The role of thalamic pulvinar in eye-hand coordination for goal-directed actions

Dissertation

zur Erlangung des mathematisch-naturwissenschaftlichen Doktorgrades

"**Doctor rerum naturalium**"

der Georg-August-Universität Göttingen

vorgelegt von

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Göttingen, 2017

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Dedication

This thesis is dedicated to my *f*amily

and to the people who made me love learning.

Acknowledgements

I am grateful to many people whose influence allowed me to complete this project. I am thankful for the enrichment they provided me, professionally and personally.

I would like to thank Dr. Igor Kagan and Prof. Dr. Melanie Wilke for their supervision, along with the several funding sources that allowed the development of this thesis and are acknowledged in each one of the corresponding chapters.

I thank my thesis committee, Prof. Alexander Gail and Prof. Hansjörg Scherberger, for all their valuable suggestions and scientific discussions, as they greatly helped me ground the scope of my project and whose valuable feedback I tried to reflect in the current version of this document.

I thank Prof. Andrea Antal and Prof. Tim Gollisch for participating in my examination board.

Upon my arrival to the Primate Center I met wonderful people: Ira Panolias, Olga Dyakova and Malte Köster, Ira was a fantastic officemate, colleague and outstanding multitasker that kept our lab running at all times. Olga is one of the nicest and hardest working people I met in our group, her motivation and good attitude was contagious. Malte was the best first impression I could get from my lab mates, smart, driven, curious and with a balanced life, and I am very grateful to have had them around at the beginning of my path in the laboratory.

I would like to thank Lukas Schneider, Danae Theodosopoulou, Kathleen Williams Yuranny Cabral and Enrico Ferrea for their invaluable company. Lukas was my main collaborator during later stages of my Ph.D., in addition to being my lab mate, flat mate,

officemate and closest friend during my stay in Germany. Danae and Katie made my life here so much better; they are both unique remarkable individuals and I hope to encounter them again. Conversations with Yuranny helped me keep perspective of our goals in such a foreign place. I was lucky to have found you guys.

I had the opportunity to work with fun and smart students during their laboratory rotations, Annika Grass, Kirsten Emmert, and Uwe Zimmermann. Each in their own way contributed to the thinking of the work presented here.

My longtime friends and colleagues must be acknowledged, as they are part of the reason why neuroscience is so precious to me.

I thank my past and present colleagues at the German Primate Center for all the fun times and for their valuable input for the improvement of my projects.

I thank three furry characters that evoked a great joy and empathy in me, Linus, Flaffus, and Curius, I will greatly miss them.

Most importantly, I thank my wonderful family for their unconditional support.

“I am not interested in how people move, but what moves them.”

Pina Bausch

Index

Preface

The thalamic pulvinar, the largest and one of the most diversely interconnected subcortical regions in primates is a mysterious one, and perhaps, one of the most difficult brain regions to describe functionally. Streams of interest on the region have sparked and faded from time to time. Early last century, for example, a lesion study hinted a potential link of pulvinar (together with other thalamic nuclei) to goal-directed behavior in primates (Walker, 1938). In his study, Walker reported complete degeneration of pulvinar cells after ipsilateral hemidecortication of a chimpanzee. Based on his, and previous findings of cortical and pulvinar size increase in primates, he speculated that there could be a link between complex upper limb behavior and the notable growth of cortex and thalamic nuclei in primates.

Recent ongoing efforts from several branches of neuroscience are providing a more comprehensive view of thalamic nuclei within the rich circuitry of the brain. These new functions of the thalamus span well beyond the relay of information from peripheral organs to the cortex as it was once thought to be. The functions of the pulvinar, however, remain underexplored. Taking another look at the pulvinar in action during goal-directed behaviors might help us illuminate questions that have lingered in the mind of neuroscientists for several decades.

Introduction

i.1 Visual processing streams

The primate brain has grown and developed during evolution as have our complex interactions with the environment. Primates, particularly humans, have extensive association cortices whose defining feature is the lack of direct inputs from sensory areas or projections to motor command centers. Association areas are interconnected to each other, but also share projections to subcortical regions; in particular, dense connectivity has been reported to dorsal sub regions of the pulvinar nuclei of the thalamus (Asanuma et al., 1985; Kaas and Lyon, 2007; Buckner and Krienen, 2013). The functions observed in association cortices often reflect integration of information that is used to generate future actions.

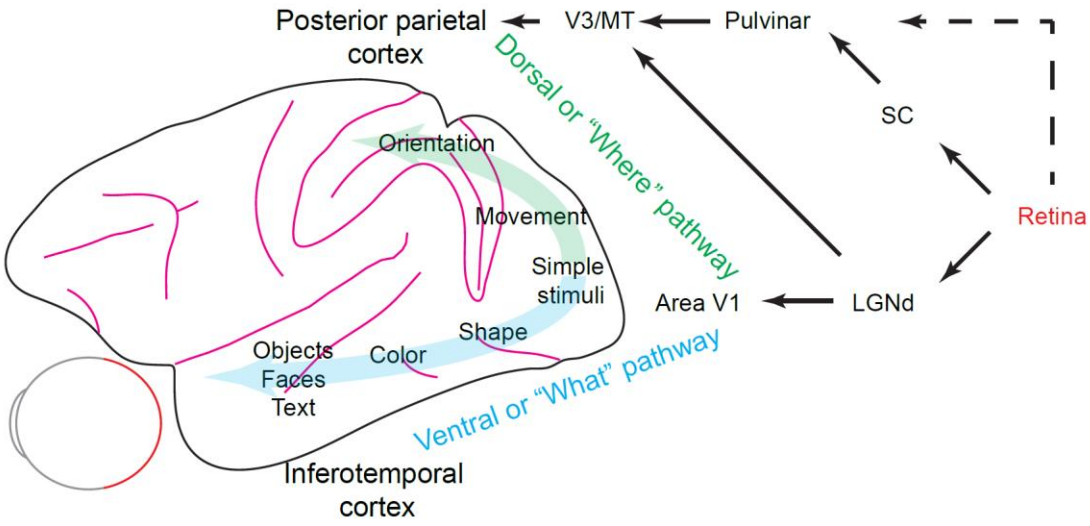
A large part of the information to be integrated by association cortices comes via the visual system. Vision is represented across several cortical and subcortical regions in the brain, in the cortex only, over 30 areas are known to represent visual features (Ungerleider and Haxby, 1994). The visual information not only allows us to scrutinize and categorize our surroundings but also to interact with them in an efficient way. Many brain regions are involved in distinct aspects of visual processing e.g. its meaning, retrieval, and emotional content. Other areas are involved in the use of such information for the planning and execution of actions (Goodale and Milner, 1992). Of particular interest for primates are the parietal and frontal cortices, as they have been identified to be linked in the planning of eye and hand movements (Snyder et al., 1997, 2000a; Battaglia-Mayer et al., 2003; Caminiti et al., 2015)

The widely accepted canonical nature of the visual system (Mishkin et al., 1983; Ungerleider and Haxby, 1994) has allowed vision researchers to study simple to increasingly complex features of the visual world across the information flow hierarchy. In general terms, the early visual pathway drives information from the visible part of the electromagnetic spectrum, detected by the ganglion cells in the retina, through the optic tract to the lateral geniculate nucleus of the thalamus, while in parallel, other fiber bundles

project to tectal and pretectal areas of the midbrain. These multiple projections are thought to be part of a system that also optimizes motor commands (Guillery, 2003, 2005). From the thalamus, the information continues through optic radiations to its first cortical target, the striate visual cortex.

The primary visual cortex routes visual information to two functionally distinct visual streams (**Figure i.1**): The first stream includes the primary visual area (*V1*), V2, V4, as well as occipital temporal and inferior temporal cortices while 2) a second stream includes mainly occipital parietal cortices, V1 and V2 in addition to areas V3, MT, MST, and regions in the posterior parietal cortex and superior temporal sulcus. The functional characterization of the ventral and dorsal streams was made possible largely by the observation of deficits after brain to distinct brain regions in both humans and monkeys. Damage to regions in the ventral stream was found to cause extensive deficits in object discrimination and retrieval, while damage to areas in the dorsal stream mainly impaired performance in tasks with spatial-relevant components (Mishkin et al., 1983; Ungerleider and Haxby, 1994). Initially, the classification of the distinct visual areas was proposed to heavily depend on the presence of strong perceptual or spatial properties in the area, i.e. the widely known “what” and “where” pathways. Further study of perceptual and motor deficits in patients with parietal and temporal damage contributed to refining the role of the dorsal stream as one with not only spatial components but action-oriented properties, i.e. the ¨how¨ pathway (Goodale and Milner, 1992; Goodale et al., 2005).

Figure i.1 **Organization of the visual streams in the macaque brain**



Visual inputs travel from the retina via the optic tract to the LGNd, and then to V1 via optic radiations. From early visual areas V1 and V2, the visual information diverges in two pathways. The first one modulated by the physical properties of objects which contribute to the creation of semantic representations of our visual surroundings at multiple processing levels in the visual hierarchy (ventral stream). The second stream is more sensitive to spatial and goal-directed properties of our visual environment (dorsal stream). An additional input to the dorsal stream emerges from the retina and bypasses the LGNd, relaying information to V3/MT through the SC and pulvinar or directly via pulvinar. Composite figure from and with permission of (de Haan and Cowey, 2011) and (Goodale, 2011). *LGNd, Dorsal lateral geniculate nucleus; MT, Medial temporal area; V1, Visual area 1 (primary visual cortex)*

In addition to the often encountered canonical nature of the visual system (Mishkin et al., 1983; Ungerleider and Haxby, 1994), there are processes for which the parallel recruitment of different cortical areas, in addition to the known sequential processing in the visual streams might be required (de Haan and Cowey, 2011). As our brains need not only to integrate complex visual inputs but also to generate visually-guided motor commands, a visuo-motor network that dynamically and rapidly is able to recruit neural populations across several brain regions seems to be a cost-effective solution. Brain regions with extensive bidirectional connections with the central nervous system are of relevance, as they might act as hubs that facilitate the generation and integration of visually-guided actions.

Multiple brain regions are involved in the active exploration of the environment. A structure with a central position, both physically and more importantly, functionally, is the thalamic complex. The thalamus is in a privileged position to participate in information modulation, as all sensory modalities (except for the olfactory) possess a thalamic relay.

i.2 Thalamus

The thalamus, a group of several nuclei of diencephalic origin is crucially involved in the relay of information from peripheral sensory organs to the cortex and in addition, in the transfer of information between different cortical areas. The relevance of the thalamus, at least an obvious one, is that most sensory inputs coming from the sensory organs will reach the cortex through it. An exception are the olfactory inputs which relay directly to the olfactory bulb, a structure whose functions resemble those of thalamic nuclei (Kay and Sherman, 2007). In other words, our representation of the world is, at some level, relayed and modulated through the thalamus.

Relay neurons in the thalamus display two types of channel gates, Na^+ and T-type Ca^{2+} gates (Sherman, 2009). Depending on their gate type, thalamic neurons also present different refractory periods and resting potentials. This diversity of gate types contributes to the complex burst and tonic firing patterns in thalamic neurons, these firing patterns likely add up computation power to the processes involving these nuclei.

Inputs to the thalamus differ in nature. In general, thalamic inputs can be classified in one of two categories: drivers and modulators (Sherman and Guillery, 2002; Guillery, 2005; Sherman, 2009). A driver input is one that carries a message from one region to another. A modulator, on the other hand, has the function of modifying the message carried by a driver without having a message on its own. Modulators represent most of the synaptic inputs to the thalamus from the cortex. Driver information can come from peripheral systems, as the

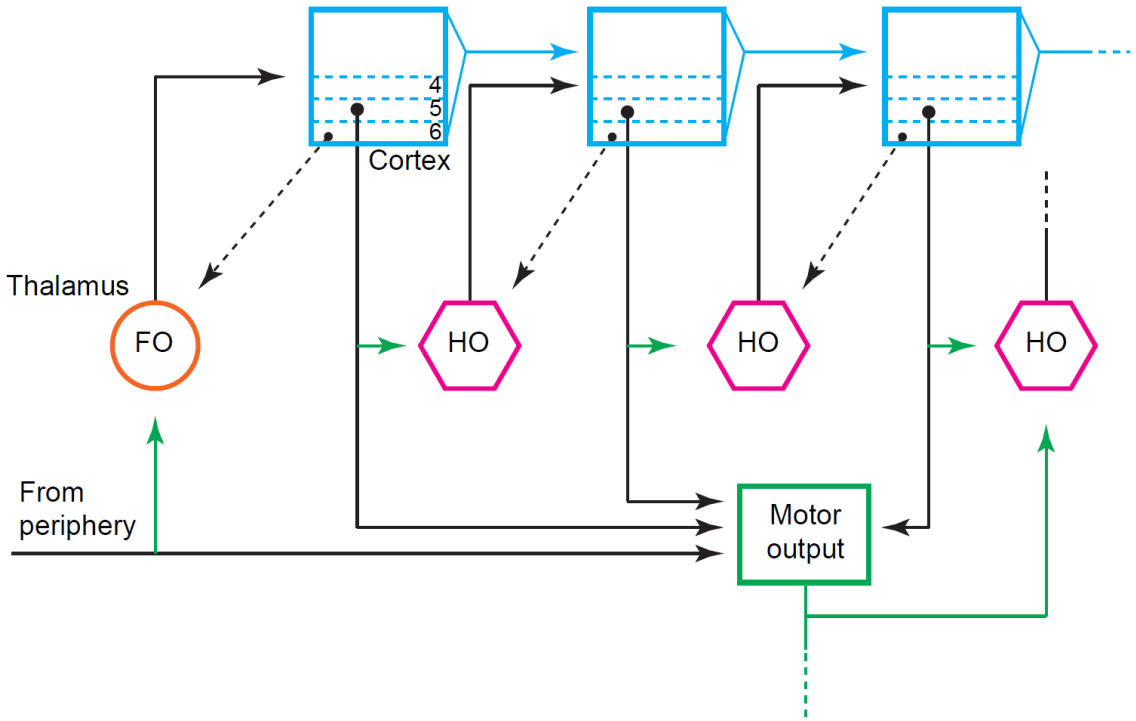
retinal inputs that reach the lateral geniculate nucleus (LGN) for vision, or directly from the cortex, creating cortico-thalamo-cortical loops.

Inputs to the thalamus from the cortex or peripheral nervous system end up in two types of nuclei, first order and higher order nuclei. First order thalamic nuclei carry information that will reach the cortex for the first time, either from sensory organs or from other sub-cortical structures. A classic example of first order thalamic nuclei is the LGN, whose driving input comes directly from the ganglion cells in the retina. In contrast, higher order thalamic nuclei receive driving inputs directly from the cortex and not from the peripheral nervous system. An example of higher order thalamic nuclei is the pulvinar complex, whose anatomical connectivity largely comprises cortical areas belonging to the ventral and dorsal visual streams. Even though both, first and higher order thalamic nuclei, receive cortical inputs, the nature of such inputs varies. The LGN as well as other first order (and higher order) thalamic nuclei receive inputs from layer 6 of the cortex, which are of modulatory nature, while the pulvinar and other higher order thalamic nuclei additionally receive inputs from layer 5, which carries driving information via the thalamus to cortical areas and also branches off to subcortical motor regions such as the basal ganglia, and the amygdala.

For some time now, there has been an effort to leave behind the preconception of thalamic nuclei acting solely as relay areas, and they are now seen to be involved in other functions, such as a central role in cortico-cortical communication (Sherman and Guillery, 2002; Guillery, 2005; Sherman, 2009; Saalmann and Kastner, 2015; Sherman, 2016). It has also been shown that sensory-motor pathways involving the thalamus present an additional pathway to motor centers, e.g. spinal cord and the brain stem (Sherman and Guillery, 2011) (**Figure i.2**). The duplication of information might be the basis for the optimization of complex sensory-motor commands. Under the perspective of optimization and integration, of interest is the thalamic pulvinar, connected to practically all areas in the primate cortex, which can serve as a good proxy for the study of complex and goal-directed processing taking place in the thalamus. It is important to note however that a large portion of the anatomical work providing insights in thalamic function (from Sherman, Guillery and

colleagues) has been performed in rodents, and further exploration of thalamic properties in primates are still highly valuable.

Figure i.2 **Corticocortical and cortico-thalamo-cortical pathways**



Information from the periphery travels to the cortex and to motor centers via the thalamus. For first and high order thalamic nuclei (orange circle and magenta hexagons respectively) there are projections from cortical layers 6, and 5 and 6 respectively. An additional pathway sends projections directly to motor centers (brain stem and spinal cord). From (Sherman and Guillery, 2011). *FO, first order thalamic nuclei; HO, high order thalamic nuclei; black solid arrows, feedforward connections; black dotted arrows, feedback projections (these are also modulatory inputs to thalamus); green solid arrows, inputs to the thalamus*

i.3 Pulvinar complex

The thalamic pulvinar is located in the posterior pole of the thalamus. In primates, it shares broad connectivity to association areas in the cortex. The pulvinar has greatly

expanded during primate evolution in comparison to other thalamic nuclei. It represents about a quarter of the total mass of the thalamus. The development of both association areas in the cortex and the pulvinar has been proposed to be linked to enhanced cognitive functions in primates (Stepniewska, 2004). Even though the pulvinar has expanded in primates, pulvinar-resembling structures, particularly of visual nature, can be found in all mammals under different names, e.g. the lateral posterior nucleus (Kaas and Lyon, 2007).

Pulvinar is regarded as a high order thalamic nucleus; however, in some animals it has been found that pulvinar also receives input from peripheral systems. In the galago, the superficial layers of the superior colliculus carry information from the optic tract to the caudal pulvinar (Harting et al., 1973). Along the same line, there have been observed in the common marmoset anatomical connections from the retina to the inferomedial subdivision of the pulvinar. In the macaque retinal inputs have been traced to the inferior pulvinar (O'Brien et al., 2001). These findings are interesting as they position the pulvinar not only as a high order but also a first order thalamic nucleus (Warner, 2010). As the evolutionary development of the pulvinar came hand in hand with the parallel development of the neocortex in primates (Ogren, 1982) and complex behaviors, it is interesting to hypothesize about which of pulvinar populations were of most recent development.

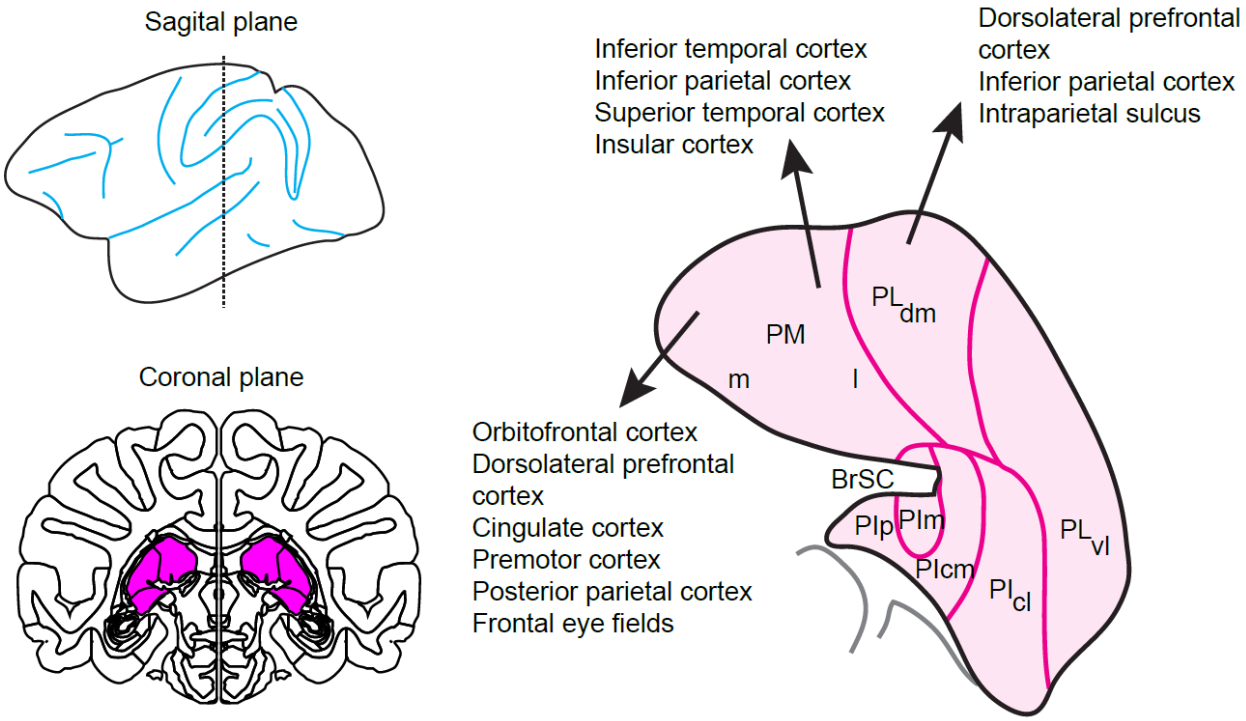
A recurrent problem for the characterization of pulvinar functions arises from the extensive connectivity of pulvinar to the cortex, and its lack of clear anatomical organization (**Figure i.3**). There have been efforts to understand how the pulvinar is organized at a microscopic and macroscopic level. Early on, the pulvinar was anatomically divided into: 1) an anterior region connected to somatosensory cortical areas, the oral or anterior pulvinar. More caudally, the pulvinar was divided to 2) inferior, 3) lateral, 4) and medial nuclei. The more ventral and lateral regions of the pulvinar are mainly connected to early visual cortical areas. Mediodorsally, the pulvinar shows stronger connectivity to a multitude of cortical areas in the parietal, frontal, orbital, and cingulate cortices (Grieve et al., 2000).

The division of pulvinar to a ventral and a dorsal region is anatomically facilitated by a dense branch of fibers known as the "*brachium*" of the superior colliculus. This separation

however is not clear-cut, as according to certain parcellations (Gutierrez et al., 2000; Kaas and Lyon, 2007), the inferior pulvinar slightly extends above the brachium. The dorsal pulvinar is the least understood of the two largely due to the complexity of its anatomical organization. The dorsolateral pulvinar is connected to parietal cortex and dorsolateral prefrontal cortex. The dorsomedial pulvinar is connected to extrastriate area V4, inferior temporal cortex, and posterior parietal cortex as well as auditory and somatosensory areas. In addition, the dorsomedial pulvinar also connects to higher order processing areas such as the superior polysensory cortex and the amygdala (Gutierrez et al., 2000; Kaas and Lyon, 2007).

Recently, immunohistochemistry has helped refine anatomically-described segmentations of pulvinar made possible by cytoarchitectonics, as it can aid to the targeting of specific molecules and neurotransmitters from the area of interest, which potentially share similar functional properties (Stepniewska, 2004). By its chemoarchitecture, up to nine different subdivisions have been identified in pulvinar. Regardless of the classification method, it has been consistently reported that there is a gradient of connectivity in the pulvinar. The ventrolateral region is bidirectionally connected to early striate and extrastriate visual areas while the most dorsomedial regions are connected to higher processing cortical areas.

Figure i.3 **Subdivisions of the macaque pulvinar**



Localization of the thalamic pulvinar and main connectivity of its dorsal subdivisions in the macaque. Left: sagittal (top) and coronal (bottom) views of the location of the pulvinar complex in the macaque brain. Right: pulvinar subdivisions and connectivity of the dorsal subdivisions. Macaque brain sagittal template from: (Culham and Kanwisher, 2001). Pulvinar modified from: (Stepniewska, 2004; Kaas and Lyon, 2007). Macaque brain coronal template from: https://scalablebrainatlas.incf.org/macaque/CBCetal15. Connectivity also from (Asanuma et al., 1985; Romanski et al., 1997; Cappe et al., 2009). *BrSC, Brachium of the superior colliculus; PMm, medial subdivision of medial pulvinar; PMl, lateral subdivision of medial pulvinar; PLdm, dorsomedial subdivision of lateral pulvinar; PLvl, ventrolateral subdivision of lateral pulvinar; PIcl, central lateral nucleus of the inferior pulvinar; PIcM, central medial nucleus of the inferior pulvinar; PIm, medial nucleus of the inferior pulvinar; PIp, posterior nucleus of the inferior pulvinar.*

In addition to its bidirectional projections to the cortex, the pulvinar also receives inputs from the superior colliculus. The superficial layers of the superior colliculus project to the more ventral parts of pulvinar while intermediate layers project to the dorsal regions (Grieve et al., 2000).

Regarding pulvinar's development, pulvinar seems to have different ontogenetic origin in humans and monkeys (Rakić and Sidman, 1968; Ogren and Rakić, 1981). In humans, the ontogenetic development of pulvinar starts late compared to other thalamic nuclei, with the largest stream of pulvinar cell increase around the gestational weeks 31 to 37. The late development of pulvinar comes as the result of late migration of telencephalic cells from the *corpus ganglio thalamicus*, a temporary brain structure in the human fetus. Reports from experiments using supravital incorporation of triated thyamidine into DNA in human embryonic cells of 18.5 week fetuses, showed that the ependymal of the third ventricle of the diencephalon ceases neuron production while the human pulvinar is only recently developing (Rakić and Sidman, 1969). In other words, the diencephalic structure participating in pulvinar's development does not account for the large mass of cell bodies that it encompasses in humans.

In the Rakić study of 1968 it was shown that the *ganglionic eminence* of the telencephalon, which gives rise to the basal ganglia, also sends migrating cells to a temporary structure, the *corpus gangliothalamicus*. In the *corpus gangliothalamicus* a second stream of pulvinar development begins later; at 13 weeks there is a small pulvinar which development accelerates from weeks 16 to 37 (Rakić and Sidman, 1969). This late stream of telencephalic pulvinar cells has been found only in humans, as experiments using *Macaca mulatta* have shown that all cells in their pulvinar are of diencephalic origin (Ogren and Rakić, 1981).

The difference in developmental origin of pulvinar poses an interesting question of how much is possible to extrapolate structural and functional findings of the macaque pulvinar to the one in humans. Still, even with differences in ontogeny, the pulvinar in different primate species seems to have similar connectivity properties, which agree with a shared evolution and recent separation from a common primate ancestor no more than 25 million years ago (Buckner and Krienen, 2013).

Speculation of pulvinar relevance and function has been around for some time. The chimpanzee's pulvinar, both in absolute and relative terms, has an intermediate size

between macaques and humans. Connectivity data obtained after chimpanzee hemidecortication has shown complete degeneration of three main subnuclei of the pulvinar after 69 days of survival (Walker, 1938). This is anatomical evidence of a shared connectivity between the neocortex and the pulvinar. Even when in this study other nuclei in the thalamus suffered similar degeneration, this was not a generalized effect. Additional reports have demonstrated pulvinar connectivity to high order brain areas such as the temporal lobe, assessed by retrograde degeneration of medial pulvinar after localized cortical lesions. These results have been shown to be true in non-human primates, as well as in humans as noted by pathology observations (Simpson, 1952).

The rich connectivity of the pulvinar, particularly of the dorsal region with the fronto-parietal network, makes the pulvinar outstandingly interesting to explore in the context of visually influenced goal-directed behavior. Even with its anatomical connectivity to high level processing areas, still not much is known about pulvinar´s role in relation to its cortical counterparts.

i.4 Pulvinar functions

It is a hard task to characterize a structure such as the pulvinar. Different, thoughtful and elegant experimental designs have been used and their findings have been broad but not conclusive. A multitude of functions have been correlated to pulvinar, mainly involving the use of visual information. For the ventral pulvinar, its functional properties seem to reflect visual properties coming from its inputs in early visual cortices. For the dorsal pulvinar on the other hand, its functions seem to be broad, and behavior dependent.

Some of the most relevant findings of pulvinar function for this thesis are described in this section; however, it is worth pointing out that especially when talking about cell encoding properties, a great diversity is the common denominator.

The ventral subdivision of the pulvinar presents visual related activity (Petersen et al., 1985; Robinson et al., 1991) congruent to its connectivity to striatal, extra striatal, and to superficial layers of the superior colliculus. Such activity seems to exist under the influence of a retinotopically organized reference frame that is additionally modulated by the eye position (Robinson et al., 1990). It is important to note that even in this subdivision of the pulvinar, connected to early visual areas, and to a lesser extent, to higher order brain regions, the influence of reference frames (other than eye-centered) is already present. It would be interesting to explore if there is any specificity of the influence of reference frames in ventral and dorsal subdivisions of the pulvinar according to their connectivity. If connectivity plays a role in the coordinate system influencing pulvinar it would not be surprising that for example dorsal pulvinar shared similar characteristics as posterior parietal or frontal cortices. Biological systems are usually not compartmentalized in functions however, for example, area 7a and LIP in the macaque, strongly connected to dorsal pulvinar, share similar gaze position influences in firing rate to ventral pulvinar (Asanuma et al., 1985; Andersen et al., 1990).

At least two retinotopic maps in the ventral pulvinar have been found, in the ventrolateral nucleus of the lateral pulvinar, and in the central lateral nucleus of the inferior pulvinar. The retinotopic maps in the lower part of the pulvinar mainly represent contralateral upper visual quadrants. Still, it is possible to find non-retinotopically organized regions. These regions correspond to the posterior, medial and central medial nuclei of the inferior pulvinar which are connected to the dorsal stream. Retinotopic maps have not been found in dorsal pulvinar. Even with a gradient that favors ventral pulvinar connections to the ventral stream and dorsal pulvinar to the dorsal stream, there are areas in both subdivisions connected to the opposite stream.

In the greater galago, *Otolemur garnettii*, pulvinar exerts strong influences in early stages of visual processing (Purushothaman et al., 2012). By pharmacologically inactivating lateral pulvinar neurons with matching receptive fields in supra granular layers of V1, V1´s receptive fields become unresponsive to visual stimulation. Additionally, lateral pulvinar has differential modulatory effects on V1´s receptive fields according to their level of overlap. When pulvinar-V1´s receptive fields overlap there is an enhancement of responsivity in V1. When pulvinar receptive field is stimulated but only partially matches V1´s receptive field, the V1 cell modifies its receptive field to one resembling the one in pulvinar. If on the other hand the receptive field of V1 is stimulated but does not match the one with excited pulvinar there is a suppression in V1, demonstrating strong modulation of pulvinar in early visual cortices.

It has been proposed that two subcortical regions are involved in the control of attention, the superior colliculus and the pulvinar (Shipp, 2004). There is evidence that ventral pulvinar regulates information transmission between cortical areas by regulating brain oscillations. In an attentional task, pulvinar exerted influence in cortical alpha oscillations in areas V4 and TEO as assessed by conditional Granger causality (Saalmann et al., 2012). After ventral pulvinar inactivation, cortical area V4 showed a reduction of gamma frequency oscillations during an attentional task as well as of visual stimulation responses. Additionally, inactivation of pulvinar increased low frequency oscillations in V4 in the range of 0.5 Hz to 20 Hz (Zhou et al., 2016) which the authors suggest could be linked to a

role of pulvinar on alertness. Taken together these results provide correlational and causal evidence on the influence of pulvinar-driven oscillations on attentional and alertness processes.

Petersen and collaborators (Petersen et al., 1985) characterized the neuronal activity of multiple regions of the pulvinar nuclei in the macaque. They found that the inferior and lateral pulvinar, and the dorsomedial pulvinar had marked differences in their responsiveness to visual stimuli and to behavioral tasks. The dorsomedial pulvinar had longer latencies to visual stimuli and a stronger modulation to attentional tasks when compared to the more ventral subdivisions. A later study from Petersen and collaborators (Petersen et al., 1987) looked at the causal participation of the dorsomedial pulvinar in behavioral tasks. Monkeys were tested in fixation, saccade, and target detection tasks using bar releases. As the experimenters aimed to see if dorsomedial pulvinar was causally linked to attentional performance they used GABA agonists and antagonists (muscimol and bicuculline respectively) to assess changes in attention. Using a task which involved a congruent or incongruent spatial presentation of cues and targets before and after the injection of drugs, it was observed that muscimol had an impeding role in attentional shifts while bicuculline had a facilitatory effect. The findings from the electrophysiological and causal studies from Petersen and collaborators suggest that the functions of the different subdivisions of pulvinar well correspond to the established ventral and dorsal visuo-motor streams.

Opposite to the strong influence of visual inputs to the ventral pulvinar, in the dorsal pulvinar of monkeys goal-directed behavior seems to play a larger role than vision. In the caudal lateral part, receptive fields tend to be large (>12°) and often extend from foveal vision to the periphery (Benevento and Miller, 1981). Receptive fields are commonly found in the contralateral hemispace to the recorded pulvinar and in fewer cases they are bilateral or ipsilateral. Visual responsivity of pulvinar neurons vary, neurons have been found to either be enhanced or suppressed in their firing rate, and even vary according to the type of visual stimulation (monocular or binocular).

The importance of goal-directed behavior for dorsal pulvinar is exemplified by findings in oculomotor tasks. In the dorsolateral and medial parts of the pulvinar of macaques, neuronal firing is modulated during purposeful saccades in either light or dark conditions, but not during spontaneous saccades (Benevento and Port, 1995). In this saccade or stay task there was firing attenuation to visual stimuli when the saccade was not required, on the other hand, when monkeys were presented with the same visual stimuli, but a saccade was not part of the task contingency, such attenuation was not present. The observation of goal dependent modulation in dorsal pulvinar will be of great importance in the context of results described in Chapter II. It seems from Benevento & Port's results, as well as from our own, that the task contingencies strongly influence the type of tuning that pulvinar cells display.

Another oculomotor study exemplifying the diverse tuning properties of dorsal pulvinar showed that around sixty percent of the cells in dorsomedial pulvinar are responsive to saccades in light (Robinson et al., 1986). Most of the cells responded with excitatory modulation, while some presented either inhibitory or biphasic modulation. Saccade related cells often had visual responsivity and some also responded to saccades in the dark. It has also been reported that both dorsal and ventral pulvinar, but not LGN, encode for the perceptual offset of a target evoked by a generalized flash suppression paradigm (Wilke et al., 2009). There seems to be a selective and more cognitively-driven modulation in pulvinar firing than in LGN.

As stated before, dorsal pulvinar neurons are responsive to visual stimulation, but more than that, they are sensitive to the behaviorally relevant parts of it. A recent line of research has focused on the effects of visual stimuli that might have been of evolutionary relevance for primate-specific behavior in pulvinar. It has been observed that neurons in the medial and dorsolateral pulvinar of monkeys are more responsive and show shorter visual response latencies when subjects are presented with threatening stimuli in comparison to neutral stimuli (Van Le et al., 2013). These neuronal properties could have the purpose of facilitating the generation of an appropriate motor response.

A few studies have focused on the functions of the pulvinar in humans, mostly by means of neuroimaging. Using fMRI it has been shown that in inferior pulvinar of humans there is contralateral representation of visual stimuli, whether attended or unattended (Cotton and Smith, 2007), resembling findings of the visually responsive and retinotopically organized inferior pulvinar in the macaque. Additionally, a different fMRI study (Li et al., 2012) showed that pulvinar activation and connectivity is likely to be linked to attention related changes in children. Subjects with ADHD which performed a sustained visual attention task displayed decreased connectivity of both left and right pulvinar to the right prefrontal lobe. Also, the connectivity between right pulvinar and both occipital cortices was increased, suggesting a circuit of attention that requires pulvinar function. Not only visual representation and attention have been linked to pulvinar function. Arend and collaborators have explored different behavioral aspects of patients after pulvinar damage. They have found that that the medial subdivision of the pulvinar is related to emotional features of working memory updating (Arend et al., 2015). They have also proposed a separation of temporal and spatial deficits depending on the anterior-posterior location of the damage, i.e. greater spatial deficits after anterior pulvinar damaged and greater temporal deficits after posterior pulvinar damage (Arend et al., 2008). It is particularly difficult to investigate deficits that are specific to pulvinar in humans because patients with such lesions commonly have damage extending to other thalamic nuclei or even to the cortex. Van der Stigchel and collaborators tested pulvinar-damaged patients with a distractor task. There, there was decreased filtering of distractors in the contralesional hemispace when simultaneously presented with an ipsilesional target, as well as increased reaction times for target captures in both hemispaces. Additional deficits were found while exploring saccade inhibition. In this task, normal subject saccade trajectories usually go “away” from distractors when acquiring targets. Here, it was found that there was reduced inhibition to distractors presented in the contralesional hemispace (Van der Stigchel et al., 2010).

In addition to correlation studies using single cell recordings, a few inactivation studies have been performed to study the causality of pulvinar function on behavior. Interestingly, in the two studies that will be described below, the effects seemed to be context-dependent. First, researchers inactivated the dorsal region of the pulvinar with either muscimol or

THIP while monkeys performed 1) visually-guided, direct or delayed, instructed or choice, saccades to peripheral targets, 2) reaches to food items with either the ipsilesional, contralesional, or either hand, relative to the inactivated pulvinar and 3) visual exploration in an illuminated room (Wilke et al., 2010). It was observed that after inactivation there is facilitation of reaction times to saccades to the ipsilateral hemispace of the lesion. In addition, there is increased target selection of ipsilesional targets even when the acquisition of non-preferred targets in single-target trials is unaffected. These inactivation effects suggest that dorsal pulvinar is indeed not causally involved in visual perception but that it participates in target selection. In the same study, during free visual exploration, the ipsilesional hemispace was explored for longer periods of time, which might reflect reduced desirability of the contralesional hemispace. Furthermore, when choosing between hands, after pulvinar inactivation, monkeys preferred reaching and grasping items by using their ipsilesional hand rather than the contralesional one, and items in the ipsilesional hemispace were more often acquired first. Grasping made with the contralesional hand was observed to be more impaired than when using the ipsilesional hand, Errors in grasping included abnormal hand pre-shape when reaching for food items, and frequent drop of such items.

As a follow up, the authors performed a memory-guided saccade choice paradigm study, where monkeys' dorsal pulvinar was inactivated with THIP as they were allowed to choose between target options with different reward amounts (Wilke et al., 2013). The observation of ipsilesional bias after inactivation was present as in the previous study, however, the deficit was alleviated by offering higher-reward targets in the contralesional hemispace. Again, these effects seemed to be particular of the choice condition, since monkeys were still able to perform saccades to the contralesional hemispace in single target trials. To differentiate between desirability or motivational and saliency effects of the reward the authors performed an additional experiment. In this experiment they modified the luminance of the two saccade targets without modifying the reward that each target provided. They observed that even though there was partial alleviation of the deficit, the effect was not as large as when the parameter modulated was the reward amount. It is important to note that the authors did not try to match the bias created by the luminance

change and reward modulation, which makes a direct comparison of results difficult, especially under the light that both modulations caused at least at some degree, an alleviation of the ipsilesional bias. Wilke and collaborators' findings suggest that goal-directed related mechanisms and not bottom-up ones better explain the deficits observed after pulvinar disruption.

Finally, two groups have worked on the characterization of the neuronal properties of pulvinar during reach behavior, one in monkeys and one in humans.

In *Macaca fascicularis*, the lateral posterior-pulvinar complex has reach-related neurons (Acuña et al., 1986). But even when the lateral posterior nucleus and the pulvinar present reach-related neurons, they do not show similar characteristics. While lateral posterior neurons were found to be largely active not only during active but also during passive reaches, pulvinar firing rate was only increased while the monkeys were actively performing the task. The pulvinar cells responsive to reaches in this study were located mainly in the oral and lateral pulvinar, and in smaller proportion in the medial pulvinar. In *Cebus apella* the oral, lateral and medial pulvinar are also responsive to reaches and hand manipulation (Acuña et al., 1983), particularly, cells were modulated when the object to be reached and grasped was of behavioral interest, like a piece of fruit or a target that would be followed by reward. In *Macaca nemestrina*, a small group of cells in the pulvinar-lateral posterior complex precede activity in the parietal and motor cortices, potentially indicating intentionality to perform a movement (Cudeiro et al., 1989)

There is one early electrophysiological study from preoperative recordings of medial pulvinar units from seven patients (Martin-Rodriguez et al., 1982). The authors recorded spontaneous spike activity, as well as LFPs during manual manipulations. The authors found different patterns of pulvinar bursts depending on if the patients performed active or passive grasping. Forty six percent of the units (13/28) showed firing modulation during voluntary handle presses but not during passive presses. These results in humans are in line with the findings of Acuña's group in monkeys.

The purposeful performance of reaches could be an appropriate tool to study higher pulvinar functions. Thus, a general understanding of reach-related circuits and the transformations of the visual information guiding it might prove to be enlightening.

1.5 Eye-hand representations and interactions

Our ability to perform goal-directed actions requires a broad brain circuitry. A s*imple* task such as turning a page from a newspaper requires in broad terms: 1) Spatial information about the location of the newspaper. 2) Information about our own position in respect to the newspaper to reach and grasp it. 3) Spatial transformations from a purely retinotopic representation of the newspaper in the visual cortex to one that considers the eye position in the orbit, the head position in respect to the body, the body in respect to the hand, and the hand in respect to the object. 4) And a central motor system that delivers precise signals to motor neurons in the periphery and can be updated by feedback according to the current state of the action.

The reach system of primates, often guided by visual information, as the rest of the brain, was optimized by interactions with our environment. In lemurs for example, the development of occipital and temporal lobes is linked to an increased use of their visual system as arboreal organisms requiring intensive processing of visual information (Harting et al., 1973). Likewise, efficient prehension is achieved by our primate-shared skills for reaching and grasping, for which we do not rely only on visual inputs, but also on the functional and semantic properties of the objects to be manipulated. It has being proposed that the temporal cortex could be linked in the determination of causality of object-function interactions, while parietal areas is more involved in a broader manipulation of unfamiliar tools (Johnson-Frey, 2003). Brain areas linked to efficient usage of objects are widely represented in frontal, parietal and temporal cortices (Frey, 2007). Actions that involve complex behavior where semantic information is needed to interact with the objects to be grasped have been found to be lateralized to the left hemisphere in humans in areas such as

the inferior frontal, inferior parietal, and posterior temporal cortices. Shared skills in humans and other primates for the planning and execution of reaches make the reach system an attractive one to study purposeful actions in a monkey model. Reaches might function under general primate-specific rules, not as strongly influenced by further specializations like semantic information of the object to be grasped as proposed for humans (Johnson-Frey, 2003; Frey, 2007).

Within the flow of information for the planning of visually- and internally-guided purposeful movements the parietal cortex is of special interest. Areas in the posterior parietal cortex (PPC) encode effector specific movements i.e. eye movements in the lateral intraparietal area (LIP) (Colby et al., 1996; Snyder et al., 1997), visually-guided reaches in area V6A (Galletti et al., 1997) and parietal reach region PRR (Snyder et al., 1997), and grasping, area 7 (Taira et al., 1990), and AIP (Sakata et al., 1995; Murata et al., 1996, 2000). In parallel, areas in the prefrontal cortex with broad connectivity to the motor cortex and to parietal areas are involved in effector specific motor preparation (Caminiti et al., 2015).

Visual information coming from the environment is represented in a retinotopic fashion in the primary visual cortex. In other words, there is a relation between neighboring parts of the visual field and their representation in neighboring areas in the retina responsive to these visual stimuli. As visual information travels through the dorsal stream, this representation will be transformed to account for the location of the image in relation to the eye position, the eye position to the head, the head to the body, and the body to the physical location of the object. All these transformations seem to be well distributed across the primate's brain circuitry.

In visually-guided reaches, it is especially relevant to assess at which level of the visuo-motor hierarchy the spatial transformations take place. It has been proposed that association areas might account for such transformations. Eye- and hand-movement-related activity can be found in the parietal cortex, and the spatial transformations influencing such movements have been explored. It has been shown that area LIP has a retinotopic organization which is

influenced by the gaze position of the observer, showing transformations accounting for more than pure visual information (Andersen et al., 1990). Oculo-motor activity in areas 7a and LIP of the macaque are modulated by the current and even future gaze location of the target in respect to the cell's receptive field (Andersen et al., 1985; Duhamel et al., 1992). Furthermore, a large proportion of movement related neurons in the ventral premotor cortex (PMv), around 40%, are modulated by the direction of gaze during the execution of memory-guided reaches (Mushiake et al., 1997). There, the modulation of neuronal firing was influenced by the position of the target in respect to the eye fixation and not to the position of the target in respect to the center of the trunk of the subject.

These findings could be seen as a consequence of strong visual influences acting on posterior parietal cortex, which seem to integrate retinal and orbital signals, as during reaches, parietal cells better correlate with eye-centered than with limb-centered reference frames (Batista et al., 1999). Importantly, as neuronal signals travel upstream to motor and premotor regions like dorsal premotor cortex, the reference frames better represent the specific effector that will be used for performing a reach, i.e. using a body centered reference frames (Beurze et al., 2010).

Effector specificity is very relevant in association areas, neurons in PPC are highly specific and even encode the limb used when one of two arms is instructed to perform a reach (Chang and Snyder, 2012). This limb specific preference is higher for the limb contralateral to the recorded hemisphere (approximately one third versus one sixth of cells for the contralateral and ipsilateral limb respectively). Interestingly, this firing rate enhancement has also been correlated to the reaction time of contralateral but not ipsilateral reaches.

Movement related neurons in the posterior parietal cortex reflect intentional components of performing an effector-specific movement (Snyder et al., 1997). The neuronal coding of a preferred direction for a saccade or a reach in monkeys trained to perform dissociated saccades and reaches can be dissociated for one of the effector-specific movements. This result shows that activity in the posterior parietal cortex best reflects motor intention than visual stimuli or spatial attention. Finally, neurons in effector-specific regions of the

parietal cortex that encode properties of different effectors but do not reflect planning of such movements could indicate a crosstalk between effectors that is potentially relevant for eye-hand coordination or to help represent movement goals (Snyder et al., 2000b).

Some relevant behavioral observations are that for our reach system to efficiently work, it requires to integrate visual, and proprioceptive information (Prablanc et al., 1979b). In a series of experiments in humans set to test how the availability of visual information modifies the execution of eye- and arm-movements, Prablanc and collaborators made several valuable observations. Both eye and hand reaction time increase with eccentricity of the targets to acquire, and for targets located farther than 30 deg the coordination properties of eye and limbs seem to differ, i.e. eye movements tend to start later than the reach. Also, there is a decreasing performance depending on the availability of visual information of the target and the hand while performing a reach. If the hand and eye are visible during the full trial there is a better performance than if the information is available only from the start of the movement and finally better than performing a reach with proprioceptive information only (Prablanc et al., 1979a, 1979b). Visual information optimizes the reach and this optimization can be further improved by adding visual information of the effector used to the proprioceptive inputs. The idea of multimodal integration as a way to improve goal-directed behavior has recently been confirmed by Dadarlat and colleagues (Dadarlat et al., 2014). By stimulating primary somatosensory cortex, it has been shown that monkeys have more accurate reaches when they use a combination of artificial proprioceptive and visual information than when using either type of information in an individual manner.

Under most conditions reach behavior is linked to oculomotor behavior, and there are influences of one on the other. An interesting example of these influences has been shown with a look and point, versus look and grasp paradigm in humans, where the purpose of a limb movement influenced saccadic performance (Bekkering and Neggers, 2002). Subjects presented with a rectangular sample block with a certain color and with orientation were required to find a match among distractors for either one or both parameters and either grasp the target or point at it. Subjects did more saccade orientation errors, more saccades to non-matching orientation distractors, when the task required them to point in comparison

to when they were asked to grasp the object. In contrast, subjects had similar errors to saccade to erroneous colors regardless of if the task required them to point or grasp the target. These results show that the cognitive weight of the arm movement can improve the performance of saccadic behavior. The involvement of a reach also dominates which targets will be looked at (Horstmann and Hoffmann, 2005). It has been shown that the selection to a target with a coordinated reach-saccade is more strongly correlated to the selection to a target doing dissociated reaches than using dissociated saccades. These findings stress the notion that a saccade is not an independent movement once it's coupled to a reach. Also, it has been shown that humans' reach reaction time to a congruent side of the reaching limb is shorter and deviate less from the target center than when the reach is performed to the contralateral side (Carey and Liddle, 2013). The parameter that better explains such ipsilateral reaction time advantage is the hemispace to which the reach is performed and not the hemifield. This result shows that the biomechanical restrains to the ipsilateral hemispace are smaller than the ones to the contralateral hemispace, regardless of where the visual stimuli are presented.

I have stressed the tight interactions of different effectors involved in visuomotor behavior, and how several regions in association cortices are involved in the planning and generation of visually-guided reaches. A convincing case to link neural and behavioral findings on reach-related behavior is the observation of visuo-motor deficits after parietal disruption like the ones reported by Hwang and collaborators in a monkey model of optic ataxia (Hwang et al., 2012). Optic ataxia is a deficit often present in patients who suffered damage to the parietal cortex (Andersen et al., 2014). Optic ataxia´s defining characteristic is an increased difficulty to perform extra foveal reaches, while foveal reaches appear less impaired, and it often involves damage to the parietal cortex in human patients.

In addition to the classic characteristics of optic ataxia, patients with posterior parietal damage present difficulties for rapid visuomotor control (Gaveau et al., 2008). In a task looking at the role of timing on reach performance control subjects and optic ataxia patients were asked to acquire a target whose location was synchronously updated either with the onset or offset of a saccade. If the visual update occurred by the onset of the saccade

controls could reprogram their movement while optic ataxia patients could not; presenting 1) hypometric saccades followed by additional corrective saccades or 2) delayed saccades with slightly better accuracy. In contrast, when the target update happened at the offset of the saccade, both controls and patients presented the same deficits as the patients in the first experiment, showing that optic ataxia is likely linked to a disruption in the update of visual information. It has also been reported that in optic ataxia the absolute location of the target is not the determining factor for the strength of the deficit (Khan et al., 2005). By asking patients to either reach to a remembered target in the ipsilateral or contralateral side, subjects had more reach errors to the contralesional side of space. However, when subjects were asked to make a reach to a remembered location from an updated eye position in the opposite hemispace it was observed that the errors depended on where the target was relative to the updated location of the eye. This finding showed how parietal cortex plays a role in the integration of dynamic multi-effector actions. In summary, ataxia seems to reflect a disruption in the dynamic integration of eye position and reach planning, and it's heavily dependent on damage to brain regions within the dorsal stream

I have elaborated on how the parietal cortex is part of a complex limb and eye movement network. I have focused on this lobule and not on the prefrontal cortex as the parietal cortex is located earlier in the visuo-motor hierarchy, thus being strongly influenced by early sensory and proprioceptive inputs (Snyder et al., 1997; Caminiti et al., 2015). Parietal cortex is tightly linked to several brain areas linked to eye and hand movement generation, and some of these connections are likely to have relays via the dorsal pulvinar. This connectivity suggests that similar functions might be encountered in both regions. Finding how the least understood region of this circuitry, the dorsal pulvinar, functions and interacts with the cortex will help us to add more pieces to our understanding of how goal-directed behavior is generated in the primate brain.

i.6 Chasing function || Research rationale

Even though the initial purpose of my thesis was to explore the role of the dorsal pulvinar in eye-hand coordination, pulvinar is a region of possibilities for cognitive exploration and as it's often the case in science, a finding leads to new questions. To address my main aim, I narrate different aspects of pulvinar function in three independent but interconnected chapters.

In *Chapter I*, we[1] aimed to confirm pulvinar's involvement in saccade target selection. For this purpose, we electrically stimulated dorsal pulvinar, which is known to bias saccades to the ipsilesional hemispace when inactivated (Wilke et al., 2010, 2013). Our findings, however, revealed a more complex and time-dependent effect that varied with the time of the stimulation. There was indeed a target selection bias effect, but this effect ranged from increasing choices to the ipsiversive hemifield when stimulating early in the trial to the expected contraversive choice increase when stimulating closer to the saccade execution. The choice findings were accompanied by biphasic reaction time effects that were specific for dorsal pulvinar as compared to other pulvinar subdivisions. These results suggest that the participation of dorsal pulvinar in purposeful behavior might be more complex than previously thought. To test this hypothesis, we made an initial assessment of the electrophysiological properties of pulvinar cells during saccades. We found a great variety of firing rate modulation properties in dorsal pulvinar. Neurons were modulated around the saccade onset, during the saccade, or around the offset, as well as during the movement preparation. This activity could be larger to the contralateral or ipsilateral hemispace and could be of facilitatory or suppressive nature with a great diversity of receptive fields. A similar range of neuronal responses as the ones in our study has been reported in early studies (Benevento and Port, 1995). In addition to the inspection of pulvinar properties during saccades to single targets we recorded the same units while monkeys performed saccade choices and saccades to single targets from different starting fixation positions to

[1] Chapter 1 is a joint project with equal contribution of Adán Ulises Domínguez-Vargas and Lukas Schneider

deepen our knowledge on the participation of the region in target selection and spatial transformations.

In *Chapter II* and *III* I tackle the unified question of if and how pulvinar is involved in visually-guided reaches from two fronts. Chapter *II* is devoted to characterizing the electrophysiological properties of dorsal pulvinar neurons during volitional eye- and hand-movements. Previous studies have provided some evidence of pulvinar's involvement in motor behavior in different species. Such studies include models like *Felis catus* (Wei and Marczynski, 1979), *Macaca nemestrina* (Acuña et al., 1986; Cudeiro Mazaira et al., 1989; Acuña et al., 1990), *Cebus apella* (Acuña et al., 1983), *Macaca fascicularis* (Magariños-Ascone et al., 1988), and in human (Martin-Rodriguez et al., 1982). Even when previous studies have approach the question of how pulvinar encodes motor commands or the interactions between motor and visual (or auditory) stimulation, the topic is far from being fully resolved. In this study, the functional properties of pulvinar cells during coordinated and dissociated eye-hand tasks will be described, and it will probe if the region is involved not only in the representation of movements and visual inputs but potentially in the integration of such behavior. I will describe hand-specific tuning when a particular hand is expected to perform the movement and in some cases, complex interaction of eye and hand movements.

Finally, in *Chapter III* I will revisit the results of a study from our group (Wilke et al., 2010), with a similar array of tasks as for *Chapter II* to provide quantifiable data on the effects of dorsal pulvinar inactivation on the coordination of eye and hand movements.

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Chapter I

Electrical Microstimulation of the Pulvinar Biases Saccade Choices and Reaction Times in a Time-Dependent Manner

Contributions

AUDV, LS, MW and IK designed the project, AUDV and LS performed the stimulation sessions in monkey L and C. AUDV and LS performed analysis of behavioral data, AUDV and LG collected and sorted electrophysiological data, LS performed analysis of electrophysiological data, LG and IK collected imaging data, IK performed analysis of imaging data, IK and MW supervised the project, AUDV, LS, MW and IK wrote the manuscript.

Adán Ulises Domínguez Vargas (AUDV); Lukas Schneider (LS); Lydia Gibson (LG); Igor Kagan (IK); Melanie Wilke (MW).

