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Functional Neuroanatomy of Visual Pathways Involving the Pulvinar

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Résumé

Les neurones du cortex visuel primaire (V1) peuvent emprunter deux voies de communications afin d'atteindre les aires extrastriées : une voie cortico-corticale, et une voie cortico-thalamo-corticale à travers des noyaux thalamiques de haut niveau (HO) comme le pulvinar. Les fonctions respectives de ces deux voies restent toujours méconnues. Un pas vers une meilleure compréhension de celles-ci seraient d'investiguer la nature des signaux qu'elles transmettent. Dans ce contexte, deux grands types de projections cortico-thalamiques (CT) ont été identifiés dans le système visuel : les neurones de type I (modulator) et type II (driver) caractérisés respectivement par des axones minces dotés de petits boutons terminaux et par des axones plus épais et de plus grands boutons respectivement. Une proposition récente a aussi émis l'hypothèse que ces deux types pourraient également être distingués par leur expression de transporteur de glutamate vésiculaire. Cette hypothèse suggère que les projections de type II et de type I peuvent exprimer sélectivement VGLUT2 et VGLUT1, respectivement (Balaram, 2013; Rovo et al, 2012).

Chez le chat, les projections de V1 vers le pulvinar se composent principalement de terminaux de type II, tandis que celles de l'aire PMLS présentent une combinaison de terminaux de type I et II suggérant ainsi que, la proportion de terminaux de type I augmente avec le niveau hiérarchique cortical des zones visuelles. Afin de tester cette hypothèse, nous avons cartographié la distribution des terminaux CT du cortex AEV (article 1) ainsi que de l'aire 21a (article 2). Nous avons aussi étudié l'expression de VGLUT 1 et 2 dans le système visuel du chat afin de tester si leurs expressions corrèlent avec les sites de projections de neurones de type I et II (article 3).

Nos résultats indiquent que la grande majorité des terminaux marqués dans le pulvinar provenant de l'AEV et de l'aire 21a sont de type I (Article 1 et 2) alors que ceux de V1 sont majoritairement de type II. Une comparaison de la proportion des projections de type I à travers les aires V1, PMLS, 21a et AEV révèlent une corrélation positive de sorte que celle-ci augmente avec le degré hiérarchique des aires visuelles. De plus, nos résultats indiquent que VGLUT 1 et 2 présentent une distribution complémentaire et que leur localisation dans des sites connus pour recevoir une projection de type 'modulateur' et 'déclencheur' proéminente suggère que leurs expressions peuvent montrer un biais pour celles-ci dans la voie géniculo-strié.

Les résultats de cette thèse ont permis de mieux connaitre la nature des projections CT des aires visuelles extrastriées. Ces résultats sont d'autant plus importants qu'ils établissent un lien entre la nature de ces projections et le degré hiérarchique des aires visuelles, suggérant ainsi l'existence une organisation anatomofonctionnelle des voies CT passant par le pulvinar. Enfin, les résultats de cette thèse ont aussi permis une meilleure compréhension des vésicules VGLUT 1 et 2 dans le système visuel du chat et leurs affinités respectives pour les sites de projections de neurones de type I et II.

Mots-clés : Aire 21a, cortico-thalamique, thalamus, cortex, pulvinar, VGLUT

Abstract

Visual signals from the primary visual cortex (V1), can take two main communication routes in order to reach higher visual areas: a corticocortical pathway and a cortico-thalamo-cortical (or transthalamic) pathway through high-order thalamic nuclei such as the pulvinar. While these pathways are receiving an increasing interest from the scientific community, their respective functions still remain largely unknown. An important step towards a better understanding of these pathways would be to investigate the nature of the signals they transmit. In this context, two main types of corticothalamic (CT) projections have been identified in the visual system: type I projections (modulators) and type II (drivers) characterized respectively by thin axons with small terminal and by thicker axons and larger terminals. A recent proposal has also hypothesized that these two types can also be distinguished by their expression of vesicular glutamate transporter (VGLUT) in their respective synaptic terminals such that type II (driver) and type I (modulator) projections can selectively express VGLUT 2 and VGLUT 1, respectively (Balaram, 2013; Rovo et al, 2012).

In cats, projections from V1 to the LP-pulvinar are mainly composed of type II terminals, while those from the Posteromedial lateral suprasylvian (PMLS) cortex present a combination of type I and II terminals. This observation suggests that, in higher-order (HO) thalamic nuclei, the proportion of type I terminals increases with the hierarchical level of the visual areas. To test this hypothesis, we charted the distribution of CT terminals originating from the Anterior EctoSylvian visual cortex (AEV) (article 1) and from area 21a (article 2). We also studied the expression of VGLUT 1 and 2 in the cat's visual system in order to test whether their expressions correlate with the projection sites of type I and II axon terminals (article 3).

Our results from article 1 and 2 indicate that the vast majority of terminals sampled in the pulvinar from the AEV and area 21a are of type I while projections from V1 projections to the pulvinar were mostly composed of type II terminals. A comparison of the proportion of type I projections across areas V1, PMLS, 21a and the AEV revealed a positive correlation such that its proportion increased with the hierarchical rank of visual areas.

Our results also indicate that VGLUT 1 and 2 have a complementary distribution pattern which matches prominent projection of type I and II respectively in ascending visual projections but does not in extra-geniculate pathways involving the pulvinar (Article 3).

Taken together, results from this thesis have allowed a better understanding of the nature of cortico-thalamic projections originating from extra-striate visual areas (21a and AEV). These results are all the more important in that they establish a link between the nature of these projections and the hierarchical degree of their cortical area of origin, thus suggesting that there is a functional organization of CT pathways passing through the pulvinar. Finally, results of this thesis also enabled a better understanding of the expression of VGLUT 1 and 2 in the visual system and their possible respective biases for type I and type II projections.

Keywords : Aire 21a, corticothalamic, thalamus, cortex, pulvinar, VGLUT

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ARTICLE #1

Abbreviations

AchE: Acethylcholinesterase

AEV: Anterior ectosylvian area

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

LGN: Lateral geniculate nucleus

LP: Lateral posterior nucleus

LPl: Lateral part of LP

LPm: Medial part of LP

MT: Middle temporal area

NMDA: N-Methyl-D-aspartic acid

PMLS: Posteromedial lateral suprasylvian cortex

RF: Receptive field

RGC : Retinal Ganglion Cell

SEM: Standard error of the mean

TEO: Posterior part of the inferotemporal cortex

V1, V2, …: cortical visual areas (primary, secondary...)

XC: Extra striate cortex

Acknowledgements

This may sound very *cliché,* but in these few years during which I completed this PhD thesis, there have been many moments (more like weeks and months…) when I thought about quitting. Those moments were at their peak when in 2013, after two and half years of continuously trying to replicate some preliminary results, Christian called me in his office to tell me that, despite how much he valued my talent and passion for vision sciences, he wouldn't be able to let me continue without results for much longer. He gave me one last shot at a project, and should it fail, I would have to quit for my own sake. I carefully listened to the project and to his instructions and effectively started another PhD project, from scratch, almost three years after I had joined the PhD program.

You may now begin to understand how I feel writing these words of acknowledgements, words I never thought I would be able to write for the longer part of my PhD. And since we have to give credit where it is due, I would like to officially start by thanking my supervisor, Dr. Christian Casanova.

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seen him take holidays for himself, so that we could live a normal life. And my mom, always making sure we did well at school, struggling to talk to our teachers in a language so foreign to her. To my parents I want to say, this a gift for you. For all your selflessness and sacrifice, your love and dedication.

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Asking those who know. What I learnt from a congregation of Greats[1].

Should I pursue knowledge or the comfort of a wealthier lifestyle?

> Ali : [I will answer this one.] *"Knowledge. Knowledge is better than wealth for knowledge guards you while you guard wealth; wealth is reduced by spending whereas knowledge increases by sharing (teaching). The treasurers of wealth perish during their own lifetimes, whereas the knowledgeable ones remain alive for all time, their individual selves may pass away, but the likes of them continue to remain in the hearts."*[2]

> Socrates : I agree with Ali, one should*"Prefer knowledge to wealth, for the one is transitory, the other perpetual."* [3]

Where does the journey of knowledge begin?

> Muhammad: Seek it in yourself. *"One [only] has to question. The question is half of knowledge*[4].

What is so unique about the pursuit of knowledge?

> Ali: Knowledge has many benefits, but it suffices to say that *'Every container becomes cramped by what is placed therein, except the container that holds knowledge, for verily it expands because of it.'*[1]

And where shall I seek this knowledge from, How should I choose the right teacher?

> Nikos: A *true teacher* does not need an introduction. *"True teachers are those who use themselves as bridges over which they invite their students to cross; then, having facilitated their crossing, joyfully collapse, encouraging them to create their own."*[5]

Should I find such a teacher, how should I regard him in my life?

> Ali: *'It is [your] teacher's right upon you that you greet people in general as a whole but single him out with your greeting, [...] nor insist for him to continue if he is tired, nor show disinclination at the length of his speech, for verily it [i.e. his speech] is as a palm tree from which you [should] anxiously wait for something to fall from it for you.'*[2]

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[1] It is customary to begin a thesis with a quote. I couldn't pick only one that has truly inspired me. A collection seemed more appropriate.
[2] Ali ibn Abi Talib (601-661 CE), in *Nahj al-Balagha (The peak of Eloquence),* from Sharif Razi
[3] Socrates (470-399 BCE)
[4] Muhammad ibn Abdullah (570-632 CE), in *Kanz-ul-Amal* from Al-Hindi
[5] Nikos Kazantzakis (1883-1957)

Sadiq: *'The right of the one who trains you in knowledge is to magnify him, to frequent his sessions, to listen to him attentively, and to attend to him with devotion. You should not raise your voice to him, nor answer a question that someone has asked him about something. […] You must defend him if anyone ever speaks ill of him in your presence, conceal his faults and publicise his virtues.*[6]

What is the role of reason, in the pursuit of knowledge?

Gautama: *"Do not believe in anything simply because you have heard it. Do not believe in anything simply because it is spoken and rumored by many. Do not believe in anything simply because it is found written in your religious books. Do not believe in anything merely on the authority of your teachers and elders. Do not believe in traditions because they have been handed down for many generations. But after observation and analysis, when you find that anything agrees with reason and is conducive to the good and benefit of one and all, then accept it and live up to it."*[7]

What if this (PhD) journey seems to become endless?

Leo: *"A man on a thousand-mile walk has to forget his goal and say to himself every morning, 'Today I'm going to cover twenty-five miles and then rest up and sleep."*[8]

There are no cells I can find in the pulvinar, and it's been a long day. Any thoughts?

Fyodor: Step out, for '*It is amazing what one ray of sunshine can do for a man!"*[9]

Where does inspiration come from?

Leo: From everything that is around you. *"If I were asked for the most important advice I could give, that which I considered to be the most useful to the men of our century, I should simply say: in the name of God, stop a moment, cease your work, look around you."* [10]

Any thought, Arhur?

Arthur. I agree with Leo. *"For where did Dante get the material for his Hell, if not from this actual world of ours*?"[11]

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[6] Jaʿfar ibn Muḥammad aṣ-Ṣādiq (700-765 CE), Treatise of rights.
[7] Gautama Buddha (480-400 BCE), Kalama Sutta
[8] Leo Tolstoy (1828-1910), War and Peace
[9] Fyodor Dostoesky (1821-1881), *Humiliated and Insulted*
[10] Leo Tolstoy, Essays (1828-1910, Letters and Miscellanies
[11] Arthur Schopenhaeur (1788-1860), *The world as will and representation*

What is the role of literature in life?

> Socrates: Read, and *'"Employ your time in improving yourself by other men's writings so that you shall come easily by what others have labored hard for."* [12]

Is reading enough?

> Epictetus: "*Don't just say you have read books. Show that through them you have learned to think better, to be a more discriminating and reflective person. Books are the training weights of the mind. They are very helpful, but it would be a bad mistake to suppose that one has made progress simply by having internalized their contents.*" [13]

So, knowledge, is more than knowing facts?

> Ibn Sina: Absolutely, for '*The knowledge of anything, since all things have causes, is not acquired or complete unless it is known by its causes.'* [14]

And about Ignorance, where does this path lead to?

> Averroes: "*Ignorance leads to fear, fear leads to hate, and hate leads to violence. This is the equation.*"[15]

And where does Yoda's dark side fit[16] in this equation?

> The congregation: Silent (in synchrony).

What is the source of ultimate joy?

> Anonymous voice: "*There is no joy in the finite; there is joy only in the Infinite.*"[17]"

You mean, my morning espresso shot is not real pleasure?

> Vyasa: "*Pleasures conceived in the world of the senses have a beginning and an end and give birth to misery.*"[18]

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[12] Socrates (470-399 BCE)
[13] Epictetus (50-135 CE), The Art of Living: The Classical Manual on Virtue, Happiness and Effectiveness
[14] Avicenna (Ibn Sina) (980-1037), *On Medicine*
[15] Averroes (Ibn Rushd)(1126-1198)
[16] Yoda: 'Fear is the path to the dark side. Fear leads to anger. Anger leads to hate. Hate leads to suffering.'
[17] Uknown, The Upanishads (6th century BCE)
[18] Ved Vyasa, The Bhagavad Gita

On est au Québec *icite*, un mot en Français?[19]

> Antoine :"*On ne voit qu'avec le cœur, l'essentiel est invisible pour les yeux.*[20]

I need to start the actual thesis, any last word, about meeting The Beloved?

> Saadi: When you meet the infinite being, you can always use my words: "*I was planning to tell you my sorrows when we meet; But no sorrow would remain upon meeting with you.*"[21]

Does the Beloved Himself want to add anything?

> "*O soul at peace! Return to your Lord, pleased, pleasing! Then enter among My servants! And enter My paradise!*"[22]

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[19] English translation : We are in Quebec here, anything to say in French?
[20] Antoine de Saint-Exupéry (1900-1944), Le Petit Prince. Translation : It is only with the heart that one can see rightly; what is essential is invisible to the eye"
[21] Saadi Shirazi (1210-1291)
[22] Quran, 89:27-30

Introduction

This introductory chapter will give the reader some background information about the cortex and the thalamus and will raise questions relevant to the subject of this thesis. It will review the anatomy and functional relationship of the visual cortex and the thalamus. The first part will mostly describe what we know about these two and their classically attributed role. The second part will put forth some pending questions on the organization of cortico-thalamic interactions. The third part will properly introduce research questions studied during this doctoral work.

1. The Corticocentric view of neural processing

1.1 An overview

Sensory systems are generally organized in a hierarchical manner from an anatomical and functional standpoint with a certain homogeneity observed across mammals (Kandel 2013). In the visual system for instance, the system on which this thesis is centered, it is conventionally considered that signals coming from the retina, after a brief relay at the level of the lateral geniculate nucleus (LGN) in the thalamus, are transmitted to the primary visual cortex where basic computations occur *en route* to higher areas where more complex features of the visual scenery is analysed (Casanova 2004).

In this classical understanding of visual (and more generally, sensory) processing, relevant neural information is transmitted across different hierarchically organized and distinct cortical areas from the occipital lobe, each area specialized in analysing a specific

characteristic of the visual scenery. In this cortico-centric view of neural processing, most, if not all neuronal analysis leading to perception and action are based on computations within and across cortical areas through direct cortico-cortical connections (Salin and Bullier 1995).

Applying this model to the cat visual system, which is the animal model studied in this thesis, the flow of visual information processing could be simplified as follows: the retina, lying in the fundus of the ocular globe transforms photons into an electrical signal, conveyed through retinal ganglion cells (RGCs) projecting to the LGN in the thalamus. Neurons from the LGN in turn project to the primary visual cortex (V1 or area 17) to layer 4 where preliminary computation for the analysis of contrast, orientation, spatial frequency occur. Information processed in V1 is then transferred through direct cortico-cortical connections to other areas, including V2 and V3 (area 18 and 19 in the cat) from where visual processing mainly follows two neural streams : the dorsal stream, also known as the where pathway and implicated in the processing of spatial information; and the ventral stream, more concerned with the processing of object recognition (Mishkin and Ungerleider 1982).

While this is a brief overview of the sequential manner in which visual processing occurs, it is important to highlight that in this understanding, the LGN in the thalamus where retinal signals first synapse when leaving the optic chiasma is seen a 'simple' relay and do not actively take part in the processing of visual information.

1.2 Neural processing of visual information

1.2.1 Visual pathways *en route* to the cortex

Effectively, visual processing begins at the retina, a multilayered sensory neural circuit whose main function is to absorb light and convert it into a neural signal, a process known as phototransduction.

After phototransduction happens at the level of photoreceptors, transduced signals are then passed synaptically to other vertically arranged cells forming the retina, namely the bipolar and the retinal ganglion cells (RGC); signals from the RGC's are sent to subcortical structure, leaving the retina through the optic nerve. In addition to this vertical pathway, important lateral connections are also provided by horizontal cells and amacrine cells (Kandel 2013).

Even if the bulk of processing of visual signals is classically attributed to the cortex, some degree of analysis and segregation of signals happen in the retina. Indeed, the very existence of different functional types of RGC's, each one of them bearing distinct RF properties and giving rise to distinct projecting pathways is a testament of this fact. In the cat, three types of RCC's have been described: W, X and Y cells (Figure X).

Table 7.2. *Classes and properties of retinal ganglion cells in the cat*

Property	Y cell (10%)	X cell (40%)	W cell (50%)
Morphology	alpha	beta	various
Receptive-field type	center/surround	center/surround	center/surround, on–off, other
Receptive-field size	large	small	intermediate
Axon conduction velocity	fast	intermediate	slow
Axon size	large	intermediate	small
Response to standing contrast	phasic (transient)	tonic (sustained)	variable
Edge null position	no	yes	variable
Motion sensitivity	fast	intermediate	slow
LGN targets	A, A1, C, MIN[a]	A, A1	C, MIN
Other targets	SC, PT, other[b]		SC, PT, other

[a]A, A1, C, MIN: See Figures 7.5 and 7.6 for LGN laminae.
[b]SC, superior colliculus; PT, pretectum.

Figure 1. Classification of RGC's from the cat retina based on anatomical and functional properties. From *An introduction to the biology of vision*, James T. McIlwain, 1996.

As can be seen in the figure W, X, Y, cells types are differentiated based on their electrophysiological responses, morphology, and projecting layer in the LGN. For instance RGC's from the Y pathway exhibit mainly a phasic response to standing contrast, while responses from the X pathway are tonic (Enroth-Cugell and Robson 1966). The functional segregation of these pathways can also be appreciated when comparing the RF size of their constituents RGC's: X cells exhibit small RF's, have slower axon conduction and are therefore involved in the processing of fine details. On the other hand, Y cells exhibit larger RF with faster responses making them more suitable for motion detection (Casanova 2004). W cells are the least studied of the three subtypes. They responses exhibit a greater variability

and project more heavily to the midbrain (Kaplan 1991). This functional segregation of RGC fibers have also been reported in the primate where M, P and K fibers differentiated based on their physiological responses and morphology are seen projecting in their respective layers of the LGN (Kaplan 2013).

From the retina, the majority of RGC's will project to the LGN in the thalamus, before projecting to the visual cortex. In the cat, the LGN is located dorso-laterally and is divided in two main anatomically distinct regions, the smaller ventral lateral geniculate and a more prominent dorsal lateral geniculate. Both these regions are comprised of several layers and receive distinct projections from the retina: the dorsal part of the LGN is divided into three layers, A, A1 and Cm; layers A and A1 receive X and Y signals (Blake 1979); Cm only receives Y signals (Wilson, Rowe, &Stone, 1976). The ventral portion of the LGN is also comprised of three layers, C1, C2 and C3. The ventral portion of the LGN is mainly innervated by W cells (Spear, Smith et al. 1977).

The segregation of inputs in the LGN is not only restricted to distinct functional pathways. There is also a segregation based on ocular inputs in each laminae of the LGN such that layers A, C, and C2 receive projections from the contralateral nasal retina while layers laminae A1 and C1 are mainly innervated by the ipsilateral temporal retina (Sherman 1993).

A similar organization is also present in the primate visual system. The LGN of the macaque is also organised through multiple layers (six), each one of them receiving inputs from specific functional RGC subtype. The ventral most top four layers are known as the

parvocellular layers and receive visual input from the P-pathway, whereas the dorsal most two layers are known as the magnocellular layers which receive inputs from the M pathway. In the macaque, cells forming koniocellular pathway project to the interlaminar spaces of geniculate layers (Kaplan 2013).

Not all the RGC's project to the LGN; a significant number of cells also project to the midbrain, towards the superior colliculus, a structure critically involved in oculomotor function. The proportion of the retinotectal projection differs across species (May 2006). They constitute approximately 70% of RGC's in the mouse (Hofbauer and Drager 1985), 50% in the cat (Wassle and Illing 1980) and only 10% in monkeys (Perry and Cowey 1984).

1.2.2 Receptive field properties

In the visual system, the receptive field (RF) a neuron is a region of the visual space where light stimulation excites or inhibits the recorded cell (Kandel 2013). Visual neurons are not only concerned with the spatial location of a stimuli; they are also selective for other properties, such as color, motion, contrast, all of which define the properties of a given visual RF (Purves 2018).

At the level of the retina, our knowledge of RF properties of RGC's is largely attributed to pioneer work undertaken by Stephen Kuffler who characterized the responses of single ganglion cells in the cat retina (Kuffler 1953). Based on electrophysiological recordings, he distinguished two classes of ganglion cells whose RF were organized in two concentric regions bearing opposite responses to light described as the center-surround organization: the

ON and OFF-center; RF with ON-centers respond to light increment on the central part of their RF, but when light is projected in their surrounding region, their firing activity is reduced. For OFF-centered RGC's the exact opposite is true in that, they are excited with increment of light in their surround, and inhibited if the same light is shown on the center of their RF (see Figure 2).

RF properties in the LGN closely resemble those from their projecting RGC's. They are also concentrically organized, with both ON-center and OFF-center types (Hubel and Wiesel 1961). A notable difference between RF properties of ganglion cells and the LGN is that a greater inhibitory surround mechanism has been observed at the thalamic level, contributing to a greater sensitivity for LGN neurons to increment of luminance gradients exerts a greater (Hammond 1973).
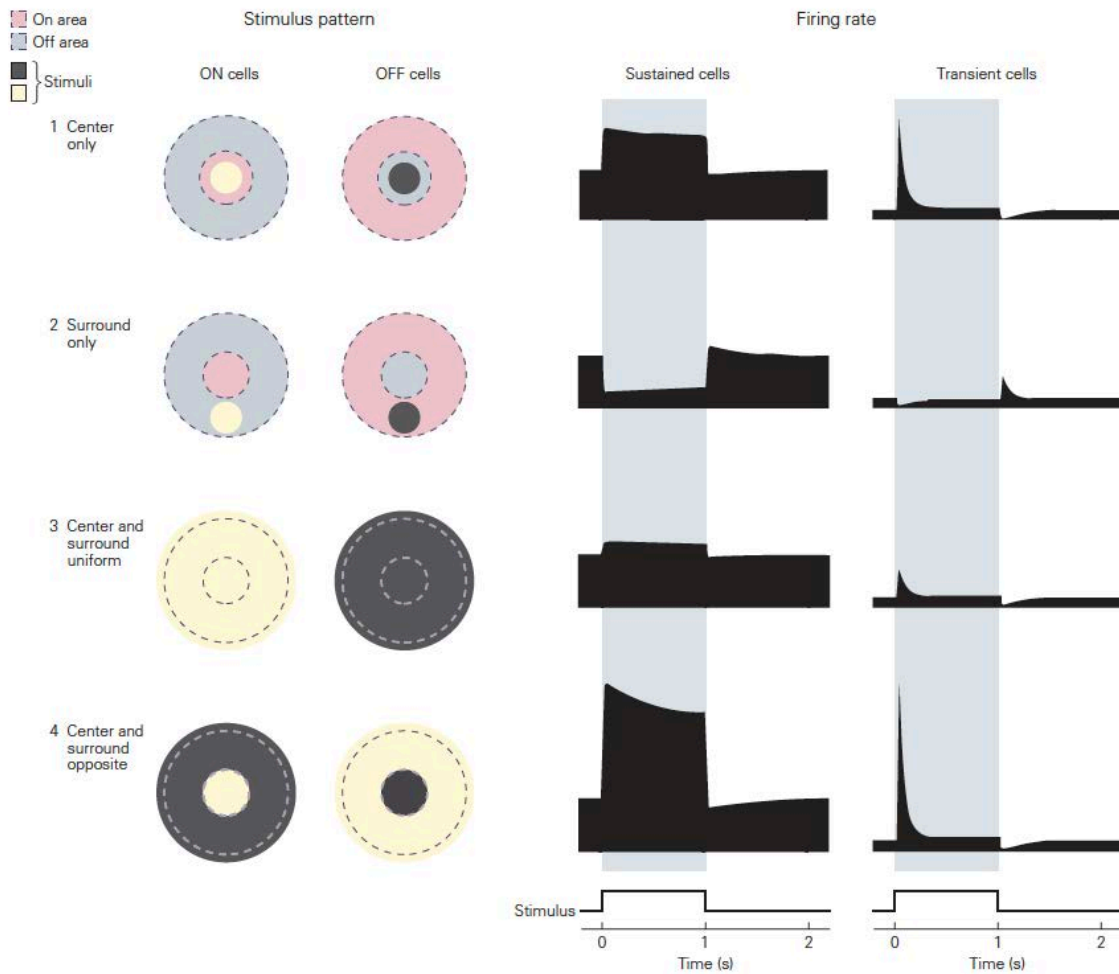
Figure 2. Center-surround organization of RGC's where On- and Off-cells are seen to respond differently when stimulated in their preferred center/surround zones through distinct stimulus patterns; taken from Principles of Neural Science, fifth edition.

.

RF properties undergo a major change from the LGN to the cortex, where cortical cells do not display a concentric organization, but a more rectangular one, making them more sensitive to lines and orientations (Figure 3). Cells from the primary visual cortex differ from those of the LGN in many ways: their RF are sensitive to orientation and can also encode the

true direction of a moving stimuli; they can be binocular and be sensitive to retinal disparity

(Hubel 1988).
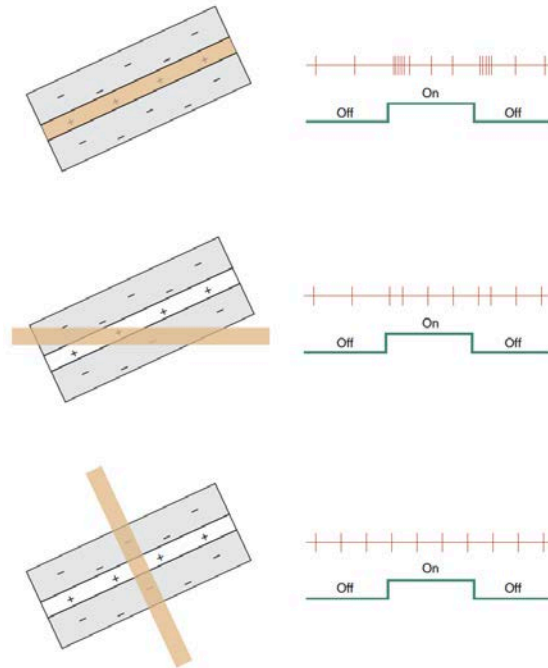
Figure 3. A representation showing the spatial organization and responses of a simple cell from area 17 of the cat bearing a rectangular shape with a middle excitatory subregion flanked by two inhibitory ones; from Visual Perception, a clinical orientation, Steven H. Schwartz, 2010.

Two main types of cortical cells have been classically described by Hubel and Wiesel, simple and complex, both of them bearing distinct RF properties. Simple cells have been described as below in the authors original article (Hubel and Wiesel 1962):

> "[The receptive fields of simple cells] were termed "simple" because like retinal and geniculate receptive fields (1) they were subdivided into distinct excitatory and inhibitory regions; (2) there was summation within the separate excitatory and inhibitory parts; (3) there was antagonism between excitatory and inhibitory regions; and (4) it was possible to predict responses to stationary

or moving spots of various shapes from a map of the excitatory and inhibitory areas.

As mentioned above, an important feature of the spatial organization of simple cells is that they are composed with relatively clear ON and OFF subregions located next to the other as can be seen in Figure 4.
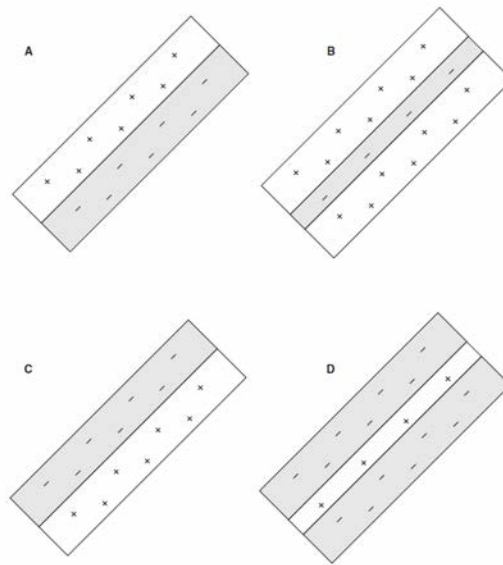
Figure 4. Representation of different spatial organization of simple cells from area 17 of the cat with distinct excitatory and inhibitory flanks. (from Visual Perception, a clinical orientation, Steven H. Schwartz, 2010)

In their 1962 paper on RF properties of area 17 neurons, Hubel and Wiesel first proposed that the RF properties of simple cells of cat area 17 could be explained through an orderly organized set of projection from multiples thalamic afferents. According to their paper, the elongated subregions of cortical cells are built from the convergence of multiple geniculate cells whose receptive-field centers are aligned in a row conferring the rectangular shape of

the newly computed cortical RF (as shown in Fig X). Simple cells are mainly found in layer III, IV and VI (Payne and Peters 2002).
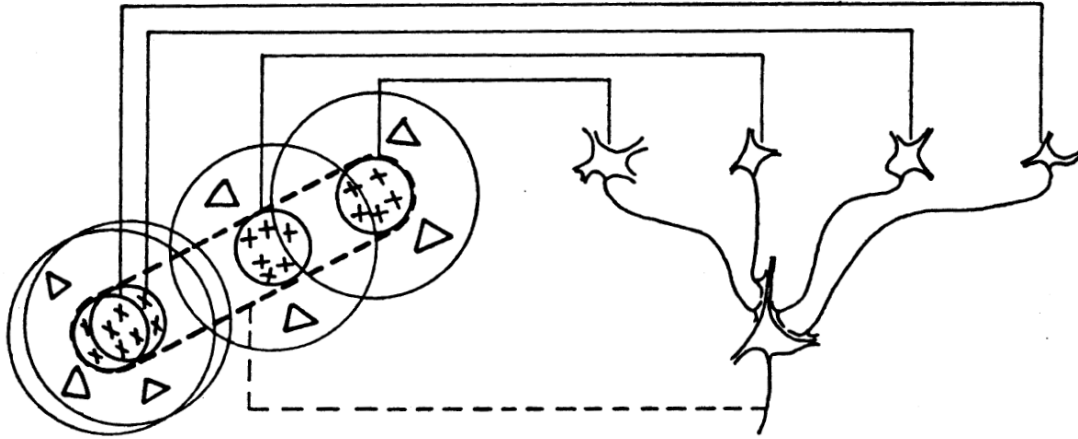
Figure 5. Drawing showing the emergence of area 17 simple cell RF properties from LGN cells, from (Payne and Peters 2002).

The other group of cells described by the authors were called complex. Complex cells are also tuned to orientation, most them to direction as well, but unlike simple cells, they have On and Off responses at each position making them less sensitive to the position of a stimulus within their RF (Figure 6). Complex cells are located in layer II, III and V.
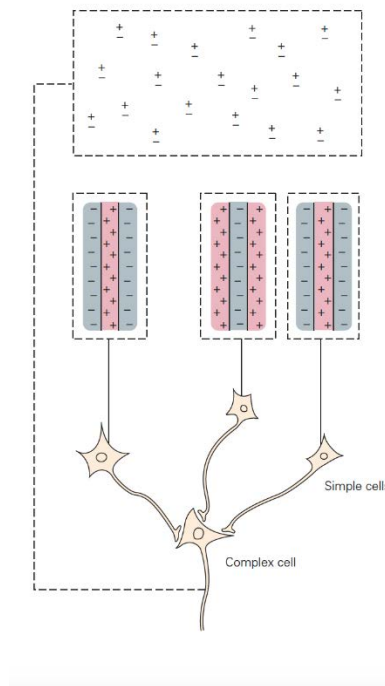
Figure 6. Spatial organization of complex cell compared to simple cell, where on and off responses are seen for each position in the RF taken from Principles of Neural Science, fifth edition, Kandel et al, 2013.

1.2.3   Modular organization of visual the cortex

Generally speaking, sensory cortices are organized in modality specific modules in which neighbouring neurons share common properties and are clustered together in order to facilitate sensory processing. In the visual system, this is translated into a precise modular organization of visual areas.

Like in the LGN for instance, the visual cortex is also organized in a retinotopic manner, defined as the two-dimensional representation of the visual field and is present in all mammals (Kaas 1997).

Earlier single cell recording studies (Palmer, Rosenquist et al. 1978, Tusa, Palmer et al. 1978, Albus and Beckmann 1980, Tusa and Palmer 1980) and more contemporary imaging techniques (Grinvald, Lieke et al. 1986, Schuett, Bonhoeffer et al. 2002, Kalatsky and Stryker 2003) have shown that, in the visual cortex, elevation is mostly represented in the antero-posterior axis, while the azimuth axis is represented in the medio-lateral one. Like in the LGN, the representation of the central visual field is amplified in that it occupies a greater cortical surface than the peripheral visual field (Figure 7).
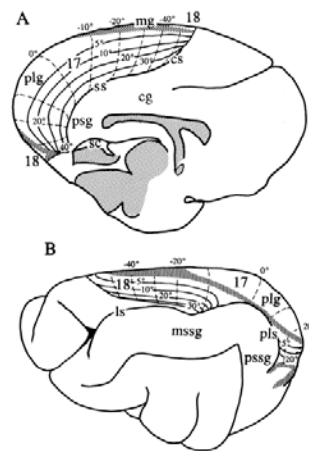
Figure 7. Representation of the retinotopic organization of area 17 and 18 for vertical and horizontal axis, from (Payne and Peters 2002).

The visual cortex is also modularly organized in columns of orientations. Indeed, in the mid 20th century, Lorente de Nó, one the most renowned disciple of Ramon y Cajal realized that

neurons had greater functional connections in vertical columns than they had through horizontal connections (De Lorente 1949). In their earlier observations, Hubel and Wiesel also reached similar conclusion when they realized that neighboring cells recorded within a single perpendicular descent shared the same orientation (Hubel and Wiesel 1962):

> "Cells with common axis orientation were therefore not scattered at random through the cortex, but tended to be grouped together. The size and shape of the regions containing these cell groups were investigated by comparing the fields of cells mapped in sequence. It was at once apparent that successively recorded cells also tended to have identical axis orientations and that each penetration consisted of several sequences of cells, each sequence having a common axis orientation."

The organization of the visual cortex in orientation domain was further demonstrated using mesoscale imaging techniques (Grinvald, Lieke et al. 1986). These imaging techniques have greatly helped to further characterize the columnar organization of the visual cortex revealing for the first time that these iso-orientation columns are organized around 'orientation centers', producing pinwheel-like patterns (Figure 8) (Bonhoeffer and Grinvald 1991). This pin-wheel like organization of iso-orientation columns has also been describe for other extra-striate areas of the cat such as area 21a (Huang, Shou et al. 2006, Villeneuve, Vanni et al. 2009).
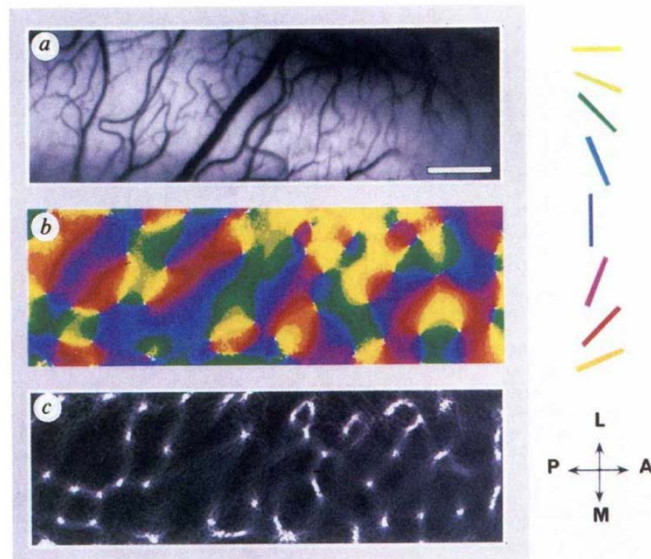
Figure 8. Original figure from (Bonhoeffer and Grinvald 1991). Using optical imaging of intrinsic signals, this was the first study to describe the pinwheel like structure of orientation columns.

