Activity-dependent regulation of thalamic interneuron and microglia in the visual thalamus

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Que Dña. *Irene Huerga Gómez* ha realizado bajo nuestra supervisión el trabajo titulado "**Activity-dependent regulation of thalamic interneurons and microglia in the visual thalamus**" conforme a los términos y condiciones definidos en su Plan de Investigación y de acuerdo al Código de Buenas Prácticas de la Universidad Miguel Hernández de Elche, cumpliendo los objetivos previstos de forma satisfactoria para su defensa pública como tesis doctoral.

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A mis padres y a mi hermano
A Alfonso

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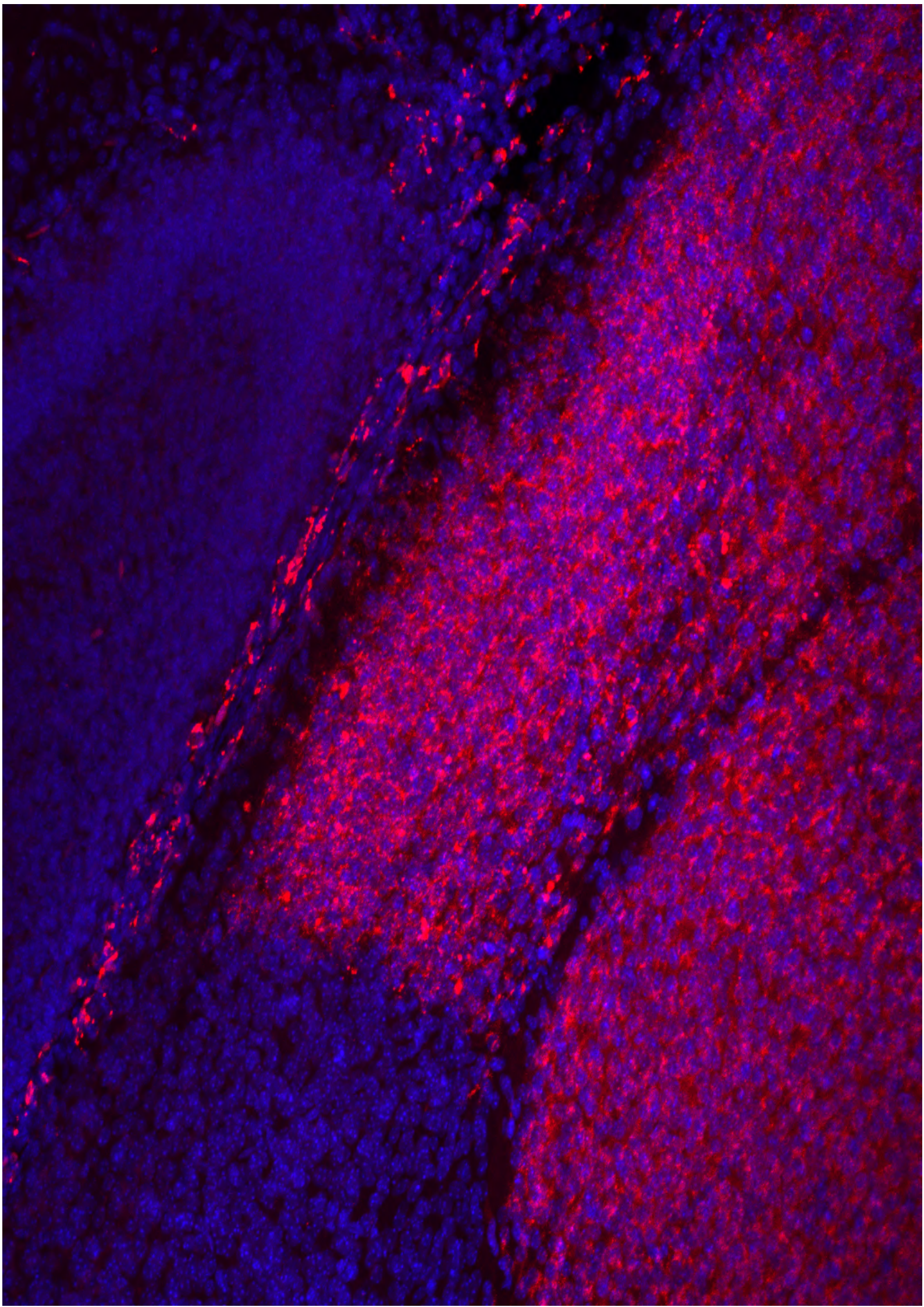
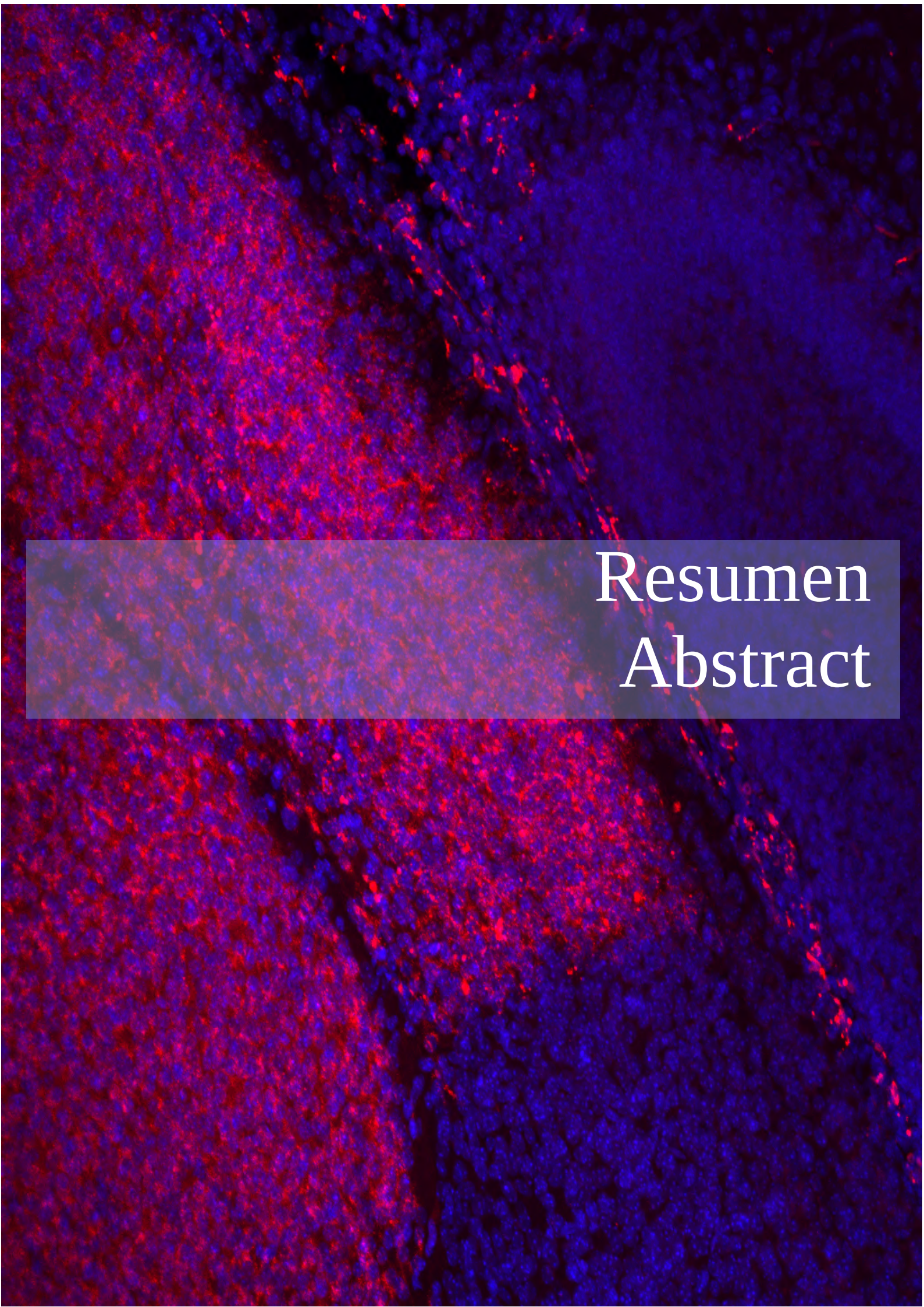
ABREVIATIONS

A1	Primary auditory cortex	**MGv**	Ventromedial Geniculate Body
Cbx	Carbenoxolone	**NMDAR**	NMDA Receptor
CTAs	Corticothalamic axons	**POm**	Posterior Medial Nucleus
DCC	Deleted in Colorectal Carcinoma	**PSPB**	Pallial-Suppallial Boundary
dLGN	Dorsal Lateral Geniculate Nucleus	**P**	Prosomere
DTB	Diencephalic-Telencephalic boudary	**PLX**	PLX3397
EmbBE	Embryonic Bienucleatioin	**PV**	Parvalbumin
Epib	Epibatidine	**RGCs**	Retinal Ganglion Cells
Fgf	Fibroblast Growth Factor	**S1**	Primary Somatosensory Cortex
FO	First Order	**SC**	Superior Colliculus
GABA	Gamma-Aminobutyric Acid	**Shh**	Sonic hedgeghog
Gbx2	Gastrulation Brain Homeobox 2	**SST**	Somatostatin
GFP	Green Fluoresscent Protein	**TCAs**	Thalamocortical Axons
HO	Higher Order	**ThKir**	Thalamic Kir
IC	Inferior Colliculus	**Th-C**	Caudal domain of the thalamic complex
INs	Interneurons	**Th-R**	Rostral domain of the thalamic complex
ION	Infraorbital Nerve	**TRN**	Thalamic Reticular Nucleus
IZ	Intermediate Zone	**V1**	Primary Visual Cortex
L	layer	**VB**	Ventrobasal Complex
LP	Lateral Posterior Nucleus	**vGLut**	Vesicular Glutamate Transporter
Ms	Microglia	**VPM**	Ventral Posterior Medial Nucleus
MGE	Medial Ganglionic Eminence	**VZ**	Ventricular zone

MGd	Dorsomedial Geniculate Body	**Wnt**	Wingless-type MMTV Integration Site Family
mGluR5	Metabotropic glutamate Receptor 5	**ZLI**	Zona limitans intrathalamica
		ZI	Zona incerta





Resumen
Abstract

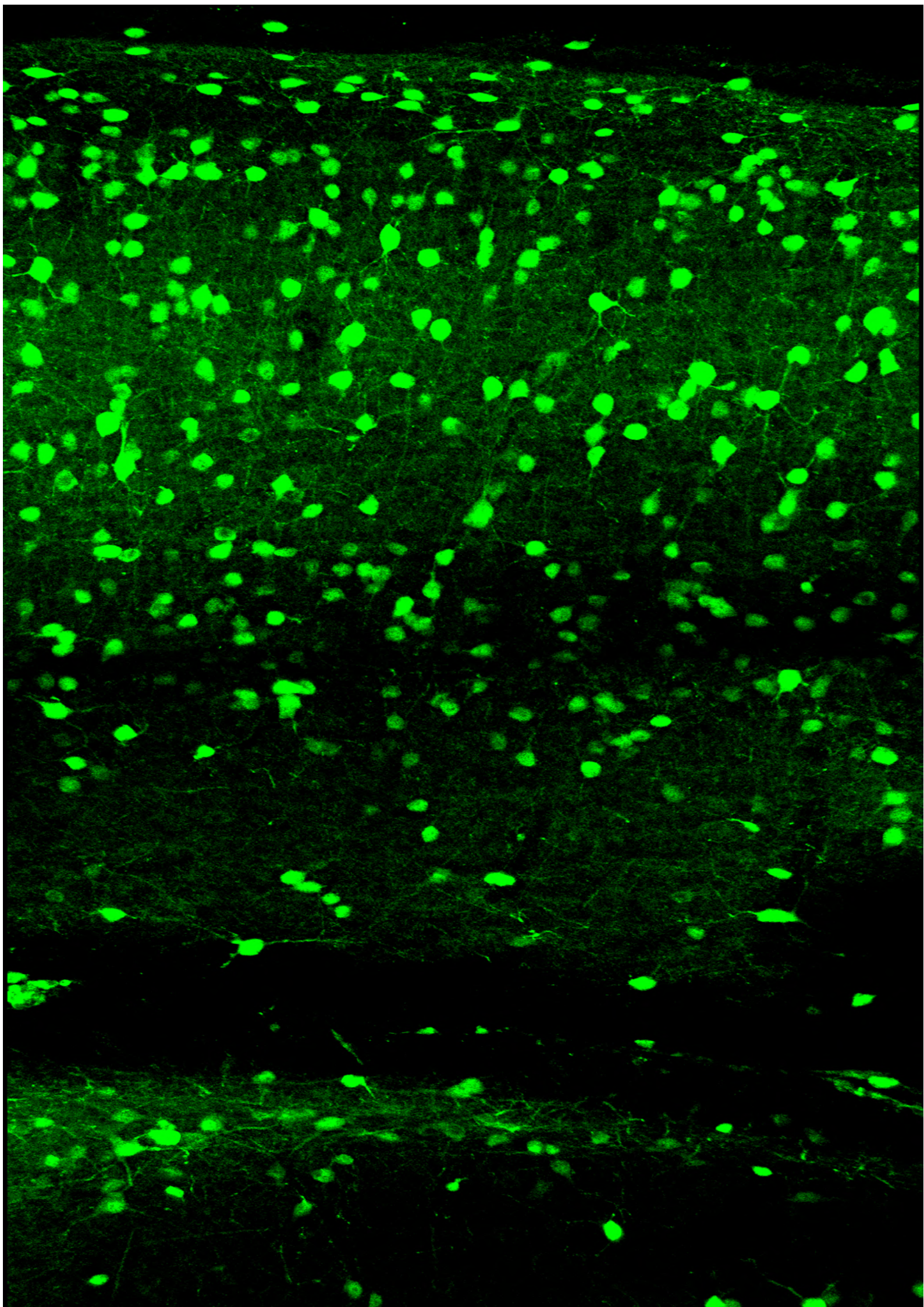
RESUMEN

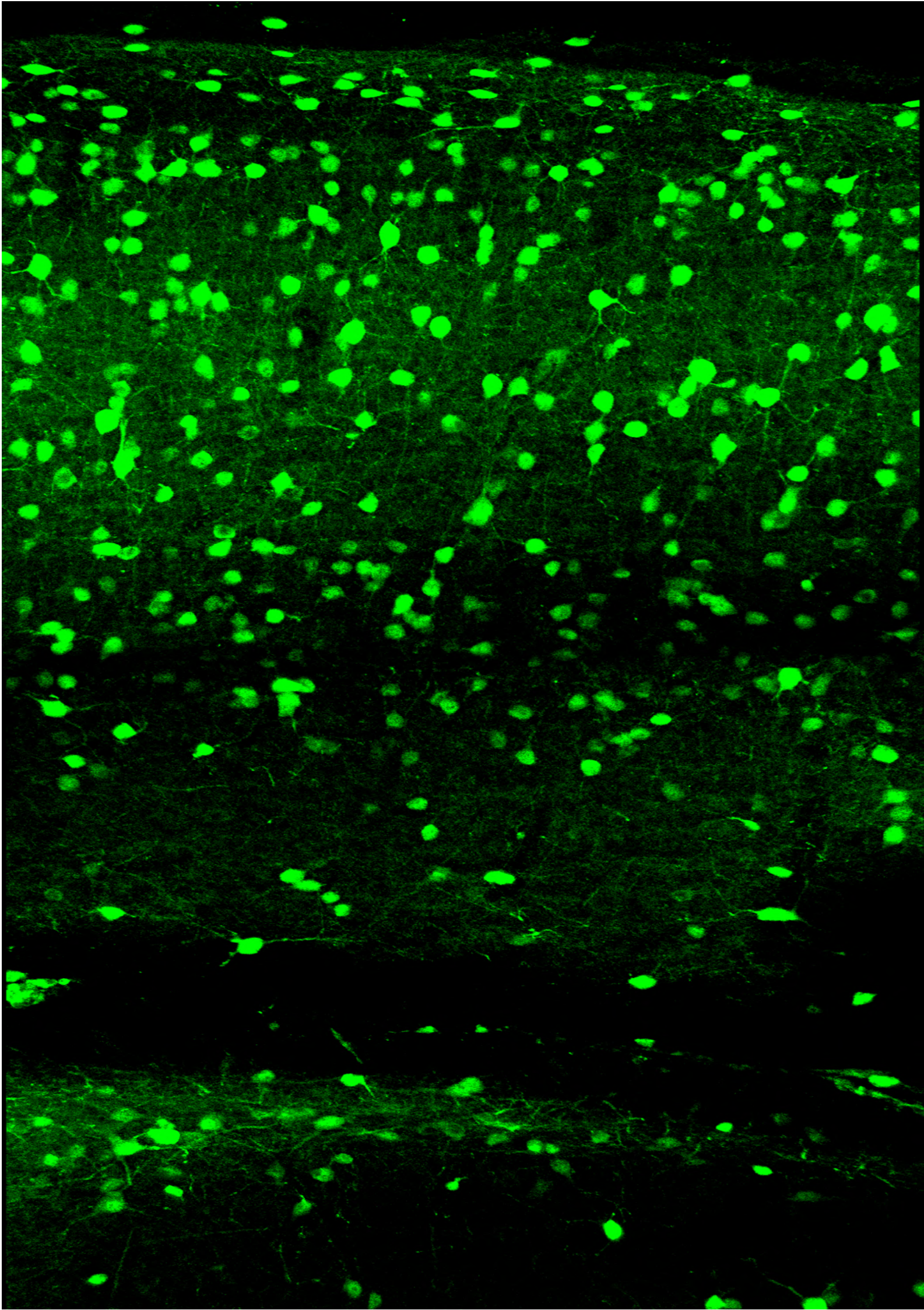
Los circuitos talámicos están formados por neuronas de proyección excitadoras y neuronas inhibidoras. De manera similar a como ocurre en la corteza, las neuronas excitadoras talámicas nacen de progenitores que se encuentran en la zona proliferativa del tálamo en desarrollo, mientras que las interneuronas inhibidoras locales nacen fuera del tálamo y necesitan migrar hasta él para integrarse en el circuito. En ratón, las interneuronas locales del tálamo se encuentran principalmente en el núcleo dorso-lateral geniculado (dLGN), el núcleo visual primario, encargado de recibir los axones retinales y proyectar a la corteza visual primaria (V1). La integración de estas interneuronas en el circuito comienza durante el desarrollo postnatal temprano en ratón. A pesar del estado inmaduro de las conexiones neuronales a esta edad, los circuitos son activos de manera espontánea, con patrones definidos de disparo. Por lo general, se ha observado que la actividad periférica es relevante para la correcta migración e integración de las interneuronas talámicas. Sin embargo, aún no está claro si estos procesos se ven afectados por patrones específicos de actividad que surgen en paralelo y son mediadas por otras fuentes. En este proyecto, describimos cómo las interneuronas se comportan de manera diferente a distintas etapas del desarrollo, desde estadíos tardíos embrionarios hasta estadíos postnatales tempranos, tras interrumpir la actividad retinal o talámica. El bloqueo de la actividad retinal mediante diferentes procedimientos confirma resultados previos que sugieren que los axones retinales son necesarios por la colocación de las interneuronas en el dLGN. Asimismo, hemos observado que la actividad intrínseca del tálamo es también importante para la velocidad de migración de estas interneuronas locales talámicas. Además, hemos visto que la actividad espontánea talámica durante el desarrollo embrionario es necesaria para el correcto posicionamiento de las interneuronas corticales en V1, principalmente aquellas que expresan SST y PV. Es interesante observar que los resultados obtenidos combinando todos los modelos muestran que el dLGN necesita llegar a un número concreto de interneuronas locales talámicas. Puesto que hay evidencias previas que sugieren que la maduración de la microglia y las interneuronas podría estar conectada, buscamos también estudiar las células microgliales en nuestros modelos. De esta forma, encontramos que la actividad intrínseca del tálamo afecta a la densidad de microglia talámica, así como a la microglia cortical en estadíos postnatales, mientras que la supresión de la actividad retinal no afecta a estas células. Así, la combinación de todos estos resultados sugiere que los patrones tempranos de actividad talámica son un

factor novedoso involucrado en la correcta integración de las interneuronas en el sistema visual.

ABSTRACT

Thalamic circuits are formed by excitatory projecting neurons and inhibitory neurons. Both cell types are essential for processing information. Similar to what happens in the cortex, thalamic excitatory neurons are born from progenitors found in the proliferative zone of the developing thalamus, while local thalamic inhibitory interneurons are born elsewhere and need to migrate in order to integrate into the thalamic circuit. In mice, local thalamic interneurons are mainly found in the dorso-lateral geniculate nucleus (dLGN), the primary visual nucleus of the thalamus, which receives retinal axons and projects to the primary visual cortex (V1). The integration of migrating interneurons into circuits starts during early postnatal development in mouse. In spite of the rather immature state of neuronal connectivity at this stage, circuits are spontaneously active with defined and different patterns of firing. In general, peripheral activity has been shown relevant to the correct migration and integration of thalamic interneurons. However, it is not clear whether these processes are differentially affected by specific patterns of activity that arise at subsequent temporal windows and are driven by different sources. Here, we describe how interneurons behave at different developmental stages ranging from late embryonic to early postnatal days, upon disruption of retinal or thalamic activity. The blockage of the retinal waves by different means have confirmed previous results suggesting that retinal axons are necessary for the correct allocation of interneurons into the dLGN. In addition, we have observed that thalamic intrinsic activity is also important for the speed of migration of local thalamic interneurons. Furthermore, we have seen that thalamic spontaneous activity during embryonic development is necessary for the correct positioning of cortical interneurons in V1, mainly somatostatin- and parvalbumin-expressing interneurons. Interestingly, the results obtained using the combination of models show that the dLGN needs to reach a given number of local thalamic interneurons. Because previous evidence suggests that the maturation of microglia and interneurons might be connected, we sought to study microglia cells in our models. We found that changes in intrinsic thalamic activity affect the density of thalamic microglia, as well as cortical microglia at postnatal stages, meanwhile the ablation of retinal activity does not affect these immune cells. Thus, all these results point at the early pattern of thalamic activity as a novel factor involved in the correct integration of interneurons within the visual pathway.





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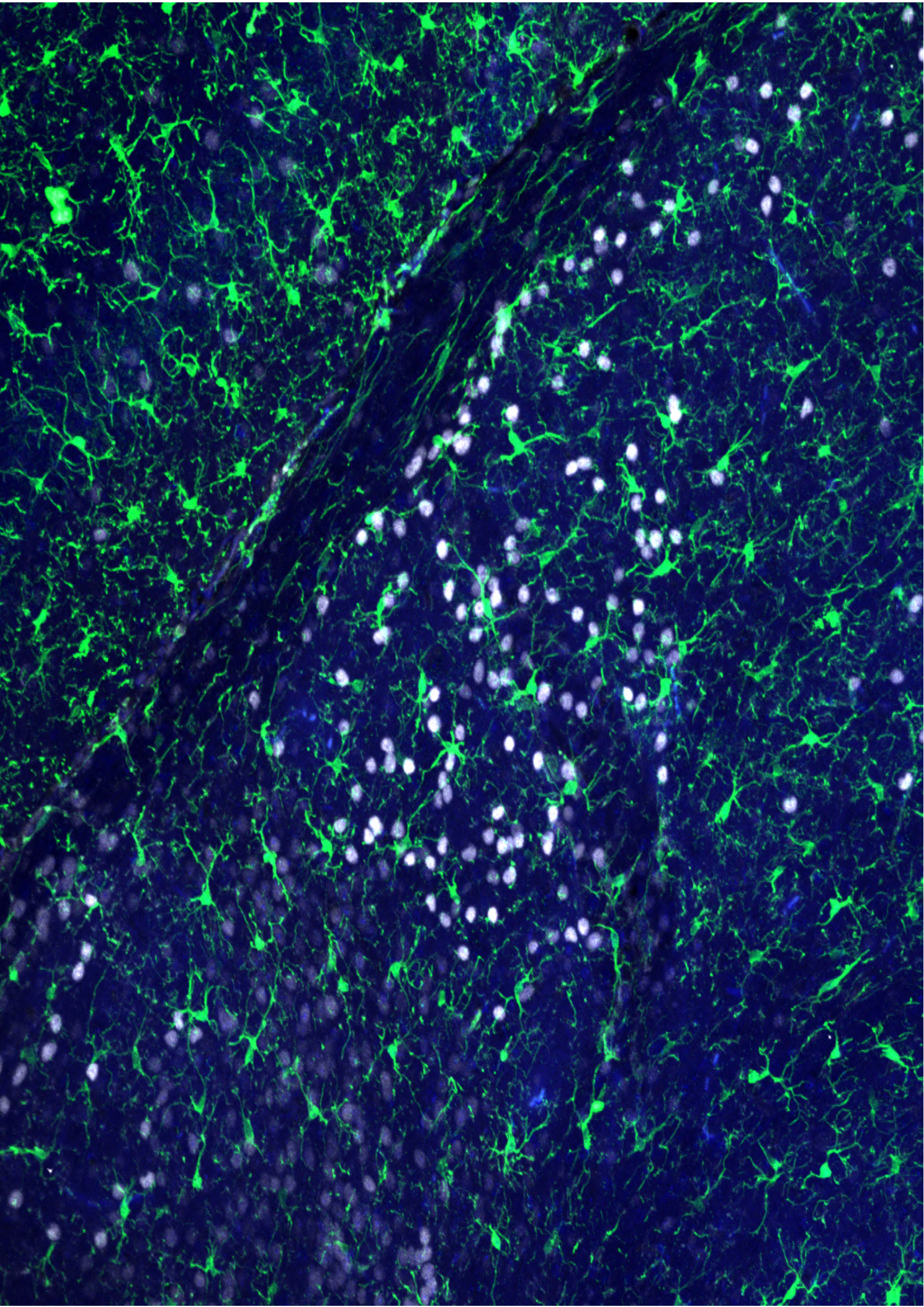
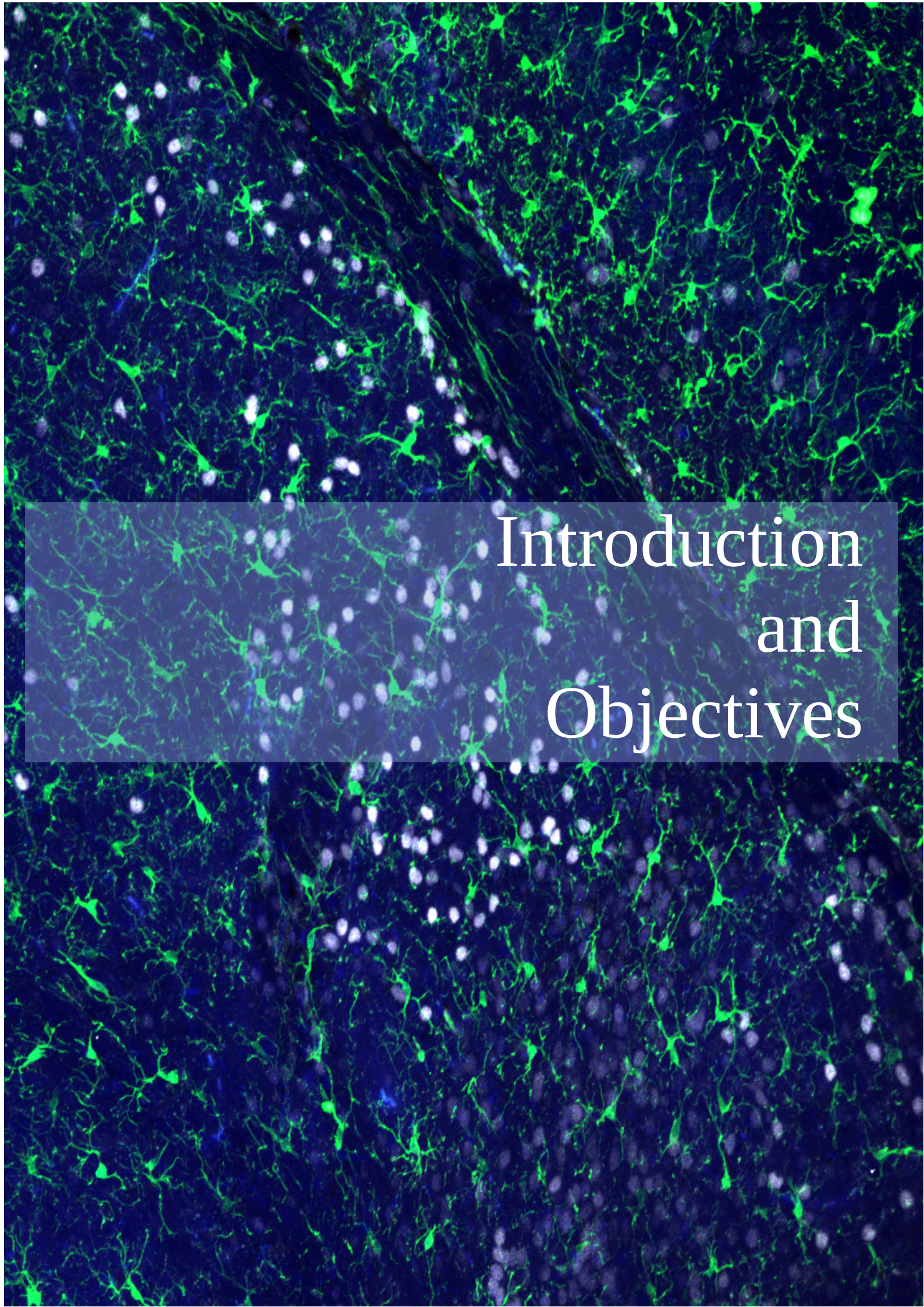




Introduction and Objectives

INTRODUCTION

1. Overview

The brain is a complex organ composed by many different structures that together conform the Central Nervous System. The neurons, which can be either excitatory or inhibitory, assemble the intricate network that directs the information coming from the environment and our own system to the respective areas where it is processed. In addition to neurons, there are other cell types that participate in this neural net, the glia. Among glia, we can find: i) astrocytes, which are stellate cells that exchange nutrients with neurons; ii) oligodendrocytes, which wrap around the neuronal axons; and iii) microglia, which are cells constantly surveilling the brain tissue.

In this thesis, we have studied two different populations of cells, inhibitory GABAergic interneurons and microglia, and how neuronal activity can affect them during development. We have focused on the thalamus, one of the main hubs for sensory processing, where interneurons are mainly found in the visual nucleus. Thus, we have directed our attention towards the visual pathway and how retinal activity or thalamic activity can influence the development of different cell-types.

2. The development of the visual pathway

2. 1. General overview

During development, sensory information passes through different sensory centers and is directed towards the cortex in a topographically organized manner. Neuronal activity plays an important role strengthening the connectivity between neighbor cells and promoting point-to-point topographical representations from the periphery to the cortex (Bednar & Wilson, 2016). Three sensory modalities that relay in the thalamus follow this principle: the somatotopic map in the somatosensory system, the retinotopic map in the visual system, and the tonotopic map in the auditory system **(Fig 1)** (Woolsey 1978, Merzenich et al., 1975; Tusa et al., 1978, respectively).

There are several mechanisms that regulate the construction of this organization, such as axon guidance cues, axon competition, and neuronal activity.

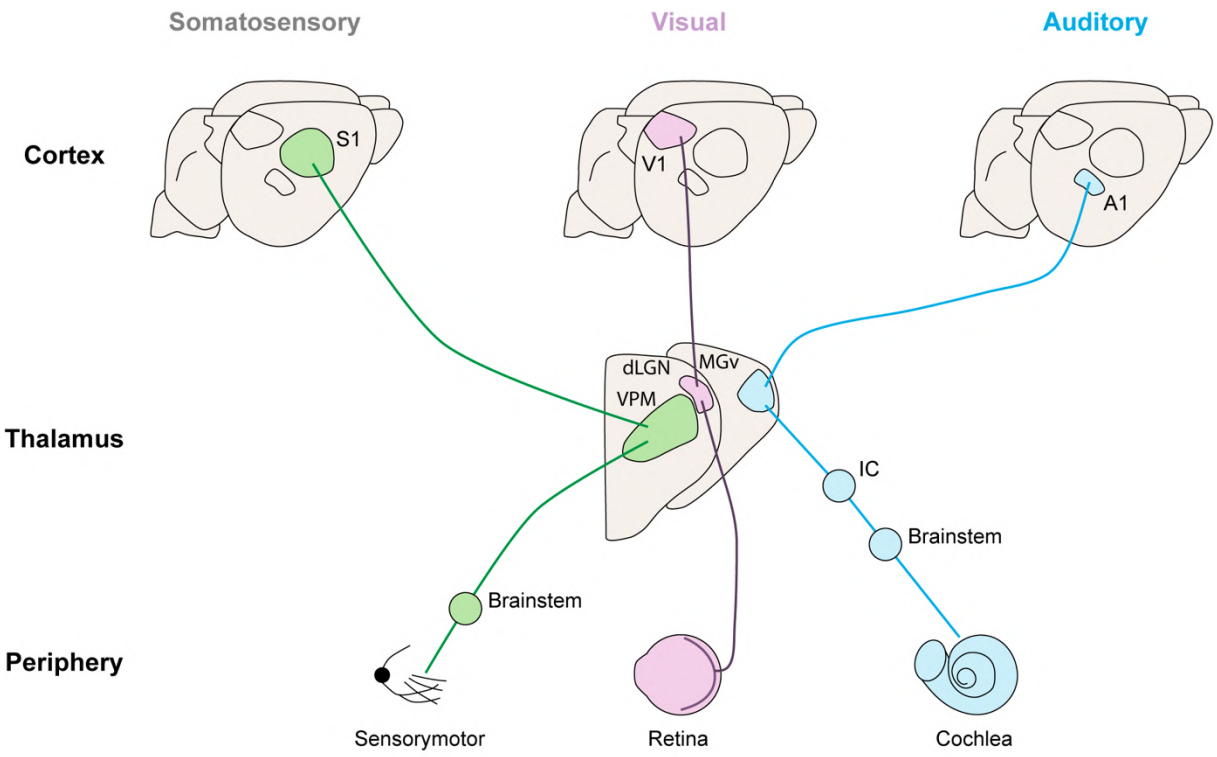


Figure 1. Connectivity from the peripheral stations to the thalamus and cortex. Schema showing how the primary sensory inputs from the peripheral sensory stations (whiskers, cochlea, and eye in mice) connect to the FO thalamic nuclei (VPM, dLGN, MGv), and the connections from the thalamus into the corresponding primary sensory cortices (S1, V1, A1). VPM: Ventral posteromedial nucleus; dLGN: dorso lateral geniculate nucleus; MGv: ventral medial geniculate nucleus; S1: primary somatosensory cortex; V1: primary visual cortex; A1: primary auditory cortex. Adapted from Martini et al., 2021.

2. 2. Neuroplasticity in the development of the sensory systems

During embryonic development, the topographical maps are established independent of sensory experience. Nevertheless, the later arrival of external stimuli varies the position and size of these areas (Huberman et al., 2008; Thompson et al., 2016). This plasticity has been described in different models of peripheral deprivation, such as blindness or deafness. In these cases, there is a compensatory mechanism increasing the processing power in the spared modalities, a

phenomenon called cross-modal plasticity. This cross-modal plasticity has been well described in different models of visual deprivation, where some existent or novel intermodal connections appear (Bavelier & Neville, 2002; Pascual-Leone et al., 2005).

2. 3. The visual system: From the retina to the visual cortex

The function of the visual system is to receive, process and interpret visual information from the environment. The retina, as a peripheral station, is the beginning of the visual pathway. Its function is translating the light into biolelectrical signals. It is formed by three layers: a layer with retinal ganglion cells, the inner nuclear layer; and the outer nuclear layer, which are intermingled with two synapse layers. From the outside to the inside, the first layer is the outer nuclear layer. Cones and rods are the photoreceptors that populate and form this layer, and they detect changes in the light. The second layer is the inner nuclear layer, which has bipolar, horizontal and amacrine cells. Amacrine and horizontal cells are in charge of filtering the visual information before it arrives to the bipolar cells. Bipolar cells, which integrate the filtered input, send the information to the layer of retinal ganglion cells (RGCs) (Masland, 2012). The RGCs are a wide population of cells comprised of more than thirty different subtypes. These cells are highly specialized, and can respond specifically to different stimuli, such as changes in light and darkness, or changes in directions (Baden et al., 2016; El-Danaf & Huberman, 2019). RGC axons bundle all together in order to form the optic nerve, which transmits the visual information to the central nervous system (Erskine & Herreral, 2014).

In mice, the axons from the RGCs start migrating towards central brain structures at E12.5. Most of the axons cross the optic chiasm and project to the contralateral side, while only a small percentage of axons stay in the ipsilateral side (Dräger & Olsen, 1980). Then, RGCs continue their journey and arrive to the visual thalamus (dorso lateral geniculate nucleus - dLGN) at around E15.5, and to the superior colliculus (SC) at E18.5 in order to control eye movement (Ackman et al., 2012; Bickford et al., 2015; Godement et al., 1984). Axons from these structures project to the cortex, from where projections are sent back to the thalamus. The visual thalamic nucleus receives both contralateral and ipsilateral projections from RGC. Retinal axons arriving to the contralateral dLGN occupy ~90% of the area, while ipsilateral axons cover ~10% of the area and reach the nucleus later, at P0-P2. This segregation is maintained throughout the entire visual pathway. Thus,

the dLGN and SC from both hemispheres have a topographical representation of the eyes, which is essential for binocular vision **(Fig 2)** (Huberman et al., 2008). However, when they arrive to the dLGN, contralateral and ipsilateral axons firstly overlap. By P4, their segregation starts out and they are refined into non-overlapping areas by eye opening (Bickford et al., 2010; Huberman et al., 2008; Pfeiffenberger et al., 2005a). Although retinal axons contribute to 5-10% of the synapses into the dLGN, they are the main excitatory drive for thalamic relay cells. The rest of the input arriving to the dLGN comes from primary visual cortex (V1) L6 projections, thalamic reticular nucleus (TRN) and nuclei in the brainstem (Bickford et al., 2010; Sherman & Guillery, 2002).

All central brain structures along the visual pathway exhibit a spatial representation of the visual field. This visual map reflects what was originally perceived by the retina, and therefore, it is known as retinotopy. Retinotopy appears at early embryonic stages thanks organized by neuronal spontaneous activity and guidance molecules, such as the pair Ephrins/Eph (Brown et al., 2000; Ellsworth et al., 2005; Huberman et al., 2005; Pfeiffenberger et al., 2005b; Vanderhaeghen et al., 2000).

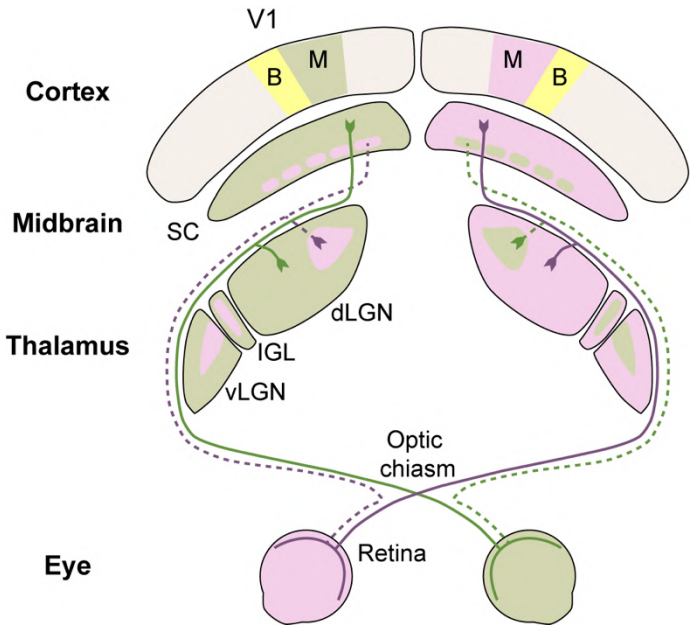


Figure 2. Development of retinal axons in the visual system. The majority of axons from the retina cross the optic chiasm, projecting into the dLGN in the thalamus. The dLGN receives binocular input and send projections towards V1, which is formed by two zones (M and B) in mice. The monocular zone (M) receives information from the contralateral eye, and the binocular zone (B) receives information from both eyes. Adapted from Seabrook et al., 2017.

3. The retina: General aspects in the development of the visual pathway

3. 1. Retinal activity during development

During the first postnatal week, the retina generates bursts of spontaneous activity that are transmitted along the circuit and help to organize the visual pathway (Torborg & Feller, 2005). By P14, when the eyelids open in mice, synchronous spontaneous activity desynchronizes and behaves similarly to the adult stage. At this point, other properties of V1, such as the retinotopic organization and the eye-specific segregation, are already established (Ko et al., 2013; Rochefort et al., 2009; Smith & Trachtenberg, 2007).

It is well known that the immature retina presents waves of spontaneous activity way before eye opening and visual experience (Akerman et al., 2002; Rochefort et al., 2011; R. O. L. Wong, 1999). In fact, retinal axons projecting to the dLGN have mature synapses capable of transmitting spontaneous activity already at perinatal stages (Ackman et al., 2012; Mooney et al., 1996). Light passes through the closed eyelid and reaches intrinsically-photosensitive RGCs, cones and rodes, which are cells that become functional after birth (Tu et al., 2005; Shen & Colonnese, 2016)). There are three types of retinal waves characterized by the molecular mechanism involved: type I, type II, and type III. Firstly, in mice, type I retinal waves appear embryonically from day E17 until ~P1, and are mediated by gap-junctions in neighbor RGCs (Bansal et al., 2000; Syed et al., 2004). They comprise large propagating events and small non-propagating events (Bansal et al., 2000; Voufo et al., 2022). Our group has recently demonstrated, for the first time, the function of these early retinal waves in sensory circuits development. We showed that visual and somatosensory circuits emerge as intermingled modules and that they become functionally segregated by a mechanism that occurs in the superior colliculus (SC) at perinatal stages in the mouse. This mechanism is triggered by the arrival of retinal axons to the SC and the activity of the type I retinal waves (Guillamón-Vivancos et al., 2022) demonstrating a crucial role of the earliest retinal spontaneous activity in circuit development and sensory-modality specification. Interestingly, it has been recently observed in vitro that type I retinal waves seem not to be completely removed upon the administration of a gap-junction blocker (Voufo et al., 2022) but further experiments need to be performed along this line to demonstrate the genesis of this retinal activity. Secondly, type II retinal waves, which are mediated by nicotin-cholinergic receptors in the amacrine cells, begin at ~P1 and last until ~P10,

and they appear in parallel to the retinotopic and eye-specific refinement (Huberman et al., 2008; Zheng et al., 2004). Finally, type III retinal waves start propagating from ~P10 until P14, and are mediated by glutamatergic transmission **(Fig 3)** (Ackman et al., 2012; Blankenship et al., 2009; Feller et al., 1996; Firth et al., 2005; Martini et al., 2021; Syed et al., 2004). Mice open their eyes between P12 and P14, and therefore, stage III retinal waves coexist with sensory experience mediated activity. Retinal waves type I, II, and III also transmit the spatial and temporal information important for the refinement of the visual pathway (Ackman et al., 2012). At the same time, they are regulating the growth velocity of the TCAs in a process mediated by DCC and Robo1 receptors (Castillo-Paterna et al., 2015; Mire et al., 2012). Strikingly, it has been observed in various *in vivo* experiments that spontaneous retinal waves propagate through the entire visual system, from the retina to the primary visual cortex (Ackman et al., 2012; Ackman & Crair, 2014; Kerschensteiner, 2016). In fact, retinal activity is transmitted to the visual thalamus, superior colliculus, the primary, and secondary visual cortices amplified by the corticothalamic loop (Murata & Colonnese, 2016). It is also involved in the map formation and dendrite refinement in the visual areas of the cortex (Burbridge et al., 2014; McLaughlin et al., 2003; Siegel et al., 2012). Both light-evoked activity in the retina and the spontaneous retinal waves interact during the development of the visual system in order to refine the retinogeniculate projections (Ackman & Crair, 2014; Renna et al., 2011; Tiriac & Feller, 2022).

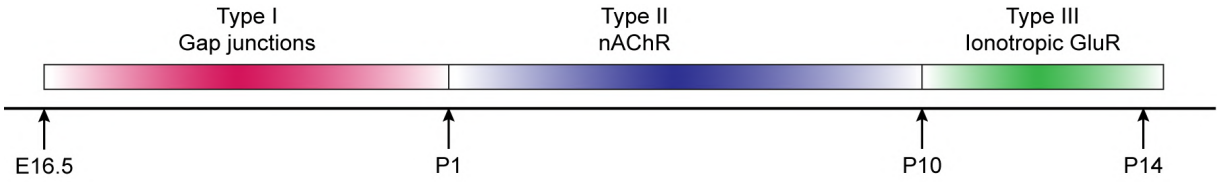


Figure 3. Retinal waves. Type I retinal waves are mediated by gap junctions, which start around E16.5 and propagate until ~P1.Type II retinal waves, on the other hand, are mediated by nicotin-cholinergic receptors, start around P1 and last until P10. Finally, Type III retinal waves start at around P10 and finish at around P14, and they are mediated by ionotropic glutamate receptors. Adapted from Ford and Feller, 2012.

Throughout the last decades, several experiments have helped to understand the role of retinal waves in the development of the visual system. For instance, tetrodotoxine (TTX) was used to block these spontaneous retinal waves by intraocular injection in cats (Shatz & Stryker, 1988). In addition, the administration of epibatidine in ferrets, which prevents specifically stage II retinal waves, affected eye segregation (Penn et al., 1998) and changed ocular dominance columns (ODC) patterning. It also increased the size of the receptive field in the visual cortex (Huberman et al.,

2006). Therefore, it is clear that retinal activity prior to eye opening is necessary and important for eye-specific segregation along the pathway and ODC formation.

Finally, a microarray of the visual cortex from monocularly enucleated mice was performed at different developmental stages and showed a group of genes that were downregulated (Majdan & Shatz, 2006). Brain-derived neurotropic factor (BDNF), transcription factor Fos, early growth factors 1 and 2 (Egr1 and Egr2), and genes involved in the regulation of the MAPK signaling pathway, were among those genes. Strikingly, it has also been observed that ephrinA5 was downregulated in the dLGN of binocular enucleated mice (Dye et al., 2012). In addition, the ablation of RGCs has shown that ADAMs metalloproteinases are differentially expressed in the dLGN of sensory deprived mice (Brooks et al., 2013a). All these experiments suggest that retinal activity is regulating the expression pattern of several dLGN genes (Brooks et al, 2013b).

3. 2. The importance of visual experience in the development of the visual system

As it has been explained above, retinotopy and eye-specific segregation are also reflected in V1. Back in the 1960s, several experiments performed by Hubel and Wiesel showed functional columns in the cortex, which were defined as ocular dominance columns (ODCs). These columns have a preference on a particular orientation of visual stimuli arriving to V1 (Hubel and Wiesel, 1963; Hubel and Wiesel, 1968; Hubel and Wiesel, 1977; Hubel et al., 1977), and have been used to study the role of visual experience in cortical plasticity (Katz and Crowley, 2002). To this aim, a plethora of invasive and non-invasive approaches have been implemented, such as eyelid suturing, intraocular injections of TTX, monocular and binocular enucleations, eye patching, dark rearing at different stages, or alternating light-dark periods have been used and have allowed to study the role of experience in the development of the visual pathway (Berlucchi & Rizzolatti, 1968; Gordon & Stryker, 1996; Morales et al., 2002; Négyessy et al., 2000; Petrus et al., 2014; Toldi et al., 1994, 1996).

Ocular dominance plasticity refers to a developmental phase particularly sensitive to changes in the visual experience that takes place from P20 to P35 (Gordon & Stryker, 1996; Sur et al., 2013). For instance, it has been described that monocular deprivation during this critical period decreases the

response from the deprived eye into the cortex and increases the response of the remaining eye. Surprisingly, there are also changes in thalamocortical axon (TCAs) connectivity, since the TCAs from the deprived eye are removed and the TCAs from the untouched eye expand (Antonini & Stryker, 1996). Moreover, visual deprivation during the critical period changes the transcriptional profile, activating genes involved in neuronal degeneration and growth factors (Lyckman et al., 2008).

4. The thalamus

4. 1. General overview

The thalamus has always been considered as a mere relay station due to its location and the input it receives. It is composed by several nuclei in charge of receiving all sensory modalities, but olfaction. Visual, somatosensory and auditory stimuli firstly convey into the thalamus, and then are sent to the respective cortical areas, where they are processed in a topographical manner (Huberman et al., 2008; Petersen, 2007; Tsukano et al., 2017; Garel and López-Bendito, 2014). The thalamus is composed mainly of projecting excitatory neurons that integrate information from different brain structures, as well as thalamic networks. In addition to excitatory neurons, there are inhibitory projecting neurons and local inhibitory interneurons which also participate in this circuit, shaping information processing.

The thalamus is composed of more than 40 anatomical nuclei or areas, each of them with different functions. These functions can be classified into motor, associative and sensory, with subdivision for each modality (visual: dLGN-LP, somatosensory: VBM-PoM, auditory: MGv-MGd). However, the nuclei can also be classified based on the origin of their input into first order nuclei (FO) and higher order (HO) nuclei. FO nuclei comprise the dorsolateral geniculate nucleus (dLGN), the ventral medial geniculate nucleus (MGv), and the ventral posteromedial nucleus (VPM), which are those that receive ascending input from the periphery **(Fig 4).** HO nuclei, on the other hand, are the lateral posterior nucleus (LP), the dorsal medial geniculate nucleus (MGd), and the posterior medial nucleus (PoM). They are considered modulators of the sensory signals since they receive direct input from neurons in layer 5 of the cortex (Bickford et al., 2015; Sherman & Guillery, 2002), and connect the thalamus with different cortical areas (Butler, 2008).

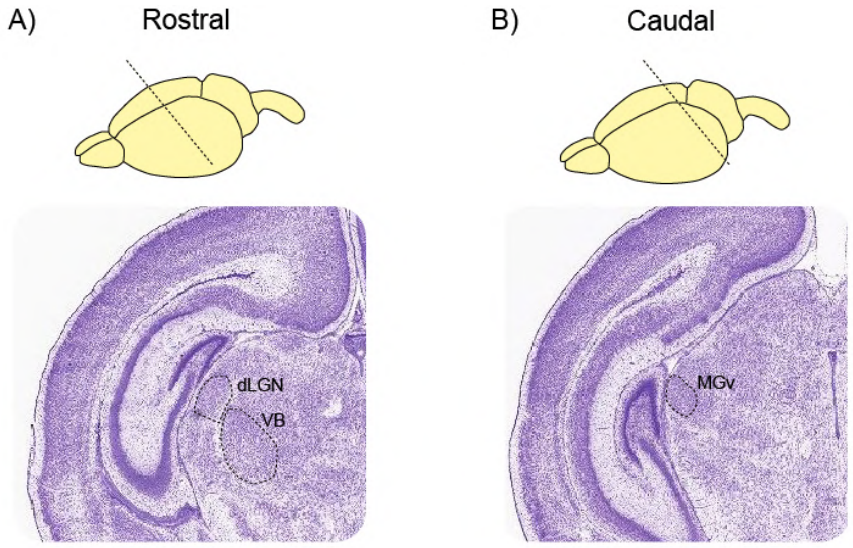


Figure 4. Coronal sections showing thalamic first order (FO) nuclei at P7. A) Coronal section at a rostral level in which the visual and somatosensory nuclei are depicted with dashed lines. B) Coronal section at a caudal level in which the auditory nucleus is marked with dashed lines. dLGN: dorso laterogeniculate nucleus; VB: ventrobasal nucleus; MGv: ventral medial geniculate.

4. 2. The development of the thalamus

The development of the thalamus is driven by genetic and activity-dependent mechanisms (reviewed in Nakagawa, 2019). The process starts following the activation of the wingless-INT proteins (Wnt), Sonic hedgehog (Ssh), and Fibroblast growth factors (FGF) signaling cascades (Kataoka & Shimogori, 2008; Martinez-Ferre & Martinez, 2009, 2012). They act as morphogenes, expressed in gradients of concentration (Martinez-Ferre & Martinez, 2012). The activation of these cascades divides the diencephalon into three regions called prosomeres. Prosomere 1 gives rise to the pretectum, in charge of the processing of visual information and the visual reflexes (Ferran et al., 2009). The thalamus and the epithalamus will develop from prosomere 2, and prosomere 3 will form the prethalamus, including the reticular nucleus (RTN) and the zona incerta (ZI) (Puelles & Rubenstein, 2003). The zona limitans intrathalamica (ZLI) separates prosomere 2 and 3, and expresses high levels of Shh, which is fundamental in prosomere 2 for the differentiation of the prospective thalamus from the epithalamus (Chatterjee et al., 2014; Chatterjee & Li, 2012; Mallika et al., 2015). The gradual exposure to these morphogenes makes, by E10.5 in mice, the prospective thalamus to divide into two progenitor domains, the rostral and the caudal progenitor domains **(Fig 5)** (R-pTh and C-pTh, respectively). Progenitors in the C-pTh are exposed to high

concentrations of Shh and differentiate into glutamatergic projecting neurons that will compose the thalamic nuclei and connect to the cortex (Price et al., 2012; Tou et al., 2007). R-pTh progenitors, on the other hand, sense low concentrations of Shh and give rise to GABAergic projecting neurons that will form the perihabenular nucleus (pHB) and the intergeniculate leaflet (IGL) (Delogu et al., 2012; Fernandez et al., 2018).

Most of the neurons that will form the adult thalamus derive from the C-pTh. Some evidence indicates that this population of progenitor cells is very heterogeneous, in order to generate the neuronal diversity that will compose the thalamic nuclei (Nakagawa & Shimogori, 2012; Tou et al., 2007; F. K. Wong et al., 2018). Moreover, it has been recently published that the cohorts found in specific regions are derived from thalamic progenitors based on their position. Nevertheless, it is also likely that the combination of the exposure to the gradient of morphogenes, and the location of the progenitors might work together in order to control the cell fate of the neurons that will populate the different thalamic nuclei (Shi et al., 2017).

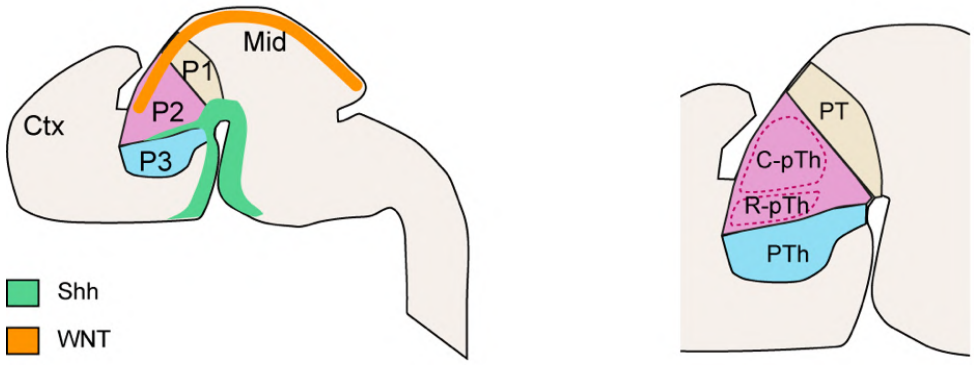


Figure 5. Domain specification in the thalamus during early development. *Left panel*: Sagital section showing the prosomeres p1, p2 and p3 early in brain development, and the distribution of the morphogenes. *Right panel*: The two progenitor domains R-pTh and C-pTh are depicted. PTh: prethalamus, PT: pretectum.

After the area of the prospective thalamus is defined, the determination of thalamic nuclei that project to the cortex is driven by the gradual expression of the *Gbx2* transcription factor, which is postmitotic. The expression of *Gbx2* starts at E9.5 and is very dynamic in time and space. It defines the borders of the thalamus, separating it from the prethalamus, the pretectum and the epithalamus, and at the same time dividing the thalamus into its different nuclei **(Fig 6)** (Chen et al., 2009; K. Li et al., 2012; Mallika et al., 2015; Nakagawa & O'Leary, 2001; Vue et al., 2009). The lack of *Gbx2* expression affects the correct formation of the thalamus, inducing a wrong migration of thalamocortical axons (TCAs) and defects in cell proliferation (Chatterjee & Li, 2012; Chen et al.,

2009; Mallika et al., 2015). Recently, there has been an increased interest in the transcription factors that give rise to the different parts of the thalamus and could be subject to genetic manipulations. During the last few years, several nuclei-specific genes have been described in our laboratory (Gezelius et al., 2017), which might be necessary for the organization of the TCAs and their topographical targeting.

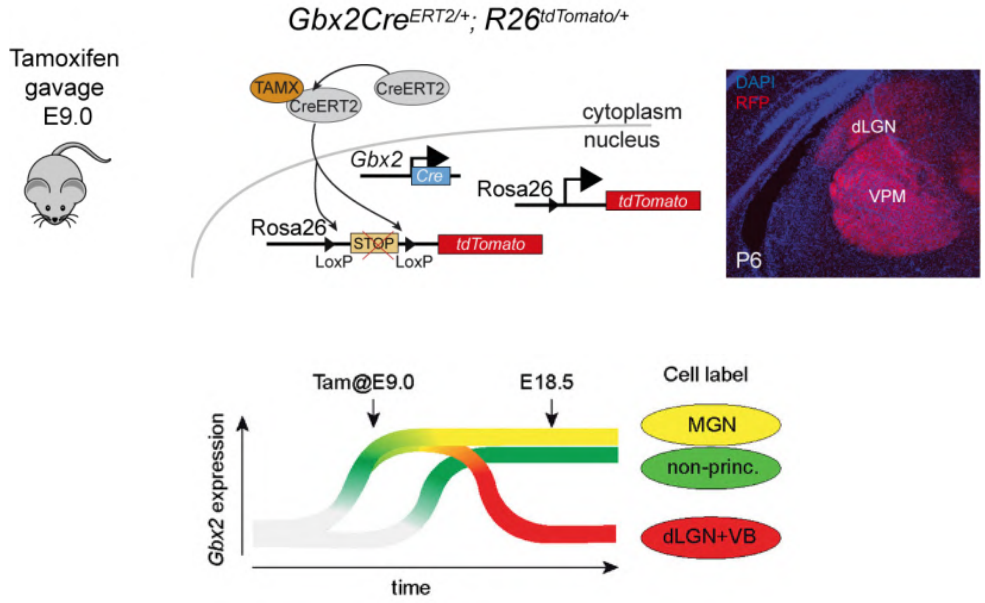


Figure 6. Gbx2 expression in the developing thalamus throughout time. A) Scheme showing the transgenic mouse line Gbx2^{CreERT2}; R26^{tdTomato} which helps to study the expression pattern of Gbx2 in the developing thalamus. B) Tamoxifen administration at E9.0 in the TdTomato Cre/Lox transgenic c57 mice labels the principal nuclei in red. If the tamoxifen is administered at E14.5, the MGv is the only primary nucleus labeled. Modified from Gezelius et al., 2016.

Neuronal activity is also important for the correct development of the thalamus, as it strengthens the connections between adjacent cells. Activity-dependent mechanisms can be divided into spontaneous intrinsic patterns of activity, and evoked activity due to an external stimulus. The waves of spontaneous activity appear at different time points during thalamic development (Martini et al., 2018) and are transmitted along the thalamocortical tracks to the neocortex, and therefore, could impact its correct development.

4. 3. Spontaneous activity in the development of the thalamus

In the developing thalamus, neuronal activity evolves at different stages. Firstly, spontaneous activity is intrinsically generated during development, independent from the external stimuli and necessary for early developmental processes. Later, peripheral sensory input evokes neuronal activity and is necessary for the final assembly and refinement of the circuits.

The first stage in the development of neuronal activity occurs at the end of the second gestational week in mice and is characterized by endogenous and uncorrelated activity. Manipulations in the activity rate have shown changes in the expression of genes that are involved in the growth and branching of thalamocortical axons (Antón-Bolaños et al., 2018; Castillo-Paterna et al., 2015; Herrmann & Shatz, 1995; Mire et al., 2012; Moreno-Juan et al., 2017; Uesaka et al., 2007). In later stages, the activity in the thalamus becomes more synchronous, and by E14 this synchronous activity takes the form of waves. These waves of spontaneous activity firstly propagate among FO nuclei, and subsequently engage HO nuclei (Moreno-Juan et al., 2017; Martini et al., 2021). Spontaneous thalamic waves of activity cease at perinatal stages, as it has been described in acute slices, first in the somatosensory and auditory nuclei, and then at P2 in the visual nucleus **(Fig 7)**. However, it remains to be determined whether thalamic waves follow this temporal pattern also *in vivo*. Thalamic waves are transmitted along the axons projecting into the cortex. Thus, early thalamocortical input could impact cortical development by means of activity-dependent mechanisms. For instance, the alteration of these patterns of activity by genetic manipulations induces cross-modal changes in the development of sensory cortical areas, which might be mediated by subplate neurons (Moreno-Juan et al. 2017; Antón Bolaños et al., 2018; Antón-Bolaños et al., 2019; Barkat et al., 2011; Hanganu et al., 2002; Molnár et al., 2020; Viswanathan et al., 2012; Zhao et al., 2009).

During the first two postnatal weeks, these electrical properties progressively mature, becoming more continuous and uncorrelated (Murata and Colonnese, 2018; Martini et al., 2021; Murata and Colonnese, 2016). This transition in thalamic activity might be critical for the processing of sensory stimuli by the cortex, and might be associated with changes in the sensory organs, synaptic maturation, or circuit remodeling due to the integration of inhibitory cells (Colonnese, 2014; Demas et al., 2003; Sokhadze et al., 2019).

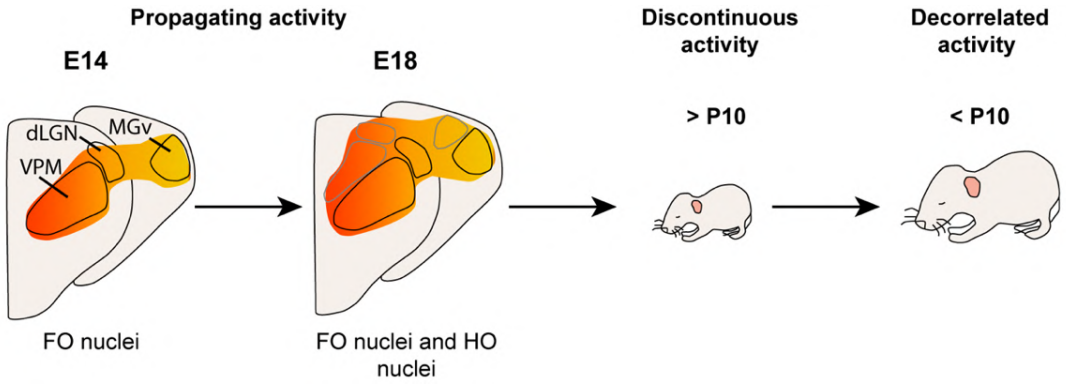


Figure 7. Thalamic spontaneous activity during development. From E14 to E18, there are thalamic spontaneous calcium waves that propagate between firstly among FO nuclei and then also to HO nuclei. Early postnatal, thalamic activity is characterized by discontinuous activity, that is transformed to decorrelated later during the second postnatal week. Adapted from Martini et al., 2021.

4. 4. Thalamocortical connections

4. 4. 1. Development of thalamocortical connections

The thalamus and the cortex are connected in both directions. These connections carry sensory and motor information from the periphery to the cortex, where it is integrated and processed.