The reader is referred to:

Appendix A (pp 182)

Electrical Microstimulation of the Pulvinar Biases Saccade Choices and Reaction Times in a Time-Dependent Manner

Manuscript as accepted by The Journal of Neuroscience
On December 30th, 2016

Chapter Ibis

(Addendum)

Electrophysiological properties of dorsal pulvinar during saccade behavior: gaze influence and target selection

Contributions

AUDV, LS, MW and IK designed the experiments. AUDV collected and sorted the dataset of Monkey L. LG collected the dataset from monkey C. AUDV and UZ sorted the dataset from monkey C. LS wrote the electrophysiology analysis scripts. AUDV and LS analyzed the electrophysiological datasets. IK supervised the project. AUDV wrote this addendum.

Adán Ulises Domínguez Vargas (AUDV); Lukas Schneider (LS); Lydia Gibson (LG); Uwe Zimmermann (UZ); Igor Kagan (IK); Melanie Wilke (MW).

Introduction

Goal-directed actions in primates often involve eye, arm and hand movements. Early visual areas follow a similar representation of the visual space as it was captured by the retina i.e. they show retinotopy. Later, this information is integrated with proprioceptive and spatial properties of the location of the objects in respect to the agent performing the action. To perform an efficient movement e.g. a visually-guided reach, we need information about the position of the target in respect to the retina, the eye in the orbit, the head on the body, and the limb position in space, and this information needs to be constantly updated as the visuo-motor plan evolves to program an efficient movement path, and kinematics (Batista et al., 1999; Snyder, 2000; Buneo et al., 2002; Crawford, 2004; Beurze et al., 2010). As primates, a large portion of our neural circuitry is devoted to vision and motor execution. It is not surprising that different brain areas represent spatial information using different reference frames as the hierarchy shifts from sensory to motor.

The thalamic pulvinar is positioned in a privileged spot, as it shares projections with a variety of visual and high order cortices as well as with subcortical brain regions. Ventrally it's connected to early striatal and extra striatal cortices, as well as to the superficial layers of the superior colliculus. Dorsally it mainly projects to areas in the fronto-parietal network and intermediate layers of the superior colliculus (Asanuma et al., 1985; Grieve et al., 2000; Stepniewska, 2004; Kaas and Lyon, 2007; Bridge et al., 2016). Fronto-parietal cortices are modulated during the planning and execution of goal-directed actions, with spatial signals encoded in an eye-, head-, or body-centered reference frames (Boussaoud and Bremmer, 1999; Andersen and Cui, 2009; Beurze et al., 2010).

Neurons in the posterior parietal cortex (area 7 and LIP) of macaques, known to present retinotopic representations of space, are also influenced by the current location of the gaze while the monkeys perform behavioral tasks (Andersen and Mountcastle, 1983; Andersen et al., 1990). This influence on the firing rate tends to be linear, and dependent on the

eccentricity and direction of the gaze location. The linear increase in the sensitivity of the response that does not influence the properties of what the neurons respond to is termed gain field (Salinas and Abbott, 2001). Gain fields are now known to be a generalized phenomenon across different cortical areas and are thought to reduce computations in areas influenced by different reference frames (Zipser and Andersen, 1988). In association areas LIP and MIP it has been observed that there can be mixed influences to visual and auditory stimulation with both eye- and head-centered reference frames (Mullette-Gillman et al., 2009). Not only cortical but also subcortical structures are part of the spatial transformations circuitry. It has been reported for example that the central thalamus also participates in the relay of eye position signals from subcortical structures to the cerebral cortex (Tanaka, 2007).

Also in the thalamus, there are reports of ventral pulvinar being relevant for spatial transformations. Robinson and collaborators (Robinson et al., 1990) found that visual responses in ventral pulvinar can be modulated by gaze position. The reference frame under which visual information is represented in dorsal pulvinar, is however still an open question. Answering this question could provide insights on how subcortical structures highly connected to association areas participate in spatial transformations for action-based behaviors.

Although there are several association cortices and subcortical regions involved in motor preparation, the processes taking place in such regions are rich and diverse, a few examples being: 1) The selection of motor plans in the frontoparietal network (Pesaran et al., 2008; Pastor-Bernier and Cisek, 2011; Shadlen and Kiani, 2013). 2) The integration of perceptual and motor properties found in the caudate nucleus (Yamamoto et al., 2012). 3) Target selection in saccade and reach tasks being related to dorsal pulvinar function (Wilke et al., 2010), 4) And the cognitive nature of such function (Wilke et al., 2013). Focusing on dorsal pulvinar, it has also being shown that dorsal pulvinar is involved in perceptual categorization tasks (Komura et al., 2013). Furthermore, it has been observed that electrical stimulation of dorsal pulvinar has time-dependent effects on saccade choices (*see Chapter I*).

Causal evidence suggests that dorsal pulvinar is involved in target selection. How is it involved in target selection at a neuronal level? And is dorsal pulvinar's firing influenced by the multiple coordinate systems that have been found to influence association areas? Here, we performed two additional experiments to characterize electrophysiological properties in dorsal pulvinar during purposeful oculomotor behavior. In the first experiment, single cells from dorsal pulvinar were recorded during a memory-guided saccade task in which the starting gaze position was shifted to one of three different locations before monkeys performed a center out saccade to one of eight peripheral positions relative to the fixation spot. Firing rates were analyzed in respect to the starting location, movement direction or physical location of the target, to speculate about pulvinar's place in visuo-motor transformation hierarchy. In a second experiment, single cells from dorsal pulvinar were recorded while monkeys performed a free-choice task like the one in *Chapter I*, to assess the role of the pulvinar in the processing of competing visual information and eye movement plans.

Materials and methods

The reader is referred to the Materials and Methods in *Chapter I* for aspects of animal preparation, as well as for general properties of the stimuli and electrophysiological analysis as Monkey C and L were also used for these experiments.

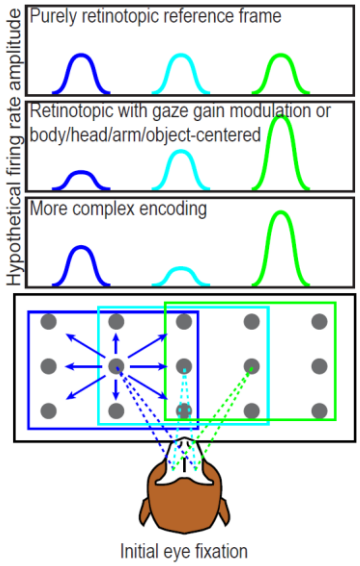
For the target selection task neurons from the left dorsal pulvinar of Monkey L are also included. For specifics of chamber planning in Monkey L's left pulvinar the reader is referred to the Materials and Methods in *Chapter II*.

Gaze modulation experiment

Trials were performed in pseudorandomized interleaved trials varying the initial fixation gaze position of the monkey **(Figure Ib.1)**. Monkeys had to perform memory-guided center out saccades to targets selected from a rectangular array with eight peripheral target positions. From the gaze center, peripheral targets were located -15 °, 0°, or 15° horizontally and -10°, 0°, or 10° vertically.

At the beginning of each trial, monkeys had to acquire a dim red fixation spot with a 1° diameter, within a 5° radius window. Once the fixation spot was acquired it brightened up and the monkey had to hold fixation for 0.5 s. Next, one peripheral cue was flashed for 280 ms at the location of a future saccade. Monkeys were required to maintain fixation throughout the cue period and the subsequent memory period of 1 s. The fixation spot offset served as a Go signal, allowing the monkeys to saccade to the instructed location. After a saccade to, and the fixation hold of the remembered location for 200 ms, the target became visible and bright. Monkeys were required to hold their gaze position for 0.5 s for the trial to be considered successful. Between trials there was an inter trial interval of 2.5 s for successful trials, and 2 s for failed trials. Monkeys had to perform 10 hits to each target location per fixation offset accounting on average for 240 hit trials per block

Figure Ib.1 **Gaze position modulation display and expectations**



Dorsal pulvinar neurons were recorded while monkeys performed memory saccades from the fixation spot to one of eight targets in the periphery. Bottom panel: the full array of targets and the fixation spot were either centered or shifted 15° to the left or right of the monkey's midline (cyan, blue and green frames respectively), creating three arrays of partially overlapping targets. The arrays allowed the examination of firing rate changes due to: the position of the target on the retina, by additional components such as the physical location of the target on the screen, the position of the eye relative to the head, or by their interactions. Upper panels: hypothesized firing rates of pulvinar when influenced by the retinotopic position of the cue, and by additional non-purely retinotopic factors.

Neuronal population

For the gaze modulation dataset 179 and 166 units for monkey L and C were recorded from their left and right pulvinar respectively. From those, 144 and 95 units were identified as stable during the recording and were classified as single units by inspection of spike clusters (comparing principal component 1 and 2 and principal component 1 vs timestamp). From those, 138 and 83 had a minimum spike count and trial number criteria (50 spikes/unit, 60 trials/task) and are reported here.

Analysis of firing activity

Neurons were sorted offline using Offline Sorter v.4.0.0 and v.2.8.8 (Plexon, USA) using either a waveform template algorithm, a principle component analysis with k-means clustering algorithm, manual contour definitions, or a combination. Spike density functions for each trial were derived by convolution of the discrete spike arrival times with a Gaussian kernel (SD 20 ms). For each trial, and each epoch of interest (**Table 1b.1**), firing rates were computed by counting the spikes in each epoch and dividing the sum by the epoch duration. Ten behaviorally relevant epochs were selected for analysis.

Table 1b.1 **Epochs of interest**

EPOCH BEING ANALYZED	Trial initialization	Fixation acquisition	Fixation hold	Cue	Memory early	Memory late	Pre saccade	Peri saccade	Target hold invisible	Target hold
REFERENCE EVENT time (s) to reference period	Fixation spot onset -0.4 -0.1	Fixation acquired 0.05 0.15	Cue onset -0.3 0	Cue onset 0.05 0.15	Cue offset 0 0.2	Go (fixation spot offset) -0.3 0	Saccade onset -0.1 -0.01	Saccade onset -0.01 0.05	Target acquired 0 0.1	Target offset -0.3 0

For each of the epochs of interest (top row) the table shows a reference event (bottom row) and the time window relative to the event for the epoch definition and calculation of average firing rates.

Electrophysiological analysis of gaze modulation task

For each task epoch, a two-way ANOVA with factors initial fixation and target position relative to the fixation spot was performed to look for main effect of fixation, movement direction and interactions. In addition, a second two-way ANOVA with factors initial fixation (initial gaze) and physical target position on the screen was performed.

Memory-guided saccade choice task rationale

To address the question of dorsal pulvinar modulation by the presence of an additional stimulus and an additional movement option a two-alternative free choice paradigm (subset from data of *Chapter I*) was performed.

The task sequence has been reported in Chapter I and is identical to control choice trials from the memory saccade task in the microstimulation experiment, except for use of three additional target pairs with a 12 deg eccentricity.

In summary, a total of 420 single and multiunits were recorded for the memory-guided saccade task (365 right pulvinar) and 296 for the visually-guided task (230 right pulvinar).

Electrophysiological analysis of free choice task

Additionally to the 365 and 230 units recorded from Monkey's L and C right pulvinar, 55 and 66 units were added to the dataset from Monkey L's left pulvinar for the memory-guided and visually-guided saccade tasks respectively. Units maintained a spatial label to the recorded hemisphere after being compiled to assess either contralateral or ipsilateral stimulus influences in firing rate according to the recorded pulvinar.

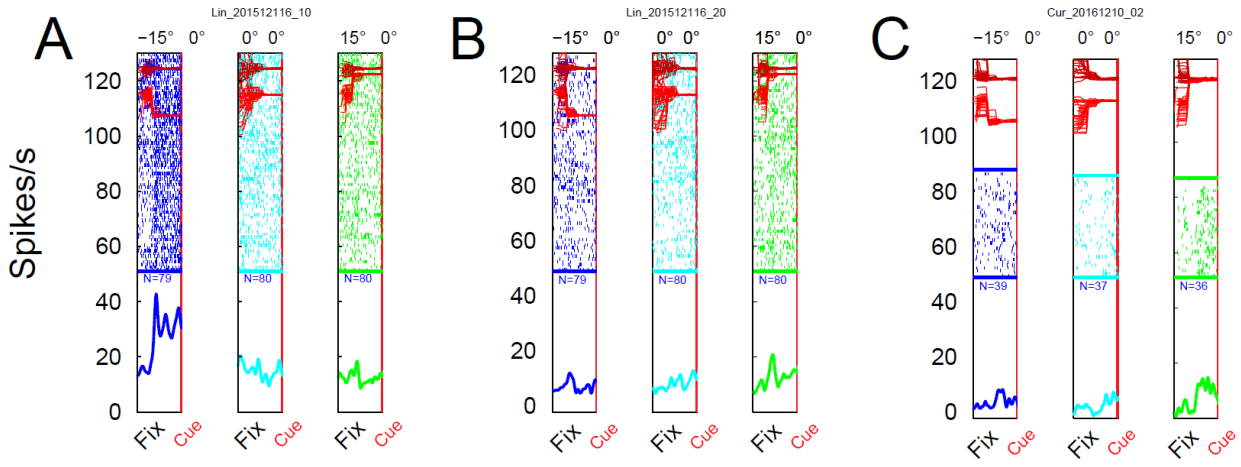
To assess influences of target selection options, a non-paired two-tailed t-test was performed for each epoch, comparing the responses to the (not necessarily significantly) preferred hemispace in instructed trials with choice trials where the same hemispace was selected (to test the effect of an additional movement option in the memory late and pre-saccadic period). In this analysis epochs where visual stimulation was present need to be taken cautiously as firing rate changes involve not only a difference in the action planning but also the physical differences of the input. To compare the firing rate of trials with choice trials to the ipsilateral and contralateral hemispace to the recorded dorsal pulvinar paired two-tailed t-tests were used.

Results

Gaze modulation effects

Does pulvinar encode space in purely retinotopic (gaze-centered) manner, or spatial transformations exert a strong influence on dorsal pulvinar firing? To address this question, monkeys performed a task in which their initial gaze position before performing a memory saccade was shifted 15° to the left or right from a central location. **Figure Ib.1** shows the raster and peri-stimulus time histograms (PSTHs) of three example units during the fixation hold period. The trials were compiled by initial gaze in this representation. In all three subpanels, the PSTH during the fixation period "Fix" is expected to look similar regardless of the initial gaze location when there are no postural effects influencing pulvinar. The neuron in **Figure Ib.1A** presents a decrease of firing rate as the fixation is shifted to the right (contralateral increase to the recorded hemisphere). This neuron represents units where postural effects exert influence an in pulvinar. The neuron in **Ib.1B** preferred the right initial gaze position and was less modulated at other eccentricities. This cell was also recorded from the left hemisphere. The neuron in **Ib.1C** recorded in the right hemisphere of monkey C had an increased firing when fixating to the right. Even with a significant initial position preference in different units, from single cell examples it was difficult to observe an apparent contralateral or ipsilateral preference to the recorded hemisphere.

Figure Ib.1 **Example neurons with gaze position preference during the fixation hold epoch**



Each colored raster plot and PSTH per panel represents action potentials and peri stimulus time histogram (PSTH) in 20 ms bins for trials with same initial gaze position in three example neurons, locations being: -15° horizontal, 0° vertical (blue), 0° horizontal, 0° vertical (cyan), and 15° horizontal, 0° vertical (green). Each panel displays the initial fixation until the onset of the movement cue. Color conventions and periods of interest are consistent across plots for this dataset. Light red traces are the horizontal gaze positions across trials; dark traces are the vertical ones. **A.** For this unit as the monkey's initial gaze went from the left to the right there was a decrease of firing rate with the strongest firing at the -15° horizontal, 0° vertical position. **B and C.** Units with a higher firing rate for the 15° horizontal, 0° vertical fixation position. Additionally, in (**C**) there was firing suppression around the saccade. *Fix, fixation*

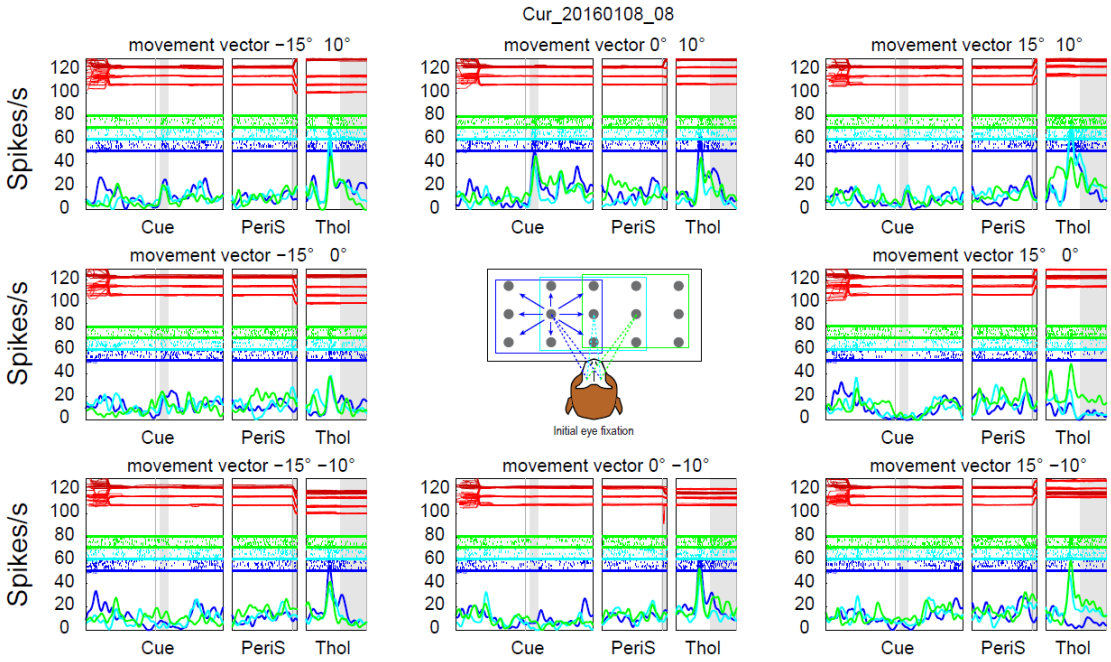
From the cells with a main effect of gaze position during fixation hold in the ANOVA (gaze position) 46% (102 out of 221 units) showed gaze position modulation. Of these 48% (49 out of 102 units) showed a monotonous increase of firing during that period. The increase was 59% towards the ipsilateral side and 41% to the contralateral. This population might reflect cells in which there is a gain field like modulation when retinotopic influences are present.

To confirm the effect of movement direction on the firing of neurons as suggested by findings in *Chapter I,* trials per neuron were resorted to represent matching trials by

movement vector relative to the initial gaze. This resulted in a matrix with a similar number of relative movement directions starting from the fixation spot.

The neuron in **Figure Ib.2** shows retinotopic tuning during the cue presentation with no further influence of gaze position. The modulation is most evident in the top panel.

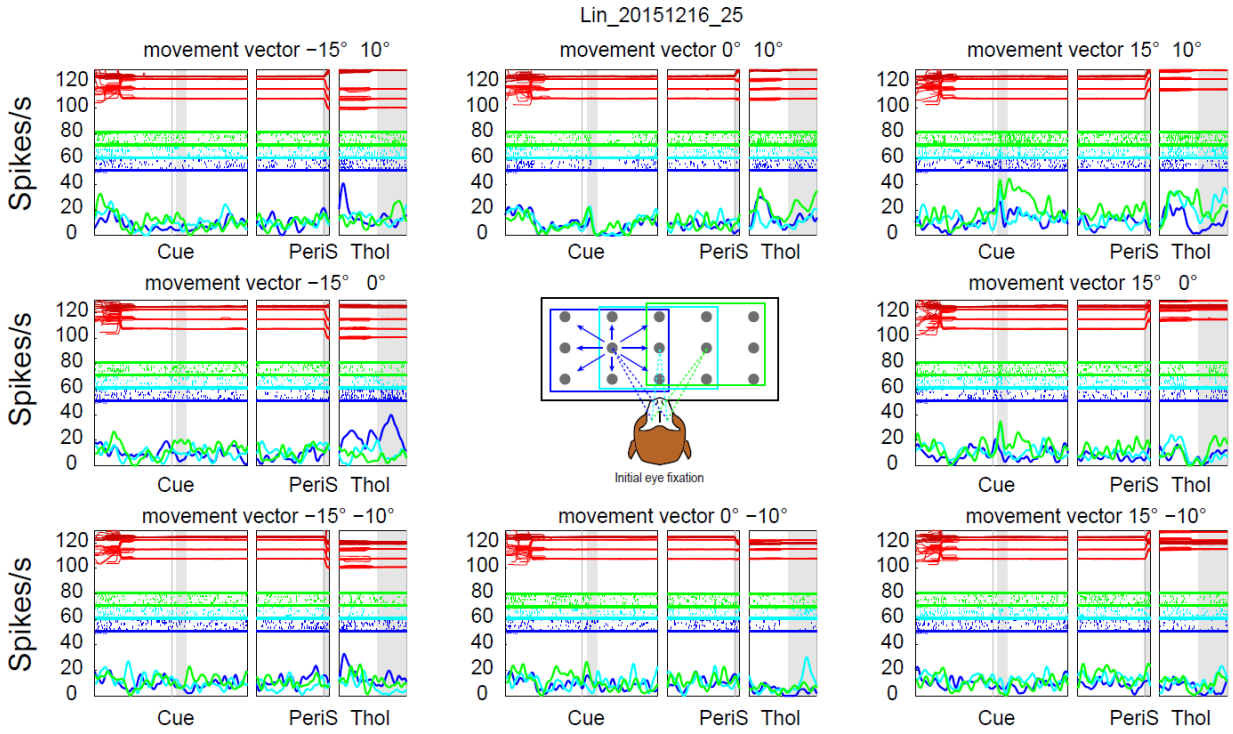
Figure Ib.2 **Retinotopic cue modulation**



Each panel represents one of the eight available movement directions sorted by initial fixation position: leftward (blue traces and raster), central (cyan traces and raster), and rightward (green traces and raster). This unit was retinotopically influenced during the cue period. The cue-related firing enhancement was mainly to the 0° horizontal, 10° vertical movement direction regardless of the initial gaze position. In addition, there was suppression for the to the 15° horizontal, 0° vertical, movement directions.

On the other hand, **Figure Ib.3** shows a neuron with firing rate enhancement during the cue period (top right, and right panels). This enhancement was not only retinotopic but also modulated by the current gaze position, commonly known as gain field.

Figure Ib.3 **Example neuron with an effect of gaze position**



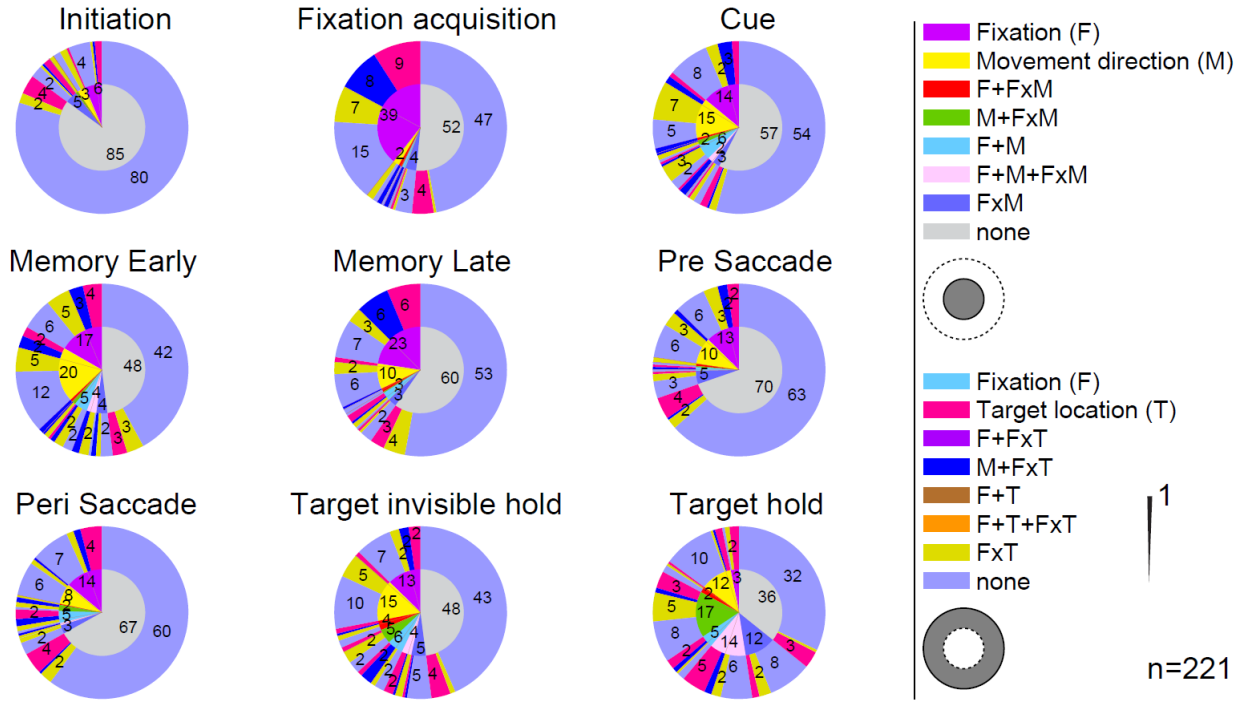
This unit was responsive during the cue period to the 15° horizontal, 10° vertical movement direction and to the 15° horizontal, 0° vertical movement direction. Crucially, not only the visual stimulation modulated this neuron, but also the initial gaze position.

Another potential scenario is that cells in dorsal pulvinar encode the physical location of a cue, and thus, the planning of a movement is influenced by its fixed location. To observe this modulation a last sorting based on the physical location of the target on the screen was performed. On a single-cell level however, this type of encoding was not visually apparent.

To assess how the population of units was represented in each of the categories observed by visual inspection, two two-way ANOVAs with factors fixation and movement direction, and fixation and physical target location were performed per epoch. The results of the ANOVAs with factors fixation and movement direction (inner circle in the pie plot from **Figure Ib.4)** revealed that during the cue period, 43% of all cells tested were modulated, 15% of the units were modulated in a retinotopic fashion, while 14% were modulated by the gaze position. This agrees with findings seen across several visuo-motor areas influenced by a retinotopically based reference frame. Additionally, 14% of the cells also

had an interaction of effects or both main effects. This indicates that dorsal pulvinar does not only encode visual information in a retinotopic reference frame but might also be influenced by gaze position, potentially facilitating the generation of gain fields. The largest percentage of task modulation was found during "target visible hold" after the monkey was given visual feedback of correct performance. In this period, there was a considerable interaction of the gaze position and the movement direction; this was possibly due to both the preferred gaze position plus the influence of the recently executed saccade. The second ANOVA (outer circle in the pie plot) revealed that, as predicted by visual inspection, only a small percentage of units were modulated by the physical location of the target in space. Only a small proportion of the cells during the cue period were uniquely modulated by the absolute target location. Supporting the idea of dorsal pulvinar sharing similar properties with the posterior parietal cortex, a retinotopic coordinate system influenced by gaze location and/or additional reference points dependent on the effector used.

Figure Ib.4 **Summary: dorsal pulvinar neurons are not purely retinotopic**



For each of the behaviorally relevant epochs the inner circle in the pie plot contains the results of a two-way ANOVA with factors Fixation (F) (initial gaze) and Movement direction (M) showing effects for F, M, both main effects F+M, and interactions (F+M+FxM, F+FxM, M+FxM, and FxM), or no effects. The outer circle shows the main effects of target position (T)

or its interactions with fixation. Inner circle: During cue presentation and subsequent memory period there was a large modulation of the starting gaze position (14% and 17% "cue" and "early memory") and by the movement direction (15% and 20% "cue" and "early memory"). An early main effect of movement direction shows that there is influence of a retinotopic-based reference frames in these cells. After the saccade, during the target hold period there was in addition to the main effects of fixation and movement direction an interaction (F+M+FxM) of 14%, potentially due to the cells´ retinotopic encoding influenced by the starting gaze position of the recently executed movement. Outer circle: There was almost no cell percentage influenced by the absolute location of the target. Around the saccade the maximum percentage of cells modulated purely by the physical location of the target was 4%. The overall encoding of these cells was mostly an interaction of initial fixation position and movement direction, suggesting an intermediate processing stage for dorsal pulvinar in spatial transformations.

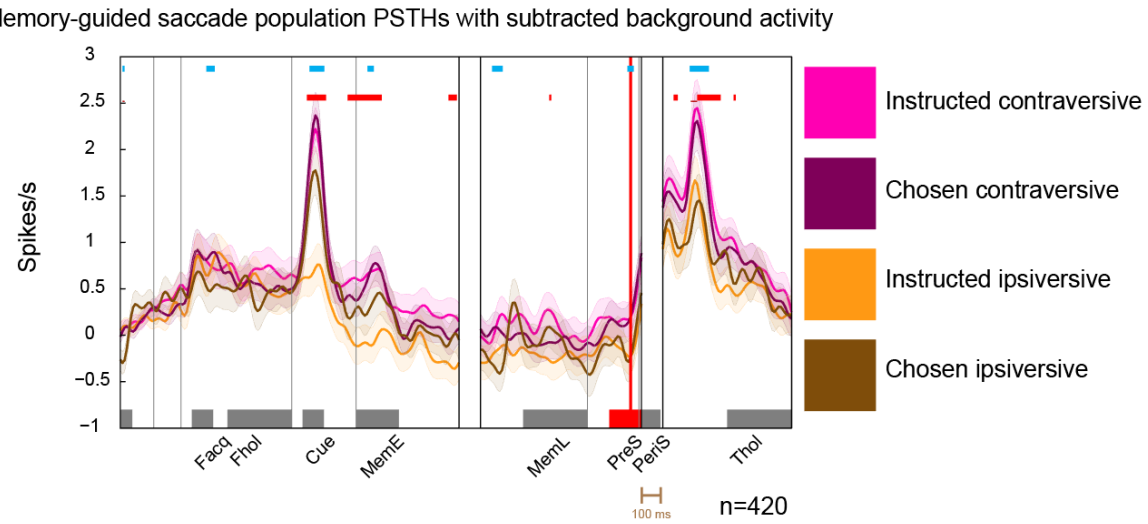
Target selection

Does the possibility of freely choosing among two saccade options influence the visual or motor firing rate in dorsal pulvinar during saccades? To address this question, a control experiment was performed where monkeys' dorsal pulvinar neurons were recorded during an equally rewarded free-choice saccade task. Responses to saccades to single targets were reported in *Chapter I.*

First, the baseline-subtracted average population PSTHs for instructed and choice memory- and visually-guided saccade trials were plotted to allow a direct comparison of potential spatial tuning differences when more than one cue/target are available. **Figure Ib.5** shows the population firing for the memory-guided saccade task. During the cue presentation, there was an increased firing if a cue was presented in the contralateral hemispace to the recorded pulvinar. Furthermore, in the cue period there was a short increment of firing rate when monkeys chose the contralateral hemispace in comparison to when the ipsilateral hemispace was chosen. Trials with ipsiversive instructed cues had smaller firing differences to baseline than the other three conditions. Later, the firing was enhanced by the direction the movement and mostly after the saccade offset. There were no apparent differences between single- or two-target trials to saccade either to the contralateral or ipsilateral hemispaces. The effects observed in dorsal pulvinar during the memory-guided task reflect

1) a broad contralateral spatial preference during the cue and early memory periods and 2) a post motor influence on firing determined by the previous action but not by the visual stimuli that evoked such action.

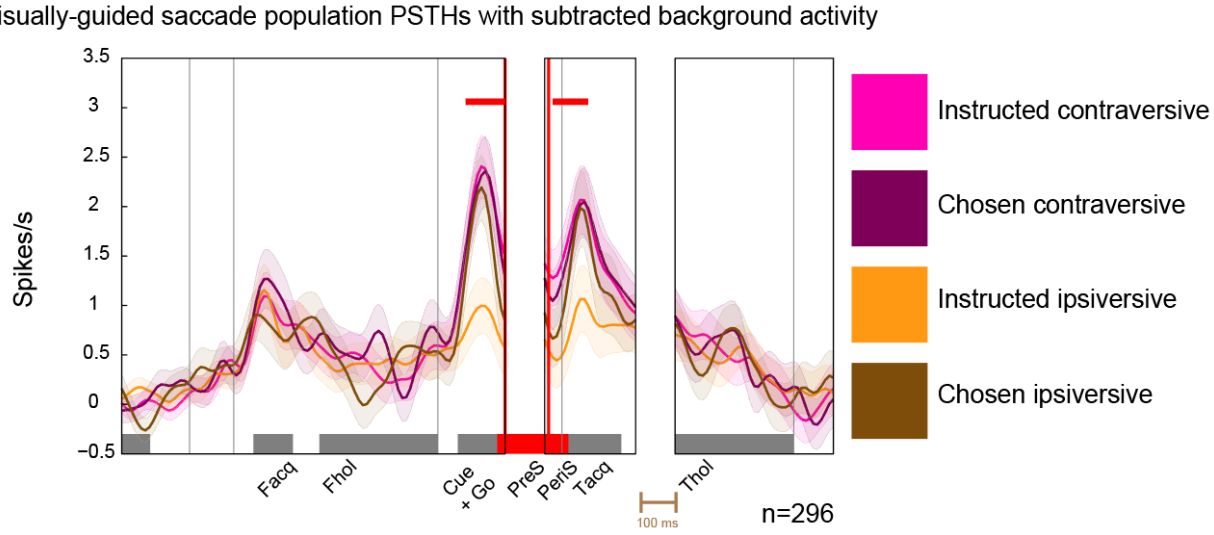
Figure Ib.5 **Memory-guided saccade task´s PSTHs for instructed and choice trials**



Baseline corrected population PSTH (solid traces), and SE (shaded bars) of instructed and choice trials. Spikes were sorted according to the target location for instructed trials and to the chosen target in choice trials. Red horizontal traces are significant differences per bin between hemispaces for instructed trials derived from two-tailed non-paired t-tests. Blue horizontal traces are significant differences per bin between choices to the contralateral hemispace and to the ipsilateral hemispace derived from two-tailed paired t-tests. During the cue presentation, there was enhanced firing to cues if they were present in the contralateral hemispace. During cue presentation there was also an increase of firing when monkeys chose a contralateral target in comparison to when they chose an ipsilateral one. When a single stimulus was shown in the ipsilateral hemispace there was only a modest increase in firing in comparison to baseline. In other words, as long as there was a target in the contraversive side of space the cells were unaffected by the presence or absence of an ipsilateral stimulus. During the memory period, there was a modest increase of firing for instructed and chosen contraversive targets, as well as for chosen ipsiversive targets. Around and after the saccade the firing reflected the hemispace of the movement and not its instructed or choice nature. Later, after the saccade the PSTHs for instructed and choice trials largely overlapped. Analysis periods: *Facq, fixation acquisition; Fhol, fixation hold; MemE, memory early; MemL, memory late; PreS, pre saccade; PeriS, peri saccade; Thol, target hold*

In the visually-guided saccade task, (**Figure Ib.6)**, the population had a similar response as in the memory task (**Figure Ib.5)** by the presence of a contralateral target. For instructed ipsiversive trials there was no big influence of the target in the firing modulation. The modulation around the saccade had a similar pattern, larger firing rate to contralateral targets than to ipsilateral targets. It is important to mention that as the onset of the target functions as the "Go" signal, the constant visual stimulation is inevitably a confound for the analysis of independent internally-generated processes in this specific task.

Figure Ib.6 **Visually-guided saccade task´s PSTHs for instructed and choice trials**



Conventions as in **Figure Ib.5.** For neurons recorded during direct saccades, at the Go signal there was a strong firing rate enhancement when the target was presented in the contralateral hemispace of the recorded pulvinar in comparison to a sole ipsilateral presentation. There was a strong visuo-motor influence of the preferred hemispace on pulvinar firing, however, the dissociation between visually-triggered and internally-generated planning or motor signals is not possible in this condition. *Cue + Go, target onset which functions simultaneously as a Go signal; Tacq, target acquisition.*

Discussion

In this addendum to *Chapter I* we aimed to address two important questions about the electrophysiological properties of dorsal pulvinar during oculo-motor behavior.
First: Are neurons in dorsal pulvinar, modulated in a 1) purely retinotopic reference frame? or 2) is dorsal pulvinar influenced by the current gaze position of the monkey?

The second question: Is there a neuronal correlate of target selection for oculo-motor behavior in dorsal pulvinar? Namely, is there visual competition, or choice selectivity during the cue period? This question was motivated by previous causal perturbation studies where after pulvinar disruption either by pharmacological inactivation (Wilke et al., 2010, 2013) or by electrical microstimulation (Chapter I) there were among other effects, changes in target selection patterns for saccade behavior. Directly assessing if target selection is in any way represented in dorsal pulvinar has not been tested before.

D.Ibis.1 A subset of dorsal pulvinar neurons showed encoding in not purely retinotopic reference frame

Visual neurons in the inferior and lateral subdivisions of ventral pulvinar of the macaque are modulated by gaze position (Robinson et al., 1990). No similar data exist characterizing gaze influences on visual responsivity in the dorsal pulvinar. For this experiment we were particularly interested in the dorsal region as it shares a broader connectivity to parietal and frontal cortices in monkeys (Grieve et al., 2000; Kaas and Lyon, 2007), and in humans[2] (Leh et al., 2008) than ventral pulvinar. We hypothesized that dorsal pulvinar could likely display complex modulations to visual stimulation influenced by gaze position. With the purpose of gaining intuition on the topic, we recorded single neurons while monkeys performed a standard center-out memory saccade task. Critically, at the beginning of each trial the monkeys held their gaze at one of three possible fixation spots, straight ahead, to

[2] In this study the authors did not distinguish between dorsal and ventral pulvinar.

the left, or to the right of the monkeys' primary gaze. The saccades were made to one of eight peripheral targets from a rectangular array, some of these locations overlapped across fixation conditions, creating an array of similar movement directions with distinct and partially overlapping target spatial locations.

Figure Ib.4 shows that neurons in dorsal pulvinar had a firing rate increase dependent on the 1) position of the visual cue on the retina (15% at "cue", main effect *Movement direction*), 2) an interaction of the movement direction and the initial gaze position, or both main effects (14% at "cue"), or in a smaller percentage a 3) an increased firing rate by the physical location of the target (2% at "cue", main effect *Target location*). There were also cells influenced in their firing solely by the gaze position (8% at "cue", main effect *Fixation*). Along this line it has been shown that there is an influence of gaze position on visual responsivity in parietal cortex (Andersen and Mountcastle, 1983). This effect now referred as ¨gain field¨ is suggested to facilitate the execution of actions (specifically, actions performed with limbs) computed in a different coordinate system (Zipser and Andersen, 1988). Also in early visual areas such as V3a, with connectivity to ventral pulvinar, neurons´ firing rate has been shown to be influenced by the eye position (Galletti and Battaglini, 1989), which could explain why gain fields are also encountered in the more visual part of pulvinar. In area V3a there is additionally preferential enhancement of firing to contralateral gaze shifts relative to the recorded hemisphere. The directionality of gain fields in our dataset is something we will explore in the future, although cells with a monotonic firing increase during the fixation hold period did not show hemispatial preference (41% and 59% of the cells to the contralateral and ipsilateral hemispace respectively).

Dorsal pulvinar neurons influenced by the gaze position only ranged from 3% to 23% across epochs (median 14%, excluding cells modulated during the fixation acquisition) and can be regarded as postural units. The large percentage of units with an effect of movement direction during the cue and early periods of the task reflects that pulvinar preferentially is influenced not only by retinotopic reference frames but also gaze-, head-, or body-centered systems. It has been shown that in brain areas involved in movement planning the reference

frame in which visual signals are processed commonly depend more on the position of the targets in the retina (Snyder, 2000) than in the head or body (Flanders et al., 1992). Using common types of coordinate systems across brain areas is a more economical solution when independent and constantly updated actions are planned and involve a high number of computations with different effectors.

Looking back at the Andersen and Mountcastle (1983) study, the gain field effect was only present when the monkeys were actively engaged in the task. Spatial transformations in the primate brain seem to depend on if there is an upcoming action. Background firing in area LIP of parietal cortex also differs dependent on the type of action expected to be performed (Colby et al., 1995). We have some evidence of dorsal pulvinar cells encoding preferentially for specific hands before the trial has started if the hand to be used can be predicted (See *Chapter II*). It is interesting to wonder if different coordinate frames would influence pulvinar's firing patterns when there is the expectancy of upcoming actions using multiple effectors.

Among the limitations of this experiment a relevant one is that due to technical limitations our target array did not fully overlap for all target locations when starting from different gaze positions. With such overlap, a target per target comparison could have been performed to assess more precisely if the physical location of the cues causes a change on the firing rate of dorsal pulvinar neurons. Interpreting such comparison in our current setup however could still prove to be a hard task. Classic visual electrophysiology studies relied on the visibility of illuminated targets in otherwise completely darkened rooms. Our current setup requires that the targets are presented in monitors. The use of monitors creates a dim but visible background illumination which might provide unwanted reference points to the monkeys which could lead to confounds. Finally, our study required the monkeys to have their head restrained. To achieve a better dissociation between a retinotopic and a head-, body-, or object-centered coordinate systems additional experiments with no head fixation, and head position manipulation are required. It has been shown even in areas like the superior colliculus that the manipulation or free movement of the head has influences on neural firing (Walton et al., 2007; DeSouza et al., 2011). The effects related to the head

position would help us create a better picture of the interactions between eye and head movement potentially represented in dorsal pulvinar.

D.Ibis.2 Dorsal pulvinar is modulated by the location of a cue in the preferred hemispace and to a target to be chosen in the preferred hemispace.

To assess the potential modulation of the selection of one freely chosen target in comparison to a movement to an instructed single target in dorsal pulvinar we trained two monkeys to perform a standard free-choice task in a visually- or memory-guided context. We did not aim to modulate their natural bias to any of the target options. The targets were always rewarded equally, as well as presented equidistantly from the viewing gaze of the monkeys.

There was contralateral spatial tuning to the recorded pulvinar **(Figure Ib.5)**. This increase in amplitude was true in instructed and less in choice trials. As reported in *Chapter I* there was a general preference for cues presented in the contralateral hemispace. In addition, during the cue period in choice trials there was higher firing rate when monkeys later chose the contralateral target than when they chose the ipsilateral one, suggesting choice processing during the cue period. However, this pattern was largely lacking during the memory period. For visually-guided trials (**Figure Ib.6**) there was a similar response favoring contralateral targets, however due to the lack of a memory period we cannot make many interpretations of that dataset.

It has been suggested for other subcortical regions like the superior colliculus (Krauzlis and Dill, 2002; Port and Wurtz, 2009) that target selection and saccade generation are intertwined and might be non-separable. If a tight choice-saccade link in the time domain is needed for dorsal pulvinar to influence decisions, it might explain the lack of an apparent change in firing rate during the late memory period and the higher firing during the cue period for choice trials where the contralateral target was later chosen.

Given reports of the role of pulvinar in attentional processes (Petersen et al., 1987; Desimone et al., 1990; Snow et al., 2009; Saalmann et al., 2012). It would be interesting to explore the differences of perceptual decisions and free choices in the dorsal pulvinar. It has been observed in FEF in perceptual tasks requiring monkeys to select targets from distractors that the firing of simultaneously recorded neurons with matching receptive fields cooperate by spike timing synchrony. Perceptual tasks would also be useful to address difficulties of estimating decision times in a free choice task, as most of the current definitions of when decisions occur at a neuronal level refer to the statistical difference in receptive field firing rate between a correct and incorrect targets (Cohen et al., 2010)

Given that our task always presented one target in the contralateral and another in the ipsilateral hemispace, and that dorsal pulvinar neurons show large receptive fields and a preference to the contralateral hemispace, it might be worth to assess choice-related firing in vertically arranged target options in each hemispace individually, so that horizontal preference is not an additional variable that might preclude the observation of choice encoding in dorsal pulvinar.

Among the analysis that is still required we need to address on a cell by cell basis if there was a different response during the cue period when a target inside the neuron's receptive field was presented with an additional target outside of the receptive field. This comparison will allow us to look for signs of visual competition or enhancement when multiple target options are available. This analysis will require offline estimation of receptive fields, as there was no mapping performed before the recordings. Additionally, choice selectivity on a cell by cell basis during the cue and memory periods need to be assessed.

Taken together these findings provide evidence of dorsal pulvinar largely encoding spatial properties of visual stimuli not only using a retinotopic reference frame, but one that is influenced by the current gaze position or by additional spatial reference points according to the behaviorally relevant epochs of the task. Additionally, further electrophysiological analyses of the influence of choice on saccade-related neuronal activity will enhance our understanding of how target selection is represented in the dorsal pulvinar.

Acknowledgements

We would like to thank Uwe Zimmermann for spike sorting of a subset of data of Monkey C. We thank Ira Panolias, Sina Plümer, Klaus Heisig, and Dirk Prüße for technical support. We also thank Stefan Treue, Alexander Gail, Hansjörg Scherberger, members of the Decision and Awareness Group, Sensorimotor Group and the Cognitive Neuroscience Laboratory for helpful discussions. Supported by the Hermann and Lilly Schilling Foundation, German Research Foundation (DFG) grants WI 4046/1-1 and Research Unit GA1475-B4, KA 3726/2-1, CNMPB Primate Platform, and funding from the Cognitive Neuroscience Laboratory.

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Chapter II

Electrophysiological correlates of pulvinar function during reach behavior

Contributions

AUDV, MW and IK designed the experiments. AUDV collected and sorted the electrophysiology dataset of Monkey L and F. LS wrote the electrophysiology analysis scripts. AUDV and LS analyzed the electrophysiological datasets. MW and IK supervised the project. AUDV wrote this chapter.

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Abstract

The pulvinar, the largest thalamic complex in primates has largely expanded during primate evolution, along with association cortices. Its dorsal part is anatomically connected to the fronto-parietal network, involved in the planning and generation of purposeful eye, arm, and hand movements. Early studies have identified neural correlates of reach-related behavior in pulvinar cells in different species. Such reports however are very sparse, even though insightful and revealing. Thus, the formulation of a hypothesis of dorsal pulvinar involvement in visually-guided reaches is largely speculative. Here, we recorded neurons from dorsal pulvinar of two *Macaca mulatta* performing delayed, visually-guided foveal and extrafoveal reaches and saccades with active hand engagement to characterize pulvinar response properties during purposeful visuo-motor behavior. We found spatial influence on firing rates with preferences to the contralateral or ipsilateral hemispace (to the recorded hemisphere), which varied according to the period of interest in the task. We found that the firing pattern for saccades was mostly suppression during the movement and strong enhancement after saccade offset. The enhancement of firing rate for reaches appeared before the movement onset and lasted during the reach. We found cells responsive to the usage of a specific effector, two effectors, specific hand usage, and space-hand interactions. In addition, we found that after repeatedly using one hand for performing the tasks in right/left hand blocks, some cells showed a hand-specific tuning even before start of the trial. These results add evidence to dorsal pulvinar involvement in visually-guided reach planning and execution, besides the now known visuospatial encoding.

Introduction

Primates excel at visuo-motor tasks. Of special interest is how and at which levels visual information is integrated with proprioceptive inputs to later be transformed into motor commands. Several cortical regions have been shown to take part in the planning and execution of grasping and reaches, e.g. areas 7, V6A and MIP (Taira et al., 1990; Galletti et al., 1997; Snyder et al., 2000a). Reaches are a good proxy to study goal-directed behavior, as they likely involve actions of similar skillfulness across most primate species. On the other hand grasping can be influenced by the semantic properties of the object of interest in humans, and such influence does not seem to be present in all primate species (Johnson-Frey, 2003; Frey, 2007). Furthermore, reaches provide a behavioral output rich enough for the analysis of spatial and temporal properties that can be traced to their neural generators.

Under natural conditions, primates perform volitional reaches guided by visual information. This guidance is additionally integrated with body, head and eye movements. Since the introduction of periods that dissociate purely visual influences from motor actions (Hikosaka and Wurtz, 1983) the visuo-motor circuitry of behaving macaques has been an important focus of study of system neuroscientists. It has been found for example that the oculomotor system involves a rich circuitry of frontal and parietal association cortices, as well as subcortical brain regions (Schiller et al., 1987; Snyder et al., 1997; Barash, 2003). For the generation of reaches several cortical association areas have been identified (Caminiti et al., 2015) however, less is known about the role of subcortical regions.

Neurons in association cortices are modulated during the planning and execution of reaches and saccades. As association cortices, the thalamic pulvinar has greatly and disproportionally expanded during primate evolution. The pulvinar is anatomically connected to the cortex in a ventro-dorsal gradient to the ventral and dorsal streams (Asanuma et al., 1985; Stepniewska, 2004; Kaas and Lyon, 2007; Bridge et al., 2016).

Dorsal pulvinar is strongly connected to the fronto-parietal network. It has been linked to processes like spatial attention (Petersen et al., 1987), target selection using saccades (Wilke et al., 2010; Dominguez-Vargas et al., 2017), modulation by motivational components (Wilke et al., 2013), confidence in perceptual categorization (Komura et al., 2013) and purposeful reach and grasping behavior (Wilke et al., 2010). Pulvinar neurons display reach-related firing in: *Macaca fascicularis* (Acuña et al., 1986; Magariños-Ascone et al., 1988), *Cebus capucinus* (Acuña et al., 1983), *Macaca nemestrina* (Cudeiro et al., 1989) and humans (Martin-Rodriguez et al., 1982). Common denominators in these studies are a higher responsiveness to active reaches in comparison to passive manipulations of the arm, and stronger firing rate for reaches to objects of interest. In *Macaca nemestrina* the firing rate enhancement of pulvinar due to reaches comes before that of parietal and motor cortices, which could mean reach preparation in pulvinar (Cudeiro et al., 1989). Also in the Cudeiro et al. study it was shown that neurons were more responsive to reaches in the lateral and oral pulvinar in comparison to the medial subdivision. It was also noted that pulvinar´s responsivity to visual stimulation does not follow a retinotopic representation (Acuña et al., 1983), this is particularly true in the mediodorsal pulvinar (Kaas and Lyon, 2007). Most importantly, in the *Cebus capucinus* study from Acuña and colleagues, in humans (Martin-Rodriguez et al., 1982) and in *Macaca nemestrina* (Cudeiro et al., 1989), the intentionality of performing a movement was crucial to modify the firing rate of pulvinar neurons, suggesting the critical relevance of pulvinar in goal-directed actions. Furthermore, another study (Wilke et al., 2010) showed that inactivation of pulvinar causes deficits in reach-to-grasp tasks, the deficits were suggested to resemble optic ataxia- and visual extinction-like symptoms in *Macaca mulatta*, although their design did not allow to delineate the deficit with such precision. Optic ataxia (OA) is of interest as it involves the planning and execution of visually-guided movements. The defining characteristic of OA is that there is difficulty to successfully perform extrafoveal reaches, while foveal reaches are less impaired (Andersen et al., 2014). The exploration of multiple eye-hand coordination and dissociation conditions while using causal techniques in dorsal pulvinar are still needed to elucidate if the region is causally involved in optic ataxia or other visuo-motor behaviors requiring a cognitive control and will be addressed in *Chapter III*.

The available literature about reach encoding in the dorsal pulvinar is scarce and additional characterization of pulvinar engagement during visually-guided reach planning and execution is needed. Many open questions remain: Is the population of dorsal pulvinar neurons preferentially modulated by visual stimulation, planning of upcoming movements, or the execution of visually-guided movements? Would all dorsal pulvinar population fire to the same events or would it reflect the diverse connectivity of pulvinar? Would saccades and reaches be encoded similarly? Would the presence of more than one active effector influence the activity of dorsal pulvinar? Would there be hand specific tuning?

Here, we analyzed single cell recordings from the dorsal pulvinar of two macaques trained to perform delayed visually-guided foveal and extrafoveal reaches as well as saccades with hand engagement to characterize the functions of dorsal pulvinar during purposeful actions.

Materials and methods

Ethics, experimental approval and disclosures

All experimental procedures were conducted in accordance with the European Directive 2010/63/EU, the corresponding German law governing animal welfare, and German Primate Center institutional guidelines. The procedures were approved by the responsible government agency (LAVES, Oldenburg, Germany).

During training or experimental days, monkeys worked for water/juice until satiated. On weekends or non-training days, monkeys had unlimited access to liquids (water and fruits/vegetables). Food with low water content was unrestricted and available at all time.

Monkey L's right pulvinar was studied in a previous report and the following procedures have partially been reported *Chapter I*. That study looked at the effects of electrical microstimulation on oculomotor behavior and free-choice decision-making. For such study, the right pulvinar of L was stimulated using bipolar trains of current ranging from 100 μA to 300 μA in 48 experimental sessions.

Animal preparation

Two adult male rhesus macaques (*Macaca mulatta*) L and F weighing 9 kg and 11 kg respectively were used. In an initial surgery, under aseptic conditions, anesthetized and monitored, monkeys were implanted with a PEEK MRI-compatible headpost. The headpost was embedded in a bone cement head-cap (Palacos with Gentamicin, BioMet, USA) anchored by ceramic screws (Rogue Research, Canada). Markers were drilled in the head-cap for the planning of recording chambers. The chamber planning was performed using an MRI-guided navigation software (Ohayon and Tsao, 2012). In a second surgery monkey L was implanted with two 22 mm inner diameter PEEK MRI-compatible chambers in both

hemispheres plus a craniotomy in the right hemisphere to allow pulvinar access (right hemisphere: center at -3.12P / 20.2L mm, tilted: -18P / 37L, left hemisphere: center at -4.3P / 21.7L mm, tilted: -18P / 38L L). Monkey F was implanted with a 22 mm chamber and craniotomy in the left hemisphere (center at -6.4P / 12.4L mm, tilted: -15P / 32L L). Monkey L underwent a third craniotomy surgery to give access to the left pulvinar. The dura mater and surrounding tissue were routinely kept sealed by a silicon elastomer (Kwik-sil, World Precision Instruments, USA) to reduce tissue growth above the dura mater. The tissue exposed inside the chambers was monitored by frequent exudate samples and maintained by a routine cleaning procedure.

Recording chambers were cleaned every third day in addition to before and after every recording session. A silicon elastomer protecting the tissue above the dura matter was removed and the chamber was flushed several times with saline solution. The chamber inner walls were cleaned with 3% H_2O_2 moistened swabs. A 7.5% Povidone-Iodine-based antiseptic solution Braunol (B. Braun Melsungen AG, Germany) was placed inside the chamber for 10 minutes before it was rinsed with saline solution. The chamber inner walls were then cleaned with alcohol moistened swabs. Surgical soap Lavasorb (Fresenius Kabi, Germany) was placed inside the chamber and remained there for additional 10 minutes. The chamber was again flushed, and finally new elastomer was placed over the tissue covering the dura matter before it was sealed.