Another important feature of the visual cortex is its organization into columns of ocular dominance, a feature only present in cortical layer IV which receives direct geniculate afferents. Like orientation columns, this organization was first revealed through electrophysiological recordings (Hubel and Wiesel 1968, Hubel and Wiesel 1972) and confirmed with anatomical studies (Hubel, Wiesel et al. 1978) using radioactive deoxyglucose (Figure 9).
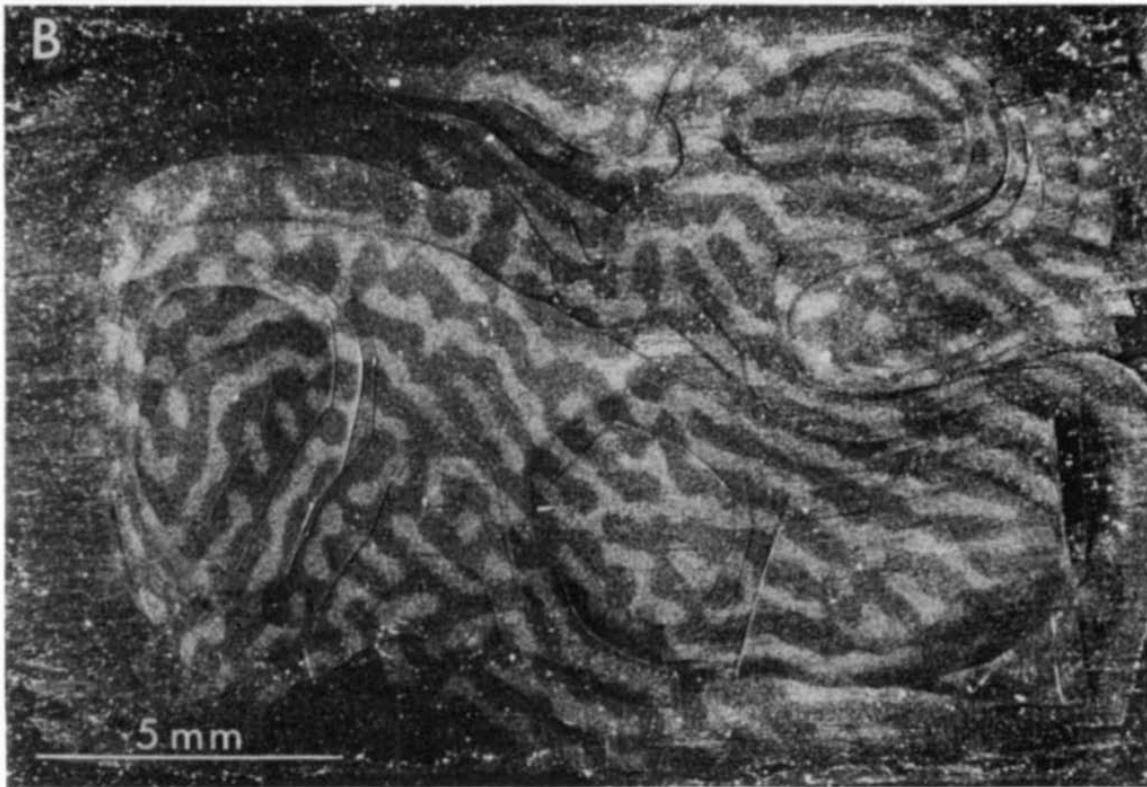
Fig. 8A   Reconstruction of orientation columns from monkey No. 2, left occipital lobe, in the same series as that of figure 7, made by cutting and mounting the parts of each section passing through layer VI.
    B   Reconstruction of ocular dominance columns in the same region as A, made from autoradiographs of ³H-proline sections following injection of the right eye; dark-field photographs. Parts of each autoradiograph passing through layer IV c were cut and mounted.

Figure 9. Original figure from (Hubel, Wiesel et al. 1978) showing alternate bands of ocular projection in layer IV of the macaque visual cortex following ocular injection of radioactive proline.

A similar organization was also found in the cat visual system using electrophysical and anatomical (LeVay, Stryker et al. 1978, Cynader, Swindale et al. 1987) as well as optical imaging techniques (Bonhoeffer, Kim et al. 1995).

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1.3   Processing beyond V1

1.3.1   Cortical hierarchy

There is more to vision than computations observed in the primary visual cortex, which is mainly involved in basic encoding while further processing takes place in more specialized extra striate areas. Indeed, the cat visual cortex is comprised by more than twenty hierarchically organized visual areas each one specialized in analysing a specific feature of the visual scenery. The prevailing view of cortical communication is that information transfer from lower visual areas to higher ones is carried through feedforward connections, while neurons from higher areas project back to areas of lower rank through feedback pathways (Salin and Bullier 1995, Casanova 2004).

This hierarchical understanding of neural processing was built upon anatomical observations that cortical projections in visual areas mostly follow a similar pattern (Felleman and Van Essen 1991, Markov, Vezoli et al. 2014) : projections from lower areas to extra striate (XC) areas originate from supragranular layers and project to their target areas in layer IV, on the other hand, projections from XC areas towards the primary visual cortex or more caudal areas mostly originate from infragranular layers and terminate outside of layer IV. Taking into account the laminar organization of geniculo-striate pathways, these observations suggest that ascending pathways from striate to XC areas are feedforward (FF) whereas, those from XC to lower areas are feedback (FB) (Salin and Bullier 1995, Markov and Kennedy 2013).

The analysis of these projection pattern enabled Felleman and Van Essen to propose a hierarchical model of visual areas in the primate brain (Felleman and Van Essen 1991), an exercise followed by (Scannell, Blakemore et al. 1995) who applied the same methodology to hierarchically ranked visual areas of the cat (Figure 10)
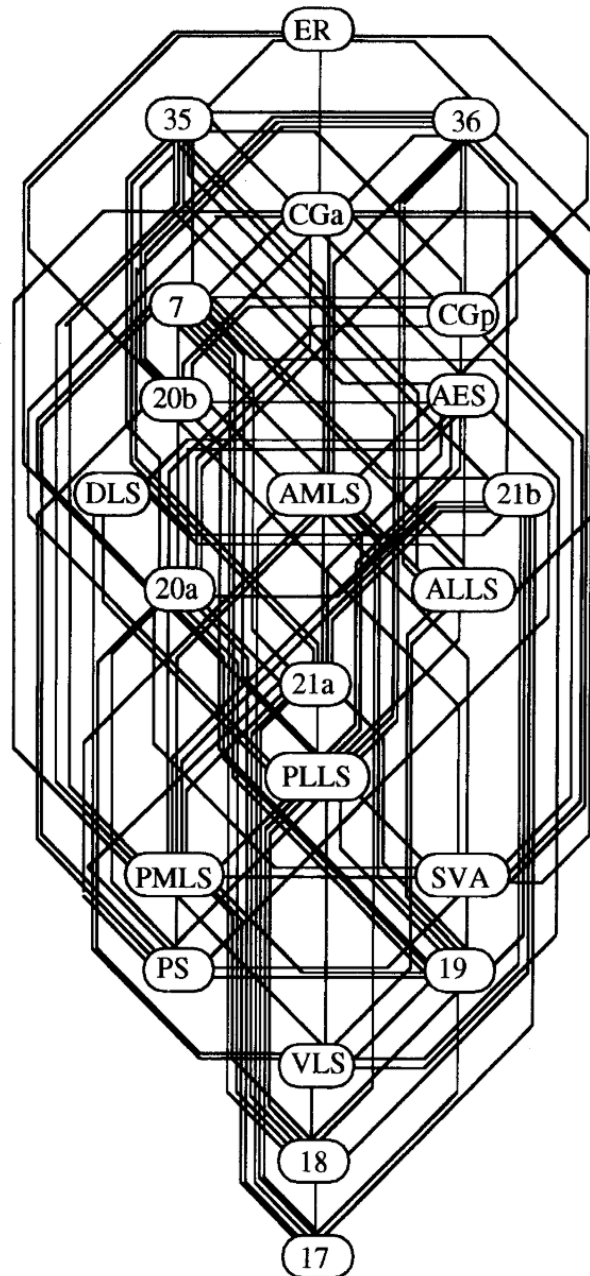
Figure 10. Hierarchical organization of visual areas based on laminar projection patterns of corticocortical connections in the cat; from (Scannell, Blakemore et al. 1995).

At the functional level, evidence suggest that there is also a hierarchical organization of the visual cortical area. The best evidence in support of this hierarchical processing comes from the observation that visual cells, from lower to higher visual areas, respond progressively to an increasing complexity of visual image properties such that higher ranked XC areas may exhibit sensitivity for complex stimuli that may be absent in lower areas (Felleman and Van Essen 1991, Ungerleider and Haxby 1994).

On the other hand, there is also evidence that in some instances, processing in the visual system follows a more parallel scheme in which areas located at a different hierarchical rank from an anatomical standpoint may exhibit at a functional level, an overlapping sensitivity for complex visual features (Hegde and Van Essen 2007). These results suggest that, at the functional level, visual processing does not always follow a hierarchical scheme and therefore, that it does not strictly follow the anatomical hierarchy flowchart of visual information (Hegde and Felleman 2007).

1.3.2   Visual Streams

As mentioned above, visual information flows from striate to extra-striate areas in a hierarchical and parallel manner along with a feedback pathway from higher to lower visual areas.

An important feature of the visual cortex in the context of cortical hierarchy and extra- striate processing is the presence of two functionally and relatively independent visual streams: the ventral pathway, also known as the 'what' pathway, associated with face, object and pattern recognition; and the dorsal pathway, also known as the "where" pathway, mainly associated with the processing of motion and spatial location of visual stimuli (Goodale and Milner 1992, Grill-Spector and Malach 2004, Schwartz 2010)

An important study providing critical evidence for the existence of these relatively independent functional pathways was undertaken by (Mishkin and Ungerleider 1982) where they observed that confined lesions to visual areas of the primate temporal cortex caused deficits in object recognition while lesions in the parietal cortex impaired spatial tasks. Similar conclusions were also reached in human studies where subjects were asked to selectively attend to the shape, color or speed of stimulus, a task which led to the activation of visual areas from functionally different pathways (Corbetta, Miezin et al. 1991).

Building on the two streams hypothesis by (Mishkin and Ungerleider 1982), Goodale and Milner (Goodale and Milner 1992) proposed that the ventral and dorsal stream theory may be better understood as different functional pathway each more concerned by perception or action. This understanding was based on evidence of a patient (patient DFF) presenting brain damage in the occipito-parietal region associated with object recognition or the 'what' pathway proposed by (Mishkin and Ungerleider 1982) who as expected, had difficulties recognize the shape and orientation of geometric shapes (in this case, the orientation of a slot). However, when the task was to insert a card inside the slot, patient DF performed it

adequately, placing the card in the correct orientation of the slot therefore suggesting that the neural substrates for the recognition of object stand in contrast to those that generate visually guided behavior (Goodale, Milner et al. 1991).

The existence of these two pathways has been recognized in primates but also in cat models. In the primate model, pathways located in the dorsal stream are mainly located in the occipito-parietal cortex. The main motion sensitive area involved in spatial localization of objects in the primate brain is MT, also known as the motion-sensitive area of the of the superior temporal sulcus (STS). From area MT, visual signals are sent forward for further processing to several other motion-sensitive areas within the STS, including area MST (Andersen 1997).

On the other hand, areas of the ventral stream are located in the occipito-temporal cortex and are implicated in the analysis of visual features including color, shape and more complex computations such as face and body recognition (Mishkin and Ungerleider 1982, Kravitz, Saleem et al. 2011). The gateway cortical area for object and form recognition in the primate brain is considered to be area V4 which sends visual signals to higher areas of the ventral streams including, area TEO (posterior, inferior temporal cortex). From V4 and TEO, visual information carrying cues critical for the analysis of object form, color, and texture is sent forward to area TE (anterior, inferior temporal cortex) and TEO which comprise the inferior temporal (IT) cortex (Ungerleider. 2013).

The functional segregation of visual areas into distinct streams has been also shown in the cat (Lomber, Payne et al. 1996, Lomber 2001). The dorsal stream of the cat is also involved in motion and visuo-motor function. Its motion sensitive are mainly located in the middle suprasylvian sulcus in the visuoparietal cortex and include area PMLS, identified as a possible homolog for primate area MT (Payne 1993). A deactivation of these areas leads to an inability of cats to detect motion and/or to orient themselves towards new stimuli (Lomber, Payne et al. 1996).

Areas of the ventral streams of the cat are located in the posterior suprasylvian (PS) cortex in temporal cortex. The gateway area involved in the ventral pathway of the cat is area 21a in the dorsal part of the posterior suprasylvian gyrus, and has been considered as an homologue of area V4 of the primate (Payne 1993). Its adjacent areas are areas 19 (medially and caudally), PMLS, VLS and 21b (Shipp and Grant 1991). Area 21a maintains cortico-cortical connections with most visual areas of the cat visual cortex, but receives its strongest input from area 17 and 18 which originate from supragranular layer III (Tusa and Palmer 1980, Shipp and Grant 1991, Scannell, Burns et al. 1999). Indeed, projections from area 17 are critical for response properties of area 21a cells as its cooling is known to significantly decrease its visual responses (Michalski, Wimborne et al. 1993).

Area 21 also maintains reciprocal projections with the LP-Pulvinar. Area 21a sends projections to both subdivisions of the LP (Abramson and Chalupa 1985, Abbas Farishta, Boire et al. 2020) while both subdivisions projects back to area 21a (Ratzlaff and Grinvald 1991). The retinotopic organization of area 21a only represent the central most part of the

visual field, covering about 20 degrees of visual axis (Tusa and Palmer 1980). Its receptive field are complex like, tuned for low spatial frequency, exhibit binocular summation and show selectivity for orientation with poor responses to specific direction (Mizobe, Itoi et al. 1988, Toyama, Mizobe et al. 1994, Vickery and Morley 1999) and are organized in functional modules (Villeneuve, Vanni et al. 2009). In line with roles attributed to areas for the ventral pathway, the deactivation of area 21a is known to disturb complex pattern and object recognition without affecting tasks requiring motion detection (Lomber, Payne et al. 1996).

Besides visual areas of the lateral suprasylvian cortex mentioned above like the PMLS and area 21a, areas of the anterior ectosylvian (AES) cortex also represent an important stage of visual processing, particularly for complex motion and oculo-motor integration. The visual area of the AES (AEV) is considered to be one of the highest in the hierarchy of the cat visual cortex (Scannell, Blakemore et al. 1995). Interestingly, despite its relatively well defined anatomical connectivity pattern, and visual response properties, no other area in the mammal visual system can be considered its homologue (Olson and Graybiel 1987, Payne 1993).The AES is a multisensory associative cortex located in the ectosylvian sulcus and comprises three modality-specific subregions: the somatosensory SIV (Clemo and Stein 1982), the auditory FAES, (Clarey and Irvine 1986) and the visual AEV (Olson and Graybiel 1987). Multisensory neurons are present in each subregion but are mainly situated at their common borders (Stein, Meredith et al. 1993, Jiang, Lepore et al. 1994, Jiang, Lepore et al. 1994).

The AEV maintains cortical projections with several visual areas of the LS (Mucke, Norita et al. 1982), which are reciprocal (Miceli, Reperant et al. 1985). Areas projecting to and

receiving afferents from the AEV are the AMLS, ALLS, and the PMLS, most of which are involved in motion processing (Ouellette, Minville et al. 2004, Villeneuve, Ptito et al. 2006, Ouellette, Minville et al. 2007). These projections may explain visual response properties of its neurons. Neurons of the AEV are relatively large, show binocular summation, are highly sensitive to the direction and velocity of moving objects and respond to complex motion (Scannell, Sengpiel et al. 1996, Nagy, Eordegh et al. 2003, Zabouri, Ptito et al. 2008). These response properties are in line with the role played by the AEV in spatial orientation (Wilkinson, Meredith et al. 1996, Jiang, Jiang et al. 2002).

The AES cortex also maintains reciprocal connection with several subcortical structures, including the LP-pulvinar complex and the LM-Sg (Mucke, Norita et al. 1982). Projections from the AEV mostly target the LPm. Significant projections from the AEV have also been reported to target the intermediate and deep layers of the SC (Fuentes-Santamaria, Alvarado et al. 2009).

2. The Thalamus, more than just a relay

In the previous chapters, we have presented an overview of the classical understanding of sensory processing taking the visual system as a model. In this corticocentric view, most processes necessary for the interpretation of relevant stimuli happen in the cortex, and the thalamus is seen as a relay whose main role is to transfer information from lower centers to the neocortex.

While this corticocentric view is partially correct and has greatly helped our general understanding of the visual system, it has also been (rightly) challenged by a growing number of studies pointing out its limitations in at least two significant ways.

First, there is a growing evidence that cortical areas do not communicate only through direct cortico-cortical connections, but also through lesser known transthalamic pathways, allowing for a cortico-thalamo-cortical transfer of information through higher order thalamic nuclei, such as the pulvinar (Sherman and Guillery 2011). The importance of these pathways has been recognized by several authors in the recent past (Shipp 2003, Casanova 2004, de Souza, Cortes et al. 2019) and while our understanding of these transthalamic routes has significantly expanded in the last decade, we still have very little information as to how they differ from their direct cortico-cortical counterpart (Sherman 2016).

Second, the very existence of these transthalamic pathways has come to question to validity of confining the bulk of neural integration, processing and interpretation of relevant stimuli to the cortex. There is a growing evidence that these transthalamic loops are not just relaying information from one area to another, but that they may be actively involved in the integration of stimuli and play a critical role in processes leading to cognition (Halassa and Kastner 2017).

Thus, the emergence of studies involving the thalamus as an *active* player capable of modulating cortical function brings several considerations for research in neurosciences, one of them being that once cannot fully understand the function of any cortical area, let alone of

the entire cortex without studying how these areas connect to and are modulated by thalamic centers (Sherman and Guillery, 2013).

2.1   Transthalamic pathways and Higher order Nuclei

The classical view of the thalamus defines this structure as a collection of nuclei primarily composed of excitatory neurons, each concerned with transmitting a specific type of afference signal (visual, auditory…) to a functionally distinct area of the neocortex. The significance of the thalamus can be better understood through this quote from Sherman and Guillery, two pioneer researchers in the field of neurosciences, particularly in studies involving the thalamus, which says:

> 'Almost everything we can know about the outside world or about ourselves is based on messages that have had to pass through the thalamus' (Sherman, Guillery et al. 2006).

Even though there is a unanimous consensus in the field of brain studies on the fact that cortical processing critically relies on information sent by the thalamus, the overwhelming majority of models of neural processing view the thalamus and its nuclei as mere relays. In the context of the visual system, this would mean that the LGN 'simply' transfers what is sent by the retina to the cortex, without altering, modifying, modulating let alone integrating signals for further processing. This view was largely based on the fact that many of the

thalamic nuclei studied were in fact 'First Order' (FO) relays, defined as those nuclei that send messages to the cortex about events in subcortical parts of the brain.

While this simple, almost machine-like relay role of transferring information from lower centers to the cortex attributed to thalamic nuclei may very well account for subcortical inputs relayed to cortical areas through FO nuclei such as the LGN, they do not take into account the existence of massive feedback projections from the cortex to the thalamus. Even in the case of a FO nuclei like the LGN, feedback CT projections from the cortex significantly outnumber its subcortical retinal inputs, thus making it possible to dynamically modulate its output to the primary visual cortex (Kawamura et al., 1974; Updyke, 1977). Furthermore, while limiting the thalamus to a role of transferring subcortical inputs to the cortex explains only part of the function of FO nuclei, they do not tell us anything about higher order (HO) nuclei whose main input and output are cortical areas providing monosynaptic trans-thalamic pathways between areas of the neocortex (Shipp 2003, Sherman and Guillery 2011, de Souza, Cortes et al. 2019).

As can be seen in Figure 11, cortical signals from a given area that need to be transferred to higher order areas can either be conveyed through direct cortico-cortical connections, or through transthalamic projections via HO thalamic nuclei. In the context of the visual system, this means that the primary visual cortex can project to higher visual areas such as V4 through the cortico-cortical connections, or through transthalamic pathways involving the pulvinar (HO nuclei in the visual thalamus). In primates, nuclei that contain higher-order circuits account for more than half of the volume of the thalamus (Sherman, Guillery et al. 2006).

Therefore, confining the thalamus to a station where signals from the periphery are relayed *en route* to cortical areas doesn't account for a significant number of thalamic areas which do not receive critical subocortical drive.
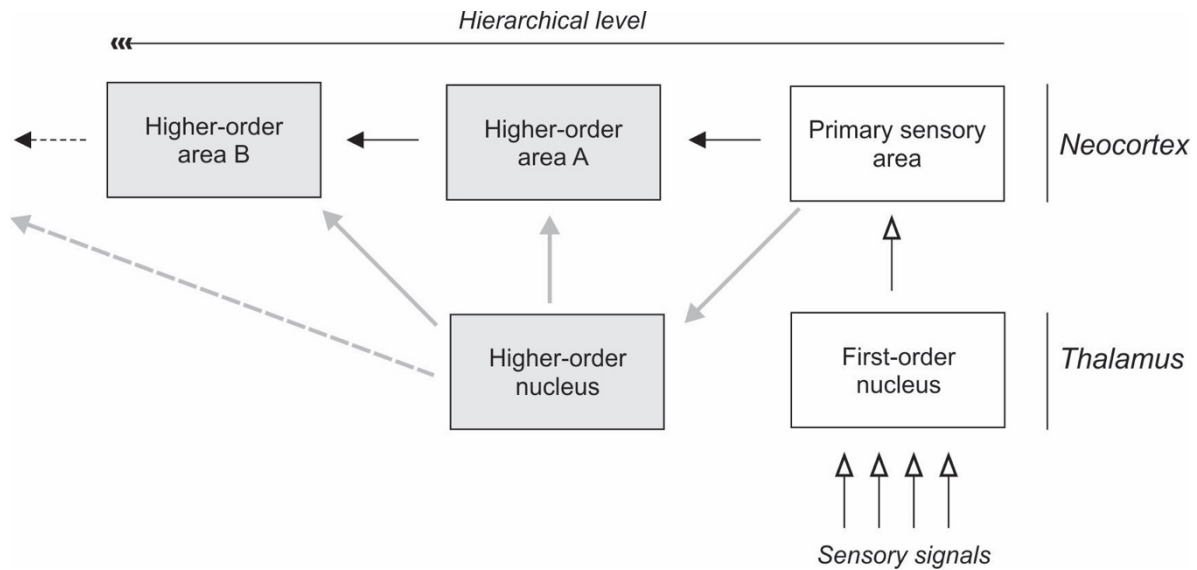
Figure 11. Diagram showing conventional view (corticocortical) and alternative view of cortical communication, C.Casanova.

2.2   Drivers and Modulators

2.2.1   Overview and definition

Even though the existence of transthalamic pathways has been increasingly acknowledged in the scientific community, little information is available about its functional significance and how it differs from direct cortico-cortical pathways. One way towards a better understanding of these transthalamic pathways and more generally, interactions between the cortex and the

thalamus, is to determine the nature of the signals they reciprocately convey. In this context, two types of connections have been described between the cortex and the thalamus (Rockland 1994, Rockland 1996). The type II projections are defined as *drivers*; they are the main carrier of sensory information and are therefore critical for the establishment of classical receptive field properties on the other hand, type I projections are known as *modulators* as they fine-tune ongoing activity of the recipient neurons. This modulation can happen in several ways : through a modulation of RF properties (tuning orientation, or center surround suppression could be such examples), through short and long term synaptic plasticity, or modulation of synchrony between connected networks of neurons. This modulation however, would not be enough to totally abolish visual responses in the recipient cell.

2.2.2   Differentiating criteria

Several functional and anatomical criteria have been listed in order to differentiate between these two types (Table 1). As mentioned above, they can primarily be distinguished on the basis of their axon morphology. As can be seen in Figure 12, type I (*modulator*) projections are characterised by long and thin axons giving rise to small boutons on short stalks or small swellings on the axon itself (*en passant*). Type II terminals are characterized by large caliber axons and clustered endings. The grouping of terminals range between lone singletons and more complex flowery forms of rosettes (Guillery, Feig et al. 2001).

Driver	Modulator
Activates only ionotropic receptors	Activates ionotropic and metabotropic receptors
Synapses show paired-pulse depression (high p)*	Synapses show paired-pulse facilitation (low p)*
Large EPSPs	Small EPSPs
Minority of inputs	Majority of inputs
Less convergence onto target	More convergence onto target
Thick axons	Thin axons
Large terminals on proximal dendrites	Small terminals on distal dendrites

*p refers to the probability of transmitter release.

Table 1. Main properties of driver and modulator projections (Sherman, 2017).

Besides terminal morphology observed with regular light microscopy, other criteria can help differentiating between these two distinct types of CT inputs. Ultrastructure analysis of CT cells using electron microcopy also reveal two major types of synaptic arrangements. RL (Round Large) profile present large synaptic terminals that contain round vesicles and contact proximal dendrites of thalamocortical relay cells. In the LGN, RL profiles have been identified to originate from the retina. They correspond to type II axons described at the light microscopy level. Another type of profile, more characteristic of striate projections to the LGN are called RS profile. They contain round synaptic vesicles, are relatively small and contact more distal portions of relay cell dendrites. RS profile are equivalent of type I axons observed in light microscopy (Sherman and Guillery, 2006).
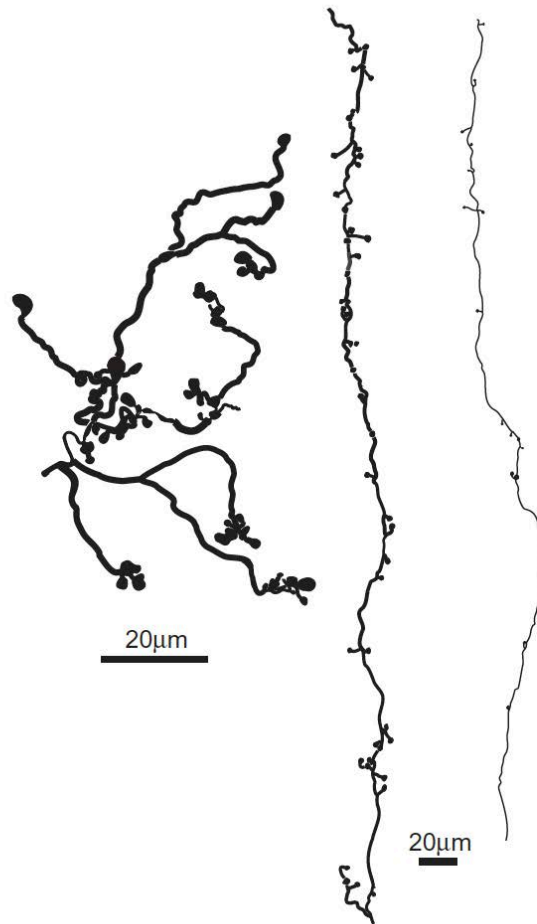
Figure 12. Morphology of type II (left) and type (I) CT axon, Sherman and Guillery, MIT Press 2006.

Another important differentiating criteria has been highlighted in a thorough review by Rouiller and Welker which compared the organization of cortico-thalamic projections of a variety of mammals in sensory and motor systems (Rouiller and Welker 2000). In this review, not only did the author mention that these two types of CT projection have been observed in sensory and motor systems of all mammals studies, they also mention an important observation : across all studies which investigated the anatomical origin of CT projections, it

is the laminar origin of these CT axons which dictates their terminal morphology such that projections originating from layer VI give rise to type I axons, where as those emanating from layer V give rise to thicker type II (Gilbert and Kelly 1975, Ojima 1994, Bourassa and Deschênes 1995, Ojima, Murakami et al. 1996).

Besides laminar origin and terminal morphology, both driver and modulators can also be differentiated on the basis of the postsynaptic glutamatergic receptor they activate or express. A relevant study shedding light on the different putative functional roles of these projections reveals that the postsynaptic elements of type I and II axons express different types of metabotropic glutamate receptors (Vidnyanszky, Gorcs et al. 1996). This study not only confirmed the existence of two distinct types of CT axons in the thalamus, they also observed that thinner type I terminals established a synaptic contact with a dendritic profile that was immune-positive for mGluR1 at the level of the postsynaptic membrane, while thicker type II axons in the pulvinar where immune-negative for this receptor, an observation which suggests that these two morphologically distinct types of projections may very well have different functional roles. This study is also in line with other reports revealing that CT projections from layer VI can activate metabotropic receptors (Godwin, Van Horn et al. 1996), while projections from layer V to HO thalamic nuclei only rely on 'faster' ionotropic, AMPA and NMDA, receptors (McCormick and von Krosigk 1992, Salt 2002, Reichova and Sherman 2004, Sherman, Guillery et al. 2006).

These differences in latencies can actually be recorded at the electrophysiological level where both types of inputs have different functional signatures at the relay neuron they contact and

can be measured through their respective excitatory post synaptic potentials (EPSPs). Indeed, driver projection elicit large EPSP with paired pulse depression while modulatory one's are characterised with smaller amplitudes and paired pulse facilitation (Li, Guido et al. 2003, Reichova and Sherman 2004). These differences can be attributed to the distinct kinetics and probability of synaptic vesicle release of both types of CT projections. Overall, fast acting driving inputs are the ideal candidate allowing for a reliable transfer of information necessary for the establishment of RF properties of a target cell. These types of projection require a high temporal resolution, a characteristic which is line with the electrophysiological features of type II CT projections. On the other hand, slower type I inputs are ideally placed to modulate the ongoing firing of the recipient cell.

2.2.3 Driver and Modulator in the visual system

In the context of the visual system, the presence of both these inputs has been well documented for retino-geniculo-striate pathways (Sherman and Guillery 1998; Sherman and Guillery 1996): retinal projections terminating in the LGN comprise large caliber axons with clustered boutons bearing type II morphology. At the ultrastructural level, they contact relatively large dendritic stems and bear an RL profile (Szentagothai 1963, Guillery 1969). Retinal projections to the LGN are considered driver, because they pass their receptive field properties to geniculate cells (Cleland, Dubin et al. 1971, Shapley and Lennie 1985, Usrey, Reppas et al. 1998, Sherman, Guillery et al. 2006). Similarly, RF properties of layer IV

neurons of V1 rely on inputs from the LGN (Alonso, Usrey et al. 1996, Sherman and Guillery 1998, Sherman, Guillery et al. 2006). They are also considered as driver inputs. On the other hand, neurons in the dLGN do not critically rely on descending projections from layer VI whose stimulation, cooling or lesion yield only mild effects (Kalil and Chase 1970, Baker and Malpeli 1977, Jones and Sillito 1994, Cudeiro and Sillito 1996). These projections are considered modulatory. Interestingly, most studies which have compared the relative proportion of both these inputs in the thalamus suggest driver type II inputs only represent a small fraction of projections to the thalamus even if they seem most critical for the firing of the recipient cell. In the LGN for instance, retinal driver input account for roughly 5-10% of the total projections while corticothalamic modulatory projections comprise 30 to 40% of inputs (Figure 10A) (Sherman 2017). Local inhibitory GABAergic neurons and brainstem projections constitute the rest (Sherman 2007; Van Horn, Erişir, and Sherman 2000).

2.2.4   Vesicular glutamate as a possible anatomical correlate

As mentioned above, the driver and modulator theory concerns itself with the classification of glutamatergic projection between the cortex and the thalamus. Amongst the differences between these two types of projections listed above is that they could be differentiated on the basis of the postsynaptic glutamatergic receptor they activate or express, suggesting that type I and type II projections may selectively interact with distinct actors of the glutamatergic system.

Based on these observations some studies in the primate have correlated VGLUT distribution with known terminating sites of driver and modulatory projections (Rovo, Ulbert et al. 2012, Balaram, Hackett et al. 2013).

Vesicular glutamate transporters (VGLUTs), which are expressed in every glutamatergic neuron across the CNS (Fremeau, Kam et al. 2004, Takamori 2006) are used in the transport of glutamate pre-synaptically, and hence, are indicative of the terminal activity of a projecting neuron.

Amongst the significant differences between these two transporters is that VGLUT1 and VGLUT2 seem to rely on distinct vesicle recycling pathways after their fusion with the presynaptic terminal membrane. These distinct pathways enable VGLUT 1 and 2 to have different temporal properties of glutamate release (Voglmaier, Kam et al. 2006, Weston, Nehring et al. 2011), due to their different affinity with endophilin A1 (which acts in a negative allosteric manner, reducing the probability of endocytosis): the higher binding affinity of VGLUT1 to endophilin A1 reduces the probability of vesicle endocytosis and thus, of glutamate release while VGLUT 2's lower affinity enables it to maintain a comparably faster rate of vesicle endocytosis (Weston, Nehring et al. 2011).

Other than having distinct membrane trafficking properties, early studies investigating their overall expression pattern across the CNS of mammals have reported that VGLUT 1 and 2 have a complementary expression patterns suggesting that they may recognize distinct types of projections (Fremeau, Troyer et al. 2001, Herzog, Bellenchi et al. 2001).

Studies investigating the expression of VGLUT 1 and 2 protein and mRNA distribution in the CNS of rodents, cats, tree shrews and primates (Fujiyama, Furuta et al. 2001, Kaneko and Fujiyama 2002, Kaneko, Fujiyama et al. 2002, Fujiyama, Hioki et al. 2003, Nahmani and Erisir 2005, Balaram, Hackett et al. 2011, Balaram, Takahata et al. 2011, Balaram, Isaamullah et al. 2015) have provided further insight for a better understanding of their functional role with respect to the 'driver-modulator' framework. In these studies, the most consistent observation reported across all mammals studied points towards a predominant use of VGLUT 2 by retinal projections to the SC and the LGN, and by projections from the LGN to geniculo-recipient layers of V1, both of which are known to be driver projections. The prominent expression of VGLUT 1 mRNA in layer VI of the primary visual cortex known to send modulatory feedback projection to the thalamus, along with the diffuse expression of VGLUT 1 protein across all layers of the LGN, known to receive layer VI modulatory striate projections further strengthened the view that VGLUT 1 and 2 may selectively identify driver and modulatory projections respectively in geniculo-striate pathways.

While there seems to be a case for VGLUT 1 and 2 to show a bias for modulatory and driver projections in the retino-geniculo-striate and retino-tectal pathway, this association is less straightforward in cortico-thalamic pathways, especially those involving higher-order thalamic nuclei. For instance, infragranular layers of V1 known to send modulatory projections to the LGN from layer VI and driver projections to the LP-Pulvinar from layer V, both express VGLUT1 mRNA, with faint to no VGLUT 2 mRNA (Balaram, Hackett et al. 2011, Balaram, Hackett et al. 2013, Balaram, Isaamullah et al. 2015), suggesting that both

modulatory and driver CT projections utilise VGLUT 1. Moreover, in primates, large 'driver' cortico-pulvinar projections from the motor cortex displayed VGLUT1 immunoreactivity suggesting that VGLUT 1 is involved in driving corticothalamic projections (Rovo, Ulbert et al. 2012). Therefore, there does not seem to be a strict association between VGLUT's and known driver and modulatory projections in cortico-thalamic pathways involving HO nuclei.

2.2.5   Beyond a strict driver/modulator framework

Even if this dichotomy of categorizing glutamatergic projections in two broad classes has helped understanding the functional relationship between the cortex and the thalamus, recent anatomical and functional evidence suggests there maybe be other types of CT projections which are not accounted for in the classical driver-modulator framework.

A recent review gathering evidence for the reconsideration of the driver/modulator framework highlighted this position (Bickford 2015). In her analysis, Bickford highlighted the fact that some projections could not be classified as either driver or modulator (named 'driver-like'). These projections which were mostly found in tecto-recipient areas had larger anatomical profile that may resemble *driver* projections but whose postsynaptic responses did not exhibit neither frequency dependent depression nor facilitation typical of *driver* and *modulator* projections respectively and fall in between these two known classes of projections.

Moreover, the initial framework provided by Sherman and Guillery created a relatively clear distinction between first order higher-order thalamic nuclei, whose relay cells would receive

their drive from subcortical and cortical projections respectively. However, there is now evidence that a single thalamic relay cell can receive driving input from both cortical and subcortical origin (Groh, Bokor et al. 2014).

3.   The Pulvinar

3.1   An overview of the pulvinar

Lying over the dorsolateral posterior thalamus and running along the medial edge of the LGN the pulvinar complex is the largest thalamic structure in the primate brain (Zhou et al 2017). In parallel with the expansion of the cortex through evolution, the primate pulvinar underwent a significant increase in size and established extensive reciprocal connections with most visual cortical areas.

The pulvinar has traditionally been neglected in the field of visual neurosciences perhaps because its exact function in the context of visual processing has been rather elusive. Most of the earlier clinical cases and reports involving pulvinar dysfunction do not report a significant loss of visual function especially when compared with dysfunction of other visual subcortical areas such as the LGN or the Superior Colliculus. Moreover, this structure has proved to be more difficult to record from in animal models, especially in acute condition as pulvinar neuron seem to be quite sensitive to anesthesia. It's connectivity pattern with higher ranked visual areas, the amygdala, and areas of the frontal lobe point towards its significant role in cognitive process and higher order visual tasks. More recent clinical reports have actually implicated pulvinar lesions with altered responses to emotional stimuli, visual

attentional tasks, (Ward, Calder et al. 2007, Snow, Allen et al. 2009, Bridge, Leopold et al. 2016) as well as psychiatric disorders such schizophrenia, attention deficit and hyperactivity disorder (ADHD) (Benarroch 2015, Dorph-Petersen and Lewis 2017, Homman-Ludiye and Bourne 2019).