The thalamocortical connectivity starts developing at the end of the second embryonic week in mice (E13.5), and it is tightly regulated by axon guidance cues, spontaneous calcium activity and patterns of gene expression (reviewed in López-Bendito, 2018; Antón-Bolaños et al., 2018; Castillo-Paterna et al., 2015; Garel & López-Bendito, 2014; Leyva-Díaz et al., 2014; López-Bendito, 2018; Marcos-Mondéjar et al., 2012; Mire et al., 2012; Molnár et al., 2012; Dufour et al., 2003; Quintana-Urzainqui et al., 2020; Callejas-Marin et al., 2022). TCAs must follow a particular path in order to reach their cortical target. Firstly, they arrive to the internal capsule (IC) after crossing the boundary between the telencephalon and the diencephalon, the diencephalic-telencephalic boundary (DTB), guided by the repulsive cues Slit1 and Slit2 (Bagri et al., 2002; Bielle et al., 2011; Braisted et al., 2009; López-Bendito et al., 2007). At this point, TCAs are already organized in a topographic manner due to the morphogenes and molecular cues (Molnár et al., 2012). They follow the path formed by the corridor cells between the proliferative zone of the medial ganglionic eminence (MGE) and the globus pallidus (Bielle et al., 2011; López-Bendito et al., 2006). The

corridor cells are guidepost cells, mostly composed by GABAergic neurons from the lateral ganglionic eminence (LGE), which secrete a gradient of Neuregulin-1 (Nrg1) that direct the axons towards the neocortex (Bielle et al., 2011; López-Bendito et al., 2006). At E14.0, thalamocortical axons continue their route towards the subpallium and reduce their speed at the pallial-subpallial boundary (PSPD), where they wait to the corticofugal axons from subplate neurons (SuPNs) (de Carlos & O'Leary, 1992; McConnell et al., 1989). This encounter of axons from both origins has been proposed as the "handshake hypothesis" (Blakemore & Molnar, 1990). TCAs use the corticofugal axons as a scaffold in order to reach their target in the cortex. Then, TCAs move dorsally and by E15.5 they arrive into the neocortex, where they stop and wait at the subplate (SuP). At this point, TCAs and SuPNs make temporary connections, a key mechanism for the formation of the early circuit, which spreads until E17.5, the time at which TCAs start invading the cortical plate **(Fig 8)** (Allendoerfer & Shatz, 1994; del Río et al., 2000; Hoerder-Suabedissen & Molnár, 2015; Kanold & Luhmann, 2010; Little et al., 2009; Viswanathan et al., 2012, 2017; Molnár et al., 2020; Pal et al., 2021; Kanold et al., 2019;). Since corticogenesis is still ongoing and granular layers are not completely formed, TCAs could act as an extrinsic influence to cortical development (Molyneaux et al., 2007). This means that TCAs could act as an extrinsic influence to cortical development. During the first postnatal week, the principal cortical target of TCAs are the granular cells from L4, although they can also send collateral projections to L5b. Finally, TCAs get organized into specialized structures, such as the barrels in the primary somatosensory cortex (S1) (López-Bendito & Molnár, 2003).

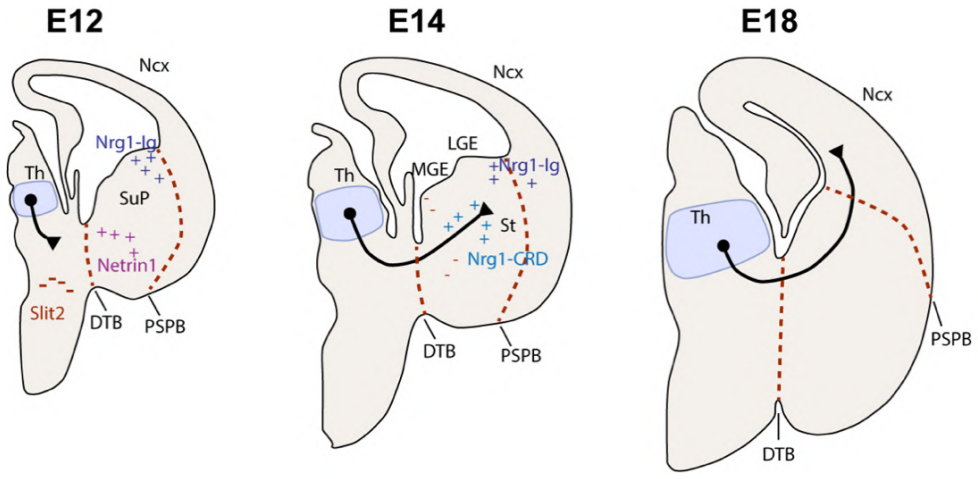


Figure 8. Development of thalamocortical connections. Schema showing the developmental time course of the TCAs (black) from E12 to E18. Axons from the thalamus cross major boundaries, such as the diencephalic/telencephalic (DTB) and the palial/subpallial (PSPB) boundaries following attractant and repulsive cues. Adapted from Garel and López-Bendito, 2014.

4. 4. 2. Feed-forward connections between the thalamus and the cortex

First order nuclei (FO) receive sensory information from the peripheral sensory centers and act as a relay station, connecting with the spiny-stellate neurons in L4, as well as neurons in L5b, and L6 (Swadlow & Alonso, 2017). Within a cortical column, information from L4 goes to L2/3 and from there to L5 and L6, layers that project out of the cortex. HO nuclei receive input from L5b and L6b, and neurons in L6a project back to FO nuclei **(Fig 9)** (Hoerder-Suabedissen et al., 2018; Sumser et al., 2017). All these connections form loops that conform the basis of sensory processing in the thalamocortical system (Viaene et al., 2011).

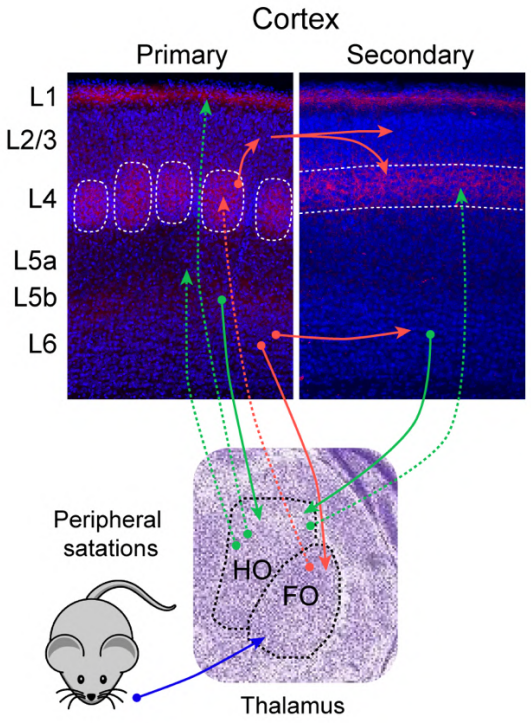


Figure 9. Thalamocortical and corticothalamic connectivity. First order thalamic nuclei (FO) receive information from the peripheral stations, and connect with neurons in L4 of the primary sensory cortex. Then, L4 neurons connect with L2/3 neurons, which send cortico-cortical projections to L2/3 and L4 in the secondary areas. The thalamo-cortico-thalamic loop is closed since high order nuclei (HO) send projections to L4 in the secondary area. On the other hand, HO nuclei receive cortical input from L5 and L6 in the secondary areas. In addition, HO nuclei connect to L1 and L5 neurons in the primary cortical areas.

Moreover, the thalamus acts as a station that can transmit information to and from different cortical areas (C. C. Lee & Murray Sherman, 2010; Reichova & Sherman, 2004; Sherman & Guillery, 2002; Theyel et al., 2010). This connection between cortices that involves the thalamus

allows the cooperation of different cortical areas in order to perform several cognitive functions (Fries, 2009; Seidemann et al., 1998; Sherman, 2016). In fact, it has been seen using optogenetics that cells from L5 can induce the appearance of waves of activity in other cortical areas (Stroh et al. 2003). Therefore, the thalamus acts both as a relay station of sensory information, and as a transmitter of this information between cortical areas, thus, contributing to information processing (Sherman, 2016).

4. 4. 3. Early thalamic input influences cortical development

The thalamus and the cortex develop at the same time, and even though they are regulated by different genetic programs, they can influence each other in the maturation process. The thalamus can influence radial organization, cell proliferation, navigation of corticothalamic axons and specification of cortical areas, as well as interneuron maturation and circuit assembly (Dehay et al., 1996; Rakic, 1991; Zechel et al., 2016). For instance, it has been suggested that the release of glutamate by TCAs is needed for the correct development of Reelin-expressing interneurons in the cortex (de Marco García et al., 2015). The cortical integration of parvalbumin (PV) and somatostatin (SST) interneurons is also regulated by thalamic input (Wamsley & Fishell, 2017). Recently, it has been observed that SST interneurons are very important during the first postnatal week for the correct arrival of thalamocortical input to L4 PV interneurons and pyramidal neurons. This way, SST interneurons contribute to the assembly of the cortical excitatory-inhibitory circuit (Che et al., 2018; de Marco García et al., 2015; Marques-Smith et al., 2016; Takesian et al., 2018; Tuncdemir et al., 2016). Not only interneurons in the cortex are regulated by TCA arrival. Pyramidal neurons in L4 need TCA input in order to correctly segregate and form the barrel walls in S1(Assali et al., 2017; H. Li et al., 2013). The close interaction between CTAs and TCAs has been demonstrated using a mouse model lacking TCAs, in which CTAs acquire an aberrant trajectory, and hence, confirming that TCAs are necessary for the correct pathfinding of the cortical counterparts (Deck et al., 2013).

TCAs are also important for the differentiation of primary and secondary cortical areas, since the genetic restriction that controls this specification relies on the arrival of thalamic input (Chou et al., 2013). Thus, the removal of FO thalamic nuclei induces changes in the primary sensory areas, which acquire the characteristic molecular and functional properties of the secondary cortical areas (Pouchelon et al., 2014; Vue et al., 2013). For instance, the ablation of the somatosensory thalamic

nucleus VPM provokes the POm to target neurons from L4 in the S1 (Pouchelon et al., 2014). These observations point out the influence TCAs have on the identity of L4 cortical neurons, and circuit assembly.

Neuronal transmission in the thalamocortical pathway can also influence the development of the cortex, since it is important for the acquisition of neuronal identity, axon refinement and neuron migration (Kirischuk et al., 2017; Luhmann & Khazipov, 2018; Martini et al., 2018). For instance, there is a synchronization of the thalamus and the counterpart cortical area during the first postnatal week led by early gamma oscillations (Minlebaev et al., 2011). In addition, the disruption of TC activity at different levels affects the correct formation of the barrel map in S1 (H et al., 2014; H. Li et al., 2013; Narboux-Nême et al., 2012; Suzuki et al., 2015). Furthermore, thalamic spontaneous activity during embryonic development is essential to maintain the homeostasis among the sensory systems. This way, cross-modal changes in the cortex might be directed by waves of activity communicating thalamic nuclei (Moreno-Juan et al., 2017). Hence, variations in thalamic activity during development can be translated into changes in the cortex.

4. 5. Thalamic interneurons

4. 5. 1. General overview

The thalamus, as other brain structures, is composed by different populations of cells. The main population is formed by glutamatergic projecting neurons. However, there are also interneurons (INs), astrocytes, and microglia.

Thalamic glutamatergic neurons receive the inhibitory input mainly from projecting GABAergic neurons that reside in the reticular nucleus (RTN), the zona incerta (ZI) and the vLGN. There are other extra-thalamic sources of inhibitory input, such as the basal ganglia, the SC, the hypothalamus, and the pontine reticular formation (Halassa & Acsády, 2016). However, in addition to the projecting GABAergic neurons, there are some local thalamic interneurons. The distribution and the number of thalamic interneurons is not conserved across species. Interneurons are sparse and mainly found in the dLGN of small mammals, such as mice and marsupials. However, in large mammals they are abundant and widely distributed throughout the whole thalamus. Strikingly, non-mammalian amniotes, such as crocodiles, lizards and snakes lack dLGN interneurons (Butler,

2008). In addition to local thalamic GABAergic cells, there is a small subpopulation of GABAergic cells that reside within the perihabenula (pHB) and intergeniculate leaflet (IGL) and project outside the thalamus (Delogu et al., 2012; Fernandez et al., 2018; Harrington, 1997; Inamura et al., 2011; Tou et al., 2007).

4. 5. 2. Origin of thalamic interneurons

In the last years several groups have focused on the development and understanding of thalamic excitatory projecting neurons, their electrophysiology and connections. However, in the thalamus there are also local GABAergic interneurons that exert an important role maintaining the balance of excitation and inhibition. Compared to the excitatory thalamic neurons, little is known about these inhibitory cells, which points towards a field that has to be explored.

In mice, thalamic INs in the dLGN receive retinal input and comprise less than 10% of the total dLGN cell population (Evangelio et al., 2018). They establish the connections after excitatory relay neurons have matured (Charalambakis et al., 2019a). Local thalamic interneurons that will integrate into the circuit are born outside of the thalamus in other brain regions, and they migrate during the last gestational week in mice from the origin towards their final destination, mostly the dLGN. On the one hand, a stream of cells that will differentiate into local GABAergic cells populates the thalamus migrating from the proliferative zone in the midbrain (Bakken et al., n.d.; Hayes et al., 2003; Jager et al., 2021; Jones, 2002). They are born between E10 and E13 and belong to the *Engrailed1* lineage, which is expressed in the midbrain and not in the forebrain (Jager et al., 2016, 2021; Sgaier et al., 2007). The neurogenesis of GABAergic neurons in this domain depends on the expression of the lineage selector genes *Gata2* and *Tal2* (Virolainen et al., 2012). They start entering the thalamus at ~E17, covering first the caudal tiers. These prospective GABAergic cells will constitute the largest fraction of local thalamic interneurons, and also express the transcription factors *Otx2*, *Sox14* and *Gata2*. Once they arrive into the thalamus, they populate mostly FO nuclei, even though they can also be found in HO and rostral nuclei (Jager et al 2021).

On the other hand, the prethalamus is a second source of thalamic interneurons. The prethalamus generates a number of GABAergic cells that will mostly populate prethalamic structures, such as the vLGN and the RTN. However, some of them will invade the developing thalamus from rostral regions (Golding et al., 2014; Jager et al., 2021). From the total number of local thalamic

interneurons found in the mature thalamus, around 20% of them are originated in the prethalamus. The fate of these GABAergic cells depends on the expression of the lineage selector genes *Dlx1/2* (Delogu et al., 2012; Le et al., 2017). These prethalamic interneurons express *Dlx5/6* and *Foxd1,* but not *Sox14,* and they are enriched in HO nuclei (Jager et al 2021). Thus, the fact that local thalamic interneurons have two different origins, prethalamic and mesencephalic, indicates that they are a cell population characterised by two large molecular identities: the *Gata2/Tal2* lineage that is enriched in FO nuclei, and the *Dlx1/2* lineage, enriched in HO nuclei.

While there are no intrinsic local inhibitory cells, the thalamus does generate projecting GABAergic interneurons. The progenitors in the proliferative neuroepithelium of R-pTh generate a stream of cells that express *Sox14*, *Nkx2.2* and *Tai1* (Tou et al., 2007; Jeong et al., 2011), and that will populate the pHB and the IGL (Anastasides et al., 2021; Fernandez et al., 2018; Moore et al., 2000).

4. 5. 3. Genetic and activity-dependent control of thalamic interneurons

GABAergic cells follow different developmental genetic programs in order to acquire their final identity. The molecular mechanisms that characterize thalamic GABAergic cells have not been specifically studied. However, it is possible that the site of origin determines the genetic programs that will confer the identity to a subpopulation. In the midbrain, for instance, the sustained expression of *Tal2* and *Gata2* by GABAergic precursors regulates the acquisition of the GABAergic phenotype after the cell-cycle exit. These transcription factors activate the expression of genes related to the maintenance of the GABAergic identity, such as *Tal1*, *Gata3*, *Six3*, *Gad1* (Achim et al., 2013; Delogu et al., 2012; Kala et al., 2009). Nevertheless, it is still unknown whether thalamic interneurons that derive from the midbrain share a developmental trajectory similar to other GABAergic neurons that also derive from this structure. Regarding thalamic interneurons that are originated in the prethalamus, there are no direct studies to help understand their developmental program. However, it is plausible that they follow a program similar to the GABAergic cells that come from the rostral GABAergic domain, either by the direct control of the expression of the GAD isoform by *Dlx1/2,* or by the indirect activation of *Dlx5* and *Dlx6* (Lindtner et al., 2019; Cobos et al, 2007; Le et al., 2017).

Interestingly, a microarray of the dLGN performed at different postnatal stages showed that local thalamic interneurons are characterized by the expression of *Otx2, Reelin, Meis2, Cplx3, α6nAChR,*

and β3nAChR (Golding et al., 2014). More specifically, *Otx2* controls the tangential migration and the specification of dLGN interneurons, since mice lacking *Otx2* present a thalamus devoid of inhibitory cells (Jager et al., 2016; Jager et al., 2021).

Apart from the genetic programs, there are extrinsic factors that can modulate and influence the development of local thalamic interneurons. In the last few years, several groups have observed that the correct location and maturation of thalamic interneurons is influenced by retinal activity (Charalambakis et al., 2019b; Golding et al., 2014; Su et al., 2020). Strikingly, visual deprivation by optic nerve section at P0 or using the anophtalmic mutant mice *Ey1*$^{-/-}$, disrupts interneuron migration, which gather in the upper tiers of the dLGN (Golding et al., 2014). Moreover, *Math5* knock-out mice, a gene needed for the differentiation of the RGCs, showed that the absence of retinal input disrupted the synaptic connectivity between thalamic interneurons and relay neurons, and also showed an accelerated corticothalamic innervation (Charalambakis et al., 2019). Lastly, it has recently been observed that FGF15 expression by astrocytes also controls the entrance of interneurons into the thalamus, which is, at the same time, induced by retinal axons. The ablation of FGF15 expression produces an impairment in the migration of GABAergic interneurons into the dLGN, which are then misrouted into the VB. Moreover, it seems that by the expression of *Shh* RGCs control the correct migration of dLGN interneurons into the nucleus (Somaiya et al., 2022). The disruption of *Shh* expression affected the expression of FGF15 by dLGN astrocytes, and as a consequence, dLGN interneuron migration was impaired (Somaiya et al., 2022).

4. 5. 4. Intrinsic properties of thalamic interneurons

Several groups have tried to classify interneurons of the thalamus. As they mature, thalamic interneurons increase the size of the soma, the number of processes, and the branches during the first two postnatal weeks. So far, the most important parameter that can be used to group these cells is the membrane capacitance. The levels of nNOS or the cell size can also help to group them (Leist et al., 2016). Local thalamic interneurons make particular synaptic contacts with pyramidal projecting neurons using dendro-dendritic synapsis called F2 terminals (Famiglietti & Peters, 1972; Guillery, 1969; Hamos et al., 1985; Lieberman, 1973; Montero, 1986; Ohara & Lieberman, 1993). Interestingly, these F2 terminals form a triadic synaptic structure that consist of the axon terminal of a retino-geniculate neuron (coming from the retina), the distal presynaptic dendrite of the

interneuron, and the proximal postsynaptic dendrite of the thalamocortical projecting neuron **(Fig 10)**. This way, the retino-geniculate terminal makes an excitatory synapse with the proximal dendrite of the thalamic projecting neuron, as well as contacts the distal dendrite of the interneuron. Therefore, this innervation produces a disynaptic inhibition at that area of the thalamocortical neuron dendrite (Cox & Beatty, 2017), shaping their receptive fields and augmenting the stimulus selectivity, as well as refining the temporal precision of action potentials (Holdefer et al., 1989; Sillito & Kemp, 1983; X. Wang et al., 2007, 2011). In addition to the F2 terminals, local interneurons also present F1 terminals, which are axo-dendritic (Cox & Beatty, 2017). Recently, it has been observed that one single local interneuron in the dLGN can participate in a number of neuronal interactions using different synaptic motifs, and thus, giving rise to distinct synaptic relationships within a single neuron (Morgan & Lichtman, 2020).

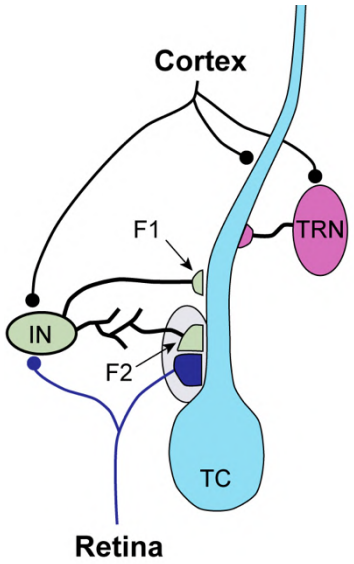


Figure 10. The triadic synapse in dLGN interneurons. Schema showing the F2 terminals formed between the retino-geniculate axon (dark blue), the interneuron dendrite (IN, green), and the axon from the thalamocortical neuron (TC, light blue). F1 monosynaptic connections come from both the TRN and dLGN IN onto the thalamic projecting neurons. Adapted from Cox et al., 2017.

5. The cortex

5. 1. General principles

In the neocortex there are two classes of neurons: glutamatergic neurons, which either project locally or establish long range connections with intracortical or subcortical targets; and GABAergic interneurons, which establish local interactions (Petreanu et al., 2009). The neocortex is divided into different cortical areas which control sensory, cognitive and consciousness functions. Each area is subdivided into six layers populated by neurons and radial glial cells. Cells in different layers tend to form vertical connections with other cells above or below forming radial columns of functional circuits. These columns are then attached into modules in order to form cortical columns with the same physiological properties (Mountcastle, 1997). This columnar organization relies on the radial glial processes, which are used as a scaffold during development by clonally related neurons (Rakic, 1988; Rakic et al., 2009). This has been recently demonstrated using viral and genetic tools that have helped tracing the progeny of a singular apical progenitor at different times during development (Guo et al., 2013; Luskin et al., 1988; Zong et al., 2005; Gao et al., 2014; Llorca et al., 2019).

The laminar organization of the cortex is developed in an inside-out manner, generating all the heterogeneity as soon as they are born (Greig et al., 2013). In mice, excitatory neurons, which are born from E10.5 to E18.5, start populating the cortex from the deepest layer (Jabaudon, 2017; Molyneaux et al., 2007; Lodato and Arlotta, 2015; Di Bella et al., 2021). Having neurons at different developmental stages in the same region has a functional consequence, as they respond differently to signalling cues. Superficial neurons, which are less mature, are more prone to plastic changes than deep layer ones (Fox & Wong, 2005).

However, it is still not well understood how newborn neurons reach their final destination and therefore, two hypotheses have been made. The first one, called The Protocortex Theory, is focused on how the environmental changes and cues could refine the final synaptic organization (O'Leary, 1989). In this case, equipotent cells would be forming the embryonic cortical plate, which receives innervation from subcortical areas modulating the final cell fate. The second option is called the Protomap hypothesis, and suggests that the progenitors are born with an intrinsic genetic program, which defines the area specification of neurons and is not based on external cues (Rakic, 1988). Nowadays, both theories are combined, and it is widely accepted that there is an interaction between intrinsic and extrinsic mechanisms during neocortical development (Oberst et al., 2019). Extrinsic mechanisms, such as the membrane potential of the apical progenitors (Vitali et al., 2018), or the thalamocortical innervation (Monko et al., 2021; Ohtaka-Maruyama et al., 2018; Vue et al., 2013), modulate spatially and temporally the rough protomap established through intrinsic genetic

mechanisms. Therefore, the specification and plasticity of cortical areas can be influenced by the manipulation of TCA innervation and peripheral input, as it was suggested by classic graft studies (Schlaggar & O'Leary, 1994). Intrinsic genetic programs are also important, since they provide identity and positional information to new-born neurons (Telley et al., 2019). Finally, it has been recently suggested that radial glial cells do not follow the exact number of cells divisions, and do not generate the exact same progenitor cells, therefore, creating collectively the diversity of neurons found in the cortex (Llorca et al., 2019).

5. 2. Corticothalamic projections

All the different cortical areas project back to the thalamic nuclei (Caviness & Frost, 1980). From all the synaptic input that arriving to the thalamus, 50% comes from corticothalamic projections (CTAs). The CTA input into the thalamic nuclei shapes the information that would be processed afterwards in the cortex (Briggs & Usrey, 2008; Olsen et al., 2012; Sherman & Guillery, 2002). The specificity of the corticothalamic projections into the thalamus depends on the laminar identity of the cortex, which are SuPNs, L6 and L5 neurons. SuPNs provide the structural scaffold for the axons coming from L5 and L6 (Kim et al., 1991; McConnell et al., 1989). L5 and L6 neurons, as soon as they are born, start projecting to their subcortical targets (Grant et al., 2012; Hoerder-Suabedissen & Molnár, 2015). L5 neurons project to FO nuclei, while L6 neurons project to HO thalamic nuclei (del Río et al., 2000; Molyneaux et al., 2007; Price et al., 2006).

Post-mitotic neurons in the cortical plate give rise to the corticothalamic projections at E10 in mice. In the corticothalamic pathway, corticofugal axons pass through the intermediate zone (IZ), where they experiment the first waiting period at E13.5 (Jacobs et al., 2007). Later, at E15.5, they continue towards the IC, where the corridor cells guide also the CTAs (López-Bendito et al., 2006). Once they cross the DTB, they arrive to the prethalamus at E16.5 through the RTN and perireticular (PRN) nuclei, where they wait until E17.5 (Deck et al., 2013; Garel & Rubenstein, 2004; Lokmane et al., 2013; Lokmane & Garel, 2014; Molnár et al., 2012; Simpson et al., 2009). At this point, corticofugal axons are sorted: the cerebral peduncle receives most of the axons from L5, while the remaining axons from L5 and those coming from L6 are directed to the respective thalamic target **(Fig 11)** (Clascá et al., 1995; Jacobs et al., 2007; Molnár & Cordery, 1999). CTAs arrive slowly to the thalamic nuclei during the first postnatal days, a process that correlates with the acquisition of the functionality and sensory processing. The VPM and ventrolateral nuclei (VL),

which are the FO nuclei involved in the reception of sensory and motor inputs, are innervated by TCAs between E18.5 and P0. The MGv and dLGN are completely the innervated by P8 (Grant et al., 2012; Jacobs et al., 2007).

The mechanisms behind the development of CTAs are still poorly understood. It has been recently observed that there is a premature entrance of L6 projections into the dLGN upon removal of retinal input. Under this condition L5 neurons, which usually innervate the HO nucleus LP, were invading the FO visual nucleus dLGN in a cross-hierarchical manner (Grant et al., 2016). Interestingly, aggrecan, a repulsive chondroitin sulfate proteoglycan, is enriched in the dLGN at perinatal stages and regulates the invasion of CTAs into this nucleus (Brooks et al., 2013b). Furthermore, the connections in the FO nuclei depend on peripheral input, since the removal of the peripheral input leads to HO transcriptional programs in the FO thalamic nuclei, and induces a rewiring of the CTAs into the FO nuclei and not the HO thalamic nuclei. This network connectivity is conserved in all the sensory modalities (Frangeul et al., 2016). Thus, peripheral sensory activity might also be important for the correct development of the cortico-thalamic circuitry.

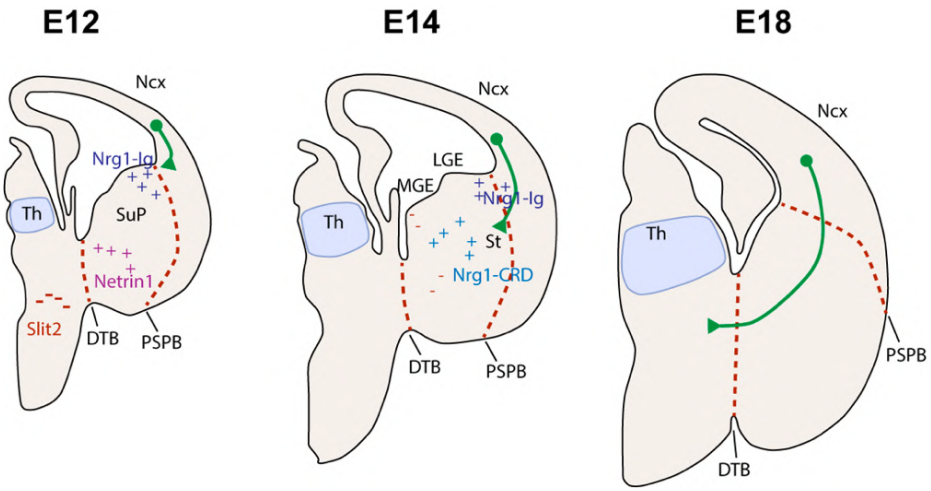


Figure 11. Development of corticothalamic connections. Schema showing the developmental time course of the corticothalamic projections (green) from E12 to E18. Axons from the neocortex cross the DTB and the PSPB boundaries. Adapted from Garel and López-Bendito, 2014.

5. 2. 1. Cortical influence on thalamic development

Cortical activity can also influence thalamic development. Cortical patterns of activity appear during development and are necessary for layer formation, and the arborization and navigation of dendrites and axons (Simi & Studer, 2018). Several studies have shown that disrupting cortical activity with knock-out models of NMDA receptor 1 (NMDAR1), metabotropic glutamate receptor 5 (mGluR5), and adenylyl cyclase 1 (AC1), generates deficiencies in TCAs, inducing smaller barrels with blurry borders in S1, and also disrupting the neuronal organization (Antón-Bolaños et al., 2019; Ballester-Rosado et al., 2010; Datwani et al., 2002; Iwasato et al., 2000, 2008; L. J. Lee et al., 2005; Martini et al., 2018). Therefore, it appears that the thalamus and the cortex have a close relationship necessary for the correct development of the functional sensory connections.

5. 3. Cortical interneurons

Excitatory cortical neurons constitute ~80% of all the neurons, while the remaining ~20% of neurons are inhibitory (INs). It is well established that INs are necessary for the maintenance of the excitation/inhibition balance in the brain. During the last decades it has become clearer that cortical interneurons are a wide and heterogeneous population. They appear with different morphologies, electrophysiological and neurochemical properties, which help classifying them (DeFelipe, 1997; Markram et al., 2004).

5. 3. 1. Origin, migration and types of cortical interneurons

The first studies revealing that there are inhibitory neurons comes from the first half of the XX century (Brown, 1914; Eccles & Sherrington, 1931; Renshaw, 1941). For a long time, it was thought that these cells derived from the same progenitors as excitatory neurons (Rakic, 1988). However, later it was described that neurons expressing GABA were actually born in the subpallium, the ventral region of the embryonic brain (Lavdas et al., 1999; Marín et al., 2001a; Pleasure et al., 2000; Wichterle et al., 1999). The origin of cortical interneurons is conserved throughout vertebrates

(Anderson et al., 2002; Brox et al., 2003; González et al., 2002). Immunohistochemical and bromodeoxiuridin (BrdU) experiments have shown that GABAergic interneurons migrate tangentially from their site of origin towards the cortex (DeDiego et al., 1994; van Eden et al., 1989) and that these cells are from a different lineage than those migrating radially, i.e., radially-migrating neurons are glutamatergic and tangentially-migrating neurons are GABAergic (Mione et al., 1997; Tan et al., 1998). During their journey towards their final destination, GABAergic interneurons acquire their biochemical markers.

Several transcriptomic analyses have shown that there are around 50 different interneuron transcriptional signatures that can be distinguished (Harris et al., 2018; Tasic et al., 2016; Zeisel et al., 2015). However, cortical interneurons can be classified into major classes depending on their morphology, the neurochemical, and electrophysiological properties (Defelipe et al., 2013; Llorca and Deogracias, 2022). Cortical interneurons are born in the medial ganglionic eminence (MGE), the lateral ganglionic eminence (LGE), the caudal ganglionic eminence (CGE), and preoptic area (POA) (D. M. Gelman & Marín, 2010). A number of evidences have shown that the MGE, LGE and CGE generate non-overlapping types of cortical interneurons. Regarding the neurochemical properties, interneurons can be divided into three big groups: parvalbumin (PV), somatostin (SST) and 5HT3a-receptor interneurons (5HT3aR). 5HT3aR interneurons are, at the same time, subdivided into Reelin and vasoactive intestinal peptide (VIP) interneurons (Rudy et al., 2011). SST- and PV-expressing interneurons are born in the MGE and the POA, and constitute 60% of the total population of interneurons (Ferrer & de Marco García, 2022; D. Gelman et al., 2011; Lavdas et al., 1999; Wichterle et al., 2001; Xu et al., 2004, 2008). The CGE gives rise to 5H3aR and neuropeptide-Y (NPY) interneurons (Butt et al., 2005; Lim et al., 2018; Nery et al., 2002; Niquille et al., 2018; Xu et al., 2004). The POA also gives rise to NPY and a subset of 5HT3aR interneurons (D. M. Gelman et al., 2009; Niquille et al., 2018), and the LGE generates interneurons that will populate the olfactory bulb (OB) and the striatum **(Fig 12)** (Bandler et al., 2017). Interestingly, cortical interneurons acquire their morphological and functional features later at postnatal stages.

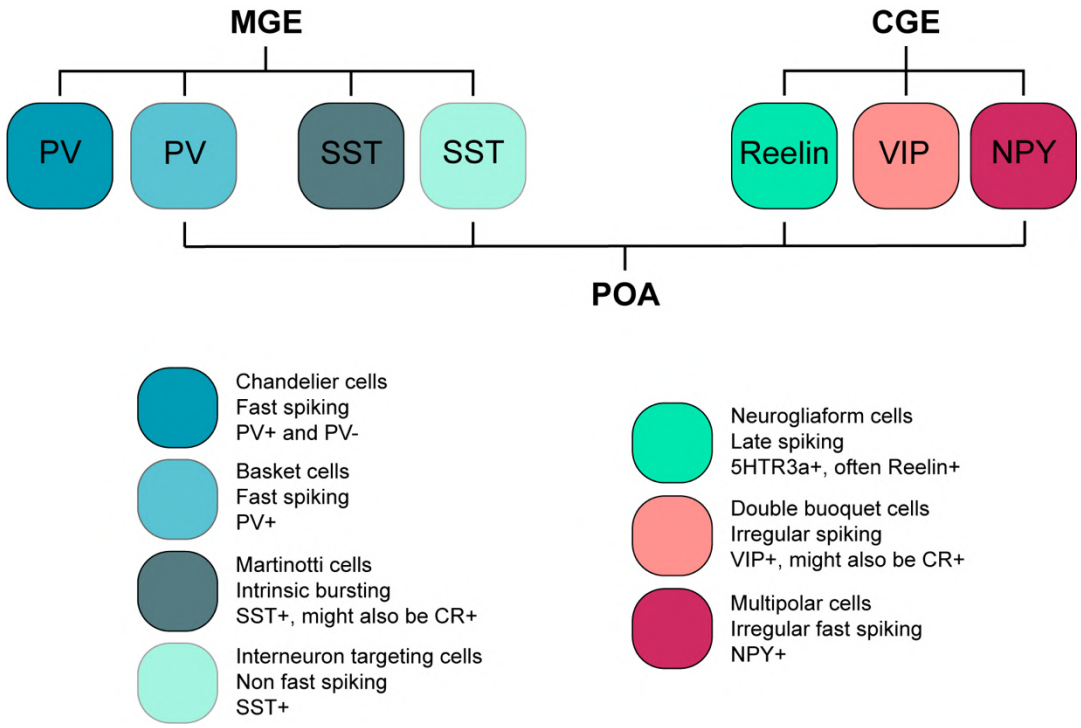


Figure 12. Main population of interneurons and their developmental origin. The main populations of cortical interneurons can be classified into PV, SST, VIP, Reelin and NPY. Adapted from Bartolini et al., 2013.

During development, cortical INs migrate tangentially from its origin towards the cortex. There are three temporal waves of migration **(Fig 13)**: 1) Around E11.5 there is a first wave of GABAergic cells that are born in the MGE (Cossart, 2011; Marín et al., 2001a), which take a superficial trajectory towards the cortex along the marginal zone (MZ) and the subventricular zone (SVZ). This migration depends on the expression of the *Satb1* and *Lhx6* genes (Babij & de Marco Garcia, 2016); 2) Between E12.5 and E14.5, there is a second big wave of tangential migration of interneurons generated in the MGE, which appears to be the principal source of cortical interneurons (Rubenstein and Marín, 2001). In fact, there is a peak of neurogenesis at E13.5 in the MGE (Mayer et al., 2018a). These cells migrate using both the superficial and the deep layer routes (Anderson et al., 2001). At the same time, at this embryonic stage, LGE-derived interneurons migrate towards the olfactory bulb; 3) Late-born interneurons, which are those generated from E14.5 to E16.5, derive from the LGE and MGE, and they mostly take the route towards the SVZ. Interneurons from the CGE are born from ~E12.5 until ~E17.5. There is a peak of neurogenesis at E14.5 in the LGE and CGE (Mayer et al., 2018).

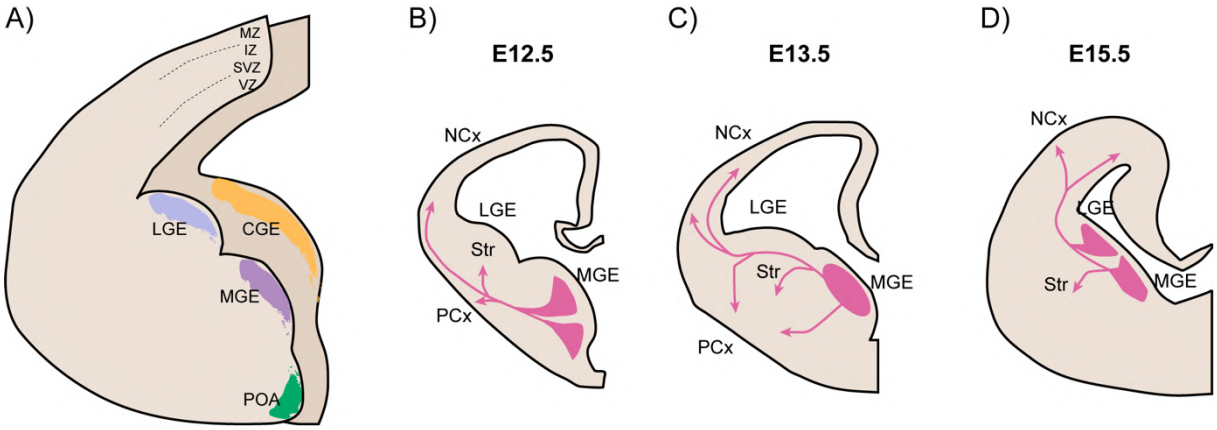


Figure 13. Temporal waves of cortical interneuron tangential migration. A) Coronal section showing the location of LGE, MGE, CGE, and POA. B) The first wave of tangential migration takes place around E11.5, and interneurons originate from the MGE and take a superficial route. C) The second wave has a peak at E13.5, when interneurons originated from the MGE take mainly the deep route. D) At E15.5 both MGE and LGE can give rise to cortical interneurons, which take the SVZ route. NCx, neocortex; POA, preoptic area; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; Str, striatum; MZ, marginal zone; IZ, intermediate zone; VZ, ventricular zone; SVZ, subventricular zone; PCx, piriform cortex. Adapted from Flames, 2005.

All interneurons take the same stereotyped tangential routes towards the cortex irrespective of their site of origin, avoiding other regions of the subpallium and the striatum. For this process they use the same molecular mechanisms (Martini et al., 2009; Yanagida et al., 2012). There are three migratory streams from the ganglionic eminences towards the neocortex: 1) the more superficial route goes near the marginal zone (MZ); 2) the deeper route goes near the subventricular zone (SVZ); 3) there is a small proportion of interneurons that migrate through the subplate (SP). Interestingly, it has been described that the expression of the chemokine Cxcl12 is required for the tangential migration of cortical interneurons (H. Li et al., 2008; López-Bendito et al., 2008; Sánchez-Alcañiz et al., 2011; Stumm et al., 2007; Tiveron et al., 2006; Y. Wang et al., 2011; Llorca and Deogracias, 2022). This process has been well studied in MGE interneurons, in which the transcription factor *Nkx2-1* controls the expression of chemorepulsive receptors Sema3A and Sema3F, which is necessary for the MGE-derived interneurons to avoid the striatum (Marín et al., 2001b). In addition to chemorepulsive molecules, interneurons also follow chemoattractant cues such as neuregulin-1 (Nrg1) (Flames et al., 2004). Strikingly, interneurons that take different migratory routes also present different transcriptional profiles, which means that they do not choose the MZ or the SVZ routes randomly (Antypa et al., 2011).

Once they reach the neocortex, interneurons switch to radial migration in order to get to their final destination. This change in the trajectory from tangential to radial could be due to the loss of

responsiveness to the Cxcl12 chemokine (Li et al., 2008). Recent work has also demonstrated that pyramidal neurons express neuregulins, such as Nrg3, which help interneurons to reach their final destination (Bartolini et al., 2017). Interestingly, at the time when the respective pyramidal cells are beginning to differentiate into a specific layer, interneurons start acquiring their laminar distribution (Hevner et al., 2004; Miyoshi & Fishell, 2011; Pla et al., 2006). Thus, the disruption in the layering of pyramidal cells could impact the laminar distribution of interneurons in the cortex (Lodato et al., 2011; Pla et al., 2006; Ye et al., 2015; Wester et al., 2019).

Neuronal activity might also have a role in the radial migration of interneurons. On this regard, the KCC2 channel, a potassium/chloride exchanger found in interneurons, has been widely studied. This channel reduces the speed and eventually stops the migration of interneurons by decreasing the frequency of the calcium transients in response to GABA (Bortone & Polleux, 2009). Therefore, the reduction in the excitability of interneurons changes their laminar positioning (de Marco García et al., 2011), and thus, suggests that the upregulation of the KCC2 channel is part of the maturation program of all types of interneurons (Inamura et al., 2012).

In the developing neocortex, there is increasing evidence that different types of interneurons are found across distinct brain regions. Not only that, but the same progenitor can generate interneurons that will populate different telencephalic areas (Harwell et al., 2015; Mayer et al., 2015). As a result, pools of progenitor cells at the ganglionic eminences can give rise to different types of interneurons. For instance, it has been shown that progenitors in the MGE generate most of the SST neurons in the first part of the neurogenic period, while PV interneurons are produced constantly. Even though the ganglionic eminences generate different types of interneurons, these cells pass through conserved precursor states once they become postmitotic. These precursor states will give rise to interneurons or projecting neurons (Mayer et al., 2018b). Related to this, it has been recently suggested that even a few hours after they are born they are already transcriptionally heterogeneous both at the MGE and CGE, and have a defined fate long before they are allocated at their final position in the neocortex (Mi et al., 2018; Miyoshi et al., 2010; Petryniak et al., 2007; Allaway et al., 2021). Interestingly, within the same cortical area a wide number of different interneuron subtypes can be found (Gouwens et al., 2020).

MGE-derived interneurons populate the neocortex in an inside-out manner following the birthdates of the pyramidal cells (Miyoshi et al., 2007). In contrast, CGE-derived interneurons

contribute in a 75% to the superficial layers and 25% to the deep layers consistently and independent on their birth (Miyoshi et al., 2010). Recently, it has been described that the final destination of cortical interneurons does not depend on their birthdate, but on the ganglionic eminence where they are born. Thus, MGE interneurons that are born at E12.5 will populate deeper layers of the neocortex, while E12.5 CGE-derived interneurons will migrate towards superficial layers (Miyoshi & Fishell, 2011). However, there is not obvious preference towards the superficial or the deep migratory route in both E12.5 MGE- and CGE-derived interneurons; it is irrespective of their site of origin (Miyoshi & Fishell, 2011). Moreover, it has been observed that different types of interneurons use different streams to get into the cortex (Lim et al., 2018).

5. 3. 2. The importance of neuronal activity and sensory input for cortical interneurons

A big proportion of all the interneurons (more than 30%) generated in the subpallium follow programmed cell death between the first and second postnatal week (Southwell et al., 2012). This cell death occurs progressively and seems to be linked to the integration of interneurons into the cortical circuits that are emerging and to the excitability of the interneurons themselves (Close et al., 2012; Priya et al., 2018; F. K. Wong et al., 2018). Several studies have shown that the maturation of interneurons requires excitatory input during the first postnatal week from local and afferent pyramidal cells, and that the integration into a functional circuit is necessary for their survival (Anastasiades et al., 2016; Cobos et al., 2005; de Marco García et al., 2011, 2015; Modol et al., 2020; Tuncdemir et al., 2016; Duan et al., 2020). For instance, in the somatosensory cortex there is a peak of interneuron apoptosis between P7 and P9. Experiments inducing the overexpression of the potassium channel Kir2.1 on interneurons have shown that they are less active in the cortical circuit and that the proportion of interneuron apoptosis is increased (Duan et al., 2020).

As it has been explained above, interneurons require neuronal activity and the emergence of sensory experience in order to mature and integrate correctly in the circuit. In fact, GABAergic cells in the cortex work as a functional network with coordinated activity and temporal dynamics (Modol et al., 2020). As an example, experiments inducing whisker deprivation have shown an impact on the functional organization of these circuits, affecting differentially deep and superficial layers (Modol et al., 2020).

In the cortex, layer 4 receives thalamocortical input, and thus, it has a significant role in the processing of sensory information (Douglas & Martin, 2004). PV basket cells from layer 4 are in charge of the feedforward inhibition, although the connectivity between the excitatory and the inhibitory neurons remains weak until the second postnatal week, when PV cells receive direct input from thalamic neurons (Chittajallu & Isaac, 2010; Daw et al., 2007). On the other hand, SST neurons in layer 5 receive dense and transient thalamic input during the first postnatal week, while innervating layer 4 and layer 5 excitatory neurons, as well as PV interneurons (Marques-Smith et al., 2016; Tuncdemir et al., 2016). Interestingly, it has been observed that these SST interneurons in layer 5 are necessary for the maturation of deep layers PV basket cells (Tuncdemir et al., 2016).

In the visual cortex, interneurons play an important role in the correct development of this cortical area. For example, it has been recently described that SST interneurons restrict the spread of low frequency events by the beginning of the second postnatal week, which would help to preserve the retinotopy and the plasticity before PV interneurons mature (Leighton et al., 2021). Furthermore, in order to acquire the binocular vision, retinal and callosal contralateral activity drive apoptosis in a subtype of PV interneurons called Chandelier cells in V1 (B. S. Wang et al., 2021). In the adult V1, the connectivity of cortical interneurons has been already established: PV cells inhibit pyramidal cells and vice versa, and are involved in cortico-cortical feed -back and -forward inhibition between V1 and higher order visual areas (Gonchar & Burkhalter, 2003; Ibrahim et al., 2021); SST cells inhibit both pyramidal cells and 5HT3aR interneurons (Adesnik et al., 2012); and VIP neurons, on the contrary, disinhibit SST cells (Pfeffer et al., 2013).

Similar to the visual cortex, there are several groups that have studied interneurons in the somatosensory cortex. For instance, it has been recently observed that sensory deprivation by whisker plucking, or the reduction of Cajal-Retzius cells (CRc) in L1 by the expression of diphteria toxin in these cells, induced a decrease in the density of PV but not of SST interneurons in L5 and L6 in the somatosensory cortex (Genescu et al., 2022).

Furthermore, it has also been described that L1 transient CRc are important for the correct allocation of interneurons found in the upper layers. In fact, embryonic thalamic activity was shown to control CRc density in L1. Transgenic mouse models lacking prenatal thalamic waves showed a reduction in the density of L1 CRc, which in turn affected the distribution of L2/3 interneurons expressing NPY, Calretinin or Reelin markers, whose densities were increased (Genescu et al., 2022).

Finally, Kastli and colleagues have described that the activation of VIP and SST interneurons in S1 depends on the arrival of stimuli. VIP cells are able to differentiate between single-whisker and multi-whisker stimulation before P14 but not after that time point. Meanwhile, SST interneurons respond to multi-whisker stimulation before and after P14. This difference in the response is due to a change in the thalamic connections that innervate these interneuron types (Kastli et al., 2020). Interestingly, it has been recently described that depending on their developmental trajectory and the cortical area they will populate, interneurons are organized in different connectivity circuits (Pouchelon et al., 2021).

6. Microglia

6. 1. General overview

The central nervous system is formed by a wide variety of cells apart from neurons. One of these populations is the microglia. Even though it was firstly thought that microglia were just the immune cells in the nervous system, it is now widely accepted that microglia present several different functions.

Firstly, microglia do not have the same embryonical origin as neurons. Back at the end of the 19th century and early 20th century, F. Nissl, W. Robertson, Ramón y Cajal and Pío del Río-Hortega proposed that microglia had a mesodermal origin (Ginhoux et al., 2013). It is now widely accepted that microglia derive from the primitive macrophages that are born in the yolk sac (YS), which is where the first hematopoiesis takes place at E7 in mice (Bertrand et al., 2005; Gomez Perdiguero et al., 2015; Hoeffel et al., 2012, 2015; Kierdorf et al., 2013; Palis et al., 1999; Schulz et al., 2012). Between E8 and E10 the circulatory system begins to appear, and the primitive macrophages, which are found in the YS, start propagating into the embryo before the closure of the blood brain barrier at E13 (Ginhoux et al., 2010; Hoeffel & Ginhoux, 2018). Once they enter the brain parenchyma, they start proliferating rapidly at the clusters that they form in the white matter (Monier et al., 2006; Swinnen et al., 2013; Varney et al., 2011). The entrance of microglia into the CNS corresponds with the vascularization of the brain tissue, E9.5 in rodents, and therefore, the interaction between microglia and vascular sprouts could facilitate microglial migration and population of the brain areas (Earle & Mitrofanis, 1998; Monier et al., 2006; Rigato et al., 2011).

However, it has also been proposed that, since there is no vascular network at this embryonic stage, microglia precursors might enter the CNS using extravascular routes (Arnold & Betsholtz, 2013; Chan et al., 2007; Streit, 2001). Between E14 and E16, there is a huge rise in microglia numbers that cannot be explained by an increase in the proliferation solely. In fact, it is thought that there is a second entrance of microglial progenitors that would contribute to this increment (Chan et al., 2007; Arnold and Betsholtz, 2013; Swinnen et al., 2013; Kierdorf et al., 2013). Microglia continues proliferating until E17.5, and by this time they migrate and scatter throughout all the regions (Santos et al., 2008; Swinnen et al., 2013).

Microglial precursors start invading the CNS from E8.5 in rodents in two phases, using tangential migration followed by radial migration (Alliot et al., 1999; Ginhoux et al., 2010; Schulz et al., 2012). First, there is an initial tangential migration along the deep layers of the neocortex in which microglial precursors move using radial glia feet and the axonal bundles of the cortex (Cuadros & Navascués, 2001; Pont-Lezica et al., 2011; Squarzoni et al., 2014a). However, before entering the CP around E16.5, microglia is found in the VZ and IZ, which are the regions with progenitor cells (Sorokin et al., 1992; Squarzoni et al., 2014b; Swinnen et al., 2013). Second, microglial precursors change from tangential to radial migration in order to reach all the regions. Therefore, in the developing cortex, they start accumulating between the cortical plate and the subplate (Monier et al., 2007), and then populate all the layers. Strikingly, in addition to the local cues that might be controlling the trajectory of microglia, it has been recently observed that this can be influenced by signals derived from the microbiota or inflammation, as well as sexual identity (Hanamsagar et al., 2017; Thion et al., 2018).

Microglia proliferation can be observed until the second postnatal week in rodents, when there is a peak in microglia density. The proliferation nearly disappears later in the adult brain (Arnoux et al., 2013; Dalmau et al., 2003; Marín-Teva et al., 1999), and there is a reduction in microglia density that is maintained throughout time (Nikodemova et al., 2015; Paolicelli et al., 2011). There is an increase in microglia apoptosis which coincides with a reduction in the proliferation, and both factors, therefore, contribute to decrease the overall microglia numbers (Askew et al., 2017; Nikodemova et al., 2015; Tay et al., 2017). Elmore and colleagues identified in 2014 the progenitor cells that in the adult brain are responsible for the repopulation of microglia when it is depleted (Elmore et al., 2014) suggesting that microglia is self-renewing throughout life (Hashimoto et al., 2013).

Some years ago, microglia was classified into three developmental stages in which the cells were expressing different set of genes, such as CSF-1, IL-34 and TGF-β, mainly due to the signals secreted by the growing CNS (Q. Li & Barres, 2018). Early microglia appears between E10.5 and E14.5, followed by a second stage that is observed between E14.5 and P9. Finally, the adult phenotype is observed after 4 weeks (Holtman et al., 2017; Matcovitch-Natan et al., 2016).

Microglia presents transcriptomic and epigenetic markers that distinguish them from other macrophages (Gosselin et al., 2014; Lavin et al., 2014). Correct microglia development depends on the expression of the transcription factor Pu.1 and the interferon regulatory factor Irf8. Both factors form a heterodimer that is essential for microglia phenotype (Beers et al., 2006; Kierdorf et al., 2013; Minten et al., 2012). In addition to Pu.1 and Irf8, the transcription factor Runx1 and microRNA *miRNA24* might also be involved in microglia proliferation (Zusso et al., 2012), motility and morphological changes (Ponomarev et al., 2011; Svahn et al., 2016), respectively. CSF1 receptor (CSF1-R) is also essential for microglial numbers, and it can bind CSF1 and IL-34. It has been observed that IL-34 is more important than CSF1 for regulating microglia density in the adult brain (Greter et al., 2012; Y. Wang et al., 2012). Most of the experiments that have helped to unravel the role of CSF1-R in microglia development and adulthood have tried to deplete microglia, such as the CSF1-R inhibitors (Elmore et al. 2014), and DAP12-deficient mice, in which the CSF1-R adapter protein DAP12 is removed (Kierdorf et al., 2013; Otero et al., 2009). Finally, microglia express exclusively the fractalkine receptor CX3CR1 (Cardona et al., 2008), which is involved in the infiltration, distribution and proliferation of microglia into the developing brain (Hoshiko et al., 2012; Paolicelli et al., 2011).

It is now clear that, in physiological conditions, microglia is not resting, but they are instead continuously extending and retracting their processes in a surveillance state. By doing this, they are able to sense and check the surrounding cells in order to respond faster to any insult or sudden change in the microenvironment (Davalos et al., 2005; Haynes et al., 2006; Nimmerjahn et al., 2005; Orr et al., 2009). For instance, right after an acute injury, microglia move towards the damage by retracting their processes and becoming more amoeboid and motile (Stence et al., 2001; Davalos et al., 2005; Nimmerjahn et al., 2005). The expression of the microglial purinergic receptor P2Y12 seems to be involved in the chemotaxis, since damaged neural cells secrete ATP or ADP (Davalos et al., 2005; Haynes et al., 2006). Moreover, by doing this surveillance, it has been suggested that microglia support the survival, proliferation and maturation of neuronal progenitors and neurons (Davalos et al., 2005; Frost & Schafer, 2016; Ueno et al., 2013).

6. 2. Microglia, apoptosis and synapsis pruning

When neurons become apoptotic, they secrete several signals that attract peripheral macrophages and can, at the same time, attract surrounding microglia. Some of these signals are fractalkine (CX3CL1), lipid lysophosphatidylcholine (LPC), sphingosine 1 phosphate (S1P), ATP and UTP (Elliott et al., 2009; Gude et al., 2008; Lauber et al., 2003; Truman et al., 2008). It has to be considered that neuronal apoptosis does not influence or mediate microglia entering into the CNS during embryonic development (Eyo et al., 2016).

Microglia are in charge of eliminating apoptotic cell debris **(Fig 14)**. Their cellular processes contact apoptotic cells expressing activated Caspase-3 during the first two postnatal days in mice (Mosser et al., 2017). This is a process likely mediated by DAP12 and CD11b, since it has been observed that DAP12 or integrin CD11b depletion induces a reduction in neuronal apoptosis. Moreover, it has been shown that microglia localize within some neurogenic niches, where they control neuronal progenitor cells (NPC) numbers by selective engulfment at the postnatal SZ and neocortex (Cunningham et al., 2013), as well as promoting active apoptosis and the removal of debris from the dying NPCs (K. Ashwell, 1990; Marín-Teva et al., 2004; Sedel et al., 2004). In the adult brain, microglia is also important for clearing dead cells and those in excess via phagocytosis. This, nevertheless, does not seem to require cell activation (Sierra et al., 2010).

Recently, it has been described the importance of microglia in synapsis refinement and pruning in the healthy brain **(Fig 14)** (Hoshiko et al., 2012; Paolicelli et al., 2011; Schafer et al., 2012; Tremblay et al., 2010; Wake et al., 2009a; Zhan et al., 2014). During CNS development, there is an overproduction of synapses. This excess of synapses is then refined in an activity-dependent manner (Hua & Smith, 2004). For instance, it has been observed that dark rearing during the critical period in mice increases the proportion of phagocytic structures in visual cortex microglia (Tremblay et al., 2010). Moreover, it has been demonstrated that during eye specific segregation, microglia in the visual cortex is carrying out a selective pruning of weak synapses through an activity-dependent process that requires the P2Y12 receptor (Schafer et al., 2012; Sipe et al., 2016). However, it seems that microglia does not only remove weak synapses, but it is also in charge of remodelling the circuits (Tremblay et al., 2010; Wake et al., 2013).

Apart from the activity-dependent removal, serotonin is also known to be involved in the refinement of the visual system (van Kleef et al., 2012). In fact, microglia express the serotonin receptor 5-HT2B, which promotes the extension of microglial processes towards a source of serotonin. This has been observed in mice lacking this receptor, which have shown defects in the refinement of the synapsis in the retinal projections (Kolodziejczak et al., 2015). In addition to serotonin, microglial expression of BDNF has also been observed to facilitate synapse formation. BDNF controls the expression of proteins involved in the synapse itself, such as AMPA and NMDA receptors subunits (Parkhurst et al., 2013; Roumier et al., 2004). Tumor necrosis factor alpha (TNFα) secreted by microglia can also impact synapses. Its expression can promote the endocytosis of $GABA_A$ receptors during inhibitory synapsis (Stellwagen et al., 2005). Finally, it has been recently shown that IL-33 secreted by astrocytes can regulate the phagocytosis of neuronal spines by microglia. This demonstrates an astrocyte-microglia interaction that has neuronal output (Vainchtein et al., 2018). Importantly, it has been also seen *in vitro* that the depletion of microglia induces a reduction in the numbers of newly generated astrocytes (Antony et al., 2011), and that astrocyte proliferation and differentiation can be stimulated by factors secreted by microglia (Giulian & Ingeman, 1988; Nakanishi et al., 2007).

An example of the role of microglia in the maintenance of neuronal circuits occurs in the barrel field of the somatosensory cortex. The barrels in layer 4 are formed at P3. Two days later, at P5, microglia enters into the barrels in order to interact with the thalamocortical synapses and favour their functional maturation (Hoshiko et al., 2012; Thion et al., 2019). Altogether, these studies suggest that changes in the normal function of microglia can have consequences in the maintenance of synaptic networks, since these cells have an important role during embryonic development of the CNS.

6. 3. Microglia and brain wiring

It has been observed in several species that microglia is found close to developing axonal tracks (K. W. S. Ashwell et al., 1989; Cuadros et al., 1993; Herbomel et al., 2001; Innocenti et al., 1983; Pont-Lezica et al., 2014; Rezaie et al., 1999; Verney et al., 2010). For instance, in mice, microglia has been found in the MZ close to the axon fascicles (Cuadros et al., 1993; Soria & Fairén, 2000), at the subpallium (Squarzoni et al., 2014), the corpus callosum (Pont- Lezica et al., 2014), and the hippocampal commisure (Dalmau et al., 1998). Moreover, microglia appear in parallel to the axonal

tracks, where they acquire the ramified phenotype and mature (Cuadros et al., 1993; Dalmau et al., 1998; Torres-Platas et al., 2014).

Back in 2014, several groups observed that apart from the role of microglia in the maintenance of cell numbers by promoting proliferation and apoptosis, and its role in circuit refinement and synapse pruning, microglia was also important in brain wiring **(Fig 14)**. This was suggested by loss-of-function and knock-out experiments of the *DAP12* and *Pu.1* genes, respectively, which led to the defasciculation of axons in the corpus callosum (Pont- Lezica et al., 2014). Moreover, it was observed that manipulating microglia during embryonic development affected the normal growth of dopaminergic axons and the distribution of a subpopulation of cortical interneurons (Squarzoni et al., 2014).

6. 4. Microglia and activity

Microglia is a population of cells that are continuously extending and retracting their processes in order to contact the synapses and check the surrounding microenvironment. Several groups have shown that the duration of these microglia-synapse contacts depends on network activity and could be influenced by the fate of these synapses (Y. Li et al., 2012; Pfeiffer et al., 2016; Sipe et al., 2016; Tremblay et al., 2010; Wake et al., 2009b). Interestingly, it has been recently observed that microglia can sense and respond to neuronal activity due to the expression of several receptors (Helmut et al., 2011). One of these receptors, P2Y12R, appears to be important in this context (Sipe et al., 2016).

Microglia can sense both hyperexcitability and hypoexcitability:

- **Hyperexcitability**:

  Under physiological conditions, microglial processes are recruited by active neurons, such as those activated upon a visual stimulation (Akiyoshi et al., 2018a; Y. Li et al., 2012). When neuronal activity is increased throughout a long period of time, neurons secrete ATP and ADP, which are sensed by the P2Y12-R, which in turn engages process extension in microglial cells (Haynes et al., 2006; L. J. Wu et al., 2007). This P2Y12/ATP-ADP signalling pathway is also part of the response of microglia to tissue damage. However, in healthy

tissue the increase in neuronal excitability eventually increases the recruitment of microglia and the interaction between microglia and neuronal synapses, therefore, reducing this neuronal activity as a consequence (Akiyoshi et al., 2018b; Y. Li et al., 2012; Wan et al., 2020). Thus, it has been observed that the ablation of microglia during seizures has deleterious effects (Badimon et al., 2020; W. Wu et al., 2020). Moreover, CX3CR1-knock out mice, which do not express the fractalkine receptor in microglia, resulted in an increased seizure severity (Eyo et al., 2016). The ablation of the P2Y12 receptor caused a reduction in the microglia-neuron interactions along with more severe seizures as well (Badimon et al., 2020; Eyo et al., 2014). Finally, it has also been seen that the close apposition between microglia and neurons reduces neuronal hyperexcitability through a mechanism that is still unknown (Kato et al., 2016).

- **Hypoexcitability:**

In addition to hyperexcitability, the hypoexcitability of neuronal networks can be sensed by microglia as well. In this case, the microglial response to hypoexcitability has been observed under anaesthetized conditions. In anaesthetized animals and with different anaesthetics, microglial dynamics are increased. Interestingly, under these circumstances, microglial cells increase the surveillance state a few minutes after the administration of the anaesthesia (Liu et al., 2019). It seems that the levels of norepinephrine (NE) regulate the motility and process extension of microglia in the hypoactive stated (Liu et al., 2019; Mishima et al., 2019; Stowell et al., 2019).

6. 5. Microglia and interneurons

As it was introduced before, it has been observed that the depletion or over-proliferation of microglia during embryonic development affects the distribution of a subpopulation of cortical interneurons postnatally (Squarzoni et al., 2014). After this first result, several studies have deepened into how microglia can affect GABAergic inhibitory neurons, and therefore, the network. In addition to this, the role of microglia in controlling and maintaining the excitatory/inhibitory balance in the brain has been widely studied in pathological conditions such as seizures.

The first findings relating microglia and interneuron at early developmental stages appeared in 2014. Squarzoni and colleagues observed that embryonic depletion or overproduction of microglia affected *Lhx6*-positive interneurons, and in particular fast-spiking interneurons. They observed that under these circumstances, fast-spiking interneurons were not correctly allocated in the developing neocortex, mainly in the upper layers (Squarzoni et al., 2014). Later, in 2019, it was described that either depletion or over-proliferation of microglia during embryonic development had an impact on PV cells in layer 4 of the barrel cortex. In fact, these PV cells had a reduced inhibitory drive onto their targets in the adult brain, while in contrast, in juvenile mice there was a higher density of this subpopulation of interneurons and the inhibitory drive onto the targets was increased (Thion et al., 2019). In addition, and supporting this microglia-interneuron interaction, it has been very recently demonstrated that during mouse postnatal development there is a subpopulation of microglia which specifically binds GABA due to the expression of the $GABA_B$ receptor on their surface, and therefore remodels inhibitory synapses, and not excitatory (Favuzzi et al., 2021).

Altogether, these studies suggest that cortical interneurons are sensitive to changes in microglia, and at the same time, microglia is important during development for the correct formation of neuronal circuits.