MR imaging

Monkeys were scanned in a 3 Tesla MRI scanner (Siemens Magnetom TIM Trio at the University Medical Center Göttingen or Siemens Magnetom Prisma at the German Primate Center Functional Imaging building). Similar MRI sequences were obtained as the ones reported in *Chapter I.*

Pulvinar targeting

The dorsal pulvinar was localized on anatomical basis using MR images. The upper, medial and lateral boundaries were readily localized by changes in contrast of grey and white matter in T1 and T2 weighted images. The lower boundary was defined by the beginning of the brachium of the superior colliculus. Even though some parcellation schemes also show a small portion of ventral (inferior) pulvinar above the brachium (Gutierrez et al., 2000; Stepniewska, 2004; Kaas and Lyon, 2007), the brachium-based dorsal/ventral division is the best anatomical pulvinar segmentation reference without histological confirmation.

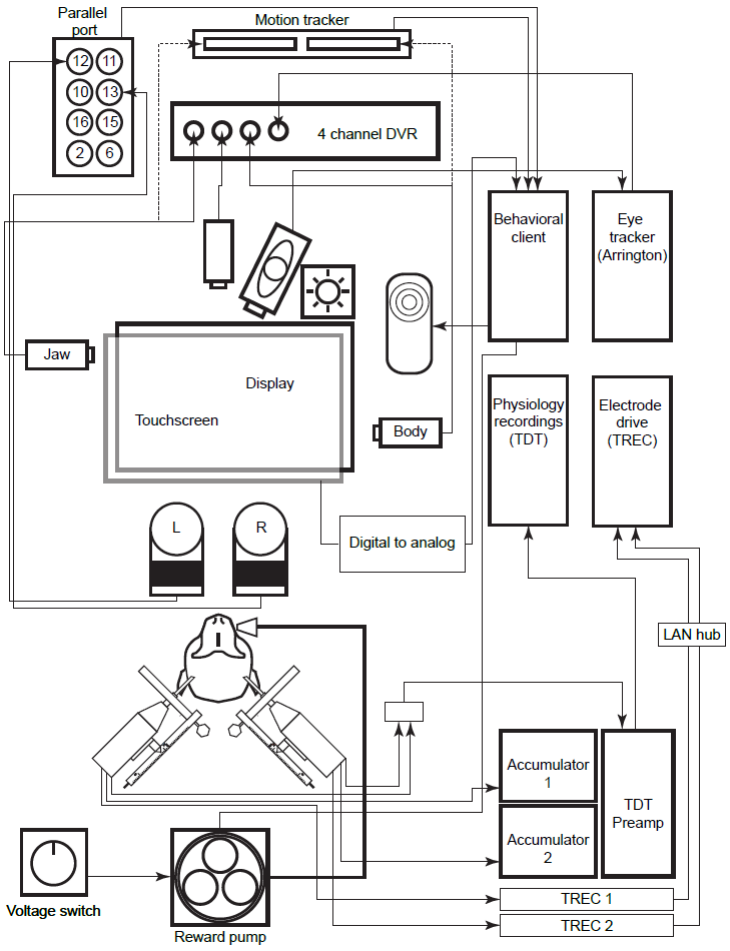
General experimental setup

Monkeys sat in a vertical primate chair facing a display with their heads restrained by a PEEK head holder attached to the implanted headpost in a darkened room with an eye-to-screen distance of 30 cm (**Figure II.1**). Infrared cameras were placed inside the room to monitor jaw movements. To maintain stable recordings a motion detection system model MD2001 single channel analog video motion detector (Pelco, USA) was used. The motion detector triggered a TTL pulse whenever motion was present, resulting in aborted trials.

Stimuli presentation and behavioral recording

Task controller and stimuli were programmed in Matlab (The MathWorks, Inc. USA) using the Psychophysics Toolbox (Brainard, 1997). Stimuli were presented on a 27'' LED display (60 Hz refresh rate, model HN274H, Acer Inc. USA). Reaches were performed to a transparent surface acoustic wave touchscreen model SCN-IT-FLT27.8-001-006 (IntelliTouch, ELO touchsystems). The touchscreen was placed in front of the 27'' stimuli display. Horizontal and vertical coordinates of finger position were recorded as analogue channels of a data acquisition card after passing a custom-made digital to analog converter. Real-time eye tracking was performed using a 220 Hz ViewPoint system, model MCU02 (Arrington Research Inc. USA) running on a separate PC. The infrared camera was placed just above the task display to get a close to straight angle from the gaze position. Before training and experimental sessions, a linear calibration was performed using the task controller to adjust the offset and gain of the eye signals.

Figure II.1 **Experimental setup**



The monkeys performed reaches and saccades inside an isolated booth next to a control room where the experimenter remotely recorded their behavioral and neuronal signals. The room's illumination was turned off and only 0.16 cd/m^2 of luminance from the monitor at monkey's viewing distance of 30 cm was available. Jaw motion was monitored to ensure that non-task related movements were kept at a minimum. Hand sensors (L and R, placed close to monkeys' left and right hands) were used to prevent the monkey from using un-cued hands. Eye, touch, initial hands resting position, and neuronal signals were recorded during the experimental sessions.

Eye-hand movement rationale

To assess neuronal properties of dorsal pulvinar cells during visually-guided behavior, monkeys were trained to acquire ipsilateral or contralateral targets using their gaze or their ipsilateral or contralateral hand (in respect to the recorded hemisphere).

Trial sequence reach task

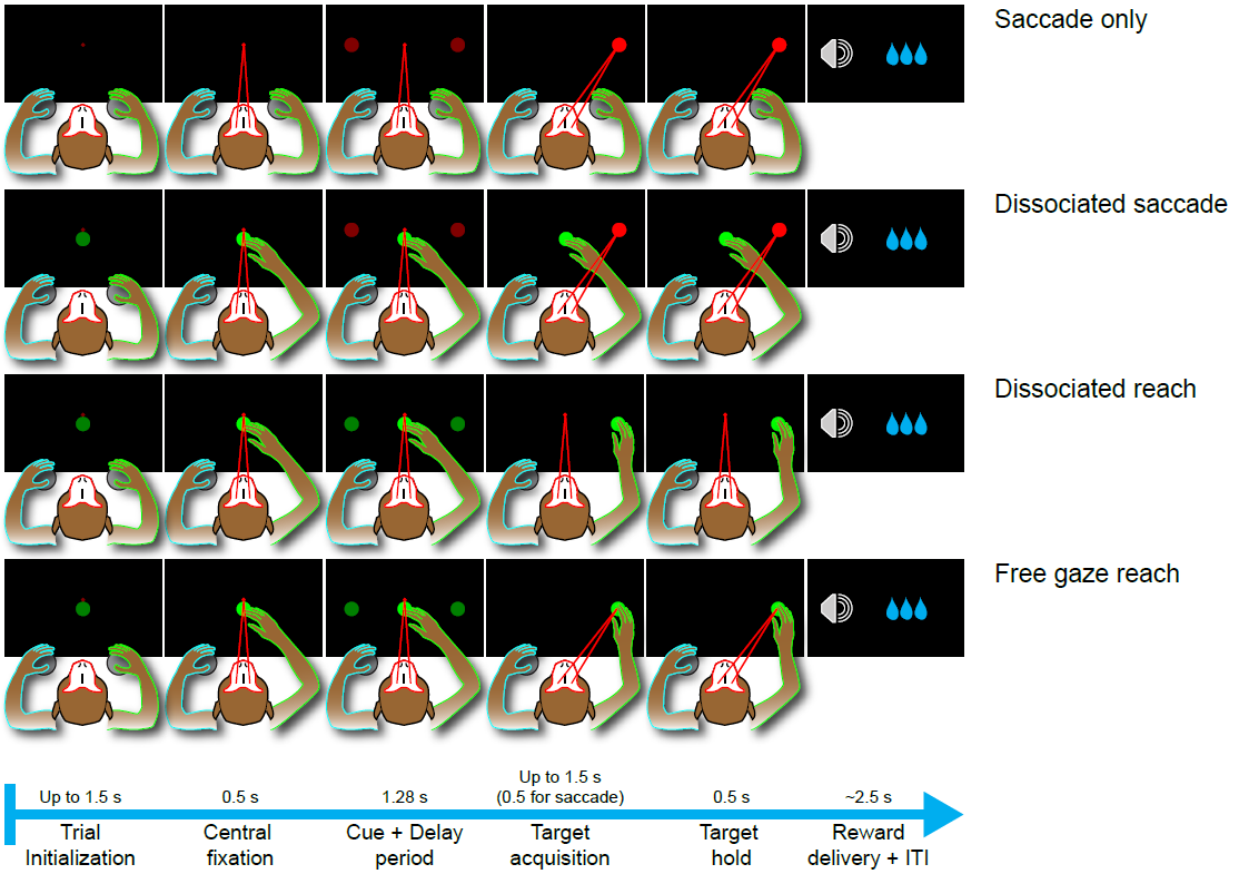
For monkey F, all tasks were performed as pseudorandomized interleaved trials of effector and required hands. For monkey L, the task was performed with the required hand blocked or interleaved. Also in monkey L task types were either blocked or interleaved.

Delayed visually-guided tasks

For all task types (**Figure II.2**), a trial started with the monkey resting both hands in two sensors positioned in front of them for 0.5 s. Next, two dim fixation spots appeared in the center of the display: a small and dim 0.5° radius red circle (*eye fixation*) above a larger and dim 4° radius green or blue circle (*hand fixation*) located at the horizontal center of the screen at monkey's gaze height. A green *hand fixation* cued monkeys to use their right arm; a blue *one* cued the left arm. If an un-cued hand was lifted from the sensor at any time during the trial such that trial was immediately aborted and went back in the pool of pseudorandomized conditions. Monkeys were required to look at the *eye fixation* and touch the *hand fixation* within 1.5 s from presentation; then the fixation circles would brighten up and the monkeys had to maintain fixation for 0.5 s to start the contingency-specific part of the trial. A dim peripheral stimulus at 24° to the right or left of the central fixation circles cued the location and effector of a future movement: a red circle indicated a future saccade, a green/blue circle a future reach. In addition to purely horizontal targets, two targets with the same eccentricity of 24° were presented 20° of angular degrees above or below the horizontal gaze center for a total of three target locations to the left and three to the right. Monkeys were instructed to respond when either one or both fixation spots disappeared in the center of the screen after a delay of 1.28 s. For reaches, if only the *hand fixation* disappeared monkeys had to make a reach while keeping their gaze at the *eye fixation* (dissociated reaches). If both *fixations* disappeared monkeys had to make a reach while they

could freely look around for the rest of the trial (free gaze reaches). For eye movements, monkeys had to make a saccade while keeping their hands at the *hand fixation* (dissociated saccades). Once the movement took place, the targets would brighten up, and the monkeys had to maintain their gaze/hand position for 0.5 s on the target. After each successful or failed trial, there was a 2.5 s or 2 s inter trial interval respectively. In total, monkeys had to achieve 10 hits to each target location for the block to be completed. The luminance of the dim/bright targets for saccade, left arm and right arm cues were: 9.4 cd/m^2 33 cd/m^2; 34 cd/m^2 87 cd/m^2; and 22.5 cd/m^2 94 cd/m^2 respectively with a 0.16cd/m^2 background.

Figure II.2. **Tasks layout**



A trial started when the monkey rested both hands in touch sensors. Then two fixation spots, a red for eye, and either a green or blue for the right or left hand respectively appeared. Monkeys could perform a peripheral movement only after at least one of the central *fixation*s spots was extinguished, signaling which effector to use to acquire a peripheral target. Monkeys had to prepare a dissociated saccade (arm resting in the center of the monitor for the duration

of the trial) or a reach. The reach could be a free gaze (likely foveally-guided) or a dissociated reach (eye *fixation* in the center).

Saccade definitions

A saccade was detected whenever there was a change in eye trace instant velocity larger than 200°/s in Euclidean distance. Saccade offsets reflected the time when saccade velocities dropped below 50°/s. Saccade velocities were derived from interpolated (220 Hz to 1 kHz) and smoothed eye position traces with a 15 ms moving average rectangular window, which was then smoothed again with a second 15 ms moving average rectangular window.

Saccade latency was defined as the time between fixation spot(s) offset and the moment when the first saccade was detected during the target acquisition period in each trial. Saccade duration was defined as the time between saccade onset and offset. Saccade peak velocity was defined as the maximum instant velocity across the duration of the saccade of interest.

Reach definitions

Reach latency (reaction time) was defined as the time between fixation spot(s) offset and the moment when the hand lost contact with the touchscreen during the target acquisition period in each trial. Reach duration was defined as the time from the reach latency to the next touchscreen contact.

Statistics

For all tasks, for behavioral analysis successful trials to targets to the left and to the right hemi spaces were combined. All data analysis was performed using MATLAB R2012b and the Statistics Toolbox. To test for changes in mean movement latency, duration, velocity, and accuracy we used independent t-tests.

Electrophysiological recordings

For the reach dataset 285 units recorded from monkey L (220 and 65 from left and right hemisphere, respectively) and 172 units from monkey F. From these 233 (182 left, 51 right, monkey L) and 145 (monkey F) were used after an exclusion criterion according to the unit stability and/or its classification as a single cell. An additional exclusion criterion was also used: if there were less than 60 trials per hand per task or there were less than 50 spikes per unit the unit was not analyzed. This led to a final dataset of 220 (168 left, 52 right) and 129 units for monkey L and F, respectively. For population analyses we combined the data from both hemispheres of monkey L. The total count of units per task was: Monkey L 180 dissociated saccades (97 blocked hands), 193 dissociated reaches (97 blocked hands), 159 free gaze reaches (87 blocked hands), and 129 for monkey F for all tasks.

Analysis of firing rate

Neurons were sorted offline using Offline Sorter v.4.0.0 and v.2.8.8 (Plexon, USA) using either a waveform template algorithm, a principle component analysis with k-means clustering algorithm, manual contour definitions, or a combination. Spike density functions for each trial were derived by convolution of the discrete spike arrival times with a Gaussian kernel (SD 20 ms). Epochs of interest are described in **Table II.1**.

Table II.1 **Epochs of interest, reach-saccade dataset**

EPOCH BEING ANALYZED	Trial initialization	Fixation acquisition	Fixation hold	Cue	Early delay	Late delay	Pre saccade	Peri saccade	Post saccade	Pre reach	Peri reach	Post reach	Target hold
REFERENCE EVENT time (s) to reference period	Fixation spot onset -0.4 -0.1	Fixation acquired -0.04 -0.1	Cue onset -0.3 0	Cue onset 0.05 0.15	Go (fixation spot offset) -0.6 -0.3	Go (fixation spot offset) -0.3 0	Saccade onset -0.1 -0.01	Saccade onset -0.01 0.05	Saccade offset 0.05 0.2	Reach onset -0.3 -0.01	Reach onset -0.05 0.15	Reach offset 0.05 0.2	Target offset -0.3 0
BASELINE	-	Trial initialization	Trial initialization		Fixation hold	Fixation hold	Early delay	Early delay	Early delay	Early delay	Early delay	Early delay	Fixation hold

For each of the epochs of interest (top row) the table shows a reference event (middle row) and the time window relative to it for the calculation of average firing rates. All firing rate epoch comparisons were done taking the reference event for each trial and comparing it to a baseline (bottom panel).

For population analysis, trials in the same hemispace relative to the recorded hemisphere were combined. For each unit, if for a given neuron information from both hands was available, a three-way ANOVA including hand, hemispace and epoch as factors was performed, otherwise a two-way ANOVA using hemispace and epoch as factors was used. The hemispace with the higher firing rate was marked if there was a significant difference determined by unpaired t-tests. A similar approach was used to determine hand specific tuning. Enhancement or suppression of neuronal firing was defined for each epoch relative to a baseline specified independently for each epoch (see **Table II.1**) using paired t-tests comparing firing rates for ipsilateral and contralateral hemifields independently and regardless of hand. Enhancement or suppression was reported if either ipsilateral, contralateral, or both types of trials showed significant difference from baseline, regardless of the used hand. In cases where one hemifield showed significant enhancement, while the other had suppression, the unit was reported to have a bidirectional response.

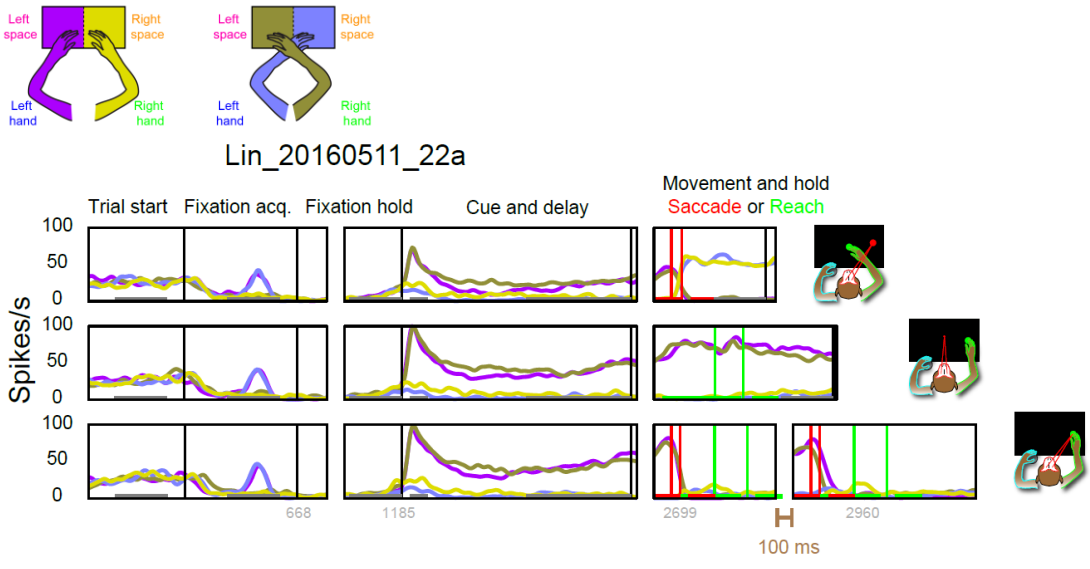
For baseline-corrected population PSTHs, the average firing rate during the late period of the inter-trial interval that immediately preceded the trial start (fixation spot onset) was subtracted from the spike density function, separately for each trial. Responses for each unit were derived by averaging raw firing rates across all trials for the respective condition.

Results

Single cell examples

To address the questions of if dorsal pulvinar participates in the planning and/or execution of upper limb movements, the integration of eye and hand movements, or even in the dissociation of movement plans into specific effectors, a series of tasks were performed by two monkeys. In these tasks, the monkeys were required to do natural free-gaze (i.e. likely foveally-guided) or dissociated (only one effector performing the action) eye-hand movements. Dorsal pulvinar neurons displayed an array of responses e.g. visual modulation, spatial tuning, pre, peri and post movement enhancement or suppression and combinations. Given that many units were recorded during all three tasks: dissociated saccades, dissociated (extrafoveal) reaches, and free gaze (likely foveal) reaches, it was possible to visually inspect putative firing differences due to different effectors. **Figures II.3** to **II.9** illustrate some of the most salient characteristics of single cell examples.

Figure II.3 **Visual unit**



Raw average peri-stimulus time histograms (PSTHs) per task per hemispace during delayed visually-guided tasks. Each row shows PSTHs from one of the three tasks presented to the

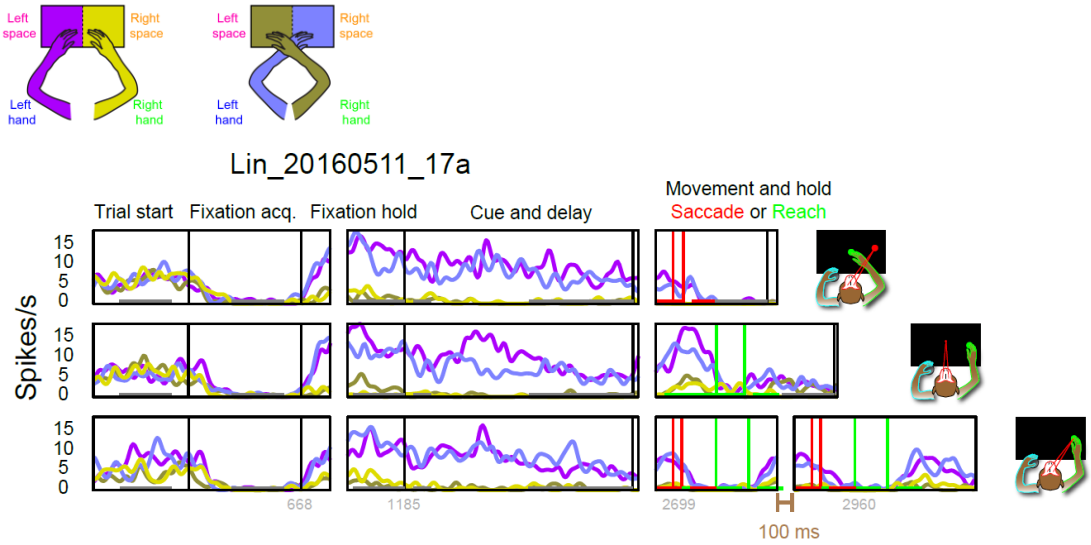
monkeys: dissociated saccades (top row), dissociated reaches (middle row), and free gaze reaches (bottom row). Trials performed to targets to the left and to the right of the *fixation* were pooled together (per hemispace) and according to the hand used for a maximum of four traces per panel. The color code used here is consistent across single cell plots: rightward trials using right hand (yellow trace), leftward trials using right hand (olive trace), leftward trials using left hand (magenta trace) and rightward trials using left hand (blue trace). Panels in each row are divided to represent the alignment to: onset of fixation hold, onset of the cue, and the onset of the movement, saccade for dissociated saccades and free gaze reaches, and reach for dissociated and free gaze reaches. Labels above the top panel mark the location of relevant task or behavioral events. Vertical red and green line-pairs are the onset plus average duration of saccade and reach respectively. This representation is consistent across single cell examples.
This example neuron increased its firing rate by visual inputs presented in the left hemispace. Top, for dissociated saccades there was enhancement of firing by the presentation of a cue to the left. After a rightward saccade, either the left or right hand remained to the left of the fovea (containing the receptive field of the unit), which made the "tuning" shift as after the eye movement to the right. Middle, in dissociated reaches as the hand moves to the left but the eye stays at the display's center it makes the cell's receptive field to keep its tuning properties consistent for the trial duration. Bottom panel, in free gaze reaches the cue tuning remains like the other tasks. At the time of the movement, once the saccade happens and brings the stimuli away from the receptive field and to the fovea there is a drop in the firing rate to baseline level. Note that a small peak during "fixation acquisition" is there for all tasks when fixing left hand. This peak revealed to be visually influenced by the movement of the hand from the resting sensor to the center of the monitor as it passed through the cell's receptive field.

Figure II.3 shows the average firing of a unit whose modulation was clearly visual and tuned to the contralateral hemispace (left visual field, right dorsal pulvinar recorded for this unit). During dissociated saccades, where the monkey was required to keep its contralateral or ipsilateral hand at the center of the display (left or right hand for this example cell) cue tuning was enhanced to the left hemispace. This pattern was consistent in all tasks. There was either an apparent shift of tuning after a saccade to the right (the hand is still at the *fixation,* so the receptive field tuned to a left cue is now responsive to the largely leftward location of the hand). If a reach was performed but no saccade the tuning was maintained to the left hemispace. If there was a free gaze (likely foveal) reach, the left receptive field of

the neuron stopped being stimulated as soon as the target was foveated, which made the unit to stop firing.

A few movement-specific units like the one in **Figure II.4** were suppressed during the reach both to the fixation spot and to the peripheral targets and had hand specific enhancement or suppression during movement preparation.

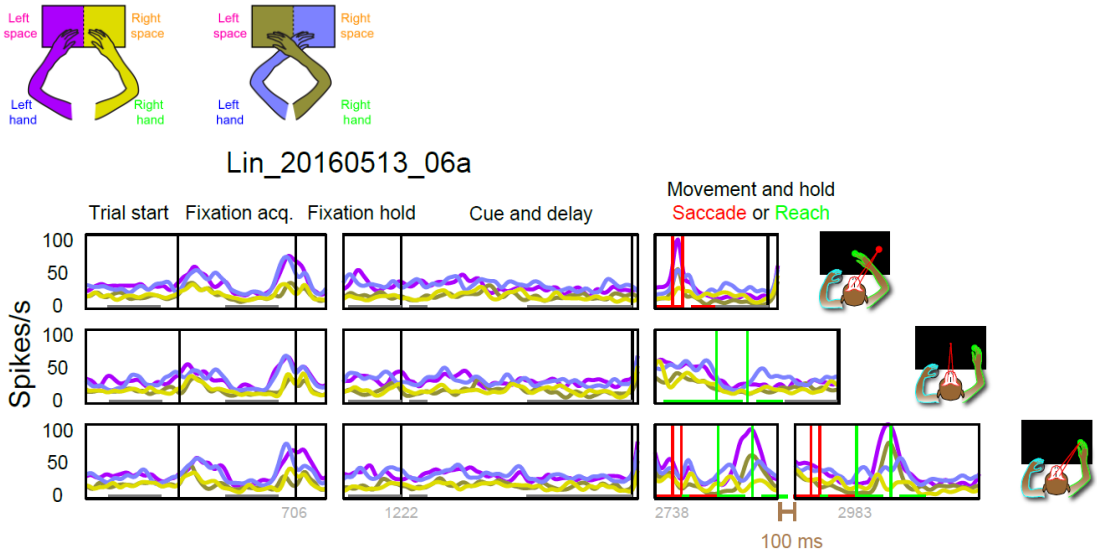
Figure II.4 **Unit suppressed by reaches**



For all tasks, there was suppression of firing while the monkey reached from the resting sensors to the central fixation spot regardless of the hand used. Later, saccade and reach cue presentation had an enhancing influence on the firing rate of the unit. This effect was highest close to the onset of the reach (middle panel), and suppressed during the actual movement. A similar effect was present when eye movements could accompany the reach (bottom panel). Such firing rate decrease did not happen during the saccade task (top panel) but a similar suppression was also seen in the post saccade period.

The unit from **Figure II.5** was enhanced by a dissociated saccade, suppressed by an extrafoveal reach and enhanced by a foveal reach but only after the reach was performed i.e. not modulated by the saccade.

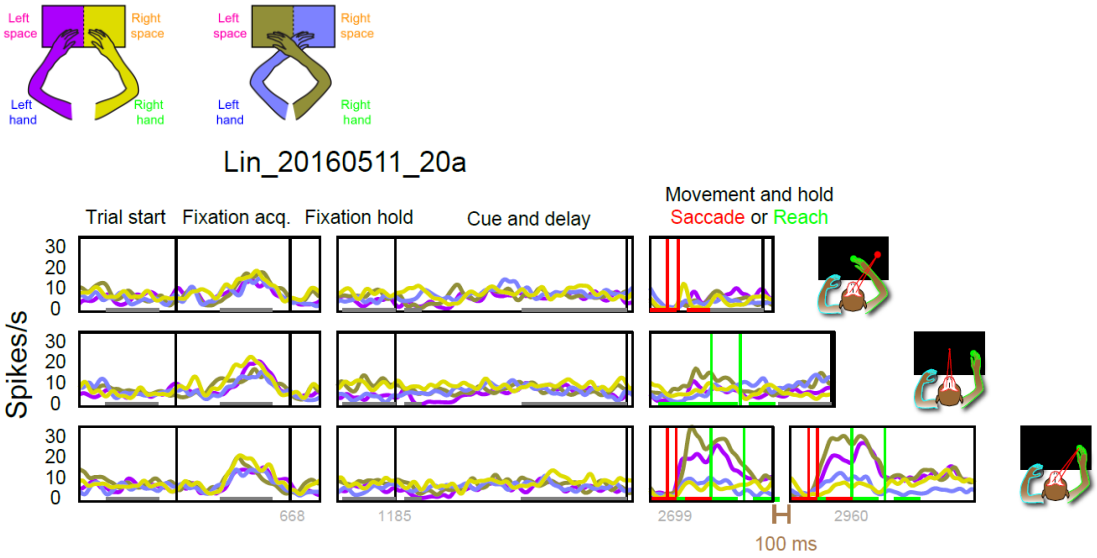
Figure II.5 **Interaction of saccade and reach, suppression for reach only**



For all panels, this unit's firing increased at the start and end of the reach to the fixation spot. During dissociated saccades (upper panel), there was a clear peri saccade increase of firing rate for leftward saccades. During dissociated reaches (middle panel), there was suppression of the firing rate around the movement. During free gaze reaches (bottom panel) there was reach and post reach enhancement of firing.

A unit modulated only by visually-guided reaches is shown in **Figure II.6**. This unit was space specific and responded for the free gaze condition before and until the end of the visually-guided reach.

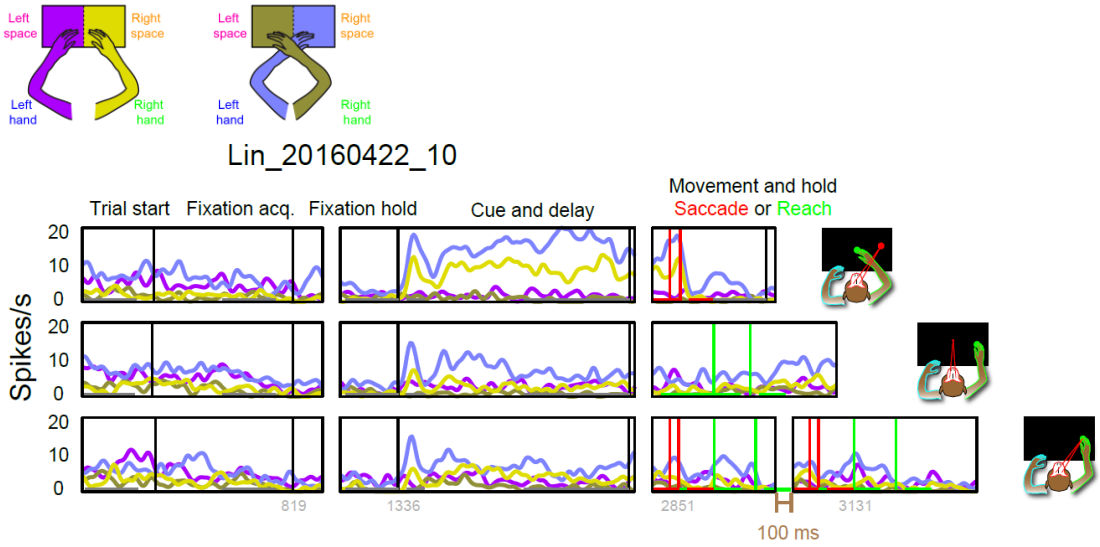
Figure II.6 **Firing rate enhancement for combined effector movements**



For all panels, there was enhancement of firing during reaches to the initial fixation spot. This increase was similar for both hands. Later and for all tasks, there was no modulation during the cue presentation and the delay period. For the free gaze reach condition (bottom panel) there was enhancement of firing rate to the left hemispace well before the reach was performed.

Other complex interactions between effector and space are shown in **Figure II.7** where an increase of firing occurred for dissociated saccades, in contrast to the dissociated reach and free gaze reach tasks. Additionally, the firing rate was higher for the ipsilateral hand (left hand for this unit).

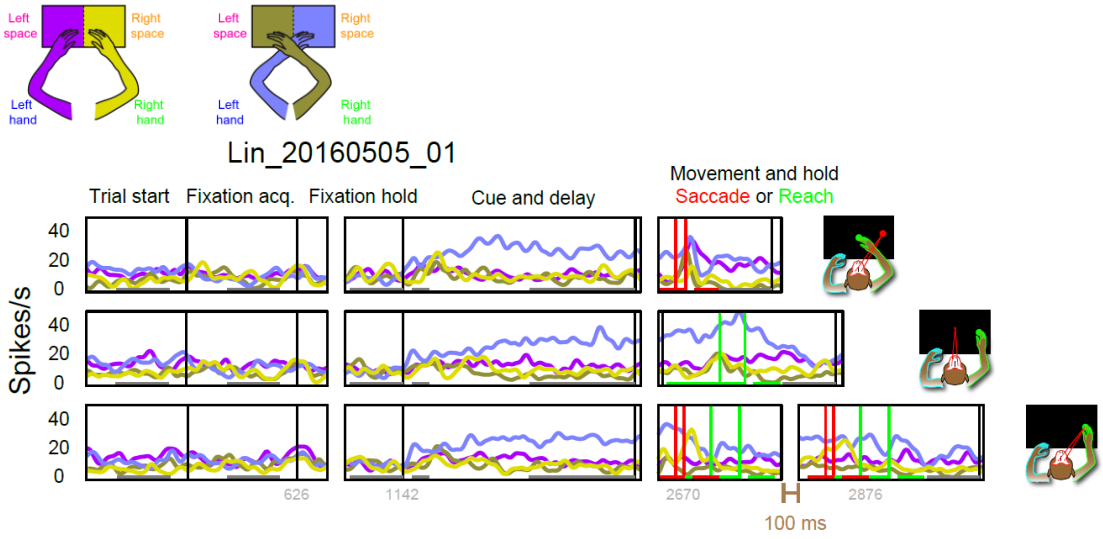
Figure II.7 **Space, hand, task and epoch delay related firing**



For all tasks, this unit was responsive to the presentation of a cue in the right hemispace. The firing to cue was different for future reach and saccade movements. Due to non-matching luminance for the saccade and reach cue the higher firing for the saccade cue due to the difference in luminance for these stimuli, however, the reach cue would likely evoke the largest response as its luminance was higher (*see Methods*). For dissociated saccades (top panel) the unit's modulation continued to increase until the saccade execution. This enhancement was higher when using the left hand than when using the right one. For both reach tasks, after the initial transient firing increase at cue's presentation there was a decrease back to baseline during the delay and movement execution.

Figure II.8 Shows the influence of hemispace during the preparation and execution of a movement to the contralateral hemispace when the ipsilateral hand (left) is used both for reaches and saccades. For this cell, the largest responsivity was not purely visual as there was no fast firing rate increase to the onset of the initial fixation spot or cue, but there was a strong slow ramping up of firing during the delay period and well until the offset of the reach.

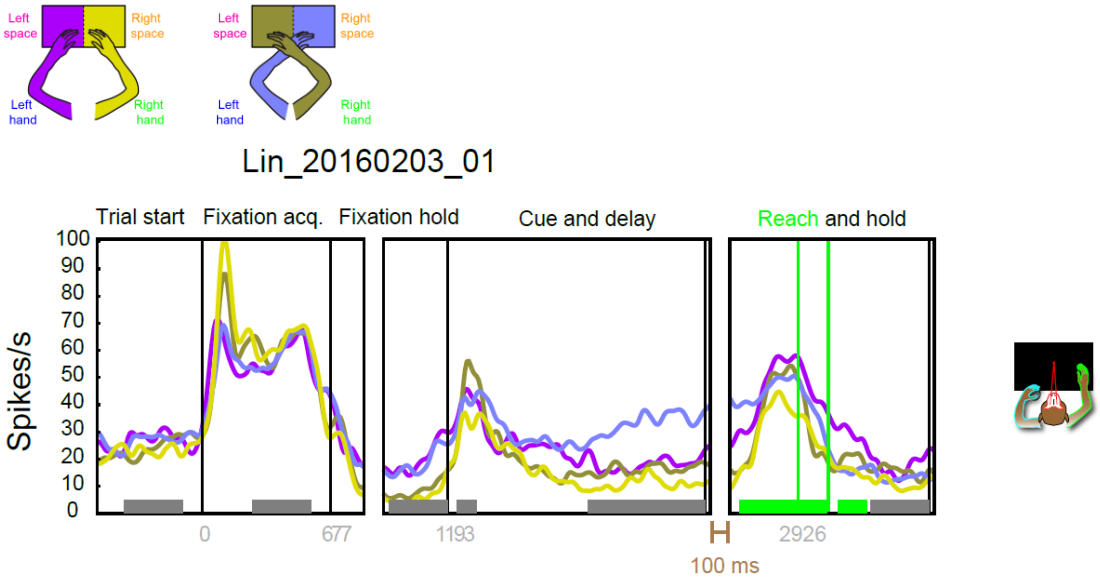
Figure II.8 **Early preparation of a movement for a specific side and effector**



For all tasks, there was a ramping up of firing after the cue's presentation until the end of the movements. This firing rate increase was specific for the left arm and the right hemispace. The enhanced firing was present for saccades and reaches.

Finally **Figure II.9** shows a high firing unit during the free gaze reach task. The unit's firing increased preferentially for the contralateral hand (right) to a reach to the *fixation* and later increased to plan a likely foveally-guided reach to the right hemispace with the ipsilateral hand. The unit was enhanced until shortly before the movement for all conditions.

Figure II.9 **Interactions**



This unit was recorded for the dissociated reach condition only. It had firing rate enhancement at fixation onset; this effect was stronger when the monkey used its right arm. During the fixation hold and before cue onset the firing was suppressed, mostly while fixating with the right arm. Suppression during the central fixation (with a residual effect of hand still apparent) was followed by increase of firing during cue presentation and a ramping up of firing for movements to the right using the left hand.

Reach population grouping

To assess how the different visual and motor responses were represented in the population firing of dorsal pulvinar two groups were made. For the first group, data from both monkeys where they performed the tasks with a randomized use of hands were combined (157 units, two monkeys combined). A second group was done to assess the effects of instructing the arm to be used in blocks (87 units recorded during the free gaze task and 97 units recorded during the dissociated saccade and reach tasks for monkey L only, as for monkey F the tasks were always performed with interleaving the hand and task).

Table II.2 and II.3 summarize behavioral patterns found in monkey L and F for reaches and saccades respectively. This summary encompasses both subgroup datasets (where the hands were blocked and interleaved) as from the moment when the hand was in the central

fixation spot monkeys' behavior is expected to be similar. Importantly, for monkey L in five sessions each block contained one effector type only, which could cause faster mean reaction times as the monkey potentially knows beforehand what movement to perform. In general, Monkey L had a tendency for longer reach latencies than monkey F in all conditions regardless of hand, hemispace, and task contingencies ($p<0.05$ in all conditions but dissociated reaches to the left hemispace with the left hand $p=0.07$).

Some of the general observations regarding the differences between monkeys were that Monkey F took up to 70 ms longer to finalize a reach in comparison to monkey L, who had mean reach duration of 171 ms $\pm$ 7 ms ($\pm$ SE) across conditions. Saccades latencies ranged between 170 ms and 210 ms across conditions and monkeys, with durations between 50 ms and 60 ms.

Table II.2 **Behavioral summary per monkey for reaches**

	Monkey L		Monkey F						Monkey L		Monkey F					
	LEFT															
Foveal reach	LH mean	LH SE	LH mean	LH SE	Effect	p	t	df	RH mean	RH SE	RH mean	RH SE	Effect	p	t	df
Latency (sec)	0.40	0.01	0.35	0.01	-0.06	**0.00**	6.54	31.00	0.43	0.01	0.35	0.01	-0.08	**0.00**	7.71	32.00
Duration (sec)	0.18	0.00	0.19	0.00	0.01	0.21	-1.27	31.00	0.18	0.00	0.21	0.00	0.03	**0.00**	-4.03	32.00
Extrafoveal reach																
Latency (sec)	0.38	0.01	0.36	0.01	-0.02	*0.07*	1.88	34.00	0.41	0.01	0.39	0.01	-0.02	**0.04**	2.10	35.00
Duration (sec)	0.16	0.00	0.18	0.00	0.03	**0.00**	-5.54	34.00	0.15	0.00	0.20	0.01	0.04	**0.00**	-4.69	35.00
	RIGHT															
Foveal reach	LH mean	LH SE	LH mean	LH SE	Effect	p	t	df	RH mean	RH SE	RH mean	RH SE	Effect	p	t	df
Latency (sec)	0.44	0.01	0.37	0.01	-0.07	**0.00**	4.43	31.00	0.42	0.00	0.35	0.01	-0.07	**0.00**	8.42	32.00
Duration (sec)	0.20	0.00	0.20	0.01	0.00	0.97	-0.03	31.00	0.18	0.00	0.23	0.00	0.06	**0.00**	-8.85	32.00
Extrafoveal reach																
Latency (sec)	0.42	0.01	0.38	0.01	-0.04	**0.03**	2.21	33.00	0.39	0.01	0.35	0.01	-0.04	**0.00**	3.40	35.00
Duration (sec)	0.18	0.01	0.16	0.00	-0.02	**0.03**	2.23	33.00	0.14	0.00	0.22	0.00	0.07	**0.00**	-11.83	35.00

Descriptive statistics for behavioral parameters in monkey L and F and inferential statistics between monkeys L and F with effect size F-L. For delayed reach tasks. inferential tests were performed to assess the similarity of reach latency and duration across monkeys. Reaches performed to the left hemispace are in the upper panel (magenta shaded) while reaches to the right hemispace are in the bottom panel (orange shaded). Trials performed with the left hand are shaded in cyan while trials performed with the right hand are shaded in green. The p-values were derived from two-tailed non-paired t-tests across sessions for all electrophysiology recording sessions (bold $p<0.05$, italics $p<0.1$). Reaches that occurred before 200 ms from the Go signal were excluded from all behavioral analysis as they are considered express movements. In general monkeys had substantial differences in both latency and duration of reaches. The periods of interest for spike analysis were defined relative to the movement or using periods suitable to both monkeys according to their behavior. *LH, left hand; RH, right hand; SE, standard error of the mean, df, degrees of freedom*

Table II.3 **Behavioral summary per monkey for saccades**

	Monkey L		Monkey F						Monkey L		Monkey F					
	LEFT															
Free gaze saccade	LH mean	LH SE	LH mean	LH SE	Effect	p	t	df	RH mean	RH SE	RH mean	RH SE	Effect	p	t	df
Latency (sec)	0.19	0.00	0.20	0.00	0.01	**0.00**	-3.90	31.00	0.19	0.00	0.19	0.00	0.00	0.65	0.46	32.00
Duration (sec)	0.05	0.00	0.05	0.00	0.00	**0.00**	-7.94	31.00	0.05	0.00	0.05	0.00	0.00	**0.00**	-6.25	32.00
Velocity (°/s)	751.76	8.30	688.31	4.77	-63.45	**0.00**	5.16	31.00	755.97	7.68	699.67	5.62	-56.30	**0.00**	4.76	32.00
Dissociated saccade																
Latency (sec)	0.20	0.01	0.19	0.00	-0.02	*0.07*	1.89	31.00	0.19	0.00	0.18	0.00	-0.01	0.19	1.35	32.00
Duration (sec)	0.06	0.00	0.05	0.00	-0.01	**0.00**	4.21	31.00	0.06	0.00	0.05	0.00	-0.01	**0.00**	4.46	32.00
Velocity (°/s)	711.93	11.25	608.28	7.96	-103.65	**0.00**	6.11	31.00	724.84	10.45	606.03	12.37	-118.81	**0.00**	6.83	32.00
	RIGHT															
Free gaze saccade	LH mean	LH SE	LH mean	LH SE	Effect	p	t	df	RH mean	RH SE	RH mean	RH SE	Effect	p	t	df
Latency (sec)	0.18	0.00	0.20	0.00	0.02	**0.00**	-5.26	31.00	0.21	0.00	0.19	0.00	-0.01	**0.02**	2.42	32.00
Duration (sec)	0.05	0.00	0.05	0.00	0.00	0.38	0.90	31.00	0.05	0.00	0.05	0.00	0.00	0.15	1.48	32.00
Velocity (°/s)	717.76	4.89	650.17	3.71	-67.59	**0.00**	9.09	31.00	730.18	5.59	650.27	3.81	-79.91	**0.00**	9.34	32.00
Dissociated saccade																
Latency (sec)	0.17	0.00	0.19	0.00	0.01	**0.00**	-3.83	31.00	0.19	0.00	0.18	0.00	-0.01	0.18	1.39	32.00
Duration (sec)	0.06	0.00	0.06	0.00	-0.01	**0.00**	4.85	31.00	0.06	0.00	0.06	0.00	-0.01	**0.00**	4.18	32.00
Velocity (°/s)	709.73	8.02	613.81	6.26	-95.92	**0.00**	7.84	31.00	707.39	8.41	623.22	5.32	-84.18	**0.00**	6.59	32.00
	LEFT								RIGHT		RIGHT					
Saccade Only	Mean	SE	Mean	SE	Effect	p	t	df	Mean	SE	Mean	SE	Effect	p	t	df
Latency (sec)	0.19	0.00	0.20	0.00	0.01	0.26	-1.15	26.00	0.18	0.00	0.21	0.00	0.04	**0.00**	-5.52	26.00
Duration (sec)	0.06	0.00	0.05	0.00	0.00	0.68	0.41	26.00	0.06	0.00	0.06	0.00	0.00	0.84	0.21	26.00
Velocity (°/s)	685.56	18.70	526.21	0.00	-159.35	0.12	1.61	26.00	658.50	15.93	509.26	0.00	-149.24	*0.09*	1.77	26.00

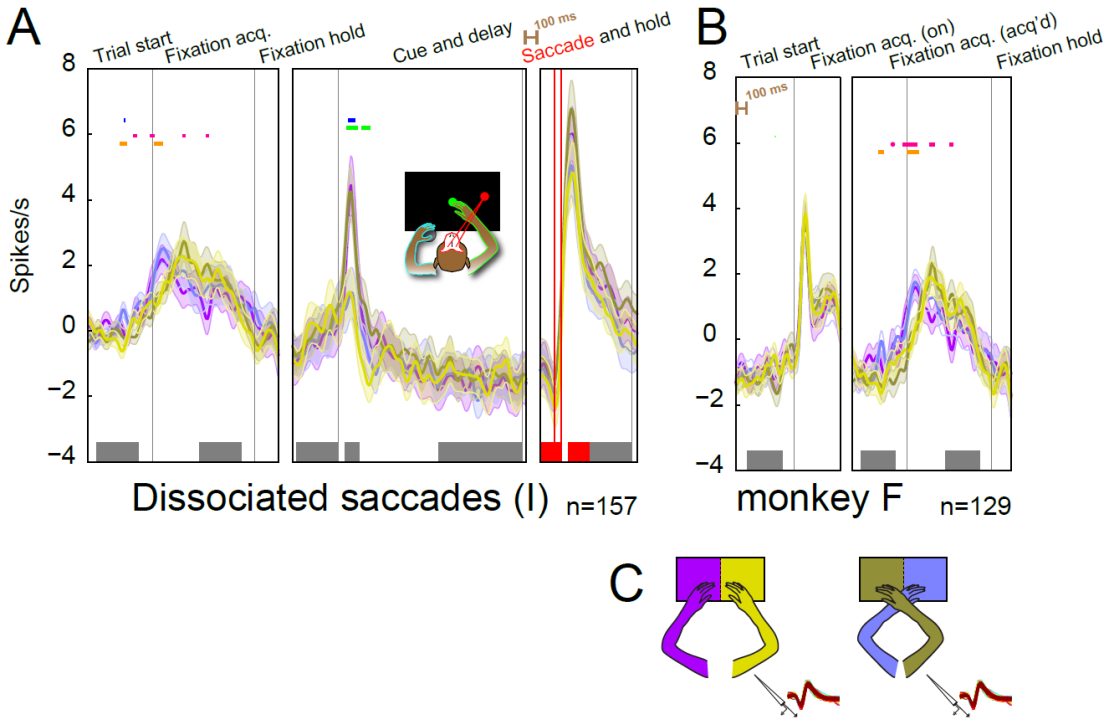
Like **Table II.2**, descriptive statistics for behavioral parameters in monkey L and F and inferential statistics for delayed saccade tasks between monkeys L and F with effect size F-L. Saccades performed to the left hemispace are in the upper and lower left panels (magenta shaded) while saccades to the right hemispace are in the middle and lower right panel (orange shaded). Trials that involved the left hand are shaded in cyan while trials involving the right hand are shaded in green. The p-values were derived from two-tailed non-paired t-tests across sessions for all electrophysiology recording sessions (bold $p<0.05$, italics $p<0.1$). Saccades that occurred before 80 ms from the Go signal were excluded from this analysis as they are considered express movements. In general monkeys had differences in both latency and duration of saccades when the usage of a hand was required for completing the trial.

Raw PSTHs during saccade and reach behavior

The baseline-subtracted raw (with no tuning property as preselection) average PSTH of dorsal pulvinar units in the interleaved hand design are plotted for the three eye-hand tasks. **Figure II.10** panel **A** shows population (n=157) properties during the dissociated saccade task. The PSTH traces are colored according to the recorded hemisphere. The largest population response was due to visual stimulation to the contralateral hemispace. In addition, peri and strong post saccade suppression and enhancement respectively were present for movements to both hemispaces and regardless of the engaged arm. As for all subsequent PSTHs, the alignment was done to the beginning of the fixation holding period. With this alignment, an initial peak of firing when using the contralateral hand (magenta

versus yellow traces) seemed to reflect an earlier response for the usage of the contralateral hand. However, realigning that period to the onset of the fixation spots in monkey F revealed that the peak to the fixation spot occurred at a similar time (as seen in **Figure II.10** panel **B).** For this comparison Monkey F was chosen because it had the largest hand reaction time difference between ipsilateral and contralateral hand, 286 ms vs 367 ms.

Figure II.10 Dissociated saccades raw PSTH

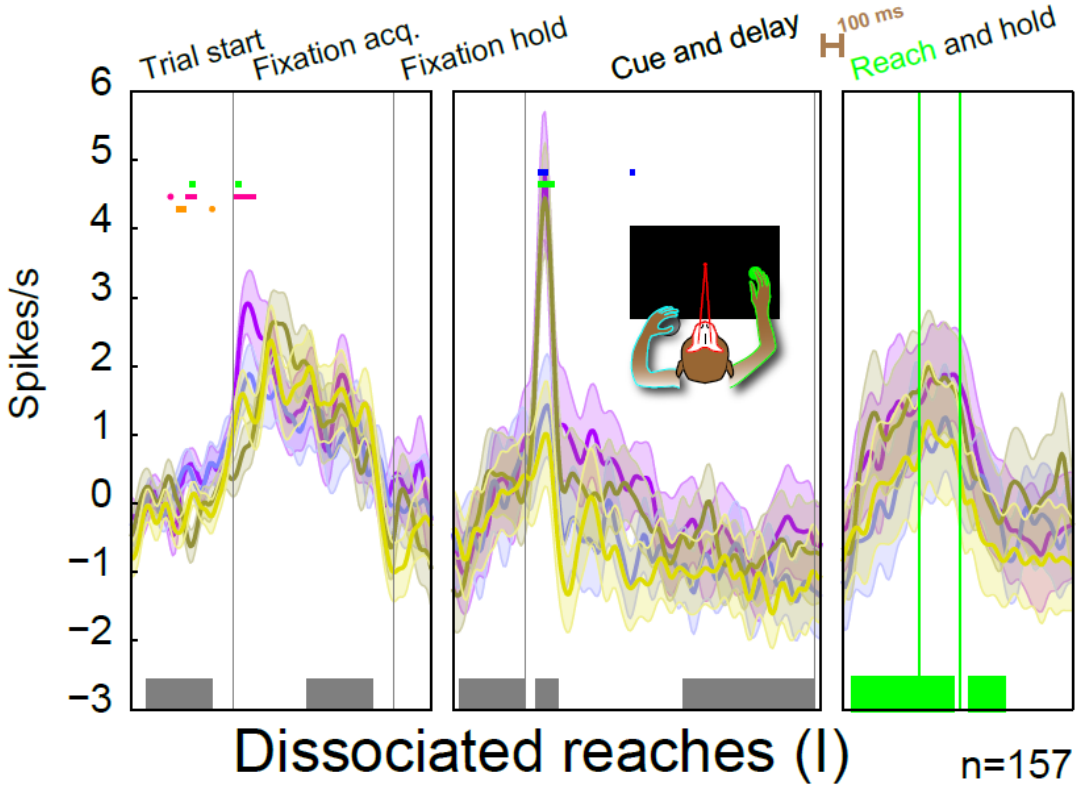


Main panels (A-B): Baseline corrected population PSTH (solid traces), and SE (shaded bars) combined for cells recorded during the dissociated saccade task. Trials were sorted according to the hemispace and hand used relative to the recorded hemisphere. Straight lines above the PSTH signify spatial tuning for the ipsilateral and contralateral hand (green and blue traces respectively) and hand tuning to the ipsilateral and contralateral hemispace (orange and magenta traces respectively). Subpanels are aligned to the fixation hold, cue onset and movement onset. Grey bars in the bottom signify analyses windows. Red bars in the bottom signify saccade analyses windows. C: PSTH line color conventions. Colors are referred in respect to the recorded hemisphere: Yellow for ipsilateral hand ipsilateral hemispace, Olive for ipsilateral hand contralateral hemispace, Blue for contralateral hand ipsilateral hemispace and Magenta for contralateral hand contralateral hemispace. A) For dissociated saccades, there was a clear firing enhancement by the cue onset as well as a spatial preference to the

contralateral hemispace. In addition, after the fixation onset, there was what appeared to be a delay in the onset of ipsilateral hand influence on the firing rate. This effect was however not present when realigning the PSTH to the fixation onset as in B. Around the saccade there was suppression of firing prior to the onset of the saccade, followed by peri and post saccade firing enhancement.

In the dissociated reach task (**Figure II.11)** there was similar tuning to the contralateral presentation of a cue. In addition, there was enhancement of firing during the late portion of the delay period, which was strengthened during and until after reach offset. In contrast to the dissociated saccade task, the firing enhancement around the reach time appeared earlier (in opposition to suppression before and during the saccade and post saccade enhancement). This finding suggests a potential participation of dorsal pulvinar in the planning of reaches.