The pulvinar receives its main driving inputs from layer V of the cortex and is therefore considered a Higher-Order nucleus (HO) as opposed to a first-order nucleus, such as the LGN, which receives its driving afferents from the retina (Sherman, Guillery et al. 2006). It is therefore not surprising that RF properties of the pulvinar resemble those of the cortex. Pulvinar RF can be binocular and sensitive to retinal disparity, they are orientation and direction selective. Typically, RF of the pulvinar area large which may reflect their ability to integrate signals from various areas, which may explain the presence of subset of pulvinar neurons which can respond to the true direction of moving plaid pattern (Merabet, Desautels et al. 1998, Casanova 2004).

3.2   The Cat pulvinar

Like in the primate the pulvinar of the cat (also named the LP-Pulvinar complex) can be parceled into several areas. Internal subdivisions of the LP-Pulvinar complex have been defined by connectivity patterns, functional properties or based on cyto- and chemo-architectonics (Updyke 1977, Berson and Graybiel 1978, Updyke 1981, Chalupa, Williams et al. 1983, Chalupa and Abramson 1988, Chalupa and Abramson 1989). For the purpose of this thesis, the histochemical boundaries of the LP-Pulvinar described by Graybiel and

Berson (Graybiel and Berson 1980) based on the revelation of thalamic acetylcholinesterase (AChE) activity has been used. This method is used by several laboratories. Typically, AChE staining for the visual thalamus enables a relatively straightforward parcellation of the LP-pulvinar into three subregions: a densely marked, medially located subdivision of the LP known as LPm; a lightly marked subdivision located more laterally known as LPl; and the pulvinar proper, located superior to the LGN. A coronal section of the visual thalamus stained for Ache and revealing LP-pulvinar's subdivision can be seen in Figure 13. The overall retinotopic organization of the LP-pulvinar is more complex than the LGN because of multiple visual field representations (Raczkowski and Rosenquist 1981, Updyke 1983); up to five have been proposed by (Hutchins and Updyke 1989). These multiple representations and the large extent of receptive fields contribute to an LP-pulvinar visuotopic organization that is also less defined than that of the LGN or area 17 (Casanova 2004).

Figure 13. Example of a coronal section stained with AChE used to identify thalamic subregions, adapted from Huppé-Gourgues et al, 2019.

3.2.1 Anatomical connectivity of cat pulvinar

The LP-Pulvinar complex receives its main inputs from visual areas of the cortex. As such, the LP-Pulvinar maintains anatomical and functional connection with virtually all cortical areas involved in visual processing such as the primary visual areas 17 and 18, area 19, 20a and b, 21a and b, PMLS, AMLS, area 5 and 7 and the AEV with the strength of each area's projection varying amongst its subdivisions (Updyke 1977, Graybiel and Berson 1980, Raczkowski and Rosenquist 1983). A relevant difference in anatomical projection pattern between subdivisions of the LP-Pulvinar complex and visual cortical areas is that the LPl remains the only recipient of direct striate projections from area 17 and 18 and is therefore known as the striate-recipient zone of the pulvinar. Striate projections to the LP-Pulvinar mostly originate from layer V with a smaller proportion coming from layer VI. On the other hand, projections from XC areas mainly arise from layer VI (Abramson and Chalupa 1985, Baldauf, Chomsung et al. 2005). Most projections from the cortex to the pulvinar are reciprocal in nature, such that the pulvinar also projects back to the cortex. LP-Pulvinar cells predominantly project to layer I in the primary visual cortex and to layer IV in extra-striate areas.

The pulvinar also receives significant subcortical input, especially from the SC which mainly projects to the LPm and is therefore known as the tecto-recipient zone of the LP. Only a subset of collicular projection to the LP-Pulvinar complex project to the LPl in a zone adjacent to the pulvinar and identified as LPl2 (Abramson and Chalupa 1988, Kelly, Li et al. 2003). Tectal projection to the pulvinar mainly originate from the lower part of the stratum griseum superficiale (SGSi) (Graybiel and Berson 1980, Kawamura, Fukushima et al. 1980).

The retina also sends direct projections to the pulvinar. But unlike for the LGN which receives its main drive from the retina, only subset of retinal projections synapse in the LP-pulvinar in mainly in the pulvinar proper (Boire, Matteau et al. 2004). The other important subcortical projection to the cat pulvinar in the context of the visual system is the pretectum a structure involed in visumotor functions including accommodation and optokinetic reflex. Pretectum-pulvinar projections terminate in the pulvinar nucleus (Berson and Graybiel 1978, Sudkamp and Schmidt 1995, Baldauf, Wang et al. 2005).

3.2.2   Functional relationship between the visual cortex and the pulvinar

An important question regarding the pulvinar and its role in cortical function is the nature of the signals transmitted between them. As mentioned earlier two types of CT connections have been identified between cortex and the pulvinar, namely type I and type II, having different functional roles (modulatory and driving respectably).

Few studies have investigated the nature (driver or /modulator) of CT projection terminating in the pulvinar. Anatomical investigations from our lab revealed that, in the cat, the morphology of CT axons differed between striate and extra striate areas, such that area 17 projections to the pulvinar consisted of mainly thick type II boutons, whereas projections from the PMLS were mainly thin type I (Huppé-Gourgues, Bickford et al. 2006). Similar observations were also reported by (Guillery, Feig et al. 2001) as they observed a greater number of type I boutons in the pulvinar following injections in XC area 19 than following injections of area 17 or 18. A similar picture also emerged from higher order area 5 and 7

which mainly sends type I projections to the pulvinar (Baldauf, Chomsung et al. 2005).

In other species, similar observations were also reported in the grey squirrel (Robson and Hall 1977) where projections from extrastriate areas mainly comprised type I terminals whereas area 17's projections to the pulvinar were of type II. In macaque monkey, (Rockland 1996) also reports that unlike V1 which sends type II terminals in the pulvinar, extrastriate areas send almost exclusively type I terminals. In the tree shrew, the same organization also emerges as the primary visual cortex sends mainly type II terminals to the pulvinar (Day-Brown, Slusarczyk et al. 2017) while extrastriate cortex send almost exclusively type II terminals (Chomsung, Wei et al. 2010).

Thus, the comparison between terminal type from striate and extra-striate areas in the cat and other species shows an interesting organization in that the proportion of type I versus type II axon terminals seems to increase with the hierarchical order or visual cortical area. In other words, higher-order cortical areas would preferentially have modulatory influences while lower visual areas would preferentially provide primary drives to thalamic cells. Even though this hypothesis has not been tested, functional studies which have investigated the nature of these projections are in line with this assumption as electrophysiological data reveals that the deactivation of area 17 significantly decreases receptive field responsiveness of a number of cells in the cat LPl (Casanova, Savard et al. 1997, Rushmore, Payne et al. 2005), mouse LP (Bennett, Gale et al. 2019) and in the inferior pulvinar of primate (Bender 1983), suggesting a common ground of organization among species.

3.3   The pulvinar and its role in cognition

Selective attention is defined as the capacity of our brain to filter relevant information amongst distractors as a strategy to process relevant stimuli taking into account the fact that our brain has limited capacities (Buschman and Kastner 2015) . In the context of the visual system, two main subtypes of visual systems have been described, spatial attention and feature based attention.

Studies have shown that visual spatial attention, or the capacity of the brain to preferentially process information coming from a specific location can improve the speed of behavioral performance at a cued location (Posner 1980), better the perception of contrast (Carrasco, Ling et al. 2004), and improve discriminability (Lu and Dosher 1998).

Traditionally, models describing networks of the brain associated with visual attention have mostly highlighted the role of the cortex, more specifically intraparietal cortex and superior frontal cortex as well as the temporoparietal and the inferior frontal cortex (Corbetta and Shulman 2002).

A growing number of studies have also implicated a subcortical involvement of attentional modulation of vision. In particular, the pulvinar, whose neurons were shown to exhibit attention-related discharge modulation similar to cortical cells (Petersen, Robinson et al. 1985) has also been shown to participate in functional networks involved in visual attention (Fiebelkorn and Kastner 2019). Indeed, the pulvinar has been shown to regulate visual

information transmission between visual cortical areas based on attentional demands (Saalmann, Pinsk et al. 2012), mediate spatial attention at behaviorally relevant locations (Fiebelkorn, Pinsk et al. 2019). These studies are in line with early studies of patients presenting a pulvinar lesion who demonstrated a slowing at engaging attention for a new target (Rafal and Posner 1987, Danziger, Ward et al. 2001, Michael, Boucart et al. 2001, Ward, Danziger et al. 2002) and with pulvinar inactivation studies in behaving monkeys showing behavioral deficits in visual attentional tasks (Petersen, Robinson et al. 1987).

In addition to a role in attention, a growing number of studies suggest that the pulvinar may be involved in social cognition (Saalmann and Kastner 2013). Neurons of the pulvinar have been shown to respond to faces or face-like stimuli; a subset of these cells are also sensitive to important cues in social cognition i.e. orientation of face and direction of gaze direction and face orientation (Nguyen, Hori et al. 2013). The sensitivity of pulvinar neurons to face related stimuli may have come from its association to several functional networks. The pulvinar maintains extensive anatomical connection with the visual cortex, especially areas involved in face processing such as the inferotemporal (IT) cortex (Shipp 2003). While the cortex maybe one source of face-like visual information transiting through the pulvinar, other studies have also implicated the superior colliculus-pulvinar-amygdala network, which would account for the some of the fast spiking face sensitive neurons recorded in the pulvinar with latencies as fast as 30ms, clearly suggesting for a quick transfer of information by passing cortical processing. This pathway functional relationship with the amygdala may also account for the responsiveness of pulvinar neurons to emotional expression of faces (Maior,

Hori et al. 2010), even in the absence of conscious vision due to primary visual cortex loss (Pegna, Khateb et al. 2005).

4.   Organizational and Functional models of corticothalamic connectivity

4.1   Matrix and Core

In 1998, Jones proposed a model shedding light on thalamo-cortical organization (Jones 1998). This model is based on the observation that there are two distinct types of pulvino-cortical projections : calbindin (CB) positive and parvalbumin (PV) positive cells. In this model, the larger PV positive cells form the *core* as they project to localized specific areas of cortical layer IV in a topographical manner, while the smaller CB positive cells form the *matrix* as they project in a diffuse manner mainly to superficial layers, especially layer I. According to this model, PV+ cells forming the core send specific sensory information from the thalamus to the cortex while the diffusely organized matrix may serve to send 'modulatory' information reflecting changes in attention or behavioral sates (Sherman, Guillery et al. 2006). Later, Jones also proposed that cells forming the matrix may also play a role in synchronizing thalamocortical network in coherent activity underlying cognitive events (Jones 2001).

This organization has been challenged based on anatomical evidence that a single neuron can exhibit both a matrix and core like projection pattern via a branching axon; moreover, anatomical study also show that a neuron can exhibit spatially diffuse projection targeting

middle layers or more focal projections targeting superficial and deep layers simultaneously (Clasca, Rubio-Garrido et al. 2012, Kuramoto, Pan et al. 2017).

4.2   Thalamocortical circuits in cognition and cortical synchrony

Traditionally, models describing networks of the brain associated with cognitive task, especially those requiring attention have mostly highlighted the role of the cortex, more specifically intraparietal cortex and superior frontal cortex as well as the temporoparietal and the inferior frontal cortex (Corbetta and Shulman 2002) where thalamic relay have a more passive role, with little influence on cognitive processes (Lehky and Maunsell 1996).

However, more recent models based on anatomical and functional studies have called for the integration of the thalamus and corticothalamic connectivity as important components of cognitive models of visual attention (Saalmann and Kastner 2009). These models are based on several observation: first, human studies show that FO thalamic nuclei such as the LGN exhibit an attention-related modulation (O'Connor, Fukui et al. 2002, Schneider and Kastner 2009), a modulation also observed in single cell recording in primate (McAlonan, Cavanaugh et al. 2008). Second, studies have shown that HO thalamic nuclei such as the pulvinar play an important role during visual cognitive tasks, ensuring proper synchronization of visual cortices and optimizing communication between cortical areas during attentive tasks. According to these models where attention is seen as a dynamic process that unfolds over a

period of time, the thalamus may play the role of a cortical timekeeper through its extensive cortico-thalamo-cortical network of loops (Fiebelkorn and Kastner 2019).

4.3   The pulvinar: a facilitator and integrator of visual information

Because of their anatomical connectivity pattern, HO thalamic nuclei such as the pulvinar may be expected to play a significant role in the processing of sensory information. Indeed, taking the visual system into account, the pulvinar enjoys a privileged situation when it comes to being able to analyze visual information coming from a variety of functional networks, which is why it may play a role as a facilitator and integrator of visual information (Saalmann and Kastner 2009).

The pulvinar may facilitate visual processing at several level : at the thalamic level, it could play a role in the filtering visual information from different inputs in order to only transmit relevant information to concerned functional sub-networks; at the cortical level, the pulvinar may facilitate and modulate the transmission of direct inputs from cortical areas (Saalmann and Kastner 2009, Saalmann, Pinsk et al. 2012).

Some evidence from functional studies also suggest that the pulvinar may be playing the role of an integrator of visual information. In this context, a groundbreaking study from (Merabet, Desautels et al. 1998) reported that a subset of neurons in the pulvinar can signal the true direction of motion of a plaid pattern, a computation which necessarily requires the integration of different visual cues into a coherent moving percept. This study was the first

to ever demonstrate the existence of such high-level analysis in a thalamic nucleus, therefore calling for a greater role for the thalamus in sensory processing. These results are very much in line with human studies in which subjects presenting a pulvinar lesion have difficulties binding visual features (Ward, Danziger et al. 2002).

5. Objectives and hypothesis

Several important points have been mentioned in the previous sections. First, despite the growing number of studies highlighting the existence of alternative transthalamic routes for cortical processing, the functional role of these cortico-thalamo-cortical routes have largely been unknown as we do not know the anatomical nature of projections from various cortical areas to the pulvinar. We also do not know if these projections are organized in a hierarchical manner in a way that would resemble their cortico-cortical counterparts. Moreover, we do not know if the nature of CT projection to the pulvinar varies between visual areas from the ventral or dorsal pathway.

In this context, the objectives of this thesis were four fold: First, to investigate the nature of the corticothalamic terminals from the AEV, one of the highest ranked visual area of the cat in order to elucidate the possible functional ways in which it relates to the cortex (Article 1). Second, to investigate the anatomical nature of CT projections from area 21a, a cortical area located in the ventral pathway (Article 2). Third, to compare the varying proportion of type I and II projections from area 17, PMLS, 21a and AEV to the pulvinar and investigate the

possibility of hierarchical organization of these projections (Article 2) . Four, to investigate the expression of VGLUT 1 and 2 in the visual thalamus in order to investigate whether their expression may reveal functional subdivisions within the pulvinar complex (Article 3).

Based on previous studies from our lab and others which revealed that, in the cat, the morphology of CT axons differed between striate and extra striate areas, such that area 17 projections to the pulvinar consisted of mainly thick type II boutons, whereas projections from the PMLS were mainly thin type I (Guillery, Feig et al. 2001, Huppé-Gourgues, Bickford et al. 2006), our hypothesis is that projections from extra-striate area 21a and AEV would also be modulatory in nature and vary in a hierarchical manner. According to this hypothesis, we expect that the proportion of type I projection from the AEV should be higher than the PMLS and area 21a, and proportion of type I inputs from area 21a to be higher than the PMLS (article 1 and 2). Overall, we expect type I projections to be higher in XC areas when compared to striate projections to the LP-Pulvinar (article 2). As for the expression pattern of VGLUT 1 and 2 in the cat, our hypothesis is that it would bear similarities with the general expression pattern reported in the macaque visual system given the fact that they share a general overall organization. From this perspective, we expected VGLUT 2 to be mainly expressed in anatomical sites known to receive major driver inputs such as the LGN and layer IV of area 17 (article 3).

3.1 Article 1: Distribution and Morphology of Cortical Terminals in the Cat Thalamus from the Anterior Ectosylvian Sulcus

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Distribution and Morphology of Cortical Terminals in the Cat Thalamus from the Anterior Ectosylvian Sulcus

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Abstract: Two main types of cortical terminals have been identified in the cat thalamus. Large (type II) have been proposed to drive the response properties of thalamic cells while smaller (type I) are believed to modulate those properties. Among the cat's visual cortical areas, the anterior ectosylvian visual area (AEV) is considered as one of the highest areas in the hierarchical organization of the visual system. Whereas the connections from the AEV to the thalamus have been recognized, their nature (type I or II) is presently not known. In this study, we assessed and compared the relative contribution of type I and type II inputs to thalamic nuclei originating from the AEV. The anterograde tracer BDA was injected in the AEV of five animals. Results show that (1) both type I and II terminals from AEV are present in the Lateral Posterior- Pulvinar complex, the lateral median suprageniculate complex and the medial and dorsal geniculate nuclei 2) type I terminals significantly outnumber the type II terminals in almost all nuclei studied. Our results indicate that neurons in the AEV are more likely to modulate response properties in the thalamus rather than to determine basic organization of receptive fields of thalamic cells.

Introduction

The thalamus has classically been viewed as a necessary relay for the transfer of sensory information from the periphery to the neocortex where complex computations take place along numerous cortical areas organized in a hierarchical manner. In the visual system, this cortico-centric view suggests that most, if not all, neuronal processing leading to perception and action is based on computations within and across cortical areas through direct cortico-cortical connections (Arend, Machado et al. 2008, Nguyen, Hori et al. 2013). This view has been challenged by a growing number of studies showing that all visual cortical areas are reciprocally connected to the main extrageniculate thalamic nucleus, the pulvinar, providing then monosynaptic trans-thalamic pathways between areas of the neocortex (Shipp 2003, Sherman and Guillery 2011).

Studies from our laboratory and others have shown that neurons in the pulvinar contribute to the processing of basic and complex visual information (Casanova and Savard 1996, Casanova and Savard 1996, Merabet, Desautels et al. 1998) and there are assumptions that trans-thalamic pathways can be used to facilitate the cortical flow of information (Theyel, Llano et al. 2010) and regulate cortical activity according to attentional demands (Saalmann, Pinsk et al. 2012). Still, the role of the cortical pathways involving the pulvinar remains to be clearly determined since there is little information about the function of the distinct trans-thalamic pathways and how they differ from their corresponding direct cortical-cortical pathways (Sherman 2017).

One way towards a better understanding of these pulvinar-cortical routes is to determine the nature of the signals they convey. In this context, two types of corticothalamic (CT) axons have been identified based on their morphology (Rockland 1994, Rockland 1996). Type I axons are thin and possess long thin branches with occasional *en passant* swellings and "drumstick-like"

side branches with small terminal endings (Szentagothai 1963) considered to be equivalent to the round small (RS) presynaptic terminals observed at the ultrastructural level (Bartlett, Stark et al. 2000, Huppé-Gourgues, Bickford et al. 2006). These small axons arise from layer VI cortical neurons (Bourassa and Deschênes 1995, Ojima, Murakami et al. 1996). Type II axons are characterized by large caliber that bear clustered endings considered equivalent to the round large (RL) presynaptic terminals (Bartlett, Stark et al. 2000, Huppé-Gourgues, Bickford et al. 2006). These large caliber axons arise from layer V cortical neurons (Ojima 1994, Bourassa and Deschênes 1995). The grouping of their terminals range between lone singletons and more complex flowery forms of rosettes (Guillery, Feig et al. 2001) and are often grouped in complex arrangements, many encapsulated within complex glomeruli, and make synaptic contact with multiple profiles (Feig and Harting 1998, Li, Wang et al. 2003, Baldauf, Chomsung et al. 2005). Importantly, different functional roles have been attributed to type I and II terminals. According to the 'driver/modulator' theory of glutamatergic pathways involving the thalamus and the cortex, type II terminals would be the main carrier of sensory information (driver inputs), while type I would fine-tune ongoing activity (modulatory inputs) (Sherman and Guillery 1998).

Several neuroanatomical studies revealed that the proportion of type I and type II cortico-pulvinar terminals vary according to the cortical areas. For instance, in the cat, the vast majority of axons coming from the primary visual cortex have type II terminals while those from the posteromedial lateral cortex (PMLS, an extrastriate area considered as the homologue of the primate area MT) exhibit type I terminals ((Huppé-Gourgues, Bickford et al. 2006)). These results suggest that the nature of the cortical projections to extrageniculate thalamic nuclei varies as a function of cortical hierarchy, characterized by an increase of the modulatory/driver inputs ratio.

In this study, we investigated the morphology and distribution of axon terminals originating from the anterior ectosylvian visual area (AEV), an associative area located in the anterior ecstoylvian sulcus (AES) considered as one of the highest area along the hierarchical organization of the cat visual system (Scannell, Burns et al. 1999). The AES comprises three modality-specific subregions: the somatosensory SIV (Clemo and Stein 1982), the auditory FAES, (Clarey and Irvine 1986) and the visual AEV (Olson and Graybiel 1987). Multisensory neurons are present in each subregion but are mainly situated at their common borders (Stein, Meredith et al. 1993, Jiang, Lepore et al. 1994, Jiang, Lepore et al. 1994). The AES is also related to the motor and limbic systems (Norita and Katoh 1986), suggesting that it might play a role in the animal's orientation and alerting behavior (Jiang, Jiang et al. 2002). Injections of an anterograde tracer were made in visual and multisensory cortical subregions of the AES and terminals reaching the main thalamic targets were characterized, i.e. in the tecto-recipient zone the pulvinar complex (namely, the medial lateral posterior nucleus (LPm), the lateral median suprageniculate complex (LM-Sg), the medial subdivision of medial geniculate (MGm) (Mucke, Norita et al. 1982, Olson and Graybiel 1983, Olson and Graybiel 1987, Clarey and Irvine 1990), and the posterior nucleus group (PO) (Clarey and Irvine 1990). Our results indicate that most CT axons are type I terminals, supporting the assumption that the proportion of driver/modulator inputs vary along the cortical hierarchy.

Materials and Methods

Animals were treated in accordance to the regulations of the Canadian Council for the Protection of Animals (CCPA). The protocols were approved by the 'Comité de déontologie de l'expérimentation sur les animaux' of the Université de Montréal. Five pigmented adult cats

were used in this study. Pre-operative anti-inflammatory agents (tolfedine 4% s.c. 0.1mg/kg) and antibiotics (tribrissen 24% s.c. 0.13ml/kg) were administered 24 hours before surgical procedures. Twenty minutes before surgery, atropine (0.1mg/kg s.c.) and Atravet (0.05mg/kg s.c.) were given to the animal. Anesthesia was induced with a mixture of 5% Isoflurane in 60% N_2O and 25% O_2 and maintained with 2% Isoflurane in the same gaseous mixture of N_2O and O_2. Animals were positioned in a stereotaxic apparatus. A craniotomy was performed over the injection sites. During all surgical procedures, animals were maintained at 38°C and heart rate, end-tidal CO_2, blood O_2 saturation and blood pressure were closely monitored.

AES craniotomies were performed according to Horsley-Clarke (H-C) coordinates from 10 to 15 mm lateral to the midline and 10 to 15 mm anterior to the interaural plane. Injections were made under electrophysiological monitoring to insure the position of the pipette into the cortex. Borosilicate pipettes (1.5 mm external diameter) were pulled to obtain a tip ranging between 20 and 30µm. Biotinylated dextran amines (BDA 3000kD) were injected (Midgard, Stoelting) in AES by iontophoresis using DC current (7 second on/off cycle 7µA) for 20 minutes. Cases in which the injections encroached the white matter were rejected (one case, AES 6 had minimal encroachment and was retained in the analysis). In order to label CT cells of the AES, WGA-HRP (3µl) was injected in the thalamus with a Hamilton syringe in one animal. Craniotomies were sealed with acrylic bone cement and the wounds were sutured in anatomical layers. Anti-inflammatory and antibiotic treatments were administered pre- and post-operatively and analgesic was applied for 48 h following surgery (temgesic 0.01 mg/kg bid).

Seven to ten days after the cortical injections, animals received an overdose of sodium pentobarbital (80 mg/kg; IP) and were perfused with phosphate buffered 0.9% saline (PBS: 0.1M, pH 7.4) followed by phosphate buffered 4% paraformaldehyde. Brains were blocked stereotaxically, removed from the cranium, post fixed overnight in the same fixation solution at 4°C, cryoprotected in 30% sucrose in 0.1M phosphate buffer (pH 7.4) and frozen until processed. The fixed brains were cut into 50 μm-thick coronal sections using a cryostat and collected in PBS. After pre-incubation in 2.5% bovine serum albumin (BSA) and normal goat serum 2% in phosphate-buffered saline (PBS; 0.01 M PB with 0.9% NaCl, pH 7.4) for 30 min, BDA was visualized with avidin-biotin-peroxidase complex (ABC; Vectastain ABC Elite kit; Vector, Burlingame, CA). Following buffer washes, sections were reacted with nickel-intensified diaminobenzidine (DAB) (Saunte, Hasselby et al.) for 10 min. After PBS washes, sections were mounted on slides, dehydrated, mounted with Depex and coverslipped for light level examination. Adjacent sections were processed for acetylcholinesterase histochemistry for the identification of cytoarchitectonic boundaries between the lateral and medial subdivisions of the lateral posterior thalamus (Graybiel and Berson 1980). Sections were incubated for six hours in an aqueous solution with 50 mM sodium acetate, 4 mM copper sulfate, 16 mM glycine, 4 mM S-acetylthiocholine and 86 μM ethopropazine adjusted to pH 5. Sections were rinsed in water and reacted for 10 min in a 1% aqueous solution of sodium sulfite and subsequently fixed in 4% paraformaldehyde for 2h.

The animal that received a thalamic injection of WGA-HRP was perfused with a short rinse of phosphate buffer (PB) 0.1 M followed by 1% paraformaldehyde 3% glutaraldehyde in PB.

Brains were cryoprotected in buffered 30% sucrose. HRP-TMB histochemistry was performed according to the method of Mesulam et al (1979).

For stereological analysis, evenly spaced sections were randomly selected. The sampling was performed according to a systematic random sampling scheme (Gundersen and Jensen 1987). Drawings of the sections were made with a 10x objective on a microscope (DMR, Leica) linked to a motorized computer-controlled stage and with a reconstruction software (Novaprime, Bioquant). Mapping of the distribution of axon terminals was carried out under a 100x,1.25 PH3 oil immersion lens. Unbiased size distribution of terminal boutons was obtained using an optical fractionators sampling scheme (West and Gundersen 1990, West, Slomianka et al. 1991). Briefly, the entire region containing axonal terminals was systematically sampled with optical dissectors ranging between 400 and 2500 μm^2 in area and 10 μm thick. Sampled area varied from 8% to 100% of the terminal fields of the selected sections. The total number of terminals is presented for every case and every nucleus (see Table 1). Care was taken to avoid sampling in the 2-3 μm immediately adjacent to the sections surfaces to avoid measuring cut or damaged terminals. For comparison purpose, previous cases from PMLS cortex and area 17 (Huppé-Gourgues, Bickford et al. 2006) were reanalyzed.

The criteria used for the morphological classification of the axon terminals were those of Guillery et al (2001). Briefly, we identified type I as small caliber axons with sparse beaded terminals linked by a small stalk (drumstick). Type I axon terminals also included small swellings on fine caliber axons. Type II axons were identified as (1) intermediate, i.e., comprising three terminals swellings in a small cluster; (2) rosette-like terminals, i.e., complex

cluster of more than 3 terminals; and singletons, i.e., single beaded axon endings–(Ridder, Nusinowitz et al.).

The proportion of each terminal type between the sampled thalamic nuclei was compared with the G-test (Sokal and Rolf 1981), while the percentage of each type of terminals arising from cortices located in the AES and PMLS cortex was compared with a Mann-Withney U test. Finally, Kendall's Tau rank correlations were computed, with the inclusion of area 17 data to further investigate the possible relationship between the cortical hierarchical order and the axon terminal types. All data presented here are available upon request.

Results

Injection sites in AES

Injections in the cortices within the AES were made at various locations to target its different sensory sub-regions(Olson and Graybiel 1987) as illustrated in figure 1 (inset Figure 1). In case AES1, the injection site was small and located in the dorsal bank of the rostral part of the AES (H-C +14) in somatosensory and visual regions (Jiang, Lepore et al. 1994, Jiang, Lepore et al. 1994). In case AES2 (not illustrated), the injection was also small and located in the dorsal bank of the caudal end of the ectosylvian gyrus. The injection in case AES3 was located in the ventral bank of the caudal ectosylvian gyrus (H-C +11) in a visual region. In case AES5, the injection was made in the dorso-caudal bank of the ectosylvian gyrus (H-C +9) in visual and auditory regions. Finally, for case AES6, a large injection was made in the dorsal bank of the rostral ectosylvian sulcus (H-C +15) in somatosensory and visual regions.

Terminals labeling following AES injections

General observations. Table 2 shows that all AES injections resulted in labeling numerous projections in the thalamus, including the LPm of the LP - Pulvinar complex.

Given its multisensory function, several other thalamic nuclei were labeled besides the pulvinar. Terminals in the MGm and MGd, first and higher order nuclei of the auditory thalamus respectively, were shown to receive almost exclusively type I terminals (AES 3, 5 and 6). Higher order thalamic nuclei such as the LM-Sg were also predominantly marked by type I terminals (except AES 5). In two cases, injections in the AES labelled cells in the PO and all projections were exclusively type I (99%). Finally, in line with our initial hypothesis, projections to the LPm subdivision of the pulvinar complex were also shown to be exclusively type I in two cases and predominantly type I in one case.

Illustrated cases.

The predominance of type I (blue dots) over type II (green and red dots) terminals across the thalamus can be better appreciated in Figure 2 and 3 (inset figure 2) where the topographical location of CT projections from four cases are represented with respect to their morphology. In all cases, nuclei, and topographical locations, labelled CT projections were either exclusively or predominantly type I.

In case AES1 (Figure 2A), the terminals were observed in the LM-Sg, in its ventrolateral tip near the border with the LPm. Some retrogradely labeled cells were also observed therein suggesting a reciprocal thalamo-cortical (TC) projection to the AES. More rostrally, terminals were also

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found in LM-Sg and PO (Figure 1A). In both thalamic regions, axon endings were almost exclusively type I terminals. The proportion of terminals in LM-Sg and PO was not significantly different (Chi-square, p=0.08).

Panel B of Figure 2 shows case AES3. In this animal, type I projections were also predominantly found in the first-order nucleus, the MGm, and in three higher-order nuclei, namely, LM-Sg, MGd and LPm. In the MGm nucleus, most axon terminals were type I and characterized by en-passant boutons (Panels A and B in Figure 4). Foci of both type I and II terminals were seldom observed in LM-Sg (Panel C). Type I terminals were occasionally found coursing within the dendritic arborization of retrogradely labeled cells in LM-Sg (Panel D). In rare occasions, complex structures corresponding to tight aggregates of more than 20 type II terminals were observed (Panel E). Figure 5 shows axons endings in LPm nuclei of the pulvinar complex and MGd in case AES3. Terminal fields in LPm contained almost exclusively type I axon terminals (Panel A and Ai of figure 5) while they were predominantly type I for the MGd (panel B and Bi). The proportion of type I and II terminals were significantly different in the labeled thalamic nuclei of each case (G=178, df=6, p<0.01). Injection of the AES in case 3 also yielded the labeling of type I -like and singleton- like terminals in the *stratum griseum intermedium* of the superior colliculus.

Panel A of figure 3 shows case AES5 where only type I terminals were found in the MGm nucleus. Type I terminals largely predominated in PO whereas type II prevailed in LM-Sg (G=267.9, df=6, p<0.05). This case contrasts with AES1 in which type I terminals predominated

in both PO and LM-Sg. Both type I and type II terminals were encountered in the LPm, the former being more prominent. Retrogradely labeled cells were observed in the PO nucleus.

In case AES6 (panel B of Figure 3), only type I terminals were observed in MGm, in close proximity to retrogradely labeled cells. Similarly, only type I endings were found in LM-Sg and LPm nuclei. The MGd nucleus contained a few type II terminals and differed significantly from the other labeled nuclei (G=53.5, df=6, p<0.01). Retrogradely labeled neurons were found in MGm, LM-Sg and PO. A small group of type I-like terminals was observed in the *stratum griseum intermediale* of the superior colliculus.

Terminals labeling following area 17 and PMLS injections

Area 17. Injection of BDA in area 17 resulted in terminal labeling in the LGNd and lateral subdivision of the LP nuclei (LPl) of the pulvinar (Table 2). In the LGNd, only type I terminals were found, while a majority of type II singletons were found in LPl (60.9%). The proportion of terminal types in LGNd and LPl were significantly different (p<0.01).

PMLS cortex. Injections in various PMLS locations resulted in terminal labeling in first-order thalamic nuclei: the MIN in cases PMLS2 and PMLS3 and the LGNv in case PMLS1 (table 2). Terminals of the cortical fibers reaching the MIN were almost exclusively type I terminals. In all five animals, axon terminals were observed in the LP nucleus: in the LPl and LPm sub-regions for PMLS 1,2 and 3 and in the LPl only for the remaining two cases. In both LPl and LPm, terminals were mostly type I, with the exception of case PMLS5 in which type II terminals prevailed. In all cases, the relative frequency of terminal types was always different between first-order and higher-order nuclei. In case PMLS1, the relative frequency of terminal types was significantly different between the LGNv, LPm and LPl (G=53.4, df=4, p<0.01). The proportion

of terminal types in LPl and LPm was also significantly different (G=31.8 df=4, p<0.01). In case PMLS2, the relative frequency of terminal types between the MIN and the LPl and LPm is significantly different (G=50.6, df=4, p<0.01). While terminals types in LPm and LPl nuclei did not differ (G=2.86, df=4, p>0.05), endings in each of the LP subdivisions were significantly different from those found the MIN (G=36.53 and 27.6, df=4; p<0.01). In case PMLS3, the percentage of terminal types was significantly different in the MIN, LPL, and LPm (G=27.75, df=4, p<0.01) and the proportion of terminals in LPl and LPm also differed significantly (G=25.8, df=4, p<0.01). Cases PMLS4 and 5 resulted in terminal labeling in the LPl nucleus only.

Overall comparison between areas.

In first-order thalamic nuclei, the percentage of each type of terminals arising from AES and PMLS cortex was not significantly different (Mann-Withney U, p>0.05). Injections in these cortices resulted mainly in the labeling of type I terminals. In higher-order thalamic nuclei, the mean percentage of type I terminals from the AES (87.88%) and PMLS (71.36%) was significantly different (Mann-Whitney U, p=0.044). This difference is enhanced if the cases in which the proportion of type I was the lowest (AES5 and PMLS 5) are held from the analysis (Mann-Whitney U, p=0.026). AES injections resulted in a mean percentage of type II singletons (3.31%) inferior to that from PMLS injections (18.76%) (Mann-Withney U, p=0.026). In other words, these results indicate that cortico-thalamic neurons from the AES comprise predominantly type I terminals.

Kendall's Tau rank correlations were computed to further investigate the possible relationship between the cortical hierarchical order and the axon terminal types with the inclusion of area 17 data. For first-order thalamic nuclei, there is no significant correlations between the cortical areas (and consequently, their hierarchical order) and the relative frequency of terminal types. For higher-order thalamic nuclei, a highly significant positive correlation was found between the cortical hierarchical order and the percentage of type I terminals (Tau=0.468, p=0.012). In addition, a very significant negative correlation was found between the hierarchical order and the percentage of singleton terminals (Tau=-0.568, p=0.006) (Figure **6**). That is to say that the number of type I terminals increases while the number of singleton (type II) decreases with increasing hierarchical levels of visual cortical area (from area 17 to PMLS to AEV).

Retrograde labeling

Corticothalamic cells in the AES were visualized following retrograde transport of WGA-HRP injected in the medial thalamus comprising the LPm, LM-Sg, and MG nuclei. Retrogradely labelled cells were predominantly found in layer VI (with rare cells in layer V) as shown in figure 7.

Discussion

The present results indicate that most CT axons of the visual and multisensory subregions located in the AES targeting higher-order nuclei exhibit type I terminals. This is in contrast with area 17 which provides mostly type II terminals (including intermediate, rosettes, and singletons) and PMLS cortex which presents a combination of all terminal types. Thus, these results are in

agreement with the initial assumption that the proportion of type I versus type II axon terminals in higher order thalamic nuclei increases with the hierarchical order of cortical visual areas. Most of the tracer injections were small in order to avoid contamination of the white matter underlying the AES. The injection and projection sites correspond to the electrophysiological distribution of sensory specific territories (Olson and Graybiel 1987, Nagy, Eordegh et al. 2003, Fuentes-Santamaria, Alvarado et al. 2009, Butler, de la Rua et al. 2018). Our injections were mainly aimed at the visual portion of the AES as confirmed by the presence of terminals in visual thalamic nuclei such as the LPm and LM-Sg. However, injections were not restricted to a single sensory modality. In cases AES5 and AES6, injections were made in the caudal part of the AES where auditory and/or visual responsive cells are present (Olson and Graybiel 1987, Meredith and Lomber 2011). The most caudal injection in AES5 resulted in more labeled terminals in the MG than any other injection sites (Roda and Reinoso-Suarez 1983). These results are in agreement with previous tracing studies (Mucke, Norita et al. 1982, Norita and Katoh 1986, Olson and Graybiel 1987, Clarey and Irvine 1990).