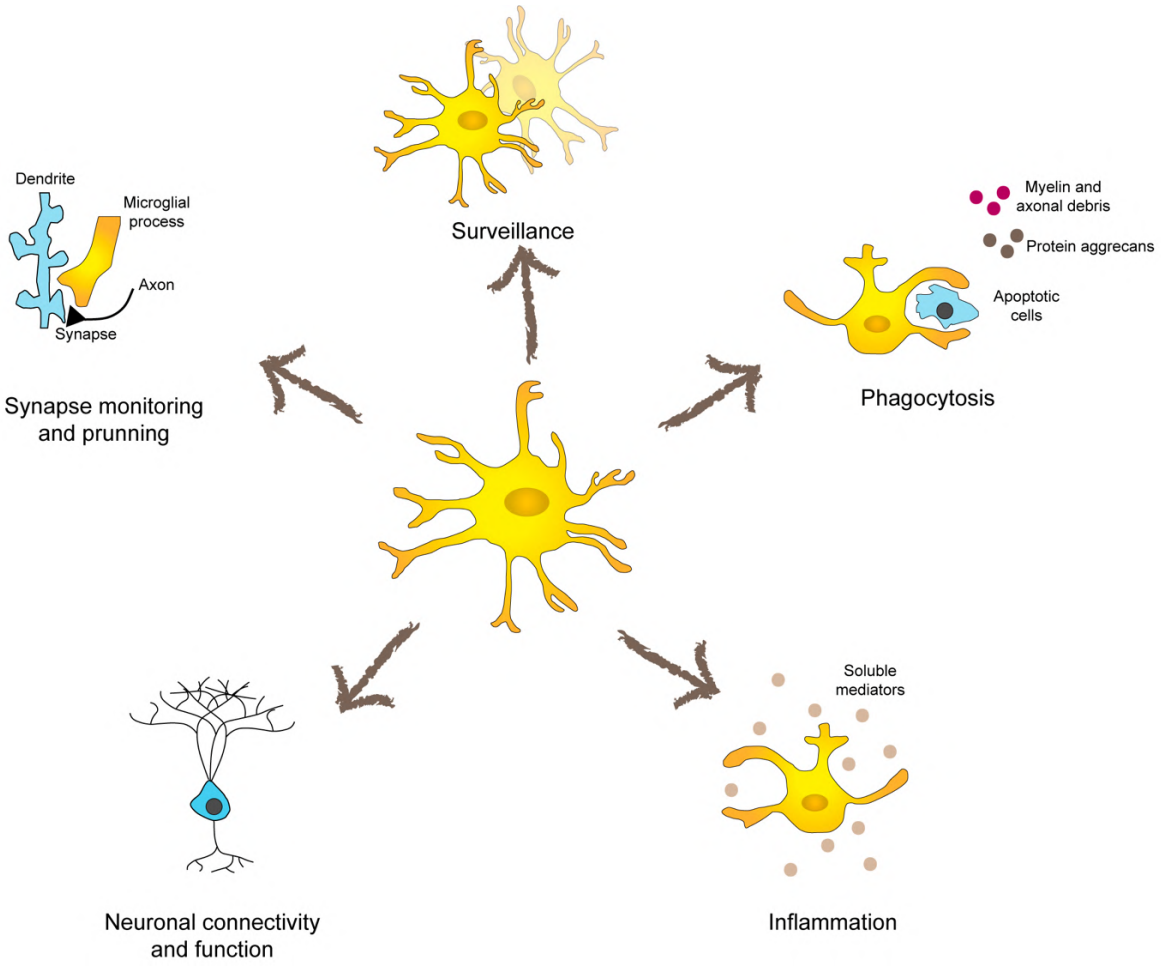


Figure 14. Some key functions of microglia. Microglia has several functions, such as surveillance and phagocytosis of apoptotic cells and debris. They are also important during inflammation and synapse pruning. Adapted from Sierra et al., 2019.

OBJECTIVES

The general objective of this thesis is to study whether local thalamic interneurons and microglia are sensitive to peripheral or thalamic activity during early stages of development in the mouse. In order to pursue this general objective, we had the following specific objectives:

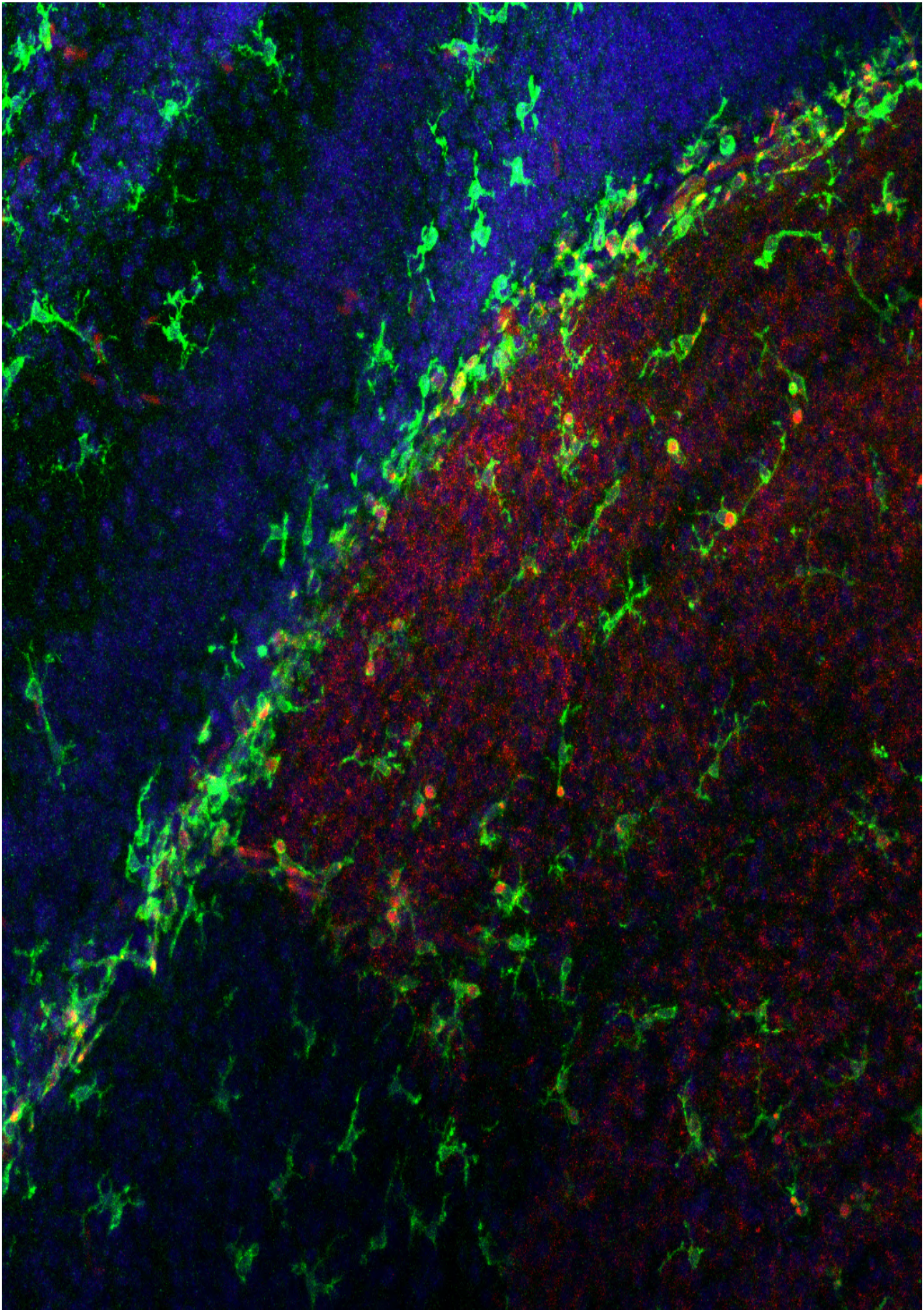
- To decipher whether peripheral spontaneous activity, in particular type I and type II retinal waves, impact thalamic interneuron migration into the dLGN during development.

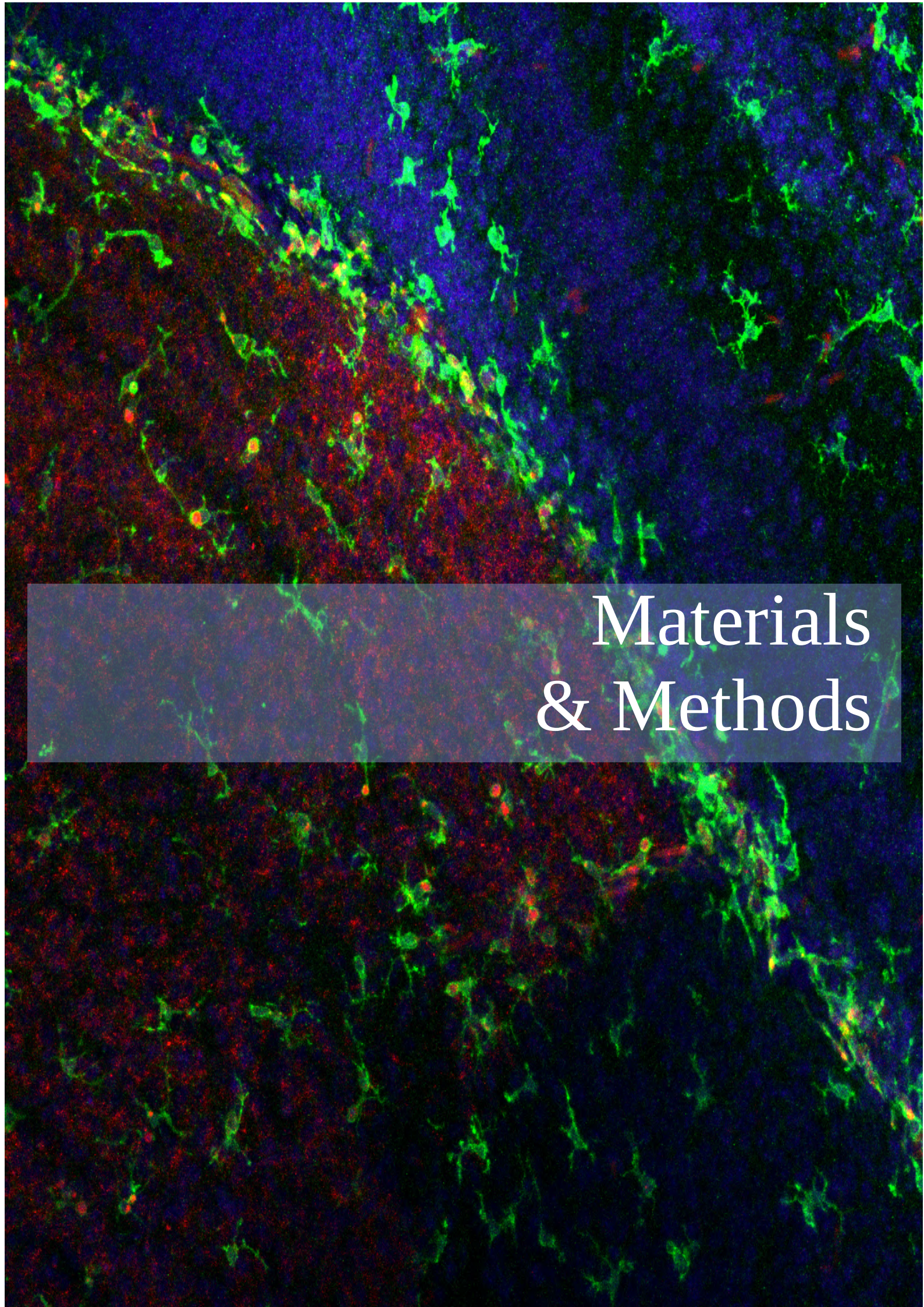
- To decipher whether thalamic spontaneous activity during embryonic development impacts thalamic interneuron migration into the dLGN.

- To study whether thalamic microglia is sensitive to changes in peripheral or thalamic spontaneous activity.

- To determine whether changes in retinal or thalamic spontaneous activity impact cortical interneurons in V1.

- To decipher whether retinal or thalamic activity would impact cortical microglia in V1.





Materials & Methods

MATERIALS AND METHODS

Mouse strains

All the transgenic mice used in this thesis were maintained in the ICR/CD-1 genetic background. They have been genotyped by PCR. We assumed E0.5 for the day of the plug. The R26tdTomato Cre-dependent mouse (stock number 007908) was from Jackson Laboratories. The GAD67-GFP mouse model expresses the fluorescent protein GFP under the interneuron promoter GAD67 (Tamamaki et al., 2003; Wu et al., 2011), which labels the whole population of interneurons in the brain. The R26Kir2.1-mCherry floxed mice were crossed with the Gbx2CreERT/+ mouse line, which is a specific thalamic promoter. Double mutants for Kir2.1 and Gbx2 are referred here as ThKir. They are obtained by tamoxifen administration (gavage, 5mg dissolved in corn oil) at E10.5, which labels all the primary sensory nuclei in the thalamus (Moreno-Juan et al., 2017; Antón-Bolaños et al., 2019). The triple mutant GAD67:GFP-Gbx2-Kir2.1 was obtained as the double mutant Gbx2:Kir2.1. Given that tamoxifen leads to early birth, progesterone was administered intraperitoneally at E14.5 (125mg/kg DEPO-PROGEVERA®). At E19.5 we performed C-sections was done on pregnant females and the pups were placed with a foster mother. All the CreERT2-negrative littermates were used as controls for all the experiments carried out for this thesis. In these animals, all the interneurons are labelled in green. On the other hand, SertCre:Kir animals express the Kir2.1 potassium channel under the serotonin transporter (Sert) promoter. In these animals, Kir2.1 is expressed gradually in the different thalamic nuclei at the end of the gestational period and extends until early postnatal days without tamoxifen administration (Antón-Bolaños et al., 2019).

In utero bilateral enucleation

The surgery was performed on pregnant females at E14.5, which were deeply anesthetized with isoflurane as previously described. The uterine horns were exposed after a midline laparotomy. Both eyes were cauterized in half of the litter, and then the embryos were placed back in the abdominal cavity. The surgical incision was closed and the embryos developed normally until birth date and postnatal stages.

All the animal procedures were approved by the Committee on Animal Research at the Universidad Miguel Hernández de Elche, and in compliance with the Spanish and European Union regulations.

Immunohistochemistry

Mice were perfused with paraformaldehyde (PFA) 4% in PBS 0.01M. The brains were dissected and postfixed overnight. In contrast, brains at embryonic stages were directly dissected and fixed in 4% PFA overnight. Coronal sections 60 μm thick were obtained with the vibratome. Brain slices were then treated with a citrate buffer at pH=6 to unmask the antigens. Then, slices were washed and the blocking solution containing 10% normal goat serum (NGS) and 0.3% Triton X-100 (Tx100) was placed for 1h. Slices were incubated overnight at 4ºC with the respective primary antibodies, 3% NGS, 0.3% Tx100 in PBS 0.01M: guinea pig anti-vGlut2 (1:5000, Synaptic Systems, #135404), chicken anti-GFP (1:2000; Aves Labs, #GFP-1020), rat anti-RFP (1:1000 Chromotek, #5F8), mouse anti-NeuN (1:1000 Merk-Millipore, #MAB377), rabbit anti-Iba1 (1:1000, Wako #019-19741), mouse anti-Otx2 (1:50, courtesy of Prosziank lab), rabbit anti-PV (1:1000, Swant #PV27), rat anti-somatostatin (1:50, Merck # MAB354), rat anti-CD68 (1:1000, Abcam # AB53444). Sections were washed several times with PBS 0.01M and incubated 2h at room temperature with seconday antibody, 3% NGS, 0.3% Tx100 in PBS 0.01M: 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). Brain slices were rinsed in PBS 0.01M and then staind with DAPI for 5 min. Finally, they were mounted with Fluoromont.

***In situ* hybridation**

Mice were perfused, dissected and postfixed as before. Coronal sections 60 μm thick were obtained with the vibratome. Brain slices are then treated with H_2O_2 1.5% for 30 minutes and washed with TNT buffer. They are treated with Proteinase K (5μg/ml) for 8 minutes, and glycine (2mg/ml). Then, the slices are washed with TNT buffer and incubated overnight with the hybridation solution containing the *Reln* probe (1:100). The next day, brain slices

are washed several times with a solution containing formamide, SSC and SDS 1%, followed by washes with another solution of formamide with SSC. After this, the tissue is washed with TNT buffer and incubated with a blocking solution with 10% FBS for 1hour at room temperature. Later, brain slices are incubated with anti-DIG-antibodies (1:500) over night. The day after, the slices are washed with TNT buffer and developed with the TSA-Cy Kit (1:500). They were washed with PBS several times and mounted with Fluoromont.
Forward primer Reelin: TCAGCTGGAGAAAATTAGAGCC. Reverse primer Reelin: CAAGCACTCAGTGTGGAGTAGG

Nissl staining

Brain slices were 60µm thick and they were mounted in Ultrafrost Super Frost. When they are completely dried, the slides are left in Cressyl violet staining for in between 30 seconds and 2 minutes. After, slides are washed for 1 min with dH_2O, and incubated in 70% EtOH for 3 minutes, followed by an incubation in 90% EtOH, and 100% EtOH, 3 minutes respectively. Finally, slides are washed with Xylol twice for 5 min. Slides are then drained on a paper and mounted with EuKitt Quick-hardening mounting medium (Sigma Aldrich).

IMARIS image processing

Sholl analysis and microglia morphology was obtained with the IMARIS 9.1 software provided by the Imaging Facility, using 63x magnification.

Confocal microscopy

Images were taken with an inverted confocal Olympus with a 20x, oil immersion objective.

Image quantification

Images were analyzed using the FIJI (FIJI is just Image J) software. The quantification of the cells in the dLGN was done with the Cell Counter plugin, selecting a particular ROI at four different rostro-caudal levels for each brain. Then, an average for the area and the total number of interneurons was calculated per brain. The same was done for microglia quantification.
For cortical interneurons and microglia, three different rostro-caudal levels were selected. In each layer, the average was done analyzing three ROIs of 100x100μm for each rostro-caudal level. Then, the average was calculated using all the levels.

Microglia depletion during development

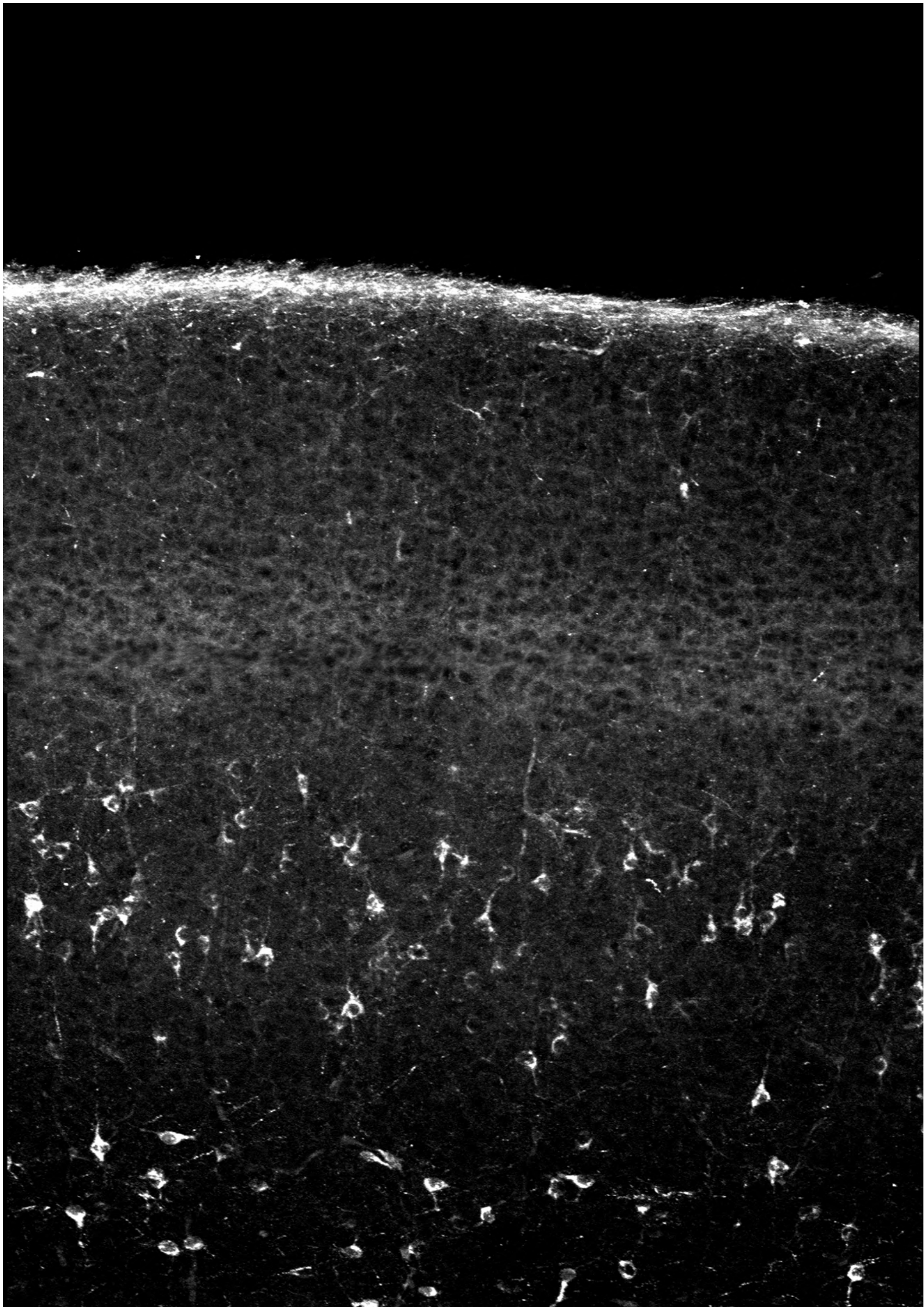
To deplete microglia during development, we administered PLX3397 (500 mg/kg) with the normal chow from E4.5 to birth to Gbx2CreERT control and Gbx2CreERT:Kir2.1 mice. Gbx2CreERT:Kir2.1 mice (ThKir) were also treated with tamoxifen at E10.5 and progesterone as explained above. Gbx2CreERT control mice were fed with PLX3397 (500 mg/kg) also after giving birth in order to deplete microglia postnatally.

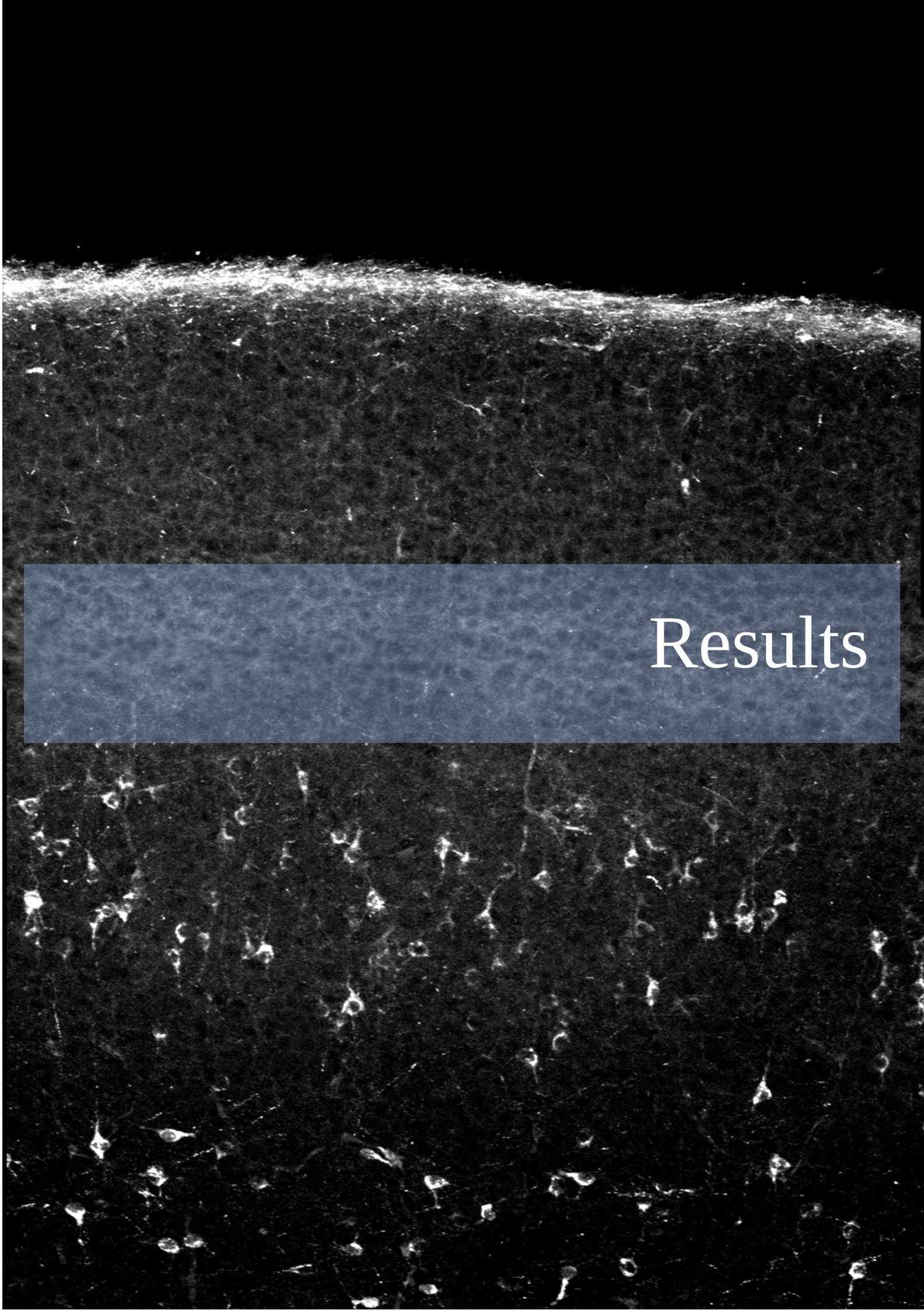
***In vivo* intraocular injections**

For the pharmacological ablation of type I retinal waves *in vivo*, Carbenoxolone (10mM; Cbx, Merck, C4790) or saline were injected into the vitreous humor of the eye both at P0 and P1 with a pulled glass micropipette. Each eye received 0.3μL of Cbx per injection through a small incision. These early postnatal pups were anesthetized using ice, and they were recovered in a heating pad. For the pharmacological ablation of type II retinal waves, Epibatidine at 0.5mM was used (Epib, Merck, E1145). 0.42-0.49 μL of 0.5mM Epib or saline were injected into the vitreous humor of the eye both at P3 and P4 with pulled glass pipettes. To do that, pups were anesthetized with ice and a small incision was performed in the eyelid to expose the eyeball. Pups recovered in a heating pad.

Statistics

The statistical analysis was carried out using SPSS and R. The statistical comparison between two specific populations was done using a two-tailed Student's t test with Welch correction (we did not assume equal variances). When data was not passing the Kolmogorov-Smirnov normality test, the Mann-Whitney test for non-parametric data was applied instead. When comparing the density of cells and the number of cells between mutant and control mice throughout ages, the data was fit to a generalized linear model with a negative binomial distribution, and analyzed using a Chi square test, followed by a Tukey post-hoc. For the area, the data fit a generalized linear model with a Gamma distribution, and was analyzed using a Chi square test and a Tukey post-hoc. For the ratios, a 2-way ANOVA was chosen. In addition, in order to compare cortical data between the genotypes, the data was fitting into a 2-way ANOVA with repeated measures. For these analyses, P values < 0.05 were considered statistically significant and set as follows: $*P < 0.05$; $**P < 0.01$ and $***P < 0.001$. The sample size was not pre-determined with a statistical method. However, the number of samples were considered adequate for the experimental design pursued and consistent with previous studies.





Results

RESULTS

CHAPTER 1: INTERNEURONS IN THE VISUAL PATHWAY

1.1. – Thalamic interneurons

1. 1. 1 Interneurons from the dLGN can be identified by Otx2 and *Reelin*

In mice, GABAergic local neurons, or interneurons, are mainly found in the primary visual nucleus (dLGN). These inhibitory cells are originated outside the thalamus and start migrating into the nucleus from ~E17, reaching their final destination by the end of the first postnatal week (Golding et al., 2014; Jager et al., 2016). As explained previously, one of the sources of local thalamic interneurons is the midbrain proliferative zone. Thalamic GABAergic interneurons from this origin are born between E10 and E13, and belong to the *Engrailed1* lineage (Jager et al., 2016, 2021). They are characterized by the expression of *Otx2, Gata2*, and *Sox14* transcription factors, and are mainly found in FO nuclei, constituting the vast majority of local interneurons in the adult thalamus (Jager et al., 2021). These interneurons migrate from the midbrain into the thalamus from the caudal tiers. The second source of local thalamic interneurons is the prethalamus. The stream of cells coming from the prethalamus populates the thalamus from the rostral tiers at the end of embryonic development (Golding et al., 2014; Jager et al., 2021). These interneurons that are originated in the prethalamus constitute 20% of the total number of local thalamic interneurons in the adult thalamus, and express *Foxd1, Dlx1/2*, and *Dlx5/6*. In contrast to the thalamic interneurons that come from the midbrain, this population is enriched in HO nuclei (Jager et al., 2021). Given this, in order to reveal thalamic interneurons, we used a transgenic mouse line that expresses GFP fused to GAD67, the enzyme responsible for the synthesis of GABA. To corroborate their identity as midbrain- and prethalamic-derived cells, we used known markers that label both subpopulations of thalamic interneurons: Otx2 and *Reelin* (Golding et al., 2014). In order to confirm that these markers can be used at different developmental stages, we chose two different postnatal time points, P6 and P20. We observed that all dLGN interneurons, and some from the vLGN, were exclusively labelled by the anti-Otx2 antibody (**Fig. 1**). In addition, we observed by *in situ* hybridization (ISH) that these interneurons contained *Reelin* mRNA. Finally, by doing a double immunostaining, we confirmed that all $Otx2^+$ cells in the dLGN were also positive for *Reelin*. Thus,

we corroborated that thalamic interneurons from the visual nucleus, and not excitatory projecting neurons, express both Otx2 and *Reelin* and, thus, they can be used as specific markers.

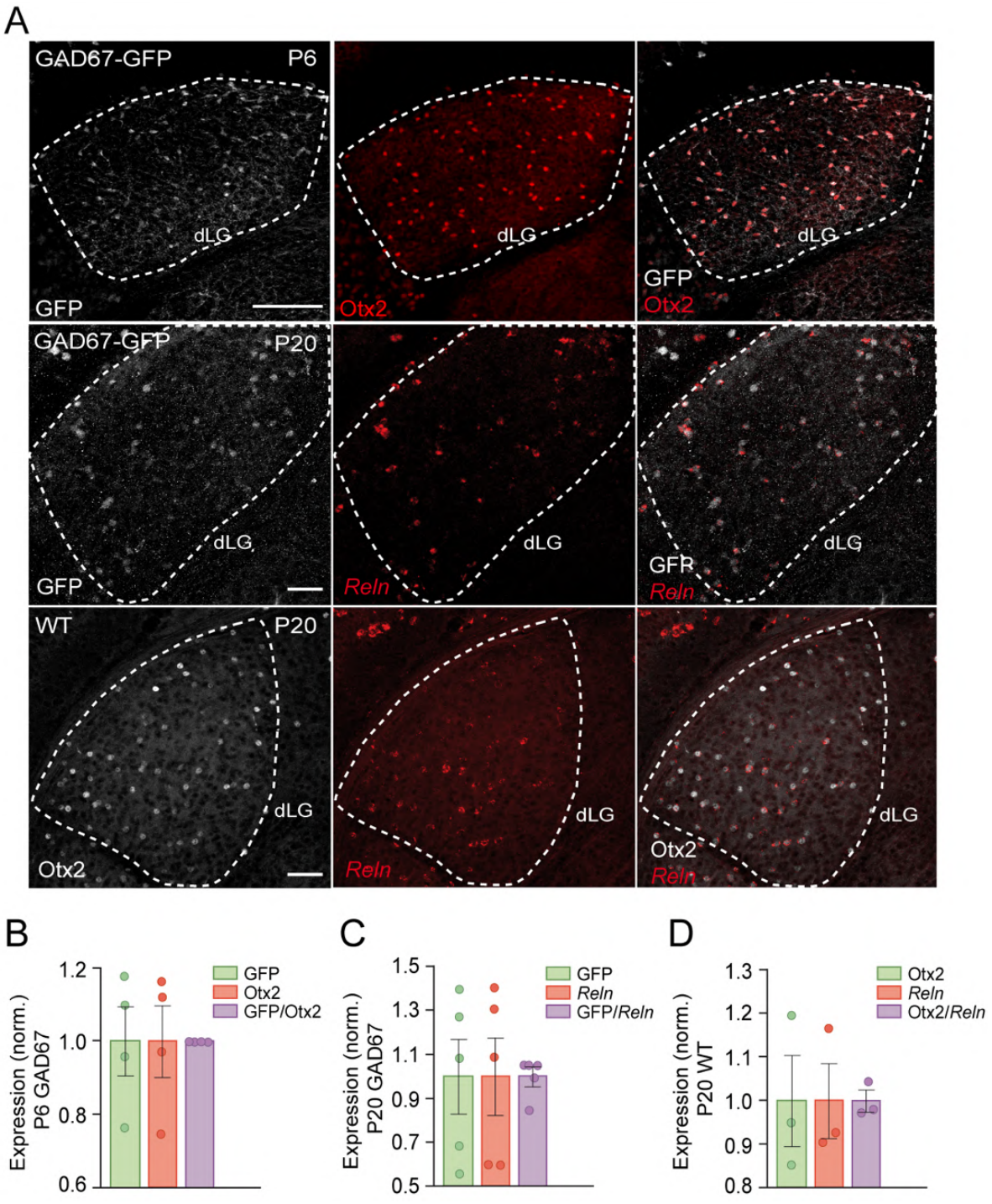


Figure 1. Thalamic interneurons in the dLGN express OTX2 and *Reelin*. A) Double staining with immunohistochemistry and ISH shows that thalamic interneurons express Otx2 and Reelin. B) Graph showing the proportion of GFP and Otx2 positive cells at P6 in the GAD67-GFP model (n=4). C) Graph showing the proportion of GFP and *Reln* positive cells at P20 in the GAD67-GFP model (n=5). D) Graph showing the proportion of Otx2 and *Reln* positive cells at P20 in a WT animal (n=3). Scale bar = 100µm.

1. 1. 2. Retinal input influences the distribution of interneurons in the dLGN

It has been previously reported that the absence of retinal input affects the migration of thalamic interneurons in the dLGN. Thereby, after optical nerve section at P0, or in *Ey1*$^{-/-}$ mutant mice, it was observed that interneurons do not distribute evenly throughout the dLGN; they remain in the upper tiers of the dLGN (Golding et al., 2014). Abnormal retinal input also disrupts the morphology and the synaptic connectivity of dLGN interneurons (Charalambakis et al., 2019).

One of main objectives of our project is to find out the contribution to the development and integration of thalamic interneurons of peripheral input/activity versus central electrical activity. To study the effect of peripheral input, we performed bilateral enucleation in mice at embryonic stages (EmbBE). This way, we remove the retinal axons before they innervate thalamic cells, which are then deprived of receiving retinal input from early stages of development. We compare here the effects with previous reports from anophthalmic mice or upon retinal input removal at later stages (Golding et al., 2014).

We cauterized the eyes of GAD67-EGFP mice at E14.5. Brain tissue was collected at different developmental stages, from E18.5 – time at which interneurons normally start migrating into the dLGN – to P15, when eyes are already open. We firstly measured the dLGN area and observed that there was a reduction in the size of the dLGN in EmbBE compared to control animals as early as P2, a difference that was maintained along the first two postnatal weeks (**Fig 2C**). Looking at the average density of interneurons at different rostro-caudal sections of the dLGN, we observed that there were significantly less interneurons in the dLGN of EmbBE mice compared to control littermates at P6 (**Fig 2D**). Strikingly, the difference observed at P6 was due to a significant reduction in the total number of interneurons (**Fig 2E**), which were accumulating in the upper tiers of the visual nucleus (**Fig 2F**), as it has been previously described (Golding et al., 2014). However, there were no significant differences at P15 between EmbBE and control animals, suggesting that the reduction in the number of interneurons was proportional to the reduction of size of the dLGN.

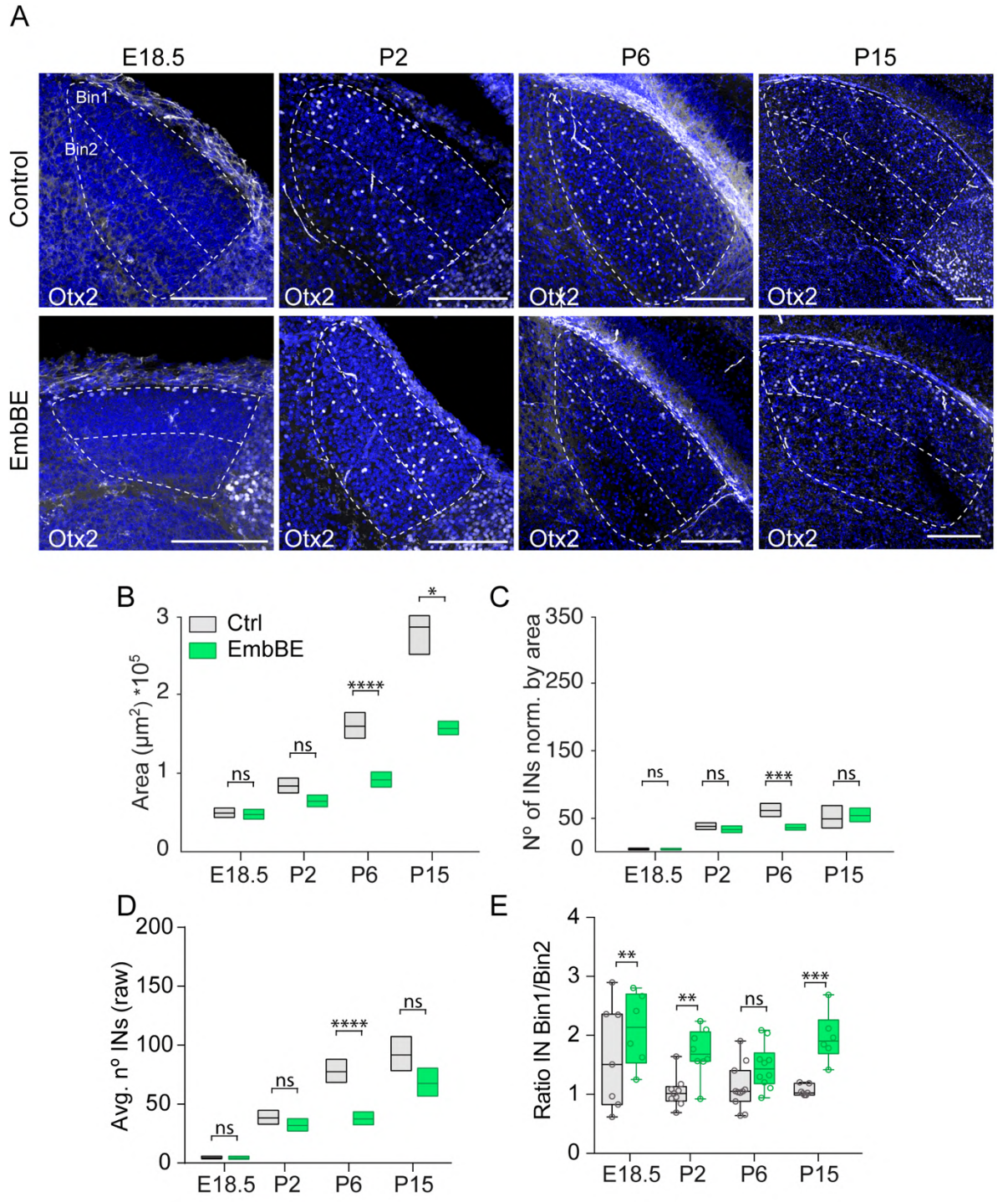


Figure 2. Embryonic bilateral enucleation changes de distribution of dLGN INs but not the density. A) Coronal sections showing the distribution of thalamic interneurons at different time points. B) Graph showing the differences in the area of the dLGN between Ctrl and EmbBE littermates (GLM Gamma distribution). C) The density of interneurons is significantly low in EmbBE at P6 compared to Ctrl animals (GLM Negative Binomial). D) Graph showing the average total number of interneurons per section of dLGN at different developmental stages in Ctrl and EmbBE littermates (GLM Negative Binomial). E) Graph showing the ratio between cells in Bin1 and Bin2 (2-way ANOVA). E18.5 n=7, P2 n=8, P6 n=10, P15 n=6. B, C, and D represent the mean and the confidence intervals. E represents the mean and the SEM. Scale bars = 100µm.

1. 1. 3. Interneuron migration into the dLGN does not depend on neonatal spontaneous retinal activity

There are three different types of retinal waves in mice: type I (~E16 - P1), type II (~P1-P10), and type III (~P10 until eye opening at ~P14). Interneurons invade the dLGN while type I and type II retinal waves are activating the cells of the visual thalamus. In the EmbBE model, both patterns of spontaneous activity are suppressed due to the early removal of the eyes. To separately assess their role, we decided to abolish type I or type II retinal waves using a pharmacological approach, without damaging the axons. In order to study whether type I retinal waves are specifically involved in interneuron migration, we used carbenoxolone (Cbx), a drug that has been widely used on brain slices *in vitro* to remove activity. We decided to use Cbx *in vivo* at 10mM, which blocks type I retinal waves while maintaining the morphology of the eye (Guillamón-Vivancos et al., 2022). Cbx was injected in both eyes at P0 and P1, when thalamic interneurons start migrating into the dLGN. In brains collected at P2 and P6, we observed that neither the average area, nor the average density of interneurons were affected (**Fig 3C** and **3D**). In addition, interneurons distributed evenly throughout the nucleus (**Fig 3E**). Thus, our results showed that blocking type I retinal waves does not affect neither interneuron density nor their location in the visual nucleus.

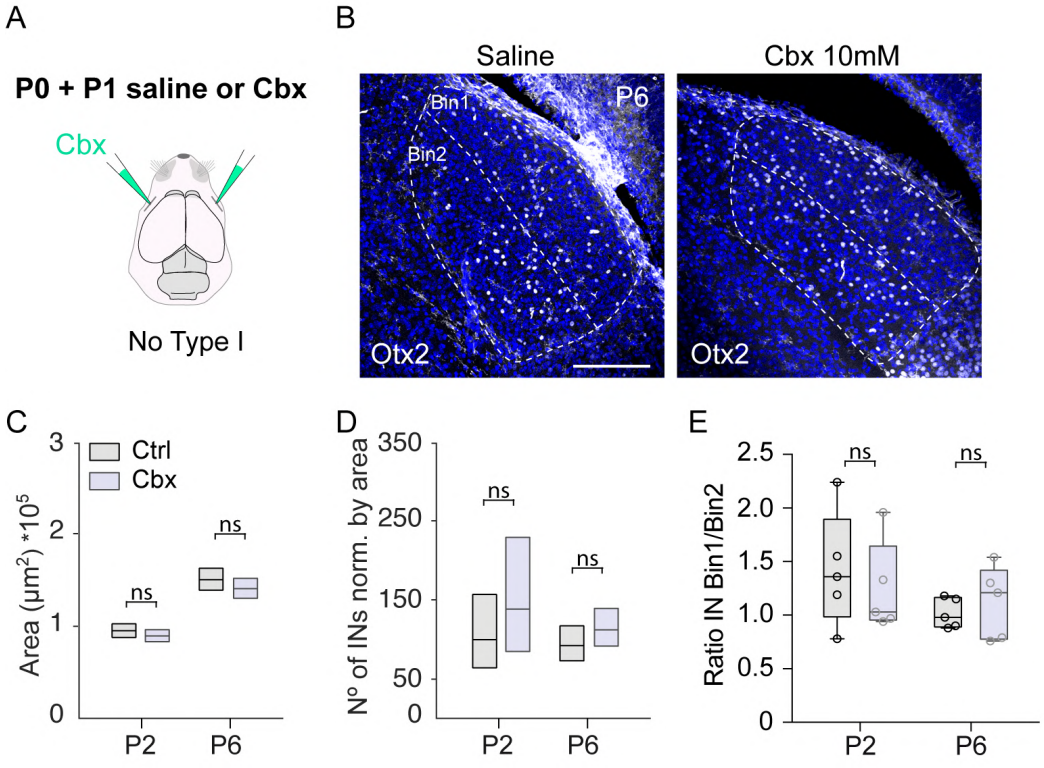


Figure 3. Blockage of type I retinal waves does not affect interneuron migration into the dLGN. A) Schematic representation of the surgery performed at P0 and P1. B) Coronal sections showing the distribution of thalamic

interneurons at P6. C) Graph showing the differences in the area of the dLGN between Saline and Cbx animals (GLM Gamma distribution). D) The density of interneurons does not change between Saline and Cbx littermates (GLM Negative Binomial). E) Interneurons distribute similarly in saline and Cbx injected animals (2-way ANOVA). P2 n=5, P6 n=5. C, and D represent the mean and the confidence intervals. E represents the mean and the SEM. Scale bar = 100µm.

1. 1. 4 Interneuron migration into the dLGN does not depend on type II retinal waves

Epibatidine (Epib) is a drug widely used to block type II retinal waves (Rossi et al., 2001; Huberman et al., 2002; Penn et al., 1998; Cang et al., 2005; Pfeiffenberger et al. 2005; Sun et al., 2008; Ackman et al., 2012). In mouse, Epib binds to the nAchRs decorrelating spontaneous activity and, therefore, disrupting waves. We injected Epib in both eyes at P3 and P4 when type II retinal waves are already established and interneurons massively invade the dLGN. Immunostaining for interneurons showed no significant changes in their density between animals injected with saline or epibatidine (**Fig.4C** and **4D**), suggesting that blocking type II retinal waves acutely does not affect the migration and distribution of interneurons into the dLGN.

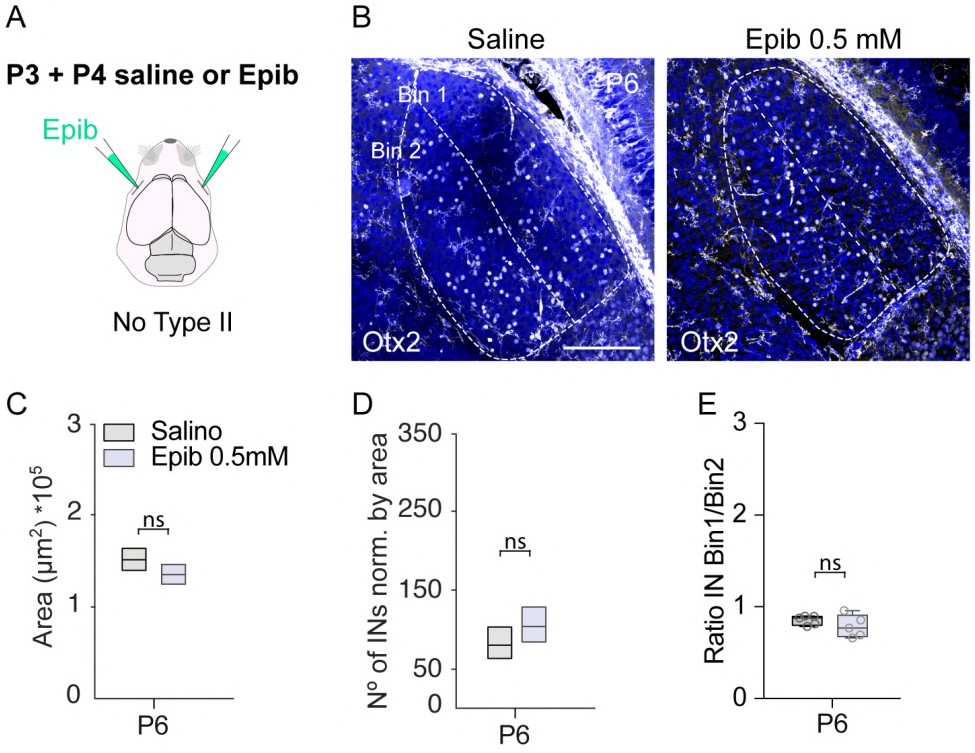


Figure 4. Blockage of type II retinal waves does not affect interneuron migration. A) Schematic representation of the surgery performed at P3 and P4. B) Coronal sections showing the distribution of thalamic interneurons at P6.

C) Graph showing the differences in the area of the dLGN between Saline and Epib injected animals (GLM Gamma distribution). D) The density of interneurons does not change between Saline and Epib littermates (GLM Negative Binomial). E) Interneurons distribute similarly in saline and Cbx injected animals (2-way ANOVA). P6 n=5. C, and D represent the mean and the confidence intervals. E represents the mean and the SEM. Scale bar = 100μm.

1. 1. 6 Interfering with the thalamic spontaneous activity perturbs the migration of interneurons into the dLGN

Retinal axons project mainly to the primary visual nucleus of the thalamus, the dLGN. The developing thalamus exhibits spontaneous patterns of synchronous activity in the form of waves from very early stages (Moreno-Juan et al., 2017; Antón-Bolaños et al., 2019). Therefore, we wondered whether this intrinsic activity would have any effect on interneuron migration into the dLGN. To that end, we took advantage of the Th^{Kir} model in which the inward rectifying potassium channel 2.1 (Kir2.1) is overexpressed in thalamic cells after cell cycle exit, driven by the Gbx2 promoter (Antón-Bolaños et al., 2019). In this mouse, thalamic calcium waves are eliminated at the embryonic life and thus, activity in the thalamus is switched from synchronous to an asynchronous mode before birth (Antón-Bolaños et al., 2019). Using this model, we analyzed the migration of dLGN interneurons at different time points (E18.5, P2, P6 and P15) in control and Th^{Kir} littermates. We firstly looked at the area of the dLGN and we observed that it was significantly reduced in Th^{Kir} animals compared to the controls as soon as P2, and this reduction was maintained throughout time (**Fig 5B**). In fact, the nucleus was not growing from P2, keeping the same size two weeks later, at P15. Moreover, we observed a significant increase in the density of these inhibitory cells at P6 and P15 in Th^{Kir} animals compared to control littermates (**Fig 5C**). This increase was mainly due to a significant difference in the total number of interneurons, which was reaching the peak already at P6 (**Fig 5D**). Interestingly, interneurons in Th^{Kir} animals were evenly distributed throughout the dLGN (**Fig 5E**). Given that the density of interneurons was higher in Th^{Kir} animals, we then quantified the ratio of excitatory versus inhibitory cells by analyzing the proportion of $Otx2^{+}$ cells (interneurons) compared to $NeuN^{+}$ cells (excitatory projecting neurons). We observed that there was a significant decrease in the excitatory versus inhibitory ratio in Th^{Kir} animals compared to control littermates (**Fig 6B**). However, the overall density of cells was unaffected, as we could observe by Nissl staining (**Fig 6D**).

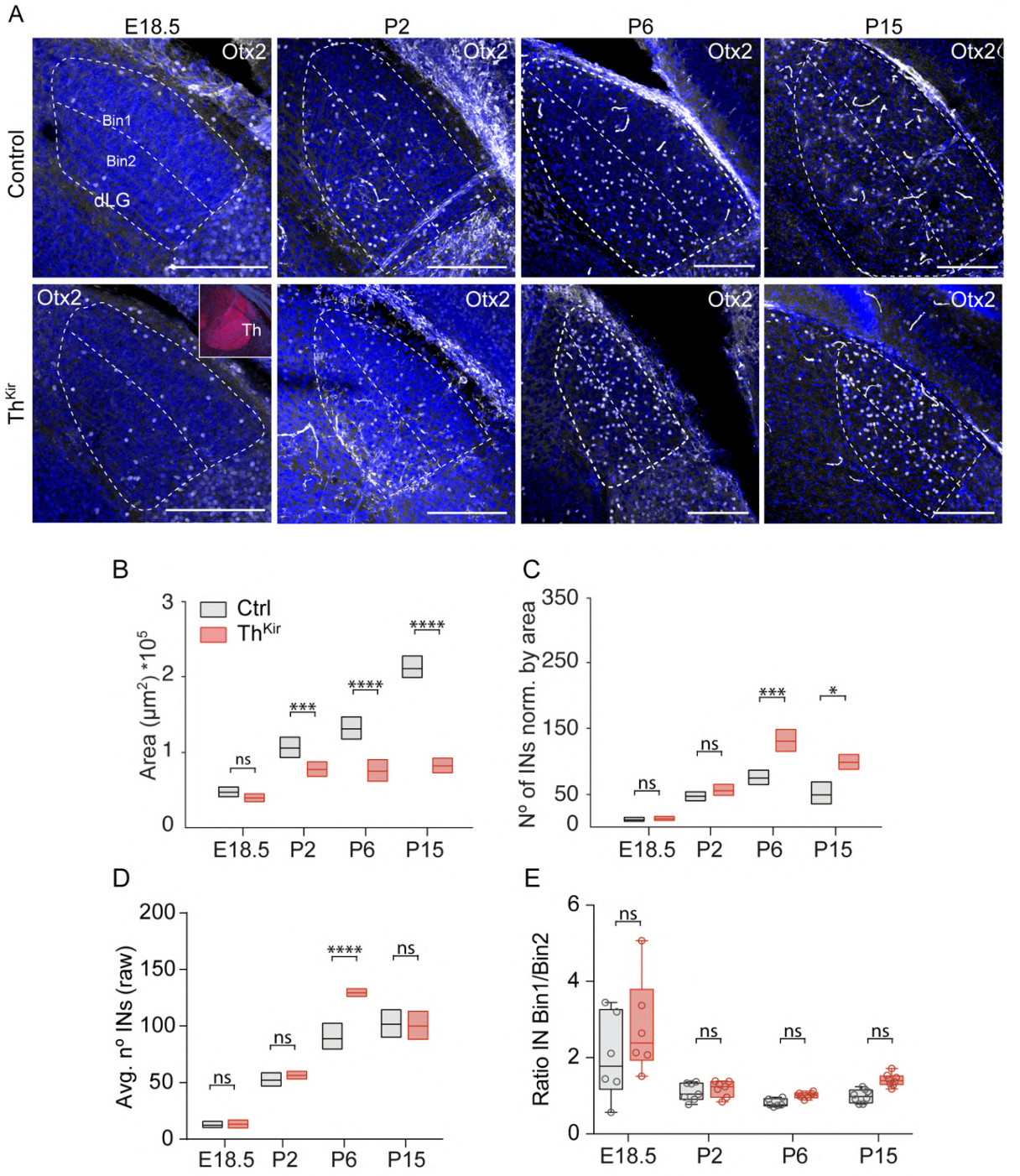


Figure 5. Perturbing embryonic thalamic activity affects dLGN interneurons. A) Coronal sections showing the distribution of thalamic interneurons at different time points. B) Graph showing the differences in the area of the dLGN between Ctrl and Th^{Kir} animals (GLM Gamma distribution). C) The density of interneurons is significantly increased in Th^{Kir} littermates (GLM Negative Binomial). D) Graph showing the average total number of interneurons per section of dLGN at different developmental stages in Ctrl and Th^{Kir} littermates (GLM Negative Binomial). E) Interneurons distribute similarly in Ctrl and Th^{Kir} animals. E18.5 n=6, P2 n=8, P6 n=8, P15 n=10. B, C, and D represent the mean and the confidence intervals. E represents the mean and the SEM. Scale bars = 100µm.

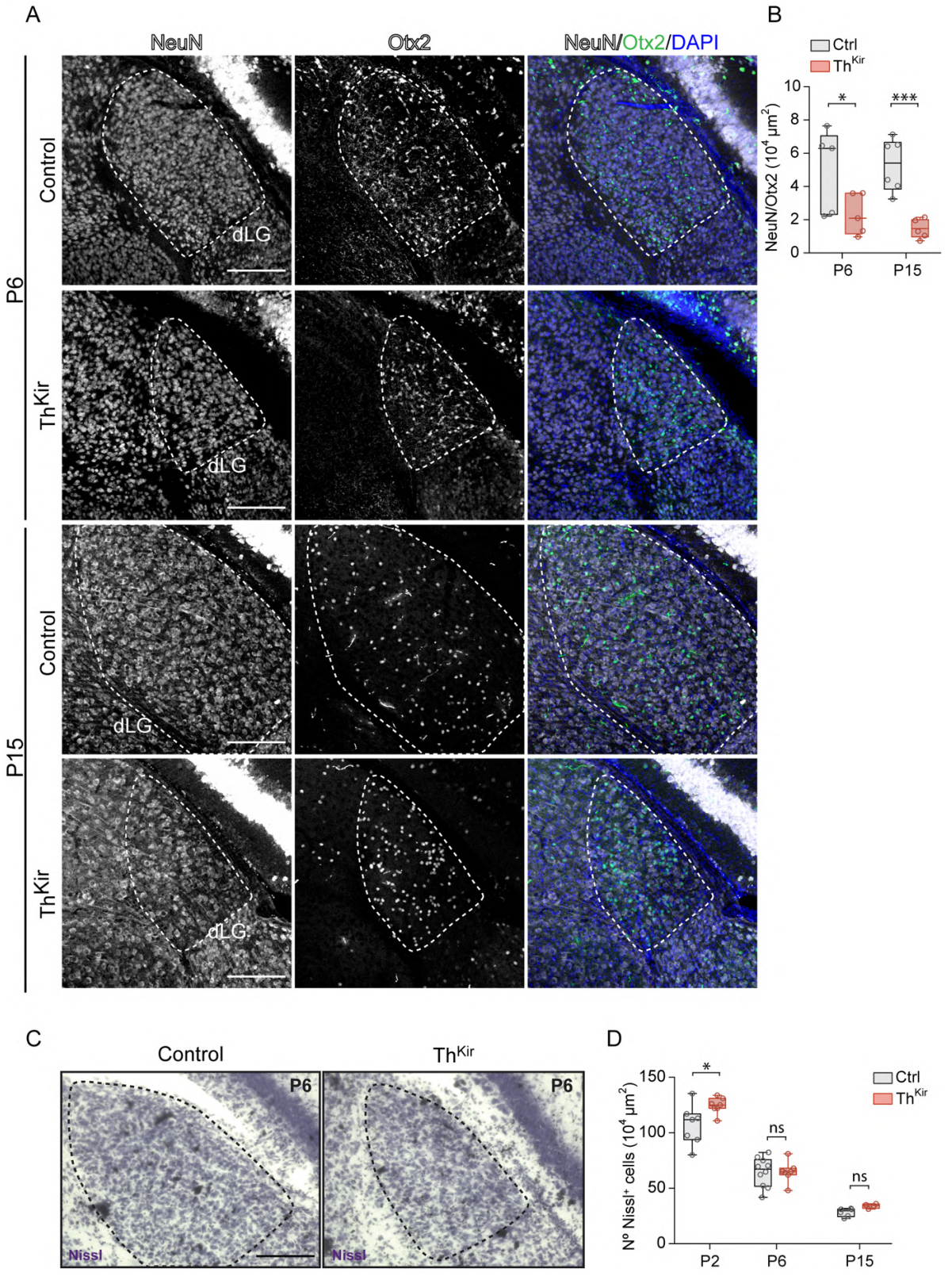
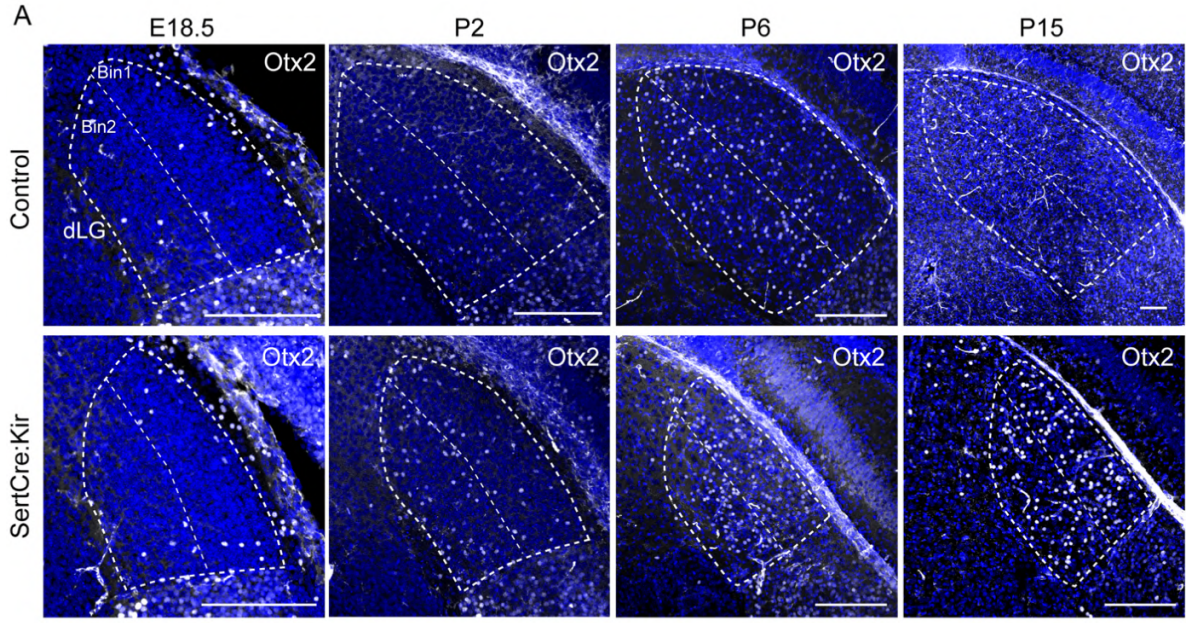


Figure 6. Th^{Kir} animals have decreased E/I ratio but no differences in cell density. A) Immunostaining for NeuN and Otx2 at P6 and P15. B) Th^{Kir} animals present a significant decrease in the E/I ratio compared to control littermates (P6 n=5, P15 n=5, 2-way ANOVA). C) Coronal sections of dLGN dyed with Nissl. D) Nissls staining

shows that the density of cells is comparable in the dLGN of Ctrl and Th^{Kir} animals (P2 n=7, P6 n=7, P15 n=5, 2-way ANOVA, representing the mean and the SEM. Scale bar = 100µm.

To determine whether the changes we were observing were driven by the embryonic or the postnatal perturbation of the thalamic activity, we decided to use the SertCre:Kir mouse model. These animals overexpress the Kir2.1 potassium channel in the thalamus gradually, in accordance with the expression of the serotonin transporter in thalamic relay neurons. As a result, and as a difference with the Th^{Kir} model, the SertCre:Kir mice overexpress the Kir2.1 channel from E17.5 (in the VP) and progressively cover all thalamic nuclei at postnatal stages (Antón-Bolaños, 2019). In the visual thalamus, its expression begins at E18.5 in caudal regions of the nucleus, and by P4 it covers the whole dLGN. We observed that, like in Th^{Kir} mutants, the area of the dLGN did not grow in SertCre:Kir animals compared to controls from P2 (**Fig 7B**). Furthermore, SertCre:Kir animals showed a significant increase in the density of INs at the end of the second postnatal week (**Fig 7C**), later than in the Th^{Kir} model, most likely due to the small area, since the number of INs did not change between SertCre:Kir and control littermates (**Fig 7D**). Similarly, dLGN INs were widely distributed throughout the nucleus (**Fig 7E**).



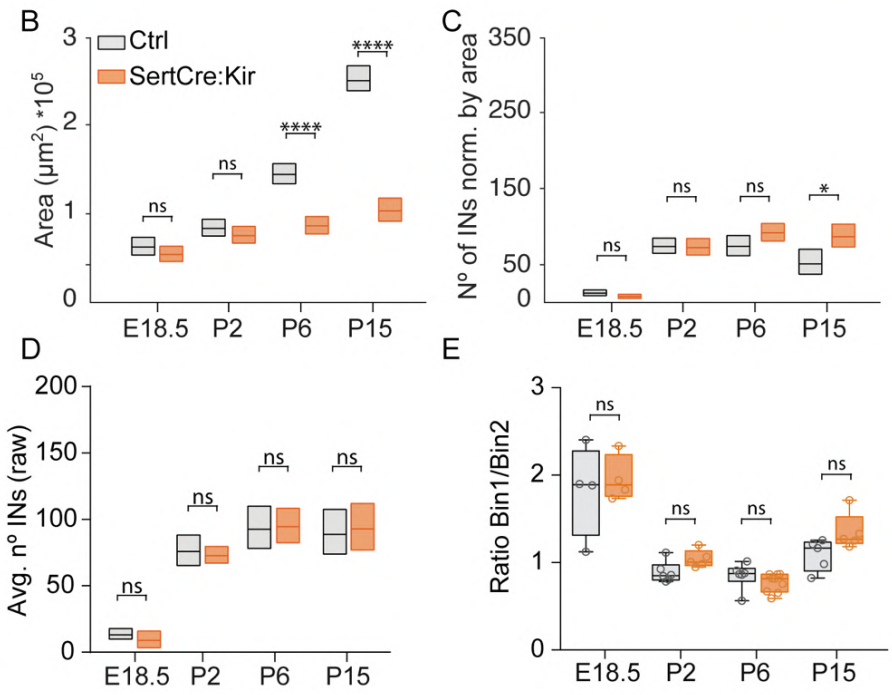


Figure 7. Postnatal ablation of thalamic activity does not affect dLGN interneurons as much as embryonic activity. A) Coronal sections showing the distribution of thalamic interneurons at different time points. B) Graph showing the differences in the area of the dLGN between Ctrl and SertCreKir animals (GLM Gamma distribution). C) The density of interneurons is significantly increased in SertCreKir littermates at the end of the second postnatal week (GLM Negative Binomial). D) Graph showing the average total number of interneurons per section of dLGN at different developmental stages in Ctrl and SertCreKir littermates (GLM Negative Binomial). E) Interneurons distribute similarly in Ctrl and SertCreKir animals (2-way ANOVA). E18.5 n=4, P2 n=7, P6 n=7, P15 n=5. B, C, and D represent the mean and the confidence intervals. E represents the mean and the SEM. Scale bars = 100µm.