Figure II.11 **Dissociated reaches raw PSTH**

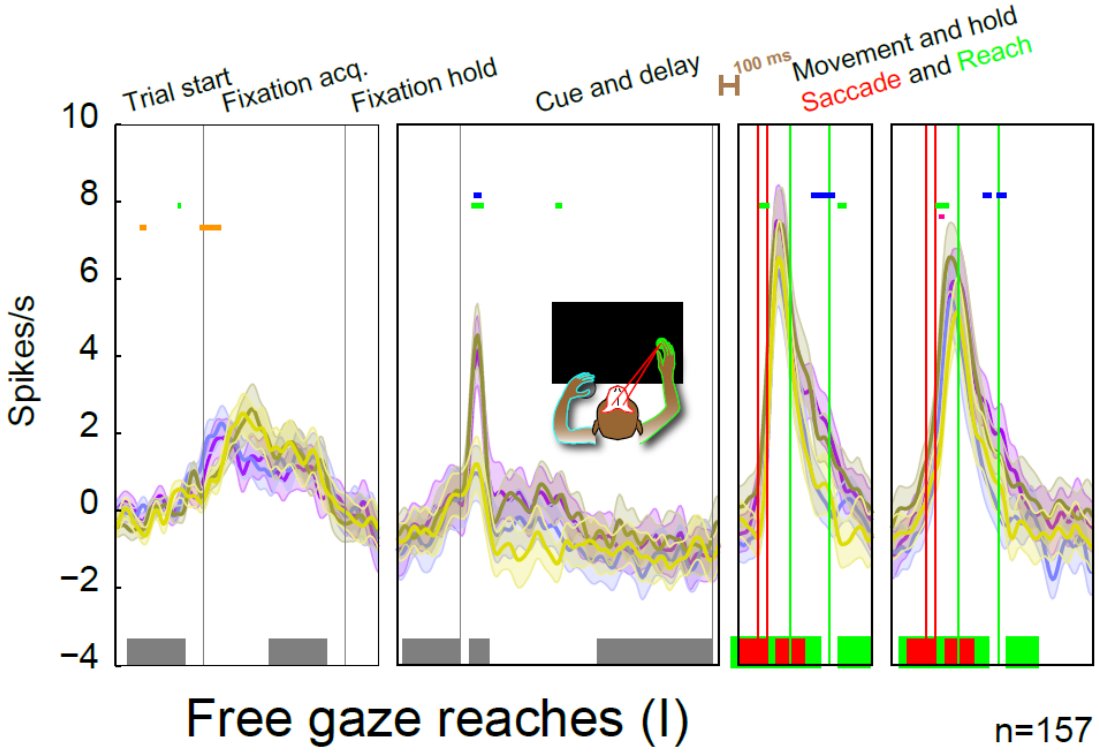


During dissociated reaches, there was cue-related firing rate enhancement to the contralateral hemispace. The main difference to dissociated saccades was an earlier onset of firing rate

increase to perform a reach. In addition, the firing was sustained until the offset of the reach, and there was no suppression of firing around the movement as for dissociated saccades. Green bars in the bottom signify reach analyses windows. Other conventions as in **Figure II.10.**

Finally, during free gaze reaches, where the monkey could perform a foveally-guided reach, the tuning largely resembled the saccade tuning (movement panel aligned to saccade in **Figure II.12**) as well as having a modest modulation for reaches to the contralateral hemispace using the contralateral hand. These results are consistent with our previous finding of strong spatial contralateral tuning in the cue response in *Chapter I* and *Ibis*. In addition, these results confirm the presence of reach related neurons in dorsal pulvinar, whose firing comes earlier and has a different pattern than the firing preceding saccade-only behavior.

Figure II.12 **Free gaze reaches raw PSTH**



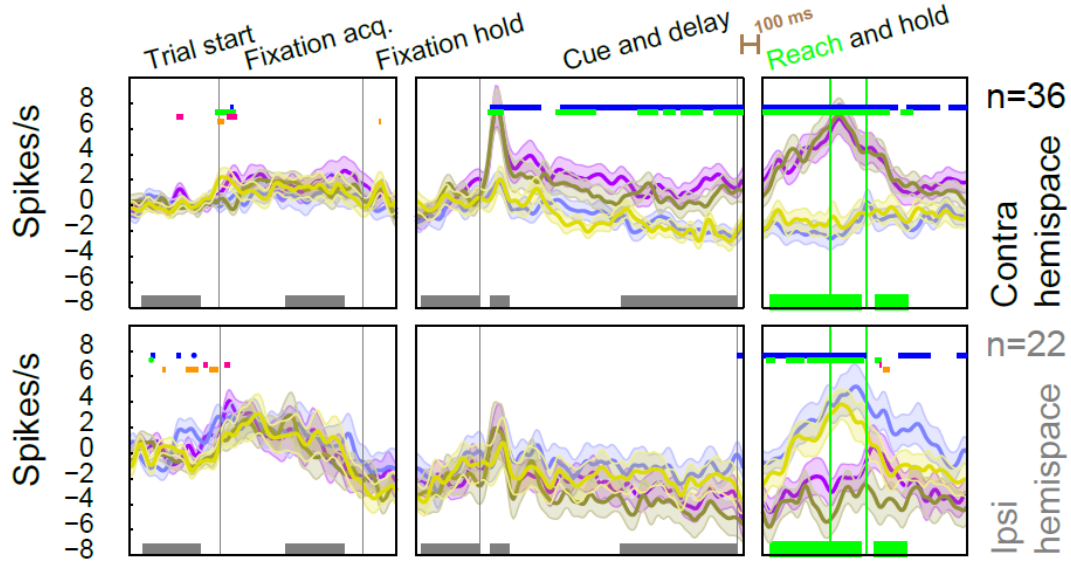
In the free gaze reach task, there was no evident firing rate suppression before saccade onset. Also, when compared with dissociated reaches (Figure II.11) the firing rate enhancement returned to baseline earlier, which could reflect a mixed influence of both saccades and reaches

in dorsal pulvinar. Red and Green bars in the bottom signify saccade and reach analyses windows respectively. Other conventions as in **Figure II.10.**

PSTHs grouped by spatial tuning properties

The combined dataset from both monkeys from hand interleaved trials was resorted to represent dorsal pulvinar subpopulations which were spatially-tuned at different task-relevant epochs (main effect of space for the epoch of interest in the corresponding ANOVA). For reaches, the period selected was the peri reach. Reach-related firing enhancement was found in subpopulations tuned to each hemispace (n=36 and n=22 cells tuned to the contralateral and ipsilateral hemispaces respectively, **Figure II.13)** well before the onset of the reach. It is important to note that for dissociated reaches it is possible that the planning of small saccades within the fixation window contributed to firing changes before the reach. This is an unlikely possibility however, as saccade related changes in dorsal pulvinar had a different pattern which was not sustained until after the reach offset.

Figure II.13 **Dorsal pulvinar neurons spatially tuned in the peri reach epoch**

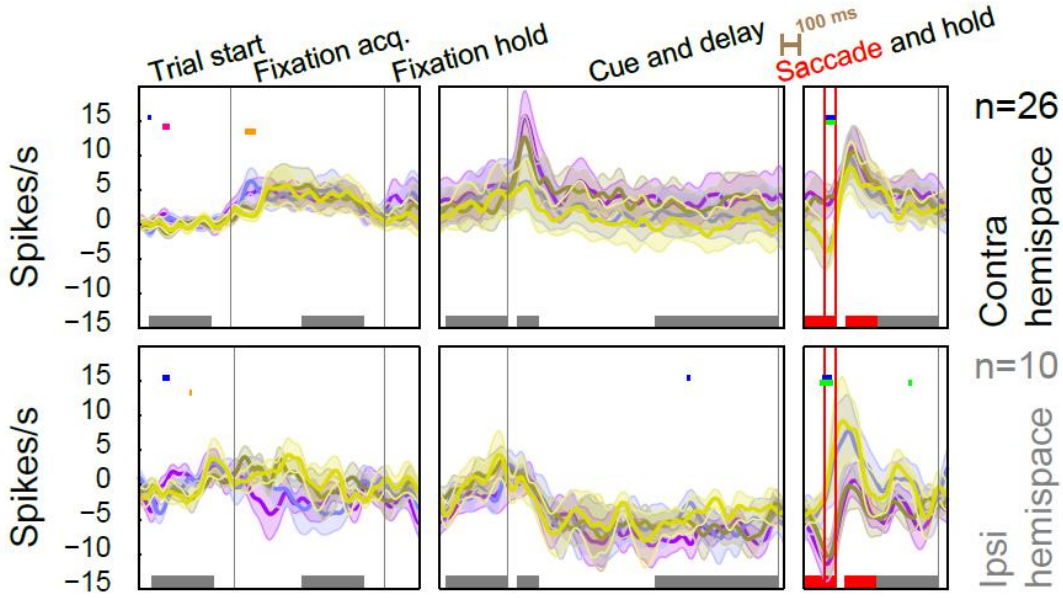


This and subsequent population plots represent cells that had a significant increase of firing rate in the epoch of interest (here, around the reach). For units with an ANOVA main effect of space in this epoch, the hemispace for which the neuron had the highest firing determined the

spatial preference. The spatial preference could be either contralateral or ipsilateral (top and bottom panels respectively) to the recorded hemisphere. In dissociated reaches with spatial tuning in the peri reach period, neurons displayed a larger increase of firing around the cue onset to the contralateral hemispace. In addition, there was enhanced firing before, during and after the reach for reaches to both hemispaces.

For the dissociated saccade task when aligning to the peri saccade epoch (**Figure II.14)** only a modest enhancement of firing in units which preferred the contralateral hemispace was observed. For units which preferred the ipsilateral hemispace there was in addition to the enhancement to the ipsilateral hemispace a marked suppression of firing rate when the stimulus was presented in the contralateral hemispace.

Figure II.14 **Dorsal pulvinar neurons spatially tuned in the peri saccade epoch**

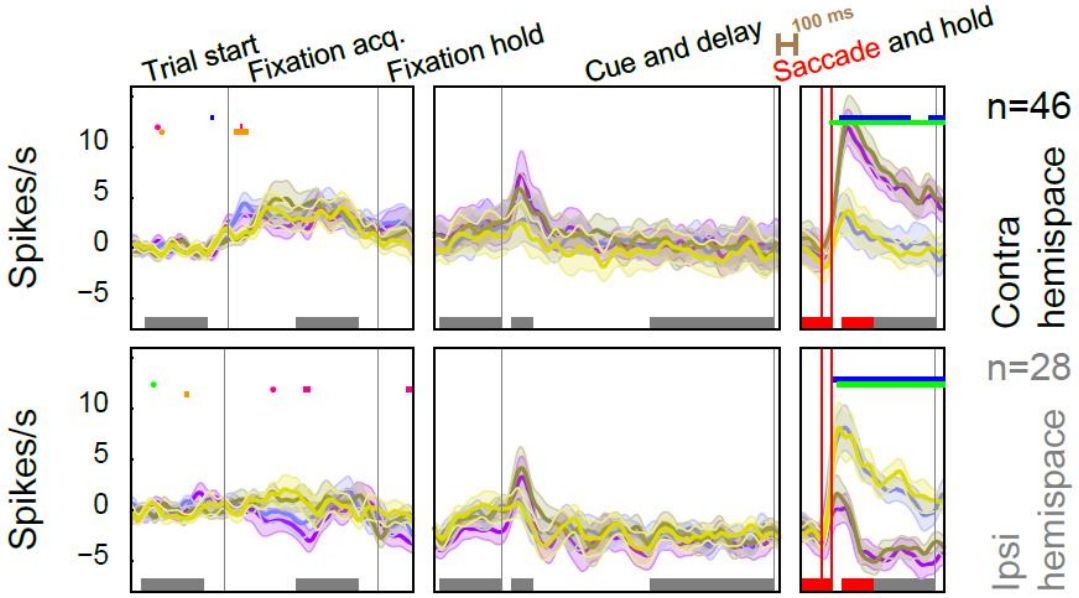


Firing rate enhancement in neurons with spatial preference in the peri saccade period showed a strong suppression to the non-preferred hemispace in the same time window, followed by enhancement after the saccade. This suppression is more apparent when looking at cells classified as enhanced to the ipsilateral hemispace around the saccade period when looking at the contralateral hemispace PSTHs.

When the alignment was made to the post saccade period (**Figure II.15)** there was firing enhancement for both contralaterally and ipsilaterally tuned units after the movement.

Interestingly, for cells tuned for the ipsilateral hemispace as soon as the saccade happened there was a quick drop of firing for trials to the contralateral hemispace.

Figure II.15 **Dorsal pulvinar neurons spatially tuned in the post saccade epoch**

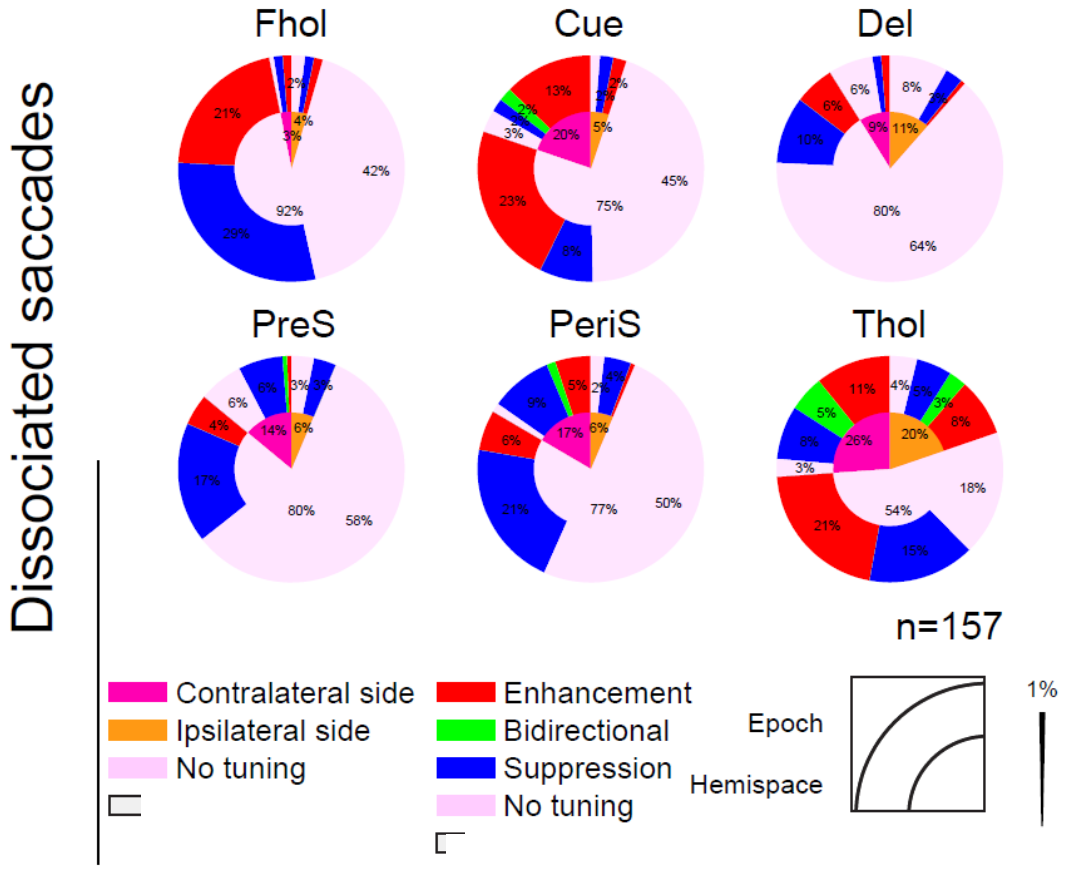


In pulvinar units with a spatial preference in the post saccade period, there was a firing enhancement to each of the hemispaces, but stronger to the preferred hemispace. In neither this nor the peri saccade subset there was significant cue response to the ipsilateral or contralateral hemispaces.

Cell counts

ANOVAs with main factor hemispace and epoch were performed to further characterize the spatial and tuning properties of pulvinar cells during the three recorded tasks. **Figure II.16** revealed that during the dissociated saccade task 25% of the cells had spatial tuning during the cue presentation (inner circle in the pie plot, 20% contralaterally tuned, 5% ipsilaterally).

Figure II.16 **Spatial tuning for the dissociated saccade task, interleaved hands**

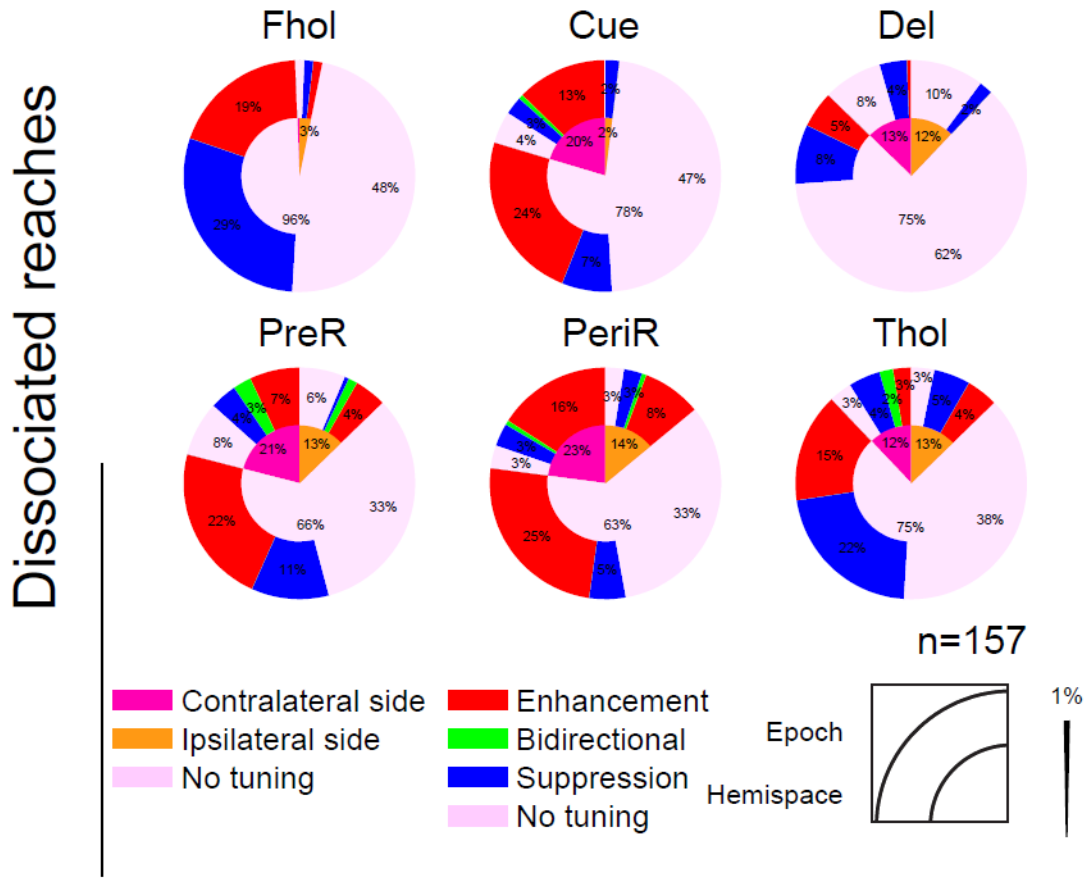


Cell percentage summary showing hemispace and firing modulation type for each epoch of interest in the dissociated saccade task. The inner pie plot contains the percentage of units with a main effect of hemispace (ipsilateral or contralateral to the recorded pulvinar) while the outer pie shows the nature of the modulation i.e. enhancement, suppression, or both effects per subpopulation. There was increased firing to the contralateral hemispace both during cue presentation and during the delay period as well as in the movement period. There was suppression of firing around the time of the saccade for spatially- but also for non-spatially-tuned units. After saccade offset there was a large enhancement of firing in the three subpopulations. This representation is consistent across similar summaries.

Later, during the delay period the tuning was equalized to the contralateral (9%) and ipsilateral (11%) hemispaces. For contralaterally tuned neurons (outer circle in the pie plot) there was in firing enhancement except for time before and around the saccade where units were strongly suppressed (6%) or showed no firing modulation. Non-spatially tuned cells were largely suppressed around the saccade (21% suppression versus 6% enhancement).

This suppression was reduced during the target hold period (15% and 21% suppression and enhancement respectively). The main difference of dissociated reaches (**Figure II.17**) to saccades (**Figure II.16**) was a large enhancement around the reach time that was present for ipsilaterally-, contralaterally- and non-spatially-tuned units (8% enhancement from 14% of ipsilaterally tuned neurons, 16% from 23%, contralaterally tuned, and 25% from 63% non-tuned units) in the peri reach period. Spatial tuning patterns were similar across tasks.

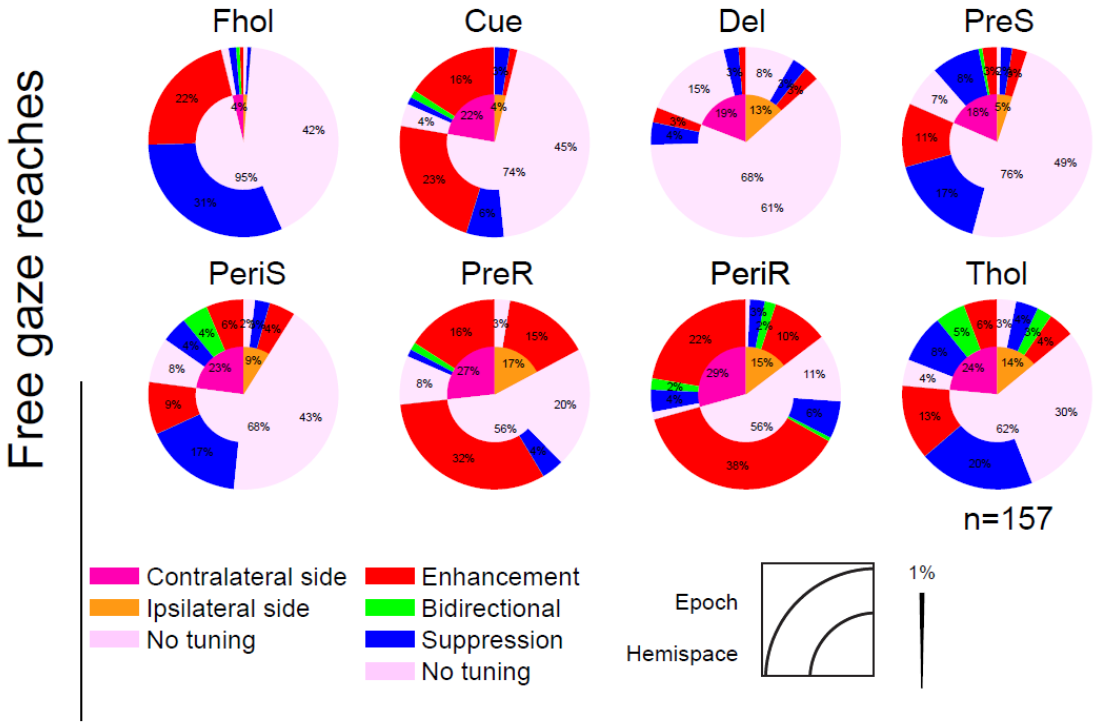
Figure II.17 **Spatial tuning for the dissociated reach task, interleaved hands**



Cell counts summary per hemispace and tuning types, for each epoch of interest in the dissociated reach task. There was a large enhancement of spatially- and non-spatially- tuned cells prior and during the reach opposite to findings in the dissociated saccade task (**Figure II.16**). The firing enhancement later decreased during the target holding period. This difference between firing across tasks might reflect a different involvement of pulvinar in the planning and execution of saccades and reaches in the visuo-motor hierarchy.

Importantly, in the free gaze reach task (**Figure II.18**) both tuning effects were present, large suppression around the saccade and enhancement around the reach.

Figure II.18 **Spatial tuning for the free gaze task, interleaved hands**

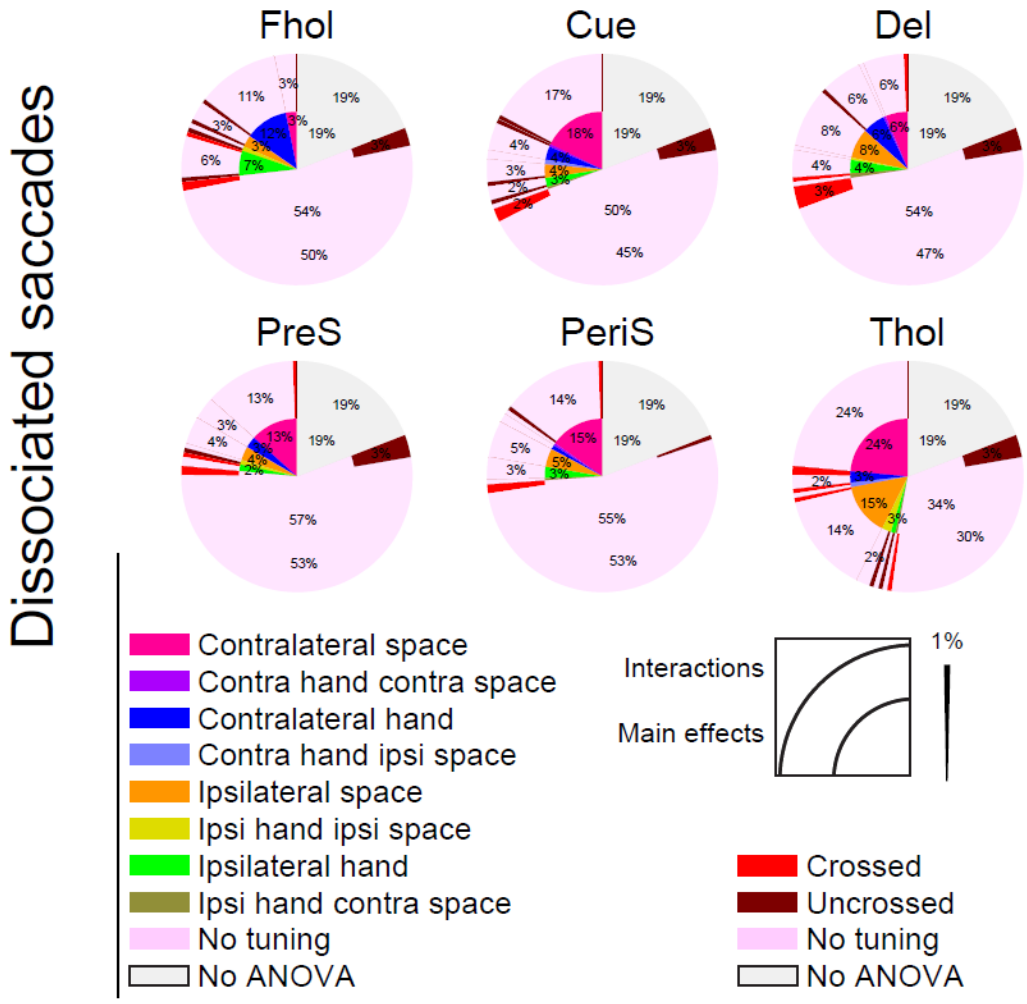


Cell counts summary per hemispace and tuning types, for each epoch of interest in the free gaze reach task. Dorsal pulvinar neurons had firing rate changes according to the movement type. In the pre and peri saccade periods there was a larger suppression than enhancement with an increased proportion of bidirectional modulation in contralaterally tuned units. In the peri reach period there was a large enhancement in spatially tuned and non-tuned units to both hemispaces.

Figures II.19-21 show for the interleaved hand design the main effects of hand and hemispace as well as their interactions during, dissociated saccades, reaches, and free gaze reaches respectively. The largest influence on the firing rate of cells prior to the onset of a cue was contralateral hand tuning in the fixation period in dissociated saccades (12%), dissociated reaches (8%), and free gaze reaches (8%). Also, there was a modest number of units enhanced to movements with engagement of the ipsilateral hand in *Fhol*. Contralateral

spatial tuning dominated during the cue presentation and was similarly tuned for the contralateral and ipsilateral hemispace around the delay period.

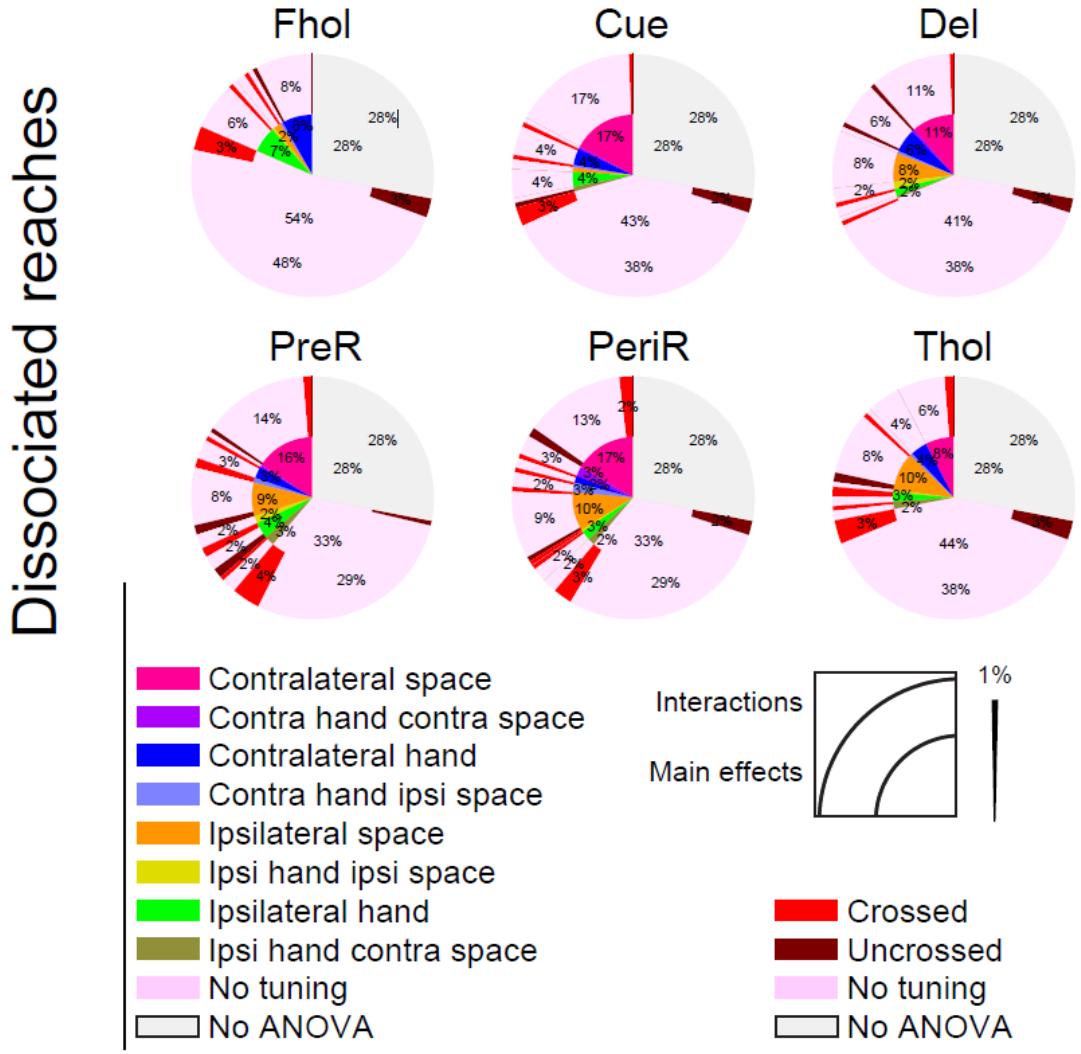
Figure II.19 **Hand-space tuning for the dissociated saccade task, interleaved hands**



Cell counts summary with hemispace, hand and interactions, for each epoch of interest in the dissociated saccade task, using interleaved hands. The inner pie plot contains the percentage of units with a main effect of hemispace (ipsilateral or contralateral to the recorded pulvinar), hand, or hand and hemispace. The outer pie shows the nature of the interactions, i.e. all uncrossed conditions: ipsi hand, ipsi space, and contra hand, contra space larger; or all crossed conditions: ipsi hand, contra space, and contra hand, ipsi space larger. A large percentage of the tuning was explained by single main effects. Particularly, the modulation to the contralateral hemispace and the usage of the contralateral hand had large influence on the

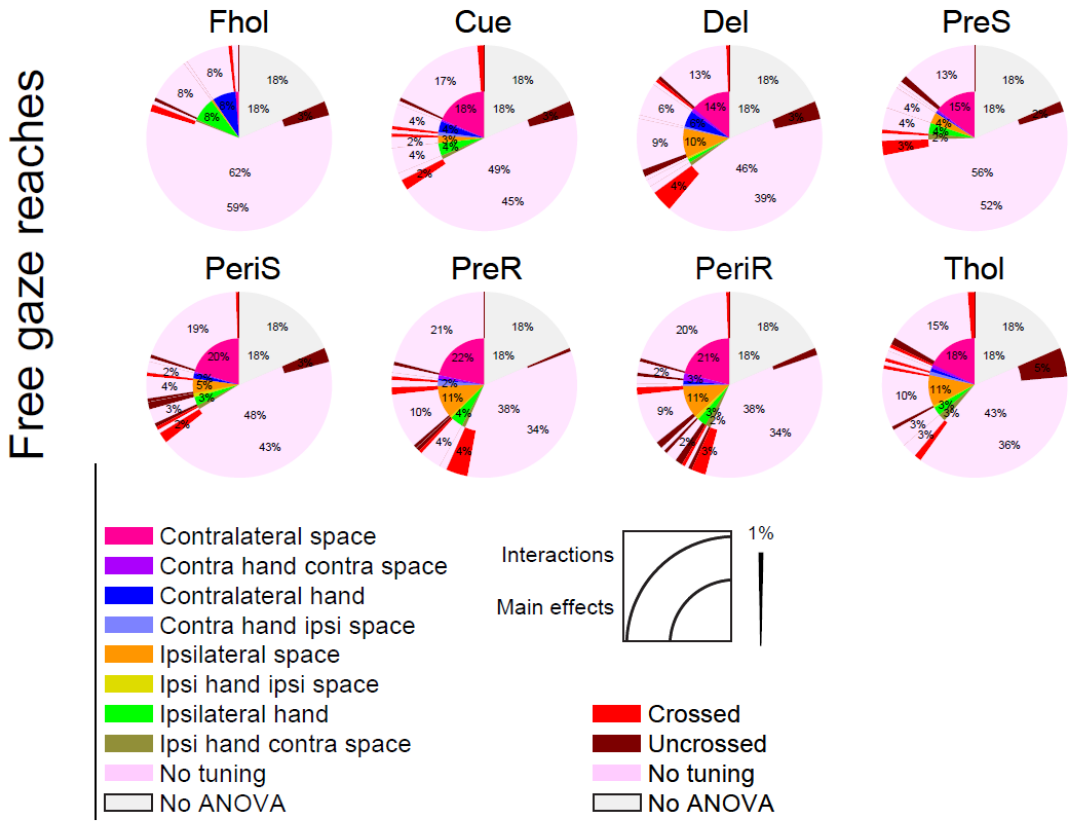
firing of a large proportion of cells. Interaction effects were minimal and benefitted both crossed and uncrossed conditions.

Figure II.20 **Hand-space tuning for the dissociated reach task, interleaved hands**



Cell counts summary with hemispace, hand and interactions, for each epoch of interest in the dissociated reach task, using interleaved hands. The largest difference when comparing to the dissociated saccade task was a larger proportion of interactions around the movement in the pre and peri reach conditions. The interaction in both periods was preferentially crossed, suggesting spatial- and effector-related influences on dorsal pulvinar. For this task involving a purposeful reach there were units modulated by hemispace (contralateral) and with the ipsilateral hand. Conventions as in **Figure II.19**

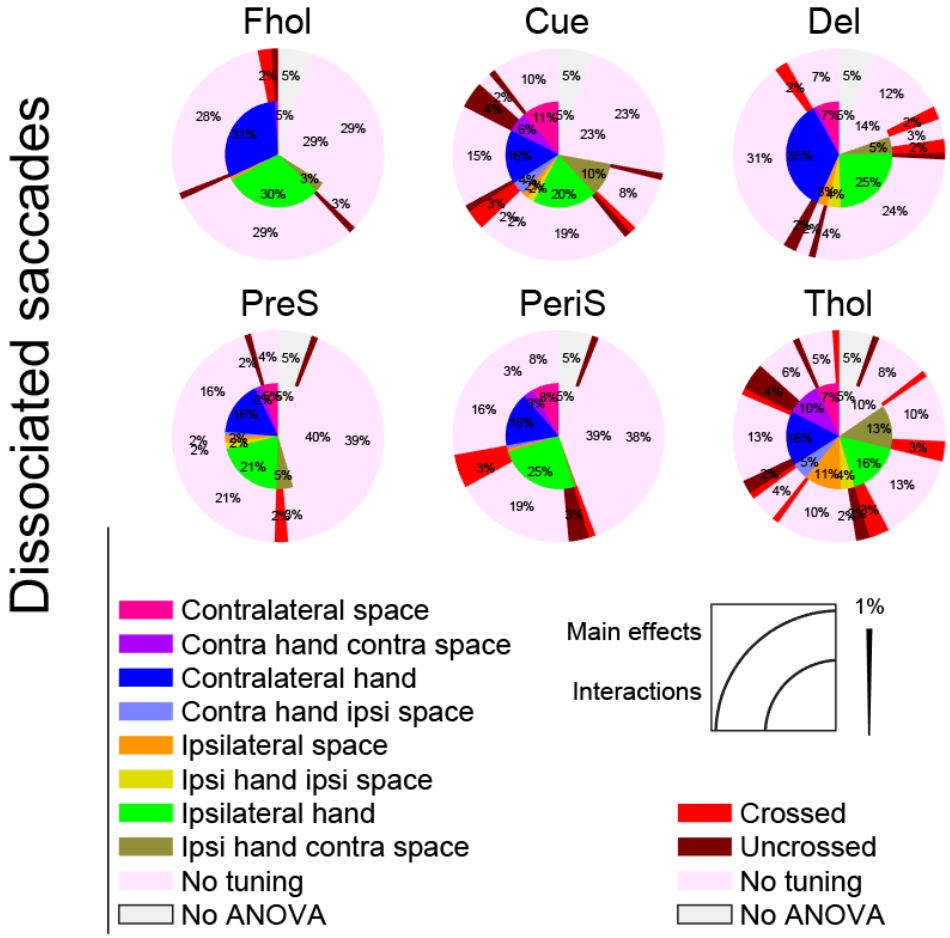
Figure II.21 **Hand-space tuning for the free gaze reach task, interleaved hands**



Cell counts summary with hemispace, hand and interactions, for each epoch of interest in the free gaze reach task, using interleaved hands. For trials where the monkey performed a reach which it could foveate the effects were like the described independently per effector, both for the spatial tuning properties and for the hand preference. Conventions as in **Figure II.19**

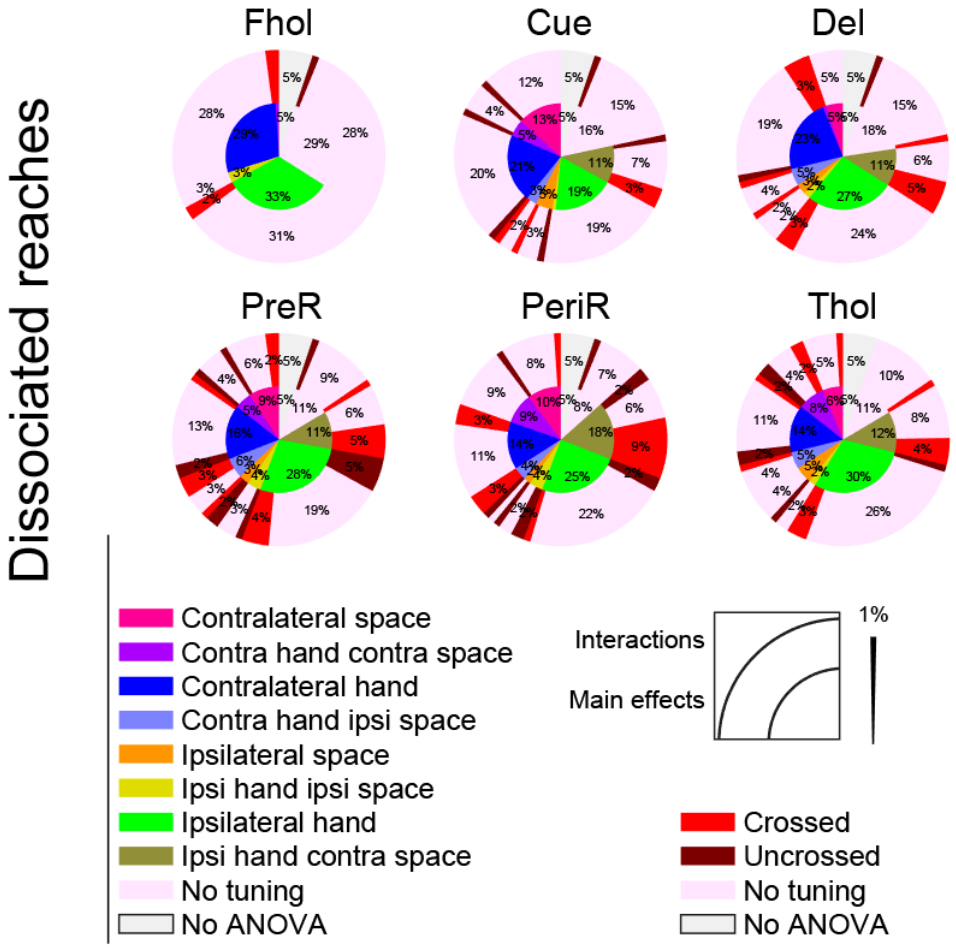
Figures II.22-24 show for monkey L ANOVA results from the blocked hand design (main effects of hand and hemispace as well as their interactions during the three visuo-motor tasks). The main difference with the interleaved hand design was that during the full duration of the trial there was a large proportion of neurons with hand preference tuning (e.g., around 30% during the *Fixation hold* period for all tasks). This hand preference was not for a specific hand relative to the recorded hemisphere. Apart from this strong hand preference, the tuning of the cells largely resembled results from the interleaved hand task.

Figure II.22 **Hand-space tuning for the dissociated saccade task, blocked hands**



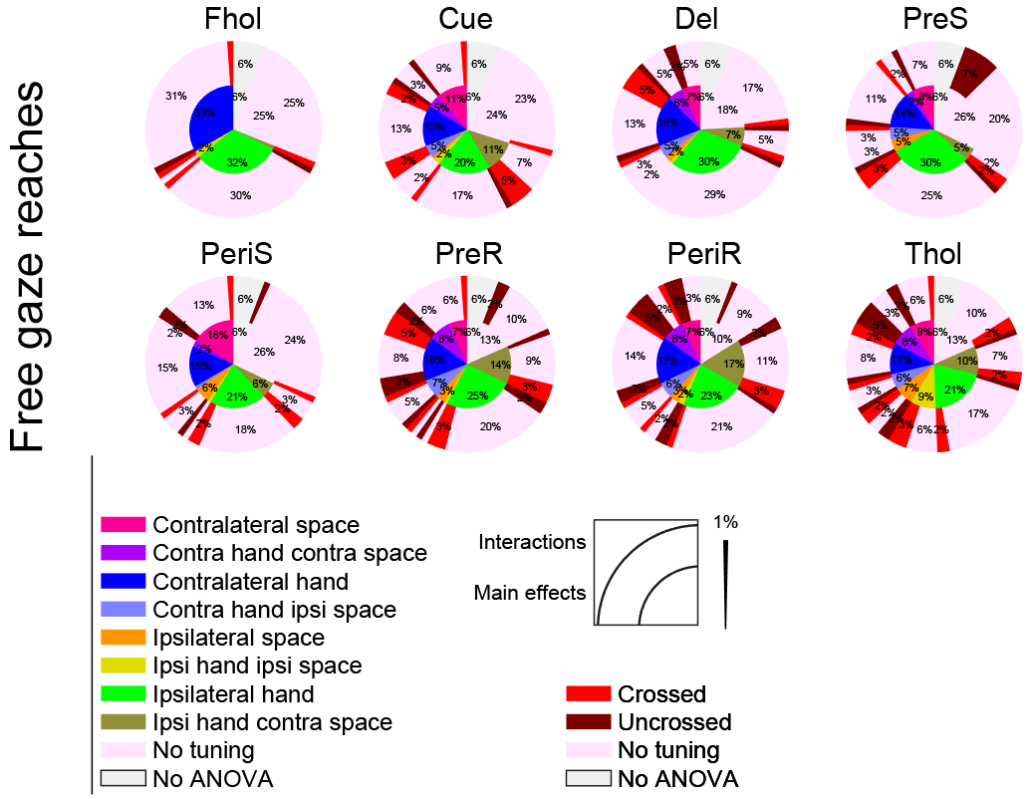
Cell counts summary with hemispace, hand and interactions, for each epoch of interest in the dissociated saccade task, for blocked hands. As in Figure II.19, the inner pie plot contains the percentage of units with a main effect of hemispace (ipsilateral or contralateral to the recorded pulvinar), hand, or hand and hemispace. The outer pie shows the nature of the interactions, i.e. all uncrossed conditions: ipsi hand, ipsi space, and contra hand, contra space larger; or all crossed conditions: ipsi hand, contra space, and contra hand, ipsi space larger. A large proportion of the neurons preferred the usage of either the ipsilateral or contralateral hand to the recorded hemisphere across the duration of the trial. This preference was also present before the hand was cued, as it would be predictable per block of trials. In this task, dorsal pulvinar cells had a slightly larger representation of the ipsilateral hand.

Figure II.23 **Hand-space tuning for the dissociated reach task, blocked hands**



Cell counts summary with hemispace, hand and interactions, for each epoch of interest in the dissociated reach task, using blocked hands. As in the interleaved hand task, there was a larger interaction of (mostly) crossed conditions around the movement, meaning an interaction of a hand and the contralateral hemispace to it.

Figure II.24 **Hand-space tuning for the free gaze reach task, blocked hands**



Cell counts summary with hemispace, hand and interactions, for each epoch of interest in the free gaze reach task, using blocked hands. As in the dissociated tasks there was a large hand preference from early stages of the trial. There were similar firing rate modulation patterns around the movements as in the dissociated tasks.

Discussion

In this chapter, we aimed to gain a better understanding of how dorsal pulvinar is involved in the execution of visually-guided reaches. As put in the *Research rationale*, the assessment of the electrophysiological properties of pulvinar during purposeful reaches is the first of two methods we used to address such question. The causal role of pulvinar in eye-hand behavior will be explored in *Chapter III*.

There are no recent systematic reports of the neuronal correlates of pulvinar function during the execution of reaches. However, early reports provided promising evidence of reach to grasp related activity across monkey species (Acuña et al., 1983, 1983, 1986; Magariños-Ascone et al., 1988; Cudeiro et al., 1989), as well as in humans (Martin-Rodriguez et al., 1982).

Dorsal subnuclei in the pulvinar are connected to different areas in the fronto-parietal network (Grieve et al., 2000; Stepniewska, 2004; Kaas and Lyon, 2007), and thus reach activity is likely to exist there. Whether this happens to be a reflection of the reach plans and actions originated elsewhere in the motor planning regions in the brain, or in the pulvinar itself as suggested by the temporal properties of such tuning (Cudeiro et al., 1989) is still a matter of debate as recent reports addressing this question are lacking.

As we have shown in *Chapter 1* and *Ibis*, dorsal pulvinar neurons are tuned to both the contralateral and less markedly to the ipsilateral hemispace to the recorded hemisphere. In addition, dorsal pulvinar neurons are modulated by saccades and by fixation; the visual or motor responsiveness of some cells is influenced by the gaze position at the time of visual stimulation and saccade execution. Similar modulation during preparation in a gaze centered frame exists in movement planning cortical areas like the posterior parietal cortex (Andersen and Mountcastle, 1983; Andersen et al., 1985; Colby et al., 1995; Snyder, 2000; Crawford, 2004). It is possible that dorsal pulvinar does not only participate in spatial

transformations for oculomotor behavior, but to also participate in the coordination of spatial transformation between distinct effector systems, or even in the generalized planning of movements.

Visual-, saccade- and reach-related firing has been reported in dorsal pulvinar. This is consistent with findings from our own results. We recorded dorsal pulvinar neurons from two monkeys while they performed delayed visually-guided saccade and reach tasks. These tasks demanded either the 1) central fixation of one of the hands to the touch display while they saccade to the periphery, a 2) central fixation of the eyes while they made a peripheral reach, or 3) the performance of a free gaze (likely foveal) reach.

The rationale behind using foveally-guided reach tasks was that one of the questions we aimed to address and have started exploring in this chapter is to assess if during periods of visual stimulation, reach planning or execution there is additional firing rate changes due to the presence of a second effector, and which is the nature of this combined activity.

D.II.1 Dorsal pulvinar's tuning is stronger for space than for hand in randomized (interleaved hands) conditions.

Across tasks, we found a significant contralateral spatial tuning during the cue presentation, regardless of the hand used (21% of the cells tuned contralaterally from ANOVA significant units**)**. Hand tuning was weaker in this period. This is evidence of dorsal pulvinar's strong spatial selectivity during early visual processing and early planning of an action. While the largest modulation for hand tuning was during the fixation hold period "Fhol" we exemplify the results from the cue period as it was the first period with spatial information. In this period, for dissociated saccades, reaches, and free gaze reaches there was a tuning of 18%, 17% and 18% of the units being spatially selective to the contralateral hemispace, and 4%, 0% and 3% to the ipsilateral space; while there was 3%, 4%, and 4%, and 3%, 4%, and 4% of units for the contralateral and ipsilateral hands respectively (n=157, interleaved hands dataset). Contralateral tuning preferences have been reported in the posterior parietal cortex during reach tasks (Hwang and Andersen, 2011; Hadjidimitrakis et

al., 2014). It is possible that the change in the influences of firing rate according to the trial epoch is a sign of pulvinar diverse and dynamics functions in the planning of actions.

D.II.2 Dorsal pulvinar's firing modulation varied according to the effector used and the current task epoch; enhancement during reach preparation and suppression before and during eye movements

For all tasks, an overall contralateral spatial preference was observed, although it was not the only finding. We observed epoch specific firing enhancement or suppression modulated by the task being performed. In dissociated saccades (**Figure II.16)** as the trial approached the execution of the movement (e.g. peri-saccade period "PeriS") the firing rate in a significant proportion of cells was suppressed. From the subpopulation of non-tuned neurons 23% and 8% neurons were enhanced or suppressed in the cue period versus 6% and 21% in the peri-saccade period respectively. This suppression might be caused by the selective activity of dorsal pulvinar neurons according to the effector.

Interestingly, a large enhancement of pulvinar firing happened only after the saccade occurred in the target hold period: 21% and 15% of non-spatially-tuned neurons were enhanced and suppressed relative to the reference period (Fixation hold). If this was an isolated result it would be fair to hypothesize that pulvinar is related to visual updating after a saccade has occurred. This explanation however, does not fully fit with our findings reported in *Chapter I.* There, we described that the disruption of the saccade behavior by the injection of current not only affected target selection but also saccade execution. It is possible that suppressed units in the pre saccade period interact with the smaller population that was enhanced in the same period for the execution of a saccade. The large firing enhancement observed after the saccade offset might be 1) signaling of the end of a saccade or 2) a corollary discharge for updating visual information as it has been proposed for saccade related cells in PRR whose largest enhancement mainly happens after the saccade (Snyder et al., 2000a).

For reaches there was no peri-reach suppression but an enhancement of the firing close to the time of the reach onset. In some single units, it was possible to see ramping up of firing

rate from the cue onset until the end of the movement which agreed with the results in the population cell counts. The firing enhancement was present well before the reach onset until the reach offset, similar to what was found in a small subset of dorsal pulvinar neurons in *Macaca nemestrina* (Cudeiro et al., 1989) and in PRR/MIP (Galletti et al., 1997; Snyder et al., 1997). Pre reach firing rate enhancement was consistent, however, this effect seems smaller than reach planning firing in the posterior parietal cortex (Snyder et al., 1997, 2000a).

D.II.3 Dorsal pulvinar is modulated by the interaction of the effectors involved in an action

At the single cell level, interesting interactions between effectors are to be mentioned. Some cells such as the one in **Figure II.6** showed clear enhancement for coordinated movements but not for the dissociated saccade or reach conditions. Other cells were suppressed for decoupled movements, but enhanced for combined movements (**Figure II.5**), among other variations. The existence of these different cell response types might indicate that there is participation of dorsal pulvinar in the integration of visually-guided reaches at different levels of movement generation.

D.II.4 Dorsal pulvinar shows strong hand preference if the hand usage is predictable

It has been observed in LIP that neurons "anticipate" and present different background firing according to the task to be performed even before a stimulus is available (Colby et al., 1995). In our study, we found that if the usage of an arm was expected in a block, tuning for a contralateral or ipsilateral arm were present in a large portion of cells in monkey L (**Figure II.22-24**). It has been reported that many cells in PRR/MIP, which is interconnected with the dorsal pulvinar, encode reaches with either contralateral or ipsilateral hand, with a mild contralateral preference on a population level (Chang et al., 2008).

Single cell examples and population data i.e. after subtracting background firing in the blocked hand condition (not shown), suggest that hand tuning can be found in dorsal pulvinar. This modulation was additionally influenced by spatial tuning and task epoch. Taken together our results show that dorsal pulvinar is involved in the preparation, execution and potentially integration of saccades and reaches, as shown by their complex modulation patterns.

Acknowledgements

We thank Ira Panolias, Sina Plümer, Klaus Heisig, and Dirk Prüße for technical support. We also thank Stefan Treue, Alexander Gail, Hansjörg Scherberger, members of the Decision and Awareness Group, Sensorimotor Group and the Cognitive Neuroscience Laboratory for helpful discussions. Supported by the Hermann and Lilly Schilling Foundation, German Research Foundation (DFG) grants WI 4046/1-1 and Research Unit GA1475-B4, KA 3726/2-1, CNMPB Primate Platform, and funding from the Cognitive Neuroscience Laboratory.

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Chapter III

Pulvinar involvement in eye-hand integration, preliminary data

Contributions

AUDV, MW and IK designed the experiments, AUDV collected the dataset and analyzed the data of Monkey L and F, MW and IK supervised the project, AUDV wrote this chapter.

Adán Ulises Domínguez Vargas (AUDV), Melanie Wilke (MW), Igor Kagan (IK).

Abstract

The dorsal pulvinar in primates is strongly connected to cortices of the fronto-parietal network. Previous studies have found reach and saccade related activity in dorsal pulvinar. Furthermore, findings from our group have shown that after dorsal pulvinar inactivation monkeys display a wide range of deficits from target selection to reach and grasp deficiencies, hinting at important roles of pulvinar in purposeful visuo-motor behavior. Here, we aimed to quantitatively characterize the aftereffects of pulvinar disruption using an array of visually-guided foveal and extrafoveal reach tasks as well as saccade tasks with the active involvement of the hand. Using MRI-guided reversible pharmacological inactivation of dorsal pulvinar with the GABA-A agonist THIP we quantified the effects of dorsal pulvinar silencing in a monkey. The main observed deficits were a decrease in the correct usage of the contralesional hand to the inactivated hemisphere, which translated to decreased hit rate, as well as a slow hand selection of the contralesional hand. In addition, we found less efficient execution of reaches to the contralesional hemispace after inactivation. There was a decreased eye-hand reaction-time correlation while eye movement properties were largely unaffected. These results suggest that dorsal pulvinar has a large involvement in the preparation of visually-guided reaches. Changes in saccade execution after inactivation might be compensated by other of the several regions known to participate in their planning.

Introduction

The dorsal aspect of the thalamic pulvinar, one of the largest and more densely connected subcortical structures to association cortices in the primate brain, i.e. frontal and parietal areas (Grieve et al., 2000; Gutierrez et al., 2000; Kaas and Lyon, 2007), might participate in the integration of eye and hand movements. Reach-related neurons have been found in the oral and lateral pulvinar across monkey species (Acuña et al., 1983, 1990). In *Macaca nemestrina* reach signals precede parietal and motor cortices signals, suggesting a role of pulvinar in the planning of reaches (Cudeiro et al., 1989). In addition, neurons in dorsal and ventral pulvinar are modulated by the planning and execution of eye movements as well as by saliency and attentional processes (Petersen et al., 1985, 1987; Robinson et al., 1986; Robinson and Petersen, 1992). The strong influence of visual, as well as of eye- and arm-movement signals in pulvinar could reflect involvement of the nucleus, particularly of its dorsal region, in the planning and execution of visually-guided reaches, the integration of visual information and proprioceptive signals for the execution of reaches, or the coordination of the oculomotor and reach systems.