First order thalamic nuclei receive their driving afferents from ascending sensory pathways and transmit information to the cortex. They also receive type I axon terminals from layer VI cells of their respective primary sensory cortices (Deschênes, Bourassa et al. 1994, Bourassa and Deschênes 1995). MGv and LGNd are the first order thalamic relays of the auditory and visual system respectively (Sherman and Guillery 2001 for the review). Guillery (1995) has defined MGm as a first order auditory relay since it receives its sensory driving input form from the inferior colliculus (Takada, Itoh et al. 1985). Our results show that MGm receives both type I and II terminals. This contrasts with the visual system organization as the LGNd does not receive

direct projections from AES. The difference between these two modalities may be explained by a different phylogenetic origin of these two nuclei (Butler 1994, b) and may represent specificities of the two modalities.

It is generally considered that higher-order thalamic nuclei receive their driving afferents from layer V of the cortex and modulatory signals from layer VI (Deschênes, Bourassa et al. 1994, Bourassa and Deschênes 1995, Li, Wang et al. 2003). Our results show that projections from the AES to the MGd, PO and the medial part of the pulvinar complex are predominately type I and originate almost exclusively from layer VI. From the 5 injections made in the AES, only one out of the 12 HO nucleus marked with BDA (LMSG of AES 5, table 2) showed a greater number of type II while other nuclei of the same animal exhibited a profile comparable with other cases (i.e a higher number of type 1), leaving only the LMSG of AES5 to stand out form others. One plausible explanation for this case could be that, because each injection were made in different areas of the AES in order to target different modalities, some projection pattern may result in a higher number of type II in rare cases (1 nuclei out of 12 in this study) , while the mast majority of projections (11 nuclei out of 12) receive type I projections suggesting that the cortical areas from the AES exert a modulatory influence on these higher-order thalamic nuclei. This is in contrast with the predominance of a driver input from the primary visual cortex on higher-order visual nuclei.

Our data also highlights the fact that the proportion of type I over type II terminals may increase along the hierarchical order of visual areas. If that is correct, the CT projections from the primary visual cortex would be the main source of driving inputs to the pulvinar while higher-order

cortical areas are mainly involved in the fine tuning of its ongoing activity. This modulatory action would be more and more prominent as one goes from lower to higher-order cortex. These results are in agreement with functional studies showing a significant decrease of receptive field responsiveness of a number of cells in the cat LPl (Casanova, Savard et al. 1997, Rushmore, Payne et al. 2005) and in the inferior pulvinar of primates (Bender 1983), following inactivation of the primary visual cortex.

The LM-Sg, one of the main targets of the subregions from the AES, is involved in multisensory information processing (Benedek, Fischer-Szatmari et al. 1996, Benedek, Pereny et al. 1997). Our results show that both types of axons terminals are located in LM-Sg but that the modulatory type I largely predominates the corticothalamic projection from the AES. This contrasts with its axons originating from cells in the superior colliculus that have type II terminals and thus exert a driver influence (Norita and Katoh 1986, Katoh and Benedek 1995). It is not known whether the corticothalamic pathway conveys multisensory information to LM-Sg. In view of the fact that LM-Sg receives a significant input from intermediate layers of the superior colliculus and that terminals are similar to type II and retinal RLP terminals, it could be proposed that multisensory properties of LM-Sg cells are provided by the superior colliculus input and that LM-Sg responses can be further modulated by AES layer VI cell signals.

The observation of terminals in close proximity to dendrites of retrogradely labeled cells raises the possibility that cortices locates in the AES participate in corticothalamocortical loops. These loops can be either strictly reciprocal loops or feedback pathways to other early visual areas (Scannell, Burns et al. 1999). Both assumptions are supported by the results of Miceli et al (1991) who reported that a group of neurons in the LPm/LM-Sg region send collaterals to both PLLS

and AES cortices. Whether cortices in the AES entertain particular reciprocal connection with such divergent relay cells is presently unknown. However, these neurons were situated in similar locations and it may be possible that the cortical areas in the AES modulate their gating properties.

To our knowledge, very few studies have investigated the proportion of type I/type II CT terminals in the pulvinar across various species, especially for extra striate areas. As highlighted in this study, in the cat model, which is perhaps the one with which we have the most data concerning the morphology of CT terminals in the pulvinar, area 17 has been demonstrated to mainly send type II (driver) input to the LPl whereas the PMLS has been shown to send both type I and type II inputs (Guillery, Feig et al. 2001, Huppe-Gourgues, Bickford et al. 2006). Projections from area 18, 19, 20a and b, AMLS, 21a, 5 and 7 have been reported in the LP pulvinar complex, but none of these studies investigated the relative nature of their driver/modulator nature.

Primate studies have also been few, and to date, have been limited to V1 and area MT. Like in the cat, primary visual cortex projection to the pulvinar have been shown to exhibit a type II like morphology whereas projections from MT are predominantly type I (Rockland 1996,1998). Since these studies were done in the context of axon and arbor reconstruction, only a relative idea on the proportion of type I/type II is known. To our knownelege, no study investigated the precise quantitative proportion of type I vs type II boutons of corticopulvinar axon terminals in the primate. It would be interesting to investigate whether the average proportions of type I/II projections reported in this study in the cat are similar in the primate as this would either generalize our hypothesis of an increasing type I input along the ascending hierarchy of visual

area; or if results were to differ from that observe in the cat, would call for another model of neuroanatomical organization of these projections that would best suit the specificities of its visual system.

In summary, the present study shows that (1) as previously reported (Bourassa and Deschênes 1995, Vidnyanszky, Borostyankoi et al. 1996), the primary visual cortex provides a modulatory input to first–order thalamic nuclei through type I terminals and conversely a much more important driving input to higher-order thalamic nuclei through type II terminals; (2) the PMLS cortex, located at a higher hierarchical level, provides mainly a modulatory input to first-order thalamic nuclei as shown by the very high proportion of type I terminals therein and both modulatory and driving signals through type I and II terminals in high-order thalamic nuclei (Ridder, Nusinowitz et al.); (3) the AES cortex, which is considered as the highest hierarchical level among the three regions (Scannell, Burns et al. 1999), provides almost exclusively modulatory inputs to both first and high-order thalamic nuclei of the thalamus via type I terminals. Thus, there seems to be a general organization in which the primary visual cortex provides a prominent driving input to high-order thalamic nuclei while higher-order cortical areas exert predominantly a modulatory influence. These last signals can be part of mechanisms subtending attention–related gating at the thalamic level (see Sherman and Guillery 2001) (Wang, Eisenback et al. 2002, Saalmann, Pinsk et al. 2012) and represent a demonstration of a top down regulation of cortical areas onto thalamic processing.

Author contributions statement :

Christian Casanova: experimental design, manuscript writing

Maurice Ptito: experimental design, manuscript writing

Denis Boire: experimental design, manuscript writing

Reza Abbas Farishta: analyses, manuscript writing

Frédéric Huppé Gourgues: experimentation, analyses, manuscript writing

Note that none of the authors has any conflict of interest.

Data Availability :

The datasets generated during and/or analysed during the current study are available from the
corresponding author on reasonable request.

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	FO MGM	MIN	LGNd	LGNv	HO MGd	LMSg	PO	LPm	LPl	LPI2
AES1						71 (100%)	81 (100%)			
AES2						8 (100%)				
AES3	25 (27%)				439 (24%)	38 (25%)		84 (23%)		
AES5	191 (12%)					36 (100%)	76 (2%)	24 (29%)		
AES6	64 100%				69 (5%)	938 (100%)		10 (100%)		
PMLS1				19 (25%)				384 (23%)	140 (15%)	
PMLS2		759 (4%)						42 (1%)	566 (1%)	
PMLS3		26 100%						60 (24%)	32 (25%)	
PMLS4									121 (8%)	36 (24%)
PMLS5									112 (17%)	10 (20%)
A17		166 (8%)							322 (26%)	

Article 1. Table 1. Stereological sampling data.

Stereological sampling data (number of counted terminals and percentage of tissue sampled) of cases by nuclei. FO: First order thalamic nuclei, HO: Higher order thalamic nuclei.

%	FO MGM	MIN	LGNd	LGNv	HO MGd	LMSg	PO	LPm	LPl
AES1 Type1 Inter/Rosette Singleton						93,4 0,0 6,6	98,8 0,0 1,2		
AES2 Type1 Inter/Rosette Singleton						100,0 0,0 0,0			
AES3 Type1 Inter/Rosette Singleton	67,9 10,5 21,6				83,4 13,8 2,8	89,9 7,6 2,5		100,0 0,0 0,0	
AES5 Type1 Inter/Rosette Singleton	100,0 0,0 0,0					21,7 56,5 21,7	99,8 0,0 0,2	71,7 23,6 4,7	
AES6 Type1 Inter/Rosette Singleton	100,0 0,0 0,0				95,9 4,1 0,0	100,0 0,0 0,0		100,0 0,0 0,0	
PMLS1 Type1 Inter/Rosette Singleton				36,8 36,8 26,3				61,2 20,8 18,0	67,9 22,1 10,0
PMLS2 Type1 Inter/Rosette Singleton		98,9 0,8 0,2						99,7 0,3 0,0	99,4 0,5 0,1
PMLS3 Type1 Inter/Rosette Singleton		100,0 0,0 0,0						54,7 7,2 38,1	81,8 0,0 18,2
PMLS4 Type1 Inter/Rosette Singleton									74,4 1,8 23,8
PMLS5 Type1 Inter/Rosette Singleton									31,8 26,3 41,9
A17 Type1 Inter/Rosette Singleton		100,0 0,0 0,0							10,9 28,3 60,9

Article 1. Table 2. Percentage of thalamic terminal types according to their cortical origin.

Percentage of thalamic terminal types according to their cortical origin. The stereologic samplings of thalamus terminal fields were calculated from injections in area17, PMLS and AES. This quantification of the different types of terminals permits comparisons according to cortical origin and thalamic localizations of corticothalamic terminals. PMLS cases are from a previous study (Huppé-Gourgues, Bickford et al. 2006).
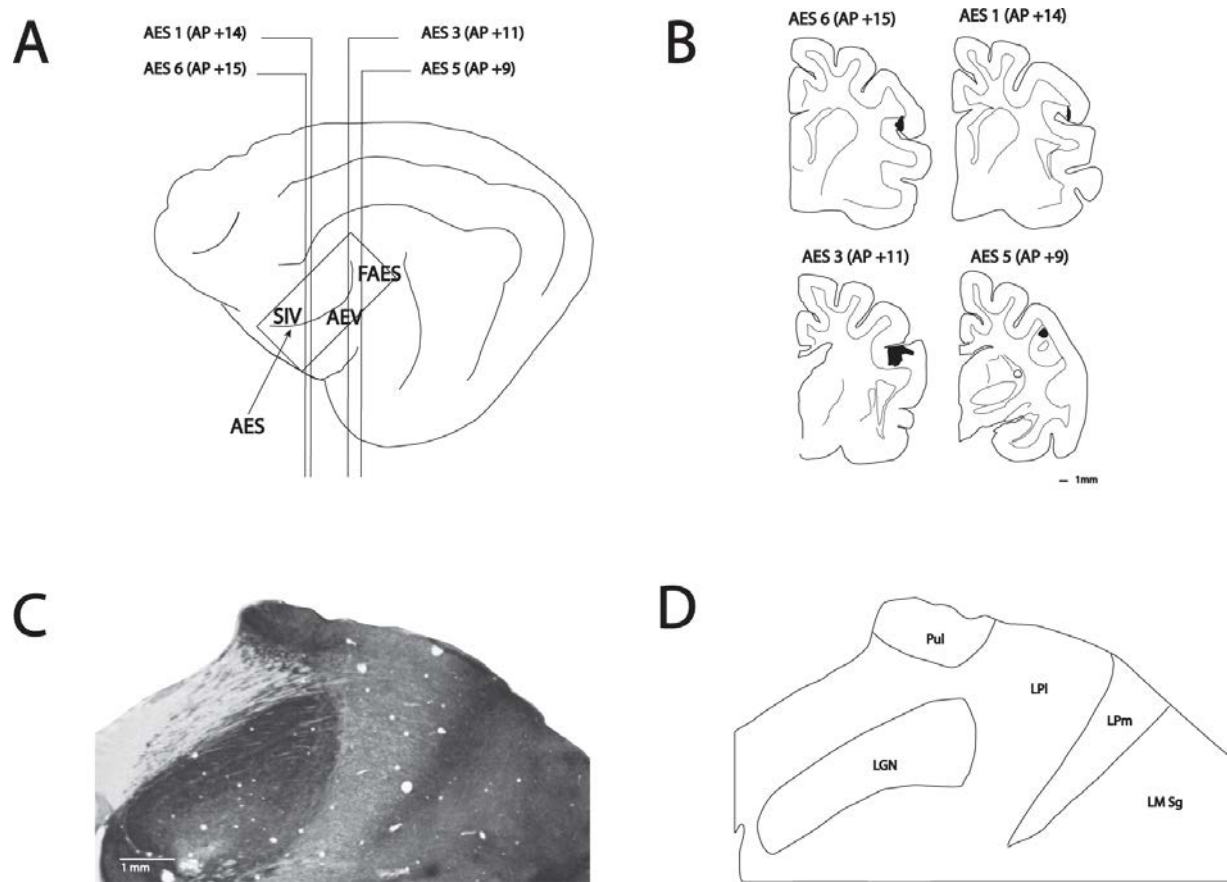
Article 1. Figure 1. Injection localization and pulvinar chemo architecture.

(**A**) Representation of the brain showing the location of the subregions of the AES cortex. (**B**) Cortical injection sites of the illustrated cases. (**C,D**) Example of a coronal section stained with AChE used to identify thalamic subregions.
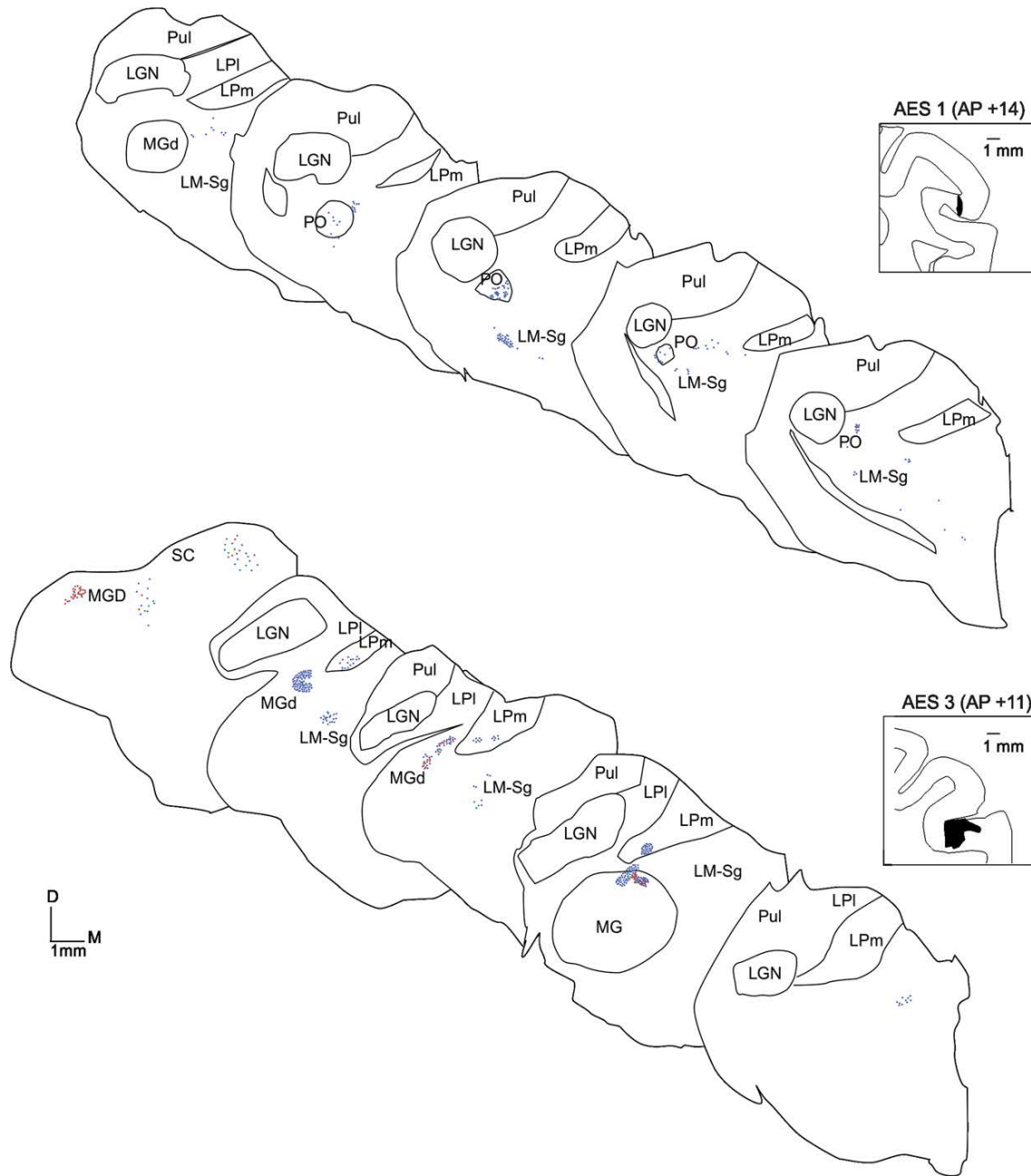
Article 1. Figure 2 Topographical representation of axon terminals in the thalamus (AES 1 and 3).

Topographical representation of axon terminals in the thalamus after injection of AES. Injection sites are presented in insets. Blue dots: type I axon terminals, Green dots: Singletons, Red dots: type II axon terminals. In Figs 2A and **3B**, one dot represent one terminal. In Figs 2B and **3A** one dot represents 5 terminals. Scale 1 mm.
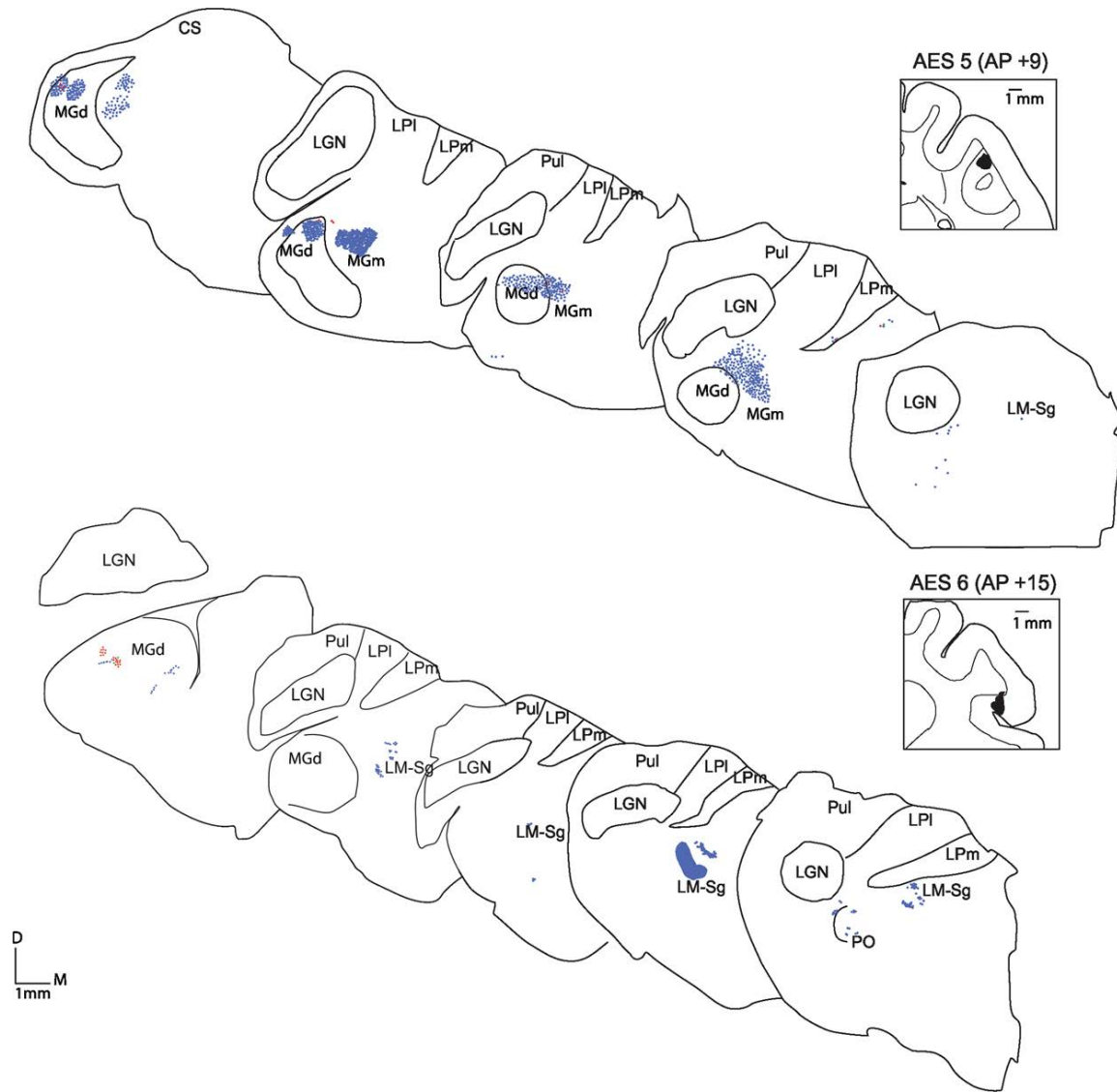
Article 1. Figure 3 Topographical representation of axon terminals in the thalamus (AES 5 and 6).

Topographical representation of axon terminals in the thalamus after injection of AES. Injection sites are presented in insets. Blue dots: type I axon terminals, Green dots: Singletons, Red dots: type II axon terminals. In Figs 2A and 3B, one dot represent one terminal. In Figs 2B and 3A one dot represents 5 terminals. Scale 1 mm
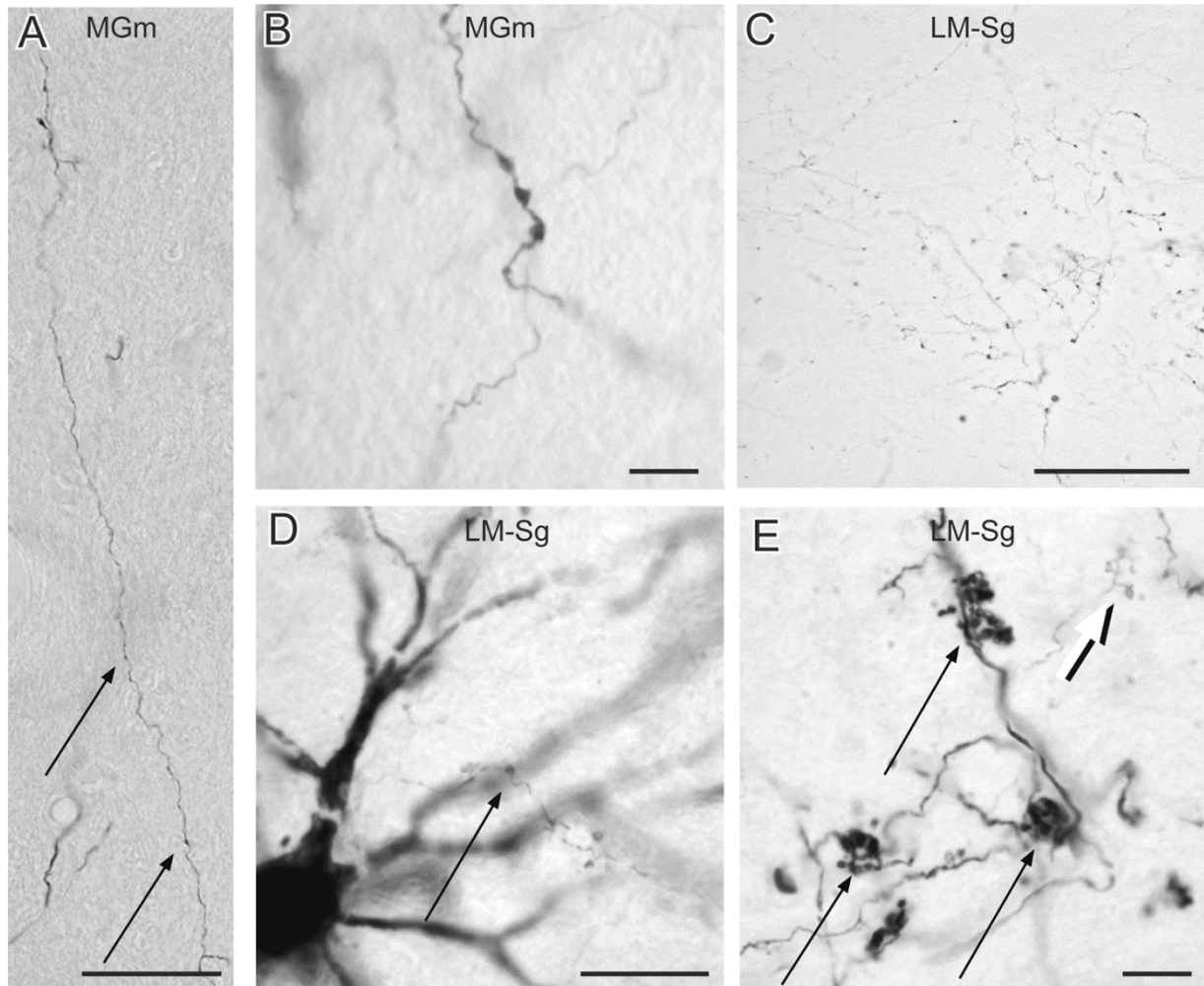
Article 1. Figure 4. Corticothalamic projection fields.

Photomicrograph of corticothalamic terminals. (**A**) Reconstruction of a typical type I axon bearing small sparse terminals (indicated by arrows) found in the MGm. (**B**) Example of en-passant boutons along a corticothalamic axon of the MGm. (**C**) Typical terminal field containing mostly type I in the LM-Sg. (**D**) Type I axon terminals in the dendritic field of a thalamocortical cell in LM-Sg. (**E**) Example of a large cluster of type II axon terminals (black arrow). For comparison, note the size of type I axon passing in the background (white arrow). Scales: A: 50 μm; B, D and E: 10 μm; C: 100 μm.
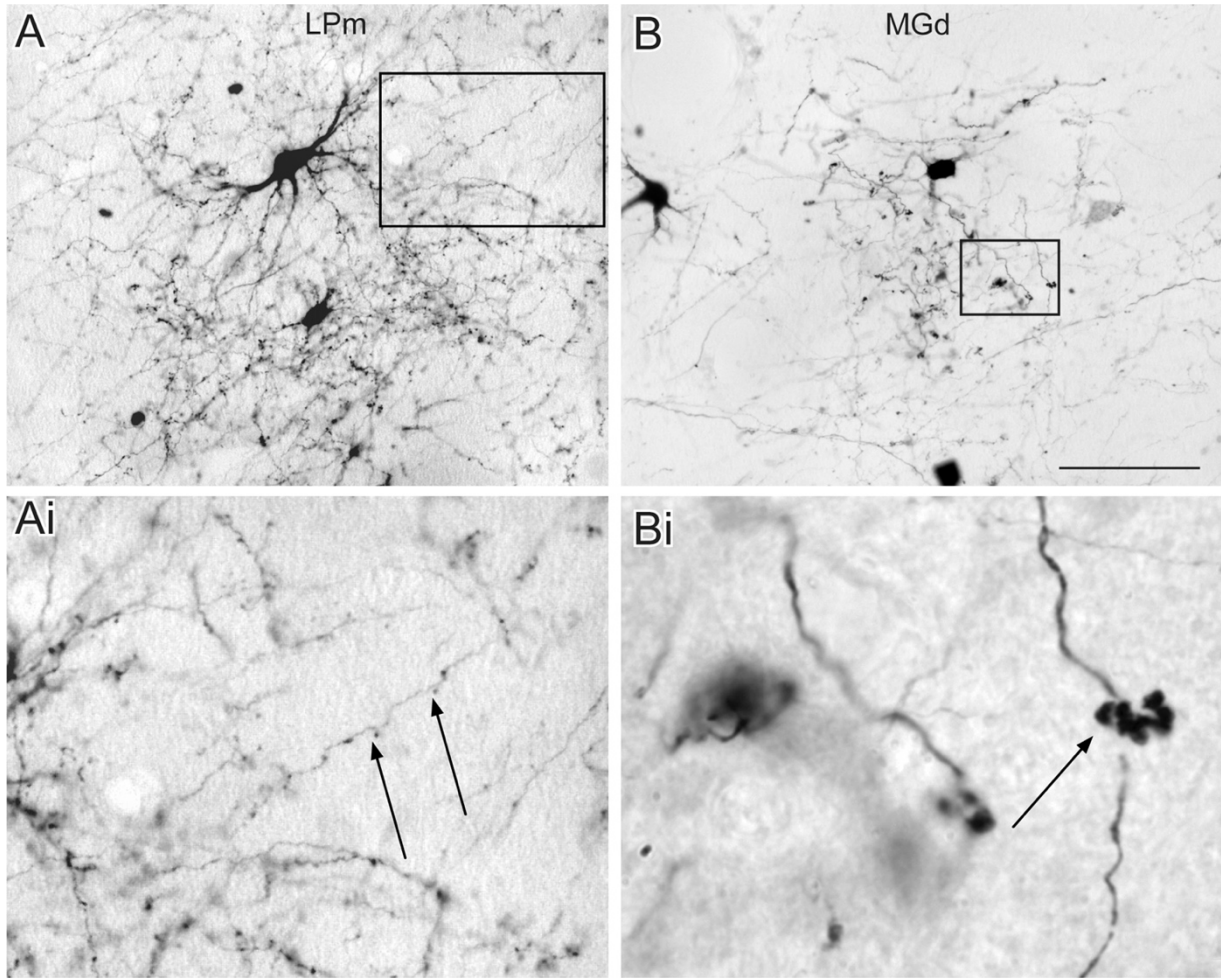
Article 1. Figure 5. Terminal fields in the thalamus following AES injections.

Example of terminal fields found in the thalamus following injection in the AES (from case No 3). (**A**) A focus containing mostly type I axon terminal in the LPm thalamic region. (**B**) A focus containing both type I and II axon terminals in the MGd. Scale in panels A and B is 100 μm.
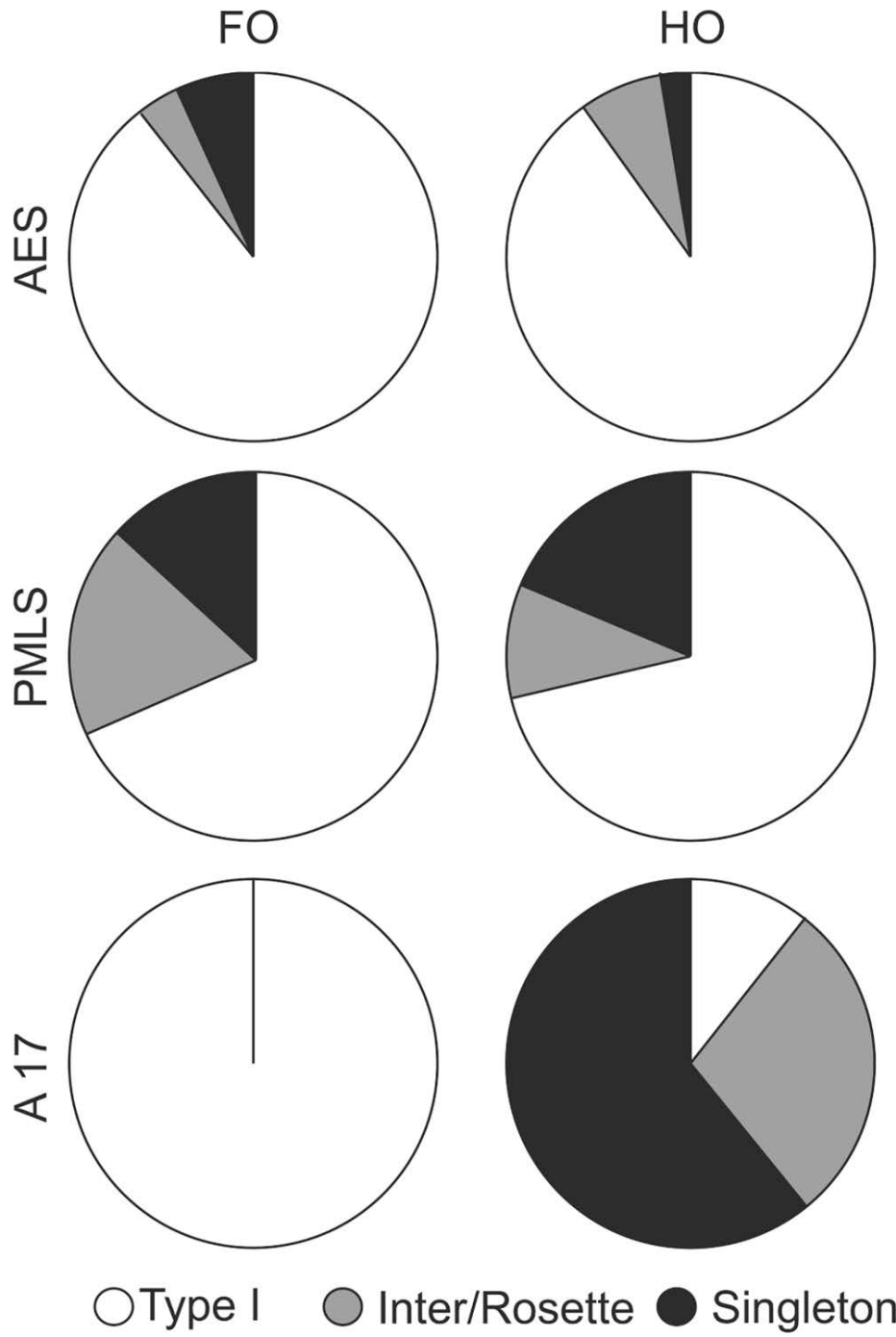
Article 1. Figure 6. Proportion (%) of terminal types according to their cortical origin and thalamic targets.
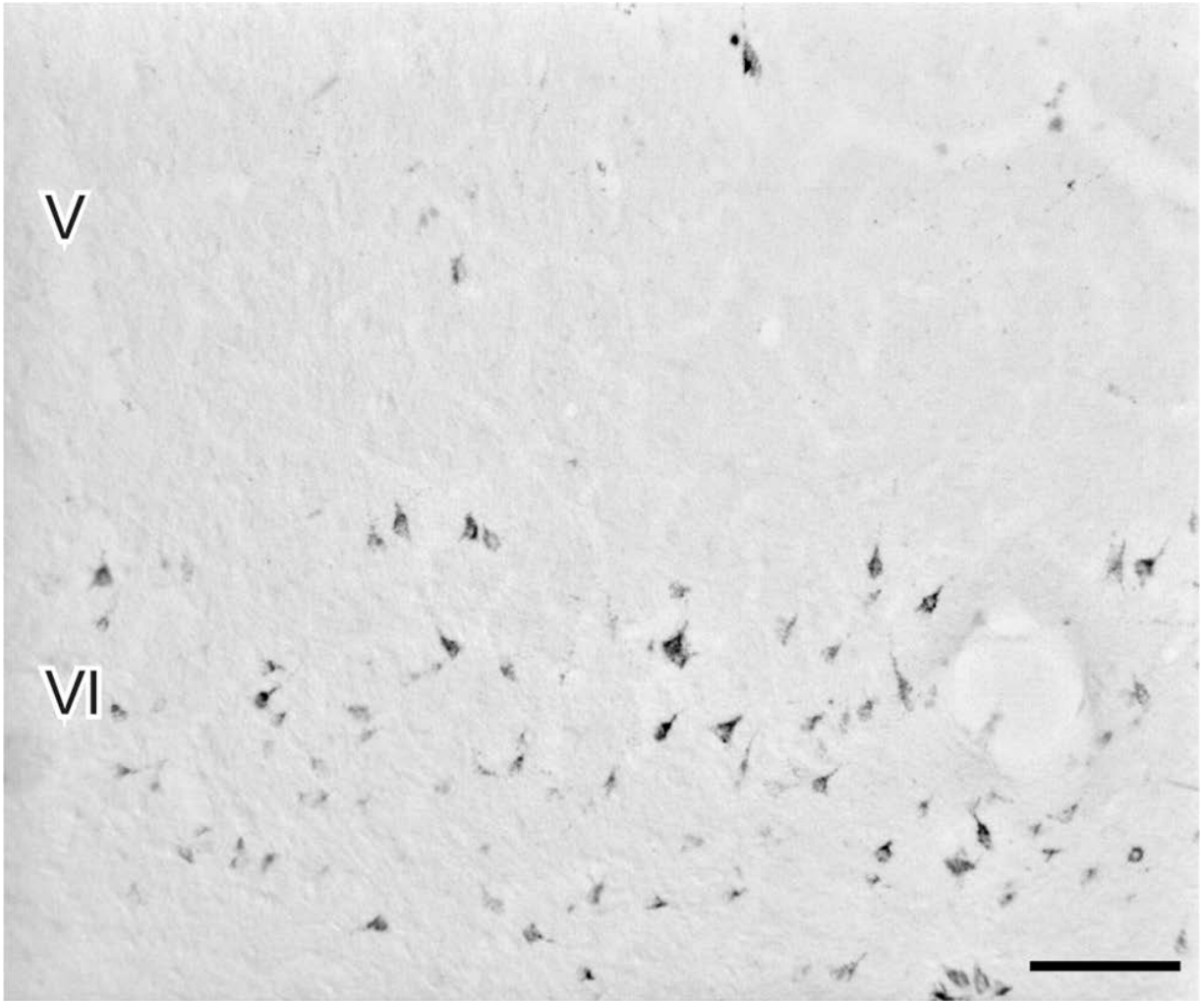
Article 1. Figure 7. Retrogradely labbeled cells in the AEV following injection in the pulvinar.