When we plot the number of interneurons versus the area of the dLGN in EmbBE, Th^{Kir} and SertCre:Kir animal models, we observe that the animals overexpressing Kir2.1 – Th^{Kir} and SertCre:Kir – follow a different tendency in comparison to controls and EmbBE animals (**Fig 8**). Ctrl and EmbBe animals present a progressive growth in the number of interneurons and area until they reach a plateau at P6 - P15, while Th^{Kir} and SertCre:Kir animal models do not increase the area meanwhile the number of interneurons rises.

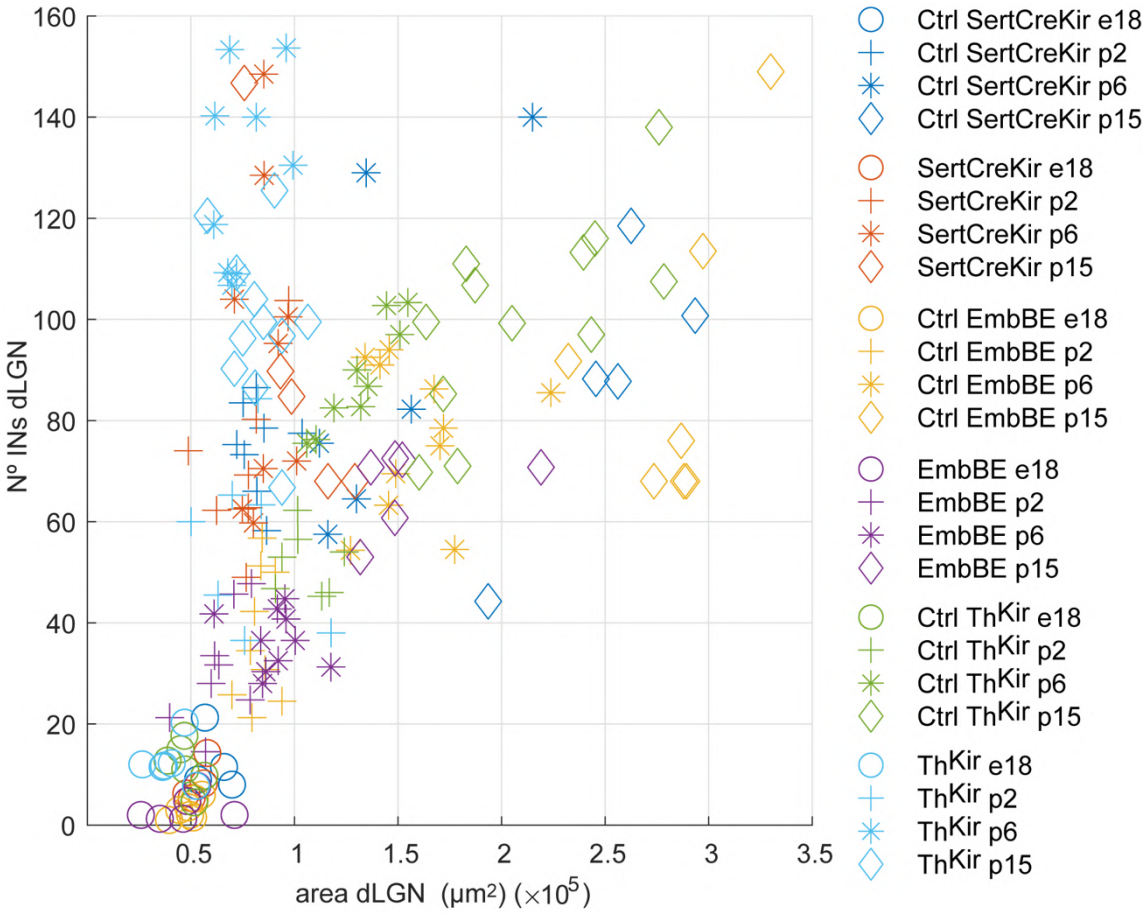


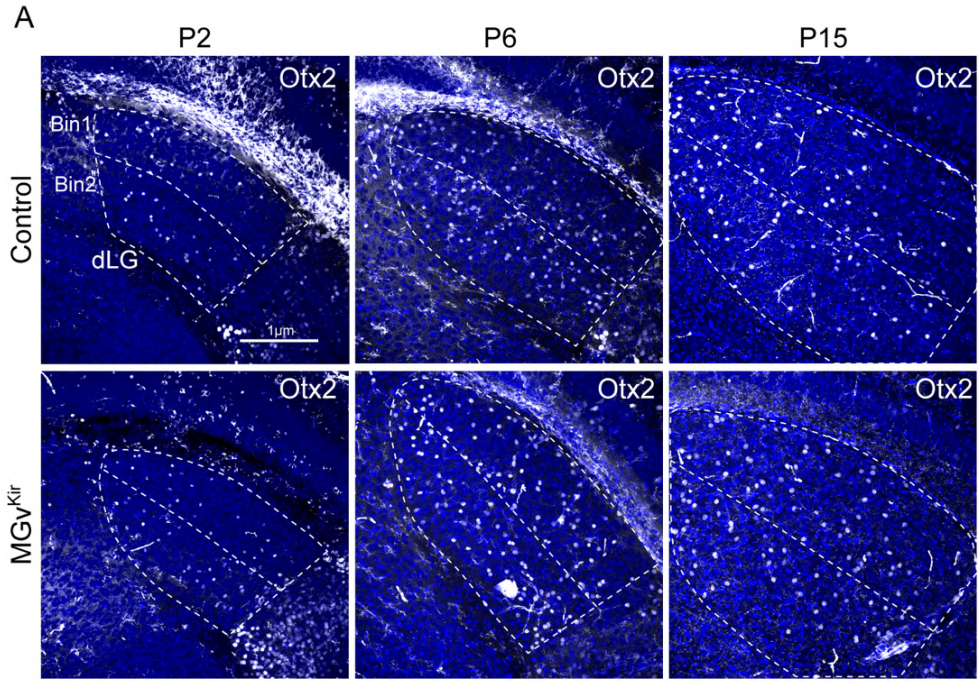
Figure 8. Temporal representation of the main changes observed in the area and the number of interneurons in the EmbBE, Th^{Kir} and SertCre:Kir models. Graph showing how the different models and their respective control littermates grow the area of the dLGN and increase the number of interneurons along time.

All these results suggest that thalamic spontaneous activity is more relevant than retinal activity for interneuron migration into the dLGN, and that it is at embryonic stages, rather than after birth, when disrupting thalamic activity causes a stronger impact into the migration of INs.

1. 1. 7. The increase in the frequency of embryonic thalamic waves does not affect dLGN interneurons

We observe a significant increase in the density of INs in the dLGN of Th^{Kir} and SertCre:Kir mouse models. Thus, we wondered whether an increase in the frequency of the waves instead of their elimination could also modify INs migration into the dLGN. A few years ago, our lab showed that the embryonic overexpression of Kir2.1 specifically in the auditory nucleus of the thalamus (MGv^{Kir}), suppresses thalamic calcium waves in this nucleus but also induces a cross-modal increase in the frequency of waves that cover the FO nuclei, including the dLGN (Moreno-Juan et al., 2017).

In this mouse model the dLGN size is not reduced, as compared to previous models. Therefore, we decided to study whether the increase in the frequency of waves in the MGv^{Kir} mouse would trigger any effect on local thalamic interneurons. However, we did not observe any significant difference neither in the area of the dLGN, the average total number of interneurons, nor the density of these inhibitory cells (**Fig 9B, 9C** and **9D**). These results indicate that, in contrast to activity suppression, the embryonic increase in the frequency of spontaneous waves does not affect the postnatal interneuron migration into the dLGN.



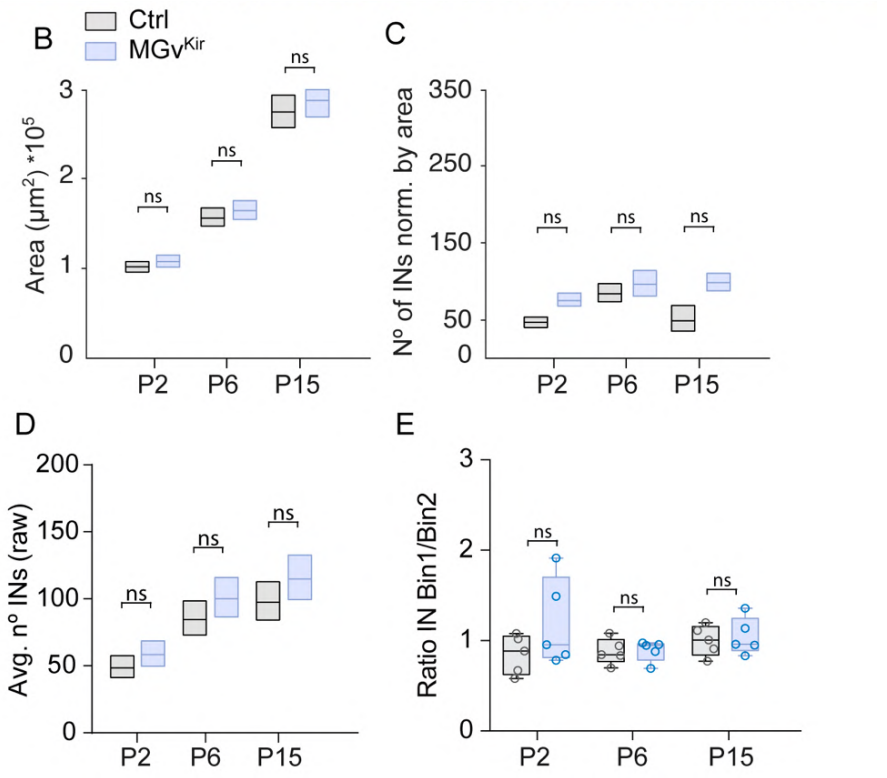


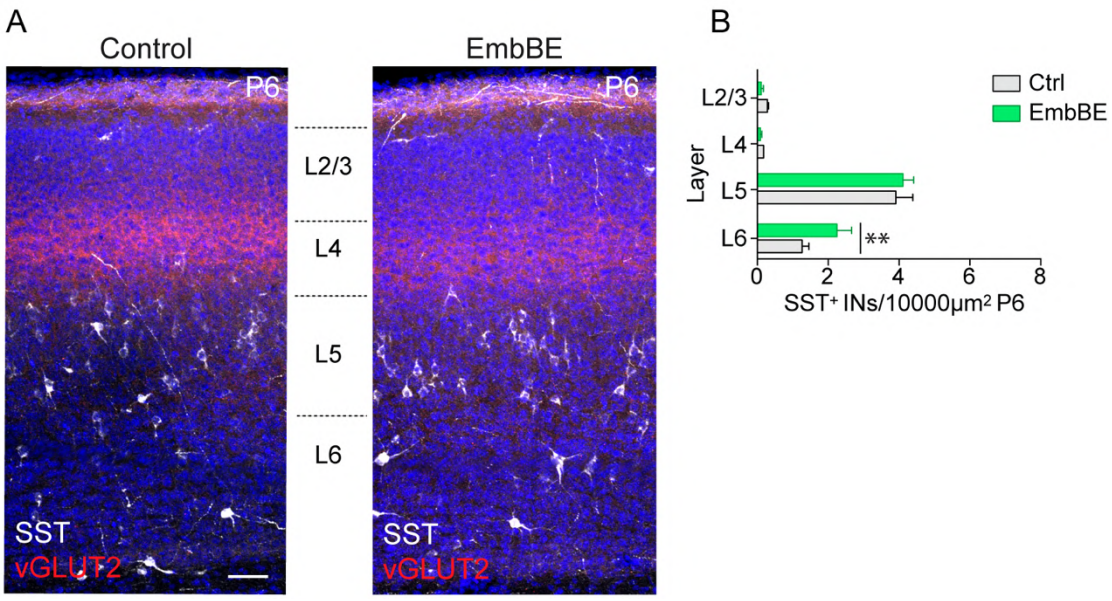
Figure 9. Increase in the frequency of waves in the dLGN does not affect the density of thalamic interneurons. A) Coronal sections showing the distribution of thalamic interneurons at different time points. B) Graph showing the differences in the area of the dLGN between Ctrl and MGv^{Kir} animals (GLM Gamma distribution). C) The density of interneurons is comparable between MGv^{Kir} and Ctrl littermates along time (GLM Negative Binomial). D) Graph showing the average total number of interneurons per section of dLGN at different developmental stages in Ctrl and MGv^{Kir} littermates (GLM Negative Binomial). E) Interneurons distribute similarly in Ctrl and MGv^{Kir} animals (2-way ANOVA). P2 n=5, P6 n= 5, P15 n= 5. B, C, and D represent the mean and the confidence intervals. E represents the mean and the SEM. Scale bar = 100µm.

1. 2 – Cortical interneurons in V1

We have seen that changes in the pattern of activity in the thalamus during embryonic development affects interneurons in the dLGN. The thalamus projects directly to the respective sensory cortices, and it has been shown that it can affect cortical development before sensory onset (Moreno-Juan et al., 2017; Antón-Bolaños et al., 2019). Hence, we wondered whether changes in peripheral and thalamic activity during embryonic development would have any effect on cortical interneurons. To do that, we decided to look at PV^{+} and SST^{+} interneurons, which are the two main populations of cortical interneurons in the primary visual cortex (V1).

1. 2. 1. Embryonic bilateral enucleation changes the proportion of SST and PV interneurons in deeper layers of V1.

First, we studied how PV^+ and SST^+ subpopulations of interneurons distribute across the cortex after embryonic bilateral enucleation. Eyes were cauterized at E14.5 and brains were collected at P6, P15 and P30, to cover from early postnatal to young adult stages. While interneurons express SST as soon as P6, however, PV starts to be expressed at ~P14 (del Rio et al., 1994). We observed that SST^+ INs increased in the L6 of P6 EmbBE mice compared to control littermates, and that this difference disappeared later at P15 **(Fig 10B** and **D)**. On the other hand, there was a significant difference in PV^+ cells at P15, also in L6, that disappeared at P30 (**Fig 10E** and **H**). Therefore, removing peripheral input at embryonic stages affected the distribution of cortical interneurons in L6, even though these changes disappear later in development.



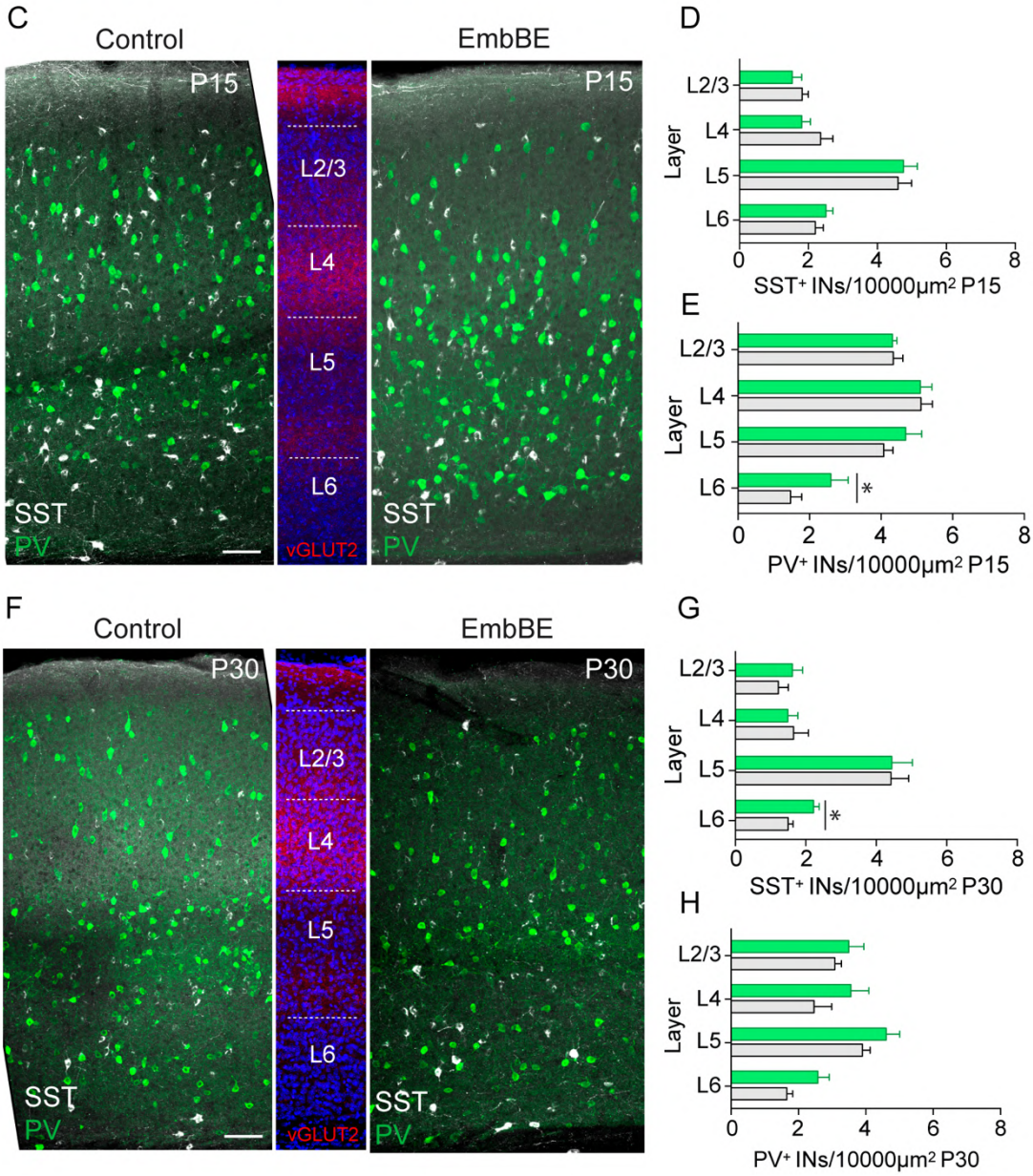
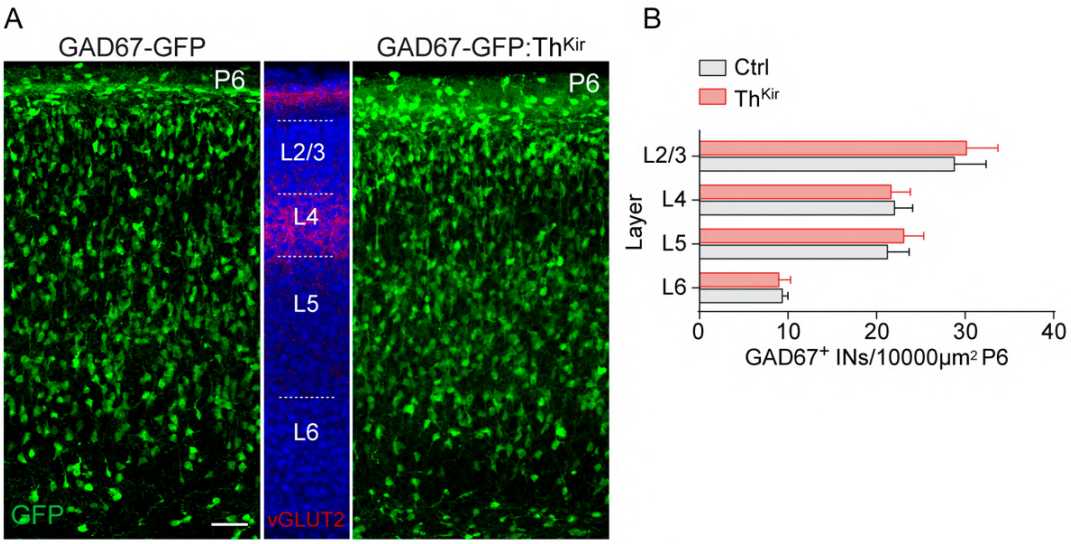


Figure 10. Embryonic bilateral enucleation changes the proportion of interneurons in L6. A) Coronal section of V1 showing the distribution of SST interneurons at P6. B) Distribution of SST interneurons at P6 in V1 of Ctrl and EmbBE animals. EmbBE present a significant increase in SST interneurons in L6 compared to Ctrl littermates. C) Coronal section of V1 showing the distribution of SST and PV interneurons at P15. D) Graph showing the distribution of SST interneurons at P15 in V1 in Ctrl and EmbBE littermates. E) Graph showing the distribution of PV interneurons at P15 in V1 in Ctrl and EmbBE littermates. There is a significant increase in PV interneurons in L6 of EmbBE littermates. F) Coronal section of V1 showing the distribution of SST and PV interneurons at P30. G) Graph showing the distribution of SST interneurons at P30 in V1 in Ctrl and EmbBE littermates. H) Graph showing the distribution of PV interneurons at P30 in V1 in Ctrl and EmbBE littermates. P6 n=5, P15 n= 5, P30 n= 5. 2-way ANOVA. Graphs represent the mean and the SEM. Scale bar = 100µm.

1. 2. 2. Lack of embryonic thalamic waves affects the distribution of PV^+ and SST^+ interneurons in V1.

When embryonic thalamic waves are experimentally removed, the cortex becomes more excitable (Anton-Bolaños et al., 2019). Therefore, we wondered whether PV^+ and SST^+ interneurons in the visual cortex were affected by changes in the thalamic activity.

Firstly, we looked at the total density of interneurons, marked by the GAD67 reporter gene. We observed a transient increase at P15, which disappeared later at P30 **(Fig 11)**. Then, looking at SST^+ interneurons, we observed that at P6 there was already a significant difference in L4 and L5, being increased in Th^{Kir} animals compared to control littermates **(Fig 12B)**. However, later at P15, this difference disappeared, and there was a similar distribution of SST^+ interneurons in both Th^{Kir} and controls that was maintained at P30 **(Fig 12D** and **G)**. On the other hand, PV^+ interneurons were not significantly different in any layer in Th^{Kir} compared to control animals at P15. Nonetheless, there was a significant difference at P30 in L4, where Th^{Kir} animals presented increased density of PV^+ interneurons compared to controls **(Fig 12E** and **H)**.



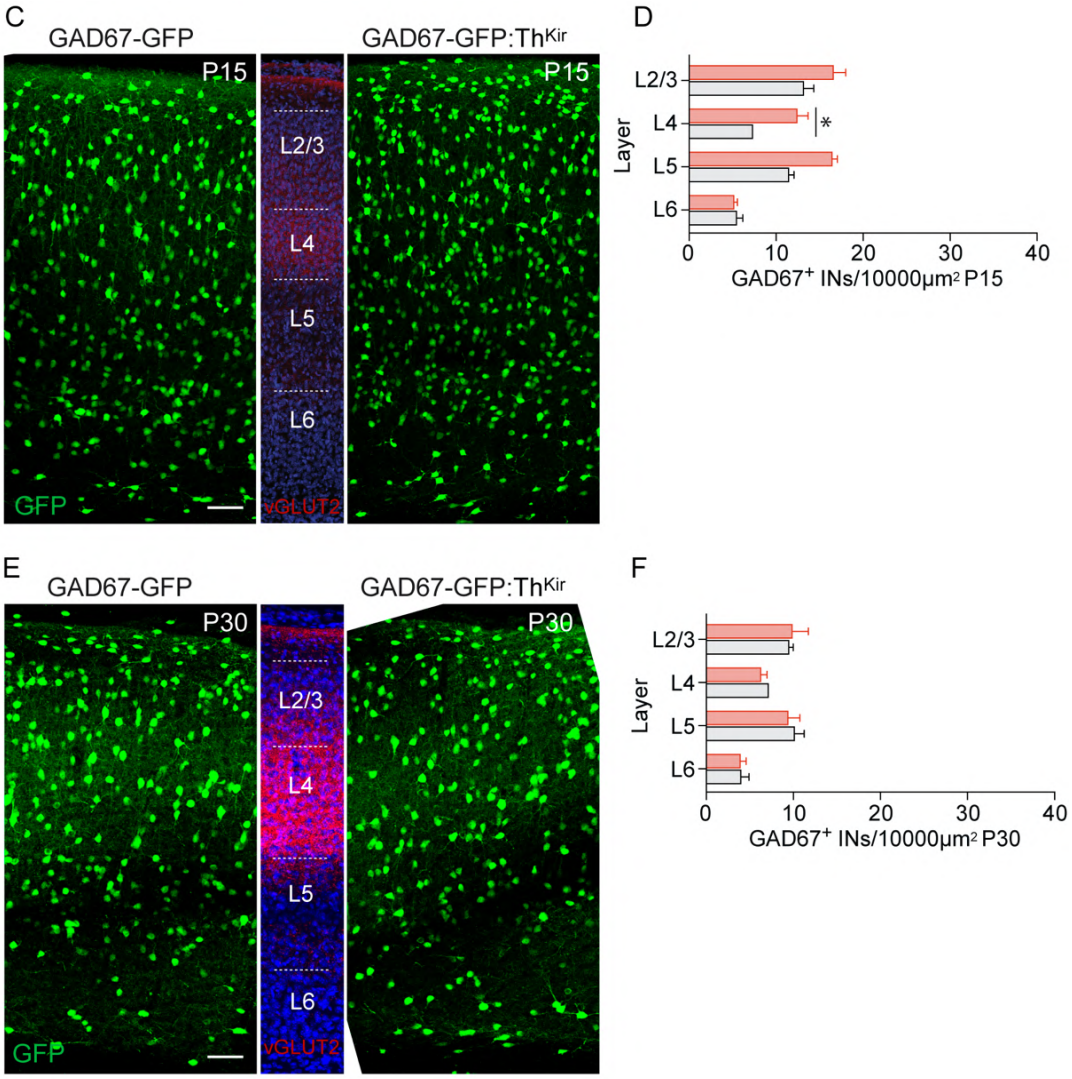


Figure 11. Ablation of embryonic spontaneuous thalamic activity does not change the overall proportion of interneurons in V1. A) Coronal section showing the distribution of GAD67 interneurons in V1 of controls (GAD67-GFP) and Th^{Kir}-GAD67-GFP littermates (GAD67-GFP:Th^{Kir}) at P6. B) Graph showing that there are no significant differences between GAD67-GFP:Th^{Kir} and control littermates at P6. C) Coronal section showing the distribution of GAD67 interneurons in V1 of controls and GAD67-GFP:Th^{Kir} littermates at P15. D) Graph showing that there are significant differences between GAD67-GFP:Th^{Kir} and control littermates in L4 at P15. E) Coronal section showing the distribution of GAD67 interneurons in V1 of controls and GAD67-GFP:Th^{Kir} littermates at P30. F) Graph showing that there are no significant differences between GAD67-GFP:Th^{Kir} and control littermates at P30. P6 n=7, P15 n=3, P30 n=3. 2-way ANOVA, showing mean and SEM. Scale bar = 100µm.

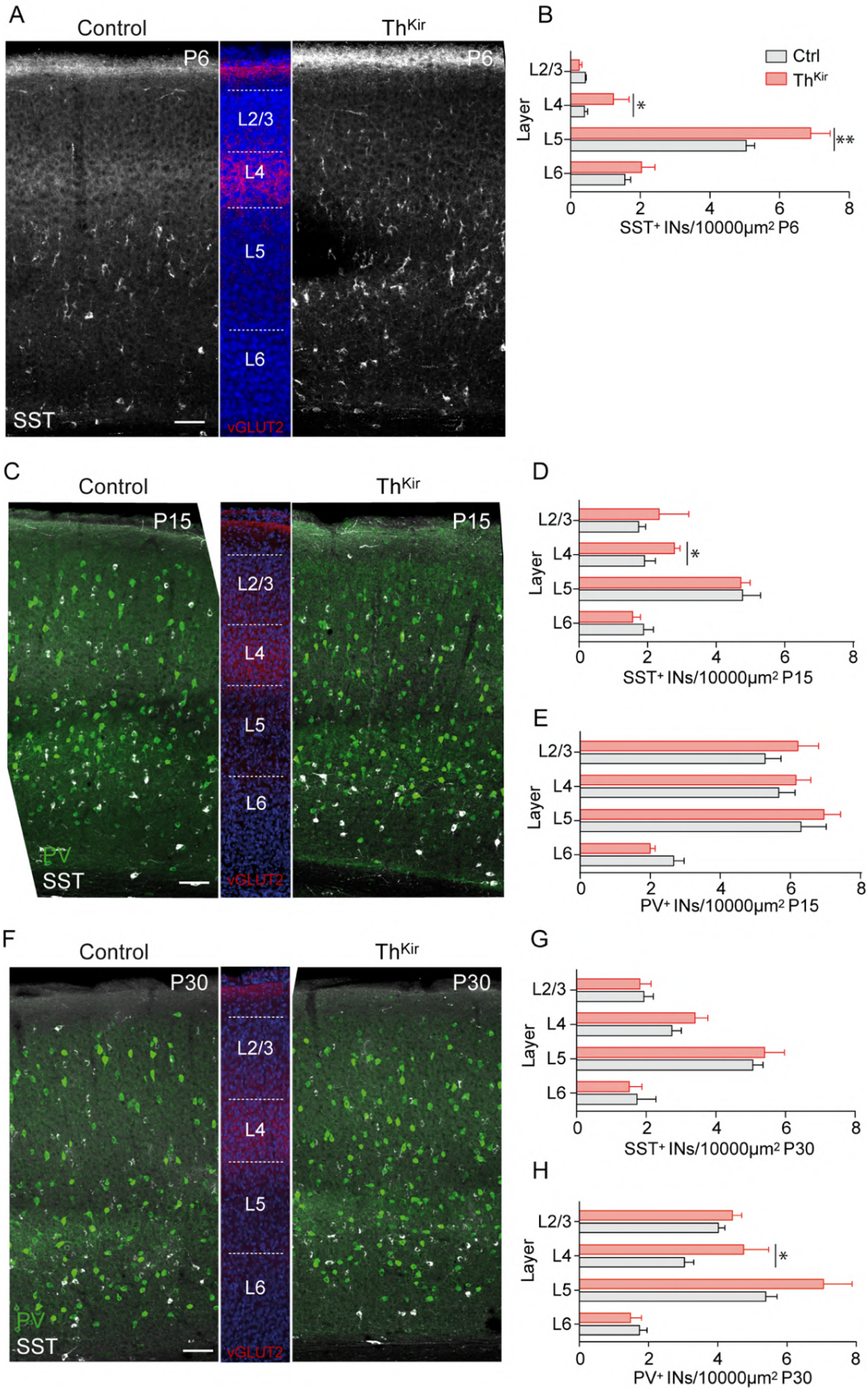


Figure 12. Elimination of embryonic spontaneous thalamic waves changes the proportion of interneurons in the postnatal V1. A) Coronal section of V1 showing the distribution of SST interneurons at P6. B) Distribution of

SST interneurons at P6 in V1 of Ctrl and Th^{Kir} animals. Th^{Kir} present a significant increase in SST interneurons in L4 and L5 compared to Ctrl littermates. C) Coronal section of V1 showing the distribution of SST and PV interneurons at P15. D) Graph showing the distribution of SST interneurons at P15 in V1 in Ctrl and Th^{Kir} littermates. E) Graph showing the distribution of PV interneurons at P15 in V1 in Ctrl and Th^{Kir} littermates. There is a significant increase in SST interneurons in L4 of Th^{Kir} littermates. F) Coronal section of V1 showing the distribution of SST and PV interneurons at P30. G) Graph showing the distribution of SST interneurons at P30 in V1 in Ctrl and Th^{Kir} littermates. H) Graph showing the distribution of PV interneurons at P30 in V1 in Ctrl and Th^{Kir} littermates. There is a significant increase in L4 PV interneurons in Th^{Kir} compared to Ctrl littermates. P6 n=7, P15 n= 6, P30 n= 5. 2-way ANOVA. Graphs represent the mean and the SEM. Scale bar = 100μm.

When we looked in an overview at how these cells behave throughout time we could observe that, although SST^+ cells begin to be more numerous in deeper layers of the cortex in Th^{Kir} compared to controls, there is a significant reduction afterwards that leads to a comparable density of SST^+ interneurons in L5 of Th^{Kir} and Ctrl animals **(Fig12B, D** and **G)**. However, SST^+ interneurons in L4 did not decrease from P6 to P15 as those in L5, and instead, they decreased later from P15 to P30 **(Fig 12D** and **G)**. Parvalbumin interneurons, however, performed differently. In the control condition, PV^+ cells covered the whole cortex and later they were removed from superficial layers, while the density was maintained throughout time in deeper layers, in accordance to their role controlling the excitation of projecting neurons (Butt et al., 2017). In Th^{Kir} mice, however, PV^+ interneurons behaved differently, this is, they were not removed from L4 at later stages **(Fig 12H)**.

These results suggest that overall, the disruption of embryonic thalamic waves did not affect the total quantity of cortical interneurons, but instead changed the layering of specific subpopulations of inhibitory cells (PV^+ and SST^+ INs) in V1.

CHAPTER 2: MICROGLIA IN THE VISUAL PATHWAY

2. 1 – Thalamic microglia

The elimination of thalamic waves, and thus, the change of the pattern of activity from mainly synchronous to asynchronous during embryonic development, modifies the expression of several genes, some of which correspond specifically to microglia (unpublished results from Lopez-Bendito's lab). As it is clear that microglia senses changes in activity (Kettenmann et al., 2011; Sipe et al., 2016), we decided to study how microglia responds to disrupting peripheral input or spontaneous thalamic waves.

2. 1. 1. Microglia in the dLGN senses changes in spontaneous thalamic activity

Unpublished results from our laboratory show that specific microglial genes are upregulated in the absence of thalamic activity during embryonic development. Thus, we decided to study how microglia cells behaved in the Th^{Kir} model. We looked in the dLGN at different time points, from embryonic development to early postnatal stages (E16.5, E18.5, P2, and P6). We decided to start looking at E16.5 because it is close to the onset of thalamic waves (Moreno-Juan et al., 2017; Antón-Bolaños et al., 2019). For each brain, we selected four rostro-caudal levels to quantify the average number and density of microglia per section. The immunofluorescence of anti-Iba 1 (Iba1), a well-known marker for microglia, showed that the density of microglia was increased in Th^{Kir} animals compared to the control littermates at different stages (**Fig 13B**).

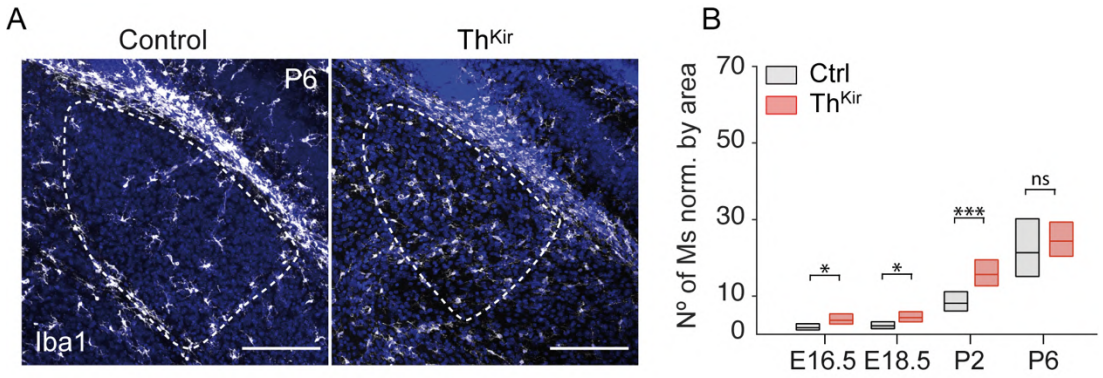


Figure 13. Th^{Kir} animals showed an increase in the density of dLGN microglia. A) Coronal sections showing the distribution of thalamic microglia at P6. B) The density of microglia is significantly increased in Th^{Kir} littermates at

early developmental stages. E16.5 n=7, E18.5 n= 7, P2 n= 7, P6 n=6; GLM Negative Binomial; B, represents the mean and the confidence intervals. Scale bar = 100μm.

Microglial morphology varies throughout time. It can be amoeboid-like with short processes and drop-like soma, or it can be highly branched, with long processes. The morphology of these cells is tightly related to their function at that specific moment (Nayak et al., 2014; Schafer and Stevens, 2015; Prinz et al., 2019). Usually, in an amoeboid form, they are either immature or more active, phagocyting cellular debris or pruning synapses, among other functions. Since we had observed an increase in the density of microglia in the Th^{Kir} mutant, we also wondered whether the morphology of microglia in this model was changed in comparison to microglia in the control condition. We used a sholl analysis to describe the morphology of microglia and found a reduction in the number and length of branches. Thus, these results suggest that the change in the pattern of thalamic spontaneous activity had an impact on dLGN microglia, increasing its density and changing its morphology (**Fig 14B** and **14C**).

As we have previously reported here when we were studying thalamic interneurons, the area of the dLGN does not grow from P2 onwards in Th^{Kir} animals. In addition, it has been recently published that dLGN in Th^{Kir} mice exhibits an increase in cell death (Moreno-Juan et al., 2022). Therefore, we wondered whether microglia in this model becomes more phagocytic compared to the control condition. To do that, we used the CD68 marker, which labels phagocytic vesicles inside microglia. We observed that CD68 increased in microglia from the dLGN of Th^{Kir} mice compared to control animals, suggesting that microglia has a more phagocytic phenotype when embryonic thalamic waves are removed (**Fig 14E** and **14F**).

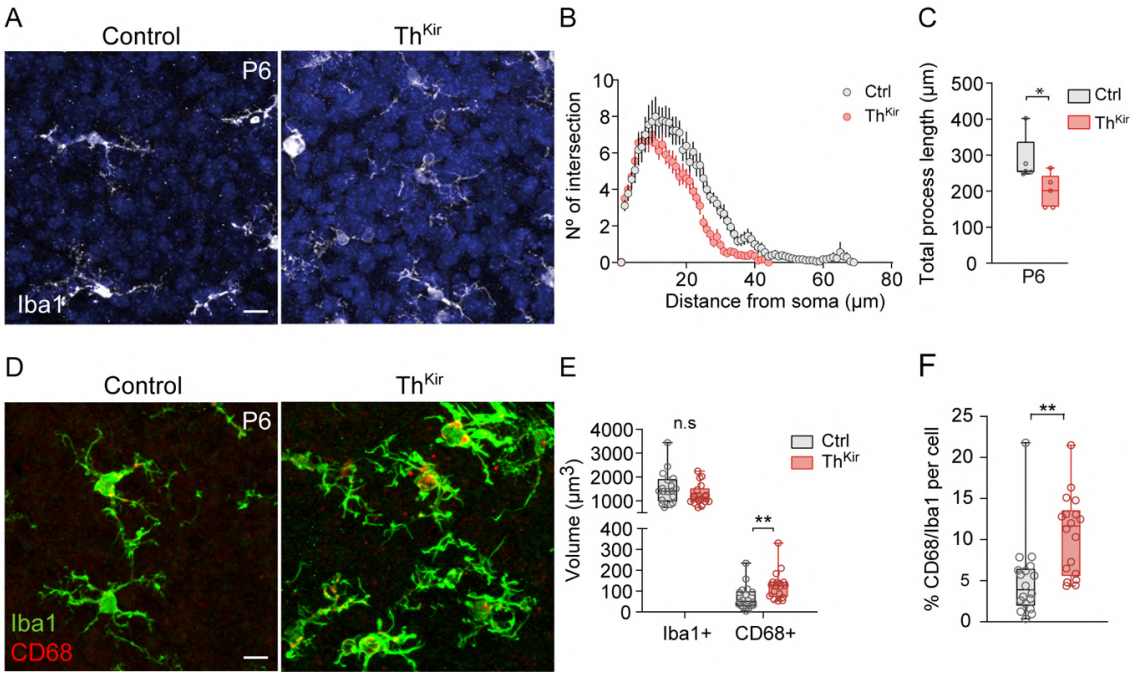


Figure 14. Microglia in the dLGN of Th^{Kir} animals is more phagocytic and presents different morphology. A) High magnification of dLGN microglia. B) Sholl analysis comparing Ctrl and Th^{Kir} microglia. C) The total process length is significantly smaller in Th^{Kir} microglia compared to Ctrl microglia. D) High magnification images of the distribution of the lysosomic marker CD68 in the dLGN. E) Graph showing a significant increase in the volume of CD68 in dLGN microglia. F) Th^{Kir} microglia has a significant increase in the proportion of CD68 inside the cells. n=18, 2-way ANOVA, representing the mean and the SEM. Scale bar = 20µm.

In order to refine the temporal window that affects microglia, we quantified the density of microglia in the SertCre:Kir model where activity becomes disrupted at later stages (perinatally) as compared to Th^{Kir} mice. In this case, we observed an increase in microglia density in the dLGN at P6, later than in Th^{Kir} animals (**Fig 15B**), suggesting that microglia is more susceptible to the elimination of embryonic thalamic waves versus the postnatal component of these activity.

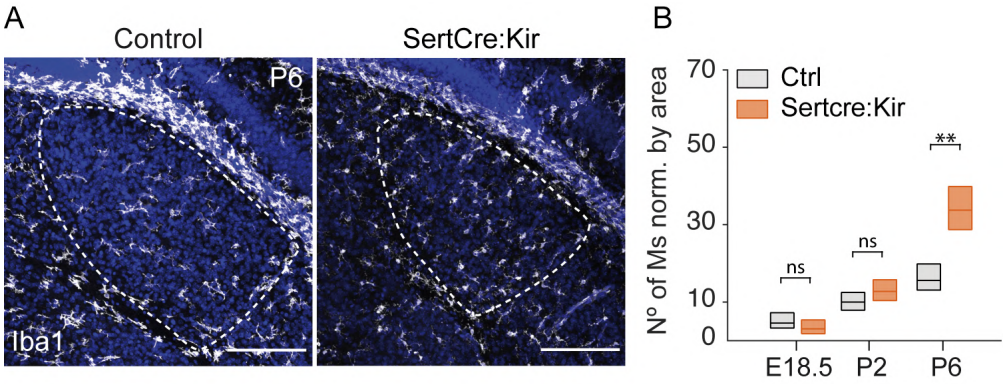


Figure 15. The effects observed in SertCre:Kir microglia appear later than in Th^{Kir} animals. A) Coronal sections showing the distribution of thalamic microglia at P6. B) The density of microglia is significantly increased in SertCreKir littermates at the end of the first postnatal week. E18.5 n=4, P2 n= 7, P6 n= 5, GLM Negative Binomial. B, represents the mean and the confidence intervals. Scale bar = 100µm.

Finally, our group has observed that there is a progressive appearance of thalamic waves when tamoxifen is diminished (unpublished). This means that the frequency of thalamic waves correlates with the dose of tamoxifen. Therefore, we were interested in studying whether microglia could sense such subtle changes. For that, we administrated tamoxifen testing two doses, 1.5mg and 2.5 mg, both lower doses than the 5mg needed to completely remove thalamic waves as in the Th^{Kir} model. Then, we analyzed microglia in the dLGN at E16.5, E18.5 and P2. We observed that the area did not change in comparison to control littermates (**Fig 16B**), but the 2.5mg of tamoxifen induced a similar phenotype to the normal dose of 5mg with increased microglial density in the dLGN at P2 (**Fig 16C**). However, when we lowered the dose to 1.5mg, the density of microglia resembled to the density found in control littermates (**Fig 16F**). Altogether, these results suggest that microglia is able to sense thalamic activity levels and/or changes in their pattern and react to them.

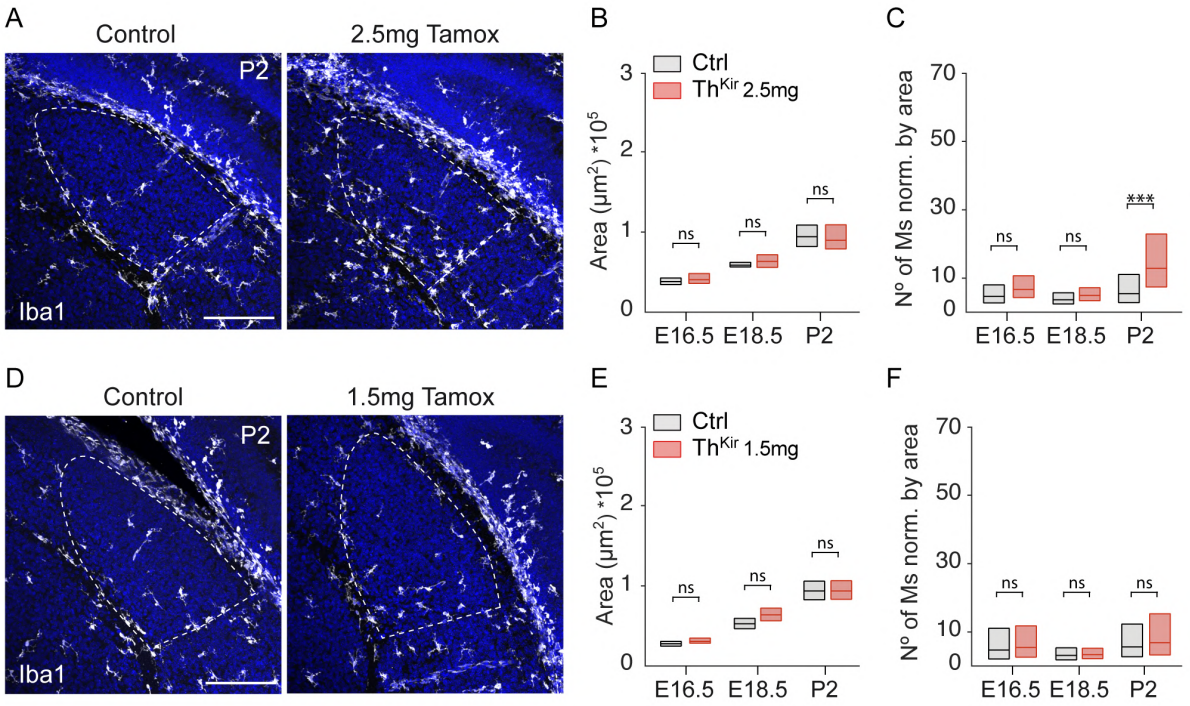


Figure 16. Microglia senses changes in the frequency of thalamic waves. A) Coronal section showing the distribution of thalamic microglia at P2 upon administration of 2.5mg of tamoxifen. B) Graph showing changes in the area of the dLGN along time administering 2.5mg of tamoxifen (GLM Gamma distribution). C) Graph showing the density of microglia in the dLGN upon administration of 2.5mg. There is a significant increase in dLGN microglia at P2 (GLM Negative Binomial). D) Coronal section showing the distribution of thalamic microglia at P2 upon administration of 1.5mg of tamoxifen. E) Graph showing changes in the area of the dLGN along time administering 2.5mg of tamoxifen (GLM Gamma distribution). F) Graph showing the density of microglia in the dLGN upon

administration of 2.5mg. The density of microglia does not change along the time (GLM Negative Binomial). 2.5mg tamox n=5; 1.5mg tamox n=5. B, C, E and F represent the mean and the confidence intervals. Scale bar = 100µm.

2. 1. 2. Thalamic microglia does not control interneuron migration into the dLGN.

There is evidence that microglia depletion or hyper-proliferation during embryonic development changes the distribution of $Lhx6^{+}$ cortical interneurons in the somatosensory cortex (S1), and decreases the inhibition of PV cells onto their targets in L4 (Squarzoni et al., 2014; Thion et al., 2019). Thus, we thought that similar effects may occur in the Th^{Kir} model. As we observed that the density of microglia increased first in the Th^{Kir} model followed by an accumulation of interneurons, we wondered whether microglia would mediate the migration of thalamic interneurons into the visual nucleus. To that end, we administered PLX3397, which binds to the microglial receptor CSF1R, in order to block microglia proliferation and survival (Elmore et al., 2014; Kuse et al., 2018). We gave PLX3397 at 500mg/kg to pregnant females from E4.5 in order to deplete microglia during the whole embryonic development. Brains were collected at P2 and P6 and the density of interneurons was quantified. We first checked that microglia was successfully depleted also at early postnatal stages (**Fig 17C**). However, we did not observe changes in interneuron density between control littermates and those fed with PLX3397 (**Fig 17D**). Therefore, it seems that microglia is not involved in the migration of interneurons into the dLGN.

We were also interested in determining whether the increase in INs density found in the dLGN of Th^{Kir} mice did depend on microglia. To that end, we fed Th^{Kir} animals with PLX3397 from E4.5 and activated the Kir.2.1 over-expression by the administration of 5mg of tamoxifen at E10.5. Unfortunately, we realized that both treatments were deleterious for the embryos and we could not test this hypothesis.

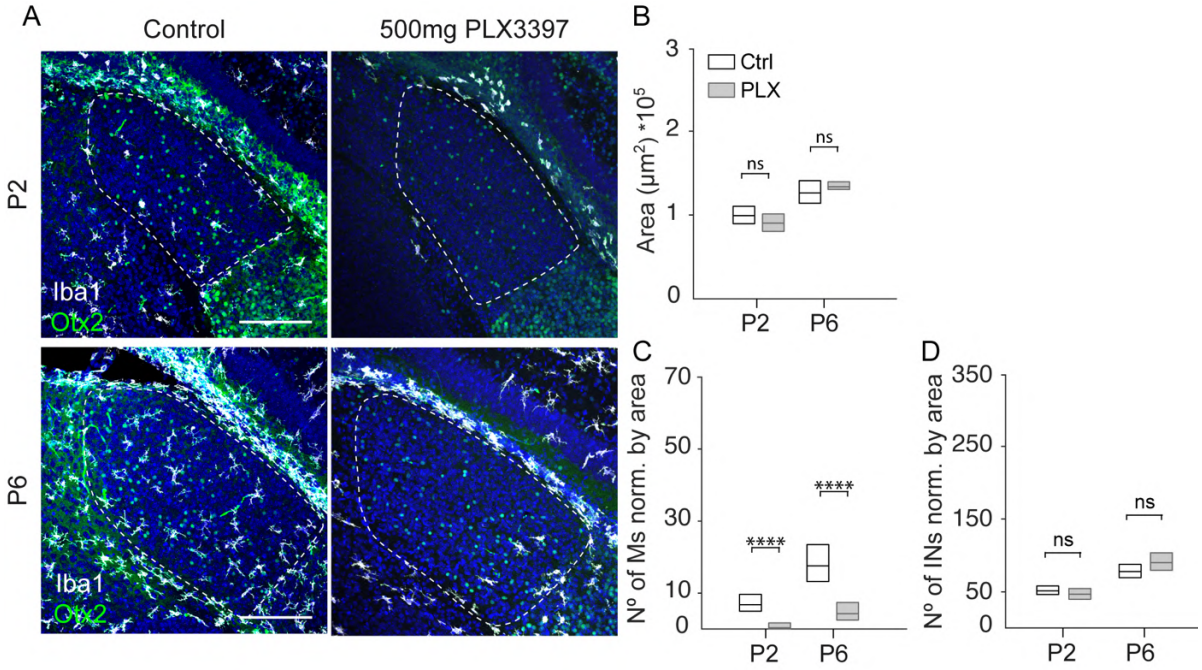


Figure 17. Microglia depletion during development does not affect thalamic interneuron. A) Coronal section showing the distribution of thalamic microglia (Iba1) and interneurons (Otx2) at P2 and P6 upon administration of PLX3397. B) Graph showing that there are no significant changes in the area between PLX3397-treated and control mice (GLM Gamma distribution). C) Graph showing no significant differences in the density of interneurons in the dLGN upon administration of PLX3397 (GLM Negative Binomial). D) Graph showing that PLX3397 depletes the brain from microglia (GLM Negative Binomial). n=5. B, C, and D represent the mean and the confidence intervals Scale bar = 100µm.

2. 2. 3. Perturbing retinal activity does not affect dLGN microglia

Embryonic bilateral enucleation changes the frequency and duration of thalamic waves (Moreno-Juan et al., 2022). Thus, we also wondered whether changes in retinal activity would affect the density or distribution of thalamic microglia in the dLGN, as we had observed in the Th^{Kir} model. To that end, we first quantified the density of microglia in the dLGN of EmbBE, and we observed an increase in the density of microglia at embryonic stages, that disappeared later at P2 and P6 (**Fig 18B**). Then, we decided to study how microglia would react upon removal of type I or type II retinal waves specifically, injecting Cbx or Epib, respectively. When we injected Cbx 10mM at P0 and P1, we did not see any significant changes in microglial cell density at P2 and P6, and the same happened after injecting Epib at P3 and P4 (**Fig 18D** and **18F**).

Therefore, perturbing retinal activity using different strategies does not affect microglia density in the dLGN.

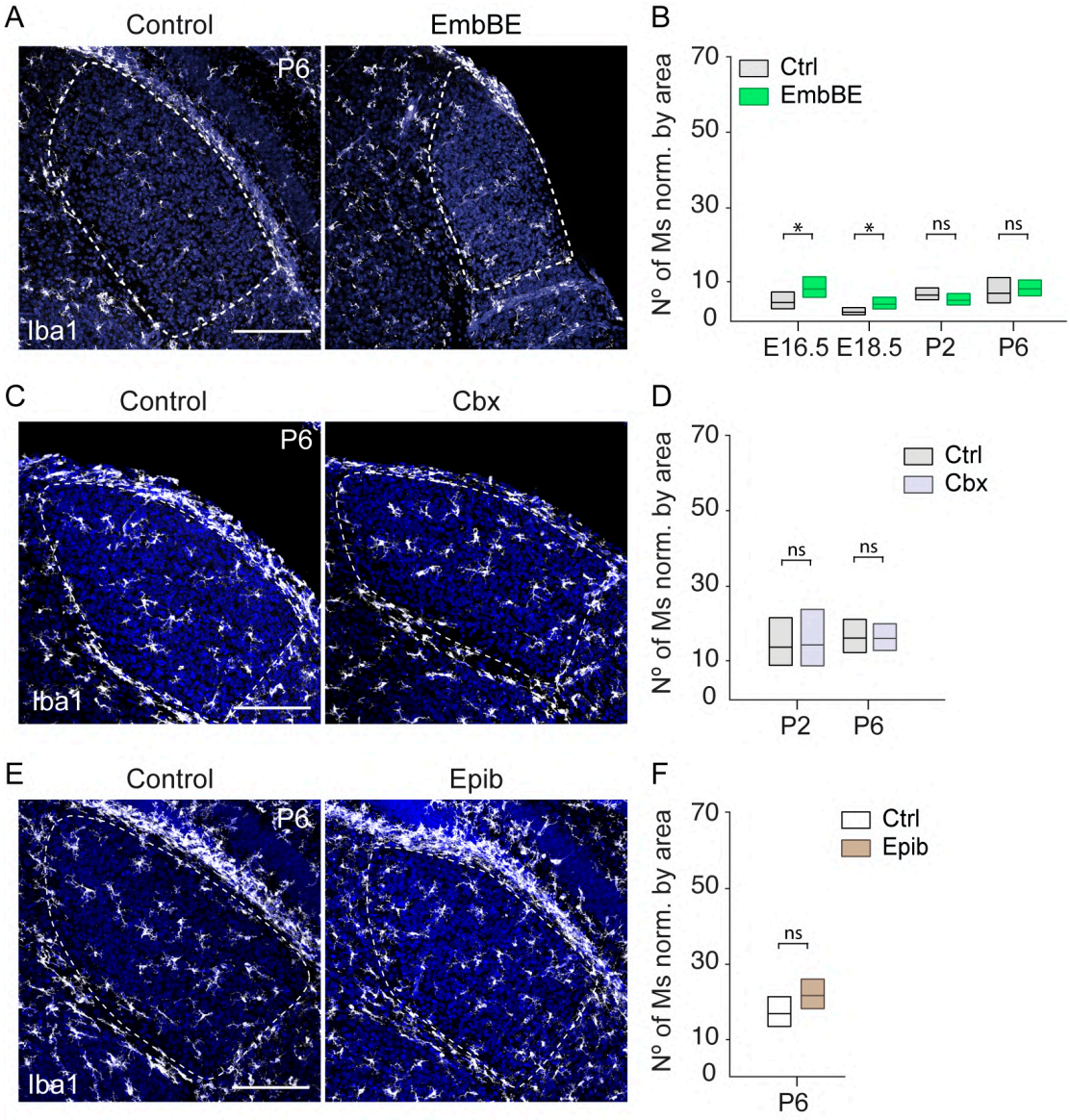


Figure 18. Perturbation of retinal activity does not affect thalamic microglia. A) Coronal section showing the distribution of thalamic microglia (Iba1) in EmbBE and control mice. B) Graph showing significant differences in the density of microglia at embryonic stages in EmbBE mice (GLM Negative Binomial). C) Coronal section showing the distribution of thalamic microglia in Cbx-injected and Saline-injected mice. D) Graph showing that there are no differences in the density of microglia between Cbx and control littermates (GLM Negative Binomial). E) Coronal section showing the distribution of thalamic microglia in Epi-injected and Saline-injected mice. F) Graph showing that there are no differences in the density of microglia between Epib and control littermates (GLM Negative Binomial). EmbBE: E16.5 n=7, E18.5 n=7, P2 n=8, P6 n=9; Cbx: P2 n=5, P6 n=5; Epib: P6 n=5. B, D and F represent the mean and the confidence intervals Scale bar = 100µm.

2. 2 – Cortical microglia

2. 2. 1. Lack of embryonic thalamic waves produces a reduction in the density of cortical microglia in V1

Since we had observed that in the thalamus the disruption of patterned thalamic activity increased the density of microglia, we wondered whether cortical microglia were also affected. We did not find changes in the density of microglia in any of the layers of Th^{Kir} mice at P6 (**Fig 19B**). However, at P10 we observed that the density of microglia was significantly decreased in all the layers in Th^{Kir} mice compared to control littermates (**Fig 20B**). Then, we decided to look at the morphology of cortical microglia. For this analysis, microglia in L5 was selected. The sholl analysis revealed that Th^{Kir} microglia had less branched processes and shorter arborization compared to control cells (**Fig 20C-F**).

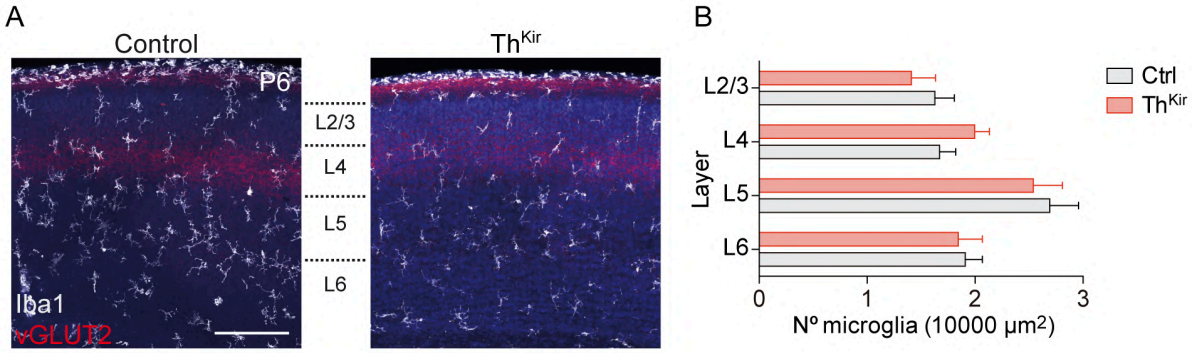


Figure 19. Lack of embryonic thalamic waves does not induce changes in the density of V1 microglia before P10. A) Coronal section showing the distribution of cortical microglia in V1 in Th^{Kir} and control littermates at P6. B) Graph showing significant differences in the density of microglia in V1 in ThKir animals compared to controls. 2-way ANOVA, n=8. Graph showing mean and SEM. Scale bar = 100µm.

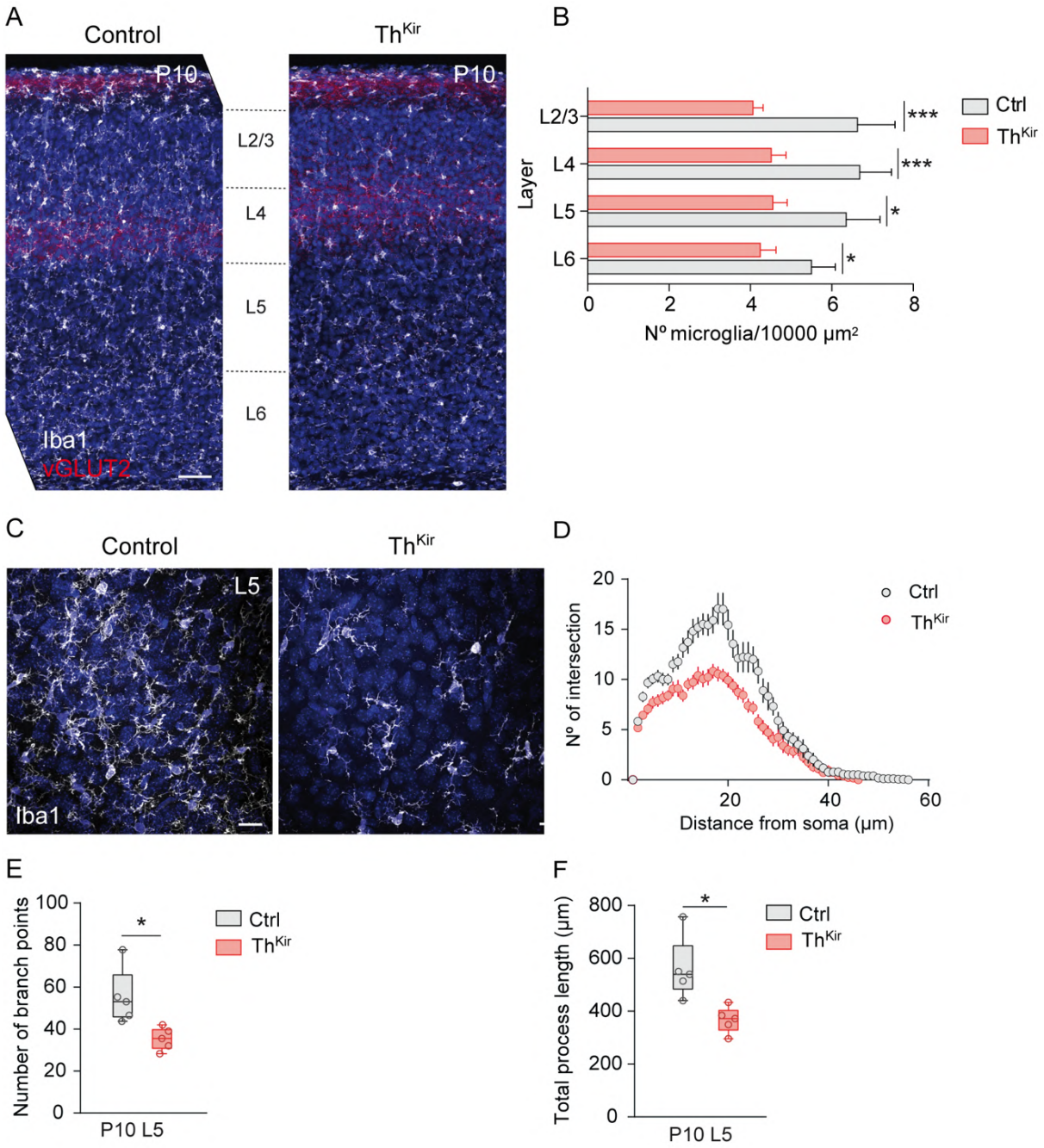


Figure 20. Lack of embryonic thalamic waves induces a reduction in the density of microglia in V1. A) Coronal section showing the distribution of cortical microglia in V1 in Th^{Kir} and control littermates at P10. Scale bar = 100µm. B) Graph showing significant differences in the density of microglia in V1 in Th^{Kir} animals compared to controls. 2-way ANOVA, mean and SEM, n=6. C) Coronal section showing high-magnification images of L5 microglia. Scale bar = 20µm. D) Sholl analysis comparing Ctrl and Th^{Kir} V1 microglia. E) Graph showing a significant reduction in the number of branch points of Th^{Kir} microglia compared to control animals (t-test, mean and SEM). F) Graph showing a significant reduction in the total process length of Th^{Kir} microglia compared to control animals (t-test, mean and SEM), n= 5.