Data from our group (Wilke et al., 2010, 2013) has shown that dorsal pulvinar inactivation with GABAergic agonists THIP, and muscimol (Krogsgaard-Larsen et al., 2002) causes strong disruption of saccades, reaching and grasping, some of which appear to be of cognitive nature. After inactivation, when monkeys were required to perform visual exploration, they developed bias to explore the ipsilesional hemispace for longer periods. There was an increased selection of ipsiversive saccade targets without impairments to acquire single contraversive targets. Finally, monkeys seemed exhibited deficiencies in reaching and grasping for food objects, especially when they used their contralesional arm and performed reaches to the contralesional hemispace. Here, we aimed to quantify the after-effects of pulvinar inactivation in tasks which require the coordination or dissociation of eye and hand, to gain further traction on the role of the thalamic pulvinar in coordinated and purposeful actions.

Materials and methods

Ethics and experimental approval and general notes

All experimental procedures were conducted in accordance with the European Directive 2010/63/EU, the corresponding German law governing animal welfare, and German Primate Center institutional guidelines. The procedures were approved by the responsible government agency (LAVES, Oldenburg, Germany).

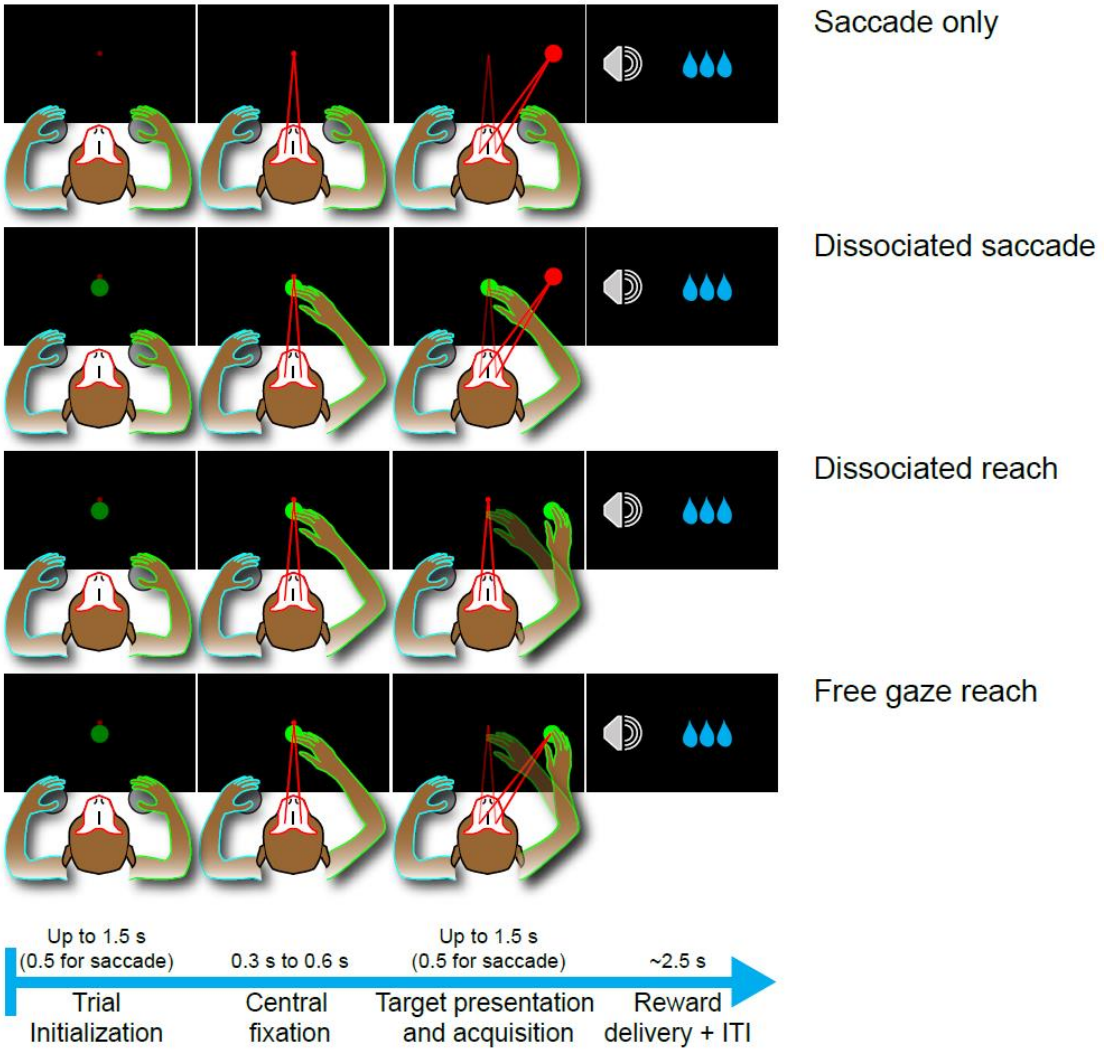
For general aspects of animal preparation, and experimental setup the reader is referred to Materials and Methods from *Chapter I* (main section) and *Chapter II* as they have been reported there.

An adult male rhesus macaque (*Macaca mulatta*) L weighing 9 kg was used. Monkey L's pulvinar nucleus was studied in two previous reports. The first study looked at the effects of electrical microstimulation in oculomotor behavior and free-choice decision-making (*Chapter I*), while the second study explored the function of pulvinar in different aspects of eye-hand encoding and integration (Chapter II). In the microstimulation study the right pulvinar of L was stimulated using currents ranging from 100 μA to 300 μA over 48 sessions. In the electrophysiology study both right and left pulvinar of monkey L were recorded acutely using Thomas Recording 5 channel mini matrix (36 successful recording sessions in the right hemisphere, 46 in the left hemisphere).

Behavioral tasks

Monkey L performed blocks of pseudorandomized, interleaved visually-guided direct saccades, dissociated saccades, dissociated reaches and free gaze reaches (**Figure III.1**), with either the right or left hand (**Figure III.2**) to single targets.

Figure III.1 **Tasks layout**



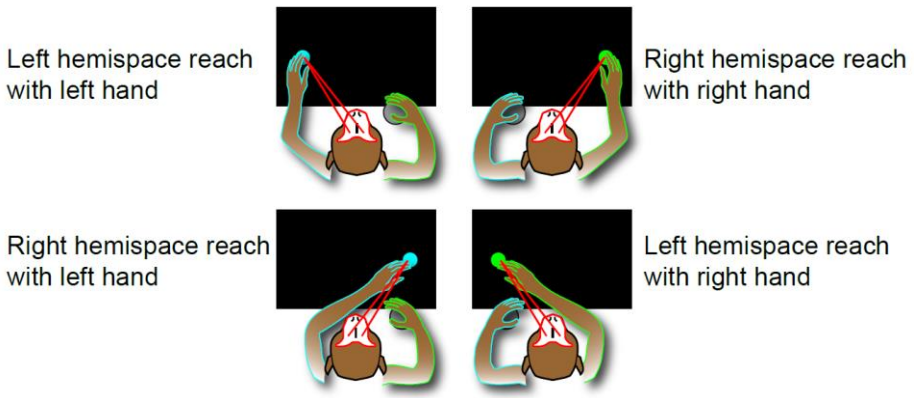
Four behavioral tasks were presented to the monkey in control and inactivation sessions. The monkey could perform a peripheral movement only after at least one of the *fixations* were extinguished, signaling which effector was allowed for acquiring the peripheral target. The monkey had to prepare a dissociated saccade (arm resting in the center of the monitor for the duration of the trial), a saccade only (both hands in sensors during the duration of the trial) or a reach. The reach could be a free gaze (most likely foveally-guided) or a dissociated reach (eye *fixation* in the center).

Direct visually-guided tasks

General aspects of the task were like the ones described in *Chapter II*, with the difference of 1) The instruction to perform the center-out eye or hand movement was done by the

onset of the peripheral cue(s) and the simultaneous offset of one or both *fixations*, 2) The peripheral stimuli were positioned at either 12° or 24° to the left or right of the central *fixations* but there were no fixed vertically offset targets. Either purely horizontally displaced targets (5 inactivation sessions) or horizontally displaced targets within a 4° radius variability window (1 inactivation session) were presented. For eye movements, the monkey had to make a saccade while keeping its hand at the resting sensors. Once the targets were acquired they would brighten up, and the monkey had to maintain its gaze/hand position for 0.5 s on the target. After each successful or failed trial, there was a 2 s inter trial interval. In total, the monkey had to achieve 10 hits to each target condition.

Figure III.2 **Visually-guided direct tasks**

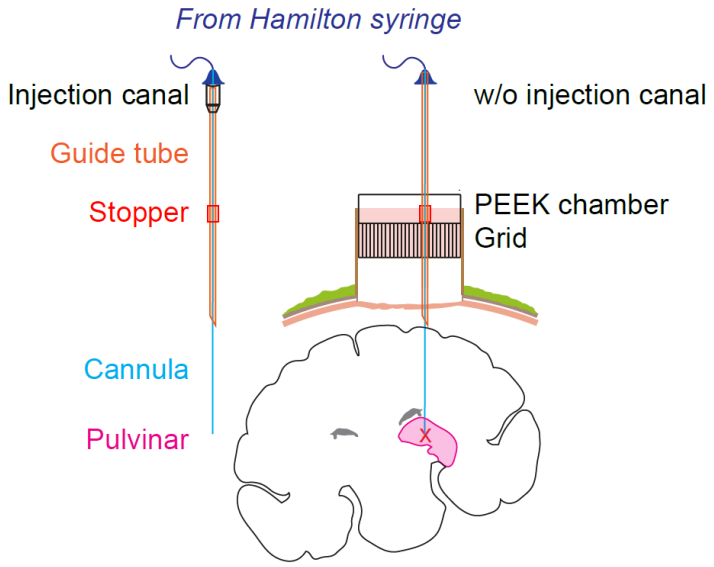


Reaches to the ipsilateral or contralateral hemispace were performed using the ipsilateral or contralateral arm depending on a green or blue fixation spot at the beginning of each trial.

Pulvinar localization and injection diffusion estimation

Before the experimental sessions, inside a custom-made MR compatible horizontal chair, Monkey L was sedated and put inside the MR scanner. T1- and T2-weighted MR images were taken before the MR contrast agent gadolinium (Magnevist, Berlex Imaging, Montville, USA) was co-injected with saline solution into dorsal pulvinar (**Figure III.3**). The injection rate was set to a constant 0.5 µl/min until 3.3 µl were injected. Immediately after injection, MR images were taken to assess the diffusion of the contrast agent in the area around pulvinar to plan future inactivation sessions. The gadolinium injection was performed using a sterile 31 gauge, 60 mm long cannula attached to a high precision microinjection syringe pump (Harvard Apparatus, USA). The cannula was placed inside a custom-made 27 gauge metallic guide tube resting on a 22 mm circular grid (with help of a silica stopper) and fixed to the chamber of the monkey's right hemisphere (**Figure III.4** and **Table III.1**). This setup allowed for a smooth and movement free targeting and injection to areas around the dorsal pulvinar.

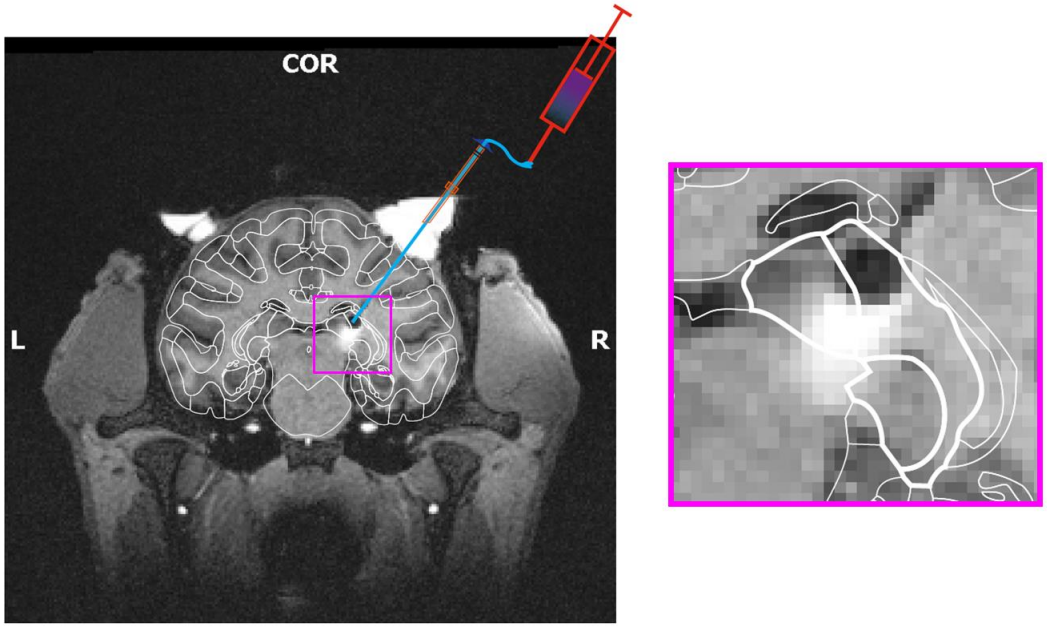
Figure III.3 **Injection approach**



An in-house built guide tube (with or without (w/o) an attached injection canal to ease the pass of a 31G cannula) was placed in the appropriate grid hole to target dorsal pulvinar through

which a microinjection cannula connected to a microinjection system delivered the liquid solution.

Figure III.4 **Injection sites**



T1 weighted MR images of injection sites in the pulvinar of Monkey L. Images are displayed in neurological orientation (image's right is head's right). Left: coronal slice showing spread of gadolinium co-injected with isotonic solution Image was taken after five inactivation sessions and the gadolinium spread is like the expected during inactivation. A lesion in the lateral border of dorsal pulvinar is observed as a black circular blob. Right: Close up of the right pulvinar showing gadolinium spread mostly encompassing the dorsal subdivisions of the pulvinar.

Table III.1 **Volume injection of GABA-A agonist THIP**

Inactivation session	THIP injected volume (µl) (& total volume if multiple injections)	Rate of volume injected (µl/s)	Waiting time after injection start before run 1 (min) (& total hits)	Waiting time after injection start before run 2 (min) (& total hits)	Waiting time after injection start before run 3 (min) (& total hits)	Baseline in same session
L20160610	3.2	0.5	27 (307)	67 (278)	-	Yes

L20160615	2, 2, 3 (7)	0.5, 1,1	49 (560)	-	-	Yes
L20160624	2.7,0.3 (3)	1,1	30 (560)	-	-	Yes
L20160701	5	0.5	42 (560)	129 (560)	-	No
L20160708	4	0.5	40 (560)	113 (560)	193 (460)	No
L20160805	3	0.5	41 (560)	131 (556)	-	No

In six inactivation sessions THIP was injected at a rate of 0.5 µl/min to 1 µl/min. Total volumes varied from 3 µl to 7 µl (mean 4.2 µl $\pm$ 1.57 µl).

Behavioral parameters

For all analyses saccades with reaction times shorter than 80 ms from target presentation and reaches with reaction times shorter than 200 ms were excluded. This criterion removed 6 trials from control sessions and 53 trials from the inactivation sessions (34 from the session six).

Saccade definitions

A saccade was detected whenever there was a change in eye trace instant velocity larger than 200°/s of Euclidean distance. Saccade offsets reflected the time when saccade velocities dropped below 50°/s. Saccade velocities were derived from interpolated (220 Hz to 1 kHz) and smoothed eye position traces with a 15 ms moving average rectangular window, which was then smoothed again with a second 15 ms moving average rectangular window.

Saccade latency was defined as the time between fixation spot(s) offset and the moment when the first saccade was detected during the target acquisition period in each trial. Saccade duration was defined as the time between saccade onset and offset. Saccade peak velocity was defined as the maximum instant velocity across the duration of the saccade of interest.

Reach definitions

Reach latency was defined as the time between fixation spot(s) offset and the moment when the hand lost contact with the touchscreen during the target acquisition period in each trial. Reach duration was defined as the time from the reach latency to the next touchscreen contact. Reach inaccuracy was defined as either the signed mean offset independently for vertical and horizontal offsets, or as the Euclidean distance: the square root of the sum of squared means for each axis. Reach imprecision was the standard deviation of the reach offsets across trials to each target, either independently for both axes or as the square root of the sum of squared standard deviations for each axis.

Statistics

For all tasks, successful trials to targets to the left and right hemispaces were combined regardless of eccentricity. All data analysis was performed using MATLAB R2012b and the Statistics Toolbox. To test for changes in mean movement latency, duration, velocity, precision, and accuracy as a result of inactivation independent t-tests were used. Means as well as t-values, and p-values are reported in tables III.2 and III.3 for all relevant comparisons. Reaction times of saccades and reaches in the free gaze reach condition were assessed using Pearson's correlation.

Results

The dorsal pulvinar of a monkey was inactivated in six sessions using the GABA-A agonist THIP to quantify effects in saccade and reach performance in tasks that allowed or prevented the use of foveal guidance for visually-guided reaches. **Table III.2** and **III.3** summarize the descriptive and inferential statistics in the control (before inactivation) and after inactivation conditions for reaches and saccades respectively.

In control conditions, which could come from runs right before inactivating dorsal pulvinar (3/6 sessions) or from the day before inactivation (3/6 sessions), the reaction times of the saccade-only task were in the range of previously reported in *Chapter I*, 166 ms $\pm$ 4 ms (SE) and 155 ms $\pm$ 6 ms for left (contralesional once inactivated) and right hemispaces respectively. There were mixed effects of hand engagement for the performance of dissociated saccades. When the monkey performed saccades during the dissociated saccade task to the left hemispace using the left hand the reaction times were shorter (152 ms $\pm$ 2 ms, $p<0.01$) than in the saccade only task, but not when the monkey used its right hand (166 ms $\pm$ 6 ms, $p>0.05$). For the right hemispace when the monkey used its left hand the reaction times were larger (179 ms $\pm$ 3 ms, $p<0.01$) and also when the monkey used its right hand (189 ms $\pm$ 6 ms, $p<0.01$). During the free gaze condition saccade reaction time for the left space with the left hand was 163 ms $\pm$ 3 ms and 159 ms $\pm$ 2 ms for the right hand. For the right space using its left hand the reaction time was 164 ms $\pm$ 4 ms and for the right hand was 168 ms $\pm$ 2 ms.

Visually-guided (likely foveal) reaches had reaction times of 376 ms $\pm$ 7 ms and 372 ms $\pm$ 7 ms to the left side using its left and right hands respectively, and 437 ms $\pm$ 7 ms and 368 ms $\pm$ 5 ms to the right side using its left and right hands respectively. For extrafoveal reaches the reaction times were no significantly different before and after inactivation with neither hand and to neither hemispace. Unexpectedly the distance to target for reaches to

the left with the right hand was more accurate when reaching without foveal guidance than with it (1.42° + 0.15° and 2.58° + 0.14° respectively, p<0.01).

A negative correlation of residual saccade and reach reaction times was significant in two out of six sessions for the left hemispace using the left hand (r=-0.96, p<0.01; and r=-0.61, p<0.01) and had a correlation trend in one session (r=-0.46, p=0.07). For movements to the left using the right hand there was a positive correlation in four out of six sessions (r=0.71, p<0.05; r=0.69, p<0.05; r=0.58, p<0.05; r=0.56, p<0.05). Similarly, a positive correlation was found in two sessions when the monkey reached to the right hemispace using its left hand (r=0.71, p<0.05, and r=0.54, p<0.05). Finally, only one session for movements to the right hemispace using the right hand had a positive correlation coefficient for saccade and reach reaction times (r=0.8, p<0.01).

The graphical representation of the main findings from table **Tables III.2** and **III.3** on the effects of THIP injection in visually-guided behavior of monkey L are presented in **Figures III.5** to **III.16.**

Table III.2 **Inactivation effects on reaches**

	Control		Inactivation						Control		Inactivation					
	CONTRALESIONAL															
Foveal reach	LH mean	LH SE	LH mean	LH SE	Effect	p	t	df	RH mean	RH SE	RH mean	RH SE	Effect	p	t	df
Latency (sec)	0.38	0.01	0.39	0.01	0.01	0.52	-0.68	10.00	0.37	0.01	0.40	0.01	0.03	**0.02**	-2.79	10.00
Duration (sec)	0.14	0.00	0.16	0.01	0.02	0.20	-1.37	10.00	0.18	0.01	0.18	0.01	0.00	0.80	-0.26	10.00
Accuracy x (°)	0.30	0.17	0.73	0.14	0.43	*0.08*	-1.97	10.00	1.43	0.18	1.41	0.17	-0.02	0.93	0.09	10.00
Accuracy y (°)	0.64	0.29	0.84	0.22	0.20	0.58	-0.57	10.00	2.11	0.14	2.30	0.18	0.19	0.42	-0.84	10.00
Hitrate (%)	0.97	0.02	0.99	0.01	0.02	0.39	-0.91	10.00	0.99	0.01	0.99	0.01	0.00	0.80	0.26	10.00
Euclidean distance acc(°)	0.80	0.28	1.14	0.24	0.33	0.38	-0.91	10.00	2.58	0.14	2.71	0.22	0.13	0.63	-0.50	10.00
Euclidean distance pre(°)	1.12	0.12	0.99	0.03	-0.13	0.33	1.02	10.00	0.92	0.06	0.88	0.09	-0.04	0.72	0.36	10.00
Extrafoveal reach																
Latency (sec)	0.35	0.01	0.37	0.01	0.02	0.37	-0.93	10.00	0.36	0.01	0.37	0.01	0.01	0.15	-1.56	10.00
Duration (sec)	0.13	0.00	0.16	0.01	0.03	**0.03**	-2.52	10.00	0.15	0.01	0.15	0.01	0.01	0.70	-0.40	10.00
Accuracy x (°)	-0.78	0.14	-0.73	0.30	0.04	0.91	-0.12	10.00	0.94	0.31	0.73	0.34	-0.21	0.66	0.45	10.00
Accuracy y (°)	0.85	0.38	1.47	0.24	0.62	0.20	-1.39	10.00	0.74	0.24	1.14	0.24	0.40	0.27	-1.18	10.00
Hitrate (%)	0.69	0.03	0.45	0.04	-0.24	**0.00**	4.75	10.00	0.66	0.07	0.53	0.10	-0.13	0.31	1.06	10.00
Euclidean distance acc(°)	1.43	0.15	1.75	0.27	0.33	0.32	-1.06	10.00	1.41	0.21	1.57	0.22	0.15	0.62	-0.52	10.00
Euclidean distance pre(°)	2.32	0.67	1.50	0.17	-0.83	0.26	1.20	10.00	1.25	0.15	1.33	0.08	0.08	0.62	-0.51	10.00
	IPSILESIONAL															
Foveal reach	LH mean	LH SE	LH mean	LH SE	Effect	p	t	df	RH mean	RH SE	RH mean	RH SE	Effect	p	t	df
Latency (sec)	0.44	0.01	0.46	0.02	0.02	0.23	-1.27	10.00	0.37	0.00	0.38	0.01	0.01	*0.07*	-2.01	10.00
Duration (sec)	0.17	0.01	0.20	0.02	0.04	*0.08*	-1.94	10.00	0.17	0.01	0.16	0.00	-0.01	0.33	1.01	10.00
Accuracy x (°)	0.24	0.18	0.61	0.27	0.37	0.28	-1.14	10.00	1.02	0.13	1.01	0.13	-0.02	0.93	0.09	10.00
Accuracy y (°)	1.21	0.17	1.71	0.16	0.50	*0.06*	-2.12	10.00	1.89	0.19	2.24	0.17	0.35	0.20	-1.37	10.00
Hitrate (%)	1.00	0.00	0.96	0.04	-0.04	0.28	1.14	10.00	1.00	0.00	0.99	0.01	-0.01	0.34	1.00	10.00
Euclidean distance acc(°)	1.29	0.18	1.90	0.19	0.61	**0.04**	-2.30	10.00	2.18	0.17	2.49	0.12	0.31	0.16	-1.50	10.00
Euclidean distance pre(°)	1.05	0.13	1.23	0.19	0.18	0.45	-0.78	10.00	0.82	0.04	0.89	0.06	0.08	0.32	-1.05	10.00
Extrafoveal reach																
Latency (sec)	0.43	0.02	0.44	0.03	0.01	0.68	-0.43	10.00	0.35	0.01	0.35	0.00	0.00	0.75	0.32	10.00
Duration (sec)	0.16	0.01	0.16	0.01	0.00	0.91	-0.12	10.00	0.15	0.01	0.15	0.01	0.00	0.80	-0.26	10.00
Accuracy x (°)	-0.47	0.28	-0.23	0.41	0.23	0.65	-0.47	10.00	0.85	0.22	0.63	0.10	-0.22	0.39	0.91	10.00
Accuracy y (°)	0.36	0.32	1.00	0.24	0.64	0.14	-1.61	10.00	1.41	0.23	2.20	0.24	0.79	**0.04**	-2.34	10.00
Hitrate (%)	0.27	0.04	0.31	0.04	0.04	0.45	-0.79	10.00	0.65	0.05	0.57	0.07	-0.08	0.38	0.93	10.00
Euclidean distance acc(°)	0.97	0.25	1.46	0.13	0.48	0.11	-1.74	10.00	1.67	0.29	2.30	0.25	0.62	0.14	-1.62	10.00
Euclidean distance pre(°)	1.25	0.26	1.36	0.10	0.11	0.71	-0.39	10.00	2.96	1.19	1.51	0.15	-1.45	0.25	1.21	10.00

Descriptive statistics for behavioral parameters in monkey L before and after dorsal pulvinar inactivation (control and inactivation) and inferential statistics between control and

inactivation sessions with effect size (Inactivation – Control); during direct reach tasks to the contralesional and ipsilesional hemispaces (magenta and orange shaded panels respectively). Reaches performed with the contralesional hand are blue shaded while reaches performed with the ipsilesional hand are green shaded. The p-values are derived from two-tailed non-paired t-tests across sessions for six inactivation sessions (bold p<0.05, italics p<0.1). Reaches that occurred before 200 ms from the Go signal were excluded from this analysis as they were considered express movements.

Table III.3 **Inactivation effects on saccades**

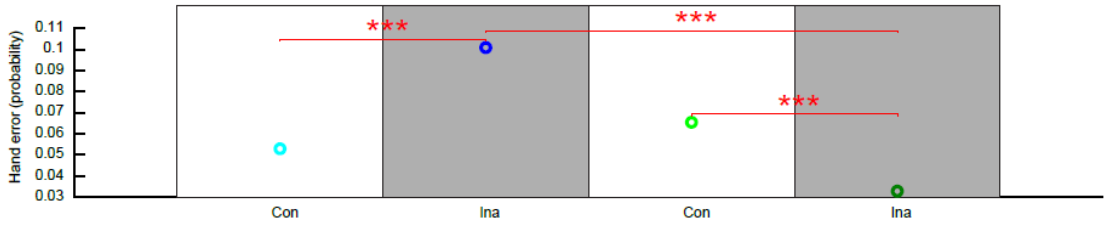
	Control		Inactivation						Control		Inactivation					
	CONTRALESIONAL															
Free gaze saccade	LH mean	LH SE	LH mean	LH SE	Effect	p	t	df	RH mean	RH SE	RH mean	RH SE	Effect	p	t	df
Latency (sec)	0.16	0.00	0.16	0.00	0.00	0.68	-0.42	10.00	0.16	0.00	0.16	0.00	0.00	0.44	-0.81	10.00
Duration (sec)	0.04	0.00	0.04	0.00	0.00	0.80	-0.26	10.00	0.04	0.00	0.04	0.00	0.00	0.99	-0.02	10.00
Accuracy x (°)	1.87	0.08	1.79	0.19	-0.08	0.71	0.38	10.00	1.79	0.08	1.73	0.11	-0.05	0.71	0.39	10.00
Accuracy y (°)	3.57	0.10	3.83	0.05	0.26	**0.04**	-2.34	10.00	3.11	0.16	3.10	0.12	-0.01	0.96	0.05	10.00
Hitrate (%)	0.97	0.02	0.99	0.01	0.02	0.39	-0.91	10.00	0.99	0.01	0.99	0.01	0.00	0.80	0.26	10.00
Velocity (°/sec)	580.71	9.47	588.50	6.11	7.80	0.51	-0.69	10.00	593.52	5.47	598.88	5.12	5.36	0.49	-0.72	10.00
Euclidean distance acc(°	4.03	0.10	4.25	0.09	0.22	0.13	-1.68	10.00	3.59	0.16	3.56	0.15	-0.04	0.86	0.18	10.00
Euclidean distance pre(°	1.09	0.10	1.14	0.03	0.06	0.61	-0.53	10.00	1.07	0.04	1.11	0.10	0.05	0.68	-0.42	10.00
Dissociated saccade																
Latency (sec)	0.15	0.00	0.15	0.00	0.00	0.99	0.02	10.00	0.17	0.01	0.16	0.00	-0.01	0.43	0.82	10.00
Duration (sec)	0.04	0.00	0.04	0.00	0.00	0.57	-0.59	10.00	0.04	0.00	0.04	0.00	0.00	0.78	-0.29	10.00
Accuracy x (°)	1.78	0.13	1.52	0.17	-0.25	0.26	1.20	10.00	2.22	0.28	1.60	0.15	-0.62	*0.08*	1.98	10.00
Accuracy y (°)	2.60	0.23	2.76	0.24	0.16	0.65	-0.47	10.00	2.36	0.29	2.49	0.28	0.13	0.75	-0.33	10.00
Hitrate (%)	0.94	0.03	0.95	0.02	0.01	0.76	-0.31	10.00	1.00	0.00	1.00	0.00	0.00			10.00
Velocity (°/sec)	547.37	11.66	555.37	8.90	8.00	0.60	-0.55	10.00	538.47	12.67	557.10	7.75	18.63	0.24	-1.26	10.00
Euclidean distance acc(°	3.18	0.18	3.19	0.21	0.00	0.99	-0.01	10.00	3.35	0.15	2.99	0.24	-0.35	0.24	1.25	10.00
Euclidean distance pre(°	1.50	0.10	1.40	0.13	-0.10	0.56	0.61	10.00	2.22	0.74	1.30	0.09	-0.92	0.25	1.24	10.00
	IPSILESIONAL															
Free gaze saccade	LH mean	LH SE	LH mean	LH SE	Effect	p	t	df	RH mean	RH SE	RH mean	RH SE	Effect	p	t	df
Latency (sec)	0.16	0.00	0.18	0.00	0.01	**0.04**	-2.43	10.00	0.17	0.00	0.17	0.00	0.00	0.65	-0.47	10.00
Duration (sec)	0.04	0.00	0.04	0.00	0.00	0.41	0.86	10.00	0.04	0.00	0.04	0.00	0.00	0.49	0.71	10.00
Accuracy x (°)	-1.71	0.11	-1.73	0.12	-0.02	0.90	0.13	10.00	-1.26	0.10	-1.18	0.13	0.08	0.63	-0.50	10.00
Accuracy y (°)	4.78	0.18	4.82	0.21	0.04	0.89	-0.14	10.00	4.45	0.15	4.54	0.15	0.09	0.68	-0.43	10.00
Hitrate (%)	1.00	0.00	0.96	0.04	-0.04	0.28	1.14	10.00	1.00	0.00	0.99	0.01	-0.01	0.34	1.00	10.00
Velocity (°/sec)	572.77	4.72	577.28	7.43	4.50	0.62	-0.51	10.00	592.48	8.58	592.16	4.76	-0.33	0.97	0.03	10.00
Euclidean distance acc(°	5.08	0.20	5.12	0.22	0.05	0.88	-0.15	10.00	4.63	0.16	4.70	0.16	0.07	0.77	-0.30	10.00
Euclidean distance pre(°	1.15	0.09	1.17	0.10	0.02	0.89	-0.14	10.00	1.01	0.08	1.22	0.10	0.21	0.13	-1.63	10.00
Dissociated saccade																
Latency (sec)	0.18	0.00	0.19	0.01	0.01	0.18	-1.46	10.00	0.19	0.01	0.19	0.01	0.00	0.64	-0.49	10.00
Duration (sec)	0.05	0.00	0.05	0.00	0.00	0.77	-0.31	10.00	0.05	0.00	0.05	0.00	0.00	0.48	-0.73	10.00
Accuracy x (°)	-2.04	0.20	-2.02	0.36	0.02	0.96	-0.06	10.00	-1.52	0.06	-1.42	0.22	0.10	0.68	-0.42	10.00
Accuracy y (°)	4.24	0.18	4.32	0.19	0.08	0.78	-0.29	10.00	4.19	0.11	4.36	0.03	0.17	0.16	-1.54	10.00
Hitrate (%)	0.97	0.02	0.90	0.06	-0.07	0.31	1.07	10.00	0.95	0.03	0.97	0.01	0.02	0.50	-0.70	10.00
Velocity (°/sec)	524.48	7.84	514.20	8.25	-10.28	0.39	0.90	10.00	518.99	6.13	543.67	4.15	24.67	**0.01**	-3.33	10.00
Euclidean distance acc(°	4.74	0.08	4.84	0.18	0.10	0.63	-0.49	10.00	4.46	0.11	4.61	0.08	0.15	0.29	-1.13	10.00
Euclidean distance pre(°	2.85	0.52	2.59	0.46	-0.26	0.72	0.37	10.00	1.67	0.10	1.58	0.11	-0.09	0.58	0.57	10.00
	CONTRALESIONAL								IPSILESIONAL							
Saccade Only	Mean	SE	Mean	SE	Effect	p	t	df	Mean	SE	Mean	SE	Effect	p	t	df
Latency (sec)	0.17	0.00	0.17	0.00	0.01	0.11	-1.73	10.00	0.16	0.01	0.15	0.00	-0.01	0.47	0.75	10.00
Duration (sec)	0.04	0.00	0.04	0.00	0.00	0.56	-0.61	10.00	0.05	0.00	0.05	0.00	0.00	0.67	0.44	10.00
Accuracy x (°)	1.48	0.15	1.42	0.17	-0.06	0.80	0.26	10.00	-0.80	0.10	-0.62	0.14	0.18	0.34	-1.01	10.00
Accuracy y (°)	1.85	0.22	2.25	0.24	0.40	0.25	-1.23	10.00	3.03	0.25	3.29	0.12	0.26	0.36	-0.95	10.00
Hitrate (%)	1.00	0.00	1.00	0.00	0.00			10.00	0.99	0.01	0.99	0.01	0.00	0.92	0.11	10.00
Velocity (°/sec)	574.12	5.89	572.75	6.86	-1.37	0.88	0.15	10.00	539.21	9.15	555.87	5.67	16.66	0.15	-1.55	10.00
Euclidean distance acc(°	2.42	0.13	2.71	0.19	0.29	0.24	-1.25	10.00	3.13	0.25	3.36	0.12	0.23	0.44	-0.81	10.00
Euclidean distance pre(°	1.86	0.39	1.36	0.04	-0.50	0.23	1.28	10.00	1.46	0.08	1.36	0.03	-0.11	0.27	1.17	10.00

Descriptive and inferential statistics similar to **Table III.2** during delayed saccade tasks. Saccades performed with involvement of the contralesional hand are in the upper and lower left panels (magenta shaded) while saccades performed with ipsilesional hand involvement are in the middle and lower right panels (orange shaded). The p-values are derived from two-tailed non-paired t-tests across sessions (bold p<0.05, italics p<0.1). Saccades that occurred before 80 ms from the Go signal were excluded from this analysis as they are considered express movements.

Inactivation effects

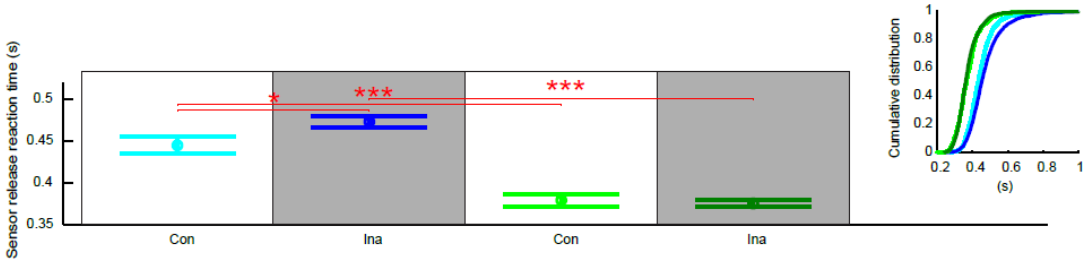
After dorsal pulvinar inactivation there were two apparent effects involving hand selection and sensor release latency. **Figure III.5** shows the probability of wrong hand selection after the initial fixation spots appeared at the display's center. After inactivation, there were increased number of attempts to use the ipsilesional hand when the contralesional hand was cued (5% erroneous attempts and 10% in control and inactivation conditions respectively, $p<0.001$). Correspondingly, errors of incorrectly releasing the ipsilateral sensor decreased, as the monkey attempted to use this hand more often regardless of the cued hand (7% erroneous attempts and 3% in control and inactivation conditions respectively, $p<0.001$). This agrees with previous findings of monkeys' decreased selection of the contralesional hand to reach and grasp for objects if they are free to use any hand (Wilke et al., 2010). When the monkey correctly selected the hand to be engaged in the trial there was an increased reaction time for the initiation of the reach to the center of the screen with the contralesional hand (**Figure III.6**) (445 ms $\pm$ 10 ms vs 473 ms + 7 ms before and after inactivation respectively, $p<0.05$) but not for the ipsilesional hand (379 ms $\pm$ 8 ms and 375 ms $\pm$ 4 ms before and after inactivation respectively, $p>0.05$). In free gaze reaches there was an increased latency for movements to the contralesional hemispace with the ipsilesional hand (**Figure III.7)** (Effect size *Inactivation - Control* (I-C) 27 ms, $p=0.02$). A similar but milder effect was found when the monkey reached to the ipsilesional hemispace using its ipsilesional hand (Effect size I-C 15 ms, $p=0.07$). The duration of the reach (**Figure III.8)** when the monkey had to perform an extrafoveal reach to the contralesional hemispace with the contralesional hand was larger after inactivation (Effect size I-C 27 ms, $p=0.03$).

Figure III.5 **Hand selection error**



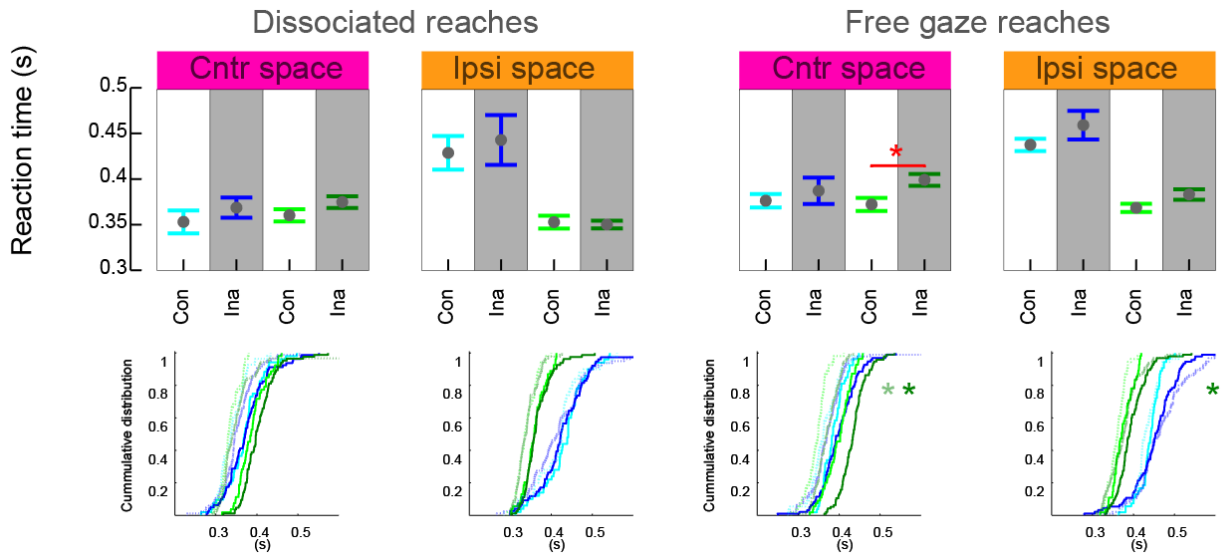
Hand selection errors per hand (blue and green circles for contralesional and ipsilesional hands respectively) defined as the probability of using wrong (an un-cued) hand before and after inactivation (light and dark colors respectively). Control data comes from blocks of trials in either the same day and before the inactivation took place (n=3), or from an immediate session before (n=3). For contralesional hand trials there was an increase of wrong ipsilateral sensor releases ($p<0.001$). For ipsilesional hand trials there was a decrease of wrong contralesional sensor releases ($p<0.001$). For all panels, statistical differences between groups were tested using a Fisher´s exact test across trials. * $p<0.05$, ** $p<0.01$, *** $p<0.001$; *con, control; ina, inactivated*

Figure III.6 **Sensor release reaction time**



Effects of dorsal pulvinar inactivation on sensor release reaction time. Sensor release reaction time after inactivation was larger for the contralesional hand. For the ipsilesional hand, there were no effects of dorsal pulvinar inactivation. For this representation, all trial types were compiled as at this stage no task-specific information (besides the hand usage) was provided to the monkey. The color conventions are as in Figure III.5. For all panels, statistical differences between groups were tested using a two-tail independent t-test across sessions. Insert shows the cumulative distribution function of reaction times per condition. * $p<0.05$, ** $p<0.01$, *** $p<0.001$

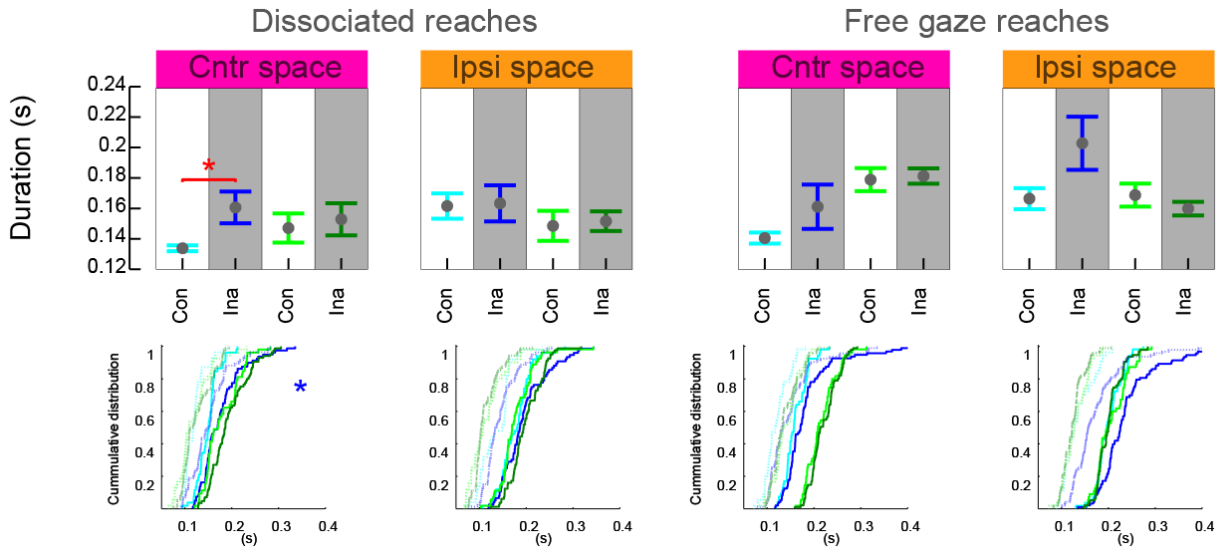
Figure III.7 **Reach reaction time**



Upper panel: Mean and SE reach reaction time, from the *fixation* towards peripheral targets sorted by hemispace (magenta and orange shades for contralesional (*Cntr*) and ipsilesional (*Ipsi*) hemispaces respectively). Color conventions and testing as in Figure III.5. Bottom panel: Cumulative distribution of reaction times across trials for control and inactivation sessions displayed by hemispace but maintaining the four potential eccentricities, -24°, -12°, 12°, 24°. Closer target trials are shown as dim and dotted traces while far ones are shown continuous and bolder. If statistical differences were found for an eccentricity this one is also displayed in the corresponding panel and with the matching brightness. Representation of reach and saccade are consistent across plots (for saccade plots eye movements in the saccade-only task are displayed in shades of red).

There were larger reaction times after inactivation in the free gaze reach task, specifically to the contralesional hemispace when using the ipsilesional hand for both close and far targets. In addition, there was reaction time delay for reaches to the ipsilesional hemispace with the ipsilesional hand for far targets.

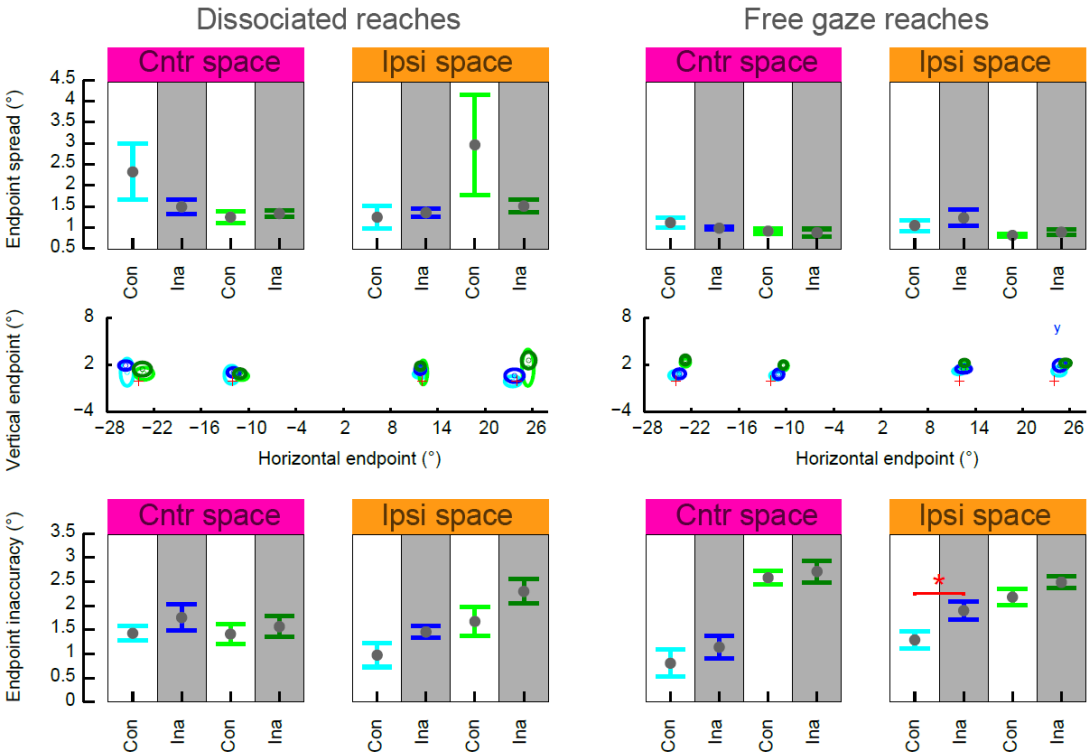
Figure III.8 **Reach duration.**



Reaches performed with the contralesional hand had a larger duration across conditions. This effect was significant when the monkey could not foveate the target in the same hemispace as the hand used. When separating the data by eccentricity, the effect was true for far eccentricities only.

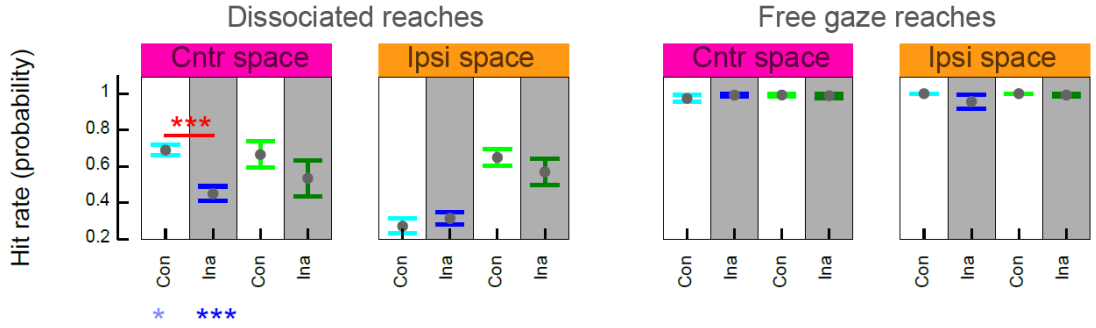
Even though the precision of reaches was unaffected after the THIP injection (**Figure III.9** middle and top panel**)** there was a small drop in accuracy for free gaze reaches which was significant for the ipsilesional hemispace with the contralesional hand (bottom panel), where there was an overshooting in the vertical plane. There was a drop of performance for extrafoveal reaches to the contralesional hemispace using the contralesional hand **(Figure III.10)**. This effect was present both for close and far targets ($p<0.05$, and $p<0.001$ respectively)

Figure III.9 **Reach accuracy**



Upper panel: endpoint spread or "precision" as the Euclidean distance. Middle panel: endpoints of reaches in the extrafoveal (dissociated) and likely foveal (free gaze) reach conditions. The ellipses represent the horizontal and vertical endpoint mean standard deviations per target across sessions. Bottom panel: endpoint inaccuracy as the Euclidean distance. There was no difference in precision for any of the tasks after inactivation. The reach inaccuracy to target, however, increased when the monkey used the contralesional hand to reach for the target in the ipsilesional hemispace.

Figure III.10 **Reach hit rate**

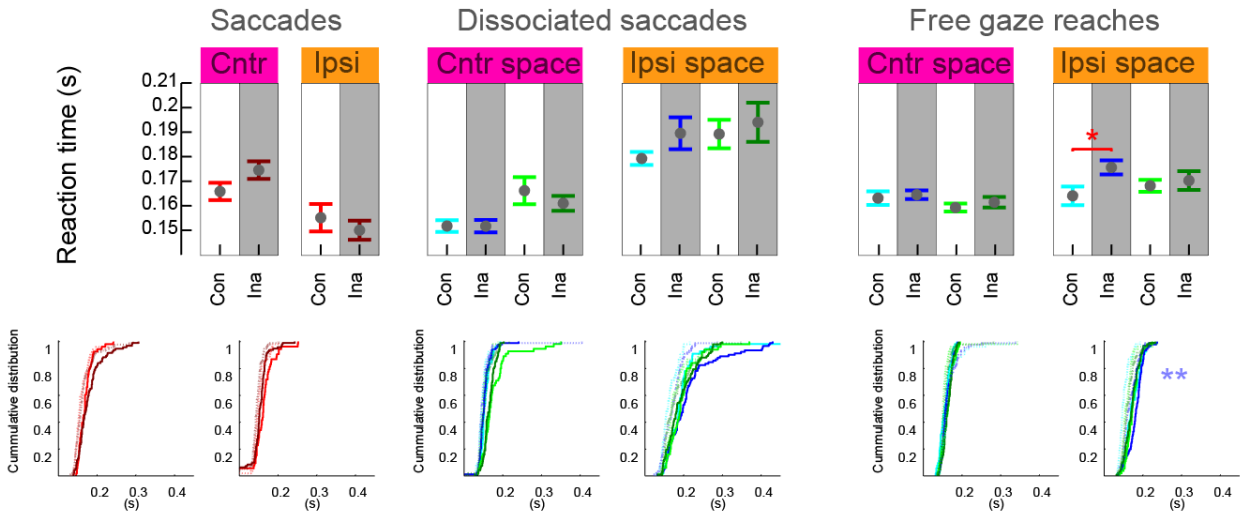


Probability of successful trials in the foveal and extrafoveal reach tasks. When the monkey could guide its reaches by visual information, there was a performance above 95% across all

conditions and there was no difference in success probability before and after inactivation ($p>0.05$). Performance for reaches in the dissociated task dropped after inactivation, particularly for reaches to the contralesional hemispace using the contralesional hand, where there were 24% more errors after inactivation ($p<0.01$).

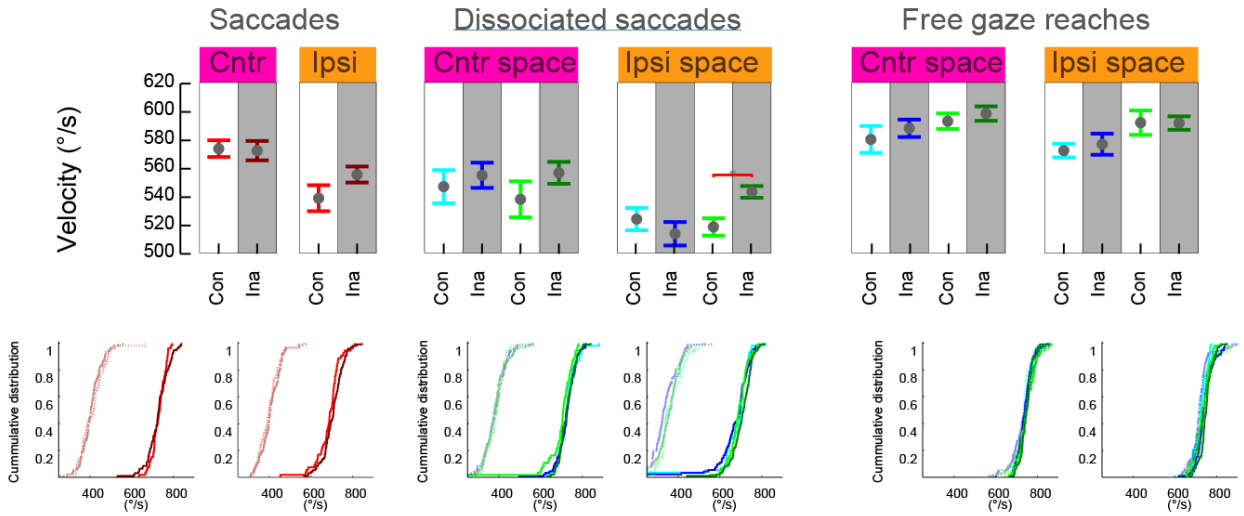
For saccades (**Figure III.11)** there were longer reaction times in the free gaze reach task to the ipsilesional hemispace when the contralesional hand was engaged (Effect size I-C 12 ms, $p=0.04$). In addition, there was a greater velocity (**Figure III.12)** when performing dissociated saccades to the ipsilesional hemispace while the ipsilesional hand was engaged (Effect size I-C 24.7°/s, $p<0.001$). For the rest of the conditions, duration, accuracy, and precision (**Figures III.13** and **III.14**) there were no effects after inactivation. However, it was observed in one session that three hours after inactivation rightward nystagmus occurred, and the last trials of a run were collected with this condition.