A large injection of WGA-HRP in the LPm reveals retrograde labeled cells in the AES. These neurons were mainly found in layer VI. Scale 100 µm.

3.2 Article 2 : Hierarchical Organization of Corticothalamic Projections to the Pulvinar

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Hierarchical Organization of Corticothalamic Projections to the Pulvinar

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Abstract

Signals from lower cortical visual areas travel to higher order areas for further processing through corticocortical projections, organized in a hierarchical manner. These signals can also be transferred between cortical areas via alternative cortical transthalamic routes involving higher order thalamic nuclei like the LP-pulvinar. It is unknown whether the organization of transthalamic pathways may reflect the cortical hierarchy. Two axon terminals types have been identified in cortico-thalamic (CT) pathways: the types I (modulators) and II (drivers) characterized by thin axons with small terminals and by thick axons and large terminals, respectively. In cats, projections from V1 to the LP-pulvinar comprise mainly type II terminals whereas those from extrastriate areas include a combination of both terminals suggesting that the nature of CT terminals varies with the hierarchical order of visual areas. To test this hypothesis, distribution of CT terminals from area 21a was charted and compared with three other visual areas located at different hierarchical levels. Results demonstrate that the proportion of modulatory CT inputs increases along the hierarchical level of cortical areas. This organization of transthalamic pathways reflecting cortical hierarchy provides new and fundamental insights for the establishment of more accurate models of cortical signal processing along transthalamic cortical pathways.

Introduction

The receptive field (RF) properties of neurons in sensory cortices undergo major transformation as they progress along ascending pathways (Stone, Dreher et al. 1979). This growing complexity is generally considered to originate from processing between anatomically and functionally organized cortical areas classified in a hierarchical manner according to the laminar pattern of their originating and terminating projections (Felleman and Van Essen 1991, Markov, Vezoli et al. 2014).

While models using this classification of cortical processing have greatly advanced our understanding of the visual system (Scannell, Blakemore et al. 1995), they remain incomplete as they restrict complex visual processing to direct cortico-cortical pathways only while it is known that all visual cortical areas are also reciprocally connected to the main extra−geniculate thalamic nucleus, the pulvinar, providing then monosynaptic trans-thalamic pathways between areas of the neocortex (Shipp 2003, Theyel, Llano et al. 2010, Sherman and Guillery 2011, Sherman 2016).

The role of the associated trans−thalamic pathways remains unclear and it is still unknown how their functions differ from that of the corresponding direct cortical-cortical pathways (Casanova, Merabet et al. 2001, Sherman 2017). Studies suggest that these pathways can be used to facilitate the cortical flow of information (Theyel, Llano et al. 2010), modulate contrast sensitivity (Cortes and van Vreeswijk 2012, de Souza, Cortes et al. 2019), and subtend attentional processes (Saalmann, Pinsk et al. 2012, Ni, Wunderle et al. 2016, Roth, Dahmen et al. 2016, Zhou, Schafer

et al. 2016).

One way to increase our understanding of these pathways is to determine whether they parallel the hierarchical organization of the direct cortico-cortical connections. In this context, two types of corticothalamic (CT) axons connecting cortical areas to the pulvinar have been identified based on their morphology. Type I axons are thin and possess long thin branches with occasional *en passant* swellings while type II axon possess larger terminals ranging between lone singletons and more complex flowery forms of rosettes (Ojima, Murakami et al. 1996, Vidnyanszky, Borostyankoi et al. 1996, Feig and Harting 1998, Guillery, Feig et al. 2001, Huppe-Gourgues, Bickford et al. 2006, Huppe-Gourgues, Abbas Farishta et al. 2019). According to the 'driver/modulator' theory of glutamatergic pathways involving the thalamus and the cortex, type II terminals (driver) are believed to be the main carrier of sensory information, while type I (modulator) are assumed to fine-tune ongoing activity (Sherman and Guillery 1998).

Studies have revealed that the proportion of type I and type II cortico-pulvinar terminals varies between cortical areas (Huppe-Gourgues, Bickford et al. 2006, Huppe-Gourgues, Abbas Farishta et al. 2019). For instance, in the cat LP-pulvinar complex, the vast majority of axons coming from V1 have type II terminals, while those from the PMLS and the AEV, mainly exhibit type I terminals. This suggests that the nature of cortical projections to extra−geniculate thalamic nuclei varies as a function of the cortical hierarchical level characterized by an increase of the modulatory/driver inputs ratio as one progresses from lower to higher-order cortical areas.

To further test this hypothesis, we charted the distribution of projections from area 21a, a cortex of higher rank than the PMLS, but lower than the AEV in hierarchy of visual areas proposed by (Scannell, Burns et al. 1999). Area 21a is located along the ventral visual stream and is mainly involved in pattern and form recognition and visual cognitive processes (Dreher, Michalski et al. 1993, Dreher, Wang et al. 1996, Villeneuve, Vanni et al. 2009). The objectives of this study were threefold: (1) to determine the morphology of CT projections from area 21a and (2) to compare these projections to the known type II projections from area 17 (Guillery, Feig et al. 2001, Huppe-Gourgues, Bickford et al. 2006) and those from cortical areas of the dorsal stream in our previous study (PMLS and AES from (Huppe-Gourgues, Abbas Farishta et al. 2019)), (3) to validate the hypothesis that there is a hierarchical organization of CT projections where modulatory inputs from the cortex to the pulvinar increase as a function of cortical hierarchy.

Materials and Methods

Animal Preparation and Injections.

Animals were treated in accordance to the regulations of the Canadian Council for the Protection of Animals (CCPA) and the experimental protocols were approved by the 'Comité de déontologie de l'expérimentation sur les animaux' of the Université de Montréal. Five normally pigmented adult cats were used in this study (n=4 injections in area 21a and n=1 in area 17). Pre-operative anti-inflammatory agents (metacam s.c. 0.1mg/kg) and antibiotics (tribrissen 24% s.c. 0.13ml/kg) were administered 24 hours before surgical procedures. Twenty minutes before surgery, atropine (0.1mg/kg s.c.) and Atravet (0.05mg/kg s.c.) were given to the animal. Anesthesia was induced with a mixture of 5% Isoflurane in 60% N_2O and 25% O_2 and

maintained with 2% Isoflurane added to the same gaseous mixture. Animals were positioned in a stereotaxic apparatus. A local anesthetic (lidocaine hydrochloride 2%) was injected subcutaneously and a craniotomy was performed over the injection site. During all surgical procedures, animals were maintained at 38°C and heart rate, end-tidal CO_2, blood O_2 saturation and blood pressure were closely monitored.

Area 21a craniotomies were performed according to Horsley-Clarke (H-C) coordinates 6 - 12 mm lateral to the midline and 0 - 8 mm posterior to the interaural plane. For 17, the craniotomy was performed according to Horsley-Clarke (H-C) coordinates 0- 4 mm lateral to the midline and 0 - 9 mm posterior to the interaural plane. Borosilicate pipettes (1.5 mm external diameter) were pulled to obtain a tip ranging between 20 and 40µm. Biotinylated dextran amines (BDA 10kD) were injected (Molecular Probes, Thermo Fisher Scientific, MA, USA) in area 21a by iontophoresis using a positive DC current (7 second on/off cycle 7µA) for 25 minutes at depths of 1.2 mm and 0.8 mm in order to target all cortical layers. In all cases, the injection sites never encroached the underlying white matter. Craniotomies were sealed with acrylic bone cement and the wounds were sutured in anatomical layers. Anti-inflammatory and antibiotic treatments were administered pre- and post-operatively and analgesic was applied for 72 h following surgery (Metacam/ Boehringer-Ingelheim.ca; 0.01 mg/kg bid).

Tissue Processing.

Ten to fourteen days after the cortical injections, animals received an overdose of sodium pentobarbital (80 mg/kg; IP) and were perfused with phosphate buffered 0.9% saline (PBS: 0.1M, pH 7.4) followed by phosphate buffered 4% paraformaldehyde. Brains were blocked

stereotaxically, removed from the cranium, post fixed overnight in the same fixation solution at 4°C, cryoprotected in 30% sucrose in 0.1 M phosphate buffer (pH 7.4) and frozen until processed. The fixed brains were cut into 40 µm-thick coronal sections and collected in PBS. After pre-incubation in 2.5% bovine serum albumin (BSA) and normal goat serum 2% in phosphate-buffered saline (PBS; 0.01 M PB with 0.9% NaCl, pH 7.4) for 30 min, BDA was visualized with avidin-biotin-peroxidase complex (ABC; Vectastain ABC Elite kit; Vector, Burlingame, CA). Following buffer washes, sections were reacted with nickel-intensified diaminobenzidine (0.5% nickel, 0.035 % diaminobenzidine and 0.002% H_2O_2) for 10 min (Hsu and Soban 1982). After PBS washes, sections were mounted on slides, dehydrated, mounted with Depex and cover slipped. Adjacent sections were processed for acetylcholinesterase (AChE) histochemistry for the identification of cytoarchitectonic boundaries between the lateral (LPl) and medial (LPm) subdivisions of the lateral posterior thalamus of the LP-Pulvinar complex (Graybiel and Berson 1980). Sections were incubated for six hours in an aqueous solution with 50 mM sodium acetate, 4 mM copper sulfate, 16 mM glycine, 4 mM *S*-acetylthiocholine and 86 µM ethopropazine adjusted to pH 5. Sections were rinsed in water and reacted for 10 min in a 1% aqueous solution of sodium sulfite and subsequently fixed in 4% paraformaldehyde for 2h. The location of area 21a was confirmed by staining slides adjacent to the injection site with the monoclonal antibody SMI 32 known to reveal the parcellation of visual cortical areas (van der Gucht, Vandesande et al. 2001). Briefly, free-floating tissue sections were preincubated for 1 h in Tris-buffered saline (TBS) containing 0.1% Triton X-100 and 5% normal goat serum. Sections were then incubated overnight in TBS containing 0.1% Triton X-100, 5% normal goat serum and primary monoclonal antibody (mouse monoclonal anti-SMI32 specific for nonphosphorylated Neurofilament H, (Catalog number 801701, 1 :2000, Biolegend, CA). On

the following day, immunoreactivity was revealed using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA) and chromogen 3,3′-diaminobensidine (DAB) with peroxide.

Analysis.

For stereological analysis in the pulvinar, evenly spaced sections (one of every five sections) were selected and projection fields were outlined under a 10x objective using a microscope (DMR, Leica) equipped with a three-axis computer-controlled stepping motor system coupled to a computer and to a color Optronix CCD camera and driven by the Neurolucida software (MBF Biosciences, Williston, VT, USA). Contours of each thalamic area in presenting anterogradely labeled axons and terminals were charted. These contours were superimposed on the images of adjacent AChE-reacted sections and resized for shrinkage differences between the AChe and BDA sections. This allowed CT projections to be assigned to either the LPl or LPm.

In order to provide an unbiased mapping of the distribution of axon terminals from areas 21a and 17 to the pulvinar and their size frequency, a systematic serological random sampling of these projection fields was performed using the Stereo Investigator software (MBF Bioscience). Projections fields in which anterograde labeling was observed were sampled under a 100x oil immersion lens (HC PL APO 100x/1.25 PH3, Leica, Germany) using the optical fractionator workflow (in Stereo Investigator) (West and Gundersen 1990, West, Slomianka et al. 1991) on approximately 10 equidistant sections covering the full anteroposterior range of the projection, except for one case injected with BDA in area 21a in which sampling was done on 5 sections. Axonal swellings were then counted in square disectors that were 15 x 15 μm and 15 μm height. Care was taken to avoid sampling in the 2-3 μm immediately adjacent to the sections surfaces to

avoid measuring cut or damaged terminals. The maximum diameter was measured for each sampled swelling. This optical fractionator sampling strategy allowed for the estimation of the total number of swellings in the pulvinar. The total numbers of swellings (N) were calculated using the following equation provided by (West et al. 1991).

$$N=\sum Q \times ssf^{-1} \times asf^{-1} \times tsf^{-1}$$

where ΣQ is the total number of swellings counted within the disectors and ssf is the section sampling fraction (number of sampled sections over the total number of sections on which the terminal projection field appears); asf is the area sampling fraction (ratio of the frame area/the total area of the reference space on the section) and tsf is the thickness-sampling fraction (disector height/measured section thickness). The overall sampling faction (see Table 1) is the product of ssf, asf and tsf. Coefficients of error (CEs) were calculated (West and Gundersen 1990, West, Slomianka et al. 1991) in order to determine whether the sampling effort was sufficient. It is widely accepted as a rule of thumb that CEs below 0.1 are indicative of a sufficient sampling (West and Gundersen 1990). Stereological sampling parameters and number of objects counted for every case are shown in table 1. For injections made in area 21a, all sections used for systematic stereological sampling of boutons in the thalamus were also carefully scanned at high magnification (40 and 100x) for the mapping of rare objects (rosette-like terminals and singleton), which could have been underestimated by the optical fractionator workflow.

The criteria used for the morphological classification of the axon terminals were those of (Guillery, Feig et al. 2001). Briefly, we identified type I as small caliber axons with sparse beaded terminals linked by a small stalk (drumstick). Type I axon terminals also included small swellings on fine caliber axons (en passant). Type II axons were identified as (1) intermediate,

i.e. comprising three terminals swellings in a small cluster; (2) rosette-like terminals, i.e. complex cluster of more than three terminals; and singletons, i.e. single beaded axon endings. For comparison purpose, previous cases from area 17, PMLS cortex and area AEV projections were reconsidered (Huppe-Gourgues, Bickford et al. 2006, Huppe-Gourgues, Abbas Farishta et al. 2019).

Statistical Analyses.

Statistical analyses were performed using SPSS v 16.0 software (SPSS, Chicago, IL, USA). Differences in the proportions of projection types from both sub nuclei in each and across all animals were compared with the Wald Khi-square test. Differences observed in the average size of boutons from the LPl and LPm and from area 17 and 21a were compared with student *t*-test. The association between the hierarchical rank of visual areas and the increasing number of type I projection to the pulvinar was tested using Spearman rank correlation coefficient. All tests were performed with a significance level of $p < 0.05$.

Results

Injection Sites in Area 21a and 17.

All injections in area 21 a were aimed at its crown to avoid spilling over in adjacent area 19 (Fig1 A). The injection in area 17 also avoided adjacent area 18 by targeting its medio-posterior part. Moreover, because type I projections arise from layer VI while type II from layer V (Ojima 1994, Bourassa and Deschênes 1995), all injections were performed at two depths (0.8 and 1.2 mm), with an angle perpendicular to the cortical surface. Careful inspection of injection sites

confirmed the targeting of all layers, but layer V and VI in particular, while no contamination of the white matter was observed. SMI-32 staining patterns (Fig 1A) on adjacent sections confirm the localization of the injection sites (Fig 1B). An example of an injection site centered on the suprasylvian gyrus and targeting all layers of cortical area 21a can be seen in (Fig 1A). SMI 32 staining patterns (on adjacent sections confirm the localization of the injection sites (Fig 1B).

Area 21a and 17 Projection Sites in the LP-pulvinar

AChE histochemistry was used to distinguish the medial (LPm) and lateral (LPl) divisions of the LP (Fig.2A). Significant labeling of cortical terminals in the LP-Pulvinar complex was observed following injections in area 21a (Fig. 2B). Cortical projections were observed in both the LPl, as well as in the LPm, the striate- and tecto-recipient zones of the LP respectively (Updyke 1977, Abramson and Chalupa 1985, Kelly, Li et al. 2003)(Fig 2B). While some projection foci were strictly located in the LPl or the LPm, some were seen targeting their border and extending in both subnuclei. Most projections observed were also spatially restricted to confined foci suggesting a relatively precise topographic organization. In all cases, several distinct foci of projections were observed within one subdivision of the LP, each one spreading over several sections (Fig 3). Most projection foci in both nuclei were loosely organized in a columnar manner that runs from the ventral to the lateral tip of the LP in an oblique manner (Fig 2B)

Significant labeling of CT axons was also observed following injection in area 17 but in contrast to area 21a, labeled terminals were confined in a single focus in the more ventral and caudal portions of the Lateral LP nucleus, immediately medial to the dLGN. This region corresponds to the striate recipient zone of the LP as originally described by (Graybiel and Berson 1980) or

the LPL 1 described by (Chalupa, Williams et al. 1983).

Classification of Anterogradely Labeled Axons From Area 21a and Area 17

Following the localization of projection foci in the LP-pulvinar complex originating from area 17 and 21a, axons in both sites were studied and classified according to their morphology. An example of a CT projection focus from area 21a to the LP is shown in Fig 4A, where a high magnification photomicrograph reveals the morphology of terminals and their overall axonal branching pattern. The vast majority of axons observed in the LP were of thin caliber (table 2), giving rise to small boutons that emanated from thin side branches that were classified as 'thin short stalks'. Swellings on the axons were classified as '*thin en passant*'. These axons were linear and poorly branched (Fig. 4 A-C). Using the nomenclature of (Guillery, Feig et al. 2001), these projections were classified as type I. A typical CT type I axon with both *en passant* and short stalk boutons is presented in panel B. In some cases, thicker axons were observed traveling amongst thinner type I projections (Fig. 4 C) and they were classified as type II. They also had either short stalk boutons (classified as 'thick short stalk') or *en passant* boutons (classified as 'thick en passant').

In contrast with CT axons from area 21a, projections from area 17 displayed a more complex arborization and a wide variety of bouton morphologies and sizes (Fig 4. D). Moreover, unlike projections from area 21a, which mostly exhibited typical type I thin caliber axons, type I inputs were less numerous following area 17 injections (25%). Also, while large type II rosette like structures were not observed, and that large singleton boutons were scarce in the projection originating from area 21a, these terminal morphologies were frequently observed following injections in area 17 (Fig. 4 D, E). Injections in the striate cortex resulted in the labeling of

complex terminals formed of multiple large boutons (rosette) as well as the presence of single large swellings at the end of axonal side branches (Fig. 4 E).

Quantitative Analysis

Proportion and frequency distribution of terminals from area 17 and area 21a

To better compare the contribution of CT projections from areas 21a and 17, every bouton sampled in the LP was measured and classified according to its morphology for each LP subdivisions and for both cortical areas. As expected, type I boutons represented the vast majority (81%) of boutons counted in the LPl, (table 2) significantly outnumbering type II boutons (19%) (Wald Khi-square test, P<0.001); type I boutons were found to be significantly smaller than boutons from type II axons (Fig 5A). The average bouton size from type I CT axons originating from area 21a in the LPl was 0.49 μm (± 0.01) and 0.99 μm (± 0.03) for type II axons (Student's *t*-test, P<0.001).

In the medial part of the LP (Fig. 5 B), a similar size distribution and proportion of axonal types were observed. Indeed, type I boutons (79%) (table 2) significantly outnumbered those from thicker type II axons (21%) (Wald Chi-square test, P<0.001). Similarly, in the LPl the average bouton diameter of type I (0.49 μm, ± 0.01) axons was also significantly smaller than that of type II axons (1.00μm; ± 0.04;(Student's *t*-test, P<0.001).

Both subdivisions of the LP have different anatomical and functional connectivity patterns: in essence, the LPl is the only recipient zone of striate projections and the LPm, the most prominent recipient zone of collicular input (a small zone in the LPl, named LPl2, received collicular inputs (Abramson and Chalupa 1985, Kelly, Li et al. 2003). Area 21a, however, projects to both

subdivisions. We thus investigated whether the strength and distribution profile of area 21a projections to the two subnuclei differed by comparing the estimated number of boutons (N), the proportion of type I and type II boutons, and their size distribution. Even though the number of labeled neurons varied between cases and is dependent upon injection size, for each case, all axons labeled in the LPl and LPm originated from the same injection and therefore, the estimated number of boutons (N) in both subdivisions of the LP is instructive of the strength of the projection. The average number of estimated objects N was 68772 for the LPl and 66776 for the LPm. No difference was observed in the strength of these projections from area 21a to both subdivisions of the LP (Student's *t*-test P=0.33). No difference was also observed in the proportion of type I vs. type II boutons in the LPl and LPm (Wald Chi-square test, P=0.976). The average size distribution of sampled boutons in both sub-nuclei was also compared: in all cases no difference was seen between the size distribution of counted boutons in both subdivisions. The average size was 0.59 (± 0.01) and 0.60μm (± 0.01) for the LPl and LPm, respectively (Student's *t*-test, P=0.521).

The frequency distribution of bouton's largest diameter was also plotted for projections originating from area 17 (Fig. 5 C). Unlike boutons from area 21a whose size was mostly found to be below 1 μm, a significant number of boutons from area 17 were larger than 1μm, many of them having a diameter greater than 3μm. Type II terminals were again found to be larger than type I terminals. The average bouton diameter for type I was 0.46 μm (± 0.01) and 1.44 μm (± 0.07) for type II terminals). When the average bouton diameter was compared between CT axons from area 17 and 21a, average bouton diameter from area 17 were found to be significantly greater (1.04μm ± 0.04) than area 21a terminals (0.6μm ± 0.01) (Student's *t*-test, P<0.001). When compared with projections from area 21a which were mostly of type I (80%), the latter

represented only a fraction (25%) of the total projections coming from area 17 (Fig 6).

<u>Type I Terminals Increase as a Function of Cortical Hierarchy.</u>

To test the hypothesis that the type I projections from the cortex to the LP-pulvinar complex increases as a function of cortical hierarchical levels, the proportion of type I terminals sent by various cortical areas was plotted as a function of the hierarchy of the cat cortical areas (Scannell, Blakemore et al. 1995) (Fig 7). Data from the present study were compared with those obtained in our laboratory using the same experimental approaches (table 3) (Huppe-Gourgues, Abbas Farishta et al. 2019). The proportion of type I projection to the LP increases as one ascends along the visual cortical hierarchy, with type I proportion around 25%, 66%, 82% and 91% for area 17, PMLS, area 21a and the AEV projections, respectively (Fig . 7A). There was a strong positive correlation (r=0.76) between the proportion of type I and the assumed cortical hierarchy (Spearman's rank correlation, p=0.002).

Discussion

The present results indicate that the majority of CT axons from area 21a projecting to each subdivision of the LP pulvinar complex exhibit type I terminals. Moreover, we show here that the morphology of cortical afferents to the pulvinar varies as a function of the hierarchical level of the visual cortical areas, providing a new framework for a better understanding of the organization and role of transthalamic cortical pathways.

Topographic Organization of CT Projections

We found projection foci in both subdivisions of the LP that spread over several sections.

(Hutchins and Updyke 1989). Other tracing studies using similar protocols also reported that injections made in area 17, 18, 19, the PMLS, areas 5 and 7 resulted in distinct projections patches spread over several sections (Guillery, Feig et al. 2001, Baldauf, Chomsung et al. 2005, Huppe-Gourgues, Bickford et al. 2006). Our observation suggests that retinotopically specific zones of area 21a are likely to be represented several times in the LP, in line with the multiple visual field representations described in electrophysiological studies (Raczkowski and Rosenquist 1981, Updyke 1983).

Morphology of Area 21 Projections to the Pulvinar

We show here that most axons originating from area 21a were type I axons presenting beaded terminals linked by a small stalk and small swellings on fine caliber axons. Thicker type II axons were also found with morphologies and boutons resembling those of type I but only bigger. In a previous study using retrograde tracer (Abramson and Chalupa 1985) found that area 21a sends projections to both LP subnuclei in a similar fashion and that these axons arise from layer V and VI. While they do not report quantitative data regarding the cortical layer of origin of these cortico-pulvinar inputs, they mention that projections from layer VI outnumbered those of layer V in extra-striate areas including area 21a. Other studies have also confirmed the existence of two distinct laminar origins of CT projection in the visual system and other sensory and motor systems in several animal models (Ojima 1994, Bourassa and Deschênes 1995, Rockland 1996, Rouiller, Tanne et al. 1998, Darian-Smith, Tan et al. 1999). They all support the observation that projections from layer VI to HO thalamic nuclei bear Type I projections, while those from layer V are of type II. Therefore, results from (Abramson and Chalupa 1985) which reported a greater number CT cells from XC areas including area 21a originating from layer VI than layer V are in

line with our observation of a greater proportion of type I terminals in the LP-pulvinar complex while further strengthening the view that type I and II CT projections arise from layer VI and V respectively.

We did not find any significant difference between the strength, bouton size and proportion of terminal types of area 21a projection to the two subdivisions pf the LP nucleus, the LPl and LPm, These results are in line with prior qualitative observations made by (Abramson and Chalupa 1985) which reported that cortical areas beyond areas 17 and 18 had a similar organizational projection patterns, irrespective of their thalamic target (tecto- or striate-recipient zones of LP).

Functional Significance of Type I and Type II Projections.

The existence of two types of CT projections in the LP-pulvinar and other HO thalamic nuclei has been demonstrated in several anatomical studies (Bourassa and Deschênes 1995, Rockland 1996, Rouiller and Welker 2000, Guillery, Feig et al. 2001, Baldauf, Chomsung et al. 2005). While few of these studies have investigated the functional role of the CT projections, there is evidence that the 'driver and modulator' function attributed respectively to type II and type I terminals along the retino-geniculo-cortical route may be generalized to extrageniculate pathways.

For instance, most projections from the primary visual cortex to LP-pulvinar complex have type II terminals (Rockland 1996, Feig and Harting 1998, Guillery, Feig et al. 2001, Huppe-Gourgues, Abbas Farishta et al. 2019). According to the 'driver/modulator' framework proposed by Sherman and Guillery, these type II projections from V1 should have a driver like influence on

pulvinar neurons and therefore be critical for the establishment of their receptive field properties. Electrophysiological data confirm this assumption since the deactivation of area 17 decreases receptive field responsiveness of neurons in the lateral posterior nucleus (LPN) of mice, the LPl of cats and inferior pulvinar of primates (Casanova, Savard et al. 1997, Rushmore, Payne et al. 2005, Bennett, Gale et al. 2019) (Bender 1983), suggesting a common ground of functional organization among species.

We expect that, CT projections from area 21a, which mainly arise from layer VI neurons, will have a different functional role than those coming from layer V area 17 neurons, thus supporting the view that corticofugal pathways originating from layers V and VI have distinct influences on thalamic activity (Li, Guido et al. 2003, Van Horn and Sherman 2004, Usrey and Sherman 2019). To our knowledge, no study investigated the impact of deactivating area 21a (or V4 in primates) on the visual responsiveness of LP-pulvinar neurons. Additional physiological experiments are thus required to determine whether this pathway carries modulatory signals. The fact that the pulvinar deactivation has different functional effects on visual responses of area 17 and 21a (de Souza, Cortes et al. 2019) may suggest that, in return, the deactivation of both these areas would have a different effect on visual responses of pulvinar neurons.

CT Axon Morphology Differs in Striate and Extra Striate Areas

Previous studies have shown that the morphology of CT axons varies between striate and extrastriate areas. While area 17 projections to the LPl consisted of mainly thick type II boutons, projections from the PMLS cortex and the AEV were mainly thin type I. Similar observations were also reported by Guillery et al., 2001 who observed a greater number of type I boutons in

the LP-pulvinar following injections in area 19 than following injections of areas 17 or 18. A similar picture also emerged from higher order area 5 and 7 which mainly sends type I projections to the LP-pulvinar (Baldauf, Chomsung et al. 2005). This difference was observed in other species such as grey squirrels (Robson and Hall 1977), macaques (Rockland 1996), and tree shrews (Chomsung, Wei et al. 2010, Day-Brown, Slusarczyk et al. 2017), suggesting a common pattern of CT projection amongst species.

Towards a Hierarchical Organization of CT Input

Altogether, results from this study and previous ones yield an interesting question: does the number of type I terminals vary as a function of cortical hierarchy? Our previous results and those described here reveal a positive correlation between the percentage of type I terminals and the level of cortical hierarchy such that the proportion of type I terminals increases as one travels along the hierarchy of visual areas. These results therefore suggest that, like cortico-cortical connections (Salin and Bullier 1995, Scannell, Blakemore et al. 1995, Markov, Vezoli et al. 2014), CT projections also follow a hierarchical model when projecting to HO thalamic areas.

As mentioned above, anatomical observations from other species performed in the visual system are also in line with our results in the cat suggesting that the hierarchical organization of CT projection in the visual system may very well be applicable to species other than the cat. There are also some indications suggesting a common organizational framework across sensory systems. First, according to our data, the projection pattern of CT projections in the visual system is based upon the hierarchical organization of cortical visual areas (Scannell, Blakemore et al. 1995). This suggests that CT projections are organized in a way which follows the organization

of their cortico-cortical counterparts. In this context, it is important to mention that other sensory systems, such as the auditory and somatosensory ones, are also organized in hierarchical manner (Iwamura 1998, Kaas and Hackett 2000, Whitehead, Papadelis et al. 2019), a pattern which may then be replicated in their respective CT connectivity. Furthermore, a thorough review authored by (Rouiller and Welker 2000) suggested the existence of a common framework for the organization of CT projections across species and systems. Indeed, in all studies reported in their review, both type I and II CT projections have been observed and like the visual system, they emanate from layer VI and V respectively.

The fact that CT projection pattern follows the anatomical hierarchical organization of cortical areas may bring interesting functional implications. Should the organization of CT projections be built upon that of corticocortical ones, one may also expect to find, within the pulvinar, an internal organization that would reflect the existence of dorsal and ventral functional pathways. In the macaque, evidence suggests that the anatomical connectivity pattern of specific subnuclei of the pulvinar show a bias towards areas of the ventral or dorsal stream (Kaas and Lyon 2007, Kaas and Baldwin 2019), an organization which was also recently revealed in the mouse (Bennett, Gale et al. 2019) further strengthening the emerging view that fundamental units of corticothalamic computations are not individual thalamic nuclei but more precise thalamic networks linking functionally related cortical areas (Shipp 2003, Halassa and Kastner 2017).

Conclusion.

Results from this study provided new information regarding the nature of CT projections

originating from area 21a. Perhaps more importantly, when these data are added to those obtained in other areas (Huppe-Gourgues, Bickford et al. 2006, Huppe-Gourgues, Abbas Farishta et al. 2019)) using the same methods, a general organizational scheme emerges : the proportion of cortical modulatory inputs reaching the LP-pulvinar increases as a function of the cortical hierarchy. This finding provides the basis for the establishment of more accurate models of corticothalamic flow of information in the visual system, and potentially in other sensory systems.

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<u>Table and Figure legends</u>

Table 1. Stereological sampling parameters for the estimation of the number of anterogradely labeled axonal swellings in each subdivision of the LP Pulvinar after BDA injections in area 21a and 17.

Table 2. Percentage of CT terminal types following injections in area 21a in the LPl and LPm.

Table 3. Percentage of CT terminal types according to cortical origin for four hierarchically organized area (area 17, PMLS, area 21a, AEV).

Figure 1. Low power photomicrographs of A. SMI-32 immunostained coronal section showing localization of areas area 21 a and surrounding visual cortical areas. B. the localization of a BDA injection in area 21a. Scale 500µm.

Figure 2. Low power photomicrographs of coronal sections of the thalamus stained for AChE (A) showing the dark staining in LPm compared to LPl. and BDA histochemistry showing the terminal fields of anterogradely labeled cortical projections from area 21 (B). Scale 250 µm

Figure 3. Schematic representation of the distribution of stereologically estimated BDA labeled terminals in coronal sections of the thalamus of two cases showing projections from area 21a to the LP in grey. Blue dots: type I axon terminals, Red dots: type II axon terminals, one dot represents five terminals, Scale(trace of the ML axis) 1 mm .

Figure 4. A. High-power photomicrograph from the boxed area of Fig 2A showing projection foci of anterogradely labeled CT axon terminals in the LPl and the LPm from area 21a. B. High-power photomicrograph showing CT axons from area 21a exhibiting a typical type I morphology with (thin) short stalks and occasional (thin) en passant boutons. C. A typical type I axon (white arrow) alongside a thicker axon (black arrow) with a similar morphology of (thicker) short stalks and occasional (thicker) en passant swellings on the axon itself classified as type II. D. Projection foci of CT axons from area 17 in the LPl displaying complex arborization with several terminals formed of multiple large boutons in a rosette-like structure (stars) as well as singletons which are large single swellings at the end of axonal side branches or en passant boutons (circles). E. Magnifed high-power photomicrograph from the boxed area of panel D displaying singletons (circles) and rosette (stars) terminals. Scales: A: 50µm; B and C : 25µm; D: 40 µm; E: 10µm.

Figure 5. Frequency distribution of CT bouton diameter from area 21a in the LPl (A), the LPm (B) and from area 17 to the LPl for type I (blue) and type II (red) terminals.

Figure 6. Pie chart representation of % of bouton types for area 21a and area 17.

Figure 7. A. Proportion in % of type I terminals as a function of the cortical hierarchy. Proportion of type I inputs from the PMLS and the AEV were reconsidered from (Huppé-Gourgues et al, 2019). B. Schematic summary of corticothalamic circuitry involving the pulvinar where the thickness of grey and black arrows represent the proportion of type I and type II projections respectively. C. A general schematic of the changing proportion of type I and type II CT projections as a functional of cortical hierarchy.