2. 2. 2. Lack of retinal input does not affect microglia distribution in V1

We also wondered whether the disruption of retinal input from very early in development would have any impact on cortical microglia. To do that, we analyzed this population of cells directly at P10 in the bilateral enucleated model. We did not observe differences between EmbBE and control animals. Therefore, these results suggest that intrinsic thalamic activity has a bigger effect on cortical microglia than peripheral activity.

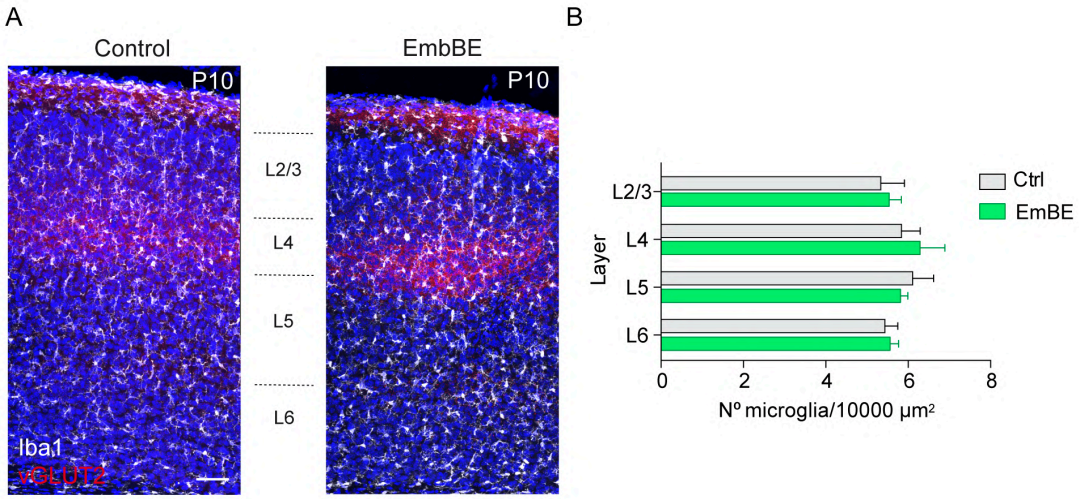
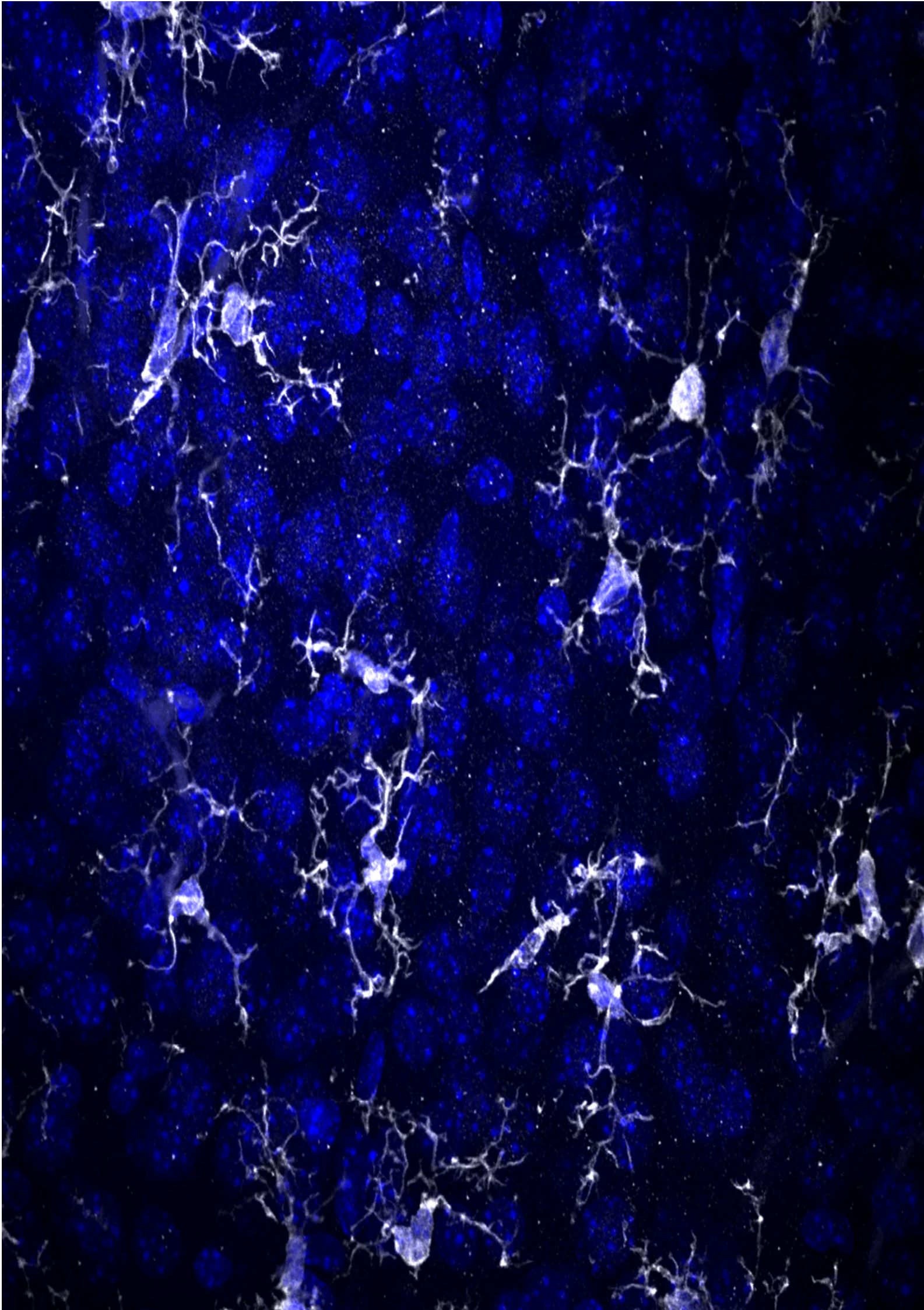
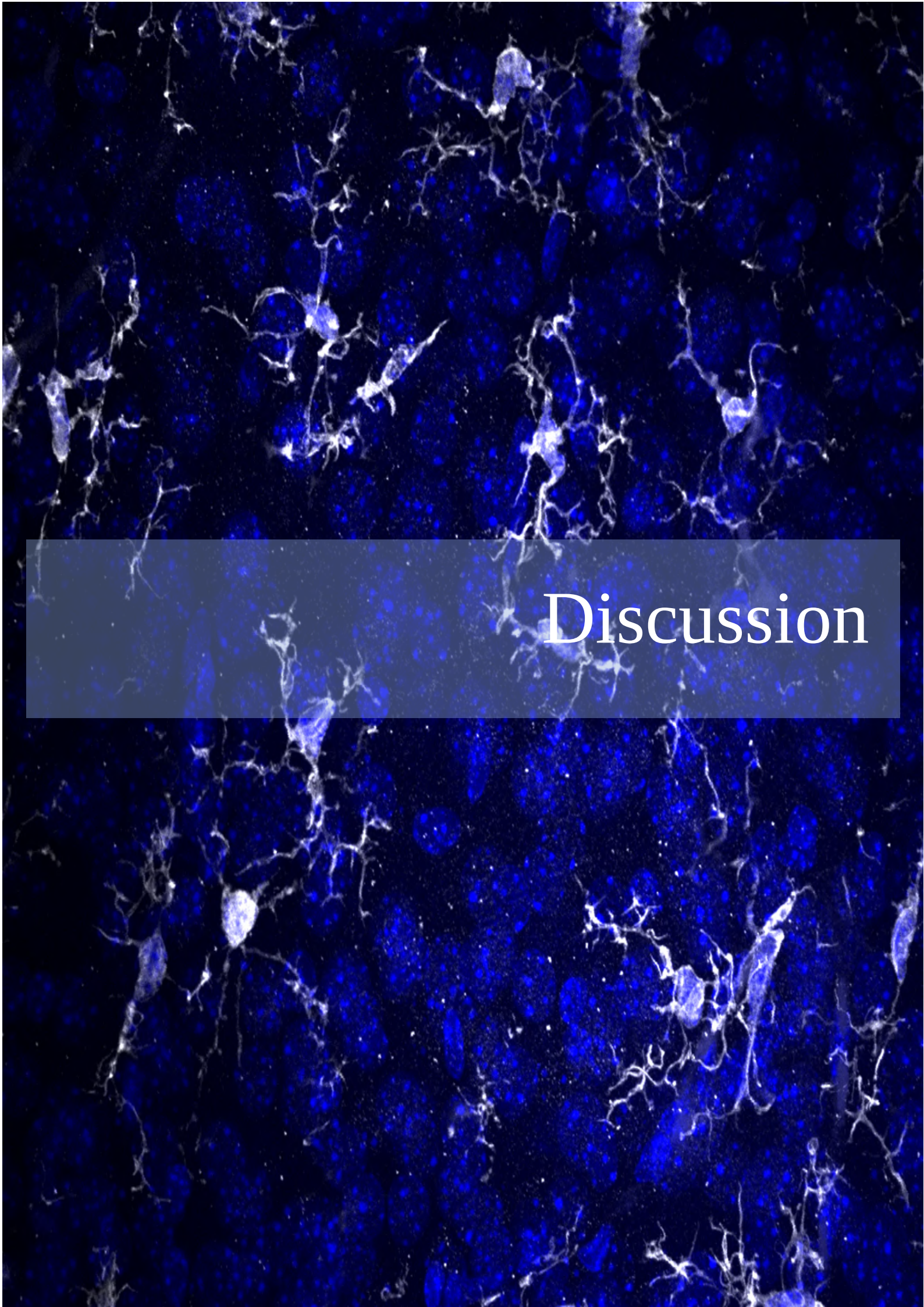


Figure 19. Lack of retinal input does not affect cortical microglia in V1. A) Coronal section showing the distribution of cortical microglia in V1 in EmbBE and control littermates at P10. B) Graph showing no significant differences in the density of microglia in V1 in EmbBE animals compared to controls. 2-way ANOVA, n=4. Scale bar = 100µm.





Discussion

DISCUSSION

The thalamus is a key element in the sensory pathway: it is in charge of receiving sensory inputs and has a direct connectivity with the cortex (Petersen, 2007; Huberman et al., 2008; Tsukano et al., 2017). In the thalamus, excitatory "relay" neurons convey the information into the sensory. However, there is also a small population of local inhibitory interneurons that also plays an important role in the processing of sensory information (Hirsch et al., 2015).

The thalamus is formed by a number of nuclei with different functions. In small mammals, such as the mouse, the visual nucleus dLGN has most of the population of local interneurons. However, interneurons are found throughout the whole thalamus in higher mammals. It has been recently described that the thalamus presents waves of spontaneous activity during embryonic development that are necessary for the correct connectivity between the thalamocortical system and the sensory cortices (Moreno-Juan et al., 2017; Antón-Bolaños et al., 2019). However, the visual pathway starts already in the retina, where there are bursts of spontaneous activity generated also during development (Torborg and Feller, 2005). Therefore, we found interesting to understand to what extent peripheral and thalamic activities were important in the migration and integration of thalamic interneurons.

During this project, we first focused on how interneuron respond to changes in peripheral or central thalamic activity. In addition, we have also studied the changes in microglial cells, the immunity cells in the brain. In sum, our results point out to the importance of neuronal activity in the organization of the nervous system and all of its different cell types, not only neurons.

In the following paragraphs I will proceed to discuss the results obtained.

Are thalamic interneurons more affected by changes in peripheral or central activity?

During the last few years there has been an increase on the interest about thalamic interneurons, especially on their morphology, hodology, and the region of birth. Several groups have suggested that while migrating, immature local thalamic interneurons of the visual nucleus dLGN respond to changes in retinal activity, which affects their migratory route into the nucleus (Golding et al., 2014; Su et al., 2019; Charalambakis et al., 2019). However, we wanted to decipher whether changes in intrinsic thalamic activity would also affect thalamic interneurons.

To that end, we firstly confirmed whether thalamic interneurons do respond to changes in peripheral activity, as it had been published. For that we used the EmbBE model, in which both eyes are removed embryonically and therefore, the retino-thalamic axons do not grow. According to previous findings, we observed that the distribution of interneurons in the dLGN was changed, gathering in the upper tiers of the nucleus. However, we also observed a significant reduction of the total number of interneurons at P6, which was translated into a reduction of interneuron density at this age. This is not in controversy with previous published results, since we have analyzed late embryonic and early postnatal stages (E18.5, P2, P6, and P15) instead of focusing on young adult animals (Golding et al., 2014). Interestingly, our results are in accordance to what has been recently published in *Math5*$^{-/-}$ mice, a model that presents an overall reduction of thalamic interneurons in the dLGN and vLGN (Su et al., 2019; Charalambakis et al., 2019). This reduction in the number of interneurons could be due to a lower speed of migration, since interneurons keep increasing, but at a slower pace in comparison to control mice. It would be interesting to analyze the number of interneurons at adult stages in order to see whether they reach a quantity comparable to control littermates, or whether they stay in lower numbers.

Furthermore, the injection of Cbx in both eyes at P0 and P1 in order to remove type I retinal waves *in vivo* did not change the distribution nor the density of interneurons in the dLGN. However, we cannot discard that a continuous injection of Cbx over several days would be necessary to have an effect. In addition, it has been recently published that visual thalamo-cortical circuits are altered in Cbx-injected mice (Guillamón-Vivancos et al., 2022). Whether retino-thalamic axons are correct in this scenario remains to be determined.

In order to remove type II retinal waves specifically, we injected epibatidine at P3 and P4 and we did not observe any changes, in contrast to what has been previously suggested

(Golding et al., 2014). It is possible that a sustained blockade of type II retinal waves with epibatidine might be necessary in order to affect thalamic interneurons, instead of an acute injection for two days.

The combination of these results suggests that the arrival of retinal axons into the dLGN is necessary for the correct allocation of thalamic interneurons in the nucleus, more than retinal activity. It is possible that retinal axons secrete several trophic factors that interneurons might sense and help them migrate into the thalamus, which we are removing with the embryonic bilateral enucleation. Nevertheless, the ablation of retinal waves either by Cbx or Epib does not perturb the arrival of retinogeniculate projections (Guillamón-Vivancos et al., 2022) and therefore, might explain the lack of changes in the distribution of dLGN interneurons.

Finally, previous work from our lab has shown that embryonic bilateral enucleation increases the frequency and duration of thalamic thalamic waves (Moreno-Juan et al., 2017). This way, it could also be thought that the changes we see in thalamic interneurons in EmbBE animals could be related to changes in thalamic activity. This way, an increase in the frequency of thalamic waves due to the bilateral enucleation could be reducing the speed of migrating interneurons and their distribution into the dLGN. However, the results obtained with the MGv^{Kir} model, in which the frequency of thalamic waves is increased similar to the EmbBE model (Moreno-Juan et al., 2017), show that thalamic interneurons do not respond to this increase in the frequency of waves. Therefore, it is possible that the combination of the lack of retinal axons, plus the increase in the frequency of thalamic waves might be causing the reduction of interneurons at P6 in EmbBE.

Related to this, it could be interesting to study whether there are changes in thalamic spontaneous activity upon Cbx or Epib injection. This could help us understand whether ablating specifically type I or type II retinal waves has an effect on thalamic activity similar to the removal of retino-geniculate axons.

On the other hand, in order to study whether local interneurons could sense changes in thalamic activity, we used two mouse models: Th^{Kir}, in which the embryonic over-expression of the potassium channel Kir2.1 makes thalamic spontaneous waves disappear, inducing a change in the activity from synchronous to asynchronous; and SertCre:Kir, in which thalamic spontaneous activity is altered at late embryonic and early postnatal stages. In the Th^{Kir}

model, but not in the SertCre:Kir model, we observe a significant increase in the density of interneurons from P6. This change at P6 could be related with the speed of migration, i.e., in the absence of embryonic spontaneous waves, thalamic interneurons migrate faster into the dLGN. It has been already published that the frequency of calcium waves is associated with the speed of the growing TC axons in their journey towards the cortex. The reduction in the frequency of calcium waves induces the expression of Robo1, which at the same time functions as a break for these TC axons (Mire et al., 2012). Following this thought, it could be possible that in the Th^{Kir} model the calcium waves were acting as a "water dam", controlling the migratory speed of interneurons. The lack of these waves would function as opening the gates of the water dam, increasing the speed of migration of thalamic interneurons. It is also noteworthy that both models, Th^{Kir} and SertCre:Kir, present a reduction in the size of the dLGN from perinatal stages. This reduction is, surprisingly, since the nucleus is not growing throughout time, which can be observed in the graph that shows the evolution of the area at P2, P6 and P15 (**Fig 5B** and **Fig7B**). This might be caused by the overexpression of the potassium channel Kir2.1 at postnatal stages, which might induce morphological changes in the excitatory cells where it is expressed, and might also increase the proportion of cell death. It has already been published that, indeed, Th^{Kir} dLGN has increased Casp3 from P2 to P7 (Moreno-Juan et al., 2022). However, the quantity of $Casp3^{+}$ cells in the Th^{Kir} model might not explain fully the huge size difference that we observe compared to control animals. In fact, we do not know whether there are changes in the arborization and the shape of the cells that compose this thalamic visual nucleus, glia and neurons.

Th^{Kir} animals present an augmented density of interneurons in the dLGN. However, the Nissl staining at different time points suggests that there are no changes in cell density in the nucleus. If there are more interneurons and the size is smaller, what is it happening with the surrounding cells that compose the nucleus? Is the shape of the cells the same under these conditions? How does this affect the E/I balance in the circuit? It is possible that: 1) there is more cell death that we are not observing with the Casp3 immunostaining, which only labels apoptotic cells. It would be convenient to do TUNEL staining in order to study all types of cell death and see which cells are dying apart from the excitatory neurons; 2) in addition to the cell death, there is a reduction of proliferation. Preliminary experiments done with the Ki67 antibody suggest that Th^{Kir} dLGN presents a lower ratio of positive cells at P2, which is the time point at which we start seeing this difference in the size of the nucleus.

At this point in development neurons are already post-mitotic in the thalamus, so it is possible that glia cells are the ones that are not proliferating enough; 3) there are morphological changes in the cells that conform this nucleus, performing smaller dendrite trees and branches, which in combination with the higher cell death ratio, could reduce the size of the nucleus.

Looking at the results obtained with all these different animal models, in which we remove peripheral or central activity, it is feasible to think that the dLGN has a genetic program that establishes its final number of interneurons. Peripheral or central changes might modulate the speed of migration but at the same time, the system will try to reach a given number of interneurons, similar to the control conditions. Moreover, the EmbBE and Th^{Kir} models point at P6 as a key age in the establishment of the local inhibitory circuit in the dLGN, since it is the time at which we see the major changes in both models.

Nevertheless, the important outcome is the resultant density of interneurons in the dLGN, since it can be related to the E/I balance. In the models of peripheral input deprivation, such as the EmbBE, Cbx, and Epib (**Fig 2, 3** and **4**) the density of interneurons is maintained throughout the first two postnatal weeks, similar to the control condition. However, the Th^{Kir} model, and the SertCre:Kir to a lesser extent, exhibit a remarkable increased density of interneurons in the dLGN (**Fig 5** and **7**). These results indicate that the perturbation of the wave patterns, rather than the higher wave frequency, has a larger impact on thalamic interneurons.

Are cortical interneurons in V1 sensitive to changes in peripheral and thalamic activity?

During brain development, the thalamic nuclei send projections and makes connections with the respective sensory cortices. It is now well stablished that changes in the thalamus during embryonic stages can affect the correct development of the cortex (Moreno-Juan et al., 2017; Antón-Bolaños et al., 2019). We have seen that the ablation of spontaneous thalamic waves during embryonic development changes the morphology of the visual nucleus dLGN and the density of interneurons in this nucleus. In comparison, perturbation of peripheral input from the retina, did not affect the density of interneurons even though there was a significant

reduction of these cells at the end of the first postnatal week. Thus, we wondered whether the blockade of peripheral activity from the retina or spontaneous thalamic waves would produce changes in the distribution and the number of cortical interneurons in V1, focusing on the two main subpopulations, SST- and PV-expressing interneurons. We consider that the number of interneurons in V1 stabilizes around the third postnatal week (Williams et al., 2021).

Firstly, in the Th^{Kir} model, we observed a significant increase in L4 and L5 SST interneurons as soon as P6 (**Fig 11**). It is known that SST interneurons in L5 are very important during the first postnatal week for the correct development of PV cells that will integrate into the circuitry (Tuncdemir et al., 2016). In the Th^{Kir} model, it has been previously described in the lab that TCA arrive intermingled to S1 and do not refine to form the barrels (Antón-Bolaños et al., 2019). Something similar could also be occurring in the visual cortex. It might be possible that thalamic axons would arrive to V1 intermingled, and therefore this could affect the distribution of SST in L4 and L5. It is now known that there are two types of SST in L5, Martinotti cells, which inhibit pyramidal cells in L5, and non-Martinotti cells, which make connections with L4 pyramidal neurons in order to be activated (Naka et al., 2019; Nigro et al., 2018). Therefore, the potential incorrect arrival of thalamocortical axons into the cortex might affect both the number and the activation of SST cells in L4 and L5.

It has been described that the Th^{Kir} cortex is more hyperexcitable under a certain stimulus (Antón-Bolaños et al., 2019). L4 SST interneurons are known to make a disinhibitory circuit, inhibiting L4 PV interneurons and therefore, enhancing the output from the pyramidal neurons (Xu et al., 2013). Thus, the early increase in the density of SST, when the cortical connections and maps are being formed, might be related to the hyperexcitability we see in the cortex of Th^{Kir} animals: the hyperexcitability could be a consequence of the increase in the SST subpopulation in L4. On the other hand, it has also been observed that non-Martinotti cells in L4 and L5 increase their activity when the cortex enters the active state in adult mice (Muñoz et al., 2017; Pala and Petersen, 2018). Hence, it could also be an opposite effect, the hyperexcitability of the cortex could be leading to an increase in this subpopulation of cortical interneurons in these specific layers. However, it has now been described that most of the SST interneurons in mouse V1 are Martinotti cells (Scala et al., 2019), and therefore, it would be interesting to confirm whether the SST interneurons increased in the V1 of ThKir animals are Martinotti or non-Martinotti cells.

Furthermore, SST cells make connections with PV interneurons in L5 and L6 already at the end of the first postnatal week, and thus, changes in SST in these layers could affect the input onto PV cells and involve a general change in the circuit.

On the other hand, it could also be possible that the increase in this subpopulation of interneurons were associated with the role of projecting neurons found in these layers. SST interneurons could be inhibiting the projecting neurons in L5 and L6, which would affect the feedback connection to the thalamus.

Moreover, the differences observed in Th^{Kir} SST disappear at later stages. It could be feasible to think that eye opening is a key moment during development, since it is well known that visual experience is very important for the maturation of the visual system (Hensch, 2005; Hofer et al., 2009). This way, eye opening might activate a compensatory mechanism, helping SST to reach the homeostasis and be comparable to the control condition.

Finally, if we see more SST already at P6 and the total amount of GAD67 interneurons does not change in the Th^{Kir} condition, what is it happening with other populations of interneurons? Do they decrease? Is the connectivity among the subpopulations of interneurons well maintained?

PV interneurons display a different behavior compared to SST cells. In the adult mouse cortex, PV cells tend to gather in L4 and L5 (Almási et al., 2019). It is well stablished that inhibitory interneurons in the neocortex undergo programmed apoptosis, firstly in deep layers, and some days later in supragranular layers (Southwell et al., 2012; Bartolini et al., 2013; Wong and Marín, 2019). Interestingly, the lack of input from pyramidal neurons induce programmed cell death in interneurons, while those that receive the input survive and integrate into the circuit (Anastasiades et al., 2016; Wong et al., 2018). Here, in the control condition we observe that PV cells start occupying upper as well as deep layers, but they remain numerous in L5 and undergo apoptosis in L2/3 and L4 at young adult stages (P30). In contrast, in Th^{Kir} animals we observe that PV cells are abundant in L4 at P15 and later at P30. It is possible that in Th^{Kir} animals PV interneurons do not mature correctly and they do not undergo programmed cell death, remaining in high numbers at supragranular layers like the immature circuit. This could also be related to the intermingled arrival of TCAs into L4,

as previously explained, or maybe due to the increase in SST during the first postnatal week, since it has been described that L5 SST interneurons are very important during these days making connections with PV cells, an interaction that is necessary for the correct integration of PV interneurons into the circuit (Marques-Smith et al., 2016).

Strikingly, we observe that the overall density of $GAD67^+$ interneurons increases particularly at P15 in L4 in Th^{Kir} animals compared to control littermates. This could be due to the changes related to eye opening and the arrival of visual evoked input into the circuit.

On the other hand, the ablation of peripheral input and retinal activity using the EmbBE mouse model induces different results in V1 interneurons. In the EmbBE we observe changes in the interneurons from L6. EmbBE animals present an increase in SST in L6 that disappears later, and an increase in L6 PV cells at P15 which also disappears later at P30 (**Fig 10**). Cortical PV interneurons are important for the closure of the critical period; an increase in the inhibition from these cells accelerates the closure of the critical period of plasticity, and a decrease in the inhibition from these cells delays it (Fagiolini et al., 2004; Hensch et al., 2005). Moreover, it has been observed that ocular deprivation changes the onset of this critical period through the perineural nets of these PV cells (Faini et al., 2018). Therefore, we might be able to observe consequences in the critical period of these animals through the changes happening in PV interneurons. However, it is also possible that these results might be related to the feedback loop from the cortex to the thalamus right after eye opening. In addition, embryonic bilateral enucleation also affects how CTAs arrive to the dLGN; CTAs cover a bigger area of the dLGN during the first postnatal week in EmbBE animals compared to control littermates (Moreno-Juan et al., 2022). Thus, there might be a compensation in the system, i.e., CTAs arrive earlier to the dLGN covering a wider area, and the system tries to overcome this early entrance by an increased inhibition in deep layers of the cortex. Finally, it should also be considered that the EmbBE has an increased frequency of waves in the dLGN that are also prolonged in time in comparison to control littermates, and therefore, the effect we see on cortical interneurons could be due to the changes in the frequency of thalamic activity during embryonic development.

Analyzing the total $GAD67^+$ population of cells helps us to understand whether the changes we see in the distribution of specific subpopulations of interneurons are related to global changes in the whole $GAD67^+$ population. Therefore, it would have been interesting to

perform a GAD67 immunostaining at P6, P15, and P30 in the EmbBE mouse model in order to see whether the total number of cortical interneurons would change in distribution or number along time, as we have done in the Th^{Kir} model.

Finally, as it has been previously explained, interneurons require neuronal activity and the emergence of sensory experience in order to mature and integrate correctly in the circuit. In fact, GABAergic cells in the cortex work as a functional network with coordinated activity and temporal dynamics (Modol et al., 2020). It has been observed that whisker deprivation impacts the functional organization of these circuits, affecting differentially deep and superficial layers (Modol et al., 2020). Hence, something similar might be happening upon bilateral enucleation in V1.

The results obtained in the primary visual cortex of Th^{Kir} and EmbBE models suggest that the perturbation of the visual pathway both at its peripheral station (eye) and at its central station (thalamic activity) has a consequence in the development of the cortical circuit in V1, changing the distribution of the two main populations of interneurons, SST and PV, in different cortical layers: embryonic bilateral enucleation affects mainly the distribution of these cells in L6, while the removal of embryonic thalamic waves affect L4 and L5.

How does microglia sense changes in retinal and thalamic activity?

Recently, there has been an increasing interest on studying microglia. Now, it is widely accepted that they have many different functions: they are capable of sensing changes in neuronal activity, they interact with neurons, they are involved in the correct wiring of the brain during development, they are in charge of synapse pruning, phagocytosis of debris, and they are monitoring the system to maintain its good function (Nayak et al., 2014; Thion et al., 2014; Favuzzi et al., 2021; Umpierre et al., 2020; Thion et al., 2019). Therefore, given that we are affecting thalamic activity during development when we perturb retinal input and thalamic activity itself, we wondered whether microglia would sense these changes. We found surprising that microglial density was not changing in the dLGN upon bilateral embryonic enucleation, Cbx, and Epib injections. However, we did see an increase in microglial density in the Th^{Kir} model from early in embryonic development. This increase was accompanied by changes in microglial morphology. Since the data in the Th^{Kir} model suggests that there are

changes in microglia before the effect we observe in interneurons, and in addition, it has been published that microglia is involved in interneuron location in the cortex (Thion et al., 2014), we thought that it was likely that similar effects could be observed in the thalamus. However, the depletion of microglia did not show any defect on thalamic interneurons, suggesting that the effect we saw in microglia was independent of that observed in interneurons.

Interestingly, we could observe that the depletion of microglia plus the blockade of spontaneous thalamic waves during embryonic development was deleterious. If we consider our previous results, we see that microglia reacts to the absence of spontaneous thalamic waves and all the morphological changes related to it increasing its density, most likely trying to maintain the homeostasis in the system. Therefore, it could be possible that the ablation of microglia during development was too much for the system, which would not be able to counteract the changes in thalamic activity and morphology. Thus, this suggests that, indeed, microglia might be involved in some homeostatic processes during brain embryonic development.

In the Th^{Kir} model microglial cells suffer morphological changes. Under this condition, their arborization and their total length of the processes is smaller in dLGN microglia. It is well described that morphological variations are related to microglial function (reviewed in Nayak et al., 2014). The changes we observe in microglia at P6 in the dLGN of the Th^{Kir} model might be related to the function they are performing at that moment. In fact, during these early postnatal stages there are morphological changes occurring in the dLGN itself, and microglia could be more active at that moment, maintaining the system. On the other hand, during embryonic stages, the dLGN does not present morphological alterations in the Th^{Kir} model compared to the control condition. Therefore, the increase in the density of microglia this early during development could be related to the changes in the pattern of spontaneous activity. This way, it could be suggested that microglia is taking two different functions at different developmental times in the Th^{Kir} model: at embryonic stages microglia would respond to the lack of spontaneous waves of activity and therefore the change in the pattern of spontaneous activity, while during the first postnatal week it would be responding to the morphological changes occurring in the dLGN. Moreover, at the end of the second postnatal week, we observe that the density of microglia stabilizes and is comparable to the control

condition, which starts to be anticipated at P6. Thus, it is possible that after the first postnatal week the system reaches the homeostasis and microglia returns to a surveillance state.

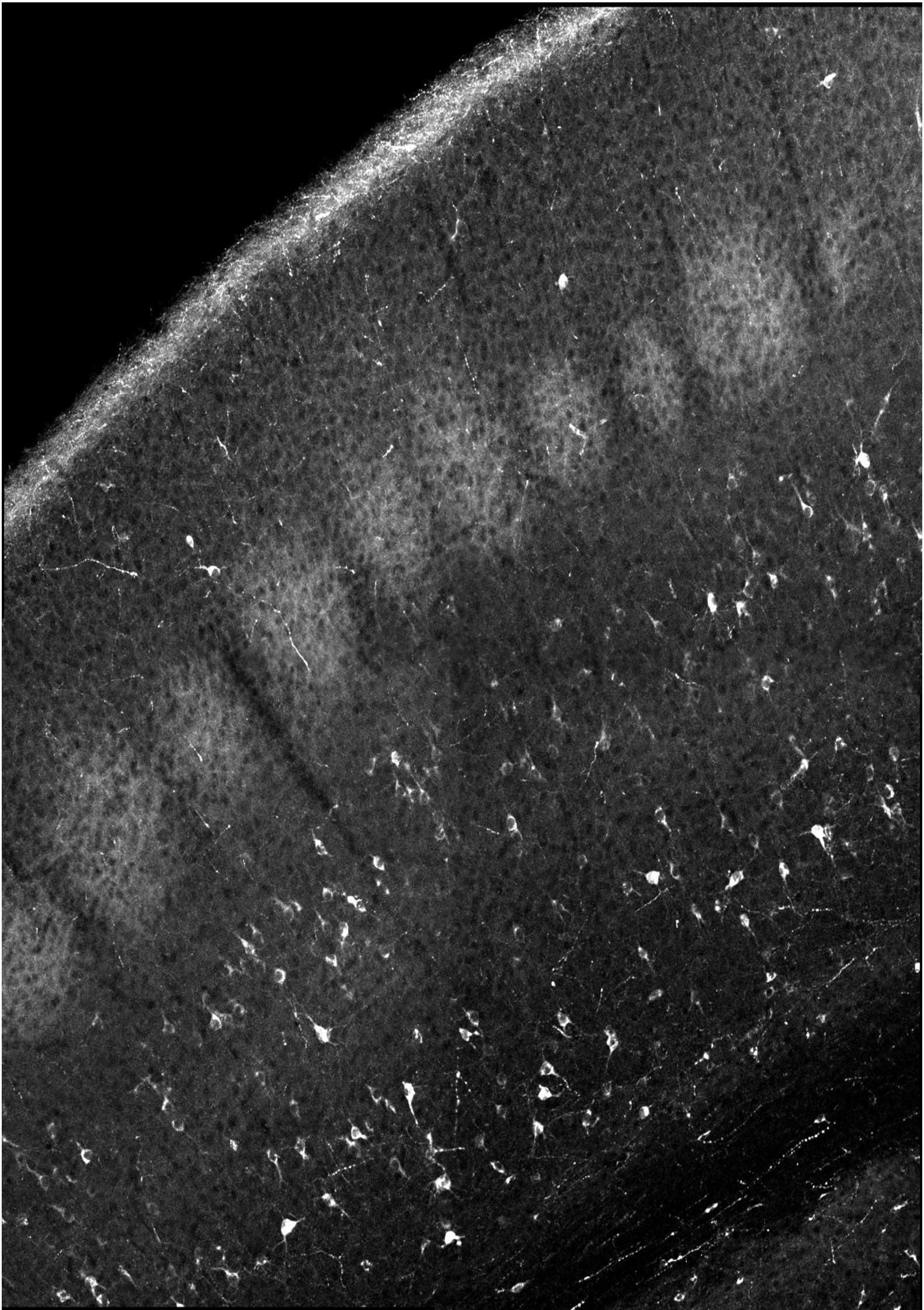
On the other hand, embryonic bilateral enucleation affects microglia only at embryonic stages, and neither the injection of Cbx or Epib changes microglial density in the dLGN. It is plausible to think that the increase in microglia density at embryonic stages in EmbBE is related to the lack of retinal afferents reaching the dLGN, and the subsequent cell death in the nucleus due to the lack of input. In addition, EmbBE animals present an increased frequency of thalamic waves and these waves are present in the thalamus until around P2, in contrast to the control condition in which thalamic waves disappear at around P0. The fact that we see less changes in the density of microglia upon peripheral activity deprivation (EmbBE, Cbx and Epib models) compared to the results obtained in Th^{Kir} animals suggests that microglia is more sensitive to changes in the pattern of activity (no waves) rather than in the frequency (waves with increased frequency).

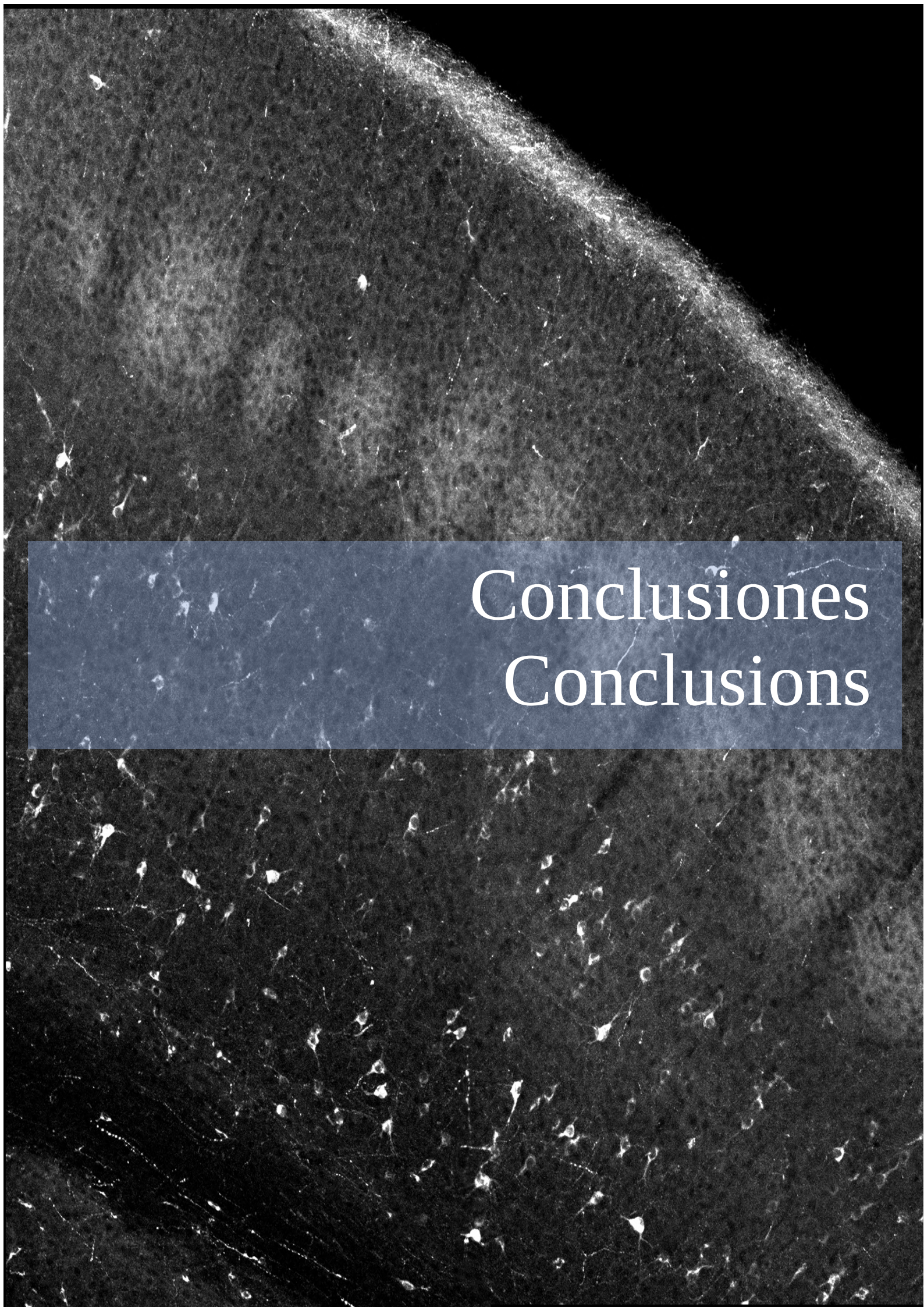
Following this thought, we were interested in studying to what extent microglia senses the changes in the pattern of activity in the thalamus. To that end, we did a titration experiment in which we gave lower doses of tamoxifen to the pregnant Th^{Kir} females. Previous findings in the lab have shown that lower doses of tamoxifen do not totally block thalamic waves, and that the frequency of these waves is proportional to the given dose of tamoxifen, i.e., a bigger dose corresponds to a lower frequency of waves (unpublished results). While the standard dose to activate the Th^{Kir} condition, which is 5mg, totally blocks thalamic waves, 3mg gives rise to a thalamus with a lower frequency of waves. Reducing gradually the dose of tamoxifen, we observed that microglia also responded. With low doses of tamoxifen (1.5mg) microglia behaved comparable to the control condition; however, with half of the total dose (2.5mg), it was responding similarly to a total blockade of waves. This led us think that microglia might have a threshold to sense activity, i.e., there is a frequency of activity that microglia senses as "control" and therefore do not respond to it, and if the frequency goes below that threshold, it senses as "no activity" and reacts trying to maintain the homeostasis.

Finally, we were also interested in studying how cortical microglia was behaving in the EmbBE and Th^{Kir} conditions. We observed that embryonic bilateral enucleation did not change the density or disposition of cortical microglia. However, in the Th^{Kir} model we

observed a reduction in microglia in V1. Surprisingly, this reduction appeared at P10, but not before. It could be feasible to think that the hyperexcitable cortex in Th^{Kir} animals might be affecting microglia, which also presents a different morphology compared to the control condition. Moreover, it has been recently published that changes in the composition of layer specific pyramidal cells in the cortex is associated to changes in the density of subpopulations of microglia (Stogsdill et al., 2022). Therefore, it could be interesting to study whether the Th^{Kir} model has a different distribution of pyramidal neuron subtypes to understand whether the changes we see in cortical microglia are related to the hyperexcitable properties of cortical neurons, or to a new distribution of these neurons.

The combination of these results suggests that upon peripheral deprivation, the system has enough time and space to counteract the alterations. However, modifications in the activity of a central station like in the Th^{Kir} model, leads to a bigger effect in the system and cortical microglia is one of the populations that responds to this alteration.





Conclusiones
Conclusions

CONCLUSIONES

1. Las interneuronas talámicas del dLGN se identifican por la expresión de Otx2 y *Reelin*.
2. La enucleación bilateral embrionaria reduce el área del dLGN y la densidad de las interneuronas a P6, y provoca que las interneuronas talámicas se acumulen en las capas superiores del núcleo visual.
3. La supresión de las ondas de actividad retinal tipo I mediante la inyección de Cbx a P0 y P1 no afecta a la migración de las interneuronas en el dLGN.
4. La supresión de las ondas retinales tipo II de manera aguda no afecta a las interneuronas del dLGN.
5. La modificación de los patrones de actividad espontánea en el tálamo mediante la eliminación de las ondas de calcio talámicas durante el desarrollo embrionario reduce el área e incrementa la densidad de las interneuronas del dLGN.
6. Los animales Th^{Kir} muestran un incremento del ratio E/I sin cambios en la densidad de células.
7. La modificación de los patrones de actividad espontánea en el tálamo a estadíos perinatales en el modelo SertCre:Kir incrementa la densidad de interneuronas en el dLGN al final de la segunda semana postnatal sin afectar al número total de interneuronas.
8. El incremento en la frecuencia de las ondas talámicas sin modificar la morfología del dLGN no afecta a la densidad de interneuonas.
9. La enucleación bilateral embrionaria incrementa temporalmente las interneuronas SST y PV en la capa 6 de la corteza visual primaria.
10. La población general de interneuronas GAD67 en la corteza visual primaria de los animales Th^{Kir} solo cambia a P15 en la capa 4.
11. Sin embargo, el cambio en el patrón de la actividad talámica en el ratón Th^{Kir} altera la proporción de interneuronas SST y PV de capa 4 en la corteza visual.
12. Hay un incremento en la densidad de la microglia a estadios embrionarios y tempranos postnatales en el dLGN de los ratones Th^{Kir}.
13. La microglia del modelo Th^{Kir} presenta una morfología diferente y parece más fagocítica.
14. La microglia es más sensible a cambios en el componente embrionario de la actividad talámica en comparación al componente postnatal.

15. La microglia no controla la migración de las interneuronas en el dLGN.
16. La alteración del input y actividad periféricos no afecta a la microglia talámica.
17. Hay una reducción en la densidad de microglia y hay cambios en su morfología a P10 en la V1 de los ratones Th^{Kir}.
18. La falta del input retinal no afecta a la microglia en V1.

CONCLUSIONS

1. Thalamic interneurons in the dLGN are identified by Otx2 and *Reelin*.
2. Embryonic bilateral enucleation reduces the area of the dLGN, and the density of interneurons at P6, and allocates thalamic interneurons in the upper tiers of the visual nucleus.
3. Ablation of type I retinal waves by Cbx injection at P0 and P1 does not affect interneurons migration into the dLGN.
4. Ablation of type II retinal waves acutely does not affect interneurons in the dLGN.
5. Modifying the pattern of spontaneous activity in the thalamus by eliminating thalamic calcium waves during embryonic development reduces the area and increases the density of interneurons in the dLGN.
6. Th^{Kir} mice showed a decreased E/I ratio with no changes in the overall cell density.
7. Modifying thalamic spontaneous activity at the perinatal stage as in the SertCre:Kir model increases the density of interneurons in the dLGN at the end of the second postnatal week, without affecting the overall number of interneurons.
8. Increasing the frequency of thalamic waves without changing the morphology of the dLGN does not affect the density of thalamic interneurons.
9. Embryonic bilateral enucleation increases temporally the SST and PV interneurons in L6 in the primary visual cortex.
10. The overall population of GAD67 interneurons in the primary visual cortex of the Th^{Kir} animals only changes at P15 in L4.
11. However, the change of thalamic patterned activity as in the Th^{Kir} mouse, alters the proportion of L4 and L5 SST and PV interneurons in the visual cortex.
12. There is an increase in the density of embryonic and early postnatal microglia in the dLGN in the Th^{Kir} mouse.
13. dLGN microglia in the Th^{Kir} model present different morphology and seems to be more phagocytic.
14. Microglia is more sensitive to changes in the embryonic component of thalamic activity, rather than in the postnatal component.
15. Microglia is not controlling interneuron migration into the dLGN.
16. Perturbation of peripheral input and activity does not affect thalamic microglia.
17. There is a reduction in the density of microglia and a change in its morphology at P10 in the V1 of Th^{Kir} mouse.

18. Retinal input deprivation does not affect cortical microglia in V1.

Bibliography

BIBLIOGRAPHY

Achim, K., Peltopuro, P., Lahti, L., Tsai, H. H., Zachariah, A., Åstrand, M., Salminen, M., Rowitch, D., & Partanen, J. (2013). The role of Tal2 and Tal1 in the differentiation of midbrain GABAergic neuron precursors. *Biology Open*, *2*(10), 990–997. https://doi.org/10.1242/bio.20135041

Ackman, J. B., Burbridge, T. J., & Crair, M. C. (2012). Retinal waves coordinate patterned activity throughout the developing visual system. *Nature*, *490*(7419), 219–225. https://doi.org/10.1038/nature11529

Ackman, J. B., & Crair, M. C. (2014). Role of emergent neural activity in visual map development. *Current Opinion in Neurobiology*, *24*(1), 166–175. https://doi.org/10.1016/J.CONB.2013.11.011

Adesnik, H., Bruns, W., Taniguchi, H., Huang, Z. J., & Scanziani, M. (2012). A neural circuit for spatial summation in visual cortex. *Nature*, *490*(7419), 226–230. https://doi.org/10.1038/NATURE11526

Akerman, C. J., Smyth, D., & Thompson, I. D. (2002). Visual experience before eye-opening and the development of the retinogeniculate pathway. *Neuron*, *36*(5), 869–879. https://doi.org/10.1016/S0896-6273(02)01010-3

Akiyoshi, R., Wake, H., Kato, D., Horiuchi, H., Ono, R., Ikegami, A., Haruwaka, K., Omori, T., Tachibana, Y., Moorhouse, A. J., & Nabekura, J. (2018a). Microglia Enhance Synapse Activity to Promote Local Network Synchronization. *ENeuro*, *5*(5). https://doi.org/10.1523/ENEURO.0088-18.2018

Akiyoshi, R., Wake, H., Kato, D., Horiuchi, H., Ono, R., Ikegami, A., Haruwaka, K., Omori, T., Tachibana, Y., Moorhouse, A. J., & Nabekura, J. (2018b). Microglia Enhance Synapse Activity to Promote Local Network Synchronization. *ENeuro*, *5*(5). https://doi.org/10.1523/ENEURO.0088-18.2018

Allendoerfer, K. L., & Shatz, C. J. (1994). The subplate, a transient neocortical structure: its role in the development of connections between thalamus and cortex. *Annual Review of Neuroscience*, *17*, 185–218. https://doi.org/10.1146/ANNUREV.NE.17.030194.001153

Alliot, F., Godin, I., & Pessac, B. (1999). Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Developmental Brain Research*, *117*(2), 145–152. https://doi.org/10.1016/S0165-3806(99)00113-3

Anastasiades, P. G., Marques-Smith, A., Lyngholm, D., Lickiss, T., Raffiq, S., Katzel, D., Miesenbock, G., & Butt, S. J. B. (2016). GABAergic interneurons form transient layer-specific circuits in early postnatal neocortex. *Nature Communications*, *7*. https://doi.org/10.1038/NCOMMS10584

Anderson, S. A., Kaznowski, C. E., Horn, C., Rubenstein, J. L. R., & McConnell, S. K. (2002). Distinct origins of neocortical projection neurons and interneurons in vivo. *Cerebral Cortex (New York, N.Y. : 1991)*, *12*(7), 702–709. https://doi.org/10.1093/CERCOR/12.7.702

Anderson, S. A., Marín, O., Horn, C., Jennings, K., & Rubenstein, J. L. R. (2001). Distinct cortical migrations from the medial and lateral ganglionic eminences. *Development (Cambridge, England)*, *128*(3), 353–363. https://doi.org/10.1242/DEV.128.3.353

Antón-Bolaños, N., Espinosa, A., & López-Bendito, G. (2018). Developmental interactions between thalamus and cortex: a true love reciprocal story. *Current Opinion in Neurobiology*, *52*, 33–41. https://doi.org/10.1016/J.CONB.2018.04.018

Antón-Bolaños, N., Sempere-Ferràndez, 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 (New York, N.Y.)*, *364*(6444), 987–990. https://doi.org/10.1126/SCIENCE.AAV7617

Antonini, A., & Stryker, M. P. (1996). Plasticity of geniculocortical afferents following brief or prolonged monocular occlusion in the cat. *The Journal of Comparative Neurology*, *369*(1), 64–82. https://doi.org/10.1002/(sici)1096-9861(19960520)369:1<64::aid-cne5>3.0.co;2-i

Antony, J. M., Paquin, A., Nutt, S. L., Kaplan, D. R., & Miller, F. D. (2011). Endogenous microglia regulate development of embryonic cortical precursor cells. *Journal of Neuroscience Research*, *89*(3), 286–298. https://doi.org/10.1002/JNR.22533

Antypa, M., Faux, C., Eichele, G., Parnavelas, J. G., & Andrews, W. D. (2011). Differential gene expression in migratory streams of cortical interneurons. *The European Journal of Neuroscience*, *34*(10), 1584–1594. https://doi.org/10.1111/J.1460-9568.2011.07896.X

Arnold, T., & Betsholtz, C. (2013). The importance of microglia in the development of the vasculature in the central nervous system. *Vascular Cell*, *5*(1). https://doi.org/10.1186/2045-824X-5-4

Arnoux, I., & Audinat, E. (2015). Fractalkine signaling and microglia functions in the developing brain. In *Neural Plasticity* (Vol. 2015). Hindawi Limited. https://doi.org/10.1155/2015/689404

Arnoux, I., Hoshiko, M., Mandavy, L., Avignone, E., Yamamoto, N., & Audinat, E. (2013). Adaptive phenotype of microglial cells during the normal postnatal development of the somatosensory "Barrel" cortex. *GLIA*, *61*(10), 1582–1594. https://doi.org/10.1002/glia.22503

Ashwell, K. (1990). Microglia and cell death in the developing mouse cerebellum. *Brain Research. Developmental Brain Research*, *55*(2), 219–230. https://doi.org/10.1016/0165-3806(90)90203-B

Ashwell, K. W. S., Holländer, H., Streit, W., & Stone, J. (1989). The appearance and distribution of microglia in the developing retina of the rat. *Visual Neuroscience*, *2*(5), 437–448. https://doi.org/10.1017/S0952523800012335

Askew, K., Li, K., Olmos-Alonso, A., Garcia-Moreno, F., Liang, Y., Richardson, P., Tipton, T., Chapman, M. A., Riecken, K., Beccari, S., Sierra, A., Molnár, Z., Cragg, M. S., Garaschuk, O., Perry, V. H., & Gomez-Nicola, D. (2017). Coupled Proliferation and Apoptosis Maintain the Rapid Turnover of Microglia in the Adult Brain. *Cell Reports*, *18*(2), 391–405. https://doi.org/10.1016/J.CELREP.2016.12.041

Assali, A., le Magueresse, C., Bennis, M., Nicol, X., Gaspar, P., & Rebsam, A. (2017). RIM1/2 in retinal ganglion cells are required for the refinement of ipsilateral axons and eye-specific segregation. *Scientific Reports*, *7*(1). https://doi.org/10.1038/S41598-017-03361-0

Babij, R., & de Marco Garcia, N. (2016). Neuronal activity controls the development of interneurons in the somatosensory cortex. In *Frontiers in Biology* (Vol. 11, Issue 6, pp. 459–470). Higher Education Press. https://doi.org/10.1007/s11515-016-1427-x

Baden, T., Berens, P., Franke, K., Román Rosón, M., Bethge, M., & Euler, T. (2016). The functional diversity of retinal ganglion cells in the mouse. *Nature*, *529*(7586), 345–350. https://doi.org/10.1038/NATURE16468

Badimon, A., Strasburger, H. J., Ayata, P., Chen, X., Nair, A., Ikegami, A., Hwang, P., Chan, A. T., Graves, S. M., Uweru, J. O., Ledderose, C., Kutlu, M. G., Wheeler, M. A., Kahan, A., Ishikawa, M., Wang, Y. C., Loh, Y. H. E., Jiang, J. X., Surmeier, D. J., … Schaefer, A. (2020). Negative feedback control of neuronal activity by microglia. *Nature*, *586*(7829), 417–423. https://doi.org/10.1038/S41586-020-2777-8

Bagri, A., Marín, O., Plump, A. S., Mak, J., Pleasure, S. J., Rubenstein, J. L. R., & Tessier-Lavigne, M. (2002). Slit proteins prevent midline crossing and determine the

dorsoventral position of major axonal pathways in the mammalian forebrain. *Neuron*, *33*(2), 233–248. https://doi.org/10.1016/S0896-6273(02)00561-5

Bakken, T. E., van Velthoven, C. T. J., Menon, V., Hodge, R. D., Yao, Z., Nguyen, T. N., Graybuck, L. T., Horwitz, G. D., Bertagnolli, D., Goldy, J., Garren, E., Parry, S., Casper, T., Shehata, S. I., Barkan, E. R., Szafer, A., Levi, B. P., Dee, N., Smith, K. A., … Tasic, B. (n.d.). *Single-cell RNA-seq uncovers shared and distinct axes of variation in dorsal LGN neurons in mice, non-human primates and humans*. https://doi.org/10.1101/2020.11.05.367482

Ballester-Rosado, C. J., Albright, M. J., Wu, C. S., Liao, C. C., Zhu, J., Xu, J., Lee, L. J., & Lu, H. C. (2010). mGluR5 in cortical excitatory neurons exerts both cell-autonomous and -nonautonomous influences on cortical somatosensory circuit formation. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *30*(50), 16896–16909. https://doi.org/10.1523/JNEUROSCI.2462-10.2010

Bansal, A., Singer, J. H., Hwang, B. J., Xu, W., Beaudet, A., & Feller, M. B. (2000). Mice lacking specific nicotinic acetylcholine receptor subunits exhibit dramatically altered spontaneous activity patterns and reveal a limited role for retinal waves in forming ON and OFF circuits in the inner retina. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *20*(20), 7672–7681. https://doi.org/10.1523/JNEUROSCI.20-20-07672.2000

Barkat, T. R., Polley, D. B., & Hensch, T. K. (2011). A critical period for auditory thalamocortical connectivity. *Nature Neuroscience*, *14*(9), 1189–1996. https://doi.org/10.1038/NN.2882

Bartolini, G., Sánchez-Alcañiz, J. A., Osório, C., Valiente, M., García-Frigola, C., & Marín, O. (2017). Neuregulin 3 Mediates Cortical Plate Invasion and Laminar Allocation of GABAergic Interneurons. *Cell Reports*, *18*(5), 1157–1170. https://doi.org/10.1016/j.celrep.2016.12.089

Bavelier, D., & Neville, H. J. (2002). Cross-modal plasticity: where and how? *Nature Reviews. Neuroscience*, *3*(6), 443–452. https://doi.org/10.1038/NRN848

Bednar, J. A., & Wilson, S. P. (2016). Cortical Maps. *The Neuroscientist : A Review Journal Bringing Neurobiology, Neurology and Psychiatry*, *22*(6), 604–617. https://doi.org/10.1177/1073858415597645

Beers, D. R., Henkel, J. S., Xiao, Q., Zhao, W., Wang, J., Yen, A. A., Siklos, L., McKercher, S. R., & Appel, S. H. (2006). Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(43), 16021–16026. https://doi.org/10.1073/PNAS.0607423103

Berlucchi, G., & Rizzolatti, G. (1968). Binocularly driven neurons in visual cortex of split-chiasm cats. *Science (New York, N.Y.)*, *159*(3812), 308–310. https://doi.org/10.1126/SCIENCE.159.3812.308

Bertrand, J. Y., Jalil, A., Klaine, M., Jung, S., Cumano, A., & Godin, I. (2005). Three pathways to mature macrophages in the early mouse yolk sac. *Blood*, *106*(9), 3004–3011. https://doi.org/10.1182/BLOOD-2005-02-0461

Bickford, M. E., Slusarczyk, A., Dilger, E. K., Krahe, T. E., Kucuk, C., & Guido, W. (2010). Synaptic development of the mouse dorsal lateral geniculate nucleus. *The Journal of Comparative Neurology*, *518*(5), 622–635. https://doi.org/10.1002/CNE.22223

Bickford, M. E., Zhou, N., Krahe, T. E., Govindaiah, G., & Guido, W. (2015). Retinal and tectal "Driver-Like" inputs converge in the shell of the mouse dorsal lateral geniculate nucleus. *Journal of Neuroscience*, *35*(29), 10523–10534. https://doi.org/10.1523/JNEUROSCI.3375-14.2015

Bielle, F., Marcos-Mondejar, P., Keita, M., Mailhes, C., Verney, C., Nguyen Ba-Charvet, K., Tessier-Lavigne, M., Lopez-Bendito, G., & Garel, S. (2011). Slit2 activity in the

migration of guidepost neurons shapes thalamic projections during development and evolution. *Neuron*, *69*(6), 1085–1098. https://doi.org/10.1016/J.NEURON.2011.02.026

Blakemore, C., & Molnar, Z. (1990). Factors involved in the establishment of specific interconnections between thalamus and cerebral cortex. *Cold Spring Harbor Symposia on Quantitative Biology*, *55*, 491–504. https://doi.org/10.1101/SQB.1990.055.01.048

Blankenship, A. G., Ford, K. J., Johnson, J., Seal, R. P., Edwards, R. H., Copenhagen, D. R., & Feller, M. B. (2009). Synaptic and extrasynaptic factors governing glutamatergic retinal waves. *Neuron*, *62*(2), 230–241. https://doi.org/10.1016/J.NEURON.2009.03.015

Bortone, D., & Polleux, F. (2009). KCC2 Expression Promotes the Termination of Cortical Interneuron Migration in a Voltage-Sensitive Calcium-Dependent Manner. *Neuron*, *62*(1), 53–71. https://doi.org/10.1016/j.neuron.2009.01.034

Braisted, J. E., Ringstedt, T., & O'Leary, D. D. M. (2009). Slits are chemorepellents endogenous to hypothalamus and steer thalamocortical axons into ventral telencephalon. *Cerebral Cortex (New York, N.Y.: 1991)*, *19 Suppl 1*(Suppl 1). https://doi.org/10.1093/CERCOR/BHP035

Briggs, F., & Usrey, W. M. (2008). Emerging views of corticothalamic function. *Current Opinion in Neurobiology*, *18*(4), 403–407. https://doi.org/10.1016/J.CONB.2008.09.002

Brooks, J. M., Su, J., Levy, C., Wang, J. S., Seabrook, T. A., Guido, W., & Fox, M. A. (2013a). A molecular mechanism regulating the timing of corticogeniculate innervation. *Cell Reports*, *5*(3), 573–581. https://doi.org/10.1016/J.CELREP.2013.09.041

Brooks, J. M., Su, J., Levy, C., Wang, J. S., Seabrook, T. A., Guido, W., & Fox, M. A. (2013b). A molecular mechanism regulating the timing of corticogeniculate innervation. *Cell Reports*, *5*(3), 573–581. https://doi.org/10.1016/J.CELREP.2013.09.041

Brown, A., Yates, P. A., Burrola, P., Ortuo, D., Vaidya, A., Jessell, T. M., Pfaff, S. L., O'Leary, D. D. M., & Lemke, G. (2000). Topographic mapping from the retina to the midbrain is controlled by relative but not absolute levels of EphA receptor signaling. *Cell*, *102*(1), 77–88. https://doi.org/10.1016/S0092-8674(00)00012-X

Brox, A., Puelles, L., Ferreiro, B., & Medina, L. (2003). Expression of the genes GAD67 and Distal-less-4 in the forebrain of Xenopus laevis confirms a common pattern in tetrapods. *The Journal of Comparative Neurology*, *461*(3), 370–393. https://doi.org/10.1002/CNE.10688

Burbridge, T. J., Xu, H. P., Ackman, J. B., Ge, X., Zhang, Y., Ye, M. J., Zhou, Z. J., Xu, J., Contractor, A., & Crair, M. C. (2014). Visual circuit development requires patterned activity mediated by retinal acetylcholine receptors. *Neuron*, *84*(5), 1049–1064. https://doi.org/10.1016/J.NEURON.2014.10.051

Butler, A. B. (2008). Evolution of the thalamus: A morphological and functional review. In *Thalamus and Related Systems* (Vol. 4, Issue 1, pp. 35–58). https://doi.org/10.1017/S1472928808000356

Butt, S. J. B., Fuccillo, M., Nery, S., Noctor, S., Kriegstein, A., Corbin, J. G., & Fishell, G. (2005). The temporal and spatial origins of cortical interneurons predict their physiological subtype. *Neuron*, *48*(4), 591–604. https://doi.org/10.1016/J.NEURON.2005.09.034

Cardona, A. E., Sasse, M. E., Liu, L., Cardona, S. M., Mizutani, M., Savarin, C., Hu, T., & Ransohoff, R. M. (2008). Scavenging roles of chemokine receptors: chemokine receptor deficiency is associated with increased levels of ligand in circulation and tissues. *Blood*, *112*(2), 256–263. https://doi.org/10.1182/BLOOD-2007-10-118497

Castillo-Paterna, M., Moreno-Juan, V., Filipchuk, A., Rodríguez-Malmierca, L., Susín, R., & López-Bendito, G. (2015). DCC functions as an accelerator of thalamocortical axonal

growth downstream of spontaneous thalamic activity. *EMBO Reports*, *16*(7), 851–862. https://doi.org/10.15252/EMBR.201439882

Caviness, V. S., & Frost, D. O. (1980). Tangential organization of thalamic projections to the neocortex in the mouse. *The Journal of Comparative Neurology*, *194*(2), 335–367. https://doi.org/10.1002/CNE.901940205

Chan, W. Y., Kohsaka, S., & Rezaie, P. (2007). The origin and cell lineage of microglia: new concepts. *Brain Research Reviews*, *53*(2), 344–354. https://doi.org/10.1016/J.BRAINRESREV.2006.11.002

Charalambakis, N. E., Govindaiah, G., Campbell, P. W., & Guido, W. (2019a). Developmental remodeling of thalamic interneurons requires retinal signaling. *Journal of Neuroscience*, *39*(20), 3856–3866. https://doi.org/10.1523/JNEUROSCI.2224-18.2019

Charalambakis, N. E., Govindaiah, G., Campbell, P. W., & Guido, W. (2019b). Developmental remodeling of thalamic interneurons requires retinal signaling. *Journal of Neuroscience*, *39*(20), 3856–3866. https://doi.org/10.1523/JNEUROSCI.2224-18.2019

Chatterjee, M., Guo, Q., Weber, S., Scholpp, S., & Li, J. Y. H. (2014). Pax6 regulates the formation of the habenular nuclei by controlling the temporospatial expression of Shh in the diencephalon in vertebrates. *BMC Biology*, *12*. https://doi.org/10.1186/1741-7007-12-13

Chatterjee, M., & Li, J. Y. H. (2012). Patterning and compartment formation in the diencephalon. *Frontiers in Neuroscience*, *6*(MAY). https://doi.org/10.3389/FNINS.2012.00066

Che, A., Babij, R., Iannone, A. F., Fetcho, R. N., Ferrer, M., Liston, C., Fishell, G., & de Marco García, N. v. (2018). Layer I Interneurons Sharpen Sensory Maps during Neonatal Development. *Neuron*, *99*(1), 98-116.e7. https://doi.org/10.1016/J.NEURON.2018.06.002

Chen, L., Guo, Q., & Li, J. Y. H. (2009). Transcription factor Gbx2 acts cell-nonautonomously to regulate the formation of lineage-restriction boundaries of the thalamus. *Development (Cambridge, England)*, *136*(8), 1317–1326. https://doi.org/10.1242/DEV.030510