Figure III.11 **Saccade reaction time**



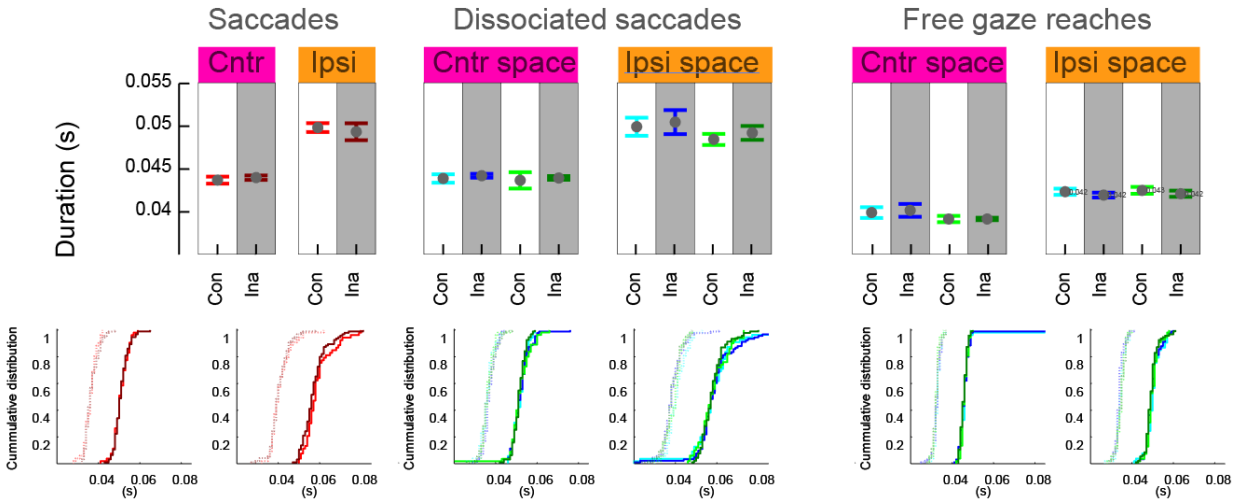
In the saccade-only task, there were no effects of inactivation in reaction times. Saccades to the contralesional side had a weak trend to be delayed ($p=0.11$). For dissociated saccades, there were no reaction time effects of inactivation. For free gaze movements, saccades to the ipsilesional hemispace were delayed when also using the contralesional hand.

Figure III.12 **Saccade velocity**



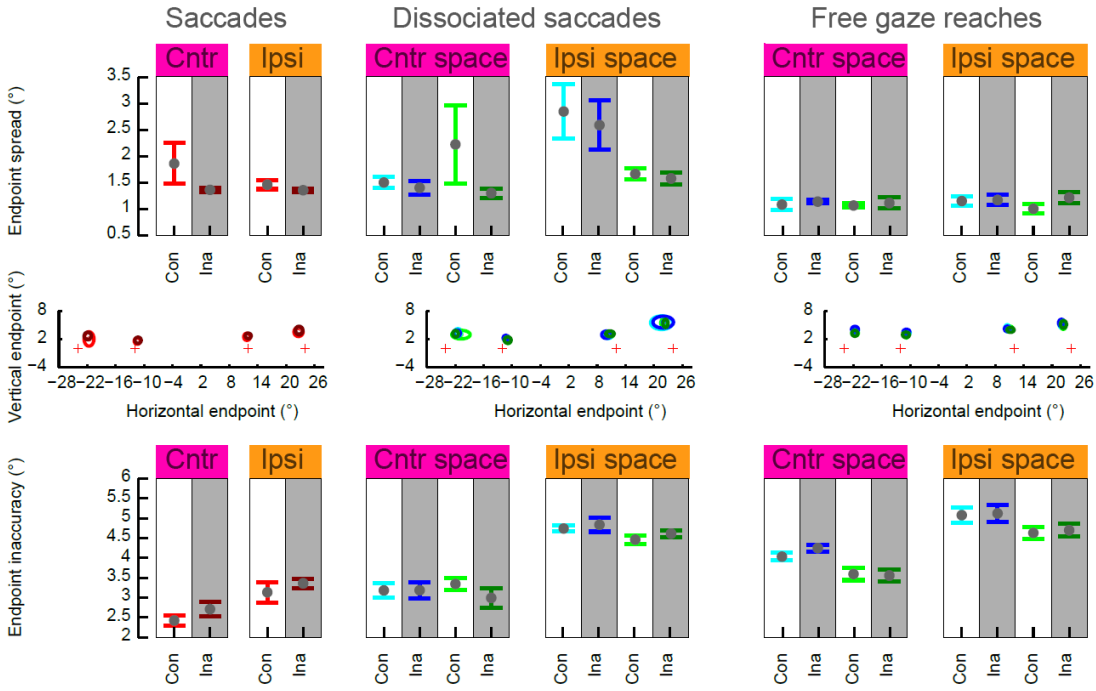
Saccade peak velocity tended to be higher after inactivation. There was a significant increase for the dissociated saccade task when the movements where performed to the ipsilesional hemispace using the ipsilesional hand.

Figure III.13 **Saccade duration**



The duration of saccades was unaffected by the inactivation regardless of the eccentricity of the target, the presence of a hand engaged in the trial, and the condition type.

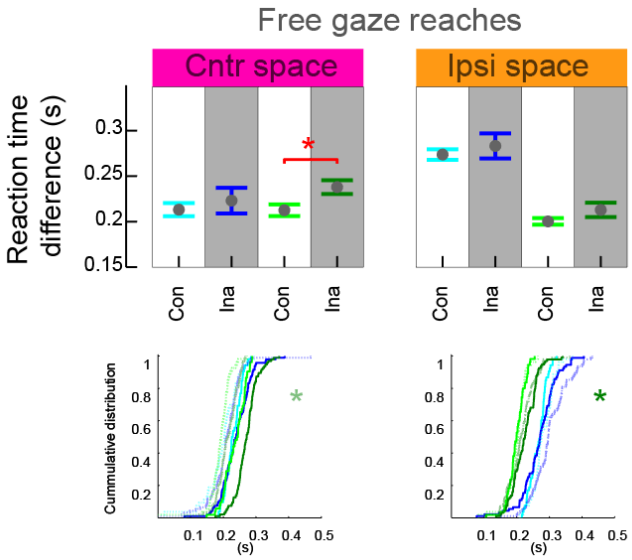
Figure III.14 **Saccade accuracy**



Similar conventions as in **Figure III.9**. Saccade Euclidean distance accuracy and precision was unaffected after inactivation. This was true for when combining data per hemispace and for target-wise comparisons.

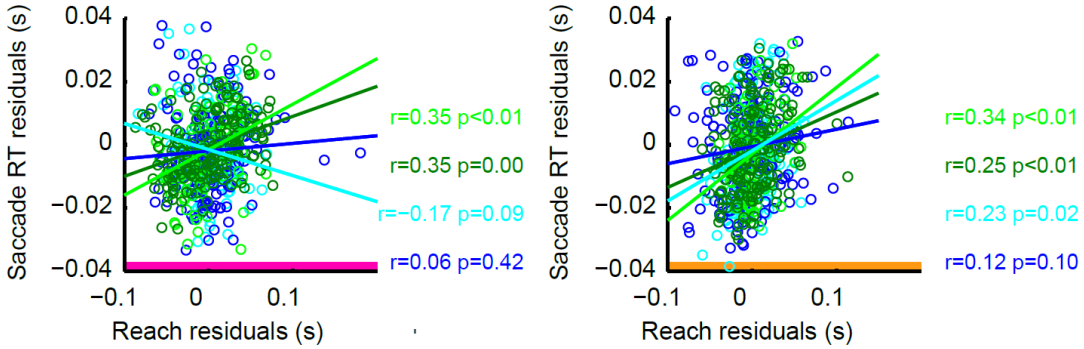
Two effects related to eye-hand coordination disruption were observed. First, by subtracting the saccade reaction time to the reach in the free gaze reach condition an increase in the difference was present for the contralesional hemispace when using the ipsilesional hand (**Figure III.15)**. In addition, the eye-hand trial by trial correlation of reaction time residuals dropped for both the ipsilesional and contralesional hands (**Figure III.16)**.

Figure III.15 **Reaction time difference (Reach – saccade)**



Eye-hand coordination as seen by the relationship of saccade and reach reaction time. The average saccade reaction time per session was subtracted from the reach reaction time to observe if the effects found for reaches were accompanied by similar changes in saccades. There was a larger reaction time difference in the free gaze reach condition to the contralesional hemispace with the engagement of the ipsilesional hand. Although there was a similar pattern for the rest of the conditions, these did not reach significance.

Figure III.16 **Saccade-reach correlations**



Correlation coefficients from trial by trial residuals of reach and saccade reaction times. The RT data was linearly detrended per condition and per session. Saccades which started earlier than 80 ms after target onset, and reaches that started earlier than 200 ms after target onset, were excluded as they were considered express movements. Left: correlation of residuals for saccades and reaches to the contralesional hemispace (green and blue: ipsilesional and contralesional hand, respectively; light and dark shading: control and inactivation conditions

respectively). There was a loss of correlation trend when the monkey used the contralesional hand after inactivation. Right: correlation of residuals for saccades and reaches to the ipsilesional hemispace. There was a slight decrease of correlation when the monkey used the ipsilesional hand and a loss of correlation when it used its contralesional hand.

The effects that we observed after the injection of THIP in dorsal pulvinar were mainly on the selection and execution of reaching movements. Saccades were largely unaffected in their latency, duration, velocity and accuracy. However, there were changes in eye-hand coordination which could indicate a disruption in either the integration of such movements supported by dorsal pulvinar function or just because one of the effectors i.e. reaches was impaired and this caused changes in the estimation of coordination even when not both effectors were affected.

Discussion

In this chapter, we aimed to revisit and expand previous findings from our group in which after dorsal pulvinar inactivation there were a series of deficits when monkeys performed reach to grasp tasks as well as saccades and visual exploration. The effects observed in this study made the authors to speculate that the dorsal pulvinar might be a subcortical center involved in eye and hand coordination (Wilke et al., 2010).

We have presented in *Chapter II* that dorsal pulvinar neurons have strong firing well before the onset of purposeful reaches and only after the offset of saccades in dissociated saccade (e.g. Figures **II.13 II.15** and **II.18)**. Similar findings have been reported in of a subset of cells of the pulvinar latero-posterior nuclei where pulvinar neurons respond up to 495 ms before reach onset (Cudeiro et al., 1989). On the other hand, during saccade tasks, pulvinar neurons also show suppression before and around the movement, when no hand was involved (*Chapter I*) or when a hand was engaged in the task (*Chapter II*). These findings, in addition to open question from previous reports of inactivation effects (Wilke et al., 2010, 2013) (e.g. mixed effects regarding the presence or absence of contralesional increase of reaction times), make the re-exploration of behavioral deficits caused by dorsal pulvinar inactivation necessary.

The effects of inactivating dorsal pulvinar in the 2010 study resembled deficits found in patients with two separate conditions, optic ataxia, and hemispatial neglect. Optic ataxia is characterized by difficulties to perform peripherally visually-guided reaches, particularly when there are time constraints to perform the action (Rossetti et al., 2003). By temporary silencing the posterior parietal cortex of monkeys it has been possible to cause deficits that resemble those of ataxic patients (Hwang et al., 2012). This makes the characterization of potentially similar deficits caused by subcortical damage so relevant. Neglect on the other hand is a deficit where there is lack of awareness of the contralesional hemispace after damage to one of the brain hemispheres and often involves the parietal cortex (Karnath et

al., 2002). Visual extinction, a condition similar to neglect manifests only when two target options are presented, one in each hemispace, only then the contralesional hemispace is ignored. In our study we did collect data during choice conditions, but we do not present the results in the context of this thesis.

In the current study, we addressed methodological concerns that facilitate the dissection and nature of the observed deficits in the previous report from our group.
However, as additional data from the studied monkey and a second dataset are to be added we will try to keep this discussion succinct to effects likely to be true after additional data has been collected.

We discuss preliminary results from one monkey trained to perform direct visually-guided foveal and extrafoveal reaches as well as saccades and dissociated saccades to targets in a touch display.

D.III.1 Impairment in hand selection

One of the conditions used by Wilke and collaborators (Wilke et al., 2010) was to present monkeys either with a free choice between arms to perform a reach to grasp food pieces, or the forced use of one of the hands by placing a barrier between the non-wanted hand and the food pieces. So, a free choice plus a motor component where involved in the task, or only the latter. In our study, we trained the monkey to perform a movement with the arm that was cued by the color of the fixation spot. The initial performance of the monkey to release the correct sensor for the contralesional hand dropped from 95% to 90% and the usage of the ipsilesional hand improved from 93% to 97% across trials. In addition, there was an increase of sensor release reaction time when the monkey used correctly the contralesional arm (contralesional hand 445 ms $\pm$ 10 ms to 473 ms + 7 ms before and after inactivation respectively, $p<0.05$; and no effect in ipsilesional hand 379 ms $\pm$ 8 ms and 375 ms $\pm$ 4 ms, $p>0.05$). It is likely that the system guiding movement the contralesional hand was impaired. As seen in *Chapter II* we did not find a general hand preference when looking at firing rate, but we did find units with preference to either hand. The arm specific

effect in reaction time could have alternatively involved other processes tangentially manifested in the sensor release latency. These effects could have been related for example to a decrease of the desirability, or awareness of the contralesional effector. It has been suggested that motivational factors modulate the effect of inactivation in saccade tasks (Wilke et al., 2013), and a similar behavioral effect might be present when there is a hand instruction. Alternatively, changes in attentional processes could also be the cause of these behavioral effects. It has been reported that the lateral pulvinar participates in attentional processes (Desimone et al., 1990) but this effects have been reported by the competition of two stimuli. If the selection of an effector, or other processes benefiting from embodied actions, share circuits with decision making systems, attentional changes might also be observed after pulvinar inactivation. Embodiment has been shown to exist during free choices in monkeys and perceptual decision in humans (Filimon et al., 2013; Kubanek and Snyder, 2015). If dorsal pulvinar shares or reflects properties of posterior parietal cortex, it is possible that its modulation is linked to action selection, integrated on basis of visual properties and the action goal. A way to address how the motor and attentional networks are represented in dorsal pulvinar would be by observing BOLD activity while monkeys perform goal-directed actions before and after inactivation of pulvinar using fMRI.

D.III.2 Mixed effects after pulvinar inactivation

After inactivation of dorsal pulvinar we encountered different deficit levels for reaches and saccades. For saccades, there were no significant effects when dividing our dataset by hemispace for reaction time, duration, imprecision magnitude and velocity. For reaches on the other hand the reaction time of the ipsilesional hand significantly increased in the free gaze task when reaching to the contralesional hemispace (**Figure III.7**). The overall effect in reach duration was an increase in the dissociated reach task to the contralesional space using the contralesional hand. This suggests that the spatial component of the task is strongly represented in the pulvinar as also shown by our electrophysiological findings. The difference in saccade and reach effects seem to agree with a stronger participation of pulvinar in the generation of reaches than in saccades.

It has been found that inactivation of the parietal reach region (PRR) produces similar effects to the ones found in patients with optic ataxia, such as decreased accuracy to the contralesional side, specifically undershooting (Khan et al., 2005; Hwang et al., 2012; Andersen et al., 2014). We hypothesized that pulvinar inactivation could cause similar deficits in accuracy. However, we did not find such effects. The only difference between inactivation and control sessions was a slight overshoot in the upward direction for the free gaze reach task when the monkey performed a reach to the ipsilesional hemispace with the contralesional hand (**Figure III.9)**. In our data we did not find undershooting in extrafoveal reaches after inactivation as we would expect from optic ataxia. Furthermore, striking differences between foveal and extrafoveal performance after inactivation were lacking. In other words, the changes in performance according to hemispace, direction of effect, or task type do not reflect ataxia-like deficits.

We also observed a decrease in the hit rate for performing dissociated reaches. This decrease was largely caused by the break fixations during the delay period towards the cue. In previous studies measuring reaction times of saccades there have been either increased latencies to the contralateral hemispace or decreased to ipsilateral targets. Although we did not find significant effects to any of the directions across sessions the average reaction times for both hemispaces have the corresponding directionalities, increased reaction time for contralateral saccades and decrease for ipsilateral.

D.III.3 Dorsal pulvinar reduces eye-hand coordination

In order to perform visually-guided reaches primates need to prepare a motor plan using the target location in respect to the current position and orientation of the limbs (Desmurget and Grafton, 2000; Gaveau et al., 2003). This motor plan is optimized by the availability of visual information and the evaluation of motor errors between target and limb computed in the posterior parietal cortex (Gaveau et al., 2003). The availability of visual information of the limb and target strongly influences the performance of reach tasks (Prablanc et al., 1979a, 1979b). In our study we found deficits after dorsal pulvinar inactivation not only on extrafoveal but also on foveal reaches and thus, we looked at the reaction time difference of

saccades and reaches and at the eye-hand reaction time correlation of residuals. Both parameters were disrupted by the inactivation. By subtracting the reaction time of the saccade to the reach we observed an increased reaction time difference to the contralesional hemispace using the ipsilesional hand. This was likely influenced by the increase reaction time of reaches after inactivation for that condition but not for saccades. The trial by trial reaction time correlation of residuals for reaches and saccades was lower (Spearman correlation coefficient $r=0.17$, $p=0.09$ to $r=0.06$, $p=0.42$ for the contralesional hemispace with the contralesional hand; $r=0.34$, $p<0.01$ to $r=0.25$, $p<0.01$ for the ipsilesional hemispace with the ipsilesional hand; $r=0.23$, $p=0.02$, and $r=0.12$ $p=0.1$ to the ipsilesional hemispace with the contralesional hand).

A final aspect to consider is the drug spread. Even when in our own assessments the drug diffusion seen with Gadolinium shows a reliable spread of the drug in dorsal pulvinar it is standard to be cautious about the time range from which the data collected is used for the analysis, to ensure that the effects found are mostly related to functions of the area of study (see (Purushothaman et al., 2012)). A comparison of effects separated in several periods after the inactivation and the online monitoring of drug diffusion over a long period of time might be of use to test for effects specificity.

Acknowledgements

We thank Ira Panolias, Sina Plümer, Klaus Heisig, and Dirk Prüße for technical support. We also thank Stefan Treue, Alexander Gail, Hansjörg Scherberger, members of the Decision and Awareness Group, Sensorimotor Group and the Cognitive Neuroscience Laboratory for helpful discussions. Supported by the Hermann and Lilly Schilling Foundation, German Research Foundation (DFG) grants WI 4046/1-1 and Research Unit GA1475-B4, KA 3726/2-1, CNMPB Primate Platform, and funding from the Cognitive Neuroscience Laboratory.

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General discussion: Summary, limitations, and project outlook

The aim of this thesis was to explore the putative participation of the dorsal subdivision of the thalamic pulvinar during eye-hand coordination. Several new questions arose during the development of our research. These questions led to a series of behavioral, electrophysiological, and causal experiments and to the collection of multiple but conceptually interconnected datasets, addressing now a more global question: What is the role of dorsal pulvinar in goal-directed behavior? The results of this thesis have been described and discussed in previous Chapters. Taken together, our findings provide novel evidence of the participation of dorsal pulvinar in goal-oriented behavior, namely target selection, spatial transformations, saccade and reach generation and likely their integration. These results opened many experimental questions, some of them already being addressed by our ongoing research. In the following section, I will briefly revisit our main findings and expand on the limitations from each study exploring pulvinar function.

Main findings

Target selection and saccade behavior

In *Chapter I* we aimed to understand how dorsal pulvinar might be involved in saccade generation and oculomotor target selection. This was motivated by studies from our group (Wilke et al., 2010, 2013), where inactivating dorsal pulvinar biased target selection to the ipsilateral hemispace from the inactivated hemisphere. To address this question, we applied trains of biphasic pulses of electrical stimulation in dorsal pulvinar starting at different task periods: before, around or after the onset of the target for a visually-guided saccade task. Additionally, we performed similar stimulations before or after the onset of a visual cue, or before or after a "Go" signal during a memory-guided saccade task. We also performed control stimulations at different depths in ventral pulvinar during visually-guided saccade tasks to test for the specificity of the effects we will describe. Finally, we characterized the visual and motor properties of dorsal pulvinar neurons during the execution of similar visually- and memory-guided saccade tasks.

Our findings revealed that dorsal pulvinar stimulation exerts influence in target selection and saccade generation. This influence was time, site, and task specific. First, in the visually-guided task we found that the selection of targets in the ipsilateral hemispace to the stimulated hemisphere was increased after early pulvinar stimulation i.e. when the stimulation started before the onset of the saccade target. Importantly, for all conditions, the stimulation trains lasted 200 ms, so in all conditions there was overlap of the stimulation with the visual stimuli onset, and in some cases with the normal saccade reaction time. Similar enhancement of ipsiversive target selection during early stimulation periods has been reported for perceptual decisions in the caudate nucleus (Ding and Gold, 2012), but not for free choices as in our study. Also for early stimulation periods, we found a decrease of reaction time to saccades to the ipsilateral hemispace but not for the contralateral hemispace.

When we stimulated later, i.e. starting after the onset of the targets, we found an increased bias to targets in the contralateral hemispace as well as increased saccade reaction time to either hemispace. Delays of reaction times regardless of hemispace after late stimulation have been reported in oculomotor areas, e.g. frontal eye fields (Izawa, 2004) and superior colliculus (Munoz and Wurtz, 1993). The delay effect could be either facilitation of holding the current gaze position or an inhibition of saccade generation. The saccade delay for late stimulation was stronger for ipsilateral saccades. This delay as discussed in *Chapter I,* has been found in cortical but not subcortical brain regions. It is interesting to hypothesize that the resemblance of the effect of pulvinar stimulation to that of cortical structures might be related to similar roles in purposeful behavior. When the task was memory-driven, saccade reaction times were affected, but target selection was not. This could indicate that either *1)* the presence of a visual influence is needed for the selection network to be affected, or that *2)* the time dissociation between the cue presentation and the Go signal disrupt an otherwise integrated motor and target selection system in the pulvinar which is not present individually. One way to address these possibilities would be to perform a similar behavioral paradigm where instead of a memory-guided saccade; a visually-guided but delayed saccade is required. This would allow having the same time dissociation with the availability of visual information.

Coming back to the target selection, one of our most interesting findings was that the stimulation effect was not purely contralateral, but a smooth transition from ipsilateral to contralateral preference. In addition, the electrophysiological tuning properties of the dorsal pulvinar also varied according to the period of interest. In general, we observed contralateral spatial tuning during cue period and more balanced spatial tuning during the peri saccade and target hold periods. This dynamic tuning was a repeated finding across our electrophysiological studies (See *Chapter I, Ibis, and II*). For stimulation happening closer to the saccade onset, the tuning was still contralateral, which could have created facilitation to select targets in the corresponding contralateral hemispace. The fact that the spatial tuning and the behavioral effects of microstimulation in the late stimulation periods correspond suggests that the stimulation enhanced normal firing activity (Clark et al., 2011) instead of disrupting it.

In *Chapter Ibis,* we continued exploring the electrophysiological properties of dorsal pulvinar in oculomotor behavior. We addressed two specific questions, one looking at target selection and the second one at spatial transformations.

Electrophysiological findings on target selection

We aimed to asses if the target selection effects from *Chapter I* had a neural correlate in dorsal pulvinar. We recorded pulvinar responses while monkeys performed visually- and memory-guided free-choice tasks. At a population level, there was a short increase of firing rate for choices to the contralateral hemispace during the cue period in comparison to choices to the ipsilateral hemispace (**Figure Ib.5**). This suggests that there might be target-selection influence from dorsal pulvinar on free choices when visual information is still available. In our stimulation experiment there was a change in target selection patterns only when the cue acted also as a Go signal, i.e. no memory processes involved. The electrophysiological evaluation of firing rate changes during the visually-guided task are however challenging as the visual inputs are always present along with the internally/generated processes of our interest. This visual confound makes it difficult to assess at which point the firing rate corresponds to each process. As discussed in *Chapter*

Ibis, it is possible that the time dissociation between the cue presentation and the Go signal account for differences in the firing patterns. Alternatively, if the constant availability of visual information during the target selection is the determining factor for dorsal pulvinar to participate in target selection in the visually-guided task, this might explain the lack of effects or firing rate modification in memory periods. There was lower firing in the single-ipsilateral-cue condition than in the rest of the conditions, not only during the cue period, but also during the memory epochs. At this stage the neural firing enhanced in choice conditions is likely to represent the spatial preference instead of an early signal of target selection (Shadlen and Kiani, 2013). Dorsal pulvinar might participate in multiple visuo-motor processes, e.g. target selection and saccade generation, which cannot be decoupled unless a temporal offset between individual processes. Alternatively, some processes involving dorsal pulvinar might emerge only when a specific set of conditions arise, e.g. a role in target selection when visual information is actively involved. It has also been shown that colliculi cells with projections to the pulvinar show target selection activity close to the onset of the stimuli, the action, and even before the target onset (Port and Wurtz, 2009) and this target selection can hardly be dissociated from the action itself. The execution of a visually-guided and delayed tasks will contribute to the disentanglement of the participation of dorsal pulvinar on target selection at a single cell level.

Gaze effect

Are the visual and oculomotor properties of dorsal pulvinar influenced by shifts in the gaze position? As the main purpose of this thesis was to explore the properties of dorsal pulvinar during eye and hand movements and their interactions, the coordination system under which they operate is a particularly interesting topic. The proficiency of primates to perform visually-guided reaches relies on an elegant system that transforms visual information captured by the retina with an organization similar to that of the visual field to one which considers the current position of the limb in respect to the object of our interest (Gaveau et al., 2003). These transformations are influenced by the properties of visuo-motor hierarchies and also require the computation of the position of the orbit in the head, the position of the head on the body, and the body relative to the limb (Flanders et al.,

1992). In association areas such as the parietal cortex, strongly connected to dorsal pulvinar, eye-centered coordinate systems are the most common (Snyder, 2000). However, as noted in the introduction of *Chapter Ibis* an eye-centered coordinate system is not the only one influencing the firing of neurons in association cortices. It has been reported for example that mixed reference frames influence the activity of visually (and auditory) signals in the posterior parietal cortex (Mullette-Gillman et al., 2009).

We wanted to assess if dorsal pulvinar neurons encode visual information using a retinotopic coordinate system or potentially a more complex one. Indeed, we found a subset of dorsal pulvinar neurons that encode visual information in a purely retinotopic way. But more interestingly, we also found that pulvinar neurons were modulated by additional factors. Due to experimental constraints in which neurons might have been activated by spatial static cues from the dimly lit setup itself, and not only by the spatial location of the targets, the non-purely eye-centered firing rate modulation is difficult to categorize, but likely involves the monkeys' gaze position, and the absolute location of the target. Gaze position firing rate modulation has been well documented in association areas (Andersen and Mountcastle, 1983; Andersen et al., 1985, 1990). The earliest reports of visually-driven firing influenced by the position of the eye in the orbit were done from studies in the parietal cortex (Andersen and Mountcastle, 1983). This influence has been hypothesized to help reduce computational costs of performing actions under separate coordinate systems (Zipser and Andersen, 1988). The fact that pulvinar neurons showed similar modulation by gaze position as parietal cortex does suggests the existence of shared functional roles between pulvinar and the high order cortical areas with shared connectivity.

Electrophysiological properties during reaches

In *Chapter II* we looked at pulvinar function during the planning and execution of reaches. We did so by recording single cells while monkeys performed delayed visually-guided dissociated, and free gaze reaches, in addition to dissociated saccades with engagement of each arm. We observed a large variety of neuronal response modulations on a single cell level. There were cells modulated by the visual stimuli only, by the space only, or by the

combination of hand and eye, among others. The main difference between saccade and reach related firing was in the timing and the directionality of the effects (even when there were subpopulations modulated differently at particular task periods). In general, while for dissociated saccades, there was suppression of firing before and around the saccade, followed by a large enhancement after saccade offset, for dissociated reaches there was a modest enhancement before and until the offset of the reach. Even when this was the overall population trend it was possible to identify dorsal pulvinar subpopulations with different tuning properties e.g. with pre saccade enhancement, this diversity can be seen in single cell examples from **Figures II.3** to **II.9**.

In some cells, ramping up of firing rate started soon after the onset of the cue and lasted until reach offset. Even on a population level the ramping up activity was visible a couple of hundred of milliseconds before the mean onset of the reach. Importantly, for free gaze reaches both main types of modulation were present, suppression followed by enhancement in saccades, and the enhancement for reaches. Could these firing rate patterns during saccades and reaches be part of a single process under natural conditions? In other words, could the post-saccade firing in pulvinar aid the optimization of a reach? It has been shown in the parietal reach region that saccade related activity exists (Snyder et al., 2000a), and that this activity, observed around and after the movement is potentially participating in the coupling of the saccade and reach systems. Our data from the dorsal pulvinar might prove to be a similar signature of eye-hand integration however additional analyses need to be performed, amongst these are the study of the properties of local field potentials and how they relate to actions potentials in dorsal pulvinar. Additionally, we need to look at brain networks using functional connectivity and combinations of disruptive techniques and observation of changes in neuronal activity.

Behavioral findings after pharmacological reversible inactivation

Finally, we collected preliminary data from one monkey performing tasks such as in *Chapter II* (and in addition a saccade only task) but without the delay component, before and after inactivation of dorsal pulvinar. This experiment was conceived as a follow up on a previous study from our group (Wilke et al., 2010). In that experiment heads were

unrestrained while monkeys performed reach to grasp movements to food objects; the experimenters reported apparent changes in the patterns of reach execution after inactivation. The monkeys did not seem to direct their heads and eyes to guide reaches as they did before the inactivation. It could have been that monkeys were unable to perform foveally-guided movements, or that postural, spatial or motor deficits were present after pulvinar disruption. In either case, an evaluation of their performance while explicitly instructing them to perform dissociated or coordinated movements was lacking. Our hypothesis supported by previous findings was that after inactivating dorsal pulvinar, potentially involved in the integration of eye and hand movements, there would be a decreased coordination of eye-hand movements and potentially of the overall performance of visually-guided, foveal reach tasks. Alternatively, if dorsal pulvinar was involved in the generation or execution of only one of the movements, reaches or saccades but not in their integration, we would expect a decrease in the performance of that specific movement with a mostly unaffected movement with a different effector.

In the 2010 paper there were behavioral changes both for the execution of saccades and reaches, however our study found mainly changes in reach performance, although in one session after more than three hours of inactivation nystagmus was observed.

There were four clear effects after inactivation: 1) an increased proportion of errors for the usage of the correct contralesional hand cued at the beginning of the trial; 2) an increased sensor release reaction time in cases where that hand was correctly selected; 3) a decrease of hit rate in the dissociated reach task with the contralesional hand to the contralesional hemispace and a 4) drop of performance for dissociated, extrafoveal reaches but not for free gaze, foveal reaches. This last finding suggests that the disruption of dorsal pulvinar does not affect foveally-guided reaches as much as it does the non-foveally-guided ones. At this point however, this is largely speculative as there was a lower hit rate for extrafoveal reaches in control sessions compared to foveal reaches, which might result in different effects post inactivation. We did not find reach undershooting in extrafoveal reaches, as it would be expected from patients with optic ataxia. The post inactivation effects we found could be due to the lack of online update of the hand position in respect to the targets in the affected hemispace. This however seems unlikely as most errors were caused by the break of eye fixation during the delay period in that condition and not by misreaches. This effect

could also be a manifestation of attentional disruptions. It has been shown that pulvinar damage increases the capture of contralateral distractors in patients (Van der Stigchel et al., 2010) and pulvinar is involved in attentional processes in monkeys (Petersen et al., 1987; Desimone et al., 1990). However as mentioned before, the specificity of this deficit is not only linked to the hemispace, but inherently to the arm used. Damage to the parietal cortex also causes spatial deficits, some of which resemble clinical manifestations of ataxic patients (Rossetti et al., 2003; Hwang et al., 2012; Andersen et al., 2014). Up to which point is dorsal pulvinar involved in eye hand integration and coordination like association cortices is a question that will be the focus of our research in the immediate future.

General conclusion

We have provided evidence suggesting the participation of dorsal pulvinar in saccade generation (*Chapters I, Ibis,* and *II),* and target selection (*Chapters I).* Spatial tuning in dorsal pulvinar was mainly to the contralateral hemispace of the recorded hemisphere, but dependent on behavioral contingencies, e.g. neurons with strong contralateral tuning around cue periods and neurons with both contralateral and ipsilateral spatial tunings closer to saccade and reach execution (*Chapters I, Ibis, and II*). In addition, gaze position influenced cue-related activity in a subpopulation of dorsal pulvinar neurons. Other subpopulations encoded visual stimuli with a classical retinotopic reference frame (*Chapter Ibis*). Pulvinar participation in target selection during free choices might be dependent on the coupling or de-coupling of target presentation and the execution of oculomotor actions (*Chapters I* and *Ibis*). Dorsal pulvinar showed different patterns of firing rate for the execution of saccades and reaches. There was enhancement of firing prior and during the execution of reaches (*Chapter II*), and mainly suppression prior and during saccade execution, followed by enhancement at saccade offset (*Chapters I, Ibis, and II*). Pulvinar might exert different roles in the planning and execution of effector specific actions, or more appealingly, in the integration of multi-effector, or coordinated behavior. Although probably not directly (*see Chapter II*), dorsal pulvinar inactivation disrupted the use of the contralateral limb (see *Chapter II and III* for arm specificity findings or the lack of thereof). Finally, dorsal pulvinar disrupted the performance of non-foveally-guided (extrafoveal) reaches when the

action was directed with the contralesional hand and to the contralesional hemispace. The coordination of eye and hand movements appeared to be correlated to normal pulvinar function. In summary, this thesis contributes from multiple angles to the hypothesis of dorsal pulvinar playing a crucial in purposeful actions. It also opens several research lines that will help enrich our understanding of how visually-guided goal-oriented behavior is encoded in the primate brain.

Limitations

Characterizing the neuronal properties of a brain region with complex connectivity to cortical and subcortical brain areas such as the pulvinar (Clark and Northfield, 1937; Asanuma et al., 1985; Grieve et al., 2000; Shipp, 2003; Stepniewska, 2004; Cappe et al., 2007; Kaas and Lyon, 2007; Cappe et al., 2009; Bridge et al., 2016) is not an easy task. The initial purpose of this study was to provide further insights about the nature of potential eye and hand movement disruptions motivated by findings from Wilke and collaborators (2010). As several additional lines of research were derived from our initial questions new experimental and interpretation considerations emerged.

Looking at our microstimulation experiments the stimulation parameters themselves are worth considering (Tehovnik, 1996). As we produced trains with a fixed duration, frequency and polarity we also reduce the possibility of generalization of our results to studies in other brain areas using different methodology. A study addressing the characterization of behavioral changes produced by the sole manipulation of stimulation protocols for dorsal pulvinar would prove a great usefulness. Even when in our study we did not systematically vary all stimulation parameters we did not only evaluate how stimulation affected our area of interest, the dorsal pulvinar, but also the ventral pulvinar. Ventral pulvinar stimulation did not evoke the paradoxical effects on behavioral target selection nor reaction time patterns as in dorsal stimulation. This specificity supports the idea that the effects observed were particular of dorsal pulvinar and not an effect of the stimulation paradigm. Moreover, the reaction time effects and target selection changes were specific for the visually-guided and memory-guided tasks, suggesting that the cognitive

demands were important for observing specific behavioral changes. The current strength is crucial but widely variable across experiments as well as the effects evoked e.g. in the thalamus (Crommelinck et al., 1977; Maldonado et al., 1980). For our study, the current strength used was operationally defined to match the one used in our neuroimaging studies looking at pulvinar connectivity. However, the estimation of different stimulation strengths and frequencies that evoke behavioral responses would enrich the interpretation we can derive from the behavioral patterns evoked.

In our gaze modulation experiment a technical concern was that opposite to early electrophysiological experiments addressing coordinate systems using tangent screens in fully darkened rooms, we used monitors to display the visual stimuli. Even when our monitors had a very low background luminance (0.16cd/m^2), we cannot completely rule out the possibility that the brightness of the display could create additional reference points in a coordinate system relevant for pulvinar neurons. An additional and important limitation was that the tasks required the monkeys to be fixated with the head straight ahead to the monitor. In previous studies it has been shown that subcortical regions such as the superior colliculus respond not only to eye position modifications, but also to the location of the head in respect to the targets. Head and eye manipulation would have greatly improved our data interpretation as it reflects a more natural way in which primates interact with their surroundings. In our recordings from the eight independent movement types only a small subset of target locations was acquired from all three distinct starting gaze locations. These target locations were selected because of the distance range under which the eye movements could still be reliably tracked with our recording system and displayed on our 27 inch monitor. A larger set of targets would allow performing target per target comparisons of the influence of target locations in a more comprehensive manner.

For our main question we looked at electrophysiological properties of the dorsal pulvinar, previous reports have related pulvinar firing to reach behavior (Martin-Rodriguez et al., 1982; Acuña et al., 1983, 1983, 1986; Magariños-Ascone et al., 1988; Cudeiro Mazaira et al., 1989). Importantly it has been shown that dorsal pulvinar does not present a retinotopy as areas in the ventral subdivisions (Benevento and Miller, 1981; Petersen et al., 1985;

Robinson et al., 1991). In our study, we did not perform an online estimation of receptive fields before the visuo-motor experiments. Additionally to the lack of retinotopy a technical reason not to estimate the receptive fields was due to the large number of trials required to assess receptive field of each neuron. As our tasks involve the execution of reaches, more demanding and time consuming than fixation or saccade only tasks, monkeys would not have performed enough trials for electrophysiological analysis. Along the same line, the isolation of neurons in dorsal pulvinar usually required a significant amount of time, which resulted in a small timeframe in which useful datasets could be collected. The lack of mapping represents a problem for data analysis as many established methods rely on clear responses inside and outside of the receptive fields for vision and decision-making experiments. We currently address this methodological concern by the offline evaluation and classification of cell subgroups based on their firing responsivity. As the firing patterns of dorsal pulvinar are likely to be as diverse as its connectivity, studying the response of different pulvinar subpopulations is undoubtedly advantageous. Currently only the electrophysiological characterization of dorsal pulvinar cells is being studied, however we did not only collect action potentials but also local field potentials. The relation between action potentials and oscillations will likely reveal different aspects of pulvinar function in visuomotor behavior.

A particularity of our studies in target selection is that our model of decision relies on free choices and not on correct responses to perceptual discrimination. We think that one of our defining characteristics as primates is based in our free exploration and will, and so, free choices are a natural output of fully internally generated decisions in the brain. In this type of decisions however, is easy for the monkeys to develop a bias to the selection of specific targets. This bias makes it difficult to compare behavior performed across different task contingencies e.g. when looking at the firing rate across choice conditions per trial when there is a preference for a subset of targets. This problem could be addressed by modulating the expected values in value-based choices, although the validity of the term free choice would become more difficult to justify.

In the inactivation experiment we performed six THIP injections in dorsal pulvinar. The tissue damage by repeated injections could affect the normal function of the pulvinar over time or lead to gradual changes in brain networks making the estimation of behavioral changes before and after inactivation difficult. Also, the time frame for the observation of inactivation effects is critical, as the spread of THIP to neighboring regions might lead to spurious effects. It has been estimated that the solution spread in other subcortical tissues is around 1 mm/hr (Hikosaka and Wurtz, 1985). For our dataset, we considered trials up to four hours after injection to dorsal pulvinar´s center, which roughly fall within the limit of estimated spread (five millimeters diameter in pulvinar´s widest dimension). A separation of trials into different time windows might be helpful for a better characterization of effects according to the drug's spread.

In both the reach electrophysiology and inactivation studies we constantly recorded the eye position of the monkeys, however the kinematics of the arm were not, and thus a full characterization of purely reach-related deficits is not present. We currently rely solely on reach endpoints, making hypothesis related to reach execution or kinematics relationships between saccades and reaches not possible to address.

Lastly, the large set of tasks and methodological approaches used for the realization of this thesis made the data collection a priority that now must be matched by several approaches of data analysis. This will add more levels of richness to our data. Among others, a systematic classification of our electrophysiological datasets is needed as pulvinar cells displayed a broad range of firing patterns. Additionally, we need to perform correlation tests to assess the links of our electrophysiological and behavioral datasets. We need to expand our behavioral analysis, as this is the foundation for the interpretation of all our datasets, particularly our causal experiment. Finally, looking at the properties of local field potentials in dorsal pulvinar and their relation to action potentials in the same region or other brain regions during goal-oriented behavior will provide insights to our current interpretation of pulvinar functions.

Project outlook

Our findings leave a series of open questions to be addressed in the future regarding the variety of processes in which dorsal pulvinar has shown to be involved. Some of these processes in which pulvinar is involved are: saccade and reach generation and integration, target selection, and potentially spatial transformations. I will list a few of interesting questions some of which are currently being addressed by our own research or research from our collaborators:

1) Are the dorsal pulvinar time-dependent microstimulation effects on target selection and saccade generation driven by motor vectors or by attentional and/or saliency properties in the region?

As our microstimulation study looked at free gaze target selection it is interesting to speculate about how the influence of pulvinar would be if there was a correct target to be selected and additional targets were meant to distract. Would different periods of stimulation in the pulvinar enhance or suppress the saliency or attention of specific targets or specific hemispaces in a time dependent manner?

2) Would the coordinate system for spatial transformations in dorsal pulvinar display similar characteristics if not only the eye but also a hand was involved in the task? Would pulvinar firing reflect the engagement of the arm for how it encodes reference frames?

Because of pulvinar's connectivity to the fronto-parietal network it is reasonable to think that it could also be influenced by reference frames that encode stimuli relative to the limbs and not to the retina or gaze. The modulation of the head and trunk position of the monkey would of course provide very valuable information about pulvinar's function in spatial transformations, but in the immediate future the hand involvement would be an insightful addition to our datasets.

3) Expanding in our results from Chapter I, would target selection modulation in dorsal pulvinar neurons be similar when the visual information is available for the whole duration of the trials but there is de-coupling of the cue presentation to the action in oculomotor behavior?

This question is a straightforward one to answer and of paramount importance for our current interpretation of how pulvinar is involved in oculomotor decision making.

4) How does dorsal pulvinar interact with association cortices during the planning of purposeful actions?

To answer this question an elegant approach would be to pharmacologically inactivate dorsal pulvinar unilaterally while the neuronal activity of areas in the ipsilateral fronto-parietal network is recorded using multielectrode arrays to allow the evaluation of spike and LFP activity potentially modulated by the disruption of pulvinar as the monkey performs saccade and reach tasks. The disruption of the pulvinar might cause increase, decrease or mixed effects on LFP power at different frequencies. In addition, looking at the opposite dorsal pulvinar with single cell recordings would help complete the picture of how not only interareal interactions within hemisphere exist but also interhemispheric ones.

5) How are brain networks modified by the unilateral and bilateral disruption of dorsal pulvinar?

A combination of behavioral and inactivation experiments performed in the context of functional imaging would likely enrich our electrophysiological findings on a network level.

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Academic Resume

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Research Experience

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Techniques Employed: *Direct current microstimulation, extracellular recordings, pharmacological inactivation, psychophysics, eye tracking, MATLAB programming*

2009-2011 Neurodevelopment Research Unit, Cognitive & Behavioral Neurobiology Department, Neurobiology Institute, UNAM, Querétaro, México.
Techniques Employed: *Electro-oculography, video-oculography.*

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Publications

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2017 Wilke M, Schneider L, **Dominguez-Vargas AU**, Cabral-Calderin Y, Miloserdov K, Schmidt-Samoa C, Dechent P, Scherberger H, Kagan I and Bähr M Postural and Reaching Deficits following Damage to the Pulvinar. Under review, Cortex

Selected Abstracts

2016 Annual Meeting of the Society for Neuroscience, San Diego, USA
Poster: *"Contribution of dorsal pulvinar to visuo-motor behavior and spatial decision-making" Kagan I.,* ***Domínguez Vargas A. U.****, Schneider L., Gibson L., Wilke M.*

2014 Annual Meeting of the Society for Neuroscience, Washington, USA.
Poster: *"Time-dependent effects of pulvinar microstimulation on visually-guided saccades and target selection "****Domínguez Vargas A. U.****, Schneider L.*, Kagan I., Wilke M.*

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2013 10th Göttingen meeting of the German Neuroscience Society, Göttingen, Germany

Poster: *"Risk-seeking behavior in monkeys is modulated by effort in a spatial decision task"* ***Domínguez Vargas A. U.****, Grass A., Wilke M., Treue S., Kagan I.*

2011 Biannual Conference of The Association for Research in Vision and Ophthalmology (ARVO - Chapter Asia), Sentosa, Singapore.

Poster: *"Electro-oculographic analyses of eye movements in infants with diffuse periventricular leukomalacia, healthy infants and adults".* ***Domínguez Vargas A. U.****, Santiago Rodríguez E. Harmony T.*

Selected Courses

2013 FENS-IBRO-Hertie Winter School, Thalamus and thalamo-cortical Interactions: Cells, Networks, Dynamics and Disease. Obergurgl, Austria.

Oral Presentation: *"Time-Dependent Effects of Pulvinar Microstimulation on Spatial Target Selection "****Domínguez Vargas A. U.****, Schneider L.*, Kagan I., Wilke M*

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2013 BCF/NWG-Course: Analysis and Models in neurophysiology, Freiburg, Germany.

Participation *in Hands-On Course of Analysis of Electrophysiological Neuronal Data and the Theoretical Concepts behind them*

2011 Multi-Modality Short-Course. MGH/MIT/HMS Athinoula A. Martinos Center for Biomedical Imaging, Charlestown Massachusetts, USA

Participation *in the NIH funded neuroimaging course, gaining experience on functional brain imaging techniques such as fMRI, MEG, TMS, and NIRS.*

2011 Bioethics Certification. Cellular Physiology Institute, UNAM, México City, México.

Bioethics Certification *120 Hours on Human and Animal Bioethics from The Cellular Physiology Institute in Coordination with The Philosophical Research Institute of The National Autonomous University of Mexico.*

Affiliation

2013- Neurowissenschaftliche Gesellschaft (NWG)
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Student Member

2013 - Sociedad Mexicana De Ciencias Fisiológicas (SMCF)
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2011- Society for Neuroscience (SfN)
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2008-2012 Association for Research in Vision and Ophthalmology (ARVO)
Predoctoral Member

2007-2009 American Academy of Optometry (AAOPT)
Student Member

2006-2008 Academic Council for the Health and Biological Science Areas, (Formerly CAAByS, Now CAABQyS) National Autonomous University of Mexico
Student representative at the main council, and for the commissions of academic personal, and elections vigilance

Scholarships

Since 2012 Deutsches Primatenzentrum, Leibniz-Institut Für Primatenforschung (DPZ)
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2009-2011 Consejo Nacional De Ciencia Y Tecnología (CONACyT)
(National Council of Science and Technology, Mexican Government)
Master's tuition and living expenses for high academic grades

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Professional Experience

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International Master's Neuroscience program, Max Planck Research School (IMPRS)

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2009 Rehabilitation Center for the Blind and Visually Impaired (CRECIDEVI), at the public assistance foundation Conde de Valenciana, Ophthalmology Hospital México City, México

Vision Rehabilitator

Instructed Visually Impaired Patients with the Use of Optical, Non-Optical and Electronically Controlled Visual Aids

Seminar presentations at the low vision department

2007-2008 Ophthalmology department at the Social Security Institute of Mexico State and Municipalities (ISSEMyM), México State, México (Bachelor's Program Social Service).

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Appendix A

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Research Articles: Systems/Circuits

Electrical Microstimulation of the Pulvinar Biases Saccade Choices and Reaction Times in a Time-Dependent Manner

Adan-Ulises Dominguez-Vargas[1,2,4,*], Lukas Schneider[1,2,*], Melanie Wilke[1,2,3,4,+] and Igor Kagan[1,2,4,+]

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DOI: 10.1523/JNEUROSCI.1984-16.2016

Received: 20 June 2016

Revised: 21 December 2016

Accepted: 30 December 2016

Published: 24 January 2017

Author contributions: A.U.D.-V., L.S., M.W., and I.K. designed research; A.U.D.-V., L.S., and I.K. performed research; A.U.D.-V., L.S., and I.K. contributed unpublished reagents/analytic tools; A.U.D.-V., L.S., and I.K. analyzed data; A.U.D.-V., L.S., M.W., and I.K. wrote the paper.

Conflict of Interest: The authors declare no competing financial interests.

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Supported by the Hermann and Lilly Schilling Foundation, German Research Foundation (DFG) grants WI 4046/1-1 and Research Unit GA1475-B4, KA 3726/2-1, CNMPB Primate Platform, and funding from the Cognitive Neuroscience Laboratory, German Primate Center. We thank Lydia Gibson for collecting a subset of neuronal recordings; Sebastian Moeller for sharing the design of an MRI-compatible drive and many valuable suggestions; Hansjörg Scherberger for valuable discussions and suggestions regarding target selection equalization; Stefan Treue, Alexander Gail, members of the Decision and Awareness Group and the Cognitive Neuroscience Laboratory for helpful discussions; Ira Panolias, Sina Plümer, Klaus Heisig, Dirk Prüße and Ralf Brockhausen for technical support. We also thank two anonymous reviewers for their fast, valuable and constructive comments.

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Cite as: J. Neurosci ; 10.1523/JNEUROSCI.1984-16.2016

Behavioral/Systems/Cognitive

Electrical Microstimulation of the Pulvinar Biases Saccade Choices and Reaction Times in a Time-Dependent Manner

Abbreviated title: Time-dependent effects of pulvinar stimulation

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Number of Pages: 56 excluding the Title page and this page (including Figure legends and Tables)

Number of Figures: 11

Number of Tables: 4

Number of Words: Abstract: 250, Introduction: 667, Discussion: 1724

Conflict of Interest: The authors declare no competing financial interests.

Acknowledgements: Supported by the Hermann and Lilly Schilling Foundation, German Research Foundation (DFG) grants WI 4046/1-1 and Research Unit GA1475-B4, KA 3726/2-1, CNMPB Primate Platform, and funding from the Cognitive Neuroscience Laboratory, German Primate Center. We thank Lydia Gibson for collecting a subset of neuronal recordings; Sebastian Moeller for sharing the design of an MRI-compatible drive and many valuable suggestions; Hansjörg Scherberger for valuable discussions and suggestions regarding target selection equalization; Stefan Treue, Alexander Gail, members of the Decision and Awareness Group and the Cognitive Neuroscience Laboratory for helpful discussions; Ira Panolias, Sina Plümer, Klaus Heisig, Dirk Prüße and Ralf Brockhausen for technical support. We also thank two anonymous reviewers for their fast, valuable and constructive comments.

Abstract

The pulvinar complex is extensively interconnected with brain regions involved in spatial processing and eye movement control. Recent inactivation studies have shown that the dorsal pulvinar plays a role in saccade target selection. However, it remains unknown whether it exerts effects on visual processing or at planning/execution stages. We employed electrical microstimulation of the dorsal pulvinar while monkeys performed saccade tasks towards instructed and freely-chosen targets. Timing of stimulation was varied, starting before, at, or after onset of target(s). Stimulation affected saccade properties and target selection in a time-dependent manner. Stimulation starting before but overlapping with target onset shortened saccadic reaction times for ipsiversive (to the stimulation site) target locations; while stimulation starting at and after target onset caused systematic delays for both ipsiversive and contraversive locations. Similarly, stimulation starting before onset of bilateral targets increased ipsiversive target choices, while stimulation after target onset increased contraversive choices. Properties of dorsal pulvinar neurons and stimulation effects were consistent with an overall contraversive drive, with varying outcomes contingent upon behavioral demands. Reaction time and choice effects were largely congruent in the visually-guided task, but stimulation during memory-guided saccades, while influencing reaction times and errors, did not affect choice behavior. Taken together, these results show that the dorsal pulvinar plays a primary role in action planning as opposed to visual processing, that it exerts its strongest influence on spatial choices when decision and action are temporally close, and that this choice effect can be dissociated from motor effects on saccade initiation and execution.

Key words: pulvinar; microstimulation; decision-making; choice; saccades; electrophysiology

Significance

Despite a recent surge of interest, the core function of the pulvinar, the largest thalamic complex in primates, remains elusive. This understanding is crucial given the central role of the pulvinar in current

theories of integrative brain functions supporting cognition and goal-directed behaviors, but electrophysiological and causal interference studies of dorsal pulvinar are rare. Building on our previous studies that pharmacologically suppressed dorsal pulvinar activity for several hours, here we used transient electrical microstimulation at different periods while monkeys performed instructed and choice eye movement tasks, to determine time-specific contributions of pulvinar to saccade generation and decision-making. We show that stimulation effects depend on timing and behavioral state, and that effects on choices can be dissociated from motor effects.

Introduction

The ability to flexibly decide between response options is a crucial attribute of adaptive behavior. One fundamental component of this process is the guidance of eye movements exploring spatial locations of potential interest. Representations of diverse variables contributing to saccadic decisions have been found in many cortical and subcortical brain regions (Andersen and Cui, 2009; Shadlen and Kiani, 2013). Based on the extensive anatomical connectivity to those regions, the thalamic pulvinar has been suggested as a hub for the coordination of movements for goal-directed visually-guided behavior (Grieve et al., 2000; Wilke et al., 2010). In primates, the pulvinar forms the largest thalamic complex and can be coarsely subdivided into ventral and dorsal aspects (Kaas and Lyon, 2007; Preuss, 2007). The ventral aspect is retinotopically organized and is connected with striate and extrastriate visual cortices. The dorsal aspect does not seem to contain an orderly retinotopic topography and is reciprocally interconnected with areas that combine spatial attention and eye movement functions, such as parietal, superior temporal, posterior cingulate and prefrontal cortices (Gutierrez et al., 2000; Jones, 2012; Seltzer et al., 1996). Both ventral and dorsal pulvinar receive input from the superior colliculus (SC): the ventral pulvinar from the upper and the dorsal pulvinar from the lower and intermediate layers of the SC (Berman and Wurtz, 2011; Stepniewska, 2004). Thus, anatomical connectivity of the pulvinar suggests that it is involved in the selection and planning of eye movements, and spatial attention.