Figures
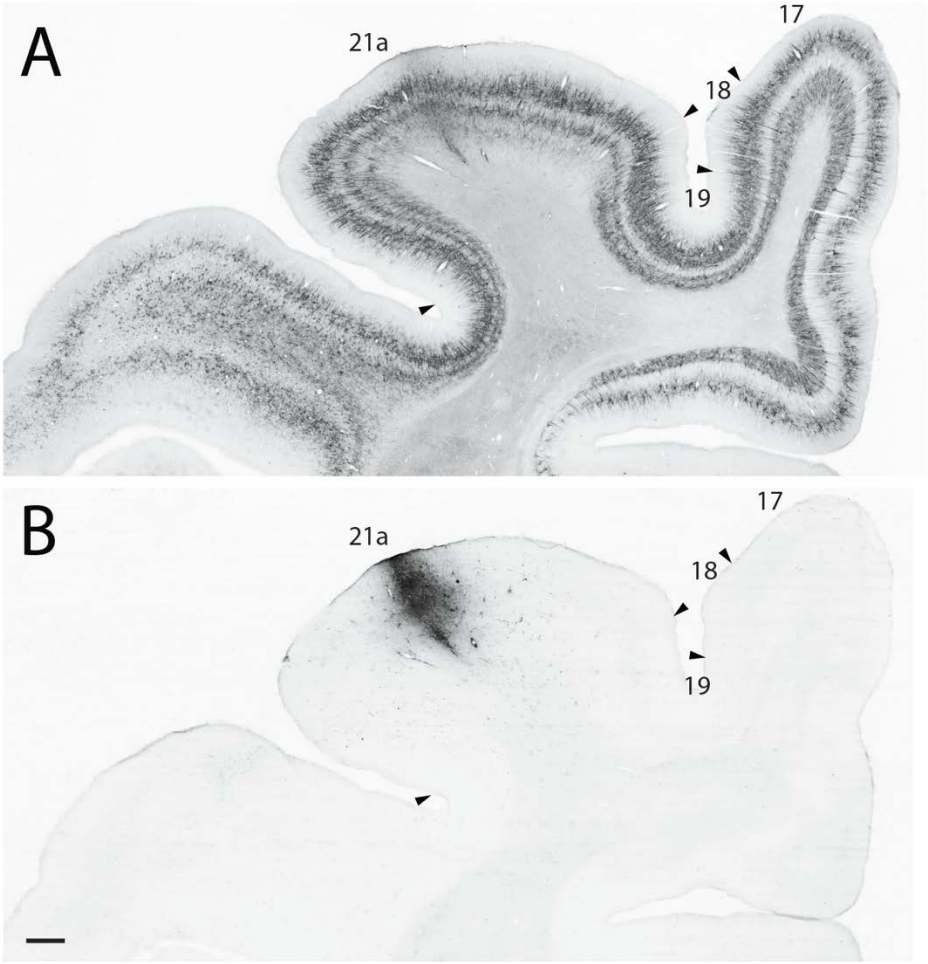
Article 2. Figure 1. Injection site for cortical area 21a
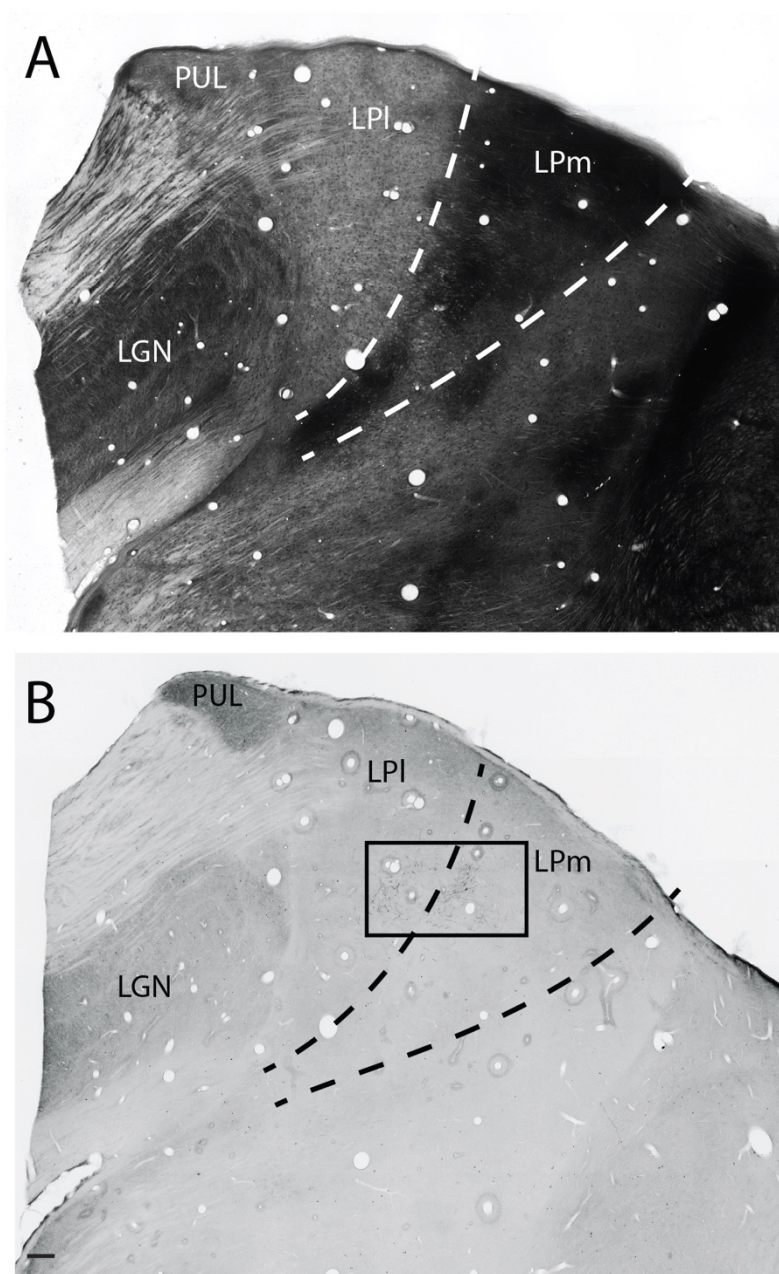
Article 2. Figure 2. Projection sites in the LP-pulvinar from CT axons of area 21a
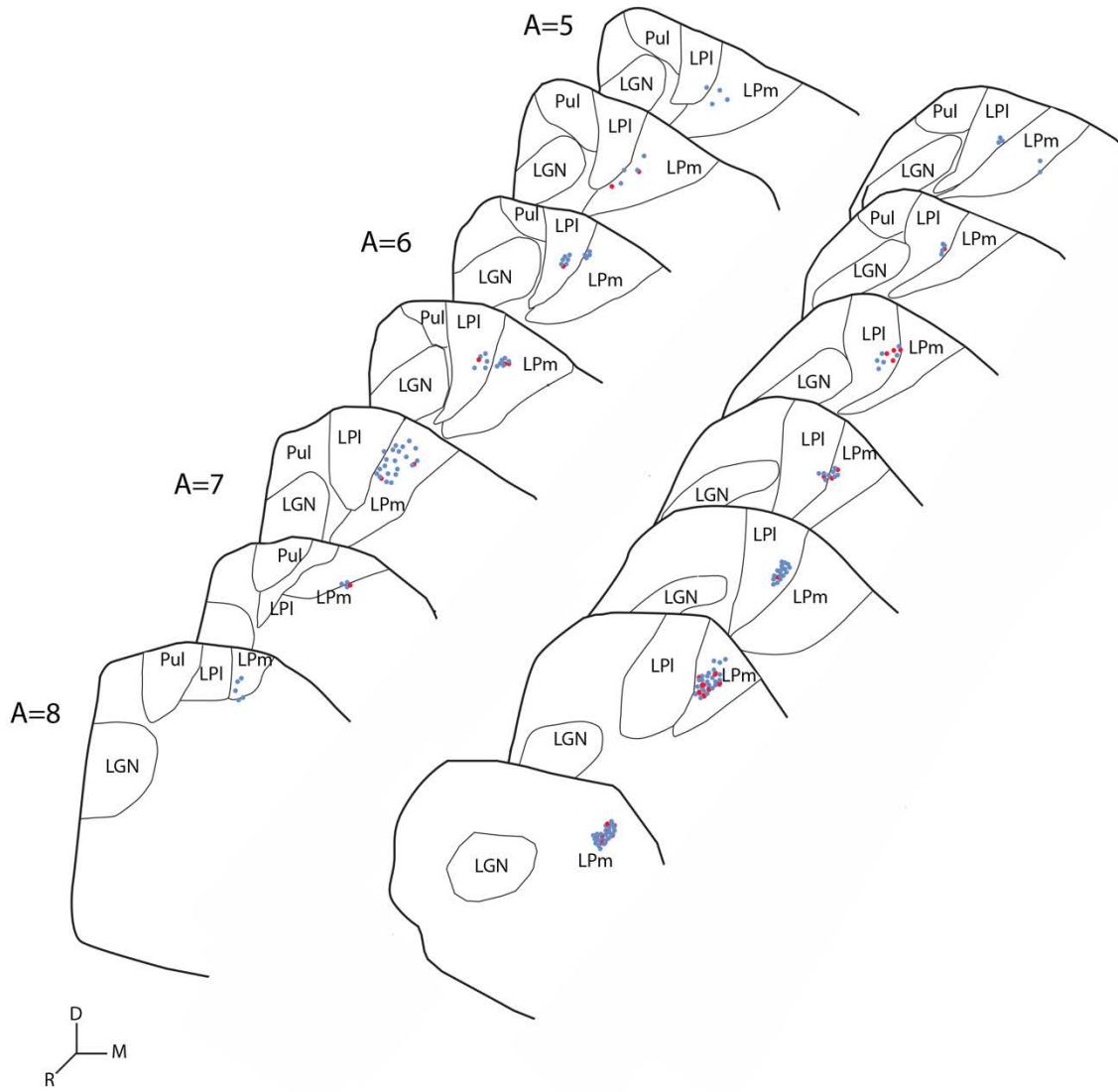
Article 2. Figure 3. Labelled terminals in the LP-pulvinar following injections in area 21a
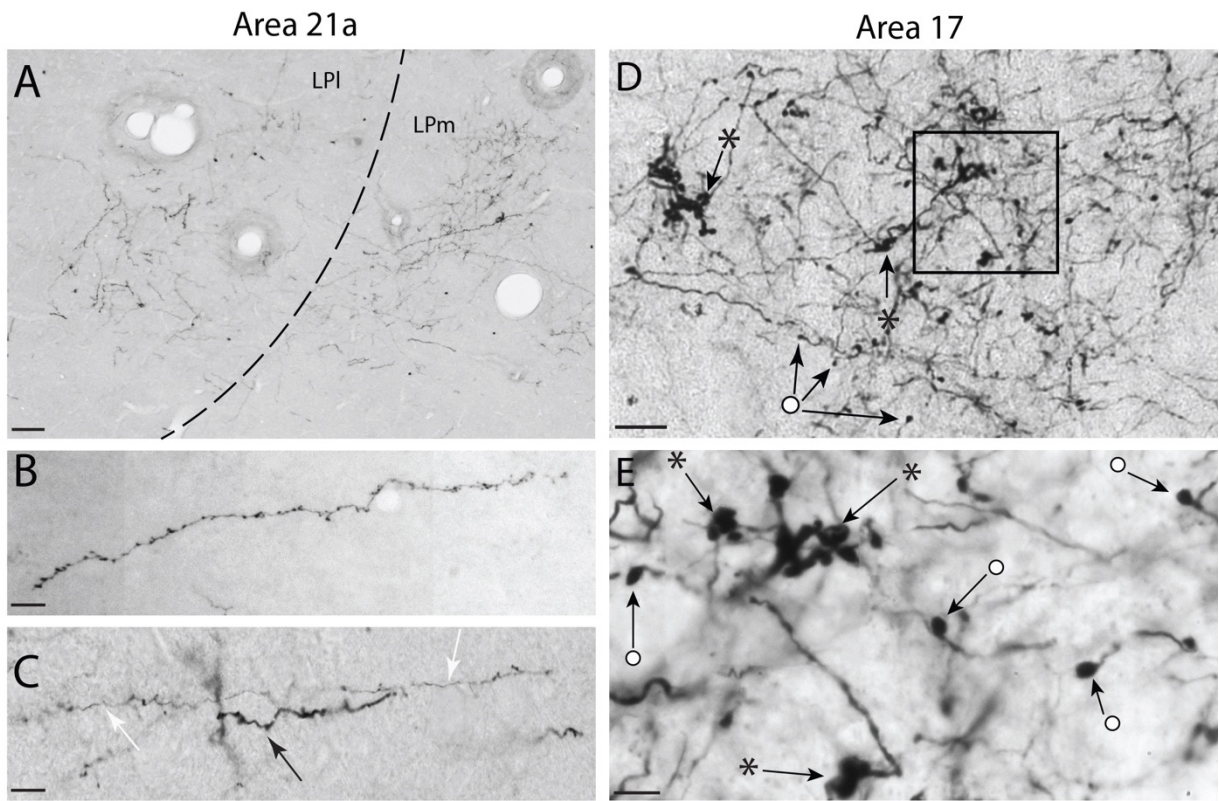
Area 21a

Area 17

Article 2. Figure 4. Terminal morphology of CT axons from area 21a and 17
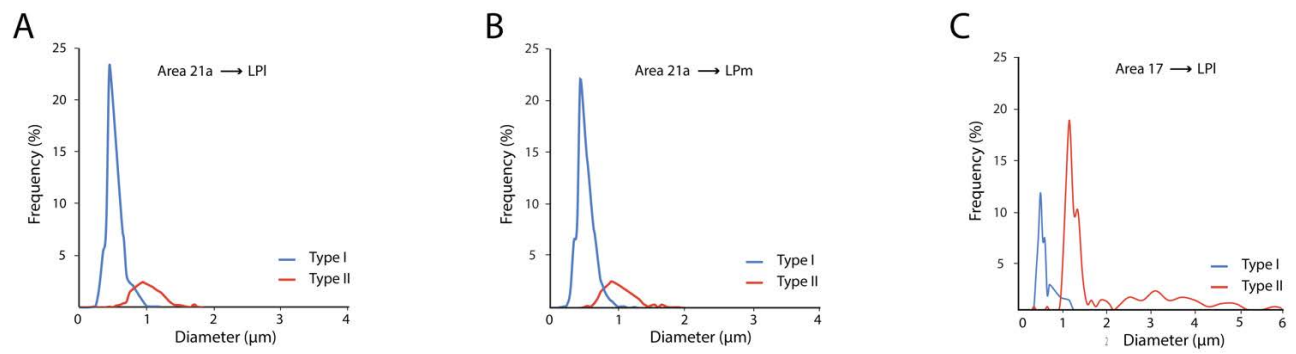
Article 2. Figure 5. Distribution of bouton size of CT projections

Area 21a
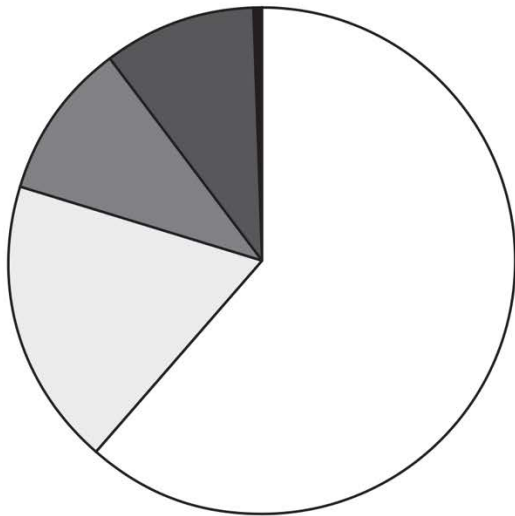
Area 17
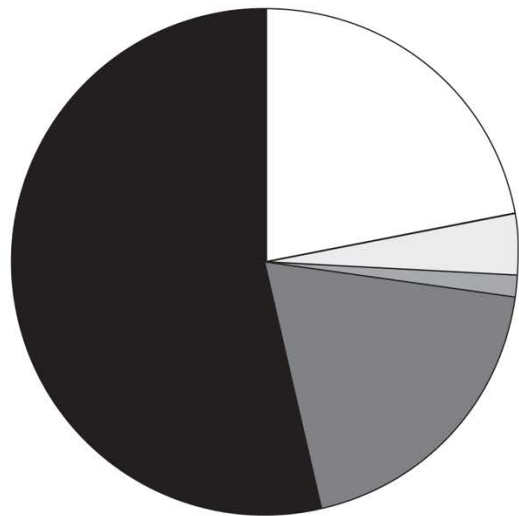
Type I

☐ Thin en Passant

☐ Thin short stalk

Type II

☐ Thick short stalk

☐ Thick en Passant

☐ Singleton or Rosette

Article 2. Figure 6. Percentage of terminal types from CT of area 17 and 21a
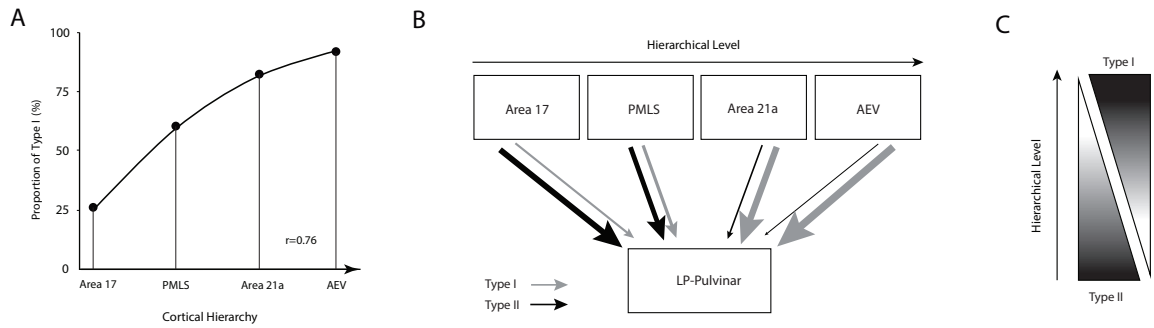
Article 2. Figure 7. The organization of CT terminals varies according as a function of cortical hierarchy

Case	Region	Number of sections	Total area (um2)	Number of disectors	Number of objects counted	Sampling fraction	Total estimation (N)	CE (N)	CE (Q)
A21a 1	LPL	15	5634351	302	798	0.007	113076	0.11	0.05
	LPM	10	4128436	209	801	0.007	122067	0.08	0.03
A21a 2	LPL	18	4975310	259	528	0.006	91347	0.10	0.05
	LPM	13	4561028	240	464	0.006	81874	0.10	0.04
A21a 3	LPL	5	1763878	100	240	0.010	22891	0.16	0.10
	LPM	6	1504564	84	157	0.010	15449	0.11	0.07
A21a 4	LPL	6	3025878	161	349	0.007	47777	0.14	0.08
	LPM	11	4086560	221	369	0.008	47717	0.15	0.09
A17	LPL	10	2044291	110	333	0.008	41372	0.05	0.02

Article 2. Table 1. Stereological sampling parameters

%	Subdivision of the LP Pulvinar	
	LPl	LPm
A21a 1		
Type I Thin en pasant	57 (452)	65 (521)
Thin short stalk	14 (115)	6 (50)
Total	71 (567)	71 (571)
type II Thick en pasant	15 (119)	16 (126)
thick short stalk	13 (107)	13 (101)
singleton/rosette	1 (5)	0 (3)
total	29 (231)	29 (230)
A21a 2		
Type I Thin en pasant	66 (349)	63 (292)
Thin short stalk	21 (112)	19 (88)
Total	87 (461)	82 (380)
type II Thick en pasant	5 (25)	8 (39)
thick short stalk	8 (41)	10 (45)
singleton/rosette	0 (1)	0 (0)
total	13 (67)	18 (84)
A21a 3		
Type I Thin en pasant	55 (131)	71 (111)
Thin short stalk	28 (67)	15 (24)
Total	83 (198)	86 (135)
type II Thick en pasant	10 (25)	11(18)
thick short stalk	7 (16)	3(4)
singleton/rosette	0 (1)	0 (0)
total	17 (42)	14 (22)
A21a 4		
Type I Thin en pasant	61 (214)	57 (212)
Thin short stalk	30 (103)	34 (124)
Total	91 (317)	91 (336)
type II Thick en pasant	2 (8)	3 (11)
thick short stalk	7 (23)	5 (19)
singleton/rosette	0 (1)	1 (3)
total	9 (32)	9 (33)
Mean A21a for All cases		
Type I Thin en pasant	60 (1146)	63 (1136)
Thin short stalk	21 (397)	16 (286)
Total	81 (1543)	79 (1422)
type II Thick en pasant	9 (177)	11 (194)
thick short stalk	10 (187)	10 (169)
singleton/rosette	0 (8)	0 (6)
total	19 (372)	21 (369)

Article 2. Table 2. Percentage of cortical terminal types

%		LPl	LPm
Area 17	Type I	25	
	type II	75	
Area PMLS	Type I	71	72
	type II	29	28
Area 21a	Type I	81	79
	type II	**19**	**21**
AEV	Type I		91
	type II		**9**

Article 2. Table 3. Percentage of cortical terminal types

3.3 Article 3 : Distributions of Vesicular Glutamate Transporters 1 and 2 in the Visual System of The Cat

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Distributions of Vesicular Glutamate Transporters 1 and 2 in the Visual System of The Cat

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Abstract

Glutamate is packaged in vesicles via two main vesicular transporter (VGLUT) proteins which regulates its storage and release in synapses of excitatory neurons. Converging studies across several species suggest that VGLUT1 and VGLUT 2, its two main isoforms which are found in most glutamatergic projections, may identify distinct subsets of excitatory projections in visual pathways. In this context, a recent proposal put forth the hypothesis that they may represent type I (modulatory) and type II (driver) projections in the visual system of primates (Balaram, Hackett et al. 2013). In order to investigate the possibility of this hypothesis being applicable to the cat visual system and how it may shed light on the function of cortico-thalamic interactions, we examined the protein distribution of VGLUT1 and VGLUT 2 in the visual thalamus, the superior colliculus and the primary visual cortex. We found that both VGLUT 1 and 2 are present in these structures, albeit with distinct expression profile suggesting that they may define distinct types of excitatory projections within the cat visual system.

Introduction

A striking feature of the mammalian brain is that it is comprised of a plethora of functionally and anatomically distinct regions, each one playing a specialized role in the analysis of external stimuli in order to allow the perception and interpretation of biologically significant patterns (Kaas 1997).

In the context of sensory systems, the prevailing view explaining how functionally distinct and relatively distant cortical areas give rise to our smooth experience of the world is that they are all interconnected and communicate synchronously with each other through an intricate and efficient micro-circuitry of corticocortical connections allowing for a rapid and synchronous transfer of relevant information within and across cortical areas (Hipp, Engel et al. 2011). In this corticocentric view, sensory signals are relayed to the cortex from the thalamus, and most if not all significant computations relevant to our perception take place therein.

While this understanding is partially correct and has greatly helped our understanding of sensory processing, it is also incomplete in at least two significant ways. First, there is a growing evidence that cortical areas do not communicate only through direct cortico-cortical connections, but also through lesser known transthalamic pathways, allowing for a cortico-thalamo-cortical transfer of information through higher order thalamic nuclei (Sherman and Guillery 2002, Sherman 2016) whose importance has been recognized by several authors in recent years (Shipp 2003, Casanova 2004, Sherman 2017).

Second, the very existence of these trans-thalamic pathways has come to question to validity of confining the bulk of neural integration, processing and interpretation of relevant stimuli to the

cortex. There is a growing evidence that these trans-thalamic loops are not just relaying information from one area to another, but that they may be actively involved in cortical processing (Saalmann and Kastner 2011, Bennett, Gale et al. 2019, de Souza, Cortes et al. 2019) and play a critical role in processes leading to visual cognition (Halassa and Kastner 2017, Rikhye, Wimmer et al. 2018).

One way towards a better understanding of these interactions between the cortex and the thalamus is to study the nature of signals they reciprocally convey. In this context, two types of connections have been described between the cortex and the thalamus relying on glutamate, the main excitatory neurotransmitter used by information bearing neurons. The type II projections are defined as drivers and are the main carrier of sensory information (driver inputs) while type I projections also known as modulators, fine-tune ongoing activity but are not critical for the establishment of receptive field properties of the target cell (Sherman and Guillery 2013).

Several functional and anatomical criteria have traditionally been used to differentiate between the two including axonal morphology: driver input exhibit thick axons, large terminal boutons, confined and complex arborization while modulatory projections exhibit longer and thinner axons, smaller boutons with very little arborization (Sherman and Guillery 2011). In the context of differentiating between type I and II projections, a recent proposal put forth the hypothesis that these two classes of connections can also be distinguished by their expression of vesicular glutamate transporters in their respective synaptic terminals (Balaram, Hackett et al. 2013). This hypothesis was based on observations that retinal ganglion cells terminating in the LGN only utilize VGLUT 2 and are devoid of VGLUT 1 (Fujiyama, Hioki et al. 2003, Bickford, Wei et al. 2008). Moreover, layer IV which is the main recipient layer of the driver geniculate projection

also exhibits strong VGLUT 2 expression (Nahmani and Erisir 2005, Familtsev, Quiggins et al. 2016). These observations prompted the hypothesis that type II (drivers) and type I (modulatory) projections may express selectively VGLUT2 and VGLUT1, respectively (Balaram, Hackett et al. 2013).

In order to investigate whether this assumption stands for other parts of the cat visual system and how it may shed light on the function of extrageniculate pathways, we examined the protein distribution of VGLUT1 and VGLUT 2 in the visual thalamus, the superior colliculus and the visual cortex. Results indicate that both VGLUT 1 and 2 are present throughout the visual system of the cat, albeit with expression profiles varying according to the region considered.

Material and Methods

Tissue acquisition

The brains of 4 pigmented cats were used in the current study. All procedures were carried out in accordance with the guidelines of the Canadian Council on Animal Care and the NIH guidelines for the care and use of laboratory animals, and were approved by the ethics committee on animal research of the Université de Montréal. Each animal received a lethal intraperitoneal dose of Sodium Pentobarbital (80 mg/kg; IP) and were perfused with phosphate buffered 0.9% saline (PBS: 0.1 M, pH 7.4) followed by phosphate buffered 4% paraformaldehyde. Brains were blocked stereotaxically, removed from the cranium, post fixed overnight in the same fixation solution at 4 °C, cryoprotected in 30% sucrose in 0.1 M phosphate buffer (pH 7.4) and frozen until processed.

Histological processing

40 µm coronal sections of the brain were acquired using a freezing microtome. Three out of every ten sections were selected and processed. Acetylcholineesterase (AchE) staining were used to distinguish layers of the superior colliculus, and to reveal the cytoarchitectonic boundaries between the lateral and medial subdivisions of the (Graybiel and Berson 1980). Other series of adjacent sections were collected for the immunohistochemical processing of vesicular glutamate transporter 1 (VGLUT1), or vesicular glutamate transporter 2 (VGLUT2), in order to identify the distribution of both proteins in synaptic terminals across the targeted visual structures. Remaining sections were cryoprotected (30% glycerol, 30 % ethylene glycol, 0.1M PBS) and stored at -20 celcius for further use.

Immunohistochemistry

Commercial antibodies used against VGLUT 1 and VGLUT2 transporter, reagents and concentrations for all immunohistochemistry preparations are listed in Table1. Protocols were adapted from similar studies (Balaram, Hackett et al. 2011, Balaram, Takahata et al. 2011, Balaram, Hackett et al. 2013, Rockoff, Balaram et al. 2014, Balaram, Isaamullah et al. 2015, Saraf, Balaram et al. 2019). Briefly, sections were rinsed three times in 0.01% Triton X-100 (Sigma, USA) in 0.01 M PBS. Endogenous peroxidase reactivity was quenched using 0.01% hydrogen peroxide in 0.01 M PBS for 30 min. Sections were rinsed again in 0.01% Triton/PBS, blocked in 5% normal serum (Jackson ImmunoResearch Labs, PA) and 0.05% Triton in 0.01 M PBS for 1 h at room temperature. Sections were then incubated overnight at 6°C with the primary antibody (see table 1 for concentrations) in a blocking solution containing 0.01 M PBS, 0.05% Triton and 5% serum. The following day, sections were rinsed three times in 0.01% Triton/PBS

to remove excess primary antibody, and then incubated in the desired secondary antibody in a blocking solution, for 1 h at room temperature. Sections were rinsed thoroughly in PBS and were incubated in an avidin-biotin-peroxidase complex (ABC; Vectastain ABC Elite kit; Vector, Burlingame, CA) with 0.05% Triton in PBS for 1 h in the dark, at room temperature. Sections were then rinsed two times in 0.01 % Triton/PBS followed by two rinses in 0.1 M Tris buffer, pH 8, to remove nonspecific binding. The stain was then visualized by reacting the tissue in 0.035 % 3.3′-diaminobenzidine Tetrahydrochloride (DAB) with 0.5 % nickel ammonium sulfate in 0.1 M Tris buffer for 10 min, followed by incubation in DAB-Nickel solution containing 0.002% H2O2. Sections were mounted on gelatin-subbed slides, dehydrated, and coverslipped with Depex (Electron Microscopy Sciences, PA).

Light microscopy and Image analysis

Photomicrograph of mounted sections were captured using a microscope (DMR, Leica) equipped with a three-axis computer-controlled stepping motor system coupled to a personal computer and to a color Optronix CCD camera and driven by the Neurolucida software (MBF Biosciences, Williston, VT, USA). Images were cropped and adjusted for brightness and contrast.

Results

The protein distribution of VGLUT 1 and 2 varied significantly amongst layers of the visual cortex, within nuclei of the visual thalamus and layers of the superior colliculus, reflecting the cortical and subcortical nature of their respective projections.

The Visual Thalamus

Here, the visual thalamic nuclei considered consisted of two main structures, namely the LGN, and the lateral posterior nucleus (LP)-pulvinar complex. Their location can be seen in Figure 1, where an AChE stained coronal section which comprises the LGN, visual cortical areas, and the LP-pulvinar complex that is lying medially next to the LGN. The two subdivisions of the LP, the lateral (LPl) and medial (LPm) nuclei can be recognized based on their distinct AChE staining pattern.

VGLUT 1 and 2 expression in the dorsal Lateral Geniculate Nucleus (LGN)

The LGN of the cat is composed of three main cell densed layers namely A, A1 and C, each one receiving its input from a specific cell type and being separated by cell sparse interlaminar spaces. Retinal ganglion cells Y and X cells predominantly target layer A and A1, whereas W cells are mostly found in layer C close to the optic tract (Rodieck 1979, Sur, Esguerra et al. 1987, Casagrande 1991). As can be seen in Fig 2B, we found that VGLUT1 was densely expressed throughout the LGN, albeit in an evenly and diffuse manner. VGLUT1 was expressed in all layers of the LGN and was also present in the interlaminar spaces. Careful examination of all slides did not reveal any strong differences in the staining intensity of VGULT 1 across all layers of the LGN.

The expression of VGLUT 2 differed notably in the LGN. As can be seen in Fig 2C, VGLUT 2 was densely expressed in geniculate layers A, A1 and C but was mainly absent from interlaminar spaces. The intensity of the expression of VGLUT 2 seemed to differ across layers as it was more densely expressed in layers A and A1 known to receive projections from X and Y cells than in layer C which mostly receive W cell inputs.

VGLUT1 and 2 expression in the LP-Pulvinar Complex

The pulvinar complex of the cat is comprised of two main nuclei, the lateral posterior (LP), itself divided in two subdivisions, the LPm and LPl and the pulvinar proper. A section of the visual thalamus strained for AChE can be seen in Fig 3A where all three nuclei can be seen next to the LGN.

Like its expression pattern in the LGN, the overall expression of VGLUT 1 in the LP-Pulvinar complex was evenly and diffusely distributed across the pulvinar, the LPl and the LPm (Fig 3B). The bottom panel of Fig 3B shows a higher magnification photomicrograph of a section stained for VGLUT 1 containing the three subdivisions of the LP-pulvinar complex. A diffuse signal was observed in all subdivisions with very little variability in intensity.

A different picture emerged when considering the expression of VLGUT 2. Contrary to the widespread and diffuse expression of VGLUT 1, VGLUT 2 staining showed a distinct staining pattern in all three nuclei of the LP-pulvinar complex. As can be seen in Fig 3C, the most intensely observed VGLUT2+ areas in the LP-pulvinar complex were located in the retino-recipient pulvinar proper. In contrast to the pulvinar, the striate-recipient zone of the LP, the LPl,

showed a lower expression of VGLUT 2 with only isolated foci of darker VGLUT 2+ patches amongst larger zones of weaker overall expression, especially when compared with intensities observed in the Pul and the LGN. This is in contrast with the medial part of the LP (LPm) whose expression pattern of VGLUT 2 showed a larger zone of darker staining, especially in its most medial part (white arrows).

VGLUT 1 and 2 expression in the Superior Colliculus (SC)

The superior colliculus is organised in a laminar fashion, each seven layers exhibiting a specific connectivity pattern and cellular type. Boundaries of its layers can be recognised based on the expression of acetylcholinesterase (McHaffie, Beninato et al. 1991).

VGLUT 1 and 2 expression in the superior colliculus showed distinct patterns. VGLUT 1 was densely expressed in superficial layers, especially in the superior part of the SGS and more moderately expressed in the SO and SGI. Indeed, a dense band of VGLUT 1 positive cells can be seen starting at the ventral edge of the SZ and extending to the ventral edge of the SGS (Fig 4B). The upper SGS stained densely for VGLUT1, whereas the lower part of the SGS was less stained. VGLUT 1 staining was fainter in the SO compared with the SGS. Most VGLUT 1 + cells were distributed in patches and located dorsally, at the border with the SGS while the more ventral part of the SO showed a moderate and diffuse staining which closely matched the faint staining patterns observed in the SGI and SGP. Overall, VGLUT 1 expression was widespread throughout the SGS from its lateral to medial edge, with fainter intensity in intermediate layers. .

On the other hand, while VGLUT 2 was also expressed in visual layers of the SC (Fig 4C), its expression pattern differed from that of VGLUT1. VGLUT2 was most intensely expressed in the SZ where strong immunoreactivity can be seen from its medial to lateral edge. The SGS also showed significant staining but with lower intensities than the SZ. The SO and SGI showed very little to no staining for VGLUT 2.

VGLUT 1 and 2 expression in the primary visual cortex

Area 17 of the cat constitutes the first cortical area of the visual cortex and the major recipient of geniculate projections which mostly synapse in layer IV for X and Y axons of LGN layer A and A1, while W cells from LGN layer C mostly terminate in layer I and III. Area 17 also receives thalamic inputs from the LPl which mostly terminate in layer I (Abramson 1985).

Like in the visual thalamus and midbrain, VGLUT 1 and 2 were seen to exhibit distinct expression patterns in the visual cortex. VGLUT1 protein was diffusely expressed in nearly all layers of area 17, with areas of slightly darker labeling in layer I and layer VI (Fig. 5). Geniculo-recipient layer IV was the most lightly stained for VGLUT 1 as can be seen in Fig 5Ai (white arrow). The remaining layers II, III and V showed a similar diffuse and abundant VGLUT1 labeling and no differences could be observed within and between these layers.

On the other hand, VGLUT2 protein distribution was mostly restricted to specific layers and varied remarkably compared to the evenly and diffuse VGLUT1 expression. Indeed VGLUT 2 was most intensely expressed throughout the geniculo-recipient layer IV and the lower part of layer III (figure 5Bi and 5Bii). VGLUT 2 staining in layer IV appeared as a densely stained band

whose staining was significantly more intense than any other area 17 layers. Besides layer IV, a thin band of densely expressed VGLUT 2 patches were also expressed in layer I, also known to receive geniculate projections from the W pathway, and layer VI which receives collaterals from X and Y geniculate cells terminating in layer IV.

Discussion

The present study examined the distribution patterns of VGLUT1 and VGLUT2 in the visual thalamus, the superior colliculus and primary visual cortex of the cat. We found that the expression pattern of VGLUT 1 and 2 varied significantly across structures of the visual pathways. Overall, VGLUT 1 was seen to be diffusely expressed in the LGN, the LP-Pulvinar complex, visual layers of the SC and across all layers of area 17. On the other hand, VGLUT 2 was mostly expressed in retino-recipient structures of the thalamus (the LGN and the pulvinar), visual layers of the SC, the tecto-recipient LPm and geniculo-recipient layer IV of the cortex. Overall, the complementary distribution pattern of VGLUT 1 and 2 suggests that may identify distinct types of projections within ascending visual pathways.

VGLUT 1 and 2 show a complementary expression pattern

In our study, VGLUT2 was seen to be densely expressed in all retinorecipient structures of the cat visual system (Figure 6). VGLUT 2 was seen to be expressed in superficial layers of the SC (SZ and upper SGS) and all layers of the LGN which are the most prominent projecting areas

for ganglion cells, suggesting that RGC's projecting to both structures mainly utilize VGLUT 2, an observation which is in line with tracing studies which reveal that RGC's projecting to the LGN and the SC express VGLUT 2 while exhibiting no immunolabelling for VGLUT 1 (Fujiyama, Hioki et al. 2003). An interesting observation following the staining of VGLUT 2 in the LGN of the cat is that its expression was more prominent in layer A and A1 known to be major recipients of medium X cells and large Y cells (Sur, Esguerra et al. 1987), while W cells mostly synapse in layer C. While these results in the LGN may suggest that VGLUT 2 is mostly expressed in X and Y channels, further tracing studies would be required to link VGLUT 2 expression with known functional RGC types, as layer C is also known to receive a subset of contralaterally projecting Y cells and W cells also project to superficial layers of the SC which show strong VGLUT 2 immunoreactivity. Interestingly, similar observations have been reported in other animal models: for instance, in the primate and tree shrew LGN which are also known to be organised in a laminar manner (Magnocellular, Parvocellular and Koniocellular of the primate correspond to X, Y and W cells of the cat respectively) VGLUT 2 staining was shown to be more prominent in magnocellular and parvocellular recipient than in koniocellular layers (or inter laminar zones) (Balaram, Takahata et al. 2011, Balaram, Hackett et al. 2013, Balaram, Isaamullah et al. 2015, Baldwin and Krubitzer 2018, Saraf, Balaram et al. 2019). Thus, our results are in line with a general observation that holds true across several animal models that, W or Koniocellular recipient layers of the LGN express VGLUT 2 with lower intensities than Magnocellular and Parvocellular ones.

Contrary to VGLUT 2, VGLUT 1 showed a remarkably diffuse and homogenous staining across all layer and interlayers of the LGN such that the laminar organization of the LGN could not be recognised with VGLUT1 staining only. VGLUT 1 expression pattern in the cat reported in this

study resembled very closely known expression of VGLUT 1 in the macaque LGN, tree shrew and Galago where it is expressed diffusely across all layer and interlaminar areas (Balaram, Hackett et al. 2011, Hackett, Takahata et al. 2011, Balaram, Isaamullah et al. 2015).

VGLUT 1 and 2 expression pattern in the LGN closely matched terminating patterns of its cortical and retinal inputs. : RGC projecting cells in the LGN are known to bear type II driver like projections, with confined terminal arbors bearing remarkably precise laminar segregation which highlights their sharp retinotopy (Sur, Esguerra et al. 1987). On the contrary, known modulatory cortico-geniculate projections have been shown to have a significantly widespread arborization (Sherman, Guillery et al. 2006) with single axons having terminating arbors and boutons in multiple laminae and in interlaminar spaces (Updyke 1975, Murphy, Duckett et al. 2000). Similarly, we found that VGLUT 2 is mainly expressed in the LGN in a segregated and laminar fashion while being absent from interlaminar spaces. On the other hand, we found VLGUT 1 to be diffusely expressed across layers and interlaminar spaces.