Chittajallu, R., & Isaac, J. T. R. (2010). Emergence of cortical inhibition by coordinated sensory-driven plasticity at distinct synaptic loci. *Nature Neuroscience*, *13*(10), 1240–1248. https://doi.org/10.1038/NN.2639

Chou, S. J., Babot, Z., Leingartner, A., Studer, M., Nakagawa, Y., & O'Leary, D. D. M. (2013). Geniculocortical input drives genetic distinctions between primary and higher-order visual areas. *Science (New York, N.Y.)*, *340*(6137), 1239–1242. https://doi.org/10.1126/SCIENCE.1232806

Clascá, F., Angelucci, A., & Sur, M. (1995). Layer-specific programs of development in neocortical projection neurons. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(24), 11145–11149. https://doi.org/10.1073/PNAS.92.24.11145

Close, J., Xu, H., García, N. D. M., Batista-Brito, R., Rossignol, E., Rudy, B., & Fishell, G. (2012). Satb1 is an activity-modulated transcription factor required for the terminal differentiation and connectivity of medial ganglionic eminence-derived cortical interneurons. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *32*(49), 17690–17705. https://doi.org/10.1523/JNEUROSCI.3583-12.2012

Cobos, I., Calcagnotto, M. E., Vilaythong, A. J., Thwin, M. T., Noebels, J. L., Baraban, S. C., & Rubenstein, J. L. R. (2005). Mice lacking Dlx1 show subtype-specific loss of interneurons, reduced inhibition and epilepsy. *Nature Neuroscience*, *8*(8), 1059–1068. https://doi.org/10.1038/NN1499

Colonnese, M. T. (2014). Rapid developmental emergence of stable depolarization during wakefulness by inhibitory balancing of cortical network excitability. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *34*(16), 5477–5485. https://doi.org/10.1523/JNEUROSCI.3659-13.2014

Cossart, R. (2011). The maturation of cortical interneuron diversity: How multiple developmental journeys shape the emergence of proper network function. In *Current Opinion in Neurobiology* (Vol. 21, Issue 1, pp. 160–168). https://doi.org/10.1016/j.conb.2010.10.003

Cox, C. L., & Beatty, J. A. (2017). The multifaceted role of inhibitory interneurons in the dorsal lateral geniculate nucleus. In *Visual neuroscience* (Vol. 34, p. E017). https://doi.org/10.1017/S0952523817000141

Cuadros, M. A., Martin, C., Coltey, P., Almendros, A., & Navascués, J. (1993). First appearance, distribution, and origin of macrophages in the early development of the avian central nervous system. *The Journal of Comparative Neurology*, *330*(1), 113–129. https://doi.org/10.1002/CNE.903300110

Cuadros, M. A., & Navascués, J. (2001). Early origin and colonization of the developing central nervous system by microglial precursors. *Progress in Brain Research*, *132*, 51–59. https://doi.org/10.1016/S0079-6123(01)32065-4

Cunningham, C. L., Martínez-Cerdeño, V., & Noctor, S. C. (2013). Microglia regulate the number of neural precursor cells in the developing cerebral cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(10), 4216–4233. https://doi.org/10.1523/JNEUROSCI.3441-12.2013

Dalmau, I., Finsen, B., Zimmer, J., González, B., & Castellano, B. (1998). Development of microglia in the postnatal rat hippocampus. *Hippocampus*, *8*(5), 458–474. https://doi.org/10.1002/(sici)1098-1063(1998)8:5<458::aid-hipo6>3.0.co;2-n

Dalmau, I., Vela, J. M., González, B., Finsen, B., & Castellano, B. (2003). Dynamics of microglia in the developing rat brain. *The Journal of Comparative Neurology*, *458*(2), 144–157. https://doi.org/10.1002/CNE.10572

Datwani, A., Iwasato, T., Itohara, S., & Erzurumlu, R. S. (2002). NMDA receptor-dependent pattern transfer from afferents to postsynaptic cells and dendritic differentiation in the barrel cortex. *Molecular and Cellular Neuroscience*, *21*(3), 477–492. https://doi.org/10.1006/mcne.2002.1195

Davalos, D., Grutzendler, J., Yang, G., Kim, J. v., Zuo, Y., Jung, S., Littman, D. R., Dustin, M. L., & Gan, W. B. (2005). ATP mediates rapid microglial response to local brain injury in vivo. *Nature Neuroscience*, *8*(6), 752–758. https://doi.org/10.1038/NN1472

Daw, M. I., Ashby, M. C., & Isaac, J. T. R. (2007). Coordinated developmental recruitment of latent fast spiking interneurons in layer IV barrel cortex. *Nature Neuroscience*, *10*(4), 453–461. https://doi.org/10.1038/NN1866

de Carlos, J. A., & O'Leary, D. D. M. (1992). Growth and targeting of subplate axons and establishment of major cortical pathways. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *12*(4), 1194–1211. https://doi.org/10.1523/JNEUROSCI.12-04-01194.1992

de Marco García, N. v., Karayannis, T., & Fishell, G. (2011). Neuronal activity is required for the development of specific cortical interneuron subtypes. *Nature*, *472*(7343), 351–355. https://doi.org/10.1038/nature09865

de Marco García, N. v., Priya, R., Tuncdemir, S. N., Fishell, G., & Karayannis, T. (2015). Sensory inputs control the integration of neurogliaform interneurons into cortical circuits. *Nature Neuroscience*, *18*(3), 393–403. https://doi.org/10.1038/NN.3946

Deck, M., Lokmane, L., Chauvet, S., Mailhes, C., Keita, M., Niquille, M., Yoshida, M., Yoshida, Y., Lebrand, C., Mann, F., Grove, E. A., & Garel, S. (2013). Pathfinding of

corticothalamic axons relies on a rendezvous with thalamic projections. *Neuron*, *77*(3), 472–484. https://doi.org/10.1016/J.NEURON.2012.11.031

DeDiego, I., Smith-Fernández, A., & Fairén, A. (1994). Cortical cells that migrate beyond area boundaries: characterization of an early neuronal population in the lower intermediate zone of prenatal rats. *The European Journal of Neuroscience*, *6*(6), 983–997. https://doi.org/10.1111/J.1460-9568.1994.TB00593.X

DeFelipe, J. (1997). Types of neurons, synaptic connections and chemical characteristics of cells immunoreactive for calbindin-D28K, parvalbumin and calretinin in the neocortex. *Journal of Chemical Neuroanatomy*, *14*(1), 1–19. https://doi.org/10.1016/S0891-0618(97)10013-8

Defelipe, J., López-Cruz, P. L., Benavides-Piccione, R., Bielza, C., Larrañaga, P., Anderson, S., Burkhalter, A., Cauli, B., Fairén, A., Feldmeyer, D., Fishell, G., Fitzpatrick, D., Freund, T. F., González-Burgos, G., Hestrin, S., Hill, S., Hof, P. R., Huang, J., Jones, E. G., … Ascoli, G. A. (2013). New insights into the classification and nomenclature of cortical GABAergic interneurons. *Nature Reviews. Neuroscience*, *14*(3), 202–216. https://doi.org/10.1038/NRN3444

Dehay, C., Giroud, P., Berland, M., Killackey, H. P., & Kennedy, H. (1996). Phenotypic characterisation of respecified visual cortex subsequent to prenatal enucleation in the monkey: development of acetylcholinesterase and cytochrome oxidase patterns. *The Journal of Comparative Neurology*, *376*(3), 386–402. https://doi.org/10.1002/(sici)1096-9861(19961216)376:3<386::aid-cne3>3.0.co;2-z

del Río, J. A., Martínez, A., Auladell, C., & Soriano, E. (2000). Developmental history of the subplate and developing white matter in the murine neocortex. Neuronal organization and relationship with the main afferent systems at embryonic and perinatal stages. *Cerebral Cortex (New York, N.Y. : 1991)*, *10*(8), 784–801. https://doi.org/10.1093/CERCOR/10.8.784

Delogu, A., Sellers, K., Zagoraiou, L., Bocianowska-Zbrog, A., Mandal, S., Guimera, J., Rubenstein, J. L. R., Sugden, D., Jessell, T., & Lumsden, A. (2012). Subcortical Visual Shell Nuclei Targeted by ipRGCs Develop from a Sox14+-GABAergic Progenitor and Require Sox14 to Regulate Daily Activity Rhythms. *Neuron*, *75*(4), 648–662. https://doi.org/10.1016/j.neuron.2012.06.013

Demas, J., Eglen, S. J., & Wong, R. O. L. (2003). Developmental loss of synchronous spontaneous activity in the mouse retina is independent of visual experience. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *23*(7), 2851–2860. https://doi.org/10.1523/JNEUROSCI.23-07-02851.2003

Douglas, R. J., & Martin, K. A. C. (2004). Neuronal circuits of the neocortex. *Annual Review of Neuroscience*, *27*, 419–451. https://doi.org/10.1146/ANNUREV.NEURO.27.070203.144152

Dräger, U. C., & Olsen, J. F. (1980). Origins of crossed and uncrossed retinal projections in pigmented and albino mice. *The Journal of Comparative Neurology*, *191*(3), 383–412. https://doi.org/10.1002/CNE.901910306

Duan, Z. R. S., Che, A., Chu, P., Modol, L., Bollmann, Y., Babij, R., Fetcho, R. N., Otsuka, T., Fuccillo, M. v., Liston, C., Pisapia, D. J., Cossart, R., & de Marco García, N. v. (2020). GABAergic Restriction of Network Dynamics Regulates Interneuron Survival in the Developing Cortex. *Neuron*, *105*(1), 75-92.e5. https://doi.org/10.1016/J.NEURON.2019.10.008

Dye, C. A., Abbott, C. W., & Huffman, K. J. (2012). Bilateral enucleation alters gene expression and intraneocortical connections in the mouse. *Neural Development*, *7*(1). https://doi.org/10.1186/1749-8104-7-5

Earle, K. L., & Mitrofanis, J. (1998). Development of glia and blood vessels in the internal capsule of rats. *Journal of Neurocytology*, *27*(2), 127–139. https://doi.org/10.1023/A:1006951423251

El-Danaf, R. N., & Huberman, A. D. (2019). Sub-topographic maps for regionally enhanced analysis of visual space in the mouse retina. *The Journal of Comparative Neurology*, *527*(1), 259–269. https://doi.org/10.1002/CNE.24457

Elliott, M. R., Chekeni, F. B., Trampont, P. C., Lazarowski, E. R., Kadl, A., Walk, S. F., Park, D., Woodson, R. I., Ostankovich, M., Sharma, P., Lysiak, J. J., Harden, T. K., Leitinger, N., & Ravichandran, K. S. (2009). Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. *Nature*, *461*(7261), 282–286. https://doi.org/10.1038/NATURE08296

Elmore, M. R. P., Najafi, A. R., Koike, M. A., Dagher, N. N., Spangenberg, E. E., Rice, R. A., Kitazawa, M., Matusow, B., Nguyen, H., West, B. L., & Green, K. N. (2014). Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. *Neuron*, *82*(2), 380–397. https://doi.org/10.1016/J.NEURON.2014.02.040

Erskine, L., & Herreral, E. (2014). Connecting the retina to the brain. *ASN Neuro*, *6*(6). https://doi.org/10.1177/1759091414562107

Evangelio, M., García-Amado, M., & Clascá, F. (2018). Thalamocortical projection neuron and interneuron numbers in the visual thalamic nuclei of the adult C57BL/6 mouse. *Frontiers in Neuroanatomy*, *12*. https://doi.org/10.3389/fnana.2018.00027

Eyo, U. B., Miner, S. A., Weiner, J. A., & Dailey, M. E. (2016). Developmental changes in microglial mobilization are independent of apoptosis in the neonatal mouse hippocampus. *Brain, Behavior, and Immunity*, *55*, 49–59. https://doi.org/10.1016/J.BBI.2015.11.009

Famiglietti, E. v., & Peters, A. (1972). The synaptic glomerulus and the intrinsic neuron in the dorsal lateral geniculate nucleus of the cat. *The Journal of Comparative Neurology*, *144*(3), 285–333. https://doi.org/10.1002/CNE.901440304

Favuzzi, E., Huang, S., Saldi, G. A., Binan, L., Ibrahim, L. A., Fernández-Otero, M., Cao, Y., Zeine, A., Sefah, A., Zheng, K., Xu, Q., Khlestova, E., Farhi, S. L., Bonneau, R., Datta, S. R., Stevens, B., & Fishell, G. (2021). GABA-receptive microglia selectively sculpt developing inhibitory circuits. *Cell*, *184*(15), 4048-4063.e32. https://doi.org/10.1016/j.cell.2021.06.018

Feller, M. B., Wellis, D. P., Stellwagen, D., Werblin, F. S., & Shatz, C. J. (1996). Requirement for cholinergic synaptic transmission in the propagation of spontaneous retinal waves. *Science (New York, N.Y.)*, *272*(5265), 1182–1187. https://doi.org/10.1126/SCIENCE.272.5265.1182

Fernandez, L. M. J., Vantomme, G., Osorio-Forero, A., Cardis, R., Béard, E., & Lüthi, A. (2018). Thalamic reticular control of local sleep in mouse sensory cortex. *ELife*, *7*. https://doi.org/10.7554/ELIFE.39111

Ferran, J. L., de Oliveira, E. D., Merchán, P., Sandoval, J. E., Sánchez-Arrones, L., Martínez-de-la-Torre, M., & Puelles, L. (2009). Genoarchitectonic profile of developing nuclear groups in the chicken pretectum. *The Journal of Comparative Neurology*, *517*(4), 405–451. https://doi.org/10.1002/CNE.22115

Ferrer, C., & de Marco García, N. v. (2022). The Role of Inhibitory Interneurons in Circuit Assembly and Refinement Across Sensory Cortices. In *Frontiers in Neural Circuits* (Vol. 16). Frontiers Media S.A. https://doi.org/10.3389/fncir.2022.866999

Firth, S. I., Wang, C. T., & Feller, M. B. (2005). Retinal waves: mechanisms and function in visual system development. *Cell Calcium*, *37*(5), 425–432. https://doi.org/10.1016/J.CECA.2005.01.010

Flames, N., Long, J. E., Garratt, A. N., Fischer, T. M., Gassmann, M., Birchmeier, C., Lai, C., Rubenstein, J. L. R., & Marín, O. (2004). Short- and long-range attraction of cortical GABAergic interneurons by neuregulin-1. *Neuron*, *44*(2), 251–261. https://doi.org/10.1016/j.neuron.2004.09.028

Fox, K., & Wong, R. O. L. (2005). A comparison of experience-dependent plasticity in the visual and somatosensory systems. *Neuron*, *48*(3), 465–477. https://doi.org/10.1016/J.NEURON.2005.10.013

Frangeul, L., Pouchelon, G., Telley, L., Lefort, S., Luscher, C., & Jabaudon, D. (2016). A cross-modal genetic framework for the development and plasticity of sensory pathways. *Nature*, *538*(7623), 96–98. https://doi.org/10.1038/nature19770

Fries, P. (2009). Neuronal gamma-band synchronization as a fundamental process in cortical computation. *Annual Review of Neuroscience*, *32*, 209–224. https://doi.org/10.1146/ANNUREV.NEURO.051508.135603

Frost, J. L., & Schafer, D. P. (2016). Microglia: Architects of the Developing Nervous System. *Trends in Cell Biology*, *26*(8), 587–597. https://doi.org/10.1016/J.TCB.2016.02.006

Garel, S., & López-Bendito, G. (2014). Inputs from the thalamocortical system on axon pathfinding mechanisms. *Current Opinion in Neurobiology*, *27*, 143–150. https://doi.org/10.1016/J.CONB.2014.03.013

Garel, S., & Rubenstein, J. L. R. (2004). Intermediate targets in formation of topographic projections: inputs from the thalamocortical system. *Trends in Neurosciences*, *27*(9), 533–539. https://doi.org/10.1016/J.TINS.2004.06.014

Gelman, D., Griveau, A., Dehorter, N., Teissier, A., Varela, C., Pla, R., Pierani, A., & Marín, O. (2011). A wide diversity of cortical GABAergic interneurons derives from the embryonic preoptic area. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *31*(46), 16570–16580. https://doi.org/10.1523/JNEUROSCI.4068-11.2011

Gelman, D. M., & Marín, O. (2010). Generation of interneuron diversity in the mouse cerebral cortex. *The European Journal of Neuroscience*, *31*(12), 2136–2141. https://doi.org/10.1111/J.1460-9568.2010.07267.X

Gelman, D. M., Martini, F. J., Nóbrega-Pereira, S., Pierani, A., Kessaris, N., & Marín, O. (2009). The embryonic preoptic area is a novel source of cortical GABAergic interneurons. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *29*(29), 9380–9389. https://doi.org/10.1523/JNEUROSCI.0604-09.2009

Genescu, I., Aníbal-Martínez, M., Kouskoff, V., Chenouard, N., Mailhes-Hamon, C., Cartonnet, H., Lokmane, L., Rijli, F. M., López-Bendito, G., Gambino, F., & Garel, S. (2022). Dynamic interplay between thalamic activity and Cajal-Retzius cells regulates the wiring of cortical layer 1. *Cell Reports*, *39*(2). https://doi.org/10.1016/j.celrep.2022.110667

Gezelius, H., & López-Bendito, G. (2017). Thalamic neuronal specification and early circuit formation. *Developmental Neurobiology*, *77*(7), 830–843. https://doi.org/10.1002/DNEU.22460

Gezelius, H., Moreno-Juan, V., Mezzera, C., Thakurela, S., Rodríguez-Malmierca, L. M., Pistolic, J., Benes, V., Tiwari, V. K., & López-Bendito, G. (2017). Genetic Labeling of Nuclei-Specific Thalamocortical Neurons Reveals Putative Sensory-Modality Specific Genes. *Cerebral Cortex (New York, N.Y. : 1991)*, *27*(11), 5054–5069. https://doi.org/10.1093/CERCOR/BHW290

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., Mehler, M. F., Conway, S. J., Ng, L. G., Stanley, E. R., Samokhvalov, I. M., & Merad, M. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science (New York, N.Y.)*, *330*(6005), 841–845. https://doi.org/10.1126/SCIENCE.1194637

Ginhoux, F., Lim, S., Hoeffel, G., Low, D., & Huber, T. (2013). Origin and differentiation of microglia. *Frontiers in Cellular Neuroscience*, *7*(MAR). https://doi.org/10.3389/FNCEL.2013.00045

Giulian, D., & Ingeman, J. F. (1988). Colony-stimulating factors as promoters of ameboid microglia. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *8*(12), 4707–4717. https://doi.org/10.1523/JNEUROSCI.08-12-04707.1988

Godement, P., Salaün, J., & Imbert, M. (1984). Prenatal and postnatal development of retinogeniculate and retinocollicular projections in the mouse. *The Journal of Comparative Neurology*, *230*(4), 552–575. https://doi.org/10.1002/CNE.902300406

Golding, B., Pouchelon, G., Bellone, C., Murthy, S., di Nardo, A. A., Govindan, S., Ogawa, M., Shimogori, T., Lüscher, C., Dayer, A., & Jabaudon, D. (2014). Retinal input directs the recruitment of inhibitory interneurons into thalamic visual circuits. *Neuron*, *81*(5), 1057–1069. https://doi.org/10.1016/j.neuron.2014.01.032

Gomez Perdiguero, E., Klapproth, K., Schulz, C., Busch, K., Azzoni, E., Crozet, L., Garner, H., Trouillet, C., de Bruijn, M. F., Geissmann, F., & Rodewald, H. R. (2015). Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature*, *518*(7540), 547–551. https://doi.org/10.1038/NATURE13989

Gonchar, Y., & Burkhalter, A. (2003). Distinct GABAergic targets of feedforward and feedback connections between lower and higher areas of rat visual cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *23*(34), 10904–10912. https://doi.org/10.1523/JNEUROSCI.23-34-10904.2003

González, A., López, J. M., Sánchez-Camacho, C., & Marín, O. (2002). Regional expression of the homeobox gene NKX2-1 defines pallidal and interneuronal populations in the basal ganglia of amphibians. *Neuroscience*, *114*(3), 567–575. https://doi.org/10.1016/S0306-4522(02)00326-3

Gordon, J. A., & Stryker, M. P. (1996). Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *16*(10), 3274–3286. https://doi.org/10.1523/JNEUROSCI.16-10-03274.1996

Gosselin, D., Link, V. M., Romanoski, C. E., Fonseca, G. J., Eichenfield, D. Z., Spann, N. J., Stender, J. D., Chun, H. B., Garner, H., Geissmann, F., & Glass, C. K. (2014). Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell*, *159*(6), 1327–1340. https://doi.org/10.1016/J.CELL.2014.11.023

Grant, E., Hoerder-Suabedissen, A., & Molnár, Z. (2012). Development of the corticothalamic projections. *Frontiers in Neuroscience*, *6*(MAY), 1–14. https://doi.org/10.3389/FNINS.2012.00053

Grant, E., Hoerder-Suabedissen, A., & Molnar, Z. (2016). The Regulation of Corticofugal Fiber Targeting by Retinal Inputs. *Cerebral Cortex (New York, N.Y. : 1991)*, *26*(3), 1336–1348. https://doi.org/10.1093/CERCOR/BHV315

Greter, M., Lelios, I., Pelczar, P., Hoeffel, G., Price, J., Leboeuf, M., Kündig, T. M., Frei, K., Ginhoux, F., Merad, M., & Becher, B. (2012). Stroma-derived interleukin-34 controls the development and maintenance of langerhans cells and the maintenance of microglia. *Immunity*, *37*(6), 1050–1060. https://doi.org/10.1016/J.IMMUNI.2012.11.001

Gude, D. R., Alvarez, S. E., Paugh, S. W., Mitra, P., Yu, J., Griffiths, R., Barbour, S. E., Milstien, S., & Spiegel, S. (2008). Apoptosis induces expression of sphingosine kinase 1 to release sphingosine-1-phosphate as a "come-and-get-me" signal. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *22*(8), 2629–2638. https://doi.org/10.1096/FJ.08-107169

Guillery, R. W. (1969). The organization of synaptic interconnections in the laminae of the dorsal lateral geniculate nucleus of the cat. *Zeitschrift Fur Zellforschung Und Mikroskopische Anatomie (Vienna, Austria : 1948)*, *96*(1), 1–38. https://doi.org/10.1007/BF00321474

H, A., F, A., & RS, E. (2014). Region-Specific Disruption of Adenylate Cyclase Type 1 Gene Differentially Affects Somatosensorimotor Behaviors in Mice. *ENeuro*, *1*(1). https://pubmed.ncbi.nlm.nih.gov/26023682/

Halassa, M. M., & Acsády, L. (2016). Thalamic Inhibition: Diverse Sources, Diverse Scales. In *Trends in Neurosciences* (Vol. 39, Issue 10, pp. 680–693). Elsevier Ltd. https://doi.org/10.1016/j.tins.2016.08.001

Hamos, J. E., van Horn, S. C., Raczkowski, D., Uhlrich, D. J., & Sherman, S. M. (1985). Synaptic connectivity of a local circuit neurone in lateral geniculate nucleus of the cat. *Nature*, *317*(6038), 618–621. https://doi.org/10.1038/317618A0

Hanamsagar, R., Alter, M. D., Block, C. S., Sullivan, H., Bolton, J. L., & Bilbo, S. D. (2017). Generation of a microglial developmental index in mice and in humans reveals a sex difference in maturation and immune reactivity. *Glia*, *65*(9), 1504–1520. https://doi.org/10.1002/GLIA.23176

Hanganu, I. L., Kilb, W., & Luhmann, H. J. (2002). Functional synaptic projections onto subplate neurons in neonatal rat somatosensory cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *22*(16), 7165–7176. https://doi.org/10.1523/JNEUROSCI.22-16-07165.2002

Harrington, M. E. (1997). The ventral lateral geniculate nucleus and the intergeniculate leaflet: interrelated structures in the visual and circadian systems. *Neuroscience and Biobehavioral Reviews*, *21*(5), 705–727. https://doi.org/10.1016/S0149-7634(96)00019-X

Harris, K. D., Hochgerner, H., Skene, N. G., Magno, L., Katona, L., Bengtsson Gonzales, C., Somogyi, P., Kessaris, N., Linnarsson, S., & Hjerling-Leffler, J. (2018). Classes and continua of hippocampal CA1 inhibitory neurons revealed by single-cell transcriptomics. *PLoS Biology*, *16*(6). https://doi.org/10.1371/JOURNAL.PBIO.2006387

Harwell, C. C., Fuentealba, L. C., Gonzalez-Cerrillo, A., Parker, P. R. L., Gertz, C. C., Mazzola, E., Garcia, M. T., Alvarez-Buylla, A., Cepko, C. L., & Kriegstein, A. R. (2015). Wide Dispersion and Diversity of Clonally Related Inhibitory Interneurons. *Neuron*, *87*(5), 999–1007. https://doi.org/10.1016/J.NEURON.2015.07.030

Hashimoto, D., Chow, A., Noizat, C., Teo, P., Beasley, M. B., Leboeuf, M., Becker, C. D., See, P., Price, J., Lucas, D., Greter, M., Mortha, A., Boyer, S. W., Forsberg, E. C., Tanaka, M., van Rooijen, N., García-Sastre, A., Stanley, E. R., Ginhoux, F., … Merad, M. (2013). Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity*, *38*(4), 792–804. https://doi.org/10.1016/J.IMMUNI.2013.04.004

Hayes, S. G., Murray, K. D., & Jones, E. G. (2003). Two epochs in the development of gamma-aminobutyric acidergic neurons in the ferret thalamus. *The Journal of Comparative Neurology*, *463*(1), 45–65. https://doi.org/10.1002/CNE.10749

Haynes, S. E., Hollopeter, G., Yang, G., Kurpius, D., Dailey, M. E., Gan, W. B., & Julius, D. (2006). The P2Y12 receptor regulates microglial activation by extracellular nucleotides. *Nature Neuroscience*, *9*(12), 1512–1519. https://doi.org/10.1038/NN1805

Helmut, K., Hanisch, U. K., Noda, M., & Verkhratsky, A. (2011). Physiology of microglia. *Physiological Reviews*, *91*(2), 461–553. https://doi.org/10.1152/PHYSREV.00011.2010

Herbomel, P., Thisse, B., & Thisse, C. (2001). Zebrafish early macrophages colonize cephalic mesenchyme and developing brain, retina, and epidermis through a M-CSF receptor-dependent invasive process. *Developmental Biology*, *238*(2), 274–288. https://doi.org/10.1006/DBIO.2001.0393

Herrmann, K., & Shatz, C. J. (1995). Blockade of action potential activity alters initial arborization of thalamic axons within cortical layer 4. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(24), 11244–11248. https://doi.org/10.1073/PNAS.92.24.11244

Hevner, R. F., Daza, R. A. M., Englund, C., Kohtz, J., & Fink, A. (2004). Postnatal shifts of interneuron position in the neocortex of normal and reeler mice: Evidence for inward radial migration. *Neuroscience*, *124*(3), 605–618. https://doi.org/10.1016/j.neuroscience.2003.11.033

Hoeffel, G., Chen, J., Lavin, Y., Low, D., Almeida, F. F., See, P., Beaudin, A. E., Lum, J., Low, I., Forsberg, E. C., Poidinger, M., Zolezzi, F., Larbi, A., Ng, L. G., Chan, J. K. Y., Greter, M., Becher, B., Samokhvalov, I. M., Merad, M., & Ginhoux, F. (2015). C-Myb(+) erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. *Immunity*, *42*(4), 665–678. https://doi.org/10.1016/J.IMMUNI.2015.03.011

Hoeffel, G., & Ginhoux, F. (2018). Fetal monocytes and the origins of tissue-resident macrophages. *Cellular Immunology*, *330*, 5–15. https://doi.org/10.1016/J.CELLIMM.2018.01.001

Hoeffel, G., Wang, Y., Greter, M., See, P., Teo, P., Malleret, B., Leboeuf, M., Low, D., Oller, G., Almeida, F., Choy, S. H. Y., Grisotto, M., Renia, L., Conway, S. J., Stanley, E. R., Chan, J. K. Y., Ng, L. G., Samokhvalov, I. M., Merad, M., & Ginhoux, F. (2012). Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *The Journal of Experimental Medicine*, *209*(6), 1167–1181. https://doi.org/10.1084/JEM.20120340

Hoerder-Suabedissen, A., Hayashi, S., Upton, L., Nolan, Z., Casas-Torremocha, D., Grant, E., Viswanathan, S., Kanold, P. O., Clasca, F., Kim, Y., & Molnár, Z. (2018). Subset of Cortical Layer 6b Neurons Selectively Innervates Higher Order Thalamic Nuclei in Mice. *Cerebral Cortex (New York, N.Y.: 1991)*, *28*(5), 1882–1897. https://doi.org/10.1093/CERCOR/BHY036

Hoerder-Suabedissen, A., & Molnár, Z. (2015). Development, evolution and pathology of neocortical subplate neurons. *Nature Reviews. Neuroscience*, *16*(3), 133–146. https://doi.org/10.1038/NRN3915

Holdefer, R. N., Norton, T. T., & Godwin, D. W. (1989). Effects of bicuculline on signal detectability in lateral geniculate nucleus relay cells. *Brain Research*, *488*(1–2), 341–347. https://doi.org/10.1016/0006-8993(89)90727-0

Holtman, I. R., Skola, D., & Glass, C. K. (2017). Transcriptional control of microglia phenotypes in health and disease. *The Journal of Clinical Investigation*, *127*(9), 3220–3229. https://doi.org/10.1172/JCI90604

Hoshiko, M., Arnoux, I., Avignone, E., Yamamoto, N., & Audinat, E. (2012). Deficiency of the microglial receptor CX3CR1 impairs postnatal functional development of thalamocortical synapses in the barrel cortex. *Journal of Neuroscience*, *32*(43), 15106–15111. https://doi.org/10.1523/JNEUROSCI.1167-12.2012

Hua, J. Y., & Smith, S. J. (2004). Neural activity and the dynamics of central nervous system development. *Nature Neuroscience*, *7*(4), 327–332. https://doi.org/10.1038/NN1218

Huberman, A. D., Feller, M. B., & Chapman, B. (2008). Mechanisms underlying development of visual maps and receptive fields. *Annual Review of Neuroscience*, *31*, 479–509. https://doi.org/10.1146/ANNUREV.NEURO.31.060407.125533

Huberman, A. D., Speer, C. M., & Chapman, B. (2006). Spontaneous retinal activity mediates development of ocular dominance columns and binocular receptive fields in v1. *Neuron*, *52*(2), 247–254. https://doi.org/10.1016/J.NEURON.2006.07.028

Huerga-Gómez I, Martini JF, López-Bendito G (2023). Building thalamic neuronal networks during mouse development. Front Neural Circuits 2023 Feb 3;17:1098913. doi: 10.3389/fncir.2023.1098913. eCollection 2023.

Ibrahim, L. A., Huang, S., Fernandez-Otero, M., Sherer, M., Qiu, Y., Vemuri, S., Xu, Q., Machold, R., Pouchelon, G., Rudy, B., & Fishell, G. (2021). Bottom-up inputs are required for establishment of top-down connectivity onto cortical layer 1 neurogliaform cells. *Neuron*, *109*(21), 3473-3485.e5. https://doi.org/10.1016/J.NEURON.2021.08.004

Inamura, N., Kimura, T., Tada, S., Kurahashi, T., Yanagida, M., Yanagawa, Y., Ikenaka, K., & Murakami, F. (2012). Intrinsic and extrinsic mechanisms control the termination of cortical interneuron migration. *Journal of Neuroscience*, *32*(17), 6032–6042. https://doi.org/10.1523/jneurosci.3446-11.2012

Inamura, N., Ono, K., Takebayashi, H., Zalc, B., & Ikenaka, K. (2011). Olig2 lineage cells generate GABAergic neurons in the prethalamic nuclei, including the zona incerta, ventral lateral geniculate nucleus and reticular thalamic nucleus. *Developmental Neuroscience*, *33*(2), 118–129. https://doi.org/10.1159/000328974

Innocenti, G. M., Clarke, S., & Koppel, H. (1983). Transitory macrophages in the white matter of the developing visual cortex. II. Development and relations with axonal pathways. *Brain Research*, *313*(1), 55–66. https://doi.org/10.1016/0165-3806(83)90201-8

Iwasato, T., Datwani, A., Wolf, A. M., Nishiyama, H., Taguchi, Y., Tonegawa, S., Knöpfel, T., Erzurumlu, R. S., & Itohara, S. (2000). Cortex-restricted disruption of NMDAR1 impairs neuronal patterns in the barrel cortex. *Nature*, *406*(6797), 726–731. https://doi.org/10.1038/35021059

Iwasato, T., Inan, M., Kanki, H., Erzurumlu, R. S., Itohara, S., & Crair, M. C. (2008). Cortical adenylyl cyclase 1 is required for thalamocortical synapse maturation and aspects of layer IV barrel development. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *28*(23), 5931–5943. https://doi.org/10.1523/JNEUROSCI.0815-08.2008

Izraeli, R., Koay, G., Lamish, M., Heicklen-Klein, A. J., Heffner, H. E., Heffner, R. S., & Wollberg, Z. (2002). Cross-modal neuroplasticity in neonatally enucleated hamsters: structure, electrophysiology and behaviour. *The European Journal of Neuroscience*, *15*(4), 693–712. https://doi.org/10.1046/J.1460-9568.2002.01902.X

Jabaudon, D. (2017). Fate and freedom in developing neocortical circuits. In *Nature Communications* (Vol. 8). Nature Publishing Group. https://doi.org/10.1038/ncomms16042

Jacobs, E. C., Campagnoni, C., Kampf, K., Reyes, S. D., Kalra, V., Handley, V., Xie, Y. Y., Hong-Hu, Y., Spreur, V., Fisher, R. S., & Campagnoni, A. T. (2007). Visualization of corticofugal projections during early cortical development in a tau-GFP-transgenic mouse. *The European Journal of Neuroscience*, *25*(1), 17–30. https://doi.org/10.1111/J.1460-9568.2006.05258.X

Jager, P., Moore, G., Calpin, P., Durmishi, X., Salgarella, I., Menage, L., Kita, Y., Wang, Y., Kim, D. W., Blackshaw, S., Schultz, S. R., Brickley, S., Shimogori, T., & Delogu, A. (2021). Dual midbrain and forebrain origins of thalamic inhibitory interneurons. *ELife*, *10*, 1–29. https://doi.org/10.7554/ELIFE.59272

Jager, P., Ye, Z., Yu, X., Zagoraiou, L., Prekop, H. T., Partanen, J., Jessell, T. M., Wisden, W., Brickley, S. G., & Delogu, A. (2016). Tectal-derived interneurons contribute to phasic and tonic inhibition in the visual thalamus. *Nature Communications*, *7*. https://doi.org/10.1038/ncomms13579

Jones, E. G. (2002). Thalamic circuitry and thalamocortical synchrony. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *357*(1428), 1659–1673. https://doi.org/10.1098/RSTB.2002.1168

Kala, K., Haugas, M., Lilleväli, K., Guimera, J., Wurst, W., Salminen, M., & Partanen, J. (2009). Gata2 is a tissue-specific post-mitotic selector gene for midbrain GABAergic neurons. *Development (Cambridge, England)*, *136*(2), 253–262. https://doi.org/10.1242/DEV.029900

Kanold, P. O., & Luhmann, H. J. (2010). The subplate and early cortical circuits. *Annual Review of Neuroscience*, *33*, 23–48. https://doi.org/10.1146/ANNUREV-NEURO-060909-153244

Kastli, R., Vighagen, R., van der Bourg, A., Argunsah, A. Ö., Iqbal, A., Voigt, F. F., Kirschenbaum, D., Aguzzi, A., Helmchen, F., & Karayannis, T. (2020). Developmental divergence of sensory stimulus representation in cortical interneurons. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-020-19427-z

Kataoka, A., & Shimogori, T. (2008). Fgf8 controls regional identity in the developing thalamus. *Development (Cambridge, England)*, *135*(17), 2873–2881. https://doi.org/10.1242/DEV.021618

Kato, G., Inada, H., Wake, H., Akiyoshi, R., Miyamoto, A., Eto, K., Ishikawa, T., Moorhouse, A. J., Strassman, A. M., & Nabekura, J. (2016). Microglial Contact Prevents Excess Depolarization and Rescues Neurons from Excitotoxicity. *ENeuro*, *3*(3), 9133–9144. https://doi.org/10.1523/ENEURO.0004-16.2016

Kerschensteiner, D. (2016). Glutamatergic Retinal Waves. *Frontiers in Neural Circuits*, *10*(MAY). https://doi.org/10.3389/FNCIR.2016.00038

Kierdorf, K., Erny, D., Goldmann, T., Sander, V., Schulz, C., Perdiguero, E. G., Wieghofer, P., Heinrich, A., Riemke, P., Hölscher, C., Müller, D. N., Luckow, B., Brocker, T., Debowski, K., Fritz, G., Opdenakker, G., Diefenbach, A., Biber, K., Heikenwalder, M., … Prinz, M. (2013). Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nature Neuroscience*, *16*(3), 273–280. https://doi.org/10.1038/NN.3318

Kim, G. J., Shatz, C. J., & McConnell, S. K. (1991). Morphology of pioneer and follower growth cones in the developing cerebral cortex. *Journal of Neurobiology*, *22*(6), 629–642. https://doi.org/10.1002/NEU.480220608

Kirischuk, S., Sinning, A., Blanquie, O., Yang, J. W., Luhmann, H. J., & Kilb, W. (2017). Modulation of Neocortical Development by Early Neuronal Activity: Physiology and Pathophysiology. *Frontiers in Cellular Neuroscience*, *11*. https://doi.org/10.3389/FNCEL.2017.00379

Ko, H., Cossell, L., Baragli, C., Antolik, J., Clopath, C., Hofer, S. B., & Mrsic-Flogel, T. D. (2013). The emergence of functional microcircuits in visual cortex. *Nature*, *496*(7443), 96–100. https://doi.org/10.1038/NATURE12015

Kolodziejczak, M., Béchade, C., Gervasi, N., Irinopoulou, T., Banas, S. M., Cordier, C., Rebsam, A., Roumier, A., & Maroteaux, L. (2015). Serotonin Modulates Developmental Microglia via 5-HT2B Receptors: Potential Implication during Synaptic Refinement of Retinogeniculate Projections. *ACS Chemical Neuroscience*, *6*(7), 1219–1230. https://doi.org/10.1021/CN5003489

Lauber, K., Bohn, E., Kröber, S. M., Xiao, Y. J., Blumenthal, S. G., Lindemann, R. K., Marini, P., Wiedig, C., Zobywalski, A., Baksh, S., Xu, Y., Autenrieth, I. B., Schulze-Osthoff, K., Belka, C., Stuhler, G., & Wesselborg, S. (2003). Apoptotic cells induce migration of phagocytes via caspase-3-mediated release of a lipid attraction signal. *Cell*, *113*(6), 717–730. https://doi.org/10.1016/S0092-8674(03)00422-7

Lavdas, A. A., Grigoriou, M., Pachnis, V., & Parnavelas, J. G. (1999). The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex.

The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, *19*(18), 7881–7888. https://doi.org/10.1523/JNEUROSCI.19-18-07881.1999

Lavin, Y., Winter, D., Blecher-Gonen, R., David, E., Keren-Shaul, H., Merad, M., Jung, S., & Amit, I. (2014). Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell*, *159*(6), 1312–1326. https://doi.org/10.1016/J.CELL.2014.11.018

Lee, C. C., & Murray Sherman, S. (2010). Drivers and modulators in the central auditory pathways. *Frontiers in Neuroscience*, *4*(MAY). https://doi.org/10.3389/NEURO.01.014.2010

Lee, L. J., Iwasato, T., Itohara, S., & Erzurumlu, R. S. (2005). Exuberant thalamocortical axon arborization in cortex-specific NMDAR1 knockout mice. *The Journal of Comparative Neurology*, *485*(4), 280–292. https://doi.org/10.1002/CNE.20481

Leighton, A. H., Cheyne, J. E., Houwen, G. J., Maldonado, P. P., de Winter, F., Levelt, C. N., & Lohmann, C. (2021). Somatostatin interneurons restrict cell recruitment to retinally driven spontaneous activity in the developing cortex. *Cell Reports*, *36*(1). https://doi.org/10.1016/j.celrep.2021.109316

Leist, M., Datunashvilli, M., Kanyshkova, T., Zobeiri, M., Aissaoui, A., Cerina, M., Romanelli, M. N., Pape, H. C., & Budde, T. (2016). Two types of interneurons in the mouse lateral geniculate nucleus are characterized by different h-current density. *Scientific Reports*, *6*. https://doi.org/10.1038/srep24904

Leyva-Díaz, E., del Toro, D., Menal, M. J., Cambray, S., Susín, R., Tessier-Lavigne, M., Klein, R., Egea, J., & López-Bendito, G. (2014). FLRT3 is a Robo1-interacting protein that determines Netrin-1 attraction in developing axons. *Current Biology : CB*, *24*(5), 494–508. https://doi.org/10.1016/J.CUB.2014.01.042

Li, H., Fertuzinhos, S., Mohns, E., Hnasko, T. S., Verhage, M., Edwards, R., Sestan, N., & Crair, M. C. (2013). Laminar and columnar development of barrel cortex relies on thalamocortical neurotransmission. *Neuron*, *79*(5), 970–986. https://doi.org/10.1016/J.NEURON.2013.06.043

Li, H., Han, Y. R., Bi, C., Davila, J., Goff, L. A., Thompson, K., Swerdel, M., Camarillo, C., Ricupero, C. L., Hart, R. P., Plummer, M. R., & Grumet, M. (2008). Functional differentiation of a clone resembling embryonic cortical interneuron progenitors. *Developmental Neurobiology*, *68*(14), 1549–1564. https://doi.org/10.1002/DNEU.20679

Li, K., Zhang, J., & Li, J. Y. H. (2012). Gbx2 plays an essential but transient role in the formation of thalamic nuclei. *PloS One*, *7*(10). https://doi.org/10.1371/JOURNAL.PONE.0047111

Li, Q., & Barres, B. A. (2018). Microglia and macrophages in brain homeostasis and disease. *Nature Reviews. Immunology*, *18*(4), 225–242. https://doi.org/10.1038/NRI.2017.125

Li, Y., Du, X. F., Liu, C. S., Wen, Z. L., & Du, J. L. (2012). Reciprocal regulation between resting microglial dynamics and neuronal activity in vivo. *Developmental Cell*, *23*(6), 1189–1202. https://doi.org/10.1016/J.DEVCEL.2012.10.027

Lieberman, A. R. (1973). Neurons with presynaptic perikarya and presynaptic dendrites in the rat lateral geniculate nucleus. *Brain Research*, *59*(C), 35–59. https://doi.org/10.1016/0006-8993(73)90252-7

Lim, L., Mi, D., Llorca, A., & Marín, O. (2018). Development and Functional Diversification of Cortical Interneurons. In *Neuron* (Vol. 100, Issue 2, pp. 294–313). Cell Press. https://doi.org/10.1016/j.neuron.2018.10.009

Little, G. E., López-Bendito, G., Rünker, A. E., García, N., Piñon, M. C., Chédotal, A., Molnár, Z., & Mitchell, K. J. (2009). Specificity and plasticity of thalamocortical connections in Sema6A mutant mice. *PLoS Biology*, *7*(4), 0756–0770. https://doi.org/10.1371/JOURNAL.PBIO.1000098

Liu, Y. U., Ying, Y., Li, Y., Eyo, U. B., Chen, T., Zheng, J., Umpierre, A. D., Zhu, J., Bosco, D. B., Dong, H., & Wu, L. J. (2019). Neuronal network activity controls microglial process surveillance in awake mice via norepinephrine signaling. *Nature Neuroscience*, *22*(11), 1771–1781. https://doi.org/10.1038/s41593-019-0511-3

Lodato, S., Rouaux, C., Quast, K. B., Jantrachotechatchawan, C., Studer, M., Hensch, T. K., & Arlotta, P. (2011). Excitatory projection neuron subtypes control the distribution of local inhibitory interneurons in the cerebral cortex. *Neuron*, *69*(4), 763–779. https://doi.org/10.1016/J.NEURON.2011.01.015

Lokmane, L., & Garel, S. (2014). Map transfer from the thalamus to the neocortex: inputs from the barrel field. *Seminars in Cell & Developmental Biology*, *35*, 147–155. https://doi.org/10.1016/J.SEMCDB.2014.07.005

Lokmane, L., Proville, R., Narboux-Nême, N., Györy, I., Keita, M., Mailhes, C., Léna, C., Gaspar, P., Grosschedl, R., & Garel, S. (2013). Sensory map transfer to the neocortex relies on pretarget ordering of thalamic axons. *Current Biology : CB*, *23*(9), 810–816. https://doi.org/10.1016/J.CUB.2013.03.062

López-Bendito, G. (2018). Development of the Thalamocortical Interactions: Past, Present and Future. *Neuroscience*, *385*, 67–74. https://doi.org/10.1016/J.NEUROSCIENCE.2018.06.020

López-Bendito, G., Cautinat, A., Sánchez, J. A., Bielle, F., Flames, N., Garratt, A. N., Talmage, D. A., Role, L. W., Charnay, P., Marín, O., & Garel, S. (2006). Tangential neuronal migration controls axon guidance: a role for neuregulin-1 in thalamocortical axon navigation. *Cell*, *125*(1), 127–142. https://doi.org/10.1016/J.CELL.2006.01.042

López-Bendito, G., Flames, N., Ma, L., Fouquet, C., di Meglio, T., Chedotal, A., Tessier-Lavigne, M., & Marín, O. (2007). Robo1 and Robo2 cooperate to control the guidance of major axonal tracts in the mammalian forebrain. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *27*(13), 3395–3407. https://doi.org/10.1523/JNEUROSCI.4605-06.2007

López-Bendito, G., & Molnár, Z. (2003). Thalamocortical development: how are we going to get there? *Nature Reviews. Neuroscience*, *4*(4), 276–289. https://doi.org/10.1038/NRN1075

López-Bendito, G., Sánchez-Alcañiz, J. A., Pla, R., Borrell, V., Picó, E., Valdeolmillos, M., & Marín, O. (2008). Chemokine signaling controls intracortical migration and final distribution of GABAergic interneurons. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *28*(7), 1613–1624. https://doi.org/10.1523/JNEUROSCI.4651-07.2008

Luhmann, H. J., & Khazipov, R. (2018). Neuronal activity patterns in the developing barrel cortex. *Neuroscience*, *368*, 256–267. https://doi.org/10.1016/J.NEUROSCIENCE.2017.05.025

Lyckman, A. W., Horng, S., Leamey, C. A., Tropea, D., Watakabe, A., van Wart, A., McCurry, C., Yamamori, T., & Sur, M. (2008). Gene expression patterns in visual cortex during the critical period: synaptic stabilization and reversal by visual deprivation. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(27), 9409–9414. https://doi.org/10.1073/PNAS.0710172105

Majdan, M., & Shatz, C. J. (2006). Effects of visual experience on activity-dependent gene regulation in cortex. *Nature Neuroscience*, *9*(5), 650–659. https://doi.org/10.1038/NN1674

Mallika, C., Guo, Q., & Li, J. Y. H. (2015). Gbx2 is essential for maintaining thalamic neuron identity and repressing habenular characters in the developing thalamus. *Developmental Biology*, *407*(1), 26–39. https://doi.org/10.1016/J.YDBIO.2015.08.010

Marcos-Mondéjar, P., Peregrín, S., Li, J. Y., Carlsson, L., Tole, S., & López-Bendito, G. (2012). The lhx2 transcription factor controls thalamocortical axonal guidance by

specific regulation of robo1 and robo2 receptors. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *32*(13), 4372–4385. https://doi.org/10.1523/JNEUROSCI.5851-11.2012

Marín, O., Yaron, A., Bagri, A., Tessier-Lavigne, M., & Rubenstein, J. L. R. (2001a). Sorting of striatal and cortical interneurons regulated by semaphorin-neuropilin interactions. *Science (New York, N.Y.)*, *293*(5531), 872–875. https://doi.org/10.1126/SCIENCE.1061891

Marín, O., Yaron, A., Bagri, A., Tessier-Lavigne, M., & Rubenstein, J. L. R. (2001b). Sorting of striatal and cortical interneurons regulated by semaphorin-neuropilin interactions. *Science (New York, N.Y.)*, *293*(5531), 872–875. https://doi.org/10.1126/SCIENCE.1061891

Marín-Teva, J. L., Almendros, A., Calvente, R., Cuadros, M. A., & Navascués, J. (1999). Proliferation of actively migrating ameboid microglia in the developing quail retina. *Anatomy and Embryology*, *200*(3), 289–300. https://doi.org/10.1007/S004290050280

Marín-Teva, J. L., Dusart, I., Colin, C., Gervais, A., van Rooijen, N., & Mallat, M. (2004). Microglia Promote the Death of Developing Purkinje Cells. *Neuron*, *41*(4), 535–547. https://doi.org/10.1016/S0896-6273(04)00069-8

Markram, H., Toledo-Rodriguez, M., Wang, Y., Gupta, A., Silberberg, G., & Wu, C. (2004). Interneurons of the neocortical inhibitory system. *Nature Reviews. Neuroscience*, *5*(10), 793–807. https://doi.org/10.1038/NRN1519

Marques-Smith, A., Lyngholm, D., Kaufmann, A. K., Stacey, J. A., Hoerder-Suabedissen, A., Becker, E. B. E., Wilson, M. C., Molnár, Z., & Butt, S. J. B. (2016). A Transient Translaminar GABAergic Interneuron Circuit Connects Thalamocortical Recipient Layers in Neonatal Somatosensory Cortex. *Neuron*, *89*(3), 536–549. https://doi.org/10.1016/j.neuron.2016.01.015

Martinez-Ferre, A., & Martinez, S. (2009). The development of the thalamic motor learning area is regulated by Fgf8 expression. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *29*(42), 13389–13400. https://doi.org/10.1523/JNEUROSCI.2625-09.2009

Martinez-Ferre, A., & Martinez, S. (2012). Molecular regionalization of the diencephalon. *Frontiers in Neuroscience*, *6*(MAY). https://doi.org/10.3389/FNINS.2012.00073

Martini, F. J., Guillamón-Vivancos, T., Moreno-Juan, V., Valdeolmillos, M., & López-Bendito, G. (2021). Spontaneous activity in developing thalamic and cortical sensory networks. In *Neuron* (Vol. 109, Issue 16, pp. 2519–2534). Cell Press. https://doi.org/10.1016/j.neuron.2021.06.026

Martini, F. J., Moreno-Juan, V., Filipchuk, A., Valdeolmillos, M., & López-Bendito, G. (2018). Impact of thalamocortical input on barrel cortex development. *Neuroscience*, *368*, 246–255. https://doi.org/10.1016/J.NEUROSCIENCE.2017.04.005

Martini, F. J., Valiente, M., Bendito, G. L., Szabó, G., Moya, F., Valdeolmillos, M., & Marín, O. (2009). Biased selection of leading process branches mediates chemotaxis during tangential neuronal migration. *Development (Cambridge, England)*, *136*(1), 41–50. https://doi.org/10.1242/DEV.025502

Masland, R. H. (2012). The neuronal organization of the retina. *Neuron*, *76*(2), 266–280. https://doi.org/10.1016/J.NEURON.2012.10.002

Matcovitch-Natan, O., Winter, D. R., Giladi, A., Aguilar, S. V., Spinrad, A., Sarrazin, S., Ben-Yehuda, H., David, E., González, F. Z., Perrin, P., Keren-Shaul, H., Gury, M., Lara-Astaiso, D., Thaiss, C. A., Cohen, M., Halpern, K. B., Baruch, K., Deczkowska, A., Lorenzo-Vivas, E., … Amit, I. (2016). Microglia development follows a stepwise program to regulate brain homeostasis. *Science (New York, N.Y.)*, *353*(6301). https://doi.org/10.1126/SCIENCE.AAD8670

Mayer, C., Hafemeister, C., Bandler, R. C., Machold, R., Batista Brito, R., Jaglin, X., Allaway, K., Butler, A., Fishell, G., & Satija, R. (2018a). Developmental diversification of cortical inhibitory interneurons. *Nature*, *555*(7697), 457–462. https://doi.org/10.1038/nature25999

Mayer, C., Hafemeister, C., Bandler, R. C., Machold, R., Batista Brito, R., Jaglin, X., Allaway, K., Butler, A., Fishell, G., & Satija, R. (2018b). Developmental diversification of cortical inhibitory interneurons. *Nature*, *555*(7697), 457–462. https://doi.org/10.1038/NATURE25999

Mayer, C., Jaglin, X. H., Cobbs, L. v., Bandler, R. C., Streicher, C., Cepko, C. L., Hippenmeyer, S., & Fishell, G. (2015). Clonally Related Forebrain Interneurons Disperse Broadly across Both Functional Areas and Structural Boundaries. *Neuron*, *87*(5), 989–998. https://doi.org/10.1016/J.NEURON.2015.07.011

McConnell, S. K., Ghosh, A., & Shatz, C. J. (1989). Subplate neurons pioneer the first axon pathway from the cerebral cortex. *Science (New York, N.Y.)*, *245*(4921), 978–982. https://doi.org/10.1126/SCIENCE.2475909

McLaughlin, T., Hindges, R., & O'Leary, D. D. M. (2003). Regulation of axial patterning of the retina and its topographic mapping in the brain. *Current Opinion in Neurobiology*, *13*(1), 57–69. https://doi.org/10.1016/S0959-4388(03)00014-X

Merzenich, M. M., Knight, P. L., & Roth, G. L. (1975). Representation of cochlea within primary auditory cortex in the cat. *Journal of Neurophysiology*, *38*(2), 231–249. https://doi.org/10.1152/JN.1975.38.2.231

Mi, D., Li, Z., Lim, L., Li, M., Moissidis, M., Yang, Y., Gao, T., Hu, T. X., Pratt, T., Price, D. J., Sestan, N., & Marín, O. (2018). Early emergence of cortical interneuron diversity in the mouse embryo. *Science (New York, N.Y.)*, *360*(6384), 81–85. https://doi.org/10.1126/SCIENCE.AAR6821

Minlebaev, M., Colonnese, M., Tsintsadze, T., Sirota, A., & Khazipov, R. (2011). Early γ oscillations synchronize developing thalamus and cortex. *Science (New York, N.Y.)*, *334*(6053), 226–229. https://doi.org/10.1126/SCIENCE.1210574

Minten, C., Terry, R., Deffrasnes, C., King, N. J. C., & Campbell, I. L. (2012). IFN regulatory factor 8 is a key constitutive determinant of the morphological and molecular properties of microglia in the CNS. *PloS One*, *7*(11). https://doi.org/10.1371/JOURNAL.PONE.0049851

Mione, M. C., Cavanagh, J. F. R., Harris, B., & Parnavelas, J. G. (1997). Cell fate specification and symmetrical/asymmetrical divisions in the developing cerebral cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *17*(6), 2018–2929. https://doi.org/10.1523/JNEUROSCI.17-06-02018.1997

Mire, E., Mezzera, C., Leyva-Díáz, E., Paternain, A. v., Squarzoni, P., Bluy, L., Castillo-Paterna, M., López, M. J., Peregrín, S., Tessier-Lavigne, M., Garel, S., Galcerán, J., Lerma, J., & López-Bendito, G. (2012). Spontaneous activity regulates Robo1 transcription to mediate a switch in thalamocortical axon growth. *Nature Neuroscience*, *15*(8), 1134–1143. https://doi.org/10.1038/NN.3160

Mishima, T., Nagai, T., Yahagi, K., Akther, S., Oe, Y., Monai, H., Kohsaka, S., & Hirase, H. (2019). Transcranial Direct Current Stimulation (tDCS) Induces Adrenergic Receptor-Dependent Microglial Morphological Changes in Mice. *ENeuro*, *6*(5). https://doi.org/10.1523/ENEURO.0204-19.2019

Miyoshi, G., & Fishell, G. (2011). GABAergic interneuron lineages selectively sort into specific cortical layers during early postnatal development. *Cerebral Cortex*, *21*(4), 845–852. https://doi.org/10.1093/cercor/bhq155

Miyoshi, G., Hjerling-Leffler, J., Karayannis, T., Sousa, V. H., Butt, S. J. B., Battiste, J., Johnson, J. E., Machold, R. P., & Fishell, G. (2010). Genetic fate mapping reveals that the caudal ganglionic eminence produces a large and diverse population of superficial

cortical interneurons. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *30*(5), 1582–1594. https://doi.org/10.1523/JNEUROSCI.4515-09.2010

Modol, L., Bollmann, Y., Tressard, T., Baude, A., Che, A., Duan, Z. R. S., Babij, R., de Marco García, N. v., & Cossart, R. (2020). Assemblies of Perisomatic GABAergic Neurons in the Developing Barrel Cortex. *Neuron*, *105*(1), 93-105.e4. https://doi.org/10.1016/j.neuron.2019.10.007

Molnár, Z., & Cordery, P. (1999). Connections between cells of the internal capsule, thalamus, and cerebral cortex in embryonic rat. *The Journal of Comparative Neurology*, *413*(1), 1–25. https://doi.org/10.1002/(sici)1096-9861(19991011)413:1<1::aid-cne1>3.0.co;2-5

Molnár, Z., Garel, S., López-Bendito, G., Maness, P., & Price, D. J. (2012). Mechanisms controlling the guidance of thalamocortical axons through the embryonic forebrain. *The European Journal of Neuroscience*, *35*(10), 1573–1585. https://doi.org/10.1111/J.1460-9568.2012.08119.X

Molnár, Z., Luhmann, H. J., & Kanold, P. O. (2020). Transient cortical circuits match spontaneous and sensory-driven activity during development. *Science (New York, N.Y.)*, *370*(6514). https://doi.org/10.1126/SCIENCE.ABB2153

Molyneaux, B. J., Arlotta, P., Menezes, J. R. L., & Macklis, J. D. (2007). Neuronal subtype specification in the cerebral cortex. *Nature Reviews. Neuroscience*, *8*(6), 427–437. https://doi.org/10.1038/NRN2151

Monier, A., Evrard, P., Gressens, P., & Verney, C. (2006). Distribution and differentiation of microglia in the human encephalon during the first two trimesters of gestation. *The Journal of Comparative Neurology*, *499*(4), 565–582. https://doi.org/10.1002/CNE.21123

Montero, V. M. (1986). The interneuronal nature of GABAergic neurons in the lateral geniculate nucleus of the rhesus monkey: a combined HRP and GABA-immunocytochemical study. *Experimental Brain Research*, *64*(3), 615–622. https://doi.org/10.1007/BF00340502

Mooney, R., Penn, A. A., Gallego, R., & Shatz, C. J. (1996). Thalamic relay of spontaneous retinal activity prior to vision. *Neuron*, *17*(5), 863–874. https://doi.org/10.1016/S0896-6273(00)80218-4

Morales, B., Choi, S. Y., & Kirkwood, A. (2002). Dark rearing alters the development of GABAergic transmission in visual cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *22*(18), 8084–8090. https://doi.org/10.1523/JNEUROSCI.22-18-08084.2002

Moreno-Juan, V., Filipchuk, A., Antón-Bolaños, N., Mezzera, C., Gezelius, H., Andrés, B., Rodríguez-Malmierca, L., Susín, R., Schaad, O., Iwasato, T., Schüle, R., Rutlin, M., Nelson, S., Ducret, S., Valdeolmillos, M., Rijli, F. M., & López-Bendito, G. (2017). Prenatal thalamic waves regulate cortical area size prior to sensory processing. *Nature Communications*, *8*. https://doi.org/10.1038/NCOMMS14172

Morgan, J. L., & Lichtman, J. W. (2020). An Individual Interneuron Participates in Many Kinds of Inhibition and Innervates Much of the Mouse Visual Thalamus. *Neuron*, *106*(3), 468-481.e2. https://doi.org/10.1016/J.NEURON.2020.02.001

Mosser, C. A., Baptista, S., Arnoux, I., & Audinat, E. (2017). Microglia in CNS development: Shaping the brain for the future. *Progress in Neurobiology*, *149–150*, 1–20. https://doi.org/10.1016/J.PNEUROBIO.2017.01.002

Mountcastle, V. B. (1997). The columnar organization of the neocortex. *Brain : A Journal of Neurology*, *120 (Pt 4)*(4), 701–722. https://doi.org/10.1093/BRAIN/120.4.701

Murata, Y., & Colonnese, M. T. (2016). An excitatory cortical feedback loop gates retinal wave transmission in rodent thalamus. *ELife*, *5*(OCTOBER2016). https://doi.org/10.7554/ELIFE.18816

Nakagawa, Y., & O'Leary, D. D. M. (2001). Combinatorial expression patterns of LIM-homeodomain and other regulatory genes parcellate developing thalamus. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *21*(8), 2711–2725. https://doi.org/10.1523/JNEUROSCI.21-08-02711.2001

Nakagawa, Y., & Shimogori, T. (2012). Diversity of thalamic progenitor cells and postmitotic neurons. *The European Journal of Neuroscience*, *35*(10), 1554–1562. https://doi.org/10.1111/J.1460-9568.2012.08089.X

Nakanishi, M., Niidome, T., Matsuda, S., Akaike, A., Kihara, T., & Sugimoto, H. (2007). Microglia-derived interleukin-6 and leukaemia inhibitory factor promote astrocytic differentiation of neural stem/progenitor cells. *The European Journal of Neuroscience*, *25*(3), 649–658. https://doi.org/10.1111/J.1460-9568.2007.05309.X

Narboux-Nême, N., Evrard, A., Ferezou, I., Erzurumlu, R. S., Kaeser, P. S., Lainé, J., Rossier, J., Ropert, N., Südhof, T. C., & Gaspar, P. (2012). Neurotransmitter release at the thalamocortical synapse instructs barrel formation but not axon patterning in the somatosensory cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *32*(18), 6183–6196. https://doi.org/10.1523/JNEUROSCI.0343-12.2012

Négyessy, L., Gál, V., Farkas, T., & Toldi, J. (2000). Cross-modal plasticity of the corticothalamic circuits in rats enucleated on the first postnatal day. *The European Journal of Neuroscience*, *12*(5), 1654–1668. https://doi.org/10.1046/J.1460-9568.2000.00057.X

Nery, S., Fishell, G., & Corbin, J. G. (2002). The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations. *Nature Neuroscience*, *5*(12), 1279–1287. https://doi.org/10.1038/NN971

Nikodemova, M., Kimyon, R. S., De, I., Small, A. L., Collier, L. S., & Watters, J. J. (2015). Microglial numbers attain adult levels after undergoing a rapid decrease in cell number in the third postnatal week. *Journal of Neuroimmunology*, *278*, 280–288. https://doi.org/10.1016/J.JNEUROIM.2014.11.018

Nimmerjahn, A., Kirchhoff, F., & Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science (New York, N.Y.)*, *308*(5726), 1314–1318. https://doi.org/10.1126/SCIENCE.1110647

Niquille, M., Limoni, G., Markopoulos, F., Cadilhac, C., Prados, J., Holtmaat, A., & Dayer, A. (2018). Neurogliaform cortical interneurons derive from cells in the preoptic area. *ELife*, *7*. https://doi.org/10.7554/ELIFE.32017

Ohara, P. T., & Lieberman, A. R. (1993). Some aspects of the synaptic circuitry underlying inhibition in the ventrobasal thalamus. *Journal of Neurocytology*, *22*(9), 815–825. https://doi.org/10.1007/BF01181326

O'Leary, D. D. M. (1989). Do cortical areas emerge from a protocortex? *Trends in Neurosciences*, *12*(10), 400–406. https://doi.org/10.1016/0166-2236(89)90080-5

Olsen, S. R., Bortone, D. S., Adesnik, H., & Scanziani, M. (2012). Gain control by layer six in cortical circuits of vision. *Nature*, *483*(7387), 47–54. https://doi.org/10.1038/NATURE10835

Orr, A. G., Orr, A. L., Li, X. J., Gross, R. E., & Traynelis, S. F. (2009). Adenosine A(2A) receptor mediates microglial process retraction. *Nature Neuroscience*, *12*(7), 872–878. https://doi.org/10.1038/NN.2341

Otero, K., Turnbull, I. R., Poliani, P. L., Vermi, W., Cerutti, E., Aoshi, T., Tassi, I., Takai, T., Stanley, S. L., Miller, M., Shaw, A. S., & Colonna, M. (2009). Macrophage colony-stimulating factor induces the proliferation and survival of macrophages via a pathway involving DAP12 and beta-catenin. *Nature Immunology*, *10*(7), 734–743. https://doi.org/10.1038/NI.1744