Converging evidence is also provided by electrophysiological and lesion/inactivation studies. Visually-responsive pulvinar neurons enhance firing for stimuli that are attended and/or are target of an upcoming saccade (Bender and Youakim, 2001; Petersen et al., 1985; Robinson and Petersen, 1992; Saalmann et al., 2012; Zhou et al., 2016). In addition, many pulvinar neurons exhibit saccade-related activity, including spatially-specific enhancement or suppression, associated with the onset of the visual target and/or onset or offset of the saccade (Berman and Wurtz, 2011; Petersen et al., 1985; Robinson et al., 1986, 1990). Studies of neural responses in eye movements tasks in the non-retinotopic, dorsal part of the pulvinar are particularly sparse, but suggest a diversity of saccade-related properties, with neurons exhibiting spatially un-tuned or direction-dependent peri- and/or post-saccadic discharges (Benevento and Port, 1995; Robinson et al., 1986). Some medial dorsal pulvinar neurons have two peak responses, one closely following the onset of the visual target and the other triggered to the onset or offset of the saccade (Benevento and Port, 1995).

Pulvinar lesions in humans or monkeys do not result in primary visual or saccade generation deficits (Bender and Baizer, 1990; Bender and Butter, 1987; Van der Stigchel et al., 2010; Wilke et al., 2010, 2013), although modest lesion-induced increase of contralesional saccade latencies has been reported (Rafal et al., 2004; Wilke et al., 2013). More pronounced are "higher-order" spatial attention and decision-making impairments (Robinson and Petersen, 1992; Saalmann and Kastner, 2011). Specifically, structural and reversible lesions in the ventral and/or dorsal pulvinar impair the ability to shift visual attention towards the contralesional hemifield, and result in an ipsilesional spatial exploration and saccade choice bias (Arend et al., 2008; Karnath et al., 2002; Rafal and Posner, 1987; Snow et al., 2009; Wilke et al., 2010, 2013; Zhou et al., 2016). While those lesion/inactivation studies provide strong evidence that normal pulvinar functioning is crucial for the selection of saccade goals in the presence of competing targets, they cannot resolve at which processing stage pulvinar exerts its effect on saccade behavior.

The aim of the current study was to investigate putative pulvinar-driven interactions between target selection and saccade generation in a temporally-specific manner. To this end, we applied transient

electrical microstimulation in the pulvinar while macaque monkeys performed visually- or memory-guided saccades to single (instructed) targets or chose between two targets in opposite hemifields. Crucially, we varied the timing of microstimulation, starting it before, at, or after onset of the saccade target(s). Our results demonstrate a temporal-specific impact of the pulvinar on spatial choices and saccade generation, further elucidating its involvement in goal-directed behaviors.

Materials and Methods

All experimental procedures were conducted in accordance with the European Directive 2010/63/EU, the corresponding German law governing animal welfare, and German Primate Center institutional guidelines. The procedures were approved by the responsible government agency (LAVES, Oldenburg, Germany).

Animal preparation

Two adult male rhesus monkeys (*Macaca mulatta*) C and L weighing 8 and 9 kg respectively, were used. In an initial surgery monkeys were implanted with a magnetic resonance imaging (MRI) compatible polyetheretherketone (PEEK) headpost embedded in a bone cement headcap (Palacos with Gentamicin, BioMet, USA) anchored by ceramic screws (Rogue Research, Canada), under general anesthesia and aseptic conditions. MR-visible markers were embedded in the headcap to aid the planning of the chamber in stereotaxic space (Monkey C, right hemisphere: center at 0.5A / 14.5L mm, tilted -11P / 27L degrees, Monkey L, right hemisphere: center at -3.12P / 20.2L mm, tilted: -18P / 37L degrees) with the MR-guided stereotaxic navigation software Planner (Ohayon and Tsao, 2012). A separate surgery was performed to implant a PEEK MRI-compatible chamber (inside diameter 22 mm) allowing access to the right pulvinar. After confirming chamber positioning with a post-surgical MRI, a partial craniotomy was made inside the chamber. The exposed dura was covered with a silicone elastomer (Kwik-sil, World Precision Instruments, USA) to reduce the granulation tissue growth and dura thickening.

MRI imaging

Monkeys were scanned in a 3T MRI scanner (Siemens Magnetom TIM Trio). Full-head T1-weighted scans (3D magnetization-prepared rapid gradient-echo, MPRAGE, 0.5 mm isometric) were acquired before and after chamber implantation, in awake (monkey C) or anaesthetized (monkey L) state, using either built-in gradient body transmit coil and custom single loop receive coil, or custom single loop transmit and 4-channel receive coil (Windmiller Kolster Scientific, USA).

In addition to pre- and post-implantation scans, similar T1-weighted scans as well as T2-weighted (rapid acquisition with relaxation enhancement, RARE, 0.25 mm in plane, 1 mm slice thickness) scans were periodically acquired during the course of experiments, either in awake (monkey C) or sedated (monkey L) state, to confirm electrode positioning. T1- and T2-weighted scans were co-registered and transformed into "chamber normal" (aligned to the chamber vertical axis) and to AP-PC space for electrode targeting and visualization. These images were acquired with the chamber and the grid filled with gadolinium (Magnevist, Bayer, Germany)/saline solution (proportion 1:200), with tungsten rods inserted in predefined grid locations, for alignment purposes.

Pulvinar targeting

The location of the electrode was estimated for every stimulation site based on anatomical MRI. Custom-made MR-compatible polyetherimide (Ultem) grids (0.8 mm hole spacing, 0.45 mm hole diameter) and custom-made plastic XYZ manipulator drives (design courtesy of Dr. Sebastian Moeller, (Moeller et al., 2008)) were used to position platinum-iridium electrodes (FHC Inc., USA, see detailed specs in the next section) in the corresponding grid hole and estimated depth. During the penetration, the electrode was protected by a custom-made MRI-compatible fused silica guide tube (320 µm inner diameter, 430 µm outer diameter, Polymicro Technologies, USA), or a custom-made stainless steel guide tube (450 µm outer diameter, 27 gauge Spinocan, Braun Melsungen AG, Germany). A stopper (530 µm inner diameter, 665 µm outer diameter, 23 gauge MicroFil, World Precision Instruments, USA) ensured that the guide

158 tube only penetrated the dura and minimally the cortex below. Prior to penetration, the electrode tip was
159 aligned to the guide tube tip and was held in place by a drop of melted Vaseline. The guide tube was filled
160 with sterile silicone oil prior to electrode insertion, to ensure smooth electrode travel and to prevent
161 backflow of cerebrospinal fluid.

162 There are multiple parcellation schemes available for the pulvinar (Jones, 2012; Stepniewska, 2004).
163 Here, the pulvinar was divided into dorsal (dPul) and ventral (vPul) aspects using the brachium of
164 superior colliculus as a landmark, as has been done in several studies (Gutierrez et al., 2000; Komura et
165 al., 2013; Wilke et al., 2010). The dorsal pulvinar (dPul) includes medial pulvinar and dorsal part of
166 lateral pulvinar (also denoted as PLdm, or Pdm in earlier papers by Robinson, Petersen and colleagues,
167 (Robinson and Petersen, 1992)), whereas ventral pulvinar contains inferior pulvinar and ventral part of
168 lateral pulvinar (also denoted as PLvl) (Kaas and Lyon, 2007; Robinson et al., 1986). Since currently
169 available online and downloadable atlases use the traditional scheme segregating medial (MPul), lateral
170 (LPul) and inferior (IPul) (and sometimes anterior/oral) nuclei (Calabrese et al., 2015; Rohlfing et al.,
171 2012), we adopted this scheme for the localization of stimulation and recording sites.

172 As can be seen in **Figure 1**, the stimulation sites in the main experiment corresponded to the dorsal
173 pulvinar, mostly to the medial part (MPul) but were also close to the dorsal aspect of the lateral part
174 (LPul). The brachium of the superior colliculus (bsc) and other neighboring structures such as reticular
175 thalamic nucleus and tail of the caudate nucleus were avoided.

176 ## Electrical microstimulation

177 An S88X dual output square pulse stimulator (Grass Products, Natus Neurology, USA) triggered by a
178 MATLAB-based task controller generated 200 ms trains of twin pulses at 300 Hz, which in turn triggered
179 a constant current stimulus isolator A365 (World Precision Instruments, USA) to produce 60 biphasic
180 pulses. The current (100-300 μA, see below) was delivered using single monopolar electrodes (100 mm
181 length platinum-iridium 125 μm thick core, initial 2 cm glass-coated with an exposed tip of 40 μm, total

thickness of 230 μm including polyamide tubing coating, UEPLEFSS (UEIK1), FHC Inc., USA); a return (reference) tungsten rod was placed in the chamber filled with saline. Voltage drop across a 10 kΩ resistor in series with the electrode was monitored using a 4 channel 1GS/s Tektronix TDS2004C oscilloscope.

The manufacturer-specified impedance of electrodes was 300-500 kΩ; the initial impedance measured at 1000 Hz before the experiment was 360-1300 kΩ. Since the impedance dropped dramatically after a few stimulation trains were applied, before each session 10 trains were delivered to the electrode immersed in saline using 300 μA current, in order to bring the electrode impedance to a more stable regime. Following this procedure, the impedance ranged from 19 kΩ to 200 kΩ for electrodes used in monkey C and 11 kΩ to 100 kΩ in electrodes used in monkey L (**Table 1**).

Electrophysiological recordings

In 19 sessions in monkey C and 28 sessions in monkey L right dorsal pulvinar neuronal activity was recorded with up to three individually-movable single platinum-tungsten (95%-5%) quartz glass-insulated electrodes with impedance ranging from 1 MΩ to 1.9 MΩ for monkey C and 1.3 MΩ to 3.5 MΩ for monkey L, using a chamber-mounted 5-channel Mini Matrix microdrive (Thomas Recording, Germany). The recording target locations were estimated similarly to the stimulation sessions, using the same grids. Similar to microstimulation experiments, single custom-made stainless steel guide tubes (27 gauge) filled with the silicone oil (Thomas Recording), with a Spinocan funnel attached to the drive nozzle were used to protect electrodes during dura penetration. A reference tungsten rod or a silver wire were placed in the chamber filled with saline, and were connected to the chassis of the drive. Neuronal signals were amplified (x20 headstage, Thomas Recording; x5, 128 or 32 channel PZ2 preamplifier, Tucker-Davis Technologies, USA), digitized at 24 kHz and 16 bit resolution, and sent via fiber optics to an RZ2 BioAmp Processor (Tucker-Davis Technologies, USA) for online filtering, display and storage on a hard drive together with behavioral and timing data streams.

Behavioral tasks

Monkeys sat in a dark room in custom-made primate chairs with their heads restrained 30 cm away from a 27'' LED display (60 Hz refresh rate, model HN274H, Acer Inc. USA). The gaze position of the right eye was monitored at 220 Hz using a MCU02 ViewPoint infrared eyetracker (Arrington Research Inc. USA). Monkey face and body were monitored with infrared cameras, to ensure that microstimulation did not elicit abrupt movements or signs of discomfort. All stimulus presentation and behavioral control tasks were programmed in MATLAB (The MathWorks, Inc. USA) and the Psychophysics Toolbox (Brainard, 1997).

Fixation task and evoked saccades

In each microstimulation session before the main visually-guided saccade task (see below) five to six blocks of 20 fixation trials (**Fig. 2A**) were performed to determine the presence/absence of evoked saccades as a consequence of electrical stimulation. A dim red spot of 1° diameter (luminance 9.4 cd/m^2) appeared in the center of the monitor (0.16 cd/m^2). Once the monkeys directed their gaze into a 5° radius window surrounding the fixation spot it became brighter (33 cd/m^2) to signal fixation acquisition. Monkeys were required to maintain their gaze position for a randomized period ranging from 1000 ms to 1300 ms to successfully complete a trial before receiving liquid reward. The inter-trial interval was 1000 ms to 2000 ms. In half of the trials of each block a stimulation was applied, starting 500 ms after fixation was acquired. Each session started with a block of 100 μA and in each subsequent block current was increased by 50 μA until the 300 μA limit was reached. This range of currents was selected to match related ongoing fMRI/microstimulation experiments in our lab. The presence or absence of evoked saccades in a given block was assessed by online monitoring and all sessions were characterized offline (see below). If no evoked saccades were observed with any of the currents the following tasks were performed with 250 μA, else the current was set to 50 μA below the lowest intensity that evoked saccades. If all current strengths evoked saccades and according to our MRI-based estimates after moving the electrode it still would be within 1 mm of the targeted pulvinar nucleus borders, the electrode was

moved by 0.5 mm or 1 mm up or down, and five blocks of fixation trials were run again (10 out of 15 sessions in monkey C, 0 out of 15 in monkey L). Alternatively, the highest current that did not evoke more saccades than the 100 μA current was used (2 out of 15 sessions in monkey C, 0 out of 15 in monkey L). In 5 out of 15 sessions in monkey C the electrode was moved and the current was lowered below 250 μA even after moving the electrode.

The offline analysis confirmed online observations. When data from all fixation trials was combined, Monkey L did not show any difference in amount of saccades during stimulation as compared to the same period during control trials (4% and 4%, respectively, 2% contraversive and 2% ipsiversive in each case). Monkey C, who incidentally had more frequent "fixational" saccades within the 5° radius fixation window even in control trials, exhibited predominantly contraversive saccades during the stimulation period (**Fig. 2C**; 60% of stimulation trials, 57% contraversive, 3% ipsiversive; 32% of control trials in the corresponding period, 22% contraversive, 10% ipsiversive). Contraversive saccades were typically followed by ipsiversive saccades (69%) within up to 200 ms after stimulation offset. Since the monkey was required to maintain fixation during the stimulation and these ipsiversive saccades were directed back to the fixation spot, we call them "return" saccades (82% of return saccades were preceded by contraversive ones). For further analysis, we classified as evoked saccades only contraversive saccades during the stimulation period that were followed by return saccades. We normalized the probabilities of evoking saccades per current strength for each site by subtracting the mean values for each site, and found that the normalized probability of evoking saccades correlated with the current strength (Spearman's $R = 0.38$, $p<0.001$). A similar analysis for evoked saccade amplitudes also showed a positive correlation with the current strength (Spearman's $R = 0.31$, $p=0.009$). Across all sites that were later used in the main experiment, and across all tested currents, the probability of evoking saccades in monkey C was 39% (40.5% for the currents selected for the main experiment, **Table 1**; for comparison, only 2% of control trials would have been classified as "evoked" using the above approach). The amplitude of evoked saccades was 1.51 ± 0.16° (mean ± SE, standard error of mean) across sites (1.9 ± 1°, mean ± SD,

standard deviation across trials), with a latency of 95 ± 39 ms after stimulation onset. Similar effects (increased probability of contraversive movements during stimulation period) were observed in several sessions where we delivered the stimulation during free-gaze exploration (*cf.* Goldberg et al., 1986; Watanabe and Munoz, 2013), with the exception of not observing ipsiversive return saccades. Our observations are in line with lateral posterior nucleus/pulvinar microstimulation studies in cats, which reported either absence of evoked saccades (Maldonado et al., 1980) or contraversive saccades with current strengths between 50-300 μA (Crommelinck et al., 1977).

Although evoked saccades were present only in some sites, with less than 50% probability, and only in one monkey, we briefly address relevant methodological considerations. Due to small amplitudes, the visual and positional consequences of these saccades are expected to be relatively minor (Carello and Krauzlis, 2004), although we cannot exclude the possibility of perceptual/attentional effects similar to consequences caused by fixational saccades (Hafed et al., 2015). Given the 24° target eccentricity in the saccade tasks (see later), these displacements were not enough to land the gaze within the target window, and they did not seem to affect the ensuing choice. For example, during choice trials, when a small contraversive shift was apparent in the online display (and later during inspection of trial eye position traces), as often as not, the monkey would go on to select the ipsiversive target even though his gaze was already closer to the contraversive target.

At those sites where microstimulation evoked small saccades, required current strength was considerably higher than reported for superior colliculus (SC), caudate nucleus or frontal eye fields (FEF) (e.g. Robinson and Fuchs, 1969; Tehovnik et al., 1999; Yamamoto et al., 2012). Instead, the range of evoked saccade thresholds between 100-300 μA was more similar to required currents in visuomotor regions such as posterior parietal cortex (Shibutani et al., 1984; Thier and Andersen, 1996) and dorsomedial frontal cortex (DMFC) (Tehovnik et al., 1999).

Visually-guided saccade task

A trial started with the onset of the fixation spot. After monkey acquired and held fixation within a 5° radius for a randomized period ranging from 400 ms to 700 ms, the fixation spot (1° diameter) was extinguished and either one target (instructed trials) or two targets (choice trials) appeared simultaneously (**Fig. 3A**). This time point will be referred to as the "Go signal". Targets (1° diameter) were presented in the left and/or right side(s) of the fixation spot, at 24° eccentricity, with three potential angles relative to the horizontal axis, 0°, 20° or -20° (0°, 8.2° and -8.2° vertical eccentricity). Monkeys had to make a saccade within 500 ms and keep their gaze position for 500 ms inside a 5° radius window surrounding the target to successfully complete a trial and obtain a liquid reward after a delay of 200 ms. In choice trials monkeys were allowed to freely choose one of the targets; both choice targets were always presented at the same height and provided equal reward. The inter-trial interval for both successful and unsuccessful trials was 1000 ms or 2000 ms. In seven out of eight trials a 200 ms stimulation train was applied at one out of seven different periods in both instructed and choice trials. The trains started either before the Go signal (-120 ms, -80 ms, or -40 ms; early stimulation periods), simultaneously with the Go signal, or after the Go signal (+40 ms, +80 ms, or +120 ms; late stimulation periods). Note that since the train duration was 200 ms, stimulation always ended after the Go signal. All trial types, target locations, and stimulation conditions were pseudo-randomized. A minimum of 15 instructed trials per stimulation period and per hemifield were collected in each session (except in one session where there was a minimum of 13 trials for the left hemifield and a session with 14 trials for the right hemifield).

Memory-guided saccade task

Similarly to the visually-guided saccade task monkeys had to acquire and hold fixation for 400-700 ms. Next, one or two peripheral cues were displayed for 280 ms at the location(s) signaling the upcoming saccade target(s). These cues had the same spatial characteristics as the targets in the visually-guided task. Monkeys were required to maintain fixation throughout the cue period and also throughout the subsequent memory period (ranging from 200 to 400 ms), after which the central fixation spot disappeared (Go signal), allowing monkeys to saccade to the instructed location, or make a decision to go to one of the two

cued locations. After the saccade to and fixation of the remembered target location for 100 ms to 200 ms the target became visible and after additional 500 ms of peripheral fixation the trial was completed. We applied stimulation in four out of five trials in one out of four periods starting before or after the cue onset (-80 ms, +80 ms), or before or after the Go signal (-80 ms, +80 ms).

Target selection equalization

During training we consistently observed a strong selection bias to the right side of space in choice trials, in both monkeys. This bias was potentially due of the fact that both monkeys were initially trained to perform reaches with their preferred right arm in the context of another experiment, in which they might have developed a strong rightward bias. To be able to assess potential target selection changes in both directions due to stimulation we used a method similar to Scherberger and colleagues (Scherberger et al., 2003) to equalize the control target selection by shifting the entire stimulus array horizontally toward the preferred right hemifield without modifying the 24° eccentricity from the fixation spot to the targets. The mean shift across visually-guided task sessions with stimulation in dorsal pulvinar was 3.2 ± 0.8° for monkey C and 4.4 ± 0.6° for monkey L, to the right (mean ± SE, **Table 1**). These shifts resulted in the 44 ± 3% and 40 ± 6% leftward selection in pre-stimulation runs that were used for the equalization procedure (monkeys C and L, respectively, mean ± SE, **Table 1**). However, during the actual stimulation experiment the leftward (contraversive) selection dropped to 29 ± 5% and 26 ± 5% in non-stimulation trials (monkeys C and L, respectively). The same target positions were used for instructed trials.

Summary of the course of a session

After advancing the electrode to the desired location, the fixation task (with the fixation spot always in the center of the screen) was used to test for evoked saccades. This procedure defined the final electrode depth and the current strength. Next, a visually-guided saccade task was performed without stimulation, and the stimulus array was shifted to find a regime in which the left and right target selection was approximately equalized (see “Target selection equalization” section above). After that the control and stimulation data for the main visually-guided task was collected. For sessions in which monkeys also

performed the memory-guided saccade task, target selection was equalized independently for the memory task, because target preference differed between the two tasks.

Data analysis

Saccade definitions

Saccade velocity was calculated sample by sample as the square root of the sum of squared interpolated (220 Hz to 1 kHz) and smoothed (12 ms moving average rectangular window) horizontal and vertical eye position traces, and then smoothed again (12 ms moving average rectangular window). Saccade onset was defined as eye position change that exceeded a starting velocity threshold and the saccade offset as reaching an ending velocity threshold. For the fixation task the starting and ending thresholds were 30°/s and 15°/s, respectively. For visually-guided and memory-guided saccades, the starting velocity of 300°/s and the ending velocity of 50°/s were used. For retrieving the saccade directions in error trials the starting threshold was lowered to 150°/s, because eye position was not recorded after fixation breaks, and thus in some cases the recorded velocity did not reach a high enough value before the trial and the recording were aborted. Saccade endpoint was defined as the eye position when the saccade velocity reached the ending threshold. In cases when several consecutive eye movements in the time interval from the Go signal until the target acquisition fitted the above criteria (e.g. due to interrupted saccades, see **Results**), the first saccade was selected for the reaction time analysis and the last one for the end-point accuracy/precision analysis.

Statistical analysis of behavioral data

All data analysis was performed using MATLAB R2012b. To test for changes in target selection preference within each session, and the hit rates, Fisher's exact test was used. For all comparisons between two conditions across sessions, non-parametric tests were used. Whenever possible (i.e. same experimental conditions/outcomes present in all stimulation periods and in all sessions), paired Friedman test with post-hoc Wilcoxon signed-rank tests were used. Otherwise, Kruskal-Wallis test with post-hoc

Mann-Whitney-U tests were used. Because the effects of multiple stimulation periods were tested against the control condition, for all post-hoc tests, and for Fisher's exact tests, the Bonferroni method was used to correct for multiple comparisons. To test for the relationship between two variables across sessions or across stimulation periods, Spearman's correlation coefficients were used. Statistical significance was reported at $p<0.05$ (*) and $p<0.01$ (**). Specific statistical tests are listed for each individual analysis. In the figures and in the text, standard deviation (SD) was used when averaging across trials and standard error of mean (SE) when averaging across sessions.

Analysis of neuronal activity

In the data from both monkeys, 230 single and multi-units for the visually-guided saccade task (140 monkey C, 90 monkey L) and 365 units for the memory-guided saccade task (251 monkey C, 114 monkey L) fulfilled analysis selection criteria (at least 50 spikes during the task periods; at least 60 instructed trials; typically 120 instructed trials, 10 instructed trials for each of the 12 targets). For recordings, the fixation hold period was 500 ms, the memory period 1000 ms, ITI period 1000 ms; other parameters were same as in the stimulation runs. Target eccentricities were 12° and 24°, arranged along the horizontal axis or at ±20° angle from the horizontal axis. Spike sorting was done using Offline Sorter v.4.0.0 and v.2.8.8 (Plexon, USA) for monkeys C and L respectively, using either a waveform template algorithm or a principle component analysis with k-means clustering algorithm.

For each trial, and each epoch of interest, firing rates were computed by counting the spikes within the epoch and dividing the count by the epoch duration. The epochs analyzed in the visually-guided saccade task were "inter-trial interval" (400 ms to 100 ms before the onset of the central fixation spot), "fixation acquisition" (50 ms to 150 ms after acquiring central fixation), "fixation hold" (last 300 ms of central fixation), "target onset" (50 ms to 150 ms after target onset), "pre-saccadic" (100 to 10 ms before saccade onset), "peri-saccadic" (10 ms before to 50 ms after saccade onset), "target acquisition" (50 ms to 120 ms after acquiring target fixation) and "target hold" (last 300 ms of fixating the peripheral target). For the memory-guided saccade task, "cue onset" (50 ms to 150 ms after onset of the cue) replaced the "target

onset“ – both will be referred to as “stimulus onset”, and “target hold invisible” (first 100 ms of fixating the invisible peripheral target) replaced “target acquisition”. Two additional epochs were also analyzed: “early memory” (first 200 ms of the memory period) and “late memory” (last 300 ms of the memory period).

For population analysis, data from 6 left and 6 right hemifield targets were combined. For each unit, a two-way ANOVA was performed across all firing rates in each of the respective epochs from successful instructed trials (same criteria as in “Behavioral tasks” section), using hemifield of the target position and epoch as factors for determining a main effect of epoch, hemifield, and interaction between the two. Spatial tuning in each epoch was determined by unpaired t-tests comparing firing rates in ipsilateral trials to firing rates in contralateral trials. The hemifield with the higher firing rate was marked, if there was a significant difference. This analysis was performed only on units that showed either a main effect of hemifield or [hemifield × epoch] interaction.

Enhancement or suppression of neuronal activity (relative to fixation baseline, “fixation hold” epoch) in each subsequent epoch was defined by paired t-tests comparing firing rates, for ipsilateral and contralateral trials independently. This analysis was only performed on units that showed either a main effect of epoch or [hemifield × epoch] interaction. Enhancement or suppression was reported, if either ipsilateral, contralateral, or both types of trials showed significant difference to fixation baseline. In rare cases in which one hemifield would show a significant enhancement, while the other hemifield showed suppression, the unit was reported to have bidirectional response (example unit counts, memory-guided task, monkey C / monkey L: cue 2/1, peri-saccade 2/0, target hold: 6/1; visually-guided task, monkey C / monkey L: target onset: 2/0, peri-saccade: 3/2, target hold: 4/4).

For response field (RF) estimation, an independent one-way ANOVA was performed on firing rates during the stimulus onset epoch for each unit to determine the effect of target position. For defining a hemifield preference, the hemifield with the higher firing rate was marked if there was an effect of target

position. For all units that showed an effect of target position, response modulation depth for each target position was calculated by averaging firing rates across trials, subtracting the lowest average firing rate across positions, and converting to percentage of maximal modulation depth. To estimate center and size of the RFs, a 2D Gaussian was fitted to the modulation depth pattern. Six fitting parameters were determined using an iterative least squares method (400 iterations), allowing elliptic RFs with peaks at the center. The size of the RF was defined by two standard deviations in each direction (semi-minor and semi-major axes). The fitting parameters were (1) the modulation depth in the center of the RF, (2) horizontal and (3) vertical location of the RF center, (4 and 5) ellipse major axis defined by 4 standard deviations, and minor axis by aspect ratio, and (6) an angle of ellipse rotation. Importantly, the RF center was always kept within the dimensions of the target array (-24° to +24° horizontally and -8.2° to +8.2° vertically). The amplitude was bounded by 50% and 150% of the original modulation depth, ellipse axes were bounded by 12° (maximum horizontal distance between targets) and 48° (target array extent), and maximum aspect ratio 4:1. Maximum modulation depth, the average of modulation-depth-weighted target positions (RF "center of mass"), intermediate major/minor axes (30°/15°), and a rotation of 0° were used as starting values for the fits.

An averaged radius (r) approximating the RF size was calculated by taking the square root of the product of the two axes of the elliptic RF. This way, r^2*pi always matches the area covered by the elliptic RF. RF size is reported as the diameter of the RF, 2*r.

For target hold and "stimulus onset" epochs (cue onset for memory-guide saccades and target onset for visually-guided saccades) contralateral tuning indexes (CI) for each unit were calculated as: $CI = (FR_{contra} - FR_{ipsi}) / (FR_{contra} + FR_{ipsi})$, where FR_{contra} and FR_{ipsi} are the average firing rate for all trials with targets in the contralateral and ipsilateral hemifield. Positive indexes indicate contralateral preference, and negative indexes indicate ipsilateral preference.

To calculate population PSTHs, spike density functions of each trial, derived by convolution of the discrete spike arrival times with a Gaussian kernel (SD 20 ms) were baseline-corrected by subtracting the average ongoing firing rate in the late period of the inter-trial interval that immediately preceded the trial start (fixation spot onset). Average responses for each unit were then derived by averaging the baseline-corrected spike density for each unit across all trials for the respective condition. Mean and SE of these baseline-corrected and averaged spike densities across units of a given sub-population were calculated to display population responses. For better visualization of target position-dependent population cue response, instead of subtracting a baseline, the response was normalized by dividing each unit's response in all conditions by the same factor. That factor was defined as the peak firing rate during the cue onset epoch calculated across all trials (regardless of target position) in the preferred hemifield.

Results

Using an MRI-guided approach (**Materials and Methods**) we stimulated the right dorsal pulvinar (dPul, **Fig. 1**, **Table 1**) and control sites in the ventral pulvinar (vPul, see later section) in two monkeys performing three oculomotor tasks: fixation, visually-guided and memory-guided saccades (to instructed or chosen locations). The fixation task (**Fig. 2A**) was used to test for occurrence of evoked saccades, and to characterize them if present. Monkey L did not exhibit evoked saccades in the regime tested; monkey C showed predominantly small (<2º) contraversive saccades at 95 ± 39 ms after the stimulation onset (**Fig. 2C**, **Materials and Methods**). The visually-guided task was used in the main experiment; and the memory-guided task was used as a control for dissociating cue processing, motor planning and execution phases (see later). Both saccade tasks included 50% single target instructed trials, and 50% choice trials between two equally rewarded targets, located equidistantly from the central fixation spot at the same height.

Visually-guided task: time-dependent reaction time facilitation and delay

In the visually-guided saccade task (**Materials and Methods**), in stimulation trials a 200 ms train was delivered at different periods relative to the target(s) onset and synchronous fixation spot offset, referred to as "Go" signal: before "Go" (*early* periods, blue-cyan colors), at "Go", or after "Go" (*late* periods, green-orange colors) (**Fig. 3A**). All trial conditions – instructed/choice, contraversive/ipsiversive in respect to the stimulated right hemisphere (left hemifield – contraversive, right hemifield – ipsiversive), stimulation/no stimulation, and different stimulation periods – were randomly interleaved.

In instructed trials (single targets), the stimulation did not affect the hit rate (the fraction of successfully completed trials), which remained consistently high (**Table 2**). However, stimulation caused mildly hypometric saccades for contraversive locations: saccades were still initiated in the correct direction but often undershot, resulting in reduced endpoint accuracy and increased scatter along the saccade trajectory axis, predominantly in late stimulation periods, during pre-movement and movement phases (**Fig. 3B**), similar to findings in SC (Schlag et al., 1989) and pre-SMA (Isoda, 2005).

The main effect of the stimulation on saccade performance consisted of changes in reaction times (RT). To illustrate this, we plotted the horizontal eye position as a function of time in the control (no stimulation) trials and in the different stimulation periods, for two target positions in two example sessions in each monkey (**Fig. 3C**). Three apparent effects of stimulation can be gleaned from these plots: 1) RT delay in most periods, with saccade onsets either stereotypically deferred until after the stimulation offset (upper example in each monkey), or delayed yet initiated during the stimulation (lower example in each monkey), for saccades in both directions; 2) RT *facilitation* for ipsiversive saccades in early stimulation periods; and 3) occurrence of interrupted saccades (movement stopping in the mid-fly) in the late stimulation periods, especially for the contraversive targets.

Figure 4 quantifies RT effects across sessions. In control trials (gray), both monkeys had comparable RTs with unimodal distributions. The RT distributions for different stimulation periods confirmed that the stimulation predominately delayed the saccade initiation (**Fig. 4A**, upper panels in each monkey). The

effect reached significance in a large proportion of individual sessions (**Fig. 4A**, lower panels in each monkey). Two distinct modes were evident upon inspection of RT distributions in Go and late period stimulation trials. As illustrated in examples shown in **Figure 3C**, the first mode contained saccades that started *during* the stimulation train, the second mode included saccades that started *after* the stimulation offset. Both effects were present in both monkeys, although monkey L had fewer sessions where the second mode was evident, especially for contraversive targets (+40 ms period: 3 sessions in monkey L, 14 sessions in monkey C; +80 ms period: 1 session in monkey L, 9 sessions in monkey C). Interestingly, there was a correlation between the depth of the microstimulation site and the probability of ipsiversive deferred saccades in both monkeys, suggesting that the occurrence of deferred saccades is site specific (but not monkey specific). In the subsequent analysis, we separated the saccades into these two categories ("during stimulation", "after stimulation") and calculated a mean RT for each stimulation period across trials in each session, and then across sessions (**Fig. 4B,C**). **Figure 4B** plots the data separately for each monkey, and for each vertical target position, demonstrating the consistency of RT effects. Monkey L showed weaker delays for saccades that started during stimulation (**Fig. 4B**, top row), especially for contraversive instructed trials, but even in his data the delay was significant across sessions (contraversive instructed: +40 ms simulation period, $p<0.05$; ipsiversive instructed: Go, +40 ms, +80 ms stimulation periods, $p<0.01$; Kruskal-Wallis followed by Bonferroni-corrected Mann-Whitney-U test). Sessions with saccades that were deferred until after stimulation offset were also present in both monkeys (**Fig. 4B**, bottom row). Therefore, in the **Figure 4C**, we combined data from both monkeys.

Across all vertical target positions, saccades that started during stimulation were delayed by 10 ms to 26 ms (min to max) in the Go and late stimulation periods, with a maximal delay occurring in the +40 ms or +80 ms stimulation periods. The saccades whose onsets were deferred until the end of the stimulation were initiated 35 ± 2 ms (contraversive) and 42 ± 2 ms (ipsiversive) following the stimulation offset (37 ± 2 ms and 48 ± 1 ms in monkey C and 30 ± 6 ms and 33 ± 3 ms in monkey L).

The main difference between the effects in the two visual hemifields was the RT *facilitation,* present only for *ipsiversive* saccades in early stimulation periods (-120 ms and to a lesser extent -80 ms), which all fell in the "after stimulation" category (**Fig. 4C**). This ipsiversive facilitation (16 ± 3 ms in the -120 ms period) was evident in the RT distributions (*cf.* gray and blue distributions), was significant in 14 out of 15 sessions in monkey C and in 8 out of 15 sessions in monkey L, and was significant in each monkey across sessions ($p<0.01$ monkey C, $p<0.05$ monkey L, Friedman test with post-hoc Wilcoxon signed-rank test). Another difference between the effects in the two hemifields was that while RT delays followed a similar pattern for contraversive and ipsiversive saccades, the effect was stronger for the ipsiversive side (individually in each monkey, $p<0.05$ for all late stimulation periods, Friedman test with post-hoc Wilcoxon signed-rank test, Bonferroni corrected). For example, in the +80 ms stimulation period, the delay was 46 ± 10 ms for contraversive and 83 ± 11 ms for ipsiversive saccades, $p<0.01$). This observation will be considered when looking at the choice behavior (see later).

The relationship of RT delays between contraversive and ipsiversive saccades is further illustrated by the scatter plot of ipsiversive vs. contraversive delays in the +80 ms stimulation period (RT difference, stimulation minus control) across sessions, showing a strong correlation between the two delays (**Fig. 5A**; Spearman's $R=0.79$, $p<0.01$). No significant contraversive-ipsiversive correlation was found for RT effects at -120 ms stimulation period.

We also tested if there was a relationship between facilitation and delay effects across sessions, in two representative early (-120 ms) and late (+80 ms) stimulation periods, for ipsiversive saccades that showed both effects. Indeed there was a strong correlation between the facilitation and the delay (**Fig. 5B**; Spearman's $R = -0.52$, $p<0.01$). Sessions that showed *more* facilitation in the early stimulation period also had *more* delay in the late stimulation period, indicating a shared influence of session-by-session variations in stimulation effectiveness. This relationship is in contrast to the opposite effect (less facilitation, more delay) found in the caudate nucleus (Watanabe and Munoz, 2011).

Finally, very similar effects on saccadic reaction times were found for choice trials, including the facilitation of ipsiversive saccades in early stimulation periods (**Fig. 4**), and a larger delay for ipsiversive choices compared to contraversive choices (+80 ms stimulation period: 55 ± 10 ms contraversive, 83 ± 12 ms ipsiversive, $p<0.01$, Kruskal-Wallis with post-hoc Mann-Whitney-U test, Bonferroni corrected).

Visually-guided task: time-dependent spatial choice modulation

In agreement with predictions from our previous pulvinar inactivation results (Wilke et al., 2010, 2013), dPul stimulation increased contraversive target selection, but only in late stimulation periods, in which the train was delivered after the target onset / Go signal, during decision and motor preparation phase (**Fig. 6A**). Surprisingly, the stimulation in early periods, which started before but ended after the target onset, led to a decrease in contraversive selection (**Fig. 6A**). This biphasic modulation of spatial choice preference was a consistent pattern across sessions (**Fig. 6B,C**), showing maximal ipsiversive bias in the -120 ms or -80 ms periods and maximal contraversive bias in the +80 ms period. Furthermore, this pattern was consistent across upper, horizontal, and lower vertical target positions (**Fig. 6D**).

Given the resemblance of the choice effect to the modulation of ipsiversive reaction times (first facilitation, then delay), we evaluated if timecourses of changes in reaction times and target selection across stimulation periods were similar. To this end, we correlated the mean percent of contraversive selection with the mean ipsiversive choice reaction time, across stimulation periods (the ipsiversive choice RT was chosen to comprise both delay and facilitation RT effects), and found a strong linear correlation (Spearman's $R = 0.99$, $p<0.001$, monkey C, $R = 0.89$, $p=0.012$, monkey L; a similar effect was found for the correlation with ipsiversive instructed RTs: $R = 0.96$, $p=0.003$, monkey C; $R = 0.93$, $p<0.001$, monkey L). This demonstrates the temporal congruency of choice and reaction time effects: in early stimulation periods, the ipsiversive choice bias was accompanied by the (ipsiversive) RT facilitation, and as stimulation onsets progressed towards the later, decision and motor planning phases of a trial, the contraversive choice bias was accompanied by the RT delay.

To investigate whether ipsiversive bias in early stimulation periods and contraversive bias in late stimulation periods might represent manifestations of the same neural mechanism, we correlated the strength of both effects across sessions. For the early -120 ms period, we found only an insignificant tendency for a stronger ipsiversive bias to be associated with a weaker contraversive bias in the late +80 ms period (**Fig.7A**; Spearman's $R = 0.29$, $p = 0.12$). We also tested the -80 ms period instead of -120 ms period, and found a stronger positive correlation (Spearman's $R = 0.65$, $p < 0.01$). Note that the positive correlation signifies an *inverse* relationship between strength of early ipsiversive and late contraversive bias. Thus, at least on a session-by-session level the relationship between the strength of the two effects is not straightforward, suggesting that factors other than overall stimulation effectiveness, for example variations of session-specific spatial preferences, might play a role. Notably, this is the only aspect we found to be incongruent between directions of the across-sessions trends for RT and choice: recall that for the ipsiversive RT a stronger early facilitation was associated with a stronger, not weaker, late delay (*cf.* **Fig. 5B**).

It is important to emphasize however that most sessions exhibited a biphasic course of choice modulation (**Fig. 6C**), and in 5 sessions both effects reached significance even after the conservative Bonferroni correction for all 7 stimulation periods (**Fig. 7A**). Therefore the biphasic choice modulation illustrated in **Figure 6A** is not a consequence of averaging across sessions with either only early or only late period effects.

Similarly, to test the relationship between the choice bias and the reaction time effects, we correlated the stimulation-induced changes in the RT with the changes in choice preference across sessions. The increase of contraversive choices in the late +80 ms period correlated with the RT delay for ipsiversive instructed saccades, but not with the RT delay for contraversive instructed saccades (**Fig. 7B**, Spearman's $R = 0.15$, $p = 0.43$; $r = 0.48$, $p = 0.01$, for contraversive instructed and ipsiversive instructed RT, respectively). The same dependency was observed for choice RT delay ($r = 0.09$, $p = 0.64$; $r = 0.50$, $p < 0.01$, for contraversive choice and ipsiversive choice RT, respectively). On the other hand, the decrease

in contraversive choices in the early -120 ms period was only weakly and insignificantly correlated with the RT changes in both contraversive and ipsiversive instructed saccades. Thus, the contraversive choice bias in the late periods was associated with stronger, likely subjectively undesirable, ipsiversive delays. This reasoning is further supported by strong positive correlations between the contraversive selection increase vs. [ipsiversive – contraversive] RT difference in all but the two earliest stimulation periods (Spearman's $R>0.5$, $p<0.01$ for -40, Go, +40, and +120 periods, $R=0.44$, $p=0.02$ for +80 period).

We interpret the opposite direction effects in the early and the late stimulation periods as different manifestations of the same stimulation-induced mechanism. Decrease of contraversive choices and shortening of ipsiversive RTs suggest an ipsiversive orienting tendency due to the stimulation in the early periods starting before the Go signal. On the other hand, increase of contraversive choices and stronger ipsiversive RT delay in the late stimulation periods point to a contraversive drive. To reconcile these findings, we propose that the effect of pulvinar activation is invariably contraversive, and the apparent ipsiversive orienting is the consequence of a compensatory process that takes place due to behavioral task demands. In brief, when the stimulation is delivered in the early periods, while monkeys are tasked with maintaining fixation, they are (at least partially) suppressing, or opposing, the detrimental contraversive eye movements, and this ipsiversive push-back against the stimulation-induced contraversive drive "spills over" beyond the stimulation offset, to the interval after the Go signal when the decision and motor preparation take place. In agreement with this interpretation, but on a longer time scale across trials, in blocks of trials without stimulation the contraversive target selection was higher than in the control (no stimulation) trials interleaved with the stimulation trials during stimulation blocks, suggesting that monkeys exhibited an ipsiversive tendency when "released" from stimulation (blocked-interleaved difference $14.6 \pm 5.3\%$, $p<0.05$ for monkey C, $14.3 \pm 6.5\%$, $p<0.01$ for monkey L, mean ± SE, Wilcoxon signed rank test on differences). This and other alternative explanations are further considered in the **Discussion**.

Visually-guided task: dorsal vs. ventral pulvinar

The effects of dorsal pulvinar stimulation were robust and consistent across multiple sites in both monkeys. To test for the site specificity of those effects, we conducted a series of control stimulation experiments in the ventral pulvinar (vPul), targeting different depths along the electrode track (**Fig. 8A,B**). **Figure 8C** summarizes the results of the experiments as a function of electrode depth. The stimulation in shallow vPul sites, which correspond to the ventro-lateral pulvinar nucleus (PLvl) according to the parcellation of Kaas and colleagues (Kaas and Lyon, 2007), resembled the patterns obtained in the main dPul experiment, with the exception of bilateral (not only ipsiversive) RT facilitation and no clear ipsiversive choice bias in the early stimulation periods. Deeper sites ("medium vPul") at the estimated border between the ventro-lateral and inferior pulvinar showed similar but weaker stimulation effects. In contrast, deepest sites ("deep vPul") in the inferior pulvinar exhibited a distinct pattern: a contraversive bias in all but very late stimulation periods, a contraversive RT facilitation (no delay) in the same periods, and only very small RT effects for the ipsiversive saccades. It is worth noting that in 3 cases for monkey C and 4 cases for monkey L at least two different electrode depths were used in the same penetration and on such days the neighboring sites were separated only by 1-2 mm but still elicited distinct behavioral patterns. This suggests that the effects of stimulation were markedly localized to specific portions of surrounding tissue.

Memory-guided task: dissociating cue processing and motor planning phases

The late stimulation periods in the visually-guided task started during the visual target presentation concurrently with the ensuing decision and saccade planning. To assess whether the stimulation effect on choices was due to affected visual, decision or motor processing stages, we employed a memory-guided saccade task and delivered the stimulation to the dPul in four different trial periods: before cue onset, after cue onset, before the Go signal, and after the Go signal (**Fig. 9A**). As shown in **Figure 9B**, there was no effect on target selection in any of the stimulation periods, although stimulation in the same sites and sessions during the visually-guided task elicited a consistent biphasic choice bias as described above (**Fig. 9C**). At the same time, and consistent with the visually-guided task, memory saccade reaction times in the

620 contraversive space were delayed with stimulation before and especially after the Go, and the ipsiversive
621 saccades were facilitated by the stimulation before the Go and strongly delayed by the stimulation after
622 the Go (**Fig. 9D**).

623 In addition, the stimulation affected the memory-guided task hit rates (**Table 3, Table 4**). Specifically, the
624 two conditions in which the hit rate dropped below 80% in the stimulation trials in both monkeys were: 1)
625 instructed *ipsiversive* trials when the stimulation started before cue (-80 ms cue); and 2) instructed
626 *contraversive* trials when the stimulation was applied after the Go signal (+80 ms from Go). Error trial
627 eye position trajectories (**Fig. 9E**) showed that most errors in the instructed ipsiversive trials were fixation
628 aborts due to saccades towards the ipsiversive cue (79% of error trials with the stimulation onset before
629 the cue), with a latency of 74 ± 14 ms (mean ± SD) after stimulation offset. The same effect was observed
630 in choice trials – even in the presence of two opposite cues, monkeys tended to break fixation by making
631 saccades to the ipsiversive side (88% of error trials with the stimulation onset before the cue). Thus,
632 monkeys had difficulty suppressing reflexive saccades to the ipsiversive cues after stimulation offset,
633 which is in line with our interpretation of ipsiversive orienting in the early stimulation periods in the
634 visually-guided task. Note that this does not contradict the absence of stimulation effect on choices: if the
635 fixation was maintained and thus trials were not aborted, subsequent choices were not affected.

636 Most errors in the second condition, the contraversive trials, were due to contraversive undershooting
637 (85% of instructed and 64% of choice error trials with the stimulation onset after the Go signal). Monkeys
638 were more severely affected by the stimulation during the motor preparation and response phase when
639 there were no visible targets to guide it, as compared to the visually-guided task which showed milder
640 hypometria with no drop in hit rates (*cf.* **Fig. 3B** and **Table 2**). But even taking into account those
641 undershooting error choice trials that were directed towards contraversive targets, the choice was not
642 significantly modulated by the stimulation in +80 from Go period, in any of the sessions ($p>0.05$, Fisher's
643 exact test).

644 Given the lack of choice effects, we considered the possibility that the delayed RTs are a consequence of
645 stimulation interfering with the processing of fixation point offset (i.e. Go signal), but we deem it unlikely
646 given the similarity to RT delays in the visually-guided task (where peripheral target onset coinciding
647 with the fixation offset served as even more apparent Go signal), and the spatially-specific difference
648 between contralateral and ipsilateral RT delays (ipsiversive delay > contraversive delay).

649 These results give rise to several important implications. First, the choice-relevant aspects of cue
650 processing seem unaffected when they are temporally dissociated from the motor response. Similarly, the
651 stimulation does not seem to affect the choice when the decision can be formed in advance of the action,
652 neither in the cue/memory period, nor just before or during the motor response. Third, the RT and the
653 choice effects, which were largely congruent in the visually-guided task, were dissociated in the memory-
654 guided task and might thus not critically depend on each other. This dissociation is reminiscent of recent
655 perceptual decision study in the caudate (Ding and Gold, 2012). Like basal ganglia, the pulvinar is
656 involved in multiple functional loops (Sherman and Guillery, 2002), and different populations or
657 pathways might encode distinct processes. Taken together, the results of visually-guided and memory-
658 guided tasks indicate that the transient pulvinar stimulation contributes to the spatial decision process
659 only when the choice must be formed and executed close in time.

660 ## Neuronal properties in the dorsal pulvinar

661 To better understand the neural contribution of dPul to the behavioral effects of microstimulation, we
662 analyzed the activity of 230 dPul units recorded in the visually-guided saccade task and 365 dPul units in
663 the memory-guided saccade task, in and around the same stimulation sites (**Materials and Methods**).
664 Dorsal pulvinar units predominantly showed low firing rates (mean firing rate across all task periods: 10
665 and 11 spikes/s, median: 6 and 7 spikes/s, SD: 10 and 11 spikes/s, for the visually-guided and memory-
666 guided task, respectively).

667 Visual response fields (RFs) were estimated offline, using an array of 12 target positions (12º and 24º
668 eccentricity). Cue responses for each target position in the memory-guided saccade task were fitted with a
669 2D Gaussian profile (**Materials and Methods**). The position of the Gaussian peak and the area covered
670 by two Gaussian SDs to each side defined center and size of the RF. **Figure 10A** illustrates firing patterns
671 and RF estimation in one example unit. Here we refer to the (visual) response fields as those computed in
672 the cue epoch, but response fields could also be computed during eye movements and peripheral fixation.
673 As can be seen in the example for the peripheral target hold epoch in **Figure 10A**, post-saccadic RFs can
674 differ from visual RFs, as has been reported in the lateral part of the dorsal pulvinar, PLdm (Robinson et
675 al., 1986).

676 The response field estimation was performed on all units showing a main ANOVA effect of stimulus
677 position during the cue period (68 units). We separated this subset further by each unit's preferred
678 hemifield and looked at the population cue response for each of the 12 target positions in contralaterally-
679 and ipsilaterally-tuned subsets (**Fig. 10B**). Even though the contralateral subset was much larger (58
680 units) than the ipsilateral subset (10 units), contralateral population cue response was more time-locked
681 than ipsilaterally-tuned responses. Additionally, contralateral responses seemed to be more consistent
682 across targets, with a small preference for lower and more peripheral targets.

683 **Figure 10C** illustrates the estimated response fields, at a scale of 1:10 in a plot representing the visual
684 target array field, with colors representing different recording sites. RF centers were scattered across the
685 entire tested visual field and their size varied substantially. Note that because RF centers were constrained
686 to the dimensions of the target array, it may seem as if many RFs are clustered along those borders.
687 However, our mapping and fitting approach did not allow drawing conclusions about potential RF centers
688 outside the target array. Typically, RF estimates were large (28 ± 9°), and most of them had their centers
689 in the contralateral hemifield (mean eccentricity=10° in the contralateral hemifield, median=11°,
690 SD=11°), with a tendency for lower peripheral positions. We did not find a consistent topographical
691 organization along the electrode recording tracts, similarly to the previous dorsal pulvinar study (Petersen

et al., 1985). The lack of retinotopic organization is consistent with fairly uniform microstimulation effects across sites and target positions. Furthermore, largely horizontal directions of small evoked saccades in monkey C might have resulted from a vector summation of upward, horizontal and downward RFs with a contralateral bias, possibly similar to a population coding in the deeper layers of the superior colliculus (Lee et al., 1988).

In the visually-guided task there was also a stronger population response for contralateral stimulus onset (target onset epoch, "T onset", **Figure 11A**). In addition, population response showed a transient and then sustained enhancement following central fixation acquisition ("Fix"), and transient post-saccadic peak ("Post-sac"), stronger for contralateral than for ipsilateral targets. Note that the weak peri-saccadic population response is due to different subsets showing either peri-saccadic enhancement or suppression (see below).

Overall, 78% of units were modulated by the visually-guided task (main effect of epoch or epoch × hemifield interaction), and 56% showed spatial specificity in at least one epoch (main effect of hemifield, or epoch × hemifield interaction). Epoch-specific enhancement or suppression, relative to the fixation baseline, was analyzed for the first subset (78%), and epoch-specific spatial tuning was analyzed in the latter subset (56%). The lower panels in **Figure 11A** summarize the main patterns of spatial tuning and enhancement/suppression in the three epochs: "target onset", "peri-saccadic", and "target hold". The significant tuning was predominantly to the contralateral hemifield in the "target onset" epoch, but became more equalized in "peri-saccadic" and especially in "target hold" epochs. Spatially-tuned units showed predominantly enhancement of firing relative to the fixation baseline (red outer sectors) for target onset epoch, while all other subsets had more equal proportions of enhancement and suppression.

Population response in the memory-guided saccade task (**Fig. 11B**) additionally revealed preference for contralateral trials during the memory period, and in the post-saccadic peripheral fixation epoch in absence of visual stimulus, before target onset ("target hold invisible"). Similarly to in the visually-guided

task, 84% of units were task-modulated (main effect of epoch or epoch x hemifield interaction), and 55% showed a main effect of hemifield, or epoch × hemifield interaction. Epoch-specific enhancement or suppression, relative to the fixation baseline, was analyzed for the first subset (84%), and the epoch-specific spatial tuning was analyzed in the latter subset (55%). The lower panels in **Figure 11B** summarize the main patterns of spatial tuning and enhancement/suppression in the three epochs: "cue onset", "peri-saccadic", and "target hold". Again, the significant tuning was predominantly to the contralateral hemifield in the "cue onset" epoch, but it became more equalized in "peri-saccadic" and especially in "target hold" epochs. Units that were contralaterally-tuned in the cue epoch predominantly showed enhancement of firing relative to the fixation baseline. Besides spatially-tuned responses, an additional 47 units (13%) showed robust cue-related enhancement that was not spatially selective (no main effect of target position and no hemifield tuning). Non-spatially-tuned units in the peri-saccadic epoch showed predominantly suppression, while all other subsets had more equal proportions of enhancement and suppression (**Fig. 11B**).

To further assess the differences in spatial tuning in "cue onset" and "target hold" epochs, population responses for subsets that showed significant tuning in those epochs were derived (**Fig. 11C**, left and right columns). The units that were contralaterally tuned in the cue epoch on average did not show spatial tuning in the post-saccadic and the target hold epochs, suggesting that the tuning in the latter intervals can be congruent or incongruent with the visual cue tuning. This is further evidenced by weak contralateral cue tuning in both subsets that showed significantly-tuned, either ipsilateral or contralateral, target hold response (**Fig. 11C**, right column).

A closer look at full-trial population responses for units showing peri-saccadic suppression (110 units, 30%, **Fig. 11C**, middle column, top row) revealed that many of these units increased firing during central fixation (58 out of 110) . Those responses might resemble so called "fixation cells" reported in the frontal eye field (FEF) and in the superior colliculus (SC) (Izawa et al., 2009; Munoz and Wurtz, 1993a). However recent work by Hafed and colleagues demonstrated that at the level of the SC, the tonic activity

741 during fixation encodes fixational microsaccades, or a retinal error by neurons tuned to foveal locations of
742 very small eccentricity (Hafed and Krauzlis, 2012); it remains to be seen whether some pulvinar neurons
743 might be similarly related to fixation maintenance. Furthermore, many "fixation response" cells in our
744 sample showed a decreased firing during the memory delay as compared to the initial fixation (45 out of
745 100 units that showed enhanced firing during fixation, relative to ITI), suggesting that spatially-specific
746 aspects such as visual memory or motor planning can modulate their activity (**Fig. 11C**, middle column,
747 top row). Conversely, units that showed peri-saccadic enhancement (**Fig. 11C**, middle column, bottom
748 row) also exhibited an increased firing in contralateral trials from the cue onset and during the memory
749 delay, ramping up prior to and peaking soon after the saccade, similar to visuomotor neurons in
750 frontoparietal areas.

