sensory representation for each modality (visual, somatosensory and auditory) is represented in both the cortex and thalamus. Primary and secondary areas are represented in both structures. (B) Ablation or reduction of first-order sensory nuclei of the thalamus engage a bottom-up plasticity, where corresponding primary cortical areas acquire properties of secondary areas. Cross-modal plastic changes are directed by embryonic thalamic activity before sensory onset. Disruption of spontaneous calcium activity in the auditory thalamic nucleus engages an increment of calcium waves in the VPM, producing an increment of the S1 size by augmenting TCA arborisation. POm, Posterior Medial nucleus; LP, Lateral Posterior nucleus; dLG, Dorsal Lateral Geniculate nucleus; MGv, ventral division of the Medial Geniculate body; VPM, Ventral Posterolateral nucleus; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; V1, primary visual cortex; V2, secondary visual cortex; A1, primary auditory cortex.

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Annotated references

(**) 1. Zembrzycki A, Chou SJ, Ashery-Padan R, Stoykova A, O'Leary DD: Sensory cortex limits cortical maps and drives top-down plasticity in thalamocortical circuits. Nat Neurosci 2013, 16:1060-1067. In this manuscript, the authors report for the first time a top-down plasticity in the somatosensory system. Generation of an animal model in which S1 remain extremely reduce during development engage top-down changes: From cortex to subcortical structures, such as the thalamus. Hence, VPM remain re-patterned due to cortical modifications.

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(**) 3. Pouchelon G, Gambino F, Bellone C, Telley L, Vitali I, Luscher C, Holtmaat A, Jabaudon D: Modality-specific thalamocortical inputs instruct the identity of postsynaptic L4 neurons. Nature 2014, 511:471-474. This novel study show for the first time that the correct development of thalamic structures is crucial for the acquisition of a precise cortical circuitry, molecularly and functionally. Cell-type specificity of layer 4 neurons is instructed by the origin of TCAs. The ablation of the VPM thalamic nucleus promotes the functional rewiring of POm projections to layer 4 neurons in S1, developing functional and molecular features of POm-target neurons.

(**) 4. Molnar Z, Adams R, Blakemore C: Mechanisms underlying the early establishment of thalamocortical connections in the rat. J Neurosci 1998, 18:5723-5745. First in vivo observations demonstrating the existence of an anatomical relationship between developing thalamic and early cortical axons. These evidences confirmed the handshake hypothesis by which thalamic axons use subplate axons as a scaffold to reach the neocortex.

(**) 5. Moreno-Juan V, Filipchuk A, Anton-Bolanos N, Mezzera C, Gezelius H, Andres B, Rodriguez-Malmierca L, Susin R, Schaad O, Iwasato T, et al.: Prenatal thalamic waves regulate cortical area size prior to sensory processing. Nat Commun 2017, 8:14172. This study describes for the very first time the existence of a prenatal intercommunication amongst developing sensory systems principal that takes place in the thalamus by means of propagating spontaneous calcium waves. This intercommunication promotes cross-modal plastic changes in the somatosensory cortex upon sensory loss.

(*) 6. Iwasato T, Datwani A, Wolf AM, Nishiyama H, Taguchi Y, Tonegawa S, Knopfel T, Erzurumlu RS, Itohara S: Cortex-restricted disruption of NMDAR1 impairs

neuronal patterns in the barrel cortex. Nature 2000, 406:726-731. In this manuscript, the authors develop an animal model in which NMDAR1 (NR1) is deleted specifically in excitatory cortical neurons. The findings indicate that cortical NMDARs are essential for the aggregation of layer 4 spiny neurons into barrels and the development of a fully normal thalamocortical pattern in S1.

(**) 7. De Marco Garcia NV, Priya R, Tuncdemir SN, Fishell G, Karayannis T: Sensory inputs control the integration of neurogliaform interneurons into cortical circuits. Nat Neurosci 2015, 18:393-401. In this paper, the authros show that superficially positioned Reelin-expressing interneurons in the mouse somatosensory cortex receive afferent innervation from both cortical and thalamic excitatory. Moreover, interfiring with thalamic transmission leads to axo-dendritic morphological defects in these interneurons.

(*) 8. Li H, Fertuzinhos S, Mohns E, Hnasko TS, Verhage M, Edwards R, Sestan N, Crair MC: Laminar and columnar development of barrel cortex relies on thalamocortical neurotransmission. Neuron 2013, 79:970-986. This study show that blocking specifically presynaptic glutamatergic transmission from the thalamus prevents the formation of barrel cortex. Indicating the importance of the thalamic activity as an extrinsic factor, which clearly influence the development and refinement of the cortex.

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(*) 10. Lokmane L, Proville R, Narboux-Neme N, Gyory I, Keita M, Mailhes C, Lena C, Gaspar P, Grosschedl R, Garel S: Sensory map transfer to the neocortex relies on pretarget ordering of thalamic axons. Curr Biol 2013, 23:810-816. This study demonstrates the importance of the fine ordering of TCAs in the correct arrangement of the functional whisker pad representation in S1. Genetic manipulations of axon guidance molecules in the subpallium lead to the scramble ordering of TCAs and the loss of a point-to-point topography between VPM and S1. TCAs failed to form a topographic barrel map.

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Prenatal thalamic waves regulate cortical area size prior to sensory processing

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