Palis, J., Robertson, S., Kennedy, M., Wall, C., & Keller, G. (1999). Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. *Development (Cambridge, England)*, *126*(22), 5073–5084. https://doi.org/10.1242/DEV.126.22.5073

Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., Giustetto, M., Ferreira, T. A., Guiducci, E., Dumas, L., Ragozzino, D., & Gross, C. T. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science (New York, N.Y.)*, *333*(6048), 1456–1458. https://doi.org/10.1126/SCIENCE.1202529

Parkhurst, C. N., Yang, G., Ninan, I., Savas, J. N., Yates, J. R., Lafaille, J. J., Hempstead, B. L., Littman, D. R., & Gan, W. B. (2013). Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell*, *155*(7), 1596–1609. https://doi.org/10.1016/J.CELL.2013.11.030

Pascual-Leone, A., Amedi, A., Fregni, F., & Merabet, L. B. (2005). The plastic human brain cortex. *Annual Review of Neuroscience*, *28*, 377–401. https://doi.org/10.1146/ANNUREV.NEURO.27.070203.144216

Penn, A. A., Riquelme, P. A., Feller, M. B., & Shatz, C. J. (1998). Competition in retinogeniculate patterning driven by spontaneous activity. *Science (New York, N.Y.)*, *279*(5359), 2108–2112. https://doi.org/10.1126/SCIENCE.279.5359.2108

Petersen, C. C. H. (2007). The functional organization of the barrel cortex. *Neuron*, *56*(2), 339–355. https://doi.org/10.1016/J.NEURON.2007.09.017

Petreanu, L., Mao, T., Sternson, S. M., & Svoboda, K. (2009). The subcellular organization of neocortical excitatory connections. *Nature*, *457*(7233), 1142–1145. https://doi.org/10.1038/NATURE07709

Petrus, E., Isaiah, A., Jones, A. P., Li, D., Wang, H., Lee, H. K., & Kanold, P. O. (2014). Crossmodal induction of thalamocortical potentiation leads to enhanced information processing in the auditory cortex. *Neuron*, *81*(3), 664–673. https://doi.org/10.1016/J.NEURON.2013.11.023

Petryniak, M. A., Potter, G. B., Rowitch, D. H., & Rubenstein, J. L. R. (2007). Dlx1 and Dlx2 control neuronal versus oligodendroglial cell fate acquisition in the developing forebrain. *Neuron*, *55*(3), 417–433. https://doi.org/10.1016/J.NEURON.2007.06.036

Pfeffer, C. K., Xue, M., He, M., Huang, Z. J., & Scanziani, M. (2013). Inhibition of inhibition in visual cortex: the logic of connections between molecularly distinct interneurons. *Nature Neuroscience*, *16*(8), 1068–1076. https://doi.org/10.1038/NN.3446

Pfeiffenberger, C., Cutforth, T., Woods, G., Yamada, J., Rentería, R. C., Copenhagen, D. R., Flanagan, J. G., & Feldheim, D. A. (2005). Ephrin-As and neural activity are required for eye-specific patterning during retinogeniculate mapping. *Nature Neuroscience*, *8*(8), 1022–1027. https://doi.org/10.1038/NN1508

Pfeiffer, T., Avignone, E., & Nägerl, U. V. (2016). Induction of hippocampal long-term potentiation increases the morphological dynamics of microglial processes and prolongs their contacts with dendritic spines. *Scientific Reports*, *6*. https://doi.org/10.1038/SREP32422

Pla, R., Borrell, V., Flames, N., & Marín, O. (2006). Layer acquisition by cortical GABAergic interneurons is independent of Reelin signaling. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *26*(26), 6924–6934. https://doi.org/10.1523/JNEUROSCI.0245-06.2006

Pleasure, S. J., Anderson, S., Hevner, R., Bagri, A., Marin, O., Lowenstein, D. H., & Rubenstein, J. L. R. (2000). Cell migration from the ganglionic eminences is required for the development of hippocampal GABAergic interneurons. *Neuron*, *28*(3), 727–740. https://doi.org/10.1016/S0896-6273(00)00149-5

Ponomarev, E. D., Veremeyko, T., Barteneva, N., Krichevsky, A. M., & Weiner, H. L. (2011). MicroRNA-124 promotes microglia quiescence and suppresses EAE by deactivating

macrophages via the C/EBP-α-PU.1 pathway. *Nature Medicine*, *17*(1), 64–70. https://doi.org/10.1038/NM.2266

Pont-Lezica, L., Béchade, C., Belarif-Cantaut, Y., Pascual, O., & Bessis, A. (2011). Physiological roles of microglia during development. *Journal of Neurochemistry*, *119*(5), 901–908. https://doi.org/10.1111/J.1471-4159.2011.07504.X

Pont-Lezica, L., Beumer, W., Colasse, S., Drexhage, H., Versnel, M., & Bessis, A. (2014). Microglia shape corpus callosum axon tract fasciculation: functional impact of prenatal inflammation. *The European Journal of Neuroscience*, *39*(10), 1551–1557. https://doi.org/10.1111/EJN.12508

Pouchelon, G., Gambino, F., Bellone, C., Telley, L., Vitali, I., Lüscher, C., Holtmaat, A., & Jabaudon, D. (2014). Modality-specific thalamocortical inputs instruct the identity of postsynaptic L4 neurons. *Nature*, *511*(7510), 471–474. https://doi.org/10.1038/NATURE13390

Price, D. J., Kennedy, H., Dehay, C., Zhou, L., Mercier, M., Jossin, Y., Goffinet, A. M., Tissir, F., Blakey, D., & Molnár, Z. (2006). The development of cortical connections. *The European Journal of Neuroscience*, *23*(4), 910–920. https://doi.org/10.1111/J.1460-9568.2006.04620.X

Priya, R., Paredes, M. F., Karayannis, T., Yusuf, N., Liu, X., Jaglin, X., Graef, I., Alvarez-Buylla, A., & Fishell, G. (2018). Activity Regulates Cell Death within Cortical Interneurons through a Calcineurin-Dependent Mechanism. *Cell Reports*, *22*(7), 1695–1709. https://doi.org/10.1016/j.celrep.2018.01.007

Puelles, L., & Rubenstein, J. L. R. (2003). Forebrain gene expression domains and the evolving prosomeric model. *Trends in Neurosciences*, *26*(9), 469–476. https://doi.org/10.1016/S0166-2236(03)00234-0

Rakic, P. (1988). Specification of cerebral cortical areas. *Science (New York, N.Y.)*, *241*(4862), 170–176. https://doi.org/10.1126/SCIENCE.3291116

Rakic, P. (1991). Experimental manipulation of cerebral cortical areas in primates. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *331*(1261), 291–294. https://doi.org/10.1098/RSTB.1991.0019

Rakic, P., Ayoub, A. E., Breunig, J. J., & Dominguez, M. H. (2009). Decision by division: making cortical maps. *Trends in Neurosciences*, *32*(5), 291–301. https://doi.org/10.1016/J.TINS.2009.01.007

Reichova, I., & Sherman, S. M. (2004). Somatosensory corticothalamic projections: distinguishing drivers from modulators. *Journal of Neurophysiology*, *92*(4), 2185–2197. https://doi.org/10.1152/JN.00322.2004

Renna, J. M., Weng, S., & Berson, D. M. (2011). Light acts through melanopsin to alter retinal waves and segregation of retinogeniculate afferents. *Nature Neuroscience*, *14*(7), 827–829. https://doi.org/10.1038/NN.2845

Rezaie, P., Patel, K., & Male, D. K. (1999). Microglia in the human fetal spinal cord - Patterns of distribution, morphology and phenotype. *Developmental Brain Research*, *115*(1), 71–81. https://doi.org/10.1016/S0165-3806(99)00043-7

Rigato, C., Buckinx, R., Le-Corronc, H., Rigo, J. M., & Legendre, P. (2011). Pattern of invasion of the embryonic mouse spinal cord by microglial cells at the time of the onset of functional neuronal networks. *Glia*, *59*(4), 675–695. https://doi.org/10.1002/GLIA.21140

Rochefort, N. L., Garaschuk, O., Milos, R. I., Narushima, M., Marandi, N., Pichler, B., Kovalchuk, Y., & Konnerth, A. (2009). Sparsification of neuronal activity in the visual cortex at eye-opening. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(35), 15049–15054. https://doi.org/10.1073/PNAS.0907660106

Rochefort, N. L., Narushima, M., Grienberger, C., Marandi, N., Hill, D. N., & Konnerth, A. (2011). Development of direction selectivity in mouse cortical neurons. *Neuron*, *71*(3), 425–432. https://doi.org/10.1016/J.NEURON.2011.06.013

Roumier, A., Béchade, C., Poncer, J. C., Smalla, K. H., Tomasello, E., Vivier, E., Gundelfinger, E. D., Triller, A., & Bessis, A. (2004). Impaired synaptic function in the microglial KARAP/DAP12-deficient mouse. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *24*(50), 11421–11428. https://doi.org/10.1523/JNEUROSCI.2251-04.2004

Rudy, B., Fishell, G., Lee, S. H., & Hjerling-Leffler, J. (2011). Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. *Developmental Neurobiology*, *71*(1), 45–61. https://doi.org/10.1002/DNEU.20853

Sánchez-Alcañiz, J. A., Haege, S., Mueller, W., Pla, R., Mackay, F., Schulz, S., López-Bendito, G., Stumm, R., & Marín, O. (2011). Cxcr7 controls neuronal migration by regulating chemokine responsiveness. *Neuron*, *69*(1), 77–90. https://doi.org/10.1016/J.NEURON.2010.12.006

Santos, A. M., Calvente, R., Tassi, M., Carrasco, M. C., Martín-Oliva, D., Marín-Teva, J. L., Navascués, J., & Cuadros, M. A. (2008). Embryonic and postnatal development of microglial cells in the mouse retina. *The Journal of Comparative Neurology*, *506*(2), 224–239. https://doi.org/10.1002/CNE.21538

Schafer, D. P., Lehrman, E. K., Kautzman, A. G., Koyama, R., Mardinly, A. R., Yamasaki, R., Ransohoff, R. M., Greenberg, M. E., Barres, B. A., & Stevens, B. (2012). Microglia Sculpt Postnatal Neural Circuits in an Activity and Complement-Dependent Manner. *Neuron*, *74*(4), 691–705. https://doi.org/10.1016/j.neuron.2012.03.026

Schlaggar, B. L., & O'Leary, D. D. M. (1994). Early development of the somatotopic map and barrel patterning in rat somatosensory cortex. *The Journal of Comparative Neurology*, *346*(1), 80–96. https://doi.org/10.1002/CNE.903460106

Schulz, C., Perdiguero, E. G., Chorro, L., Szabo-Rogers, H., Cagnard, N., Kierdorf, K., Prinz, M., Wu, B., Jacobsen, S. E. W., Pollard, J. W., Frampton, J., Liu, K. J., & Geissmann, F. (2012). A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science (New York, N.Y.)*, *336*(6077), 86–90. https://doi.org/10.1126/SCIENCE.1219179

Sedel, F., Béchade, C., Vyas, S., & Triller, A. (2004). Macrophage-derived tumor necrosis factor alpha, an early developmental signal for motoneuron death. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *24*(9), 2236–2246. https://doi.org/10.1523/JNEUROSCI.4464-03.2004

Seidemann, E., Zohary, E., & Newsome, W. T. (1998). Temporal gating of neural signals during performance of a visual discrimination task. *Nature*, *394*(6688), 72–75. https://doi.org/10.1038/27906

Sgaier, S. K., Lao, Z., Villanueva, M. P., Berenshteyn, F., Stephen, D., Turnbull, R. K., & Joyner, A. L. (2007). Genetic subdivision of the tectum and cerebellum into functionally related regions based on differential sensitivity to engrailed proteins. *Development (Cambridge, England)*, *134*(12), 2325–2335. https://doi.org/10.1242/DEV.000620

Shatz, C. J., & Stryker, M. P. (1988). Prenatal tetrodotoxin infusion blocks segregation of retinogeniculate afferents. *Science (New York, N.Y.)*, *242*(4875), 87–89. https://doi.org/10.1126/SCIENCE.3175636

Shen, J., & Colonnese, M. T. (2016). Development of Activity in the Mouse Visual Cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *36*(48), 12259–12275. https://doi.org/10.1523/JNEUROSCI.1903-16.2016

Sherman, S. M. (2016). Thalamus plays a central role in ongoing cortical functioning. *Nature Neuroscience*, *19*(4), 533–541. https://doi.org/10.1038/NN.4269

Sherman, S. M., & Guillery, R. W. (2002). The role of the thalamus in the flow of information to the cortex. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *357*(1428), 1695–1708. https://doi.org/10.1098/RSTB.2002.1161

Shi, W., Xianyu, A., Han, Z., Tang, X., Li, Z., Zhong, H., Mao, T., Huang, K., & Shi, S. H. (2017). Ontogenetic establishment of order-specific nuclear organization in the mammalian thalamus. *Nature Neuroscience*, *20*(4), 516–528. https://doi.org/10.1038/NN.4519

Siegel, F., Heimel, J. A., Peters, J., & Lohmann, C. (2012). Peripheral and central inputs shape network dynamics in the developing visual cortex in vivo. *Current Biology : CB*, *22*(3), 253–258. https://doi.org/10.1016/J.CUB.2011.12.026

Sierra, A., Encinas, J. M., Deudero, J. J. P., Chancey, J. H., Enikolopov, G., Overstreet-Wadiche, L. S., Tsirka, S. E., & Maletic-Savatic, M. (2010). Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell*, *7*(4), 483–495. https://doi.org/10.1016/J.STEM.2010.08.014

Sillito, A. M., & Kemp, J. A. (1983). The influence of GABAergic inhibitory processes on the receptive field structure of X and Y cells in cat dorsal lateral geniculate nucleus (dLGN). *Brain Research*, *277*(1), 63–77. https://doi.org/10.1016/0006-8993(83)90908-3

Simi, A., & Studer, M. (2018). Developmental genetic programs and activity-dependent mechanisms instruct neocortical area mapping. *Current Opinion in Neurobiology*, *53*, 96–102. https://doi.org/10.1016/J.CONB.2018.06.007

Simpson, T. I., Pratt, T., Mason, J. O., & Price, D. J. (2009). Normal ventral telencephalic expression of Pax6 is required for normal development of thalamocortical axons in embryonic mice. *Neural Development*, *4*(1). https://doi.org/10.1186/1749-8104-4-19

Sipe, G. O., Lowery, R. L., Tremblay, M., Kelly, E. A., Lamantia, C. E., & Majewska, A. K. (2016). Microglial P2Y12 is necessary for synaptic plasticity in mouse visual cortex. *Nature Communications*, *7*. https://doi.org/10.1038/NCOMMS10905

Smith, S. L., & Trachtenberg, J. T. (2007). Experience-dependent binocular competition in the visual cortex begins at eye opening. *Nature Neuroscience*, *10*(3), 370–375. https://doi.org/10.1038/NN1844

Sokhadze, G., Campbell, P. W., & Guido, W. (2019). Postnatal development of cholinergic input to the thalamic reticular nucleus of the mouse. *The European Journal of Neuroscience*, *49*(8), 978–989. https://doi.org/10.1111/EJN.13942

Somaiya, R. D., Stebbins, K., Gingrich, E. C., Xie, H., Campbell, J. N., Garcia, A. D. R., & Fox, M. A. (2022). Sonic hedgehog-dependent recruitment of GABAergic interneurons into the developing visual thalamus. *ELife*, *11*. https://doi.org/10.7554/ELIFE.79833

Song, H., Lee, B., Pyun, D., Guimera, J., Son, Y., Yoon, J., Baek, K., Wurst, W., & Jeong, Y. (2015). Ascl1 and Helt act combinatorially to specify thalamic neuronal identity by repressing Dlxs activation. *Developmental Biology*, *398*(2), 280–291. https://doi.org/10.1016/J.YDBIO.2014.12.003

Soria, J. M., & Fairén, A. (2000). Cellular mosaics in the rat marginal zone define an early neocortical territorialization. *Cerebral Cortex (New York, N.Y. : 1991)*, *10*(4), 400–412. https://doi.org/10.1093/CERCOR/10.4.400

Sorokin, S. P., Hoyt, R. F., Blunt, D. G., & McNelly, N. A. (1992). Macrophage development: II. Early ontogeny of macrophage populations in brain, liver, and lungs of rat embryos as revealed by a lectin marker. *The Anatomical Record*, *232*(4), 527–550. https://doi.org/10.1002/AR.1092320410

Southwell, D. G., Paredes, M. F., Galvao, R. P., Jones, D. L., Froemke, R. C., Sebe, J. Y., Alfaro-Cervello, C., Tang, Y., Garcia-Verdugo, J. M., Rubenstein, J. L., Baraban, S. C.,

& Alvarez-Buylla, A. (2012). Intrinsically determined cell death of developing cortical interneurons. *Nature*, *491*(7422), 109–113. https://doi.org/10.1038/NATURE11523

Squarzoni, P., Oller, G., Hoeffel, G., Pont-Lezica, L., Rostaing, P., Low, D., Bessis, A., Ginhoux, F., & Garel, S. (2014a). Microglia modulate wiring of the embryonic forebrain. *Cell Reports*, *8*(5), 1271–1279. https://doi.org/10.1016/J.CELREP.2014.07.042

Squarzoni, P., Oller, G., Hoeffel, G., Pont-Lezica, L., Rostaing, P., Low, D., Bessis, A., Ginhoux, F., & Garel, S. (2014b). Microglia Modulate Wiring of the Embryonic Forebrain. *Cell Reports*, *8*(5), 1271–1279. https://doi.org/10.1016/j.celrep.2014.07.042

Stowell, R. D., Sipe, G. O., Dawes, R. P., Batchelor, H. N., Lordy, K. A., Whitelaw, B. S., Stoessel, M. B., Bidlack, J. M., Brown, E., Sur, M., & Majewska, A. K. (2019). Noradrenergic signaling in the wakeful state inhibits microglial surveillance and synaptic plasticity in the mouse visual cortex. *Nature Neuroscience*, *22*(11), 1782–1792. https://doi.org/10.1038/s41593-019-0514-0

Streit, W. J. (2001). Microglia and macrophages in the developing CNS. *Neurotoxicology*, *22*(5), 619–624. https://doi.org/10.1016/S0161-813X(01)00033-X

Stumm, R., Kolodziej, A., Schulz, S., Kohtz, J. D., & Höllt, V. (2007). Patterns of SDF-1alpha and SDF-1gamma mRNAs, migration pathways, and phenotypes of CXCR4-expressing neurons in the developing rat telencephalon. *The Journal of Comparative Neurology*, *502*(3), 382–399. https://doi.org/10.1002/CNE.21336

Su, J., Charalambakis, N. E., Sabbagh, U., Somaiya, R. D., Monavarfeshani, A., Guido, W., & Fox, M. A. (2020). Retinal inputs signal astrocytes to recruit interneurons into visual thalamus. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(5), 2671–2682. https://doi.org/10.1073/PNAS.1913053117

Sumser, A., Mease, R. A., Sakmann, B., & Groh, A. (2017). Organization and somatotopy of corticothalamic projections from L5B in mouse barrel cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(33), 8853–8858. https://doi.org/10.1073/PNAS.1704302114

Sur, M., Nagakura, I., Chen, N., & Sugihara, H. (2013). Mechanisms of plasticity in the developing and adult visual cortex. *Progress in Brain Research*, *207*, 243–254. https://doi.org/10.1016/B978-0-444-63327-9.00002-3

Suzuki, A., Lee, L. J., Hayashi, Y., Muglia, L., Itohara, S., Erzurumlu, R. S., & Iwasato, T. (2015). Thalamic adenylyl cyclase 1 is required for barrel formation in the somatosensory cortex. *Neuroscience*, *290*, 518–529. https://doi.org/10.1016/j.neuroscience.2015.01.043

Svahn, A. J., Giacomotto, J., Graeber, M. B., Rinkwitz, S., & Becker, T. S. (2016). miR-124 Contributes to the functional maturity of microglia. *Developmental Neurobiology*, *76*(5), 507–518. https://doi.org/10.1002/DNEU.22328

Swadlow, H. A., & Alonso, J. M. (2017). Multielectrodes join the connectome. *Nature Methods*, *14*(9), 847–848. https://doi.org/10.1038/NMETH.4424

Swinnen, N., Smolders, S., Avila, A., Notelaers, K., Paesen, R., Ameloot, M., Brône, B., Legendre, P., & Rigo, J. M. (2013). Complex invasion pattern of the cerebral cortex bymicroglial cells during development of the mouse embryo. *Glia*, *61*(2), 150–163. https://doi.org/10.1002/GLIA.22421

Syed, M. M., Lee, S., He, S., & Zhou, Z. J. (2004). Spontaneous waves in the ventricular zone of developing mammalian retina. *Journal of Neurophysiology*, *91*(5), 1999–2009. https://doi.org/10.1152/JN.01129.2003

Takesian, A. E., Bogart, L. J., Lichtman, J. W., & Hensch, T. K. (2018). Inhibitory circuit gating of auditory critical-period plasticity. *Nature Neuroscience*, *21*(2), 218–227. https://doi.org/10.1038/s41593-017-0064-2

Tan, S. S., Kalloniatis, M., Sturm, K., Tam, P. P. L., Reese, B. E., & Faulkner-Jones, B. (1998). Separate progenitors for radial and tangential cell dispersion during development of the cerebral neocortex. *Neuron*, *21*(2), 295–304. https://doi.org/10.1016/S0896-6273(00)80539-5

Tasic, B., Menon, V., Nguyen, T. N., Kim, T. K., Jarsky, T., Yao, Z., Levi, B., Gray, L. T., Sorensen, S. A., Dolbeare, T., Bertagnolli, D., Goldy, J., Shapovalova, N., Parry, S., Lee, C., Smith, K., Bernard, A., Madisen, L., Sunkin, S. M., … Zeng, H. (2016). Adult mouse cortical cell taxonomy revealed by single cell transcriptomics. *Nature Neuroscience*, *19*(2), 335–346. https://doi.org/10.1038/NN.4216

Tay, T. L., Savage, J. C., Hui, C. W., Bisht, K., & Tremblay, M. È. (2017). Microglia across the lifespan: from origin to function in brain development, plasticity and cognition. *Journal of Physiology*, *595*(6), 1929–1945. https://doi.org/10.1113/JP272134

Theyel, B. B., Lee, C. C., & Sherman, S. M. (2010). Specific and nonspecific thalamocortical connectivity in the auditory and somatosensory thalamocortical slices. *Neuroreport*, *21*(13), 861–864. https://doi.org/10.1097/WNR.0B013E32833D7CEC

Thion, M. S., Low, D., Silvin, A., Chen, J., Grisel, P., Schulte-Schrepping, J., Blecher, R., Ulas, T., Squarzoni, P., Hoeffel, G., Coulpier, F., Siopi, E., David, F. S., Scholz, C., Shihui, F., Lum, J., Amoyo, A. A., Larbi, A., Poidinger, M., … Garel, S. (2018). Microbiome Influences Prenatal and Adult Microglia in a Sex-Specific Manner. *Cell*, *172*(3), 500-516.e16. https://doi.org/10.1016/J.CELL.2017.11.042

Thion, M. S., Mosser, C. A., Férézou, I., Grisel, P., Baptista, S., Low, D., Ginhoux, F., Garel, S., & Audinat, E. (2019). Biphasic Impact of Prenatal Inflammation and Macrophage Depletion on the Wiring of Neocortical Inhibitory Circuits. *Cell Reports*, *28*(5), 1119-1126.e4. https://doi.org/10.1016/j.celrep.2019.06.086

Tiriac, A., & Feller, M. B. (2022). Roles of visually evoked and spontaneous activity in the development of retinal direction selectivity maps. In *Trends in Neurosciences* (Vol. 45, Issue 7, pp. 529–538). Elsevier Ltd. https://doi.org/10.1016/j.tins.2022.04.002

Tiveron, M. C., Rossel, M., Moepps, B., Yong, L. Z., Seidenfaden, R., Favor, J., König, N., & Cremer, H. (2006). Molecular interaction between projection neuron precursors and invading interneurons via stromal-derived factor 1 (CXCL12)/CXCR4 signaling in the cortical subventricular zone/intermediate zone. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *26*(51), 13273–13278. https://doi.org/10.1523/JNEUROSCI.4162-06.2006

Toldi, J., Fehér, O., & Wolff, J. R. (1996). Neuronal plasticity induced by neonatal monocular (and binocular) enucleation. *Progress in Neurobiology*, *48*(3), 191–209. https://doi.org/10.1016/0301-0082(95)00038-0

Toldi, J., Rojik, I., & Fehér, O. (1994). Neonatal monocular enucleation-induced cross-modal effects observed in the cortex of adult rat. *Neuroscience*, *62*(1), 105–114. https://doi.org/10.1016/0306-4522(94)90318-2

Torborg, C. L., & Feller, M. B. (2005). Spontaneous patterned retinal activity and the refinement of retinal projections. *Progress in Neurobiology*, *76*(4), 213–235. https://doi.org/10.1016/J.PNEUROBIO.2005.09.002

Torres-Platas, S. G., Comeau, S., Rachalski, A., Bo, G. D., Cruceanu, C., Turecki, G., Giros, B., & Mechawar, N. (2014). Morphometric characterization of microglial phenotypes in human cerebral cortex. *Journal of Neuroinflammation*, *11*. https://doi.org/10.1186/1742-2094-11-12

Tou, Y. V., Aaker, J., Taniguchi, A., Kazemzadeh, C., Skidmore, J. M., Martin, D. M., Martin, J. F., Treier, M., & Nakagawa, Y. (2007). Characterization of progenitor domains in the developing mouse thalamus. *The Journal of Comparative Neurology*, *505*(1), 73–91. https://doi.org/10.1002/CNE.21467

Tremblay, M. Ě., Lowery, R. L., & Majewska, A. K. (2010). Microglial interactions with synapses are modulated by visual experience. *PLoS Biology*, *8*(11). https://doi.org/10.1371/JOURNAL.PBIO.1000527

Truman, L. A., Ford, C. A., Pasikowska, M., Pound, J. D., Wilkinson, S. J., Dumitriu, I. E., Melville, L., Melrose, L. A., Ogden, C. A., Nibbs, R., Graham, G., Combadiere, C., & Gregory, C. D. (2008). CX3CL1/fractalkine is released from apoptotic lymphocytes to stimulate macrophage chemotaxis. *Blood*, *112*(13), 5026–5036. https://doi.org/10.1182/BLOOD-2008-06-162404

Tsukano, H., Horie, M., Ohga, S., Takahashi, K., Kubota, Y., Hishida, R., Takebayashi, H., & Shibuki, K. (2017). Reconsidering Tonotopic Maps in the Auditory Cortex and Lemniscal Auditory Thalamus in Mice. *Frontiers in Neural Circuits*, *11*. https://doi.org/10.3389/FNCIR.2017.00014

Tuncdemir, S. N., Wamsley, B., Stam, F. J., Osakada, F., Goulding, M., Callaway, E. M., Rudy, B., & Fishell, G. (2016). Early Somatostatin Interneuron Connectivity Mediates the Maturation of Deep Layer Cortical Circuits. *Neuron*, *89*(3), 521–535. https://doi.org/10.1016/j.neuron.2015.11.020

Tusa, R. J., Palmer, L. A., & Rosenquist, A. C. (1978). The retinotopic organization of area 17 (striate cortex) in the cat. *The Journal of Comparative Neurology*, *177*(2), 213–235. https://doi.org/10.1002/CNE.901770204

Ueno, M., Fujita, Y., Tanaka, T., Nakamura, Y., Kikuta, J., Ishii, M., & Yamashita, T. (2013). Layer V cortical neurons require microglial support for survival during postnatal development. *Nature Neuroscience*, *16*(5), 543–551. https://doi.org/10.1038/NN.3358

Uesaka, N., Hayano, Y., Yamada, A., & Yamamoto, N. (2007). Interplay between laminar specificity and activity-dependent mechanisms of thalamocortical axon branching. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *27*(19), 5215–5223. https://doi.org/10.1523/JNEUROSCI.4685-06.2007

Vainchtein, I. D., Chin, G., Cho, F. S., Kelley, K. W., Miller, J. G., Chien, E. C., Liddelow, S. A., Nguyen, P. T., Nakao-Inoue, H., Dorman, L. C., Akil, O., Joshita, S., Barres, B. A., Paz, J. T., Molofsky, A. B., & Molofsky, A. v. (2018). Astrocyte-derived interleukin-33 promotes microglial synapse engulfment and neural circuit development. *Science (New York, N.Y.)*, *359*(6381), 1269–1273. https://doi.org/10.1126/SCIENCE.AAL3589

van Eden, C. G., zljak, L., Voorn, P., & Uylings, H. B. M. (1989). Prenatal development of GABA-ergic neurons in the neocortex of the rat. *The Journal of Comparative Neurology*, *289*(2), 213–227. https://doi.org/10.1002/CNE.902890204

van Kleef, E. S. B., Gaspar, P., & Bonnin, A. (2012). Insights into the complex influence of 5-HT signaling on thalamocortical axonal system development. *The European Journal of Neuroscience*, *35*(10), 1563–1572. https://doi.org/10.1111/J.1460-9568.2012.8096.X

Varney, M. E., Buchanan, J. T., Dementieva, Y., Elaine Hardman, W., & Sollars, V. E. (2011). A high omega-3 fatty acid diet has different effects on early and late stage myeloid progenitors. *Lipids*, *46*(1), 47–57. https://doi.org/10.1007/S11745-010-3491-3

Verney, C., Monier, A., Fallet-Bianco, C., & Gressens, P. (2010). Early microglial colonization of the human forebrain and possible involvement in periventricular white-matter injury of preterm infants. *Journal of Anatomy*, *217*(4), 436–448. https://doi.org/10.1111/J.1469-7580.2010.01245.X

Viaene, A. N., Petrof, I., & Murray Sherman, S. (2011). Synaptic properties of thalamic input to the subgranular layers of primary somatosensory and auditory cortices in the mouse. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *31*(36), 12738–12747. https://doi.org/10.1523/JNEUROSCI.1565-11.2011

Viswanathan, S., Bandyopadhyay, S., Kao, J. P. Y., & Kanold, P. O. (2012). Changing microcircuits in the subplate of the developing cortex. *The Journal of Neuroscience : The*

Official Journal of the Society for Neuroscience, *32*(5), 1589–1601. https://doi.org/10.1523/JNEUROSCI.4748-11.2012

Viswanathan, S., Sheikh, A., Looger, L. L., & Kanold, P. O. (2017). Molecularly Defined Subplate Neurons Project Both to Thalamocortical Recipient Layers and Thalamus. *Cerebral Cortex (New York, N.Y. : 1991)*, *27*(10), 4759–4768. https://doi.org/10.1093/CERCOR/BHW271

Voufo, C., Chen, A. Q., Smith, B. E., Feller, M. B., & Tiriac, A. (2022). Cellular Mechanisms Underlying Embryonic Retinal Waves. *BioRxiv*, 2022.08.14.503889. https://doi.org/10.1101/2022.08.14.503889

Vue, T. Y., Bluske, K., Alishahi, A., Yang, L. L., Koyano-Nakagawa, N., Novitch, B., & Nakagawa, Y. (2009). Sonic hedgehog signaling controls thalamic progenitor identity and nuclei specification in mice. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *29*(14), 4484–4497. https://doi.org/10.1523/JNEUROSCI.0656-09.2009

Vue, T. Y., Lee, M., Tan, Y. E., Werkhoven, Z., Wang, L., & Nakagawa, Y. (2013). Thalamic control of neocortical area formation in mice. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(19), 8442–8453. https://doi.org/10.1523/JNEUROSCI.5786-12.2013

Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S., & Nabekura, J. (2009a). Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. *Journal of Neuroscience*, *29*(13), 3974–3980. https://doi.org/10.1523/JNEUROSCI.4363-08.2009

Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S., & Nabekura, J. (2009b). Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *29*(13), 3974–3980. https://doi.org/10.1523/JNEUROSCI.4363-08.2009

Wake, H., Moorhouse, A. J., Miyamoto, A., & Nabekura, J. (2013). Microglia: actively surveying and shaping neuronal circuit structure and function. *Trends in Neurosciences*, *36*(4), 209–217. https://doi.org/10.1016/J.TINS.2012.11.007

Wamsley, B., & Fishell, G. (2017). Genetic and activity-dependent mechanisms underlying interneuron diversity. *Nature Reviews. Neuroscience*, *18*(5), 299–309. https://doi.org/10.1038/NRN.2017.30

Wan, Y., Feng, B., You, Y., Yu, J., Xu, C., Dai, H., Trapp, B. D., Shi, P., Chen, Z., & Hu, W. (2020). Microglial Displacement of GABAergic Synapses Is a Protective Event during Complex Febrile Seizures. *Cell Reports*, *33*(5). https://doi.org/10.1016/J.CELREP.2020.108346

Wang, B. S., Bernardez Sarria, M. S., An, X., He, M., Alam, N. M., Prusky, G. T., Crair, M. C., & Huang, Z. J. (2021). Retinal and Callosal Activity-Dependent Chandelier Cell Elimination Shapes Binocularity in Primary Visual Cortex. *Neuron*, *109*(3), 502-515.e7. https://doi.org/10.1016/J.NEURON.2020.11.004

Wang, X., Sommer, F. T., & Hirsch, J. A. (2011). Inhibitory circuits for visual processing in thalamus. *Current Opinion in Neurobiology*, *21*(5), 726–733. https://doi.org/10.1016/J.CONB.2011.06.004

Wang, X., Wei, Y., Vaingankar, V., Wang, Q., Koepsell, K., Sommer, F. T., & Hirsch, J. A. (2007). Feedforward excitation and inhibition evoke dual modes of firing in the cat's visual thalamus during naturalistic viewing. *Neuron*, *55*(3), 465–478. https://doi.org/10.1016/J.NEURON.2007.06.039

Wang, Y., Li, G., Stanco, A., Long, J. E., Crawford, D., Potter, G. B., Pleasure, S. J., Behrens, T., & Rubenstein, J. L. R. (2011). CXCR4 and CXCR7 Have Distinct Functions in Regulating Interneuron Migration. *Neuron*, *69*(1), 61–76. https://doi.org/10.1016/j.neuron.2010.12.005

Wang, Y., Szretter, K. J., Vermi, W., Gilfillan, S., Rossini, C., Cella, M., Barrow, A. D., Diamond, M. S., & Colonna, M. (2012). IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. *Nature Immunology*, *13*(8), 753–760. https://doi.org/10.1038/NI.2360

Wichterle, H., Garcia-Verdugo, J. M., Herrera, D. G., & Alvarez-Buylla, A. (1999). Young neurons from medial ganglionic eminence disperse in adult and embryonic brain. *Nature Neuroscience*, *2*(5), 461–466. https://doi.org/10.1038/8131

Wichterle, H., Turnbull, D. H., Nery, S., Fishell, G., & Alvarez-Buylla, A. (2001). In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. *Development (Cambridge, England)*, *128*(19), 3759–3771. https://doi.org/10.1242/DEV.128.19.3759

Wong, F. K., Bercsenyi, K., Sreenivasan, V., Portalés, A., Fernández-Otero, M., & Marín, O. (2018). Pyramidal cell regulation of interneuron survival sculpts cortical networks /631/378/2571/1696 /631/378/1934 /631/136/334/1874/345 /631/80/82/23 /631/378/1689/1373 /13/1 /13/2 /13/51 /14/19 /14/69 /38/77 /64/60 /96/63 article. *Nature*, *557*(7707), 668–673. https://doi.org/10.1038/s41586-018-0139-6

Wong, R. O. L. (1999). Retinal waves and visual system development. *Annual Review of Neuroscience*, *22*, 29–47. https://doi.org/10.1146/ANNUREV.NEURO.22.1.29

Wu, L. J., Vadakkan, K. I., & Zhuo, M. (2007). ATP-induced chemotaxis of microglial processes requires P2Y receptor-activated initiation of outward potassium currents. *Glia*, *55*(8), 810–821. https://doi.org/10.1002/GLIA.20500

Wu, W., Li, Y., Wei, Y., Bosco, D. B., Xie, M., Zhao, M. G., Richardson, J. R., & Wu, L. J. (2020). Microglial depletion aggravates the severity of acute and chronic seizures in mice. *Brain, Behavior, and Immunity*, *89*, 245–255. https://doi.org/10.1016/J.BBI.2020.06.028

Xu, Q., Cobos, I., de La Cruz, E. D., Rubenstein, J. L., & Anderson, S. A. (2004). Origins of cortical interneuron subtypes. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *24*(11), 2612–2622. https://doi.org/10.1523/JNEUROSCI.5667-03.2004

Xu, Q., Tam, M., & Anderson, S. A. (2008). Fate mapping Nkx2.1-lineage cells in the mouse telencephalon. *The Journal of Comparative Neurology*, *506*(1), 16–29. https://doi.org/10.1002/CNE.21529

Yanagida, M., Miyoshi, R., Toyokuni, R., Zhu, Y., & Murakami, F. (2012). Dynamics of the leading process, nucleus, and Golgi apparatus of migrating cortical interneurons in living mouse embryos. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(41), 16737–16742. https://doi.org/10.1073/PNAS.1209166109

Zechel, S., Nakagawa, Y., & Ibáñez, C. F. (2016). Thalamo-cortical axons regulate the radial dispersion of neocortical GABAergic interneurons. *ELife*, *5*(DECEMBER2016). https://doi.org/10.7554/ELIFE.20770

Zeisel, A., Mo͡z-Manchado, A. B., Codeluppi, S., Lönnerberg, P., Manno, G. la, Juréus, A., Marques, S., Munguba, H., He, L., Betsholtz, C., Rolny, C., Castelo-Branco, G., Hjerling-Leffler, J., & Linnarsson, S. (2015). Brain structure. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. *Science (New York, N.Y.)*, *347*(6226), 1138–1142. https://doi.org/10.1126/SCIENCE.AAA1934

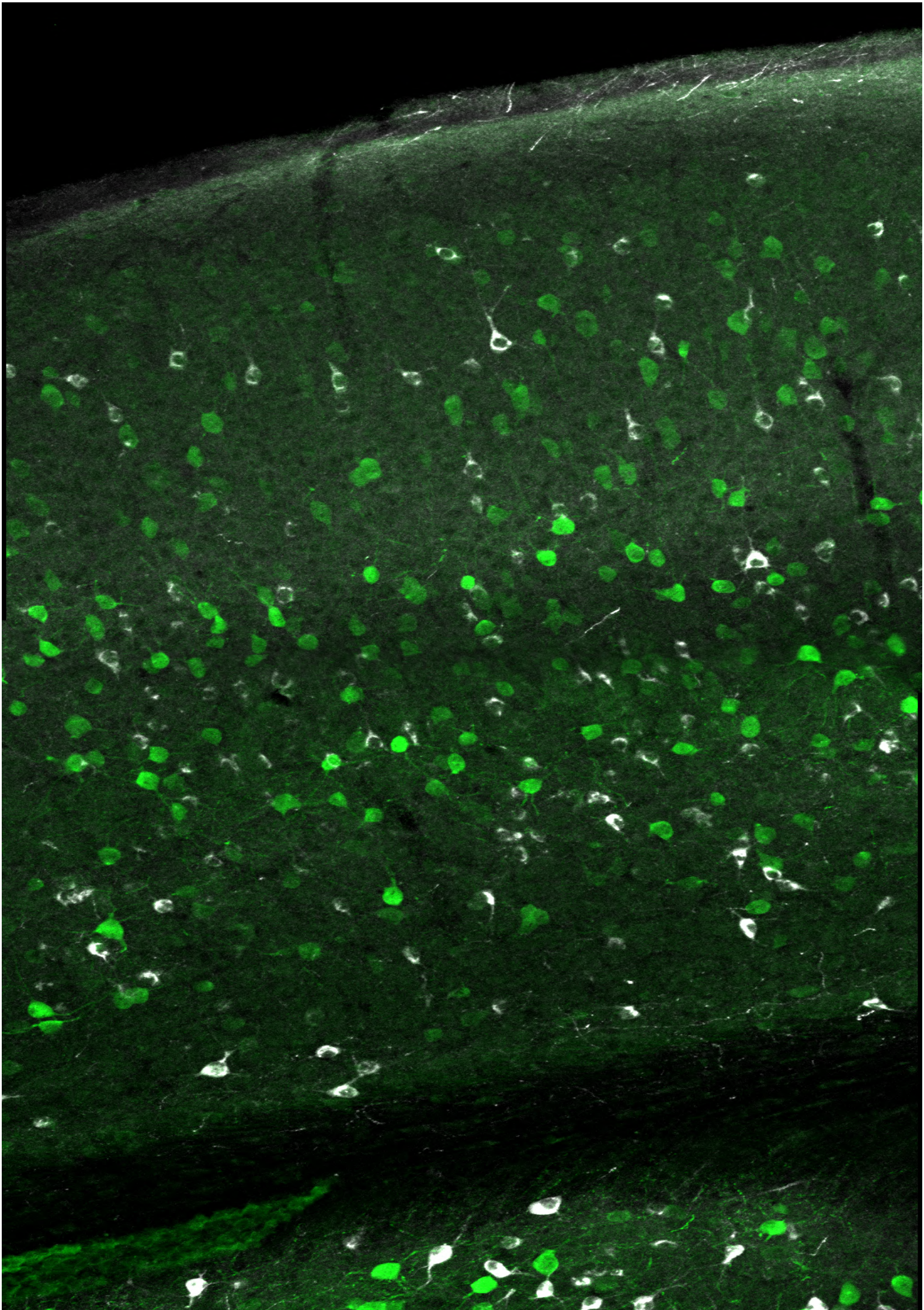
Zhan, Y., Paolicelli, R. C., Sforazzini, F., Weinhard, L., Bolasco, G., Pagani, F., Vyssotski, A. L., Bifone, A., Gozzi, A., Ragozzino, D., & Gross, C. T. (2014). Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. *Nature Neuroscience*, *17*(3), 400–406. https://doi.org/10.1038/NN.3641

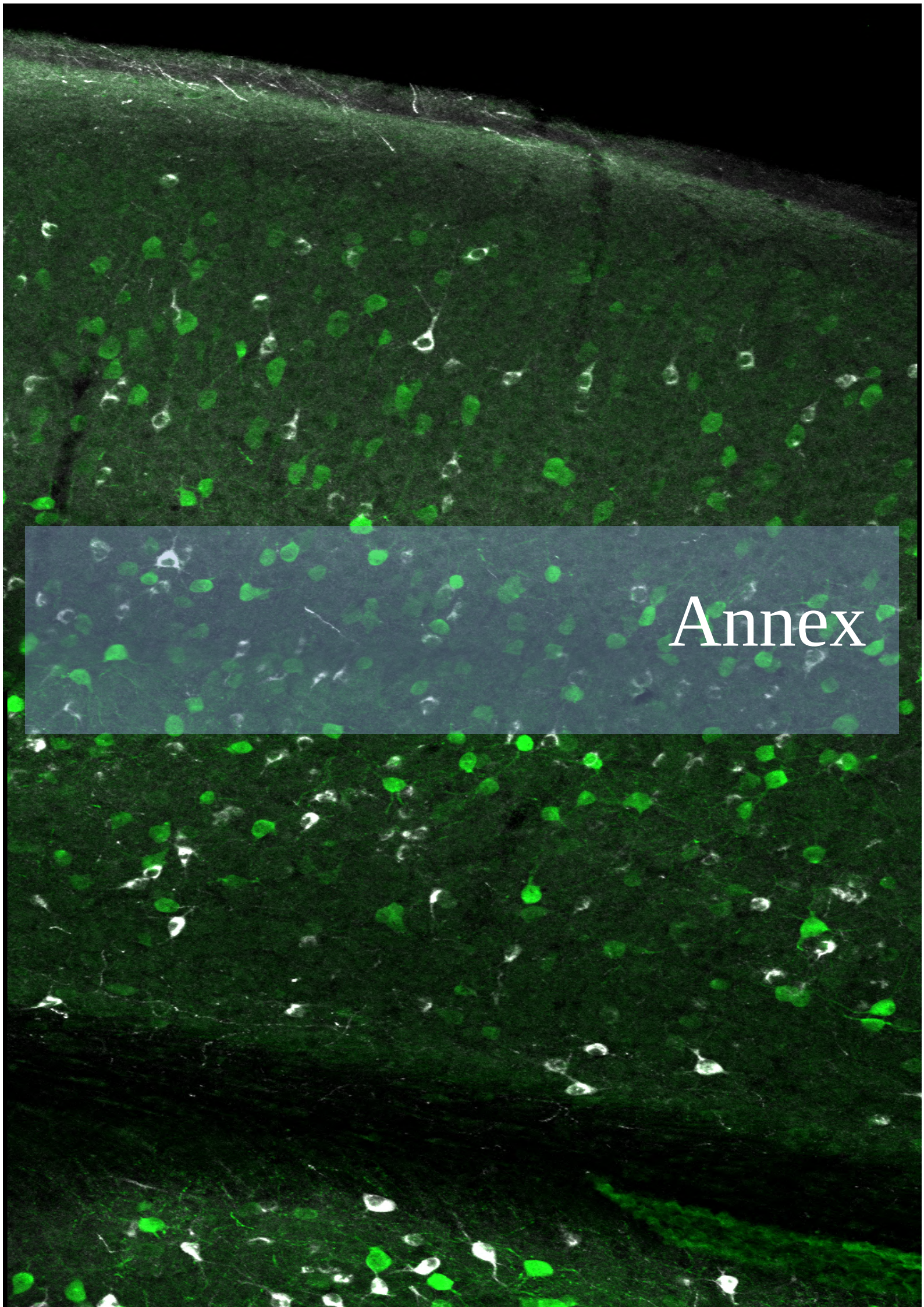
Zhao, C., Kao, J. P. Y., & Kanold, P. O. (2009). Functional excitatory microcircuits in neonatal cortex connect thalamus and layer 4. *The Journal of Neuroscience : The Official*

Journal of the Society for Neuroscience, *29*(49), 15479–15488. https://doi.org/10.1523/JNEUROSCI.4471-09.2009

Zheng, J. J., Lee, S., & Zhou, Z. J. (2004). A developmental switch in the excitability and function of the starburst network in the mammalian retina. *Neuron*, *44*(5), 851–864. https://doi.org/10.1016/j.neuron.2004.11.015

Zusso, M., Methot, L., Lo, R., Greenhalgh, A. D., David, S., & Stifani, S. (2012). Regulation of postnatal forebrain amoeboid microglial cell proliferation and development by the transcription factor Runx1. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *32*(33), 11285–11298. https://doi.org/10.1523/JNEUROSCI.6182-11.2012





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Building thalamic neuronal networks during mouse development

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The thalamic nuclear complex contains excitatory projection neurons and inhibitory local neurons, the two cell types driving the main circuits in sensory nuclei. While excitatory neurons are born from progenitors that reside in the proliferative zone of the developing thalamus, inhibitory local neurons are born outside the thalamus and they migrate there during development. In addition to these cell types, which occupy most of the thalamus, there are two small thalamic regions where inhibitory neurons target extra-thalamic regions rather than neighboring neurons, the intergeniculate leaflet and the parahabenular nucleus. Like excitatory thalamic neurons, these inhibitory neurons are derived from progenitors residing in the developing thalamus. The assembly of these circuits follows fine-tuned genetic programs and it is coordinated by extrinsic factors that help the cells find their location, associate with thalamic partners, and establish connections with their corresponding extra-thalamic inputs and outputs. In this review, we bring together what is currently known about the development of the excitatory and inhibitory components of the thalamocortical sensory system, in particular focusing on the visual pathway and thalamic interneurons in mice.

KEYWORDS

thalamus, development, mouse, thalamocortical, interneurons

Introduction

The thalamus has classically been considered a relay station in the brain due to its central location and patterns of connectivity. Excitatory neurons in sensory nuclei receive ascending information from peripheral organs and they project their axons beyond the thalamus, mainly into the sensory cortices and avoiding intrinsic connections (Petersen, 2007; Huberman et al., 2008; Tsukano et al., 2017). As the vast majority of cells in sensory nuclei are excitatory neurons, the thalamus could be considered to be merely a relay station of sensory information, transferring messages from the periphery to the cortex. However, there is an increasing body of evidence demonstrating a key role of thalamic nuclei in processing information and gating messages to the cortex. In addition to the ascending sensory information, excitatory neurons integrate signals from other brain structures and from the intrinsic thalamic networks. GABAergic neurons represent an important element in these intrinsic networks and despite their small number, the GABAergic neurons in the interconnected networks shape the output of the sensory thalamus (Hirsch et al., 2015).

Thalamic circuits develop progressively in embryonic stages and they finally assemble during postnatal life (Jhaveri et al., 1991; Schlaggar and O'Leary, 1994). Excitatory neurons are born in the ventricular zone of the developing thalamus, thereafter migrating toward the mantle zone where they start to extend dendrites and axons. Along their route toward the cortex, the axons of excitatory neurons project through different brain territories, bundling into fascicules, branching and making synaptic connections (Crowley and Katz, 2000; Hannan et al., 2001; Hevner et al., 2002; López-Bendito and Molnár, 2003; Gurung and Fritzsch, 2004; Hensch, 2004; Pfeiffenberger et al., 2005; Miko et al., 2008; Hanganu-Opatz, 2010). By contrast, the spatiotemporal developmental trajectory of local GABAergic neurons differs considerably. These GABAergic neurons are not derived from thalamic progenitors but rather, they are born and migrate from neighboring midbrain and pre-thalamic domains, invading the thalamus and integrating into its circuits some time after excitatory neurons. In this review, we bring together what is currently known about the development of the excitatory and inhibitory components of the thalamocortical sensory system, focusing particularly on the visual pathway and on thalamic interneurons in mice.

The functional organization of the sensory thalamus

Thalamic neurons are organized into spatial clusters or nuclei that can be characterized through the subcortical origin of their afferents. Some of these afferents carry sensory information derived from peripheral organs, which adopt a modal organization to define the primary sensory nuclei of the thalamus: the dorsolateral geniculate nucleus (dLG) that receives visual information; the ventral posteromedial nucleus (VPM) for somatosensory input; and the ventral medial geniculate nucleus (MGv) for auditory input. While the dLG, VPM and MGv are classified as first-order (FO) nuclei since their main driving stimuli arrive directly or indirectly from the peripheral sensory organs (Sherman, 2017; Halassa and Sherman, 2019), the thalamus also contains higher-order (HO) nuclei that receive driving inputs from subpopulations of projection neurons in Layer 5b (L5b) of the respective cortical areas (Sherman and Guillery, 2002; Bickford, 2016). HO nuclei mainly process unimodal information, even though they can integrate inputs from other modalities too. Among the HO nuclei, the lateral posterior nucleus (LP, visual), posterior medial (PoM, somatosensory), and the dorsal medial geniculate nucleus (MGd, auditory) not only further process sensory information but they also help the thalamus to connect different cortical areas (**Figure 1**; Butler, 2008; Halassa and Sherman, 2019).

The patterns of connectivity between the thalamus and cortex are similar for the different sensory modalities. Thus, sensory stimuli ascending from the peripheral organ arrives at the corresponding FO nucleus, which in turn projects to the L4, L5b, and L6 of the corresponding sensory cortex (Swadlow and Alonso, 2017). Within a cortical column, information flows from L4 to L2/3 and from there to L5 and L6, the layers that project out of the cortex. Neurons from L5b and L6b send projections to HO nuclei, and neurons in L6a project back to the FO nuclei (Sumser et al., 2017; Hoerder-Suabedissen et al., 2018). Therefore, these connections form feedback and feedforward loops that establish the basis of sensory processing in the thalamocortical system (Viaene et al., 2011). As a result, the thalamus represents a hub that can send information to and from different cortical areas (Sherman and Guillery, 2002; Reichova and Sherman, 2004; Lee and Murray Sherman, 2010; Theyel et al., 2010).

The formation of the thalamocortical system

Patterning of the diencephalon

Early in development, the diencephalon subdivides into three transverse regions called prosomeres, each of which can be further subdivided into four longitudinal bands or plates: roof, alar, basal, and floor (in a dorsoventral order, **Figure 2A**). This percolation relies on the presence of gradients of diffusible molecules, such as the wingless-INT proteins (WNTs), bone morphogenetic proteins (BMPs), Sonic hedgehog (SHH), and fibroblast growth factor proteins (FGFs) (Kataoka and Shimogori, 2008; Martinez-Ferre and Martinez, 2009, 2012). Prosomeres are evident at E10 in mice and their respective alar plates develop into different brain structures: prosomere 1 gives rise to the pretectum, which includes multiple domains of the adult brain that are involved in processing visual information and in the execution of visual reflexes (Ferran et al., 2009); prosomere 2 develops into the epithalamus and thalamus; and lastly, prosomere 3 gives rise to the prethalamus, which includes GABAergic structures like the reticular nucleus (RTN) and the zona incerta (ZI) (Puelles and Rubenstein, 2003).

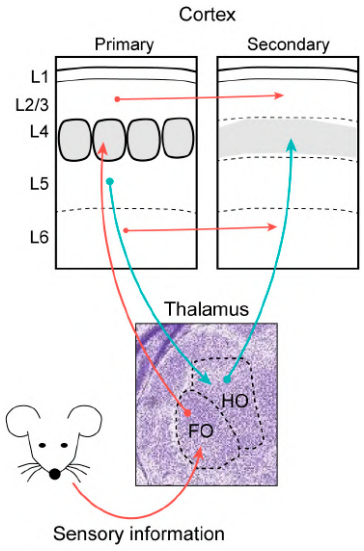

FIGURE 1
The thalamus transmits peripheral sensory information to primary sensory areas and retransmits the processed sensory message to secondary cortical areas. The schema represents the major feed-forward connections of the mouse whisker system. Stimuli from peripheral sensory organs arrive at first order thalamic nuclei (FO) which connects with L4 neurons in the primary sensory cortex. The information then spreads to other cortical areas both through direct cortico-cortical connections (red arrows) and through the trans-thalamic pathway using higher-order thalamic nuclei (HO) as relay stations (blue arrows).

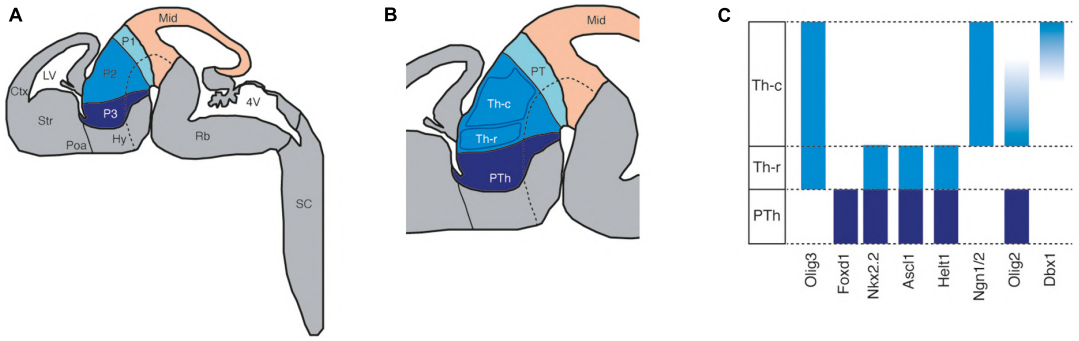

FIGURE 2
The patterning of the developing diencephalon. **(A)** The schema represents a sagittal view of the developing mouse brain highlighting the midbrain (Mid) and the subdivisions, known as prosomeres (P1, P2, and P3), of the diencephalon according to the prosomeric model. Ctx: cortex; Hy: hypothalamus; LV: lateral ventriculus; Poa: preoptic area; Rb: rhombencephalon; SC: spinal cord; Str: striatum; 4V: fourth ventriculus. **(B)** The two progenitor domains of the prosomere 2 or thalamus (Th): Th-c (caudal) and Th-r (rostral). PTh: prethalamus; PT: pretectum. **(C)** Representative expression patterns of transcription factors in the different the progenitor domains of prosomeres 2 and 3.

Prosomeres 2 and 3 are separated by the zona limitans intrathalamica (ZLI), an organization center that expresses high levels of SHH. During the early percolation of the diencephalon, SHH is a fundamental signaling molecule in prosomere 2 because it steers the differentiation of the prospective thalamus from the epithalamus (Chatterjee and Li, 2012; Chatterjee et al., 2014; Mallika et al., 2015). In the prospective thalamus, two progenitor zones are established by E10.5 in the mouse (**Figure 2B**): the rostral and the caudal progenitor domains (Jeong et al., 2011; Suzuki-Hirano et al., 2011). The progenitors in the rostral domain are exposed to higher concentrations of SHH and consequently, they express markers like *Olig3*, *Nkx2.2*, *Ascl1,* and *Olig2* (Scholpp et al., 2009). On the other hand, the progenitors in the caudal domain are exposed to less SHH, such that they express markers like *Olig3*, *Ngn1/2,* and *Dbx1* (**Figure 2C**; Barth and Wilson, 1995; Hashimoto-Torii et al., 2003; Kiecker and Lumsden, 2004; Scholpp et al., 2006; Szabó et al., 2009; Vue et al., 2009). Neurogenesis in these progenitor domains spans approximately from E10 to E13 (Wong et al., 2018) and the postmitotic progeny differentiate into two broad different cell types: the caudal domain differentiates into the glutamatergic projection neurons that populate the thalamic nuclei and form thalamocortical connections (Tou et al., 2007; Price et al., 2012), and the rostral domain differentiates into GABAergic projection neurons that populate the intergeniculate leaflet (IGL) and the perihabenular nucleus (pHB) (Delogu et al., 2012; Fernandez et al., 2018).

As most neurons in the adult thalamus derive from the caudal progenitor domain, they must undergo a complex process of specification to generate neuronal diversity and the distinct thalamic nuclei. Although it is not clear how this diversification occurs, some evidence indicates that the population of caudal progenitors is heterogeneous. Within this caudal progenitor domain, there are transcription factors that are expressed in opposite gradients through the antero-ventral to caudo-dorsal axis. For instance, the expression of *Olig2* is high at the most anterior pole and the expression of *Dbx1* in the most caudal pole. This polarization of the caudal progenitor domain has relevant implications for thalamic nucleogenesis. Lineage and birth-dating analysis demonstrated that antero-ventral progenitors give rise to neurons populating more latero-ventral nuclei, and caudo-dorsal progenitors to more medio-dorsal ones (Tou et al., 2007; Wong et al., 2018). In addition, to this spatial order, there is also a temporal sequence whereby the formation of the latero-ventral nuclei occurs earlier than the medio-dorsal nuclei, which is consistent with the earlier transition from symmetric to asymmetric division in the antero-ventral compared to the caudo-dorsal progenitors (Nakagawa and Shimogori, 2012; Wong et al., 2018).

If different subpopulations of progenitors give rise to diverse cell types populating thalamic nuclei, it is expected that they are controlled by different genetic programs. However, data from single-cell RNA sequencing of E12 mice suggests that the progenitors in the ventricular zone of the caudal domain (apical progenitors) comprises a unique cluster of cells based on their transcriptomic profile. There is, indeed, a second cluster of dividing cells that derives from the apical progenitors and corresponds to the basal (or intermediate) progenitors of the thalamus located away from the ventricular zone (Guo and Li, 2019). Apical progenitors generate larger clones than basal progenitors, 12 neurons on average, and both apical or basal-derived clones tend to occupy more than one nucleus (Wong et al., 2018). It has been suggested that sibling cells tend to occupy functionally related nuclei but further evidence is needed (Shi et al., 2017). Despite transcriptomic analysis does not reveal clear-cut internal subdivisions of progenitor domains, they do show a graded pattern of gene expression (Guo and Li, 2019), as previously observed in data obtained using labeling methods (Tou et al., 2007). In sum, to refine the classification of the apparently heterogeneous populations of progenitors in the caudal domain of the thalamus, it is necessary to generate larger databases of single-cell transcriptomic profiles, accompanied by more complete atlases of gene expression and sophisticated algorithms.

Although the projection neurons of thalamic nuclei are born in the caudal proliferative domain, it remains unclear whether their nucleus-specific identity is specified at the progenitor stage (Chatterjee et al., 2014). Rather, current evidence suggests that nucleus-specific identity is conferred when progenitors exit the cell-cycle. The gradual expression of the *Gbx2* transcription factor by post-mitotic cells is one determinant of thalamic nuclei that project to the cortex (**Figure 3B**). The expression of *Gbx2* starts at E9.5, following a very dynamic spatiotemporal pattern that ultimately defines the borders of the thalamus with the epithalamus, prethalamus and pretectum, as well as parcellating the thalamus into

distinct nuclei (Nakagawa and O'Leary, 2001; Tou et al., 2007; Chen et al., 2009; Li et al., 2012). The absence of *Gbx2* leads to a shrinking of the thalamus at its posterior and dorsal borders, enlarging the pretectum and epithalamus, respectively. In turn, there is a disruption in the histogenesis of the nuclear complexes and a significant loss of thalamocortical projections (Chen et al., 2009; Chatterjee and Li, 2012; Mallika et al., 2015; Nakagawa, 2019).

The development of thalamocortical projections

In the developing mouse thalamus, prospective thalamocortical cells begin to extend their axons toward the cortex at around E12, shortly after neurogenesis ceases (**Figure 4**). Growing thalamocortical axons are guided through different territories by molecular and cellular cues, as well as by activity-dependent mechanisms (Marcos-Mondéjar et al., 2012; Mire et al., 2012; Molnár et al., 2012; Leyva-Díaz et al., 2014; Castillo-Paterna et al., 2015). Thalamocortical axons start their journey toward the cortex by growing rostrally through the thalamus and heading toward the prethalamus. They navigate through the thalamic territory using prethalamic axons as scaffolds and following gradients of guidance cues, such as Sema3a and Netrin1 (Quintana-Urzainqui et al., 2020). Thalamocortical axons traverse the entire prethalamus and move through the Slit-free domain of the peduncular hypothalamus until they encounter the diencephalic-telencephalic boundary (DTB) (Callejas-Marin et al., 2022). Slit proteins guide thalamocortical axons out of the hypothalamus and they prevent them from crossing the midline (Bagri et al., 2002; López-Bendito et al., 2007; Braisted et al., 2009; Bielle et al., 2011). Subsequently, thalamocortical axons cross the DTB, attracted by Netrin1, and they enter the ventral telencephalon (Métin and Godement, 1996; Braisted et al., 2000).

Thalamic projections continue their journey through the telencephalon, reaching the internal capsule from where they follow a permissive corridor formed between two repulsive areas: the proliferative zone of the medial ganglionic eminence (MGE) and the globus pallidus. A large proportion of these corridor cells are GABAergic neurons derived from the lateral ganglionic eminence (LGE), cells that express membrane-bound Neuregulin1 and that migrate into the mantle of the MGE between E11-E14 (López-Bendito et al., 2006; Bielle et al., 2011). The topographic organization of thalamocortical axons is preserved throughout the ventral telencephalon, which is a result of complex interactions between cues like Netrin1, Sema3a/3f, Slit1, L1 cell-adhesion molecules and ephrinA5 (Dufour et al., 2003; Seibt et al., 2003; Mire et al., 2012; Leyva-Díaz et al., 2014; Castillo-Paterna et al., 2015).

By E14, thalamocortical axons meet corticofugal axons at the pallial-subpallial boundary (PSPB), and they use them as scaffolds to turn into the pallium and spread across the developing cortex (McConnell et al., 1989; Blakemore and Molnar, 1990; de Carlos and O'Leary, 1992). The timing of the arrival of thalamic axons is relevant for cortical development. As thalamic inputs arrive at a rather immature cortex, they can exert a major effect on ongoing processes such as neurogenesis, migration and differentiation (Cadwell et al., 2019). Thalamocortical axons start to invade the cortical plate at E17 when the granular layers are being formed (Allendoerfer and Shatz, 1994; del Río et al., 2000; Molyneaux et al., 2007; Little et al., 2009), and they finally reach their destination during the first postnatal week (**Figure 4**), mainly targeting neurons in L4 and to a lesser extent, neurons in L5b (López-Bendito and Molnár, 2003).

While invading the cortical plate, thalamic axons form functional synapses with subplate cells, a transient layer of rather mature neurons located below the cortical plate that coordinate the early maturation of thalamocortical networks (Kanold and Luhmann, 2010; Hoerder-Suabedissen and Molnár, 2015). As Cajal-Retzius cells, another transient population of cortical neurons, subplate neurons disappear by programmed cells death during the first postnatal days in mouse and thalamocortical afferents form direct contacts with neurons in L4 and deeper layers. Apart from subplate neurons, thalamocortical afferents form transient circuits with a specific subpopulation of developing interneruons. During the first postnatal week in mice, thalamocortical axons contact L5 somatostatin-positive interneurons that in turn contact spiny stellate neurons in layer 4 (Marques-Smith et al., 2016). This circuit becomes remodeled and disappears by the end of the first postnatal week. Despite its brief duration, the connection between L5 somatostatin-positive interneurons and L4 neurons orchestrates the assembly of local inhibition in layers 4. Also the density of thalamocortical input to infragranular interneurons varies during development. Somatostatin-positive interneurons receive a transient strong thalamic drive at immature stages that is required for the correct assembly of thalamic feed-forward inhibition mediated by parvalbumin-positive interneurons (Tuncdemir et al., 2016).