The other major retino-recipient structure of the mammalian visual system is the superior colliculus whose superficial layers receive significant visual inputs from RGC's and visual cortical areas with some differences in their terminating sites: corticotectal projections target all layers of the SC including intermediate and deep layers but mostly terminate in the SGS, the SO and the SGI, while retinal projections mostly target more dorsal part of the SC's superficial layers, namely the SZ, the SGS and to a lesser degree, the SO (Harting, Huerta et al. 1991, Harting, Updyke et al. 1992, May 2006, Huppe-Gourgues, Abbas Farishta et al. 2019) . In the SC, we found that both VGLUT 1 and 2 were expressed, albeit with distinct expression patterns. VGLUT 1 was seen to be diffusely and evenly expressed throughout cortico-recipient layers: the

upper SGS, with moderate staining in the SO and SGI; while VGLUT 2 was most intensely expressed in the retinorecipient and dorsal most SZ layer, evenly expressed in the SGS, and faintly expressed or absent in the SO and SGI. The presence of VGLUT 1 and 2 in the SC are in line with previous studies which have reported that the vast majority of retinotectal and corticotectal cells are glutaminergic (Binns and Salt 1996, Mize and Butler 1996), an observation which is also in line with the presence of both vesicular transporters in primates, rodents and tree shrews (Kaneko and Fujiyama 2002, Fujiyama, Hioki et al. 2003, Balaram, Takahata et al. 2011, Balaram, Hackett et al. 2013, Balaram, Isaamullah et al. 2015).

Like in the LGN and the SC, VGLUT 1 and 2 were also seen to have distinct expression patterns in the LP-pulvinar complex, likely reflecting the cortical and subcortical nature of its afferent projections. The visual cortex is one of the main afferents to the LP-pulvinar complex (Casanova 2004). While, most visual areas project to both the LPl and LPm, only the LPl receives projections from area 17 (Abramson and Chalupa 1985, Guillery, Feig et al. 2001, Huppe-Gourgues, Abbas Farishta et al. 2019, Abbas Farishta, Boire et al. 2020). The LP-pulvinar complex receives significant subcortical projections from the SC, which mainly terminate in the LPm (Kelly, Li et al. 2003), and direct retinal projections to the pulvinar proper (Boire, Matteau et al. 2004) which also receives abundant input from the pretectum (Baldauf, Wang et al. 2005). Across the LP-pulvinar complex, VGLUT 1 was seen to be diffusely expressed in all nuclei with very little variation in the intensity of its expression, likely reflecting its diffuse cortical projections. On the other hand, VGLUT 2 was more predominantly present in the pulvinar proper, likely reflecting its ascending RGC projections, which are known to rely on VGLUT 2 (Fujiyama, Hioki et al. 2003). The tecto recipient LPm was also shown to show significant expression of VGLUT 2, results which are in line with known VGLUT2- positive tectopulvinar

projections previously identified in tree shrews, rodents and primates (Hisano, Hoshi et al. 2000, Chomsung, Petry et al. 2008, Masterson, Li et al. 2009, Balaram, Takahata et al. 2011, Wei, Masterson et al. 2011, Baldwin, Balaram et al. 2013).

Along with visual subcortical structures, we also investigated the expression pattern of VGLUT 1 and 2 in area 17 of the cat. Area 17 receives its main driving input from geniculate projections with the majority of X and Y cells synapsing in layer IV and collaterals in layer III and VI, and W cells terminating in layer I and III (Boyd and Matsubara 1996). This projection pattern correlated with the VGLUT 2 activity we observed in area 17 with layer IV showing the densest and most prominent staining while fainter staining in layers I, III and VI was also observed. These results are in line with previous studies which identified VGLUT 2 as a marker of thalamocortical projections in the cat (Nahmani and Erisir 2005). Area 17 also maintains a complex architecture of intrinsic connectivity with layer IV cells sending projections to layer II and V and layer III sending projections to layer I and VI (Boyd and Matsubara 1996). Most layers outside layer IV also receive significant feedback projections from higher order extra-striate areas, suggesting that all layers of area 17 receive either intracortical or corticocortical projections. The expression pattern of VGLUT 1 in our study is very much in line with the above-mentioned known connectivity pattern of area 17 as VGLUT 1 was seen to be diffusely expressed in all layers of the striate cortex in a homogenous manner. The only layers which showed a lighter stain of VGLUT 1 activity was in fact layer IV, known to be primarily driven by subcortical geniculate projections utilizing VGLUT 2 (Nahmani and Erisir 2005).

Thus, taken together, the overall expression pattern of VGLUT 1 and 2 in the visual thalamus, the SC and area 17 reveals that both glutamate vesicular transporter are expressed in the visual

system of the cat but with distinct expression patterns. Our observations in the primary visual cortex and visual subcortical structures suggest that VGLUT 2 is mainly utilized in ascending retinofugal and geniculo-striate pathways, while corticofugal, corticocortical and intra-cortical connectivity rely more largely on VGLUT 1.

Functional significance of VGLUT's in the visual system

Our results which report distinct projections patterns for VGLUT 1 and 2 suggest that they may identify distinct types of glutamatergic projections. Similar observations in the mammalian brain of various species where VGLUT 1 and 2 were seen to be expressed in distinct sites lead to the hypothesis that they may identify distinct functional classes of projections (Herzog, Bellenchi et al. 2001, Varoqui, Schafer et al. 2002, Fremeau, Voglmaier et al. 2004) while some studies (Rovo, Ulbert et al. 2012, Balaram, Hackett et al. 2013) have hypothesised that they may identify modulatory and driving projections respectively (Sherman and Guillery 1998)

This hypothesis seems to be most valid in ascending visual pathway as retinal projections to the lateral geniculate nucleus and superior colliculus, which are all considered as driving projections (Sherman and Guillery 2002, Sherman, Guillery et al. 2006) rely on VGLUT2 (Fujiyama, Hioki et al. 2003, Land, Kyonka et al. 2004, Graziano, Liu et al. 2008, Hackett, Takahata et al. 2011). Moreover, projections from the LGN to V1 which are also considered as drivers mainly utilize VGLUT 2 (Kaneko and Fujiyama 2002, Nahmani and Erisir 2005, Wong and Kaas 2010, Balaram, Hackett et al. 2013, Balaram, Isaamullah et al. 2015). Conversely, projections from the

primary visual cortex to the LGN, which are functionally known to be modulatory, originate from layer VI which expresses VGLUT 1 but no VGLUT 2 mRNA (Ni et al., 1994, 1995; Hisano et al., 2000; Fremeau et al., 2001; Varoqui et al., 2002), suggesting that cortico-geniculate projections rely on VGLUT 1. Thus, in the retino-geniculo-striate and cortico-geniculate pathways, VGLUT 1 and 2 seem to identify modulatory and driver projections respectively.

Several observations call for more nuance in the strict association of VGLUT's with driver and modulator projections. First, the LGN of primates, rodents and tree shrews were found to express low levels of VGLUT 1 mRNA (Fujiyama, Furuta et al. 2001, Balaram, Hackett et al. 2011, Balaram, Hackett et al. 2013, Balaram, Isaamullah et al. 2015). These neurons may project intrinsically, but they may also represent a subset of geniculate projections which utilize VGLUT 1 and contribute to its diffuse expression in geniculo-recipient V1 layers (Balaram, Isaamullah et al. 2015). Moreover, the presence of driver and modulatory projections has also been described in extra-geniculate pathways involving the pulvinar (Sherman, Guillery et al. 2006) : in cats, three shews, and primates, driver projections from V1 to the pulvinar originate from layer V while modulatory ones originate from layer VI (Rockland 1996, Usrey and Fitzpatrick 1996, Day-Brown, Slusarczyk et al. 2017, Huppe-Gourgues, Abbas Farishta et al. 2019, Abbas Farishta, Boire et al. 2020). However, both layer V and VI of tree shrews and primate are mostly devoid of VGLUT 2 mRNA (Balaram, Hackett et al. 2013, Balaram, Isaamullah et al. 2015) suggesting that driver and modulatory cortico-pulvinar projections may utilise VGLUT 1, which further blurs the use of VGLUT 1 and 2 as markers of driver and modulatory projections in corticopulvinar pathways. Combined tracing and immunohistochemical studies of VGLUT's providing direct evidence of a possible association between terminal types and their bias for a given VGLUT have been few. Of interest is a study done in primates (Rovo, Ulbert et al. 2012)

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which described large 'driver' corticothalamic input to the pulvinar expressing VGLUT 1. Similarly, in galagoes, based on the expression of VGLUT 1 mRNA in V1's infragranular layers, the expression of VGLUT 1 and absence of VGLUT 2 in its pulvinar subdivisions (Balaram, Hackett et al. 2011, Balaram, Takahata et al. 2011) presumed driver cortico-pulvinar projection from layer V of V1 most likely utilize VGLUT 1. However, in the same species, pulvinar projections to V2, also described as driver projections, utilize VGLUT 2 (Marion, Li et al. 2013). Thus, results in prosimian galagos where driver cortico-pulvinar and driver pulvino-cortical projections may utilise VGLUT 1 and 2 respectively suggest that, within transthalamic pathways, there does not seem to be a strict correlation between VGLUT's and driver and modulatory projections.

Conclusion

In our study, we report the presence of VGLUT 1 and 2 throughout the visual thalamus, the SC and the visual cortex of the cat. VGLUT 1 and 2 exhibit different expression patterns, suggesting that they may be selectively expressed in functionally distinct classes of glutamatergic projections. Our results, along with those reported in other species suggests that VGLUT1 and VGLUT2 terminals originate from cortical and subcortical sources respectively which correlates largely with "modulators" and "drivers" in ascending visual projections but not in extra-geniculate transthalamic pathways.

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<u>Legends</u>

Table 1 : List and concentration of antibodies used for VGLUT 1 and 2

Figure 1 : A: Photomicrograph showing the relative position of nuclei forming the visual thalamus of the cat through a coronal section stained for AChE at the level of the lateral geniculate nucleus and the LP-Pulvinar complex. Ai: A magnified photomicrograph of the boxed area in panel (A) where the borders between the darkly stained LPm and the lightly stained LPl can be seen, along with the LGN and the Pul. Scale 1mm for panel A and 500 μm for panel Ai.

Figure 2: A, B and C: Adjacent coronal section showing the laminar organization of the LGN stained for AChE (A), VGLUT 1 (B) and VGLUT (2). The cat LGN is composed of four main layers, A, A, C and C1. Scale 1mm.

Figure 3: A, B and C: Low-power and high-power photomicrograph (from the boxed area) of adjacent coronal section showing thalamic nuclei forming the visual thalamus stained for AChE, VGLUT 1 and VGLUT 2 respectively Scale 1 mm and 250 μm for low- and high-power photomicrograph respectively.

Figure 4: A, B, C : Low-power and high-power photomicrograph of adjacent coronal section showing the laminar organization of the cat superior colliculus lying over the periaqueductal gray (PAG) with the ventral most layer SZ (stratum zonale) followed by the more dorsal SGS (stratum griseum superficiale), the SO (stratum opticum) and the SGP (stratum griseum

profundum) stained for acetylcholinesterase, VGLUT 1 and VGLUT 2 respectively. Scale bar 500 μm and 100 μm for low- and high-power photomicrograph respectively.

Figure 5: A, B, Ai, Bi: Low-power and high-power photomicrograph of adjacent coronal section at the level of the visual cortex stained for VGLUT 1 and 2 respectively showing the relative position of area 17 and 18. Aii and Bii : Magnified photomicrograph of the boxed area in panel Ai and Bi centered on the primary visual cortex. Scale bar: A, Ai, B, Bi: 500 μm; Aii, Bii : 200 μm.

Figure 6: Summary of VGLUT 1 and 2 expression profile in the visual thalamus, superior colliculus, pretectum (Pt) and area 17 along with representation of major known modulatory projections and driver projections.
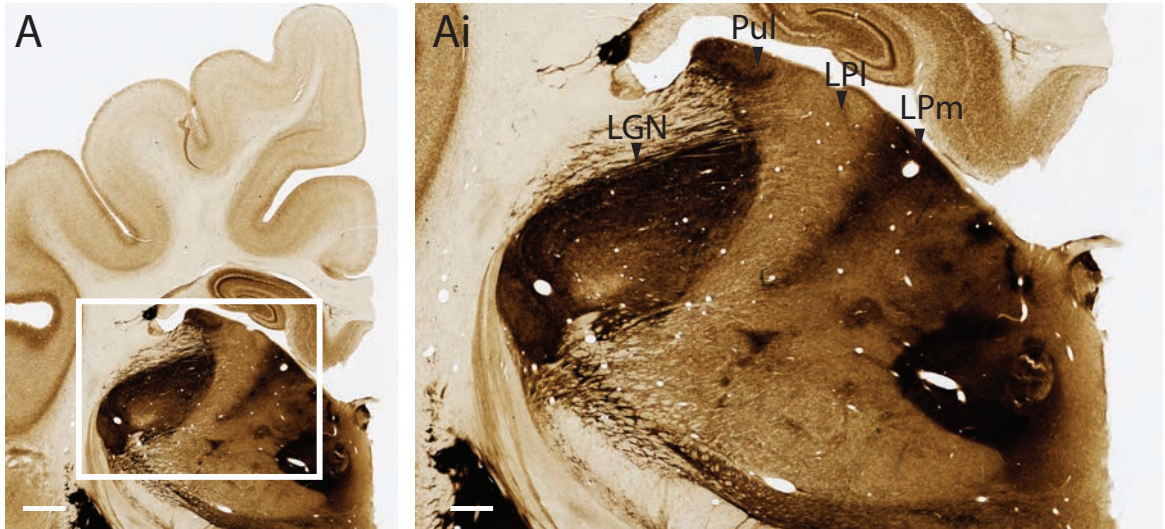
Article 3. Figure 1. Visualization of the visual thalamus of the cat
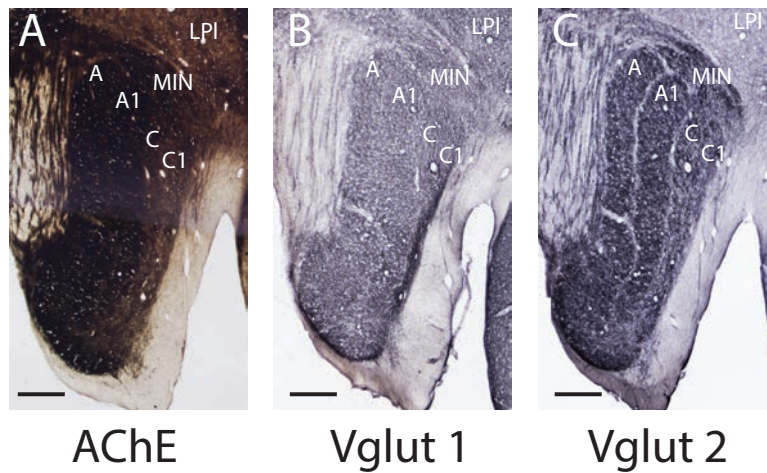
Article 3. Figure 2. Expression of VGLUT 1 and 2 in the LGN
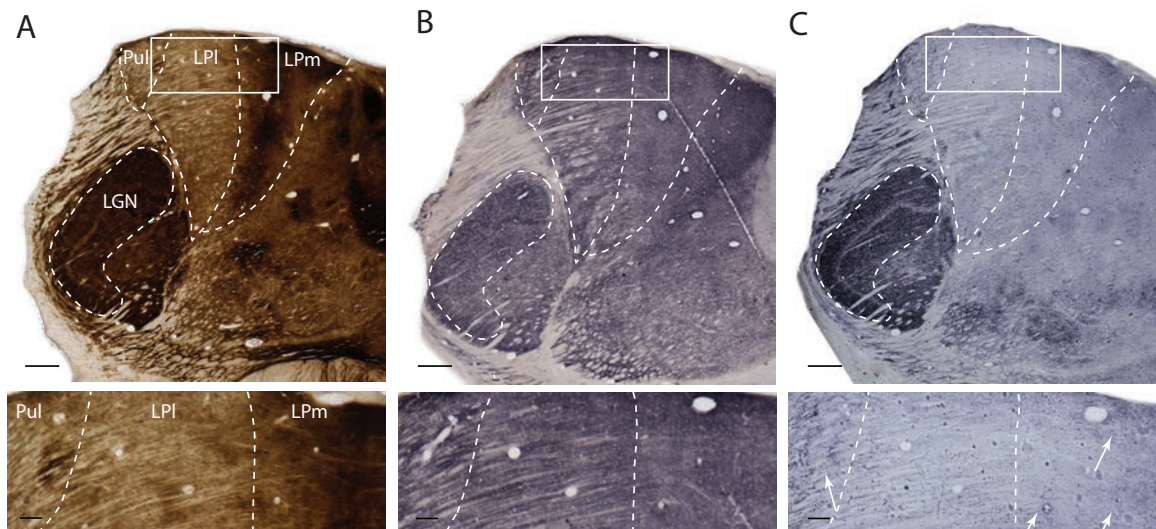
Article 3. Figure 3. Expression of VGLUT 1 and 2 in the LP-pulvinar
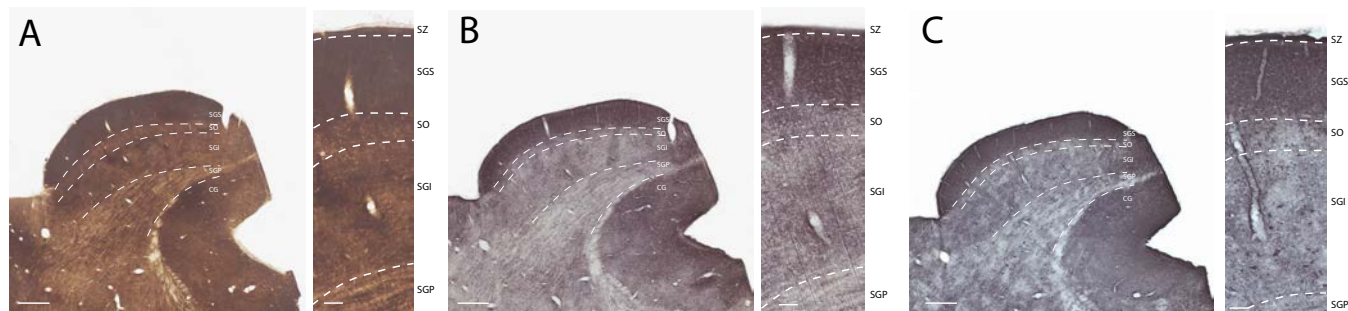
Article 3. Figure 4. Expression of VGLUT 1 and 2 in the superior colliculus
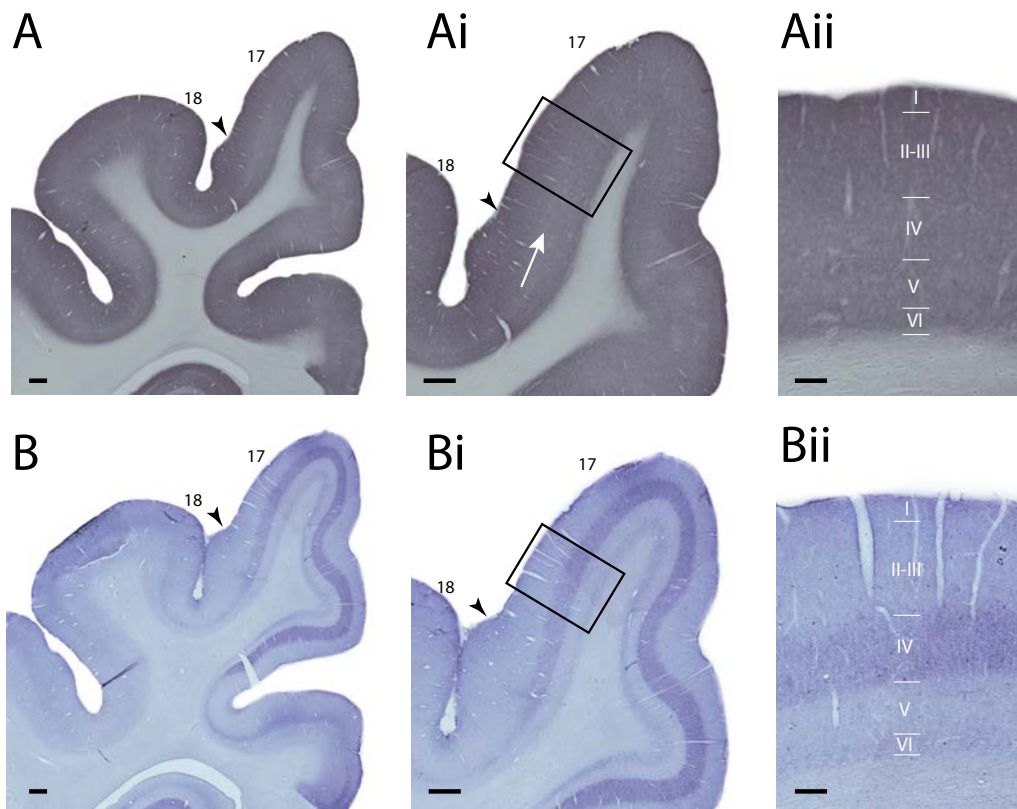
Article 3. Figure 5. Expression of VGLUT 1 and 2 in area 17
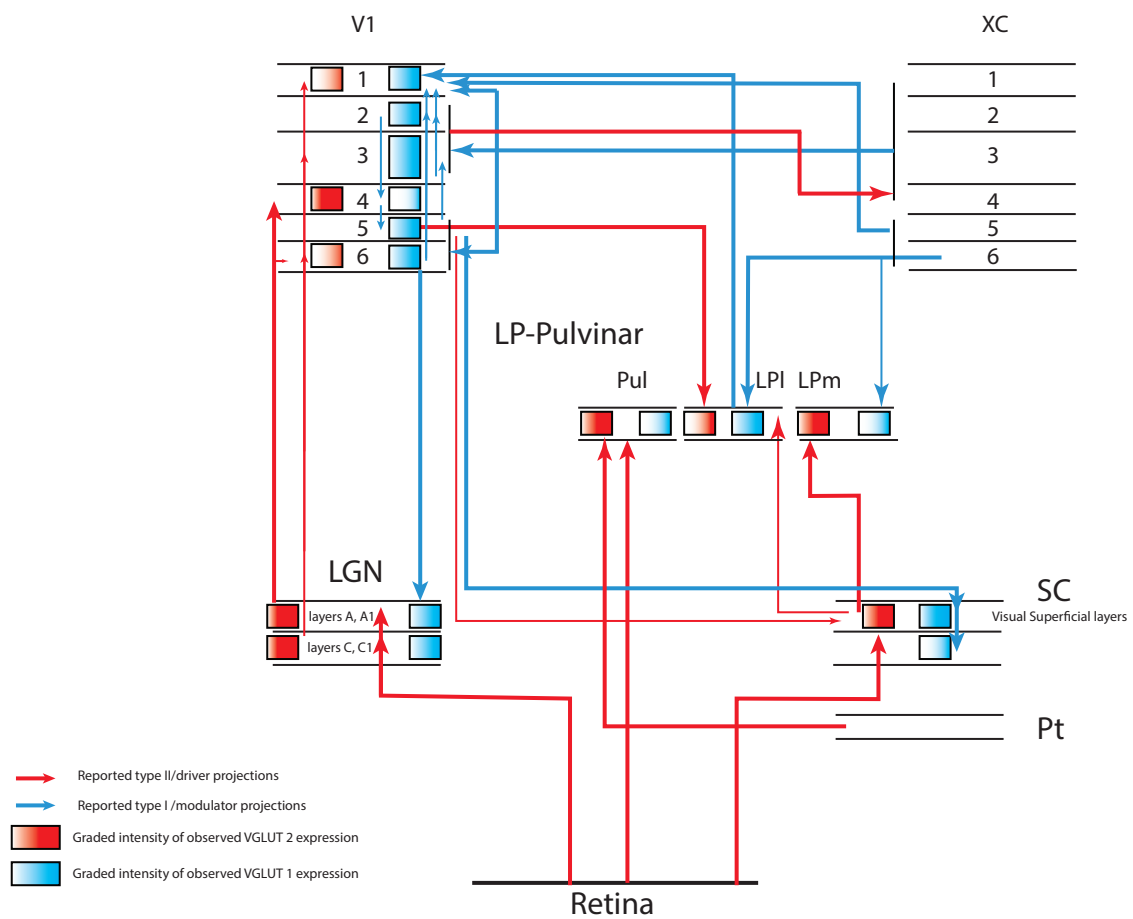
Article 3. Figure 6. Summary of VGLUT 1 and 2 expression in the visual system of the cat

Antigen	Primary Antibody	Secondary Antibody	Normal Serum
Vesicular glutamate transporter (VGLUT1)	Polyclonal antibody produced in rabbit (Synaptic Systems) Catalogue # 135 303 Dilution factor : 1:3000	Biotin-SP-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) (Jackson ImmunoResearch Labs) Dilution factor : 1:500	Normal goat serum (Jackson ImmunoResearch Labs)
VGLUT1	Polyclonal antibody produced in mouse (Biolegend) Catalogue # MMS-5245 Dilution factor: 1:3000	Biotin-SP-conjugated AffiniPure Goat Anti-Mouse IgG (H+L) (Jackson ImmunoResearch Labs) Dilution factor: 1:500	Normal goat serum (Jackson ImmunoResearch Labs)
VGLUT2	Polyclonal antibody produced in rabbit (Synaptic Systems) Catalogue # 135 402 Dilution factor: 1:5000	Biotin-SP-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) (Jackson ImmunoResearch Labs) Dilution factor: 1:500	Normal goat serum (Jackson ImmunoResearch Labs)
VGLUT2	Polyclonal antibody produced in mouse (Biolegend) Catalogue # MMS-5206 Dilution factor: 1:5000	Biotin-SP-conjugated AffiniPure Goat Anti-Mouse IgG (H+L) (Jackson ImmunoResearch Labs) Dilution factor: 1:500	Normal goat serum (Jackson ImmunoResearch Labs)

Article 3. Table 1. List of antibodies used for VGLUT 1 and 2 and their concentration

6. Discussion

6.1 Summary of results

The initial goal of this thesis was to investigate the functional neuroanatomical pathways involving the visual cortex and the pulvinar. The specific aims were to study the morphology of CT projection from the AEV cortex (1) and from area 21a (2), to investigate the possibility of a hierarchical organization of CT inputs to the pulvinar (3), and to study the expression pattern of VGLUT 1 and 2 projections and their relationship with the 'driver/modulator' theory of glutamatergic pathways (4). Our initial hypothesis was that CT projection from the AEV and area 21a would be mainly modulatory in nature (aim 1 and 2); We also expected the presence of a hierarchical organization of CT inputs in the pulvinar (aim 3); according to our hypothesis, we also expected VGLUT 1 and 2 to be selectively to show complementary expression patterns along ascending visual pathways. Our results have confirmed all our hypothesis and have given us a clearer understanding of the possible functional relationship between the cortex and the pulvinar, based on their respective anatomical organization.

In our first paper (Article 1), our main findings show that the vast majority of CT projections from the AEV to the LP-pulvinar complex consist of type I modulatory projections, whose proportion significantly outnumbers type I proportion seen in the PMLS and area 17. These results suggest that neurons from the AEV are more likely to modulate response properties in the thalamus rather than to determine basic organization of receptive fields of thalamic cells. Retrograde tracer injections in the LPm also revealed that almost all CT neurons from the AEV projecting to the LP-pulvinar were located in layer VI, further strengthening the association between layer VI - type I and layer 5 - type II projections in the thalamus (Rouiller and Welker

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2000). The proportion of type I projections was the highest in the AEV and lowest in area 17 while the PMLS exhibited a mixed proportion of type I and type II projections suggesting that striate and extrastriate areas may contribute differently to the visual responsiveness of pulvinar neurons.

In our second paper (Article 2), we investigated the morphology of CT projections from area 21a, an XC area located in the ventral stream. Area 21a is considered to be situated at a higher position than the PMLS in the hierarchy of the cat visual cortical areas (Scannell, Blakemore et al. 1995). Our main findings show that the majority of area 21a projections to the pulvinar bear a type I morphology, further strengthening the idea that extrastriate areas mainly send modulatory projection the pulvinar, while area 17 sends its driver inputs. When the proportion of type I inputs from the AEV, area 21a, the PMLS and area 17 were compared, type I inputs were seen to increase in a hierarchical manner suggesting that higher visual areas mainly modulate the activity of the pulvinar while the basic organization of its RF properties are mainly sustained through driver inputs from striate projections.

In our third paper, we investigated the possible association between type I and II projections and their bias towards utilising VGLUT 1 and 2 in their respective synaptic terminals. We found that the SC, the LGN and layer IV of V1, which are the main recipient of ascending driver projecting neurons in the visual system show a strong bias for VGLUT 2 expression. The tecto-recipient LPm of the LP-pulvinar complex was also shown to have a higher expression of VGLUT 2, as well the retino-recipient pulvinar proper which also expressed VGLUT 2. On the other hand, VGLUT 1 was diffusely expressed in the LGN and superficial and intermediate layers of the SC

in a way that resembled diffuse CT projections. VGLUT 1 was also diffusely expressed in most layers of area 17 known to receive significant feedback projection from XC areas and local vertical intracortical connection between cells of area 17's various layers. The expression pattern of VGLUT 1 and 2 showed similarities with other mammals (Fujiyama, Hioki et al. 2003, Balaram, Hackett et al. 2011, Balaram, Takahata et al. 2011, Baldwin, Wong et al. 2011, Balaram, Hackett et al. 2013, Balaram and Kaas 2014, Saraf, Balaram et al. 2019, Saraf, Balaram et al. 2019).

6.2 Methodological and technical considerations

Even if results presented in this thesis have functional implications which have bettered our understanding of transthalamic projections and the visual function of the pulvinar, they mostly remain anatomical in nature and so, several assumptions have been made which need considerations.

First, results from article 1 and 2 suggest that extra-striate areas AEV and 21a send 'modulatory' projections to the LP-Pulvinar, and that this 'modulatory' input increases as a function of cortical hierarchy. From a technical perspective, it would be more precise to say that the ratio of type I input increases, since the modulatory nature of type I input can only be confirmed functionally. Indeed, based on the modulatory effect of striate projection to the LGN which also have type I input, CT axons from layer VI projecting to the LP-Pulvinar bearing the same morphology are suspected to have a modulatory role (Sherman and Guillery 2013). Since cooling or inactivation studies of extra-striate areas on responses properties of the LP-pulvinar are scarce, this

assumption remains to be tested. What can give us an indication, however, is that striate projections to the LPl, which are mostly type II driver projections were shown to have drastic effects on LP visual responses once inactivated (Casanova, Savard et al. 1997). From this, one could infer, that those effects were mediated from layer V type II projections, and that conversely, type I projections from layer VI would have more subtle 'modulatory' effects.

The second technical consideration pertains to the overall contribution of cortical areas studied in this thesis, in cortico-pulvinar connectivity. In our experiments, BDA tracers were used to quantify the proportion of type I and II projections from several cortical areas in order to understand the type of projections they send to the LP-pulvinar. We compared the ratio of type I inputs as a function of cortical hierarchy, which is indicative of the functional impact of the overall projection of a given area to the LP-pulvinar. However, this does not give us an indication on the relative strength of the given projection to the LP-pulvinar which would likely require to have an estimation of the number of cells and boutons projecting to the LP-Pulvinar. Thus, our hierarchical organization is based on the changing nature of CT inputs to the LP-pulvinar and is not indicative the overall contribution of studied areas to the corticopulvinar connectivity as such comparative studies would likely require more controlled anterograde tracer injections, in order to minimize injection size differences, coupled with retrograde tracer injections in the LP-pulvinar, both of which were beyond the scope of this thesis.

Another technical consideration relates to the implications of these results for studies on primate and rodent visual systems. As mentioned in the discussion of article 2 and 3, there are similarities in the CT organization of visual pathways across species, one of which is the existence of type I

and II cortico-pulvinar projections across all mammals studied (Rouiller and Welker 2000). There are however several differences in the organization and functional role attributed to cortico-pulvinar and pulvino-cortical projections between cats and primates. The most relevant example for the scope of this thesis is the functional impact of inactivating the LP-pulvinar on visual responses of area 17. In the cat, a study from our laboratory revealed that the inactivation of the LP produces modulatory contrast gain changes in area 17, and greater changes in area 21a (de Souza, Cortes et al. 2019). However, in primates, visually evoked responses in supragranular layers were shown to be drastically reduced following pulvinar inactivation (Purushothaman, Marion et al. 2012), effects which are more indicative of a driver input. These results may suggest that pulvino-striate projections may subtend different functional roles across species. However, another explanation for these conflicting reports is that Purushothaman et al. studied the effect of visual responses of area 17 cells using fixed level 50% contrast, while results from our study on the cat report effects following LPl inactivation using contrast levels from about 25% to 100%. Thus, the reduction of visual responses in primate area 17 at 50% fixed contrast could be a result of a decrease in the neuron's response gain.

6.3 Function Implications

The existence of two types of CT projections originating from distinct cortical layers and bearing strikingly different morphologies prompted an initial assumption that these two types of projections would also differ from a functional standpoint (Sherman and Guillery 1998, Rouiller and Welker 2000, Sherman and Guillery 2001, Bickford 2015).

Early evidence of distinct functional roles for type I and II morphologies based on electrophysiological studies were found in the retino-geniculo-cortical pathways as RGC's synapsing at the LGN bearing type II morphologies are critical for visual responses of geniculate neurons whereas the afferents coming from neurons in layer VI of the primary visual cortex bearing a type I morphology exerted only a modulatory action on geniculate cells (Kalil and Chase 1970, Baker and Malpeli 1977, Geisert, Langsetmo et al. 1981, Crick and Koch 1998, Alitto and Usrey 2003). Moreover, LGN terminals ending in layer IV were also considered as drivers since basic properties of cortical cells are known to be built upon those from geniculate afferents (Sherman and Guillery 2002, Sherman 2016).

The existence of these two types of projections (type I and II) observed in the LGN have also been established in the pulvinar and other HO thalamic nuclei (Rockland 1994, Rockland 1996, Sherman, Guillery et al. 2006), and even though few of them have investigated their functional implication, there is some evidence that the 'driver and modulator' role attributed to type II and type I projections respectively in the geniculate system maybe generalized to extrageniculate pathways.

For instance, we have shown in article (2) that most projections from the primary visual cortex known to originate from layer V to the LP-pulvinar complex have type II terminals. These results are in line with other anatomical observations in the cat and the primate (Rockland 1996, Feig and Harting 1998, Guillery, Feig et al. 2001, Huppe-Gourgues, Abbas Farishta et al. 2019). Keeping in mind the functional significance of known driver/type II inputs observed in the LGN and according to the 'driver/modulator' framework advanced by Sherman and Guillery, these

projections from V1 to the pulvinar should have driver-like influence on pulvinar cells and should therefore be critical for the maintenance of their receptive field properties. Studies which have investigated the functional nature of these projections are in line with this assumption as electrophysiological data reveals that the deactivation of area 17 significantly decreases receptive field responsiveness of a number of cells in the cat LPl (Casanova, Savard et al. 1997, Rushmore, Payne et al. 2005), mouse LP (Bennett, Gale et al. 2019) and in the inferior pulvinar of primate (Bender 1983), suggesting a common ground of organization among species. These results suggest that type II projections, whether retino-geniculate, TC (LGN to layer IV) or CT (area 17 to LPl) may share a similar functional impact, in that their inactivation significantly alters responsiveness of their target cells. On the other hand, since type I projections from area 17 to the LGN are known to only modulate RF organization of geniculate cells without significantly altering their visual responsiveness, the assumed functional impact of type I projections on their target cells remains modulatory: this modulation can happen in several ways : through a modulation of RF properties (tuning orientation, or center surround suppression could be such examples) as observed in the LGN (Kalil and Chase 1970, Baker and Malpeli 1977, Geisert, Langsetmo et al. 1981, Crick and Koch 1998, Alitto and Usrey 2003), through short and long term synaptic plasticity (Li, Guido et al. 2003), or modulation of synchrony between connected networks of neurons (Saalmann, Pinsk et al. 2012, Fiebelkorn and Kastner 2019, Fiebelkorn, Pinsk et al. 2019). Unlike the inactivation of driver projections, this modulation however would not be enough to totally abolish visual responses in the recipient cell.

Inactivation studies are not the only ones to point towards functional differences between type I and II projections. Immunohistochemical investigations have also shed light on the different

putative functional roles of these projections revealing that their postsynaptic elements express different types of metabotropic glutamate receptors (Vidnyanszky, Gorcs et al. 1996). In their study, Vidyansky et al (1996), not only confirmed the existence of two distinct types of CT axons in the pulvinar, they also observed that thinner type I terminals established a synaptic contact with dendritic profiles that were immunopositive for mGluR1 at the level of the postsynaptic membrane, while synapses involving thicker type II axons in the pulvinar where immunonegative for this receptor. This observation further supports the notion that these two morphologically distinct types of projections may very well have different functional roles. This study is also in line with other reports revealing that CT projections from layer VI can activate metabotropic receptors, while projections from layer V to HO thalamic nuclei only rely on 'faster' ionotropic, AMPA and NMDA, receptors (McCormick and von Krosigk 1992, Salt 2002, Reichova and Sherman 2004, Sherman, Guillery et al. 2006). Taking into account the role attributed to 'driver' projections as inputs that are necessary for the establishment of the responses of its recipient cells, one may assume that these projections may require a high temporal resolution for a reliable transfer of information. In this context, the fact that type II-driver projections were seen to rely on faster ionotropic receptor and be absent from synapses involving 'slower' metabotropic mGluR1 receptors further strengthens its putative functional role.