The development of corticothalamic projections

In mice, corticothalamic projections appear at E10 from post-mitotic neurons in the cortical plate. These corticofugal projections navigate laterally through the intermediate zone until they reach the PSPB between E13 and E15 (Jacobs et al., 2007). At this boundary, corticothalamic axons interact with the ascending thalamocortical axons, facilitating their invasion of the cortical territory. The corticothalamic axons then continue their journey through the ventral telencephalon toward the internal capsule, where molecular cues and corridor cells guide them toward the diencephalon (Bagri et al., 2002; López-Bendito et al., 2006, 2007). By E15, corticothalamic axons have crossed the DTB to enter into the prethalamus, where more-or-less a day later they interact with cells from the RTN and the perireticular nucleus (PRN) (Garel and Rubenstein, 2004; Molnár et al., 2012; Deck et al., 2013). These axons are sorted in the prethalamus and the majority of axons from L5 are directed toward the cerebral peduncle, while axons from L6 and the remaining axons from L5 are directed toward their thalamic targets (Clascá et al., 1995; Molnár and Cordery, 1999; Jacobs et al., 2007). Mouse corticothalamic axons invade the thalamus just prior birth (**Figure 4**), first entering the developing somatosensory nuclei, and then invading the auditory and visual nuclei, which are fully innervated by the end of the first postnatal week (Jacobs et al., 2007; Grant et al., 2012). However, the cellular and molecular mechanisms that guide the entry of corticothalamic axons into the thalamus remain poorly understood. Nevertheless, removal of retinal input alters corticothalamic innervation of the dLGN, inducing premature entry of L6 axons and an abnormal cross-hierarchical invasion of L5 axons that would otherwise be designated to the LP (Brooks et al., 2013; Grant et al., 2016; Moreno-Juan et al., 2022).

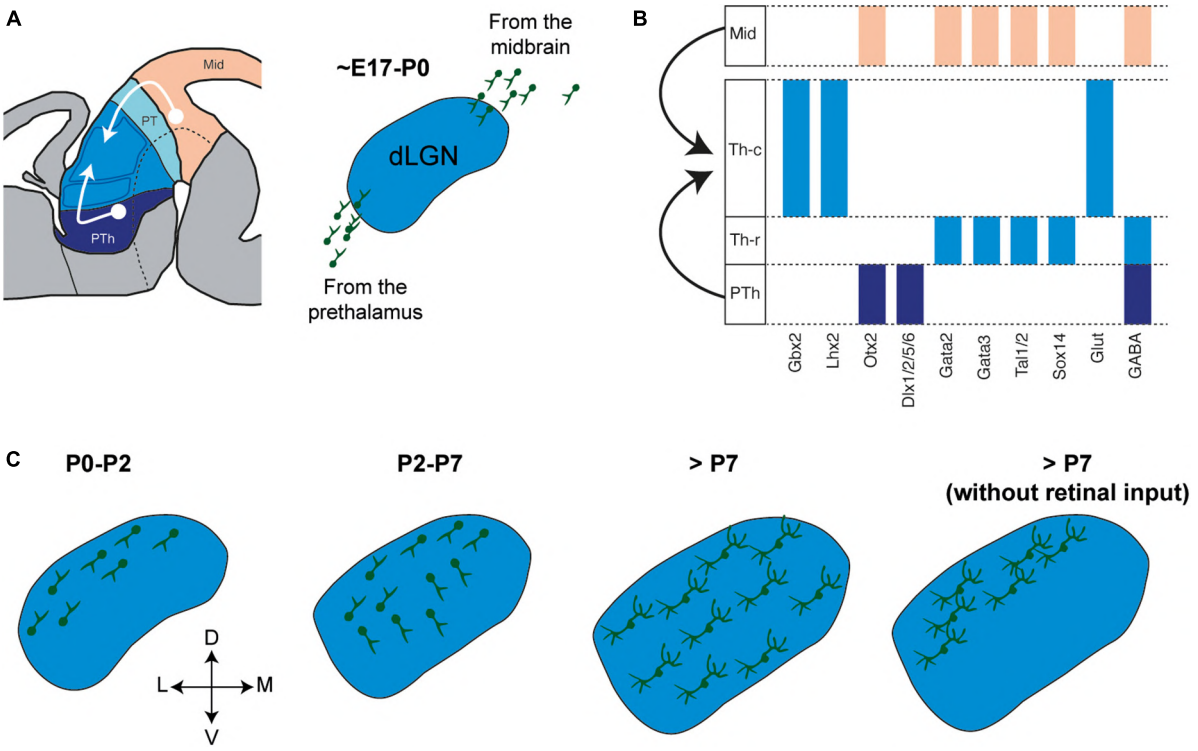

FIGURE 3
Integration of local thalamic interneurons from extra-thalamic sources. **(A)** *Right*, Schema showing the migration toward the thalamus of prospective local interneurons born in the midbrain and prethalamus. *Left*, Prospective local interneurons start invading the developing mouse dLGN shortly before birth. **(B)** Representative expression patterns of transcription factors, specially lineage selector genes, in precursor cells from prosomere 2 and GABA lineages from prosomere 3 and midbrain. **(C)** Schema of a mouse dLGN showing sequentially how local interneurons migrate and distribute into the nucleus in control conditions and upon deprivation of retinal input (Golding et al., 2014; Charalambakis et al., 2019; Su et al., 2020).

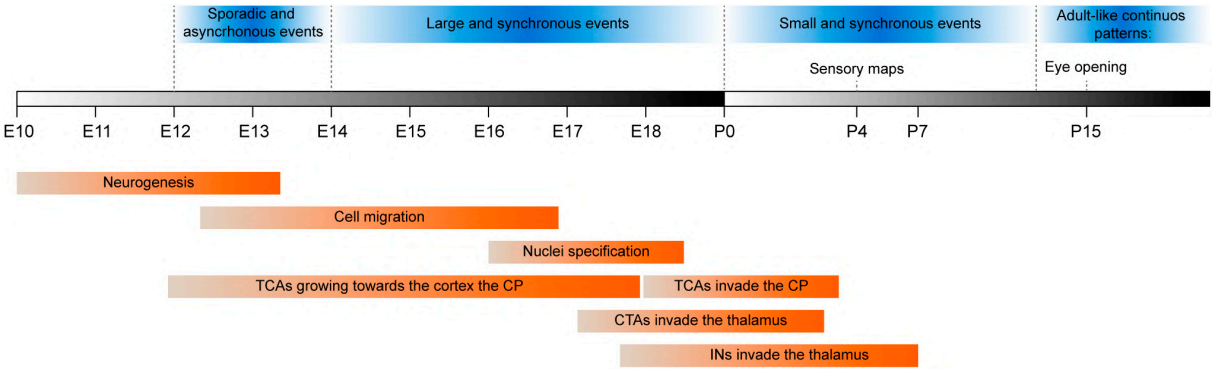

FIGURE 4
Timeline showing milestones in mouse thalamic development (red boxes). The different patterns of spontaneous thalamic activity throughout development (blue boxes). First, thalamic spontaneous activity is characterized for being scarce and the events are uncorrelated (E12-E14). This is followed by waves of spontaneous activity (E14-P0) that propagate among FO and then HO nuclei. At perinatal stages, these waves disappear and instead, there are small events most likely triggered spontaneously from peripheral organs that engage cortical areas. Before eye opening, there is a switch to an adult-like pattern where spontaneous activity becomes dense, asynchronous and continuous (Colonnese et al., 2010). CP, cortical plate; CTAs, corticothalamic axons; E, embryonic day; Ins, interneurons; P, postnatal day; TCAs, thalamocortical axons.

Early thalamic inputs affect cortical development and specification

The thalamus and cortex develop at a relatively similar pace and consequently they may influence each other's maturation. The thalamus clearly affects many aspects of cortical development, such as the radial organization, cell proliferation, specification of cortical areas, navigation of corticothalamic axons, interneuron maturation and circuit assembly (Rakic, 1991; Dehay et al., 1996; Zechel et al., 2016). More specifically, glutamate released by thalamocortical axons is required for Reelin-expressing cortical interneurons to develop (de Marco García et al., 2015). Similarly, thalamic inputs regulate the integration of somatostatin- and parvalbumin-expressing interneurons into cortical circuits (Wamsley and Fishell, 2017),

and the segregation of pyramidal neurons in the L4 of the barrel cortex (Li et al., 2013; Assali et al., 2017). Thalamic input also affects cell identity in the cortex, since differentiation into primary or higher-order cortical areas relies on the arrival of thalamocortical axons (Chou et al., 2013). Indeed, when FO nuclei are removed genetically, primary sensory areas acquire the molecular and functional properties of secondary cortical areas (Chou et al., 2013; Vue et al., 2013; Pouchelon et al., 2014). Recently, somatostatin-expressing interneurons in the cortex were seen to be necessary for the correct arrival of thalamocortical inputs onto parvalbumin-expressing interneurons and pyramidal neurons during the first week of postnatal life in mice (de Marco García et al., 2015; Marques-Smith et al., 2016; Tuncdemir et al., 2016; Che et al., 2018; Takesian et al., 2018).

Neuronal transmission in the thalamocortical system can also influence the development of the cortex, as seen when normal synaptic transmission is disrupted in knock-out mice that lack key synaptic proteins, such as NMDA receptor 1, adenylyl cyclase 1, or metabotropic glutamate receptor 5. Such disruption of neurotransmission can provoke a lack of neuronal organization and smaller barrels with blurry borders in S1 (Iwasato et al., 2000; Datwani et al., 2002; Ballester-Rosado et al., 2010; Antón-Bolaños et al., 2019).

Spontaneous activity in the developing thalamus

Neuronal activity in the developing thalamus evolves over different stages in mice (**Figure 4**). In a first stage, endogenous and uncorrelated activity spans mid-embryonic mouse development (E12-E14), which affects the expression of genes involved in thalamocortical axon growth and branching when manipulated (Herrmann and Shatz, 1995; Uesaka et al., 2007; Mire et al., 2012; Castillo-Paterna et al., 2015; Moreno-Juan et al., 2017). After this initial stage, the activity in the thalamus becomes more synchronous and by E14, spontaneous synchronic activity takes the form of waves of spontaneous activity that initially propagate through FO nuclei and that later also engage HO nuclei (Moreno-Juan et al., 2017). After birth, spontaneous activity becomes less correlated, especially in the somatosensory and auditory nuclei and at P2, in the visual nucleus as well (Colonnese et al., 2010). The waves of spontaneous activity observed in the thalamus are transmitted along thalamocortical axons to the developing cortical areas and consequently, early thalamocortical input could have an impact on cortical development through activity-dependent mechanisms. Indeed, altering patterns of activity through genetic manipulation provokes cross-modal changes in the development of sensory areas in the cortex (Moreno-Juan et al., 2017; Antón-Bolaños et al., 2018). The electrical properties of the thalamocortical circuit progressively mature during the first two postnatal weeks, undertaking more continuous and decorrelated spontaneous firing (Murata and Colonnese, 2016, 2018; Martini et al., 2021). This transition in spontaneous thalamic activity seems to be critical for the onset of the active processing of environmental information by the cortex. Moreover, it might be caused by changes in the sensory organs, synaptic maturation or circuit remodeling, such as the gradual integration of inhibitory components (Demas et al., 2003; Colonnese, 2014; Sokhadze et al., 2018).

Thalamic interneurons

General overview

Thalamic neurons receive inhibitory inputs from projecting neurons residing in the prethalamus (RN, ZI, and vLGN) and from other extra-thalamic sources, such as the superior colliculus, basal ganglia, hypothalamus and pontine reticular formation (Halassa and Acsády, 2016). In addition, they are also inhibited by local GABA-releasing neurons, although the number and distribution of these local interneurons is not conserved across species. In small mammals like mice, marsupials and bats, interneurons are sparse and mainly found in the dLGN, whereas they are abundant and widely distributed throughout the thalamus in large mammals. Inhibitory interneurons are absent from the dLGN of some non-mammalian amniotes, such as crocodiles, lizards and snakes, but they are present in birds (Butler, 2008). Local interneurons are not the only GABAergic cells in the mature thalamus, since there is a small subpopulation of GABAergic cells that reside within the IGL and pHB whose axons project to extra-thalamic targets (Harrington, 1997; Tou et al., 2007; Delogu et al., 2012; Inamura et al., 2012; Fernandez et al., 2018).

The origin of thalamic interneurons

Local interneurons that integrate into thalamic circuits are not born in the proliferative zone of the developing thalamus but rather, they migrate into the thalamus from other brain regions. Across species, some regions that generate thalamic neurons are conserved but also, additional regions are observed as thalamic circuits increase in complexity and size. The midbrain proliferative zone generates a stream of cells that colonizes the developing thalamus and that is made up of cells that differentiate into local GABAergic cells (**Figure 3A**; Jones, 2002; Hayes et al., 2003; Bakken et al., 2015; Jager et al., 2021). In the mouse, the invasion of these local interneuron precursors begins at E17, starting from the caudal tier of the developing thalamus. Fate mapping experiments confirmed the midbrain origin of these cells, showing that they are born at approximately E10-E13 and that they belong to the *Engrailed1* lineage (Jager et al., 2016, 2021), a transcription factor that is expressed in the midbrain and not the forebrain (Sgaier et al., 2007). The precursors generated from the *Engrailed1* progenitors are also characterized by the expression of the transcription factors *Sox14*, *Gata2,* and *Otx2* (**Figure 3B**). Once in the thalamus, these midbrain-derived interneurons adopt a specific spatial distribution, whereby they are enriched in FO nuclei but they also appear in HO and rostral nuclei (Jager et al., 2021). This subpopulation of GABAergic cells constitutes the largest of the local interneuron populations in the mature thalamus.

Another source of thalamic interneurons in the mouse is the developing prethalamus or prosomere 3 (**Figure 3A**). Located rostral to the thalamus, the developing prethalamus generates several GABAergic cell lineages, most of which populate prethalamic structures like the RTN and the vLGN, while others establish a stream of cells that invade the developing thalamus from its rostral tier around the time of birth (Golding et al., 2014; Jager et al., 2021). Approximately 20% of the population of local interneurons in the mature thalamus are specified in the developing prethalamus

from a lineage that expresses *Dlx1/2*, *Foxd1,* and *Dlx5/6*, and that does not express *Lhx6* or *Nkx2.1* (**Figure 3B**). The prethalamus-derived interneurons that will invade the thalamus have features complementary to midbrain-derived interneurons, for example, they do not express *Sox14* and they are enriched in HO nuclei (Jager et al., 2021).

Studying the origin of local thalamic interneurons is challenging because the thalamus does not generate local GABAergic neurons but it does generate projecting GABAergic neurons. The thalamic progenitors that give rise to projecting GABAergic neurons reside in the rostral tier of the proliferative neuroepithelium of prosomere 2, known as pTH-R (Tou et al., 2007). Indeed, the stream of cells derived from this progenitor domain can be distinguished from the neighboring caudal domain of thalamic progenitors (known as the pTH-C), and from prosomere 3, by the expression of post-mitotic markers like *Sox14*, *Nkx2.2,* and *Tal1* (Jeong et al., 2011). The cells derived from pTH-R become GABAergic projection neurons that populate diencephalic regions, such as the pHB and the IGL. While IGL neurons project to the suprachiasmatic nucleus and other hypothalamic nuclei, pHB axons target the ventromedial prefrontal cortex, the dorsomedial striatum and the nucleus accumbens, and as such, they are involved in mechanisms that regulate mood (Moore et al., 2000; Fernandez et al., 2018; Anastasiades et al., 2021).

An additional challenge is that extra-thalamic sources of local thalamic GABAergic neurons, like the prethalamus and midbrain, also generate other GABAergic neurons. As well as the *Sox14*-negative local interneurons of the thalamus, the proliferative zone of the prethalamus gives rise to many other GABAergic cells. At E10 in mice, the progenitor cells found in the ventricular zone of the prethalamus are characterized by strong expression of transcription factors like *Olig2*, *Dlx* genes and *Foxd1* (Tou et al., 2007; Blackshaw et al., 2010; Newman et al., 2018; Puelles et al., 2021). Before E14, the prethalamic lineage cells differentiate into neurons, and they start migrating laterally and dorsally to populate the nascent RTN, ZI and vLGN (Ono et al., 2008; Inamura et al., 2011). Almost all of these cells are either local or projecting GABAergic neurons, the latter targeting regions of the thalamus, pretectum and midbrain (Jones, 2007).

Genetic and activity-dependent factors control the development of thalamic interneurons

The neural tube of rodents exhibits three domains of GABAergic progenitors along its rostro-caudal axis (Achim et al., 2013), each characterized by specific genetic programs with distinct terminal selector genes and giving rise to three broad GABA lineages. The borders of these domains are defined by molecular markers and by secondary organizers. Firstly, the rostral domain expands caudally from the ganglionic eminences, through prosomere 3 up to the ZLI in the diencephalon, where GABAergic differentiation depends on the *Dlx1/2* lineage selector genes (Delogu et al., 2012; Le et al., 2017). Secondly, the ZLI separates the rostral domain from the intermediate domain, which expands caudally up to the isthmic organizer at the midbrain-hindbrain boundary. The intermediate domain includes prosomere 2, prosomere 1 and the midbrain, and GABAergic neurogenesis in the intermediate domain relies on the lineage selector genes *Tal2* and *Gata2* (Virolainen et al., 2012). Finally, the caudal domain spans through the hindbrain and spinal cord, where GABAergic fate is acquired through the expression of lineage selector genes like *Ptf1a* and *Tal1* (Hoshino et al., 2005; Muroyama et al., 2005; Fujiyama et al., 2009). Therefore, the combined mesencephalic and prethalamic origin of thalamic interneurons mean they constitute a population with two broad molecular identities: the *Dlx1/2* (enriched in HO nuclei) and the *Gata2/Tal2* lineage (enriched in FO nuclei) (**Figures 3A, B**).

The GABAergic cells derived from each lineage acquire their identity during development through different genetic programs. Although the molecular mechanisms that confer GABAergic identity have not specifically been studied in thalamic interneurons, it is likely that each subpopulation of thalamic interneurons unfolds genetic programs according to their site of origin. For instance, GABA precursors in the developing midbrain start expressing *Gata2* and *Tal2* after cell-cycle exit, fate determinants that directly regulate the acquisition of a GABAergic phenotype. These cells activate sustained expression of downstream transcription factors related to the maintenance of a GABAergic identity (*Tal1*, *Gata3*, *Six3,* and *Gad1*) and to the correct migration of midbrain precursors (*Sox14)* (Delogu et al., 2012). Indeed, in mice lacking GATA2 or TAL2, GABAergic precursors from the midbrain fail to express genes related to GABA neurotransmission and they switch fate, acquiring a glutamatergic identity (Kala et al., 2009; Achim et al., 2013). However, it remains unclear to what extent midbrain-derived thalamic interneurons share a similar developmental trajectory with other midbrain-derived GABAergic cells. A similar genetic program could also be established for the prethalamic-derived thalamic interneurons and although there are no direct studies on this subpopulation, the developmental program is likely to resemble that of other GABAergic neurons derived from the rostral GABAergic domain, such as cortical and striatal interneurons (Lindtner et al., 2019). As such, the *Dlx1/2* transcription factors may contribute to their GABAergic phenotype, either by directly controlling the expression of the GAD isoforms or by indirectly activating *Dlx5* and *Dlx6* transcription, markers of more mature GABAergic precursors (Cobos et al., 2007; Le et al., 2017).

In addition to intrinsic gene regulatory networks, extrinsic factors also influence the development of thalamic interneurons. In the mouse visual system, different developmental processes are thought to be extrinsically influenced by the input that arrives from the retina, ranging from neurogenesis to network recruitment of interneurons (Golding et al., 2014; Charalambakis et al., 2019; Su et al., 2020). This is evident in animal models where retinal projections are absent or compromised. For example, in anophthalmic mice whose optic nerves were severed at birth and in mice with abnormal spontaneous retinal activity during development, thalamic interneurons accumulate in the upper tiers of the dLGN as opposed to adopting the homogenous distribution throughout the nucleus observed in control mice (**Figure 3C**; Golding et al., 2014). In these models, the synaptic properties of thalamic interneurons were also affected due to the downregulation of presynaptic and postsynaptic proteins, enhancing the excitability of dLGN neurons and disinhibiting the visual thalamocortical system. Similar results were reported in a transgenic mouse ($Math5^{-/-}$) in which the optic tract does not develop, and following binocular enucleation in mice soon after birth (Charalambakis et al., 2019). In both scenarios, the distribution of thalamic interneurons was biased toward the dorsal part of the nucleus, failing to develop both mature intrinsic electrical properties and normal synaptic connectivity with relay neurons.

In addition to the alterations in the distribution of interneurons, there are fewer of these cells in the dLGN of *Math5*$^{-/-}$ mice than in control mice. This reduction in the number of interneurons correlates with abnormally weak FGF15 expression by some astrocytes residing in the visual thalamus (Su et al., 2020). Thus, as in other brain structures, it is likely that the release of FGFs contributes to the recruitment and maturation of inhibitory neurons. Accordingly, genetic ablation of FGF15 impairs the migration of thalamic interneurons into the dLGN and they become misrouted into the somatosensory nucleus. The expression of astrocytic FGF15 in the visual thalamus may be regulated by the SHH released from retinal axons (Deven Somaiya et al., 2022), and the expression of astrocytic FGF15 is reduced in the absence of retinal SHH, decimating the recruitment of interneurons. However, more evidence is needed regarding the interaction between SHH released from retinal axons, the SHH signaling cascades in astrocytes and FGF15 expression. Other effects of SHH might also be at play in these processes and for instance, SHH could exert a broader effect on thalamic astrocytes as it participates in astrocyte specification in brain regions like the retina (Dakubo et al., 2008).

Concluding remarks

In this review we first focus on the development of excitatory neurons of the thalamus, how they extend their axons and receive inputs from the cortex, and the role of spontaneous activity in the development of these projections. Next, we have delved into the information currently available regarding the development of the other main neuronal cell-type present in the thalamus, local GABAergic interneurons. The evidence compiled in this review establishes the state-of-the-art of the field but also, it poses important questions that need to be addressed. For instance, there are few studies that have investigated how the development of thalamocortical excitatory neurons and local GABAergic interneurons is orchestrated. Moreover, further studies are required to disentangle the precise origin of these local GABAergic neurons, as well as comparative studies using ancient species. It is still unclear what molecular signature determines the thalamic fate of interneurons derived from the midbrain, the prethalamus or other regions, as well as the guidance mechanisms that direct interneurons into the thalamus during development. Finally, since it is now well established that the thalamus presents different patterns of spontaneous activity and that changes in these patterns affect the development of other structures like the sensory cortices, it would be interesting to study the impact of this spontaneous thalamic activity on developing thalamic interneurons.

Data availability statement

The original contributions presented in this study are included in this article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

GL-B, FM, and IH-G wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Achim, K., Peltopuro, P., Lahti, L., Tsai, H. H., Zachariah, A., Åstrand, M., et al. (2013). The role of Tal2 and Tal1 in the differentiation of midbrain GABAergic neuron precursors. *Biol. Open* 2, 990–997. doi: 10.1242/bio.20135041

Allendoerfer, K. L., and Shatz, C. J. (1994). The subplate, a transient neocortical structure: Its role in the development of connections between thalamus and cortex. *Annu. Rev. Neurosci.* 17, 185–218. doi: 10.1146/ANNUREV.NE.17.030194.001153

Anastasiades, P. G., Collins, D. P., and Carter, A. G. (2021). Mediodorsal and ventromedial thalamus engage distinct L1 circuits in the prefrontal cortex. *Neuron* 109, 314–330.e4. doi: 10.1016/J.NEURON.2020.10.031

Antón-Bolaños, N., Espinosa, A., and López-Bendito, G. (2018). Developmental interactions between thalamus and cortex: A true love reciprocal story. *Curr. Opin. Neurobiol.* 52, 33–41. doi: 10.1016/J.CONB.2018.04.018

Antón-Bolaños, N., Sempere-Ferràndez, A., Guillamón-Vivancos, T., Martini, F. J., Pérez-Saiz, L., Gezelius, H., et al. (2019). Prenatal activity from thalamic neurons governs

the emergence of functional cortical maps in mice. *Science* 364, 987–990. doi: 10.1126/science.aav7617

Assali, A., le Magueresse, C., Bennis, M., Nicol, X., Gaspar, P., and Rebsam, A. (2017). RIM1/2 in retinal ganglion cells are required for the refinement of ipsilateral axons and eye-specific segregation. *Sci. Rep.* 7:3236. doi: 10.1038/S41598-017-03361-0

Bagri, A., Marín, O., Plump, A. S., Mak, J., Pleasure, S. J., Rubenstein, J. L. R., et al. (2002). Slit proteins prevent midline crossing and determine the dorsoventral position of major axonal pathways in the mammalian forebrain. *Neuron* 33, 233–248. doi: 10.1016/S0896-6273(02)00561-5

Bakken, T. E., van Velthoven, C. T. J., Menon, V., Hodge, R. D., Yao, Z., Nguyen, T. N., et al. (2015). Single-cell RNA-seq uncovers shared and distinct axes of variation in dorsal LGN neurons in mice, non-human primates and humans. *eLife* 10:e64875. doi: 10.1101/2020.11.05.367482

Ballester-Rosado, C. J., Albright, M. J., Wu, C. S., Liao, C. C., Zhu, J., Xu, J., et al. (2010). mGluR5 in cortical excitatory neurons exerts both cell-autonomous and -nonautonomous influences on cortical somatosensory circuit formation. *J. Neurosci.* 30, 16896–16909. doi: 10.1523/JNEUROSCI.2462-10.2010

Barth, K. A., and Wilson, S. W. (1995). Expression of zebrafish nk2.2 is influenced by sonic hedgehog/vertebrate hedgehog-1 and demarcates a zone of neuronal differentiation in the embryonic forebrain. *Development* 121, 1755–1768. doi: 10.1242/DEV.121.6.1755

Bickford, M. E. (2016). Thalamic circuit diversity: Modulation of the driver/modulator framework. *Front. Neural Circ.* 9:86. doi: 10.3389/FNCIR.2015.00086

Bielle, F., Marcos-Mondejar, P., Keita, M., Mailhes, C., Verney, C., Nguyen Ba-Charvet, K., et al. (2011). Slit2 activity in the migration of guidepost neurons shapes thalamic projections during development and evolution. *Neuron* 69, 1085–1098. doi: 10.1016/J.NEURON.2011.02.026

Blackshaw, S., Scholpp, S., Placzek, M., Ingraham, H., Simerly, R., and Shimogori, T. (2010). Molecular pathways controlling development of thalamus and hypothalamus: From neural specification to circuit formation. *J. Neurosci.* 30, 14925–14930. doi: 10.1523/JNEUROSCI.4499-10.2010

Blakemore, C., and Molnar, Z. (1990). Factors involved in the establishment of specific interconnections between thalamus and cerebral cortex. *Cold Spring Harb. Symp. Quant. Biol.* 55, 491–504. doi: 10.1101/SQB.1990.055.01.048

Braisted, J. E., Catalano, S. M., Stimac, R., Kennedy, T. E., Tessier-Lavigne, M., Shatz, C. J., et al. (2000). Netrin-1 promotes thalamic axon growth and is required for proper development of the thalamocortical projection. *J. Neurosci.* 20, 5792–5801. doi: 10.1523/JNEUROSCI.20-15-05792.2000

Braisted, J. E., Ringstedt, T., and O'Leary, D. D. M. (2009). Slits are chemorepellents endogenous to hypothalamus and steer thalamocortical axons into ventral telencephalon. *Cereb. Cortex* 19(Suppl. 1), i144–i151. doi: 10.1093/CERCOR/BHP035

Brooks, J. M., Su, J., Levy, C., Wang, J. S., Seabrook, T. A., Guido, W., et al. (2013). A molecular mechanism regulating the timing of corticogeniculate innervation. *Cell Rep.* 5, 573–581. doi: 10.1016/J.CELREP.2013.09.041

Butler, A. B. (2008). Evolution of the thalamus: A morphological and functional review. *Thalamus. Relat. Syst.* 4, 35–58. doi: 10.1017/S1472928808000356

Cadwell, C. R., Bhaduri, A., Mostajo-Radji, M. A., Keefe, M. G., and Nowakowski, T. J. (2019). Development and arealization of the cerebral cortex. *Neuron* 103, 980–1004. doi: 10.1016/J.NEURON.2019.07.009

Callejas-Marin, A., Moreno-Bravo, J. A., Company, V., Madrigal, M. P., Almagro-García, F., Martínez, S., et al. (2022). Gli2-mediated Shh signaling is required for thalamocortical projection guidance. *Front. Neuroanat.* 16:830758. doi: 10.3389/FNANA.2022.830758

Castillo-Paterna, M., Moreno-Juan, V., Filipchuk, A., Rodríguez-Malmierca, L., Susín, R., and López-Bendito, G. (2015). DCC functions as an accelerator of thalamocortical axonal growth downstream of spontaneous thalamic activity. *EMBO Rep.* 16, 851–862. doi: 10.15252/EMBR.201439882

Charalambakis, N. E., Govindaiah, G., Campbell, P. W., and Guido, W. (2019). Developmental remodeling of thalamic interneurons requires retinal signaling. *J. Neurosci.* 39, 3856–3866. doi: 10.1523/JNEUROSCI.2224-18.2019

Chatterjee, M., and Li, J. Y. H. (2012). Patterning and compartment formation in the diencephalon. *Front. Neurosci.* 6:66. doi: 10.3389/FNINS.2012.00066

Chatterjee, M., Guo, Q., Weber, S., Scholpp, S., and Li, J. Y. H. (2014). Pax6 regulates the formation of the habenular nuclei by controlling the temporospatial expression of Shh in the diencephalon in vertebrates. *BMC Biol.* 12:13. doi: 10.1186/1741-7007-12-13

Che, A., Babij, R., Iannone, A. F., Fetcho, R. N., Ferrer, M., Liston, C., et al. (2018). Layer I interneurons sharpen sensory maps during neonatal development. *Neuron* 99, 98–116.e7. doi: 10.1016/J.NEURON.2018.06.002

Chen, L., Guo, Q., and Li, J. Y. H. (2009). Transcription factor Gbx2 acts cell-nonautonomously to regulate the formation of lineage-restriction boundaries of the thalamus. *Development* 136, 1317–1326. doi: 10.1242/DEV.030510

Chou, S. J., Babot, Z., Leingaŕtner, A., Studer, M., Nakagawa, Y., and O'Leary, D. D. M. (2013). Geniculocortical input drives genetic distinctions between primary and higher-order visual areas. *Science* 340, 1239–1242. doi: 10.1126/SCIENCE.1232806

Clascá, F., Angelucci, A., and Sur, M. (1995). Layer-specific programs of development in neocortical projection neurons. *Proc. Natl. Acad. Sci. U.S.A.* 92, 11145–11149. doi: 10.1073/PNAS.92.24.11145

Cobos, I., Borello, U., and Rubenstein, J. L. R. (2007). Dlx transcription factors promote migration through repression of axon and dendrite growth. *Neuron* 54, 873–888. doi: 10.1016/J.NEURON.2007.05.024

Colonnese, M. T. (2014). Rapid developmental emergence of stable depolarization during wakefulness by inhibitory balancing of cortical network excitability. *J. Neurosci.* 34, 5477–5485. doi: 10.1523/JNEUROSCI.3659-13.2014

Colonnese, M. T., Kaminska, A., Minlebaev, M., Milh, M., Bloem, B., Lescure, S., et al. (2010). A conserved switch in sensory processing prepares developing neocortex for vision. *Neuron* 67, 480–498.

Crowley, J. C., and Katz, L. C. (2000). Early development of ocular dominance columns. *Science* 290, 1321–1324. doi: 10.1126/SCIENCE.290.5495.1321

Dakubo, G. D., Beug, S. T., Mazerolle, C. J., Thurig, S., Wang, Y., and Wallace, V. A. (2008). Control of glial precursor cell development in the mouse optic nerve by sonic hedgehog from retinal ganglion cells. *Brain Res.* 1228, 27–42. doi: 10.1016/J.BRAINRES.2008.06.058

Datwani, A., Iwasato, T., Itohara, S., and Erzurumlu, R. S. (2002). NMDA receptor-dependent pattern transfer from afferents to postsynaptic cells and dendritic differentiation in the barrel cortex. *Mol. Cell Neurosci.* 21, 477–492. doi: 10.1006/mcne.2002.1195

de Carlos, J. A., and O'Leary, D. D. M. (1992). Growth and targeting of subplate axons and establishment of major cortical pathways. *J. Neurosci.* 12, 1194–1211. doi: 10.1523/JNEUROSCI.12-04-01194.1992

de Marco García, N. V., Priya, R., Tuncdemir, S. N., Fishell, G., and Karayannis, T. (2015). Sensory inputs control the integration of neurogliaform interneurons into cortical circuits. *Nat. Neurosci.* 18, 393–403. doi: 10.1038/NN.3946

Deck, M., Lokmane, L., Chauvet, S., Mailhes, C., Keita, M., Niquille, M., et al. (2013). Pathfinding of corticothalamic axons relies on a rendezvous with thalamic projections. *Neuron* 77, 472–484. doi: 10.1016/J.NEURON.2012.11.031

Dehay, C., Giroud, P., Berland, M., Killackey, H. P., and Kennedy, H. (1996). Phenotypic characterisation of respecified visual cortex subsequent to prenatal enucleation in the monkey: Development of acetylcholinesterase and cytochrome oxidase patterns. *J. Comp. Neurol.* 376, 386–402. doi: 10.1002/(SICI)1096-9861(19961216)376:3<386::AID-CNE3>3.0.CO;2-Z

del Río, J. A., Martínez, A., Auladell, C., and Soriano, E. (2000). Developmental history of the subplate and developing white matter in the murine neocortex. Neuronal organization and relationship with the main afferent systems at embryonic and perinatal stages. *Cereb. Cortex* 10, 784–801. doi: 10.1093/CERCOR/10.8.784

Delogu, A., Sellers, K., Zagoraiou, L., Bocianowska-Zbrog, A., Mandal, S., Guimera, J., et al. (2012). Subcortical visual shell nuclei targeted by ipRGCs develop from a Sox14+-GABAergic progenitor and require sox14 to regulate daily activity rhythms. *Neuron* 75, 648–662. doi: 10.1016/j.neuron.2012.06.013

Demas, J., Eglen, S. J., and Wong, R. O. L. (2003). Developmental loss of synchronous spontaneous activity in the mouse retina is independent of visual experience. *J. Neurosci.* 23, 2851–2860. doi: 10.1523/JNEUROSCI.23-07-02851.2003

Deven Somaiya, R., Stebbins, K., Xie, H., Denise, A., Garcia, R., and Fox, M. A. (2022). Sonic hedgehog-dependent recruitment of GABAergic interneurons into the developing visual thalamus. *eLife* 10:e79833. doi: 10.1101/2022.02.22.481508

Dufour, A., Seibt, J., Passante, L., Depaepe, V., Ciossek, T., Frisén, J., et al. (2003). Area specificity and topography of thalamocortical projections are controlled by ephrin/Eph genes. *Neuron* 39, 453–465. doi: 10.1016/S0896-6273(03)00440-9

Fernandez, L. M. J., Vantomme, G., Osorio-Forero, A., Cardis, R., Béard, E., and Lüthi, A. (2018). Thalamic reticular control of local sleep in mouse sensory cortex. *eLife* 7:e39111. doi: 10.7554/ELIFE.39111

Ferran, J. L., de Oliveira, E. D., Merchán, P., Sandoval, J. E., Sánchez-Arrones, L., Martínez-de-la-Torre, M., et al. (2009). Genoarchitectonic profile of developing nuclear groups in the chicken pretectum. *J. Comp. Neurol.* 517, 405–451. doi: 10.1002/CNE.22115

Fujiyama, T., Yamada, M., Terao, M., Terashima, T., Hioki, H., Inoue, Y. U., et al. (2009). Inhibitory and excitatory subtypes of cochlear nucleus neurons are defined by distinct bHLH transcription factors, Ptf1a and Atoh1. *Development* 136, 2049–2058. doi: 10.1242/DEV.033480

Garel, S., and Rubenstein, J. L. R. (2004). Intermediate targets in formation of topographic projections: Inputs from the thalamocortical system. *Trends Neurosci.* 27, 533–539. doi: 10.1016/J.TINS.2004.06.014

Golding, B., Pouchelon, G., Bellone, C., Murthy, S., di Nardo, A. A., Govindan, S., et al. (2014). Retinal input directs the recruitment of inhibitory interneurons into thalamic visual circuits. *Neuron* 81, 1057–1069. doi: 10.1016/j.neuron.2014.01.032

Grant, E., Hoerder-Suabedissen, A., and Molnár, Z. (2012). Development of the corticothalamic projections. *Front. Neurosci.* 6:53. doi: 10.3389/FNINS.2012.00053

Grant, E., Hoerder-Suabedissen, A., and Molnar, Z. (2016). The regulation of corticofugal fiber targeting by retinal inputs. *Cereb. Cortex* 26, 1336–1348. doi: 10.1093/CERCOR/BHV315

Guo, Q., and Li, J. Y. H. (2019). Defining developmental diversification of diencephalon neurons through single cell gene expression profiling. *Development* 146:dev174284. doi: 10.1242/DEV.174284

Gurung, B., and Fritzsch, B. (2004). Time course of embryonic midbrain and thalamic auditory connection development in mice as revealed by carbocyanine dye tracing. *J. Comp. Neurol.* 479, 309–327. doi: 10.1002/CNE.20328

Halassa, M. M., and Acsády, L. (2016). Thalamic inhibition: Diverse sources, diverse scales. *Trends Neurosci.* 39, 680–693. doi: 10.1016/j.tins.2016.08.001

Halassa, M. M., and Sherman, S. M. (2019). Thalamocortical circuit motifs: A general framework. *Neuron* 103, 762–770. doi: 10.1016/J.NEURON.2019.06.005

Hanganu-Opatz, I. L. (2010). Between molecules and experience: Role of early patterns of coordinated activity for the development of cortical maps and sensory abilities. *Brain Res. Rev.* 64, 160–176. doi: 10.1016/J.BRAINRESREV.2010.03.005

Hannan, A. J., Blakemore, C., Katsnelson, A., Vitalis, T., Huber, K. M., Bear, M., et al. (2001). PLC-beta1, activated via mGluRs, mediates activity-dependent differentiation in cerebral cortex. *Nat. Neurosci.* 4, 282–288. doi: 10.1038/85132

Harrington, M. E. (1997). The ventral lateral geniculate nucleus and the intergeniculate leaflet: Interrelated structures in the visual and circadian systems. *Neurosci. Biobehav. Rev.* 21, 705–727. doi: 10.1016/S0149-7634(96)00019-X

Hashimoto-Torii, K., Motoyama, J., Hui, C. C., Kuroiwa, A., Nakafuku, M., and Shimamura, K. (2003). Differential activities of Sonic hedgehog mediated by Gli transcription factors define distinct neuronal subtypes in the dorsal thalamus. *Mech. Dev.* 120, 1097–1111. doi: 10.1016/j.mod.2003.09.001

Hayes, S. G., Murray, K. D., and Jones, E. G. (2003). Two epochs in the development of gamma-aminobutyric acidergic neurons in the ferret thalamus. *J. Comp. Neurol.* 463, 45–65. doi: 10.1002/CNE.10749

Hensch, T. K. (2004). Critical period regulation. *Annu. Rev. Neurosci.* 27, 549–579. doi: 10.1146/ANNUREV.NEURO.27.070203.144327

Herrmann, K., and Shatz, C. J. (1995). Blockade of action potential activity alters initial arborization of thalamic axons within cortical layer 4. *Proc. Natl. Acad. Sci. U.S.A.* 92, 11244–11248. doi: 10.1073/PNAS.92.24.11244

Hevner, R. F., Miyashita-Lin, E., and Rubenstein, J. L. R. (2002). Cortical and thalamic axon pathfinding defects in Tbr1, Gbx2, and Pax6 mutant mice: Evidence that cortical and thalamic axons interact and guide each other. *J. Comp. Neurol.* 447, 8–17. doi: 10.1002/CNE.10219

Hirsch, J. A., Wang, X., Sommer, F. T., and Martinez, L. M. (2015). How inhibitory circuits in the thalamus serve vision. *Annu. Rev. Neurosci.* 38, 309–329. doi: 10.1146/annurev-neuro-071013-014229

Hoerder-Suabedissen, A., and Molnár, Z. (2015). Development, evolution and pathology of neocortical subplate neurons. *Nat. Rev. Neurosci.* 16, 133–146. doi: 10.1038/NRN3915

Hoerder-Suabedissen, A., Hayashi, S., Upton, L., Nolan, Z., Casas-Torremocha, D., Grant, E., et al. (2018). Subset of cortical layer 6b neurons selectively innervates higher order thalamic nuclei in mice. *Cereb. Cortex* 28, 1882–1897. doi: 10.1093/CERCOR/BHY036

Hoshino, M., Nakamura, S., Mori, K., Kawauchi, T., Terao, M., Nishimura, Y. V., et al. (2005). Ptf1a, a bHLH transcriptional gene, defines GABAergic neuronal fates in cerebellum. *Neuron* 47, 201–213. doi: 10.1016/J.NEURON.2005.06.007

Huberman, A. D., Feller, M. B., and Chapman, B. (2008). Mechanisms underlying development of visual maps and receptive fields. *Annu. Rev. Neurosci.* 31, 479–509. doi: 10.1146/ANNUREV.NEURO.31.060407.125533

Inamura, N., Kimura, T., Tada, S., Kurahashi, T., Yanagida, M., Yanagawa, Y., et al. (2012). Intrinsic and extrinsic mechanisms control the termination of cortical interneuron migration. *J. Neurosci.* 32, 6032–6042. doi: 10.1523/jneurosci.3446-11.2012

Inamura, N., Ono, K., Takebayashi, H., Zalc, B., and Ikenaka, K. (2011). Olig2 lineage cells generate GABAergic neurons in the prethalamic nuclei, including the zona incerta, ventral lateral geniculate nucleus and reticular thalamic nucleus. *Dev. Neurosci.* 33, 118–129. doi: 10.1159/000328974

Iwasato, T., Datwani, A., Wolf, A. M., Nishiyama, H., Taguchi, Y., Tonegawa, S., et al. (2000). Cortex-restricted disruption of NMDAR1 impairs neuronal patterns in the barrel cortex. *Nature* 406, 726–731. doi: 10.1038/35021059

Jacobs, E. C., Campagnoni, C., Kampf, K., Reyes, S. D., Kalra, V., Handley, V., et al. (2007). Visualization of corticofugal projections during early cortical development in a tau-GFP-transgenic mouse. *Eur. J. Neurosci.* 25, 17–30. doi: 10.1111/J.1460-9568.2006.05258.X

Jager, P., Moore, G., Calpin, P., Durmishi, X., Salgarella, I., Menage, L., et al. (2021). Dual midbrain and forebrain origins of thalamic inhibitory interneurons. *eLife* 10, 1–29. doi: 10.7554/ELIFE.59272

Jager, P., Ye, Z., Yu, X., Zagoraiou, L., Prekop, H. T., Partanen, J., et al. (2016). Tectal-derived interneurons contribute to phasic and tonic inhibition in the visual thalamus. *Nat. Commun.* 7:13579. doi: 10.1038/ncomms13579

Jeong, Y., Dolson, D. K., Waclaw, R. R., Matise, M. P., Sussel, L., Campbell, K., et al. (2011). Spatial and temporal requirements for sonic hedgehog in the regulation of thalamic interneuron identity. *Development* 138, 531–541. doi: 10.1242/dev.058917

Jhaveri, S., Edwards, M. A., and Schneider, G. E. (1991). Initial stages of retinofugal axon development in the hamster: Evidence for two distinct modes of growth. *Exp. Brain Res.* 87, 371–382. doi: 10.1007/BF00231854

Jones, E. G. (2002). Thalamic circuitry and thalamocortical synchrony. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 357, 1659–1673. doi: 10.1098/RSTB.2002.1168

Jones, E. G. (2007). *The Thalamus*. Cambridge, MA: Cambridge University Press.

Kala, K., Haugas, M., Lilleväli, K., Guimera, J., Wurst, W., Salminen, M., et al. (2009). Gata2 is a tissue-specific post-mitotic selector gene for midbrain GABAergic neurons. *Development* 136, 253–262. doi: 10.1242/DEV.029900

Kanold, P. O., and Luhmann, H. J. (2010). The subplate and early cortical circuits. *Annu. Rev. Neurosci.* 33, 23–48. doi: 10.1146/ANNUREV-NEURO-060909-153244

Kataoka, A., and Shimogori, T. (2008). Fgf8 controls regional identity in the developing thalamus. *Development* 135, 2873–2881. doi: 10.1242/DEV.021618

Kiecker, C., and Lumsden, A. (2004). Hedgehog signaling from the ZLI regulates diencephalic regional identity. *Nat. Neurosci.* 7, 1242–1249. doi: 10.1038/NN1338

Le, T. N., Zhou, Q. P., Cobos, I., Zhang, S., Zagozewski, J., Japoni, S., et al. (2017). GABAergic interneuron differentiation in the basal forebrain is mediated through direct regulation of glutamic acid decarboxylase isoforms by Dlx homeobox transcription factors. *J. Neurosci.* 37, 8816–8829. doi: 10.1523/JNEUROSCI.2125-16.2017

Lee, C. C., and Murray Sherman, S. (2010). Drivers and modulators in the central auditory pathways. *Front. Neurosci.* 4:79. doi: 10.3389/NEURO.01.014.2010

Leyva-Díaz, E., del Toro, D., Menal, M. J., Cambray, S., Susín, R., Tessier-Lavigne, M., et al. (2014). FLRT3 is a Robo1-interacting protein that determines Netrin-1 attraction in developing axons. *Curr. Biol.* 24, 494–508. doi: 10.1016/J.CUB.2014.01.042

Li, H., Fertuzinhos, S., Mohns, E., Hnasko, T. S., Verhage, M., Edwards, R., et al. (2013). Laminar and columnar development of barrel cortex relies on thalamocortical neurotransmission. *Neuron* 79, 970–986. doi: 10.1016/J.NEURON.2013.06.043

Li, K., Zhang, J., and Li, J. Y. H. (2012). Gbx2 plays an essential but transient role in the formation of thalamic nuclei. *PLoS One* 7:e47111. doi: 10.1371/JOURNAL.PONE.0047111

Lindtner, S., Catta-Preta, R., Tian, H., Su-Feher, L., Price, J. D., Dickel, D. E., et al. (2019). Genomic resolution of DLX-orchestrated transcriptional circuits driving development of forebrain GABAergic neurons. *Cell Rep.* 28, 2048–2063.e8. doi: 10.1016/J.CELREP.2019.07.022

Little, G. E., López-Bendito, G., Rünker, A. E., García, N., Piñon, M. C., Chédotal, A., et al. (2009). Specificity and plasticity of thalamocortical connections in Sema6A mutant mice. *PLoS Biol.* 7:e98. doi: 10.1371/JOURNAL.PBIO.1000098

López-Bendito, G., and Molnár, Z. (2003). Thalamocortical development: How are we going to get there? *Nat. Rev. Neurosci.* 4, 276–289. doi: 10.1038/NRN1075

López-Bendito, G., Cautinat, A., Sánchez, J. A., Bielle, F., Flames, N., Garratt, A. N., et al. (2006). Tangential neuronal migration controls axon guidance: A role for neuregulin-1 in thalamocortical axon navigation. *Cell* 125, 127–142. doi: 10.1016/J.CELL.2006.01.042

López-Bendito, G., Flames, N., Ma, L., Fouquet, C., di Meglio, T., Chedotal, A., et al. (2007). Robo1 and Robo2 cooperate to control the guidance of major axonal tracts in the mammalian forebrain. *J. Neurosci.* 27, 3395–3407. doi: 10.1523/JNEUROSCI.4605-06.2007

Mallika, C., Guo, Q., and Li, J. Y. H. (2015). Gbx2 is essential for maintaining thalamic neuron identity and repressing habenular characters in the developing thalamus. *Dev. Biol.* 407, 26–39. doi: 10.1016/J.YDBIO.2015.08.010

Marcos-Mondéjar, P., Peregrín, S., Li, J. Y., Carlsson, L., Tole, S., and López-Bendito, G. (2012). The lhx2 transcription factor controls thalamocortical axonal guidance by specific regulation of robo1 and robo2 receptors. *J. Neurosci.* 32, 4372–4385. doi: 10.1523/JNEUROSCI.5851-11.2012

Marques-Smith, A., Lyngholm, D., Kaufmann, A. K., Stacey, J. A., Hoerder-Suabedissen, A., Becker, E. B. E., et al. (2016). A transient translaminar GABAergic interneuron circuit connects thalamocortical recipient layers in neonatal somatosensory cortex. *Neuron* 89, 536–549. doi: 10.1016/j.neuron.2016.01.015

Martinez-Ferre, A., and Martinez, S. (2009). The development of the thalamic motor learning area is regulated by Fgf8 expression. *J. Neurosci.* 29, 13389–13400. doi: 10.1523/JNEUROSCI.2625-09.2009

Martinez-Ferre, A., and Martinez, S. (2012). Molecular regionalization of the diencephalon. *Front. Neurosci.* 6:73. doi: 10.3389/FNINS.2012.00073

Martini, F. J., Guillamón-Vivancos, T., Moreno-Juan, V., Valdeolmillos, M., and López-Bendito, G. (2021). Spontaneous activity in developing thalamic and cortical sensory networks. *Neuron* 109, 2519–2534. doi: 10.1016/j.neuron.2021.06.026

McConnell, S. K., Ghosh, A., and Shatz, C. J. (1989). Subplate neurons pioneer the first axon pathway from the cerebral cortex. *Science* 245, 978–982. doi: 10.1126/SCIENCE.2475909

Métin, C., and Godement, P. (1996). The ganglionic eminence may be an intermediate target for corticofugal and thalamocortical axons. *J. Neurosci.* 16, 3219–3235. doi: 10.1523/JNEUROSCI.16-10-03219.1996

Miko, I. J., Henkemeyer, M., and Cramer, K. S. (2008). Auditory brainstem responses are impaired in EphA4 and ephrin-B2 deficient mice. *Hear. Res.* 235, 39–46. doi: 10.1016/J.HEARES.2007.09.003

Mire, E., Mezzera, C., Leyva-Diáz, E., Paternain, A. V., Squarzoni, P., Bluy, L., et al. (2012). Spontaneous activity regulates Robo1 transcription to mediate a switch in thalamocortical axon growth. *Nat. Neurosci.* 15, 1134–1143. doi: 10.1038/NN.3160

Molnár, Z., and Cordery, P. (1999). Connections between cells of the internal capsule, thalamus, and cerebral cortex in embryonic rat. *J. Comp. Neurol.* 413, 1–25.

Molnár, Z., Garel, S., López-Bendito, G., Maness, P., and Price, D. J. (2012). Mechanisms controlling the guidance of thalamocortical axons through the embryonic forebrain. *Eur. J. Neurosci.* 35, 1573–1585. doi: 10.1111/J.1460-9568.2012.08119.X

Molyneaux, B. J., Arlotta, P., Menezes, J. R. L., and Macklis, J. D. (2007). Neuronal subtype specification in the cerebral cortex. *Nat. Rev. Neurosci.* 8, 427–437. doi: 10.1038/NRN2151

Moore, R. Y., Weis, R., and Moga, M. M. (2000). Efferent projections of the intergeniculate leaflet and the ventral lateral geniculate nucleus in the rat. *J. Comp. Neurol.* 420, 398–418.

Moreno-Juan, V., Aníbal-Martínez, M., Herrero-Navarro, Á, Valdeolmillos, M., Martini, F. J., and López-Bendito, G. (2022). Spontaneous thalamic activity modulates the cortical innervation of the primary visual nucleus of the thalamus. *Neuroscience* 508, 87–97. doi: 10.1016/J.NEUROSCIENCE.2022.07.022

Moreno-Juan, V., Filipchuk, A., Antón-Bolaños, N., Mezzera, C., Gezelius, H., Andrés, B., et al. (2017). Prenatal thalamic waves regulate cortical area size prior to sensory processing. *Nat. Commun.* 8:14172. doi: 10.1038/NCOMMS14172

Murata, Y., and Colonnese, M. T. (2016). An excitatory cortical feedback loop gates retinal wave transmission in rodent thalamus. *eLife* 5:e18816. doi: 10.7554/ELIFE.18816

Murata, Y., and Colonnese, M. T. (2018). Thalamus controls development and expression of arousal states in visual cortex. *J. Neurosci.* 38, 8772–8786. doi: 10.1523/JNEUROSCI.1519-18.2018

Muroyama, Y., Fujiwara, Y., Orkin, S. H., and Rowitch, D. H. (2005). Specification of astrocytes by bHLH protein SCL in a restricted region of the neural tube. *Nature* 43, 360–363. doi: 10.1038/NATURE04139

Nakagawa, Y. (2019). Development of the thalamus: From early patterning to regulation of cortical functions. *Wiley Interdiscip. Rev. Dev. Biol.* 8:e345. doi: 10.1002/WDEV.345

Nakagawa, Y., and O'Leary, D. D. M. (2001). Combinatorial expression patterns of LIM-homeodomain and other regulatory genes parcellate developing thalamus. *J. Neurosci.* 21, 2711–2725. doi: 10.1523/JNEUROSCI.21-08-02711.2001

Nakagawa, Y., and Shimogori, T. (2012). Diversity of thalamic progenitor cells and postmitotic neurons. *Eur. J. Neurosci.* 35, 1554–1562. doi: 10.1111/J.1460-9568.2012.08089.X

Newman, E. A., Wu, D., Taketo, M. M., Zhang, J., and Blackshaw, S. (2018). Canonical Wnt signaling regulates patterning, differentiation and nucleogenesis in mouse hypothalamus and prethalamus. *Dev. Biol.* 442, 236–248. doi: 10.1016/J.YDBIO.2018.07.021

Ono, K., Takebayashi, H., Ikeda, K., Furusho, M., Nishizawa, T., Watanabe, K., et al. (2008). Regional- and temporal-dependent changes in the differentiation of Olig2 progenitors in the forebrain, and the impact on astrocyte development in the dorsal pallium. *Dev. Biol.* 320, 456–468. doi: 10.1016/J.YDBIO.2008.06.001

Petersen, C. C. H. (2007). The functional organization of the barrel cortex. *Neuron* 56, 339–355. doi: 10.1016/J.NEURON.2007.09.017

Pfeiffenberger, C., Cutforth, T., Woods, G., Yamada, J., Rentería, R. C., Copenhagen, D. R., et al. (2005). Ephrin-As and neural activity are required for eye-specific patterning during retinogeniculate mapping. *Nat. Neurosci.* 8, 1022–1027. doi: 10.1038/NN1508

Pouchelon, G., Gambino, F., Bellone, C., Telley, L., Vitali, I., Lüscher, C., et al. (2014). Modality-specific thalamocortical inputs instruct the identity of postsynaptic L4 neurons. *Nature* 511, 471–474. doi: 10.1038/NATURE13390

Price, D. J., Clegg, J., Duocastella, X. O., Willshaw, D., and Pratt, T. (2012). The importance of combinatorial gene expression in early mammalian thalamic patterning and thalamocortical axonal guidance. *Front. Neurosci.* 6:37. doi: 10.3389/FNINS.2012.00037

Puelles L, Diaz C, Stühmer T, Ferran JL, Martínez-de la Torre M, Rubenstein JLR. LacZ-reporter mapping of Dlx5/6 expression and genoarchitectural analysis of the postnatal mouse prethalamus. *J. Comp. Neurol.* (2021). 529:367–420. doi: 10.1002/CNE.24952

Puelles, L., and Rubenstein, J. L. R. (2003). Forebrain gene expression domains and the evolving prosomeric model. *Trends Neurosci.* 26, 469–476. doi: 10.1016/S0166-2236(03)00234-0

Quintana-Urzainqui, I., Hernández-Malmierca, P., Clegg, J. M., Li, Z., Kozić, Z., and Price, D. J. (2020). The role of the diencephalon in the guidance of thalamocortical axons in mice. *Development* 147:dev184523. doi: 10.1242/DEV.184523

Rakic, P. (1991). Experimental manipulation of cerebral cortical areas in primates. *Philos. Trans. R Soc. Lond. Ser. B Biol. Sci.* 331, 291–294. doi: 10.1098/RSTB.1991.0019

Reichova, I., and Sherman, S. M. (2004). Somatosensory corticothalamic projections: Distinguishing drivers from modulators. *J. Neurophysiol.* 92, 2185–2197. doi: 10.1152/JN.00322.2004

Schlaggar, B. L., and O'Leary, D. D. M. (1994). Early development of the somatotopic map and barrel patterning in rat somatosensory cortex. *J. Comp. Neurol.* 346, 80–96. doi: 10.1002/CNE.903460106

Scholpp, S., Delogu, A., Gilthorpe, J., Peukert, D., Schindler, S., and Lumsden, A. (2009). Her6 regulates the neurogenetic gradient and neuronal identity in the thalamus. *Proc. Natl. Acad. Sci. U.S.A.* 106, 19895–19900. doi: 10.1073/PNAS.0910894106

Scholpp, S., Wolf, O., Brand, M., and Lumsden, A. (2006). Hedgehog signalling from the zona limitans intrathalamica orchestrates patterning of the zebrafish diencephalon. *Development* 133, 855–864. doi: 10.1242/DEV.02248

Seibt, J., Schuurmans, C., Gradwhol, G., Dehay, C., Vanderhaeghen, P., Guillemot, F., et al. (2003). Neurogenin2 specifies the connectivity of thalamic neurons by controlling axon responsiveness to intermediate target cues. *Neuron* 39, 439–452. doi: 10.1016/S0896-6273(03)00435-5

Sgaier, S. K., Lao, Z., Villanueva, M. P., Berenshteyn, F., Stephen, D., Turnbull, R. K., et al. (2007). Genetic subdivision of the tectum and cerebellum into functionally related regions based on differential sensitivity to engrailed proteins. *Development* 134, 2325–2335. doi: 10.1242/DEV.000620

Sherman, S. M. (2017). Functioning of circuits connecting thalamus and cortex. *Compr. Physiol.* 7, 713–739. doi: 10.1002/CPHY.C160032

Sherman, S. M., and Guillery, R. W. (2002). The role of the thalamus in the flow of information to the cortex. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 357, 1695–1708. doi: 10.1098/RSTB.2002.1161

Shi, W., Xianyu, A., Han, Z., Tang, X., Li, Z., Zhong, H., et al. (2017). Ontogenetic establishment of order-specific nuclear organization in the mammalian thalamus. *Nat. Neurosci.* 20, 516–528. doi: 10.1038/NN.4519

Sokhadze, G., Seabrook, T. A., and Guido, W. (2018). The absence of retinal input disrupts the development of cholinergic brainstem projections in the mouse dorsal lateral geniculate nucleus 11 Medical and Health Sciences 1109 Neurosciences. *Neural Dev.* 13:27. doi: 10.1186/s13064-018-0124-7

Su, J., Charalambakis, N. E., Sabbagh, U., Somaiya, R. D., Monavarfeshani, A., Guido, W., et al. (2020). Retinal inputs signal astrocytes to recruit interneurons into visual thalamus. *Proc. Natl. Acad. Sci. U.S.A.* 117, 2671–2682. doi: 10.1073/PNAS.1913053117

Sumser, A., Mease, R. A., Sakmann, B., and Groh, A. (2017). Organization and somatotopy of corticothalamic projections from L5B in mouse barrel cortex. *Proc. Natl. Acad. Sci. U.S.A.* 114, 8853–8858. doi: 10.1073/PNAS.1704302114

Suzuki-Hirano, A., Ogawa, M., Kataoka, A., Yoshida, A. C., Itoh, D., Ueno, M., et al. (2011). Dynamic spatiotemporal gene expression in embryonic mouse thalamus. *J. Comp. Neurol.* 519, 528–543. doi: 10.1002/CNE.22531

Swadlow, H. A., and Alonso, J. M. (2017). Multielectrodes join the connectome. *Nat. Methods* 14, 847–848. doi: 10.1038/NMETH.4424

Szabó, N. E., Zhao, T., Zhou, X., and Alvarez-Bolado, G. (2009). The role of Sonic hedgehog of neural origin in thalamic differentiation in the mouse. *J. Neurosci.* 29, 2453–2466. doi: 10.1523/JNEUROSCI.4524-08.2009

Takesian, A. E., Bogart, L. J., Lichtman, J. W., and Hensch, T. K. (2018). Inhibitory circuit gating of auditory critical-period plasticity. *Nat. Neurosci.* 21, 218–227. doi: 10.1038/s41593-017-0064-2

Theyel, B. B., Lee, C. C., and Sherman, S. M. (2010). Specific and nonspecific thalamocortical connectivity in the auditory and somatosensory thalamocortical slices. *Neuroreport* 21, 861–864. doi: 10.1097/WNR.0B013E32833D7CEC

Tou, Y. V., Aaker, J., Taniguchi, A., Kazemzadeh, C., Skidmore, J. M., Martin, D. M., et al. (2007). Characterization of progenitor domains in the developing mouse thalamus. *J. Comp. Neurol.* 505, 73–91. doi: 10.1002/CNE.21467

Tsukano, H., Horie, M., Ohga, S., Takahashi, K., Kubota, Y., Hishida, R., et al. (2017). Reconsidering tonotopic maps in the auditory cortex and lemniscal auditory thalamus in mice. *Front. Neural Circuits* 11:14. doi: 10.3389/FNCIR.2017.00014

Tuncdemir, S. N., Wamsley, B., Stam, F. J., Osakada, F., Goulding, M., Callaway, E. M., et al. (2016). Early somatostatin interneuron connectivity mediates the maturation of deep layer cortical circuits. *Neuron* 89, 521–535. doi: 10.1016/j.neuron.2015.11.020

Uesaka, N., Hayano, Y., Yamada, A., and Yamamoto, N. (2007). Interplay between laminar specificity and activity-dependent mechanisms of thalamocortical axon branching. *J. Neurosci.* 27, 5215–5223. doi: 10.1523/JNEUROSCI.4685-06.2007

Viaene, A. N., Petrof, I., and Murray Sherman, S. (2011). Synaptic properties of thalamic input to the subgranular layers of primary somatosensory and auditory cortices in the mouse. *J. Neurosci.* 31, 12738–12747. doi: 10.1523/JNEUROSCI.1565-11.2011

Virolainen, S. M., Achim, K., Peltopuro, P., Salminen, M., and Partanen, J. (2012). Transcriptional regulatory mechanisms underlying the GABAergic neuron fate in different diencephalic prosomeres. *Development* 139, 3795–3805. doi: 10.1242/dev.075192

Vue, T. Y., Bluske, K., Alishahi, A., Yang, L. L., Koyano-Nakagawa, N., Novitch, B., et al. (2009). Sonic hedgehog signaling controls thalamic progenitor identity and nuclei specification in mice. *J. Neurosci.* 29, 4484–4497. doi: 10.1523/JNEUROSCI.0656-09.2009

Vue, T. Y., Lee, M., Tan, Y. E., Werkhoven, Z., Wang, L., and Nakagawa, Y. (2013). Thalamic control of neocortical area formation in mice. *J. Neurosci.* 33, 8442–8453. doi: 10.1523/JNEUROSCI.5786-12.2013

Wamsley, B., and Fishell, G. (2017). Genetic and activity-dependent mechanisms underlying interneuron diversity. *Nat. Rev. Neurosci.* 18, 299–309. doi: 10.1038/NRN.2017.30

Wong, F. K., Bercsenyi, K., Sreenivasan, V., Portalés, A., Fernández-Otero, M., and Marín, O. (2018). Pyramidal cell regulation of interneuron survival sculpts cortical networks. *Nature* 557, 668–673. doi: 10.1038/s41586-018-0139-6

Zechel, S., Nakagawa, Y., and Ibáñez, C. F. (2016). Thalamo-cortical axons regulate the radial dispersion of neocortical GABAergic interneurons. *eLife* 5:e20770. doi: 10.7554/ELIFE.20770