Taking these results into account, we expect that CT projections from area 21a which mainly arise from layer VI to have a different functional role than area 17 projections to the LP pulvinar arising from layer V and thus support the view that corticofugal pathways originating from layers

V and VI have distinct influences on thalamic activity (Li, Guido et al. 2003, Van Horn and Sherman 2004, Usrey and Sherman 2019). To our knowledge, no study ever tested the impact of deactivating area 21a or V4, a possible homologue of area 21a in the primate (Payne 1993) on pulvinar neurons and therefore, we can only assume that the impact of such a manipulation to be modulatory while its overall effect would be less drastic than the deactivation of area 17. Even though this hypothesis needs to be tested, the fact that the pulvinar deactivation has different functional effect on visual responses of area 17 and 21a (de Souza, Cortes et al. 2019) may very well suggest that, in return, the deactivation of both these areas would have a different effect on visual responses of pulvinar neurons.

6.4 CT projections and visual hierarchy

6.4.1 Corticopulvinar projections: striate vs extra striate areas

In the cat visual system, a previous study from our lab investigating the proportion and morphology of CT projection types from the PMLS hinted towards the hypothesis that these inputs may vary from striate to XC areas. Indeed, in their paper, (Huppe-Gourgues, Bickford et al. 2006) revealed that the morphology of CT axon morphology varied between striate and extra striate areas, such that area 17 projections to the pulvinar consisted of mainly thick type II boutons, whereas projections from the PMLS were composed of a combination of both. These results lead to the hypothesis that CT projections to the pulvinar may vary as a function of cortical hierarchy. Interestingly, similar observations were also reported by (Guillery, Feig et al. 2001) in their qualitative study as they reported a greater number of type I boutons in the pulvinar

following injections in area 19 than following injections of area 17 or 18. In the cat as well, a similar picture also emerged from higher order area 5 and 7 which mainly sends type I projections to the pulvinar (Baldauf, Chomsung et al. 2005). Our results from article 1 and 2 which report that the AEV and area 21a mainly send type I projections in proportions that are greater than that observed from area 17 are therefore in line with all other known observations done in this context. The functional implication of these observations in the context of the establishment of RF properties from pulvinar cells is that the overall visual responsiveness of pulvinar neurons may rely significantly on area 17 projections, while higher areas would fine-tune its properties.

In other species, similar observations were also reported in the grey squirrel (Robson and Hall 1977) where projections from extra striate areas mainly comprised type I terminals whereas area 17's projections to the pulvinar were of type II. In macaque monkey, several studies also report that unlike V1 which sends type II terminals in the pulvinar, XC areas send almost exclusively type I terminals (Ogren and Hendrickson 1979, Ogren and Hendrickson 1979, Rockland 1996). In the tree shrew, the same organization also emerges as the primary visual cortex sends mainly type II terminals to the pulvinar (Day-Brown, Slusarczyk et al. 2017) while XC cortices send almost exclusively type II terminals (Chomsung, Wei et al. 2010).

Results from articles 1 and 2 which report a greater number of type I terminals from extra striate area AEV and 21a are thus not only in line with other studies performed in the cat, but also other species suggesting a common organization of CT projection between striate and XC areas in the mammalian visual system. Moreover, because most extrastriate areas studied in other reports (TD in the three shrew, PMLS of the cat) were located in the dorsal pathway, our results which

show a greater number of type I terminals in the pulvinar from an area located in the ventral pathway (area 21a, article 2) broadens the scope of this organization of CT axons based on morphology types.

6.4.2   Is there a hierarchy of CT projections in the pulvinar?

Results from articles 1 and 2 reveal a positive correlation between the percentage of type I terminals and the level of cortical hierarchy such that the percentage of type I terminals increases as one travels along the hierarchy of visual areas. Results are in line with the qualitative observations which report that higher order area 19 sends mainly type I projections, unlike area 17 and 18 (Guillery, Feig et al. 2001). These results therefore suggest that, like cortico-cortical connection (Scannell, Blakemore et al. 1995), CT projections in the cat also follow a hierarchical model when projecting to HO thalamic areas. To our knowledge, no study directly tested the hypothesis that CT projections might follow the same hierarchical structure as corticocortical connections in any sensory system or species.

In the context of a hierarchical organization of cortical representation in the pulvinar an important review by (Shipp 2003) proposed that the pulvinar could have an internal organization which may reflect the cortical hierarchy. Even though the author mentioned differences in the morphology of projections across cortical areas reported by anatomical studies, the main focus of this review with regards to the hierarchical organization of the pulvinar was the possible existence of different sites within the pulvinar which would receive their cortical inputs according to a gradient of hierarchical rank. Based on this model, type I and II projections from

a lower visual area would project to a site of lower rank within the pulvinar while those from a higher rank would project to a higher one. Moreover, according to this model, projections from one given cortical area would target different thalamic sites in the pulvinar based on their morphology such that type I and type II projections would project to distinct foci. Therefore, even though this model touched upon a possible hierarchical model of CT projections, its main proposal was the possible existence of a spatial gradient of cortico-pulvinar projections organized hierarchically which is different from our hypothesis which suggest that the morphology of CT projections differs in a hierarchical manner regardless of their spatial organization in the pulvinar.

Whether this organization of CT axons, where modulatory type I are seen to increase along the visual hierarchy of cortical areas, can be extended to other sensory systems and species remains to be tested, as to our knowledge, no study ever explored this hypothesis. Nevertheless, a thorough review authored by (Rouiller and Welker 2000) comparing the morphology of CT projections in mammals suggests the existence of a common framework for the organization of CT projections across species and systems. Indeed, in all studies reported in their review, both types of CT projections have been observed. Taking into account the complexity and intricate manner in which the cortex and the thalamus operate, it would be unrealistic to expect that our observations could be replicated in the same exact manner across all models and systems without taking into account their own specificities. However, what may be reasonably conceivable would be that, for a given system and model, transthalamic –cortical pathways synapsing in HO nuclei would be hierarchically organized to the extent that their corresponding corticocortical would be so. In other words, because the organization of the CT projections in HO thalamic nuclei stems

from a given laminar organization in the cortex, one can expect that these transthalamic pathways would be influenced and therefore reflect or complement the corticocortical organization present in a given species. By extension, should the organization of CT projections be built upon that of corticocortical ones, one may also expect to find, within the pulvinar, an internal organization that would reflect the existence of dorsal and ventral functional pathways. Evidence reviewed by (Kaas and Lyon 2007) points towards networks of cortico-thalamo-cortical connections in the macaque primarily engaged with the dorsal and ventral stream. This functional specialization of CT projections is in line with the emerging view that fundamental units of corticothalamic computation are not individual thalamic nuclei but more precise thalamic networks linking functionally related cortical areas (Shipp 2003, Halassa and Kastner 2017).

6.5 VGLUT 2 as a possible marker of ascending driver projections

Another aim of this thesis was to investigate the relationship between VGLUT's and the driver modulator theory of glutamatergic pathways following the recent proposal put forth by (Balaram, Hackett et al. 2013) which suggested that VGLUT 1 and 2 may selectively be expressed in modulator and driver projections respectively.

Amongst the significant differences between these two transporters is that VGLUT1 and VGLUT2 seam to rely on distinct vesicle recycling pathways after their fusion with the presynaptic terminal membrane. These distinct pathways enable VGLUT 1 and 2 to have different temporal properties of glutamate release (Voglmaier et al., 2006; Weston et al., 2011; Foss et al., 2013), due to their different affinity with endophilin A1 (which acts in a negative allosteric manner, reducing the probability of endocytosis): the higher binding affinity of

VGLUT1 to endophilin A1 reduces the probability of vesicle endocytosis and thus, of glutamate release while VGLUT 2's lower affinity enables it to maintain a comparably faster rate of vesicle endocytosis (Weston et al., 2011).

Other than having distinct membrane trafficking properties, early studies investigating their overall expression pattern across the CNS of mammals have reported that the two main VGLUT 1 and 2, have a complementary expression patterns suggesting that they may recognize distinct types of projections (Fremeau, Troyer et al. 2001, Herzog, Bellenchi et al. 2001).

For instance, studies have shown that VGLUT 2 is exclusively expressed in RGCs, strongly expressed in the LGN and the SC, and exclusively expressed in thalamo-cortical projections targeting layer IV (Fujiyama, Furuta et al. 2001, Fujiyama, Hioki et al. 2003, Nahmani and Erisir 2005). Subsequent studies on Macaque (Balaram, Hackett et al. 2013), Galagos (Balaram, Hackett et al. 2011, Balaram, Takahata et al. 2011), squirrel (Baldwin, Wong et al. 2011, Balaram and Kaas 2014), tree shrew (Chomsung, Petry et al. 2008, Wei, Masterson et al. 2011, Balaram, Isaamullah et al. 2015, Familtsev, Quiggins et al. 2016), mouse lemur (Saraf, Balaram et al. 2019, Saraf, Balaram et al. 2019), cats (Nahmani and Erisir 2005, Bickford, Wei et al. 2008) and rats (Kaneko and Fujiyama 2002, Fujiyama, Hioki et al. 2003, Masterson, Li et al. 2009), have revealed striking similarity between known modulator and driver projection sites and their VGLUT 1 and 2 expression: the most evident example is that in all species studied, a strong VGLUT 2 expression is always observed in retino-recipient layers of the LGN and geniculo-recipient layer IV of V1, both of which are the primary sites of ascending driver projections in the visual system.

Moreover, amongst the criteria used to distinguish between driver and modulator projection, their ultrastructure organization and morphology of terminals is often the best anatomical criterion: driver type II inputs are known to make axosomatic contacts on proximal dendrites or soma of their postsynaptic targets (Szentagothai 1963, Pasik, Pasik et al. 1973, Li, Wang et al. 2003, Sherman, Guillery et al. 2006). They are also known to have thicker axons and bigger boutons and exhibit a higher probability of synaptic vesicular release. These features allow driver projection to elicit faster EPSPs with an increased probability of a full action potential in their target neurons (Sherman, Guillery et al. 2006, Sherman and Guillery 2011). On the other hand, modulator type I projections which have thinner axons and smaller boutons contact distal dendrites which allows for lengthier propagation times of individual EPSPs.

Taking these differences into account and comparing them with known differences between VGLUT 1 and 2 projections, a striking analogy can be observed. First, like driver projections, VGLUT2-positive terminals tend to contact the more proximal dendrites or soma of their target neurons while on the other hand, terminals stained positively for VGLUT1 make contacts along distal dendrites, which is a feature of type I modulatory projections (Masterson, Li et al. 2009) . Second, in the LGN, type I projections are known to outnumber type II driver projections (Sherman, Guillery et al. 2006). Similarly, in the LGN and LP of the rat, VGLUT 1 profile were seen to outnumber VGLUT 2 one's (Fujiyama, Hioki et al. 2003, Masterson, Li et al. 2009). Third, just like driver projections are known to rely on bigger boutons and thicker axons, VGLUT2-positive projections in the LGN were reported to be significantly larger than VGLUT1-positive terminations (Fujiyama, Hioki et al. 2003).

Even though fewer studies have investigated functional differences that exist between VGLUT 1 and 2 bearing projections and how these differences may relate to known differences distinguishing between modulator and driver projection respectively, some electrophysiological observations further validate the hypothesis that they may be expressed in different classes of glutamatergic projections. According to the driver modulator framework, driver projections terminate in synapses which bear EPSP's with paired pulse depression, whereas modulatory cortico-geniculate projections from layer VI show paired pulse facilitation (Sherman and Guillery 2013). Interestingly, retinogeniculate projections known to be VGLUT 2 positive (Fujiyama, Hioki et al. 2003) bear synapses exhibiting synaptic depression (Turner and Salt 1998) a feature known to correspond with driver projections (Sherman 2017, Usrey and Sherman 2019). This synaptic depression was also reported in geniculo-striate projections, also known to be driver projections and which were shown to be positive for VGLUT 2 alone (Turner and Salt 1998). On the other hand, cortico-geniculate projections known to express VGLUT 1 (Ni, Wu et al. 1995, Hisano, Hoshi et al. 2000, Fujiyama, Hioki et al. 2003, Land, Kyonka et al. 2004, Yoshida, Satoh et al. 2009) exhibit synapses showing a paired-pulse facilitation (Lindstrom and Wrobel 1990, Turner and Salt 1998), which is a criterion used to identify modulatory projection. Moreover, according to the driver modulator theory of glutamatergic inputs, the fact that only modulator inputs utilize slower metabotropic receptor make them more likely to produce long term changes in relay cells or induce long-term synaptic plasticity (Sherman and Guillery 1998). In this context, VGLUT 1 was shown to be more frequently expressed than VGLUT 2 in synapses showing long-term potentiation or depression (Ito and Kano 1982, Castro-Alamancos and Calcagnotto 1999, Varoqui, Schafer et al. 2002). Finally, one of the hallmarks of driver

projections is related to their fast conductance sustained through synapses that are highly reliable and exhibit efficient transmission of information. This efficient transfer of neural information is believed to take place thanks to a high probability of transmitter release (Gil, Connors et al. 1999, Sherman, Guillery et al. 2006). Again, VGLUT 2 projections were reported to have a higher transmitter release probability than VGLUT1 synapses (Fremeau, Troyer et al. 2001, Varoqui, Schafer et al. 2002, Fremeau, Kam et al. 2004). Taken together, these physiological observations along with anatomical localization of their expression, suggest that VGLUT 1 and 2 show a bias for modulatory and driver projections along retino-fugal and cortico-geniculate pathways.

While the association of VGLUT 1 and 2 with known driver and modulator sites retino-geniculo-striate pathways seems evident, several observations call for more nuance with a strict association. First, the LGN of primates, rodents and tree shrews were found to express low levels of VGLUT 1 mRNA (Fujiyama, Furuta et al. 2001, Balaram, Hackett et al. 2011, Balaram, Hackett et al. 2013, Balaram, Isaamullah et al. 2015). These neurons may project intrinsically within the LGN, but they may also represent a subset of geniculate projections which utilize VGLUT 1 and contribute to its diffuse expression in geniculo-recipient V1 layers (Balaram, Isaamullah et al. 2015).

More importantly, the presence of driver and modulatory projections has also been described in extra-geniculate pathways involving the pulvinar (Sherman, Guillery et al. 2006) : in cats, three shews, and primates, driver projections from V1 to the pulvinar originate from layer V while modulatory ones originate from layer VI (Rockland 1996, Usrey and Fitzpatrick 1996, Day-Brown, Slusarczyk et al. 2017, Huppe-Gourgues, Abbas Farishta et al. 2019). However, both

layer V and VI of tree shrews and primate are mostly devoid of VGLUT 2 mRNA (Balaram, Hackett et al. 2013, Balaram, Isaamullah et al. 2015) suggesting that driver and modulatory cortico-pulvinar projections may utilise VGLUT 1, which further blurs the use of VGLUT 1 and 2 as markers of driver and modulatory projections in extra-geniculate pathways. Combined tracing and immunohistochemical studies of VGLUT's providing direct evidence of a possible association between terminal types and their bias for a given VGLUT have been few. Of interest is a study done in primates (Rovo, Ulbert et al. 2012) which describes large 'driver' corticothalamic input to the pulvinar expressing VGLUT 1. Similarly, in galagoes, based on the expression of VGLUT 1 mRNA in V1's infragranular layers, the expression of VGLUT 1 and absence of VGLUT 2 in its pulvinar subdivisions (Balaram, Hackett et al. 2011, Balaram, Takahata et al. 2011) presumed driver cortico-pulvinar projection from layer V of V1 most likely utilize VGLUT 1. However, in the same species, pulvinar projections to V2, also described as driver projections, utilize VGLUT 2 (Marion, Li et al. 2013). Thus, results in prosimian galagos where driver cortico-pulvinar and driver pulvino-cortical projections may utilise VGLUT 1 and 2 respectively suggest that, within transthalamic pathways, there does not seem to be a strict correlation between VGLUT's and driver and modulatory projections.

6.6 Future directions

6.6.1   Functional impact of area 21a projections to the pulvinar

Results from articles 1 and 2 presented in this thesis indicate that axon terminals from area 21a and the AEV cortex mainly send type I inputs to the pulvinar, which is in contrast to area 17 which sends mostly thicker type II inputs. These data have helped us better understand the anatomical organization of both these extra-striate areas with the pulvinar and suggest that they

are most likely to modulate visual responses of pulvinar neurons rather than being critical in the establishment of their RF properties.

To our knowledge, no study investigated the functional role of CT projection of area 21a and the AEV terminating in the pulvinar. Therefore, building on the results of this thesis, one important future direction to be taken in order to further our understanding of cortico-pulvinar projections from extra striate areas would be to investigate the impact of functional of their functional inactivation on the overall responsiveness of pulvinar cells. Cortical inactivation could be done with GABA injection or even cooling probes, especially for area 21a whose cortical surface is mostly exposed. Results from these experiments on extrastriate areas could be also compared with the lens of cortical hierarchy, as it is known that the functional deactivation of the primary visual cortex since the deactivation of area 17 decreases receptive field responsiveness of neurons in the LP-pulvinar of mice (anterior part of the LP), cats (LPl) and primates (inferior pulvinar) (Bender 1983, Casanova, Savard et al. 1997, Rushmore, Payne et al. 2005, Bennett, Gale et al. 2019) suggesting a common ground of functional organization among species.

Another important point that remains to be examined is how the cat pulvinar relates to the existence of the functional dorsal and ventral stream of visual processing. In other words, whether there is in the pulvinar, functional networks that are mainly involved with a given stream of visual pathways. This is an important point as evidence reviewed by (Kaas and Lyon 2007) points towards networks of cortico-thalamo-cortical connections in the macaque primarily engaged with the dorsal and ventral stream.

6.6.2 CT projections in other systems and corticotectal pathways

Results from articles 1 and 2 presented in this thesis are also important in other ways. To our knowledge these results represent the first anatomical demonstration that the nature of the cortical afferents to a subcortical structure varies as a function of the hierarchal level of the visual cortical areas. In this way these results set the ground for a better understanding of the organization and role of the cortical projections targeting the pulvinar.

But in a broader context, these results provide new information about the functional relationship between the neocortex and higher-order thalamic nuclei that may apply to other species, especially mammals with highly developed visual system; furthermore, it may be hypothesised that this organization be somewhat similar in other sensory systems. Indeed, type I and II CT projection are not a unique feature of the visual system as they have been described in the auditory, motor, and somatosensory (Rouiller and Welker 2000).

Therefore, an important step towards the validation of this organization in other systems would to investigate the nature of CT projection from another hierarchically organized sensory system in the cat, such as the auditory one, in order to understand whether the organisation revealed in this thesis is unique to the visual cortex or if it is a feature shared with other cortices and systems. Several areas of the cat's auditory system could be targeted namely the primary and second auditory cortex (AI and AII), the dorsal zone (DZ), the posterior auditory field (PAF), ventral posterior auditory field (VPAF), ventral auditory field (VAF), temporal cortex (T), insular cortex (IN), anterior auditory field (AAF) as well as the auditory cortex of the anterior ectosylvian

sulcus (fAES), all of which can also be identified with SMI-21 staining (Mellott, Van der Gucht et al. 2010). Technically, the methodology (tracer studies) used in our studies can be used for any cortical area and can even be expended to other species as well.

Results from our tracing studies can also be taken in the context of a hierarchical organization of cortical afferents to a given subcortical structure which in this case was a thalamic nucleus, but that may be applicable to non-thalamic subcortical structure, such as the superior colliculus. In the cat visual system, prominent projections from 17 of the 25 visual cortical areas target the SC, located in brainstem. Areas 17, 18, 19, 20a and b, the PMLS and the AEV, all target superficial layers of the SC (Harting, Updyke et al. 1992), making cortico-tectal projections an ideal candidate to test the hypothesis that the functional organization between subcortical structures and series of cortical areas may reflect the hierarchical manner in which those cortical areas are organized. The fact that cortico-tectal also exhibit the two types of projections found in CT pathway (Fuentes-Santamaria, Alvarado et al. 2009) provides further ground for the relevance of widening the scope of our hypothesis to the brainstem.

Therefore, given the fact that our injections made in area 17, PMLS, 21a and the AEV not only labelled CT axons but also cortico-tectal projections, a relatively straightforward set of experiments would be to use the brainstem of cats used from article 1 and 2 and study the morphology of their cortico-collicular projections. The study of these cortico-tectal projection are in an of themselves critical of the better understanding of the role of the superior colliculus in visual functional, but they could also be examined from a comparative point of view with our results obtained from cortico-thalamic studies.

6.6.3   The role of tectopulvinar inputs and thalamic integration of sensory signals

The main finding of articles 1 and 2 are that corticothalamic projections to the LP-pulvinar complex are organized as a function of cortical hierarchy. When comparing the proportion of type I and II CT inputs to the LP-Pulvinar, both LPl and LPm were taken into account, since both were considered HO nuclei involved in transthalamic pathways (Casanova 2004). One important difference between the LPl and the LPm remains that the LPm is the main recipient of tecto-thalamic input to the LP (Graybiel 1972, Graybiel and Berson 1980).

Studies in the cat show that tecto-thalamic projections exhibit large clustered terminals that contact proximal dendrites and resemble synaptic arrangements made by retinogeniculate projections (RL type) (Kelly, Li et al. 2003) which suggest that those projection should have 'driver-like' effects and thus, significantly influence response properties of their target cell.

The presence of these 'driver-like' tecotpulvinar projections in the LPm, also known to receive driver/type II projections from extra-striate areas (Guillery, Feig et al. 2001, Huppe-Gourgues, Bickford et al. 2006, Huppe-Gourgues, Abbas Farishta et al. 2019, Abbas Farishta, Boire et al. 2020) has rightly called for a modulation of a strict classification of thalamic nuclei (Bickford 2015). As is, in the driver/modulator framework proposed by (Sherman and Guillery 2013), higher-order thalamic nuclei differ from first order ones (which receive their main driving input for the periphery/subcortical sources) in that they receive their primary driving inputs from the cortex. Therefore, in the case of the LPm, and based on anatomical data, its classification into a 'pure higher-order' thalamic nucleus seems less straightforward than the LPl, given the fact that

it receives cortical and subcortical projections which bear type II or RL driver like terminals or synaptic arrangements.

Functional studies investigating visual responses of LPm neurons have been more conclusive in determining which input, cortical or collicular, influenced the most its visual responsiveness. Extracellular recordings in the LPm have revealed that its visual response properties are significantly different from those recorded in the SC (Chalupa, Williams et al. 1983). LPm cells were found to have much bigger RF than those recorded in the SC, a subset of them requiring binocular stimulation for optimal responses, suggesting that they may integrate signals from converging inputs. Studies from our laboratory have also shown that LPm neurons can respond to complex motion stimuli such as "plaid pattern" (Merabet, Desautels et al. 1998), a computation which was only found to be present in cortical areas. Interestingly, LPm cells were still able to respond to the direction of plaids pattern after the removal of the AES cortex known to display similar pattern-motion selective cells (Scannell, Sengpiel et al. 1996) but lost its responsiveness after the removal of the lateral suprasylvian (LS) cortex was removed suggesting that the presence of complex motion sensitivity in the LPm depends on cortical signals from the LS cortex. Other studies have also revealed that visual responsiveness of LPm cells are significantly affected by cortical cooling (Hughes and Chalupa 1982), while little effect was observed after cooling the SC (Chalupa 1991). Thus, these studies suggest that from a functional standpoint and like in other HO nuclei, LPm cells are more likely driven by cortical signals (from extra-striate areas) than from their tectal driver-like projections.

While there may be a case for the functional classification of the LPm as a HO thalamic nuclei, the presence of two sources of driver inputs, both of which form RL type synaptic arrangement suggest that LPm cells may integrate cortical and subcortical signals for the emergence of novel spatial and temporal receptive field properties (Bickford 2015). And important study in the mouse from (Groh, Bokor et al. 2014) demonstrated that both first and higher order driver inputs could converge onto single cells in the somatosensory thalamus. Whether such an arrangement exists in the LPm remains an important and unresolved question. Further studies on this matter would enhance our understanding of the LP-pulvinar, especially our understanding of its tectal input, which despite bearing driver-like projections from an anatomical standpoint, does not seem to influence LPm cells with driver like effects in functional studies. The study of this question remains an important one for the general direction for thalamic studies as it would further widen the role attributed to its nuclei, from simple relays, to integrators of neural signals.

6.6.4 VGLUT immunolocalization in extra-geniculate pathways

As mentioned above, several studies have investigated functional and anatomical differences between VGLUT 1 and 2 which, when put together, suggest that both may be selectively expressed in modulatory and driver projections respectively in the ascending visual pathways. This association is however less clear in transthalamic pathways.

The most compelling evidence of such a segregation when it comes to the expression pattern of both VGLUT 1 and 2 comes from the geniculo-cortical pathway. Indeed, because the main driving input to the LGN remains the retina and because layer IV receives prominent geniculate driver projection, the clear distinction between VGLUT 1 and 2 and their association with

modulator and driver projections seems most evident. This dichotomy in the pattern of their expression is less clear for extra-geniculate pathways for understandable reasons: the pulvinar for instance, receives driver and modulatory inputs from multiple sources in a way that is more loosely organised than afferents to the LGN. For this reason, to further understand the association between VGLUT's and the driver/modulator theory, especially in pathways where a cortical or thalamic area sends and receives both type I and II projections, more precise techniques enabling to clearly distinguish axonal morphology with VGLUT colocalization would be required. A relevant study has used combined BDA injection with VGLUT immunolocalization in the primate pulvinar (Rovo, Ulbert et al. 2012), revealing that large driver like terminals show VGLUT 1 immunoreactivity, but those CT projections originated from the motor cortex. Data combining BDA tracing from the visual cortex with VGLUT immunolocalization in the LP-pulvinar is still missing, and these results would bring a decisive advantage of clearly associating the expression of a given VGLUT with a morphologically identified terminal type. These studies could also be performed in the context of the laminar origins of CT projections. Since type II projections are known to originate from layer V and type I from layer VI (Rouiller and Welker 2000), injections of BDA in layer V and VI of area 17 combined with VGLUT immunolocalization in the LP-pulvinar could further enhance our understanding of the functional significance of VGLUTs in transthalamic pathways.

7. Conclusion

To our knowledge, results from our tracing studies (articles 1 and 2) represent the first anatomical demonstration that the nature of the cortical afferents to the pulvinar varies as a function of the hierarchal level of the visual cortical areas. It thus provides a new framework for a better

understanding of the organization and role of the cortical projections targeting the pulvinar. In a broader context, these results provide new information about the functional relationship between the neocortex and higher-order thalamic nuclei that may apply to other sensory systems.

In line with the general aim of articles 1 and 2 which was to better understand relationship between the cortex and the pulvinar, the aim of article 3 was to test the hypothesis that VGLUT 1 and 2 may be complementary expressed along the visual pathway. Generally, our results confirmed the strong association between VGLUT 2 and known driving projections in ascending visual projections and were in line with other studies done in mammals. Because of its relatively well described anatomical connectivity, our results on the cat visual system may be a relevant model for future functional studies on VGLUT 1 and 2 in the context of the driver/modulatory theory.

Results from this thesis also bring forth relevant and conclusive neuroanatomical evidence on the underemphasized role played by the pulvinar, and more generally, the thalamus, in cortical function. Indeed, results from article 2 not only reveal the existence of a hierarchical organization of CT projections to HO thalamic nuclei, a level of organization which was only previously observed in the cortex, they further call for a reconsideration of the thalamus as an 'active' player of neural processing, one which cannot be dissociated with cortical function and therefore, its functional organization.

Moreover, results from our studies also tend to validate the emerging view that the classical anatomical parcellation of the thalamus into relatively homogenous and independent thalamic

nuclei may bring detrimental limitations to the understanding of its overall functional role. A clear example of this reality would be the pulvinar, whose internal connectivity pattern we have now shown to be intricately organised based on cortical hierarchy, in a manner which did not reflect its classical chemoarchitectural subdivisions. Understanding the thalamus and the pulvinar as a network of functionally connected units rather than strictly segregated nuclei defined by anatomical and chemoarchitectural techniques calls for the existence within the pulvinar, of an internal organization that would reflect other features of cortical function such as the segregation of visual processing into dorsal and ventral functional streams. Evidence reviewed by (Kaas and Lyon 2007) points towards networks of cortico-thalamo-cortical connections in the macaque primarily engaged with the dorsal and ventral stream. Thus, this functional specialization of CT projections is in line with the emerging view that fundamental units of corticothalamic computation are not individual thalamic nuclei but more precise thalamic networks linking functionally related cortical areas (Shipp 2003, Halassa and Kastner 2017).

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Wong, P. and J. H. Kaas (2010). "Architectonic subdivisions of neocortex in the Galago (Otolemur garnetti)." <u>Anat Rec (Hoboken)</u> **293**(6): 1033-1069.

Yoshida, M., T. Satoh, K. C. Nakamura, T. Kaneko and Y. Hata (2009). "Cortical activity regulates corticothalamic synapses in dorsal lateral geniculate nucleus of rats." <u>Neurosci Res</u> **64**(1): 118-127.

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9. Curriculum Vitae

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School of Optomety, Université de Montréal
Department of Ophthalmology, McGil University

EDUCATION

2020	**Université de Montréal**:	PhD, Vision science
2017	**Université de Montréal**:	Optometry Doctorate
2012	**Université de Montréal**:	MSc, Vision science
2010	**Université Paris Sud**:	MSc, Vision science
2009	**Université Paris Sud**:	MSc, Optometry
2008	**Université Paris Sud**:	BSc, Optometry

BIOGRAPHY

Visual neuroscientist and clinician. I am passionate about teaching and research in optometry and vision sciences. As a researcher in visual neurosciences, I did my PhD on corticothalamic pathways involved in the visual system. My current research interest lies in the involvement of subcortical structure in visual processes, the psychophysical study of human visual perception, including on clinical populations such as amblyopic and TBI patients. I am member of the AAO, a recipient of the Ezell fellowship in 2019, and a candidate to become a fellow of the Academy.

EXPERIENCE

2020 - Present **Postdoctoral Fellow and Clinical Scientist, McGill Department of Opthalmology**
Research focused on Human visual perception at Dr. Farivar 's Laboratory

2012 - Present **Lecturer, École d'optométrie, Université de Montréal**
Teaching students in the OD program. Course including:
Neurophysiology of vision, Ocular diopter, Eye pharmacology, ocular movements

2017 - Present **Optometrist and Clinical Consultant, Newlook Vision Group, Canada**
Private practice in the country's biggest optometry group

2017 - 2020 **Doctoral Research, Laboratory of Dr. Casanova**
Thesis on corticothalamic pawthways of the visual system

2018 - 2020 **Optometry Examining Boards of Canada, Consultant for Québec**
Review and translation of national exams to regulate optometry practice in Canada

2014 - 2018 **Clincian, École d'optométrie, Université de Montréal**
Supervision of 4th year students of the O.D program in ophthalmic optics

PUBLICATIONS

Abbas Farishta, R, Boire, D, Casanova, C, Hierarchical organization of corticothalamic projections to the pulvinar, *Cerebral Cortex Communications, 2020* tgaa030, https://doi.org/10.1093/texcom/tgaa030

Abbas Farishta, R, Zouahi, H, Casanova, C, Distributions of Vesicular Glutamate Transporters 1 and 2 in the Visual System of The Cat. *Article in preparation for the Journal of Comparative Neurology, 2020*

CS. Micaelo-Fernandes, **Abbas Farishta, R**, JF. Bouchard, J. Bouskila, M. Ptito. Expression of the CB1 receptor in the lateral inferior subdivision of the vervet monkey pulvinar, *Article in prepartion for Vision, 2020*

Huppé Gourgues, **Abbas Farishta, R** et al, Distribution and Morphology of Cortical Terminals in the Cat Thalamus from the Anterior Ectosylvian Sulcus, *Scientific Reports, 2019* Feb 28;9(1):3075 doi:10.1038/s41598-019-39327-7.

Abbas Farishta R et al, Impact of CB1 Receptor Deletion on Visual Responses and Organization of Primary Visual Cortex in Adult Mice. *Invest Ophthalmol Vis Sci. 2015* Dec;56(13):7697-707. doi: 10.1167/iovs.15-17690

PROFESSIONAL AND ACADEMIC EXPERIENCE

2012 - 2015	President of graduate students Association, School of Optometry
2012 - 2015	Member of the Scientific Committee, Vision Science Research Network
2012 - 2015	Secretary, Research Group in Vision Science, University of Montreal
2011 - 2012	Executive Member, Brain Awareness week, Society For Neurosciences
2010	Examination Jury, National Optician Entry Exam, France

PRIZES AND SCHOLARSHIP

2020	Vision Network Publication Award	$250
2019	John N. Schoen Ezell fellowship, American Academy of Optometry	$8,000
2018	Gresset-Simonet prize, Université de Montréal	$1,500
2015	Excellence merit based scholarship, Université de Montréal	$4,000
2014	Excellence merit based scholarship, Réseau Vision	$10,000
2013	Excellence merit based scholarship, Université de Montréal	$4000
2011	Best Poster award, EVER, Creta, Grece	400 Euros
2011	Vistakon travel fellowship, American Academy of Optometry	$750
2010	Travel Fellowship, Université de Montréal	$750

INVITED TALKS

R. Abbas Farishta, Functional Neuroanatomy of Visual Pathways: Current knowledge and Clinical implications. School of Optometry, Ohio State University, 2020

ORAL PRESENTATIONS

R. Abbas Farishta, , D. Boire, C. Casanova. Functional Neuroanatomy of Pathways Involving the Visual Cortex and Pulvinar. American Academy of Optometry, Orlando, Florida, October 2019

C.Casanova , M.P. Vanni, **R. Abbas Farishta,** , S.Thomas. Is The Pulvinar Driving or Modulating Responses in the Visual Cortex? Symposium on the Pulvinar. Vision Scientce Society, Naples, Florida, May 2012

R. Abbas Farishta, M.P. Vanni, C.Casanova. Responses of the striate and extra striate cortex to pulvinar and LGN stimulation in three shrews. Annual Conference, Vision Health Research Network, Montréal, Canada 2011

R. Abbas Farishta, C. Robert, M.P. Vanni, K. Minville, J-F. Bouchard, C. Casanova, Couplage hémody-namique et organisation fonctionnelle du cortex visuel primaire des souris déficientes aux récepteurs CB1 des cannabinoides. Journée scientifique du GRSV de l'Ecole d'optométrie de l'Université de Mon-tréal , Montréal (Canada), 2011

C. Casanova, N. Zabouri, **R. Abbas Farishta**, C. Robert, M.P. Vanni, K. Minville, J-F. Bouchard, Retinal and corticalfunctions in adult mice lacking cannabinoid receptors. European Association for Vision and Eye Research, Creta Maris, Greece, 2010

R. Abbas Farishta, C. Robert, M.P. Vanni, K. Minville, J-F. Bouchard, C. Casanova, Couplage hémody-namique et organisation fonctionnelle du cortex visuel primaire des souris déficientes aux récep-teurs CB1 des cannabinoides. Journée scientifique du Réseau-Vision (FRSQ), Montréal (Canada), 2010

POSTER PRESENTATIONS

R. Abbas Farishta, D. Boire, C. Casanova Modulatory signals from the visual cortex to the pulvinar in-crease along the hierarchical order of cortical areas, Society for Neurosciences, San Diego (USA) 2018

R. Abbas Farishta, D. Boire, C. Casanova, Morphology of area 21a terminals in the LP-pulvinar complex of the cat, Society for Neurosciences, Chicago (USA) 2014

R. Abbas Farishta, M.P. Vanni, C. Casanova, Spatio-temporal responses in the visual cortex evoked by visual and electrical stimulation of thalamic nuclei in cats Society for Neuroscience, Washington (USA), 2011

M.P Vanni, **R.Abbas Farishta**, C.Casanova : Spatio-temporal responses in the striate and XC cortex evoked from thalamic nuclei in tree shrews: A voltage sensitive dyes study, Society for Neurosci-ence,Washington (USA), 2011

C.Casanova, **R.Abbas Farishta**, M.P Vanni. Spatio-temporal responses in the visual cortex evoked from LGN and Pulvinar in treeshrews. European Association for Vision and Eye Research, Greece 2011

R. Abbas Farishta, M.P. Vanni, C. Casanova Effects on the visual cortex of electrical and visual stimula-tion of thalamic nuclei revealed by VSD, American Academy of Optometry, Boston (USA), 2011

R. Abbas Farishta, M.P. Vanni, C. Casanova Responses of the striate and extra striate cortex to pulvinar and LGN stimulation in three, American Academy of Optometry, Boston (USA), 2011

R. Abbas Farishta, C. Robert, M.P. Vanni, K. Minville, J-F. Bouchard, C. Casanova, Effects of Cannabinoid CB1 receptor on hemodynamic responses and functional organization of the primary visual cortex. Society for Neuroscience, San Diego, 2010

R. Abbas Farishta, C. Robert, M.P. Vanni, K. Minville, J-F. Bouchard, C. Casanova, Functional organization of the primary visual cortex in mice lacking cannabinoid CB1 receptors. American Academy of Optom-etry, San Francisco, 2010