751 To summarize spatial tuning properties, we calculated contralateral tuning indexes (CI) for stimulus onset
752 and peripheral fixation ("target hold") responses, for both tasks. **Figure 11D** shows distribution of CIs for
753 the subset of units that were recorded in both tasks, for each monkey. For both tasks, across all recorded
754 units CIs were significantly positive (i.e. contralateral) during "stimulus onset" epochs (visually-guided
755 task: 0.07±0.24; memory-guided task: 0.09±0.26; $p<0.001$, two-tailed one sample t-test); for the subset
756 recorded in both tasks, only memory-guided task indexes were significantly positive (visually-guided
757 task: 0.03±0.23, $p=0.13$; memory-guided task: 0.05±0.26, $p<0.05$). There was no significant tuning across
758 the sample in the target hold epoch, reflecting nearly equal contralaterally- and ipsilaterally-tuned
759 populations. For both epochs, there was a correlation between tuning indexes in the two tasks
760 (Spearman's $R=0.35$, $p<0.0001$ for "stimulus onset" and $R=0.59$, $p<0.0001$ for "target hold), indicating
761 that the spatial response properties in these two epochs are largely consistent across the two tasks.

762 While the full analysis of complex neuronal properties in the recorded population is beyond the scope of
763 the present study, these data provide several points aiding the interpretation of the stimulation results.
764 First, most recorded neurons were modulated by the visual and/or oculomotor contingencies of the two
765 tasks. Second, the overall contralateral tuning in response to the visual stimulus (e.g. target and cue onset

epochs), as well as in the pre-saccadic (not shown) and the peri-saccadic epochs, is consistent with the contraversive drive elicited by the stimulation, and the lack of topographic organization of RFs is in line with similar stimulation effects across different sites and target positions. Third, the fact that the population tuning is still more contralateral than ipsilateral in the peri-saccadic epochs suggests that the stronger RT delays for ipsiversive saccades in the late stimulation periods are not a direct consequence of disrupting ipsilaterally-tuned populations more than contralaterally-tuned ones. Fourth, a subset of units (*cf.* **Fig. 11A,B**, peri-saccadic epochs, outer blue sectors corresponding to spatially non-tuned populations, and **Fig. 11C**, middle top panel) discharged vigorously during fixation intervals but paused firing in the peri-saccadic period, potentially contributing to stimulation-induced saccade delays (*cf.* Yang et al., 2008). Finally, many units had spatial tuning (both contralateral and ipsilateral) in the later part of the target hold period (starting at least 200 ms after the saccade offset, when the immediate post-saccadic effects are probably gone), suggesting a contribution of the dorsal pulvinar to the encoding of gaze, similar to the retinotopic inferior/lateral pulvinar (Robinson et al., 1990)

Discussion

Electrical microstimulation of the dorsal pulvinar influenced selection and execution of goal-directed saccades in spatially- and time-dependent manner. The discussion focuses on the three main findings: 1) In the visually-guided task, stimulation starting prior to target onset (Go signal) reduced ipsiversive reaction times (RT), while stimulation at and after target onset caused a systematic increase in RT for both, ipsiversive and contraversive directions. 2) Stimulation prior to onset of targets increased ipsiversive choices, stimulation after onset of targets increased contraversive choices. 3) In the memory-guided task, stimulation exerted effects on RT, but not on choices.

Effects of microstimulation on saccade generation

Bilateral RT delays with microstimulation after the Go signal have been reported for structures involved in saccade control: *e.g.* dlPFC (Wegener et al., 2008), FEF (Izawa, 2004a), SEF and pre-SMA (Isoda,

2005; Yang et al., 2008), caudate (Watanabe and Munoz, 2010, 2011) and rostral SC (Munoz and Wurtz, 1993b). In cortex, delays were typically stronger for ipsiversive saccades (Isoda, 2005; Izawa, 2004b; Wegener et al., 2008), while the opposite pattern was observed in SC and caudate (Munoz and Wurtz, 1993b; Watanabe and Munoz, 2013). The delay can be interpreted as suppression of gaze-shifting, facilitation of gaze-holding and/or inhibition of a mechanism that switches between the two behavioral modes, and might be explained by direct or indirect, uncrossed and crossed projections to substantia nigra pars reticulata, SC and/or brain stem saccade generator nuclei (Isoda, 2005; Izawa, 2004b).

Unlike SC and caudate, and similar to frontal cortical areas, ipsiversive RT delays were stronger in the dorsal pulvinar, although ipsi- and contraversive delays were correlated, suggesting a common mechanism, e.g. the engagement of fixation neurons, or nearly balanced recruitment of ipsilateral and contralateral populations, as well as un-tuned neurons.

In contrast to stimulation after the Go signal, stimulation starting before the Go shortened the ipsiversive RT, similarly to SEF, pre-SMA (Isoda, 2005; Yang et al., 2008) and caudate (Watanabe and Munoz, 2011). However the above studies reported both contraversive and ipsiversive facilitation, indicating a general motor potentiation, or release from gaze-holding signals. In caudate, release of SC/FEF from inhibition and subsequent rebound, or interplay between direct and indirect pathways, were suggested to explain the facilitation (Watanabe and Munoz, 2011). To account for the ipsiversive-specific RT facilitation in the dorsal pulvinar, we propose a different, directional mechanism, same as for the ipsiversive choice bias (see below).

Effects of microstimulation on choices

Unlike the effects of microstimulation on saccade execution, interference on choices has been less studied, with most work focusing on perceptual decisions (Carello and Krauzlis, 2004; Cicmil et al., 2015; Fetsch et al., 2014; Hanks et al., 2006; Murasugi et al., 1993), but see (Mirpour et al., 2010; Opris et al., 2005). Therefore, one major question was whether and when pulvinar microstimulation influences

free-choice target selection. Our previous work with pharmacological inactivation of dorsal pulvinar already implicated it in the spatial decision-making (Wilke et al., 2010, 2013), but it was important to test if the stimulation potentiates the "functioning" of the pulvinar, thus biasing choices in the direction opposite to the inactivation (i.e. contraversive vs. inactivation-induced *ipsilesional* bias). Indeed, stimulation after the Go signal increased contraversive selection, and together with the contralateral neuronal tuning in the corresponding epoch this suggests that it did not merely disrupt the normal functioning (Carello and Krauzlis, 2004). This is consistent with cortical and SC studies, which typically show a correspondence between the neuronal tuning and the direction of microstimulation effects (Clark et al., 2011).

However, even assuming a facilitatory activation by stimulation, the stimulation effects were not just a "mirror image" of inactivation. When stimulation started during fixation, prior to targets onset, it caused ensuing *ipsiversive* bias, concurring with the ipsiversive RT facilitation. Such ipsiversive effects on target selection have been rarely reported. One study showed that microstimulation in upper layers of V1 biases choices away from the stimulated RFs (Tehovnik et al., 2002); similarly, caudate stimulation increased ipsiversive perceptual choices, away from contralateral RFs (Ding and Gold, 2012).

One hypothesis is that the ipsiversive selection is the manifestation of a stimulation-induced contraversive drive, which has to be counteracted during the fixation. Such putative ipsiversive compensatory mechanism might be engaged until the end of the stimulation period and extend beyond the stimulation offset (after the Go signal) into motor planning/execution epoch. Please note that this hypothesis does not necessarily imply that the monkeys were aware of the stimulation (Murphey and Maunsell, 2008) and compensated intentionally.

A related hypothesis is that the timing of stimulation *offset*, relative to saccade RTs, is important. Early stimulation periods ending ~80 ms before the typical control RTs (~160 ms) led to an ipsiversive "advantage" in RTs and choices, whereas stimulation periods overlapping with the RTs led to a

838 contraversive bias. While the stimulation causes a contraversive drive, the offset of the stimulation *per se*
839 might trigger a transient ipsiversive rebound, regardless of task requirements. To resolve whether the
840 timing of stimulation onset or the stimulation duration/offset is the crucial factor for the ipsiversive
841 facilitation, the duration of the stimulation trains should be systematically varied in future studies.

842 An even more mechanistic explanation might be that the timecourse of stimulation on the evoked activity
843 is initially excitatory and then inhibitory (Histed et al., 2013). Thus, the initial contraversive drive would
844 be suppressed by the end of the early stimulation periods, during target selection. Indeed, inhibitory
845 consequences of the thalamic stimulation on cortical activity have been reported (Logothetis et al., 2010),
846 and pulvinar stimulation study in anaesthetized treeshrews found that evoked activity in extrastriate
847 cortex consists of early and late waves, with a gap ~200 ms after the stimulation onset (Vanni et al.,
848 2015).

849 Another explanation might be that the dorsal pulvinar stimulation engages a contraversive attentional
850 shift, which acts as a "cue" in the inhibition of return phenomenon (Dorris et al., 2002). There is also a
851 possibility that the pulvinar fulfills distinct functions in different behavioral states, e.g. filtering out
852 contralateral distractors and inhibiting reflexive contraversive saccades (Van der Stigchel et al., 2010),
853 until cortical inputs signal the initiation of the active motor preparation phase. In this case, the
854 potentiation of pulvinar activity during fixation would lead to a suppression of the *currently* irrelevant
855 contraversive space. The presence of fixation-like neurons discharging persistently when monkeys
856 maintained fixation supports this notion. However the occurrence of contraversive evoked saccades
857 during fixation challenges this interpretation, unless the motor effects can be completely dissociated from
858 the attentional/target selection signals.

859 Although a combination of contraversive facilitation drive and ipsiversive compensatory/rebound effect
860 after the early stimulation offset seems most parsimonious explanation for the observed effects, the
861 question whether a given stimulation protocol leads to functionally beneficial enhancement of "normal"

neuronal activation, to a functionally detrimental disruption, or to replacement or "hijacking" (Cheney et al., 2013), is a long-standing debate, relevant for all stimulation studies (Desmurget et al., 2013). Some of our stimulation effects are consistent with the disruption of the (contraversive) pulvinar processing: ipsilateral facilitation in early stimulation periods, delayed saccades. However, this hypothesis is hard to reconcile with the contraversive choice facilitation in later stimulation periods, unless the apparent contraversive facilitation is the consequence of "less contraversive disruption than ipsiversive disruption" during saccade generation. The latter possibility is consistent with stronger ipsiversive RT delays, although the neuronal tuning in pre/peri-saccadic epochs was weakly contralateral. The contraversive disruption assumption also does not account for contraversive evoked saccades, unless the main role of pulvinar is to help maintaining fixation and ignore contralateral hemifield.

Yet another possibility is that stimulating neurons with RFs away from the target, but within the same hemifield, is more detrimental than when the RFs and the target are in opposite hemifields, thus leading to ipsiversive facilitation. However, the reasons for contraversive facilitation in later stimulation periods remain unexplained under this assumption.

Functional implications and future directions

The effect on choices was present only in the visually-guided, but not the memory-guided task. Thus, the choice bias is driven neither by purely perceptual processing (otherwise we would expect that the stimulation before or after visual cues affects subsequent choices), nor is it a purely motor consequence (otherwise we should have seen effects before and after the Go signal). We suggest that the dorsal pulvinar contribution to the decision is crucial when the visuomotor contingencies have to be rapidly integrated concomitantly with action selection. Alternatively, the pulvinar might affect the choices only when the target selection takes place in the presence of visual stimuli (which was not the case for memory-guided saccades); this conjecture needs to be tested in future experiments comparing memory-guided and visually-guided delayed saccades.

886 The interpretation of the alleged contraversive drive due to pulvinar stimulation is still open. In the
887 simplest scenario, it could relate to attentional/behavioral saliency vector in the retinotopic reference
888 frame. However, the spatial processing in the pulvinar, especially the dorsal part, might extend beyond
889 purely visual aspects, contributing to gaze and postural encoding, and perhaps to a prediction error
890 (Grieve et al., 2000; Kanai et al., 2015). For example, the stimulation could affect the perceived direction
891 of gaze, relative to the head or the body, or perceived body midline. Further experiments with
892 manipulation of visuomotor and postural contingencies will address these possibilities. Another question
893 is how general the biphasic choice effect is. Ventral pulvinar stimulation did not elicit an ipsiversive bias,
894 but it would be interesting to test the same protocol in frontoparietal cortical areas interconnected with the
895 dorsal pulvinar.

896 The inevitable conundrum of causal interference studies is to what extent the observed behavior depends
897 on the functioning of the target area, as opposed to spread of in(activation) to neighboring structures, and
898 consequences of network effects. Site-specific patterns (**Fig. 8**), and their dissimilarity from patterns in
899 adjacent SC and caudate suggest fairly localized effects, but we cannot exclude some current spread
900 through intercalated thalamo-cortical fibers, brachium of SC or neighboring PIp/m/cm subdivisions of
901 inferior pulvinar (Rosenberg et al., 2009; Stepniewska, 2004). The pulvinar stimulation with a similar
902 protocol during fMRI activates an extensive visuomotor cortical circuitry in the stimulated hemisphere,
903 with distinct patterns for dorsal vs. ventral sites consistent with anatomical connectivity (Gibson, Wilke,
904 Kagan, unpublished observations). Thus, the observed effects can be mediated by predominantly
905 contralaterally-tuned cortical areas (Kagan et al., 2010; Wilke et al., 2012). Future work combining
906 epoch-specific stimulation with fMRI and electrophysiological readouts should elucidate the neuronal
907 basis of these effects.

908

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Figure legends

Figure 1. Localization of stimulation sites in the dorsal pulvinar. **A**, Example scan of monkey C with the stimulating electrode inserted in the pulvinar (grid location: x5,y3), with the chamber and the grid filled with the MRI contrast agent (gadolinium, Gd) (T2-weighted scan, left panel) and the corresponding section of the T1-weighted scan (right panel). **B**, Electrode tip localization in individual stimulation sites (red circles), in chamber-normal coronal sections corresponding to specific grid locations (x,y; in parentheses) and depth. Pulvinar nuclei outlines (MPul, LPul, IPul) were adapted from the NeuroMaps atlas (Rohlfing et al., 2012), exported via the Scalable Brain Atlas, https://scalablebrainatlas.incf.org/macaque/DB09, https://scalablebrainatlas.incf.org/services/rgbslice.php, (Bakker et al., 2015), and LPul was further subdivided to dorsal (PLdm) and ventral (PLvl) parts. **C**, Electrode tip localization probability maps in standard AC-PC space, across all stimulation sites. The probability map was created by delineating a sphere of 0.5 mm radius around the tip in the chamber-normal space, for each stimulation session, transforming resulting volumes to AC-PC space, and converting volumes of interest (VOI) to probability map using BrainVoyager VOI functions. Pulvinar nuclei outlines from the NeuroMaps atlas (dotted white) were individually scaled in vertical and horizontal dimensions and overlaid on the corresponding anatomical sections. Right inset: standard coronal sections (indicated by the y coordinate) from the NeuroMaps atlas, going from anterior (top) to posterior (bottom). Abbreviations: bsc – brachium of the superior colliculus, blv – body of the lateral ventricle.

Figure 2. Fixation task and characterization of evoked saccades. **A**, Task layout. Monkeys fixated a central spot for a variable time to receive liquid reward. In half of the trials we applied a train of biphasic electric pulses to characterize potential evoked saccades. **B**, Stimulation parameters. Each 200 ms stimulation train consisted of 60 biphasic pulses applied at 300 Hz (two pulses are shown). Each biphasic pulse started with a 300 μs positive phase, followed by 150 μs inter-phase-interval and a 300 μs negative phase. There was a 2.58 ms interval between sets of pulses. **C**, Saccade probability distribution as a

function of time during and after stimulation (left panel), and corresponding saccade endpoints (right panel). Saccades that started during the stimulation period (purple shade) are shown in purple, saccades that started in the 200 ms window after stimulation (green shade) in green, and saccades in trials without stimulation in gray. Plotted data are from 1580 fixation trials in monkey C (15 stimulation sites). Only saccades with amplitudes >0.5° were included in this analysis. Left panel: The time axis is relative to stimulation onset or a corresponding time in control trials. The probability of contraversive or ipsiversive saccades is shown as upward and downward histograms, respectively (bin 20 ms). Right panel: saccade direction and amplitude in the fixation task. Endpoints are shown relative to each saccade starting position. We defined evoked saccades as saccades during the stimulation period that were followed by a saccade to the opposite side, returning to the fixation spot. The evoked saccades occurred mainly along the horizontal axis to the contraversive side: 83% were contained within a 30° angle below and above the horizontal axis (solid purple sector outline).

Figure 3. Visually-guided saccade task. **A**, Task layout. Stimulation was delivered in one of seven different periods: starting before the target(s) onset (-120 ms, -80 ms, or -40 ms), at the Go signal, or after the Go signal (+40 ms, +80 ms, or +120 ms). Trials without stimulation were interleaved as a control. The color code for each stimulation period is same for all following figures. **B**, Saccade accuracy and endpoint scatter. Dashed red circles represent the allowed 5° radius of target acquisition window; crosses represent the targets center. Half-axes of colored ellipses (control: gray) represent the means, across sessions, of standard deviations of radial and angular coordinates of endpoints; ellipse centers are means of mean endpoints across sessions. Both monkeys showed reduced accuracy ("Acc", the distance from the target center to the mean endpoint) and increased endpoint scatter in the contraversive side of space (precision, "Pre", the radial component corresponding to ellipse major axis). The asterisks denote significant effects on accuracy (Acc) and precision (Pre) separately for each target position (*$p<0.05$, **$p<0.01$, Friedman with post-hoc Wilcoxon signed rank test, Bonferroni corrected). These accuracy and precision effects did not impair monkeys' ability to acquire any of the targets (Fisher's exact test within each session,

Bonferroni corrected, p>0.05, see **Table 2**). **C**, Horizontal eye position traces for the different stimulation periods in two example sessions in monkey C (upper two rows) and L (lower two rows), for one pair of contraversive (left column) and ipsiversive (right column) targets, successful trials, aligned to the Go signal (0 ms, dotted vertical lines). The color of the traces represents corresponding stimulation periods, which are also shown as brackets below the traces (control: gray). The triangles below denote mean RT for each period.

Figure 4. Effect of stimulation on reaction times in the visually-guided saccade task. **A**, Stimulation effects on reaction times for contraversive (left) and ipsiversive (right) saccades, for monkey C and L, respectively. Upper panels: the reaction time (RT) histograms (10 ms bin, normalized to 100% per condition) show data across all trials for each stimulation period. For stimulation onsets at or after the Go signal, the saccade initiation was delayed compared to control (gray). The saccade initiation was either delayed or arrested until the end of the stimulation period, resulting in a bimodal RT distribution for the late stimulation periods. In ipsiversive trials for the earliest stimulation period, -120 ms to the Go signal, there was a facilitatory effect on saccade onsets. Lower panels: session by session directionality and significance of RT effect. For each stimulation period, each session reaction time that differed from control trials is shown as either a positive or negative bin, representing either delay or facilitation. Filled bins represent sessions where the change from control was significant (Friedman followed by Mann-Whitney-U test, Bonferroni corrected). It should be noted that for ipsiversive saccades, the facilitation effects at the two early stimulation periods (-120 and -80) were consistently present in both monkeys in both choice and instructed trials. **B**, Summary of reaction time effects for contraversive (left) and ipsiversive (right) saccades, separated by monkey – blue and green traces for monkey C and L, respectively – and by vertical target position (light-, medium- and dark-shaded traces denote upper, horizontal, and lower positions, see inset). Plots show saccades that started either during stimulation period (top row) or after stimulation offset (bottom row). Dashed lines in the top row connect control data with the next available stimulation data point (there were no correct saccades starting during -120 and -80

stimulation periods, since this would abort fixation). **C**, Summary of reaction time effects for contraversive (left) and ipsiversive (right) saccades, combined for two monkeys and all vertical target positions (mean and SE across sessions). Top and bottom rows show saccades that started either during the stimulation period or after stimulation offset. Note that the -40 ms period is a special case where the separation into during and after stimulation does not provide meaningful information since the offset of this stimulation period (160 ms after the Go signal) happens at the same time as the mean onset of saccades in the control condition (165±2 ms and 165±2 ms; contraversive and ipsiversive saccades respectively, both monkeys combined). Therefore, saccades that started during the -40 ms stimulation period would by definition seem facilitated when compared to control and saccades that started after stimulation would appear delayed. The reaction times in all stimulation periods were compared to control trials (marked as "C") using Kruskal-Wallis followed by Mann-Whitney-U test, Bonferroni corrected, *$p<0.05$, **$p<0.01$.

Figure 5. Reaction time correlations across sessions. Two stimulations periods, -120 ms before and 80 ms after the Go signal, in which reaction time effects (facilitation and delay respectively) were overall strongest, were selected for the correlation analysis. Data from both monkeys are combined in this and subsequent correlation plots. Filled circles indicate sessions where both effects were significant, triangles indicate that only one of the effects was significant, and open circles indicate sessions with no significant change for any of them. **A**, Contraversive vs. ipsiversive reaction time difference (stimulation – control), in each session. For the early stimulation period -120 ms before the Go signal (blue symbols), stronger facilitation (negative RT difference) in ipsiversive trials had an insignificant trend to correlate with shorter RTs in contraversive trials. For the late stimulation period +80 ms after the Go signal (orange symbols), delays (positive RT difference) in ipsiversive and contraversive trials were correlated, with most data points below the main diagonal (ipsiversive delay > contraversive delay). **B**, Reaction time delay vs. facilitation in ipsiversive saccades, in each session. The facilitation due to stimulation in the -

120 ms period and the delay due to stimulation in the +80 period ms were correlated. Black lines show best linear fits.

Figure 6. Effect of stimulation on target selection in visually-guided saccade task. **A**, Percentage of contraversive target selection as a function of stimulation periods. In control trials (marked as "C"), both monkeys showed an ipsiversive (right) target selection bias (despite initial bias equalization, see **Materials and Methods**). In stimulation trials, current applied before the GO signal further decreased the selection of contraversive targets. Late stimulation periods increased contraversive target selection. Mean and SE across sessions, p-values from Friedman test followed by Wilcoxon signed-rank test, Bonferroni corrected (*p<0.05, **p<0.01). **B**, Target selection modulation per session: direction and significance. For each session we used Bonferroni-corrected Fisher's exact test to compare target selection in stimulation trials to control trials, for each stimulation period. The direction of the effect is shown with a positive or negative vertical bar corresponding to increased or decreased contraversive selection; statistically significant sessions are filled. **C**, Percentage of contraversive target selection as a function of stimulation periods in individual sessions. Black lines connecting dots link data points from individual sessions. **D**, Target selection modulation per vertical position of left/right target pairs (right panel). The inset on the left shows the corresponding color code (monkey C – blue, monkey L green, light-, medium- and dark-shaded traces denote upper, horizontal, and lower target positions).

Figure 7. Target selection and reaction time correlations across sessions. **A**, Contraversive target selection difference (stimulation – control), in -120 ms vs. +80 ms stimulation periods, in each session. There was a weak insignificant correlation between contraversive bias at +80 ms period and ipsiversive bias at -120 ms period. **B**, Contraversive target selection difference vs. reaction time difference for the -120 ms and +80 ms stimulation periods, for contraversive (top panel) and ipsiversive (bottom panel) saccades, in each session. There was a weak insignificant correlation between ipsiversive bias and RT changes in the -120 ms period (blue symbols), for both contraversive and ipsiversive saccades, and strong

correlation between contraversive bias and RT delay in the +80 ms period, only for ipsiversive saccades (orange symbols, bottom panel).

Figure 8. Summary of stimulation effects in dorsal (dPul) and ventral (vPul) pulvinar. **A**, Top: pulvinar parcellation schemes: dorsal vs. ventral; medial, lateral and inferior; with the lateral nucleus further subdivided to dorsal (PLdm) and ventral (PLvl) parts. Bottom: stimulation sites localization in the dPul (monkey C: grid y5; monkey L: grid y3), chamber-normal coronal sections. **B**, Stimulation sites localization in the vPul (monkey C: grid y4; monkey L: grid y3) , chamber-normal coronal sections. **C**, In addition to 30 stimulation sites in dorsal pulvinar (top row), we performed control stimulations in 21 sites in ventral pulvinar (vPul) to assess the specificity of the stimulation effects on target selection and saccade generation. We binned these sites into three depth groups, shallow (6 sessions, 3 monkey C, 3 monkey L), medium (7 sessions, 2 monkey C, 5 monkey L) and deep (8 sessions, 5 monkey C, 3 monkey L). Due to the angled chamber orientation as we advanced deeper we also targeted more medial and anterior parts of the vPul. One depth group is shown per row. For all rows: left column, upper subpanel: target selection difference from control, mean and SE across sessions; bottom subpanel: direction and significance of the stimulation effect per session normalized to the number of sessions (100% scale bar). The significance in each session was assessed using Fisher's exact test, Bonferroni corrected, filled colors: $p<0.05$. Middle and right columns: data for instructed contraversive and ipsiversive reaction time effects, significance in each session assessed using Kruskal-Wallis test followed by Mann-Whitney-U test, Bonferroni corrected.

Figure 9. Stimulation effects in memory-guided saccade task (dPul). **A**, Task layout. Stimulation was delivered in one of four periods: starting before onset of the visual cue(s) (-80 ms Cue), after the onset of the visual cue(s) (+80 ms Cue), before the Go signal (-80 ms Go), or after the Go signal (+80 ms Go). Trials without stimulation were interleaved as a control. **B-C**, Target selection in the memory-guided task (**B**) and in the visually-guided saccade task in the same sites and sessions (**C**) (5 sessions, 3 sessions in monkey C, 2 sessions in monkey L), mean and SE of contraversive selection difference from control, and

direction and significance of preference change from control (Fisher's exact test, Bonferroni corrected). Insets in B for the memory-guided task show data for each monkey. **D**, Effect of stimulation on reaction time in the memory-guided saccade task for instructed contraversive (left) and ipsiversive (right) trials. For both hemifields, panels on the left show the mean and SE across sessions, and panels on the right show direction and significance of effect per session; significance in each session assessed by Kruskal-Wallis test with post-hoc Mann-Whitney-U test, Bonferroni corrected. **E**, Eye position traces during instructed memory-guided saccade error trials, two sessions combined for each monkey (monkey C left, monkey L right). Trajectories are colored according to the period in which stimulation occurred; trials in which no stimulation was delivered are gray. There were two periods in which both monkeys showed a considerable decrease in the hit rate (<80%, see **Table 3**): before the Cue onset and after the Go signal. Errors in trials where stimulation was delivered before the Cue onset were mostly fixation aborts towards the ipsiversive Cue after the stimulation period ended. Errors after the Go signal were mostly hypometric saccades that did not reach the target window.

Figure 10. Spatial response field properties in the dPul. **A**, Offline RF estimation in an example unit (Cur_20150617_03). Left: Raster plot and resulting spike density functions (SDF) separately for contralateral and ipsilateral trials, magenta and brown, respectively. Trials are grouped by hemifield, eccentricity and vertical target position, upper (UP), horizontal (HR), and lower (LW). For the raster plot and SDF black dotted lines denote events: fixation point onset ("FP onset"), acquiring fixation ("FP acquired"), cue onset, cue offset and beginning of the memory period, offset of the central fixation point (which also served as the Go signal, "Go"), saccade onset, and onset and offset of the peripheral target. Discontinuous traces indicate gaps in alignment to events: FP onset, cue onset, saccade onset, and target offset; the other event markers denote average onset relative to alignment events. Gray boxes above the time axis indicate analyzed epochs (see **Materials and Methods**). Top right panel: average firing rates for all 12 target positions during the cue and target hold epochs (upper part of the color scale). Bottom right panel: modulation depth and Gaussian fit defining the response field for that unit. Percentage

modulation depth of cue responses is displayed for each target location at its actual position on the screen (lower part of the color scale). The size of the visual stimuli is indicated by the dot in the center of each target (0.5º radius). The superimposed ellipse represents the boundaries of the Gaussian fit (two SD to each side, see **Materials and Methods**). For this unit, the response field size was estimated as 21°. **B**, Mean population response and SE across units during fixation hold, cue and early memory epochs for ipsilateral and contralateral subsets of units, orange and purple, for each target position. The two subsets represent units that had a main effect of target position during the cue epoch (gray shaded area) and were sorted into contralateral and ipsilateral populations according to the preferred hemifield. Before averaging across units, the mean peak of each unit's activity during the cue epoch across all trials to the preferred hemifield was normalized to 1. **C**, Visual response fields in the memory-guided saccade task. Upper panels: electrode tip position in individual recording sites (circles), in chamber-normal coronal sections corresponding to specific grid location (x,y; in parentheses). Recording sites where no spatially-tuned units were found are denoted by white circles, recording sites that showed tuning are shown in red, green, and blue colors representing different grid locations, with dark-to-light shades denoting recording depth. Pulvinar nuclei outlines as in **Figure 1,** colored outlines – medial dorsal pulvinar (MPul). Lower panel: RF centers and sizes for all units showing a main effect of cue location. Response field centers correspond to the center of markers, circles for monkey C, and squares for monkey L. The marker size represents RF size, scaled 10:1. The color of the markers indicates the recording sites corresponding to the site reconstruction panels above.

Figure 11. Neuronal population properties in the dPul. **A**, Units recorded during the visually-guided saccade task (N=230). Top panel: average baseline-corrected firing rate, mean (solid traces) and SE (shaded bands) across units, separately for contralateral and ipsilateral trials, in magenta and brown respectively. Same convention for alignment lines as in **Figure 10A**. Bottom panels: spatial tuning and firing rate modulation in the three epochs (target onset, peri-saccadic, and target hold). In each plot, sectors of the inner circle display the percentage of units that, in the respective epoch, preferred the

ipsilateral hemifield (orange), contralateral hemifield (purple), were not tuned (light green), or were not tested for spatial tuning because they showed neither a main ANOVA effect of hemifield nor an interaction of hemifield and epoch (light gray). The outer sectors display the percentage of units that showed enhancement or suppression in the respective epoch as compared to the fixation hold epoch, for each of the four aforementioned subsets separately: units showing enhancement for one hemifield and suppression for the other (green), only enhancement (red), only suppression (blue), neither enhancement nor suppression (dark gray), or neither a main ANOVA effect of epoch nor interaction of epoch and hemifield, and thus not tested for enhancement or suppression (white). **B**, Similar to A, for the memory-guided saccade task. Top panel: average baseline-corrected firing rate. Bottom panels: spatial tuning and firing rate modulation in the three epochs (cue onset, peri-saccadic, and target hold). **C**: Average baseline-corrected PSTHs across different subsets of units, in the memory-guided task. The subsets were defined by the classification in B: ipsilateral cue tuning (top left), contralateral cue tuning (bottom left), peri-saccadic suppression (top center), peri-saccadic enhancement (bottom center), ipsilateral tuning during target hold (top right), and contralateral tuning during target hold (bottom right). **D**, Contralateral tuning indexes (CI, see **Materials and Methods**) for visually-guided vs. memory-guided saccades, for each unit where data for both tasks was available (N=121). Filled markers denote units with significant tuning (see legend), units recorded in monkey C and L are in green and red, respectively. Left: CIs in "stimulus onset" ("target onset" / "cue onset") epochs. Right: CIs in "target hold" epoch. Lines indicate best linear fits.

Tables

(Please see next 4 pages)

Table 1. Summary of 56 stimulation datasets. First column coded as Monkey-Site-Task-Session number, C: monkey C, L: monkey L; dPul: dorsal pulvinar, v[s/m/d]Pul: ventral pulvinar (s – shallow, m – medium, d – deep); V: visually-guided task, M: memory-guided task. Offset: horizontal shift of the entire stimulus array (fixation point and targets) from the center of the screen, positive values indicate shift to the right. Current: current strength used in the visually-guided or memory-guided task. "Impedance before conditioning" refers to the electrode impedance prior to applying ten 200 ms 300 μA trains outside of the brain. First-time-use electrodes (out of the box) are marked with the asterisk. "Impedance after conditioning" refers to resulting impedance after applying conditioning stimulation trains.

Monkey-Site-Task-Session number	Trials (n)	Offset (deg)	Contraversive choice (%) in pre-stimulation runs	Current (μA)	Impedance before conditioning (kΩ)	Impedance after conditioning (kΩ)
L-dPul-V-1	566	3	80	250	-	100
L-dPul-V-2	960	3	31	250	80	60
L-dPul-V-3	546	3	22	250	80	60
L-dPul-V-4	696	6	22	250	-	50
L-dPul-V-5	625	3, 6	20	250	-	50
L-dPul-V-6	1895	0, 3	65	250	250	50
L-dPul-V-7	960	0	21	250	700*	29
L-dPul-V-8	960	5	24	250	19	14
L-dPul-V-9	960	5	21	250	160	32
L-dPul-V-10	960	8	52	250	200	60
L-dPul-V-11	960	5	45	250	100	40
L-dPul-V-12	960	5	34	250	180	25
L-dPul-V-13	480	3	41	250	65	24
L-dPul-V-14	480	7	84	250	60	28
L-dPul-V-15	480	7	36	250	600*	22
C-dPul-V-1	818	0	49	250	105	60
C-dPul-V-2	1248	5	31	100, 200	45	32
C-dPul-V-3	1276	5	27	200, 250	170	35
C-dPul-V-4	960	5	42	200	22	22
C-dPul-V-5	1440	5	25	150, 250	360*	28
C-dPul-V-6	1158	5	31	150, 250	23	23
C-dPul-V-7	960	0	62	250	1100*	33
C-dPul-V-8	960	5	49	150	1100*	33
C-dPul-V-9	1920	0, 5	38	250	1300*	65
C-dPul-V-10	960	2	43	250	390*	32
C-dPul-V-11	960	0	37	250	65	28
C-dPul-V-12	480	-2	47	250	75	20
C-dPul-V-13	480	0	51	150	600*	19.5
C-dPul-V-14	480	10	64	250	170	-
C-dPul-V-15	864	6	61	200	440*	38
L-dPul-M-1	600	-5	22	250	60	28
L-dPul-M-2	600	5	54	250	600*	22
C-dPul-M-1	600	3	89	150	600*	19.5
C-dPul-M-2	600	6	53	200	440*	38
C-dPul-M-3	240	10	93	250	170	-
L-vsPul-V-1	960	0	36	250	700*	25

L-vsPul-V-2	960	2	19	200	850*	20
L-vsPul-V-3	960	3	19	250	33	25
C-vsPul-V-1	960	2	37	250	600*	200
C-vsPul-V-2	960	5	34	250	37	35
C-vsPul-V-3	960	0	71	150	41	31
L-vmPul-V-1	960	4	11	250	140	29
L-vmPul-V-2	1920	3	22	150, 200	850*	12
L-vmPul-V-3	960	2	19	250	25	20
L-vmPul-V-4	960	3	33	250	40	21
L-vmPul-V-5	960	3	19	250	19	11
C-vmPul-V-1	960	0	66	250	41	31
C-vmPul-V-2	960	0	50	250	600*	60
L-vdPul-V-1	960	3	32	200	40	21
L-vdPul-V-2	960	3	19	250	19	11
L-vdPul-V-3	960	3	19	250	33	25
C-vdPul-V-1	960	2	46	250	28	19
C-vdPul-V-2	960	0	44	250	37	35
C-vdPul-V-3	960	0	33	250	41	31
C-vdPul-V-4	960	0	59	250	600*	60
C-vdPul-V-5	960	0	46	200	200	19

Table 2. Hit rates in the visually-guided saccade task, instructed trials, dorsal pulvinar stimulation (mean ± SE across sessions).

Stimulation period onset	Contraversive hit rate (%)			Ipsiversive hit rate (%)		
	Both monkeys	Monkey C	Monkey L	Both monkeys	Monkey C	Monkey L
Control (no stimulation)	99 ± 0	99 ± 1	100 ± 0	98 ± 1	100 ± 0	97 ± 1
-120 ms to Go	100 ± 0	99 ± 1	100 ± 0	98 ± 1	98 ± 1	98 ± 1
-80 ms to Go	99 ± 0	99 ± 1	100 ± 0	99 ± 0	99 ± 0	99 ± 1
-40 ms to Go	99 ± 0	99 ± 1	100 ± 0	99 ± 0	100 ± 0	99 ± 0
Go (target onset)	98 ± 1	98 ± 1	99 ± 0	97 ± 1	98 ± 1	96 ± 1
+40 ms from Go	97 ± 1	96 ± 1	99 ± 1	98 ± 0	98 ± 1	98 ± 1
+80 ms from Go	98 ± 1	97 ± 2	99 ± 1	97 ± 1	98 ± 1	97 ± 1
+120 ms from Go	97 ± 1	95 ± 2	100 ± 0	96 ± 1	97 ± 1	95 ± 2

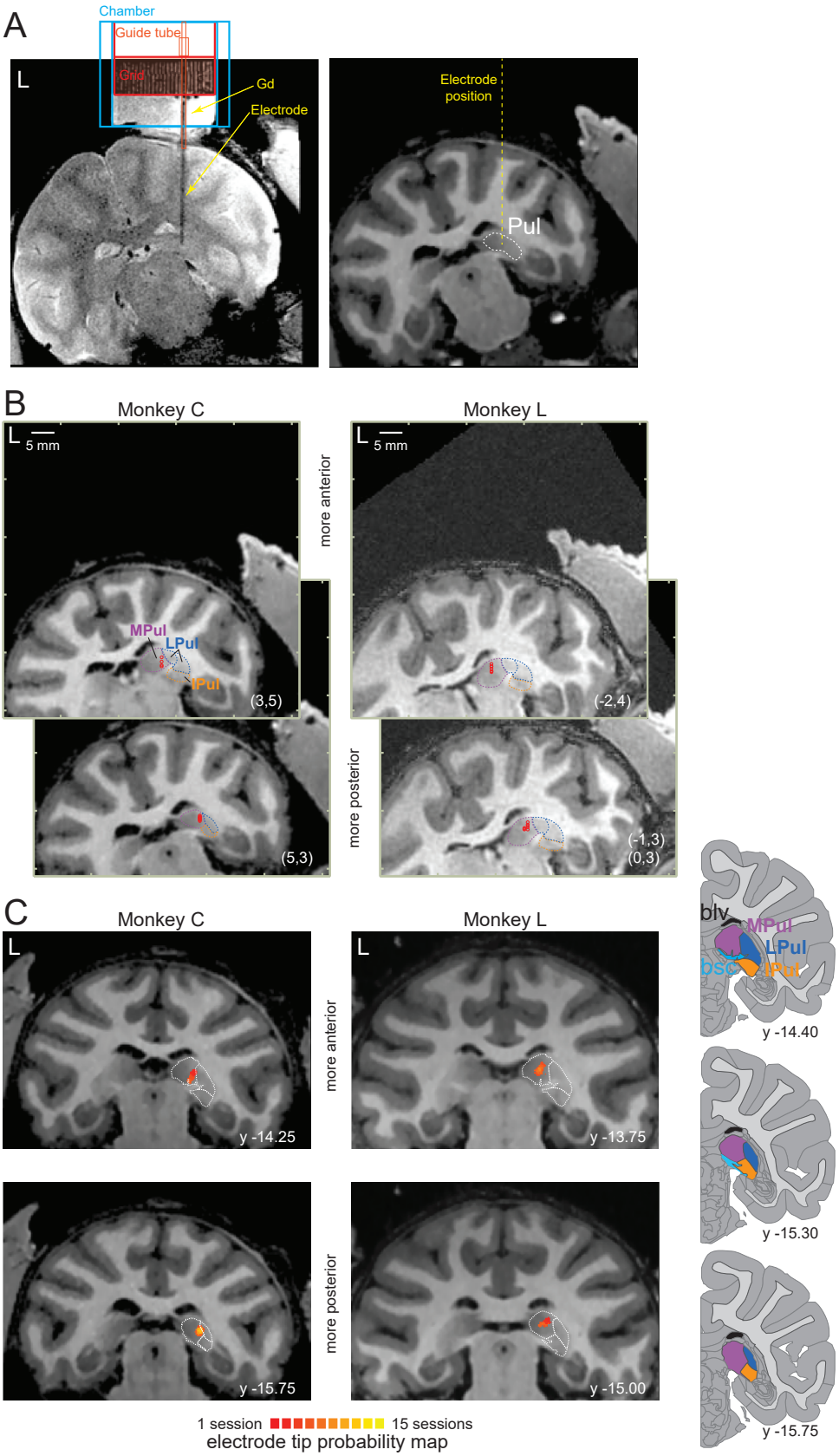
Table 3. Hit rates in the memory-guided saccade task, instructed trials, dorsal pulvinar stimulation (mean ± SE across sessions). In two stimulation periods, -80 ms to ipsiversive cue onset, and +80 ms from Go in contraversive trials, there was a drop in performance <80% (shaded; in **bold** and with asterisk, significant in at least one session, Fisher's exact test, Bonferroni corrected, $p<0.05$). Hit rates drops <80% that did not reach significance are marked in *italics*.

Stimulation period onset	Contraversive hit rate (%)			Ipsiversive hit rate (%)		
	Both monkeys	Monkey C	Monkey L	Both monkeys	Monkey C	Monkey L
Control (no stimulation)	96 ± 2	93 ± 3	100 ± 0	89 ± 8.3	82 ± 13	100 ± 0
-80 ms to Cue onset	94 ± 5	90 ± 7	100 ± 0	**73 ± 9***	*74± 14*	**71 ± 11***
+80 ms from Cue onset	96 ± 2	94 ± 3	98 ± 2	91 ± 8	84 ± 13	100 ± 0
-80 ms to Go	89 ± 5	87 ± 7	92 ± 8	84 ± 15	*74 ± 25*	98 ± 2
+80 ms from Go	**53 ± 14**	**52 ± 22***	**54 ± 21***	89± 7	84± 12	97 ± 0

Table 4. Hit rates (a fraction of successfully completed trials, regardless of chosen hemifield) in the memory-guided saccade task, choice trials, dorsal pulvinar stimulation (mean ± SE across sessions). Similar to instructed trials, in two stimulation periods, -80 ms to cue onset, and +80 ms from Go, there was a drop in performance (shaded; in **bold** and with asterisk, significant in at least one session, Fisher's exact test, Bonferroni corrected, $p<0.05$). Note that since these were two-target free-choice trials, we did not assign aborted, incomplete trials to left or right choices; therefore, here the trials are not divided into contraversive and ipsiversive. However, the plot of eye position trajectories in error trials, similar to the **Figure 9E** for the instructed trials, demonstrated similar effects: saccades to the ipsiversive cue after the offset of early -80 ms stimulation, and contraversive undershooting in +80 ms from Go late stimulation period (plot not shown).

Stimulation period onset	Hit rate (%)		
	Both monkeys	Monkey C	Monkey L
Control (no stimulation)	92 ± 4	89 ± 6	97 ± 2
-80 ms to Cue onset	**76 ± 4**	**78 ± 7**	**72 ± 1**
+80 ms from Cue onset	93 ± 6	88 ± 10	99 ± 1
-80 ms to Go	89 ± 6	85 ± 9	95 ± 0
+80 ms from Go	**74 ± 15**	**67 ± 25**	**86 ± 4**

Figure 1



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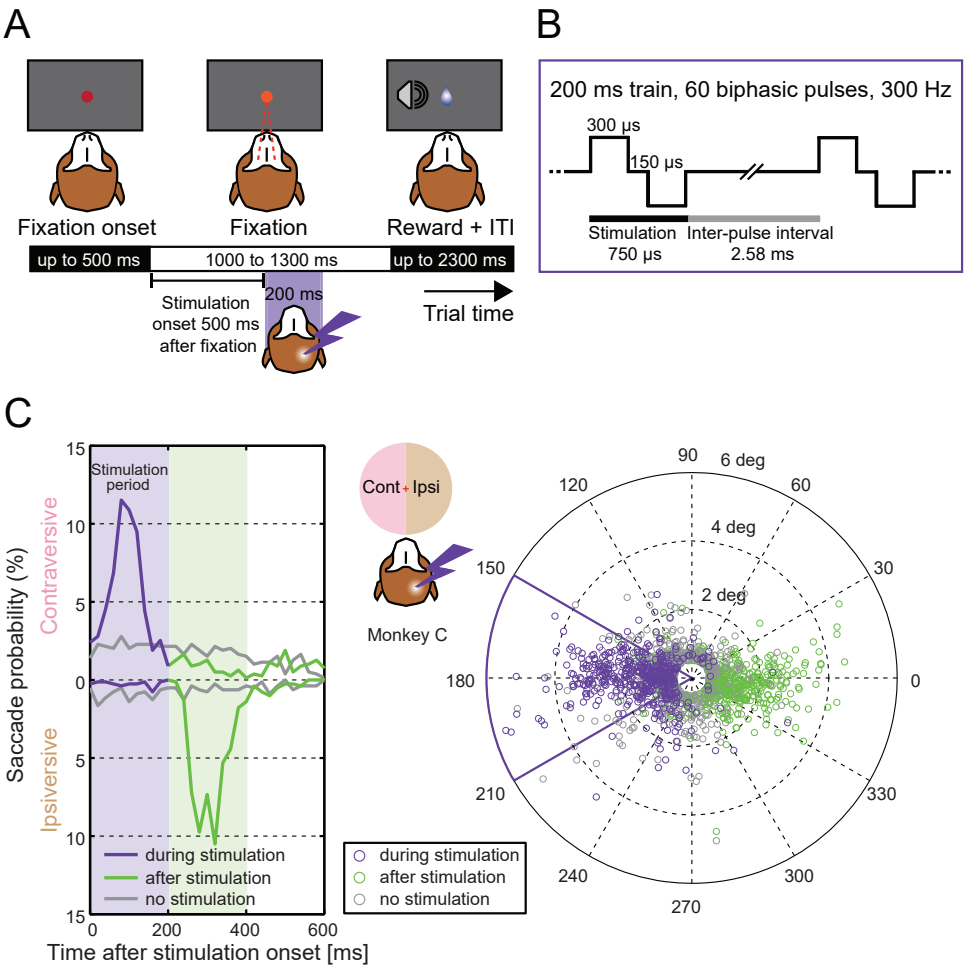

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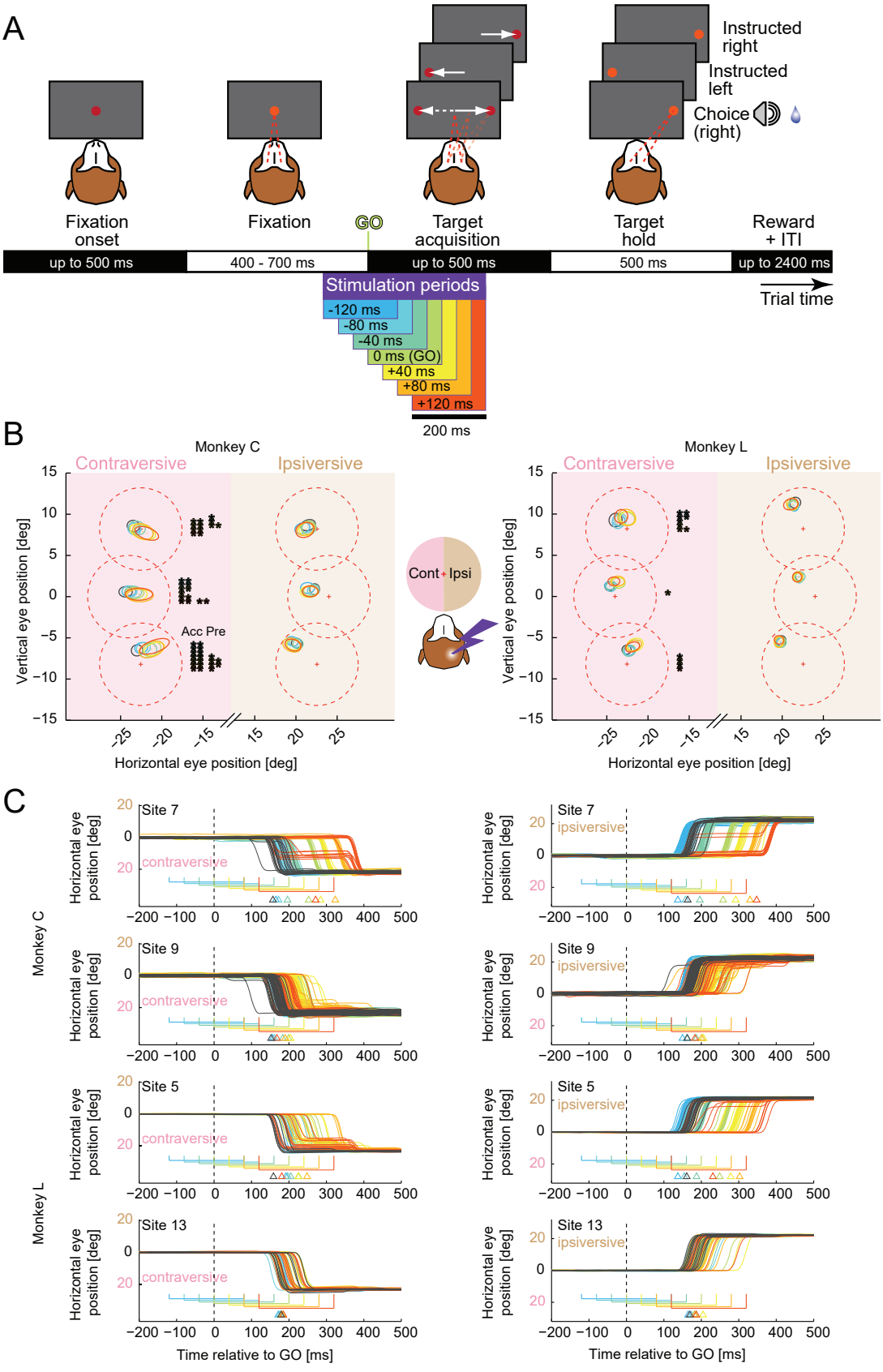

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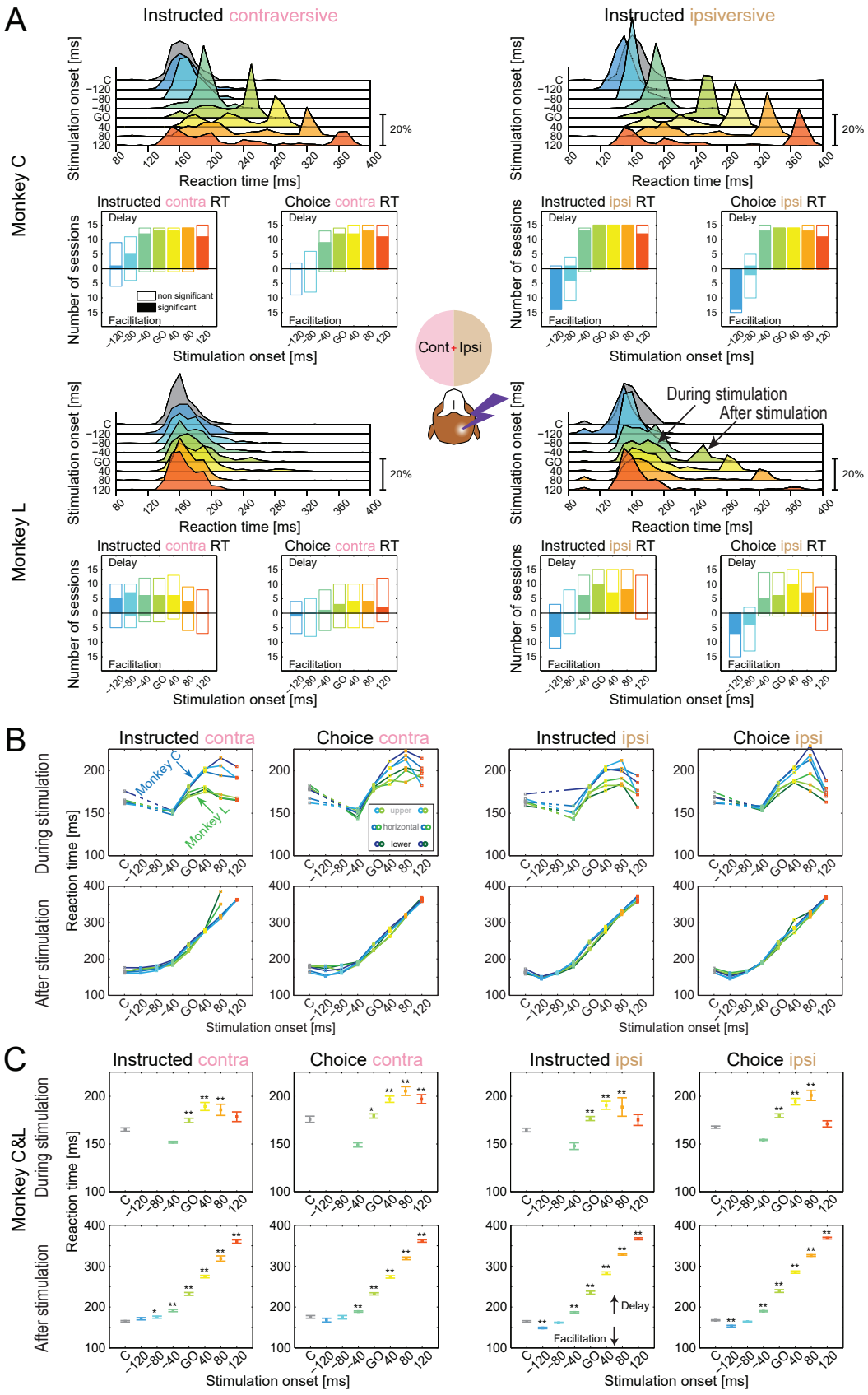

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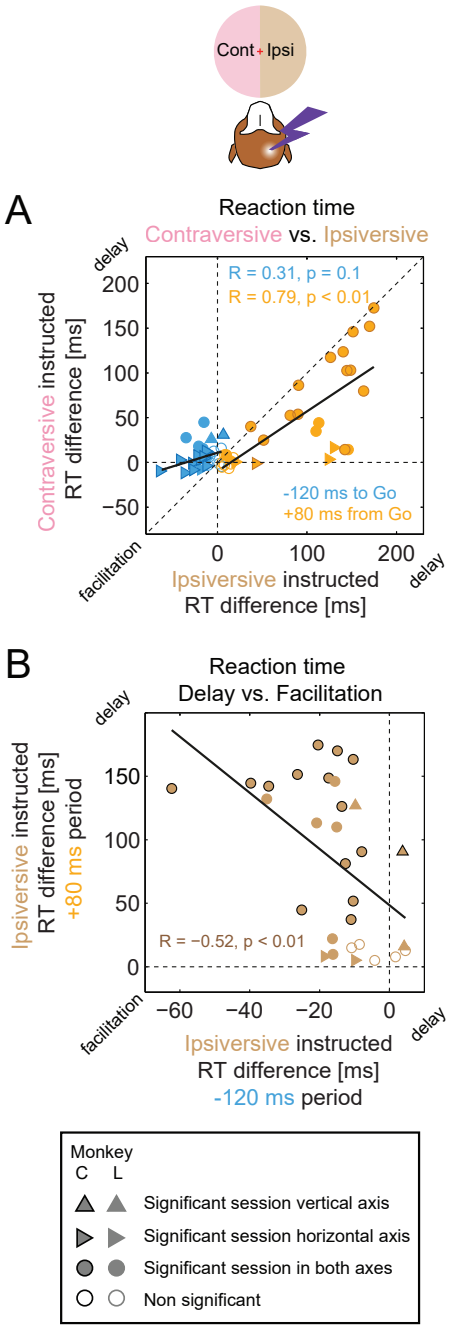

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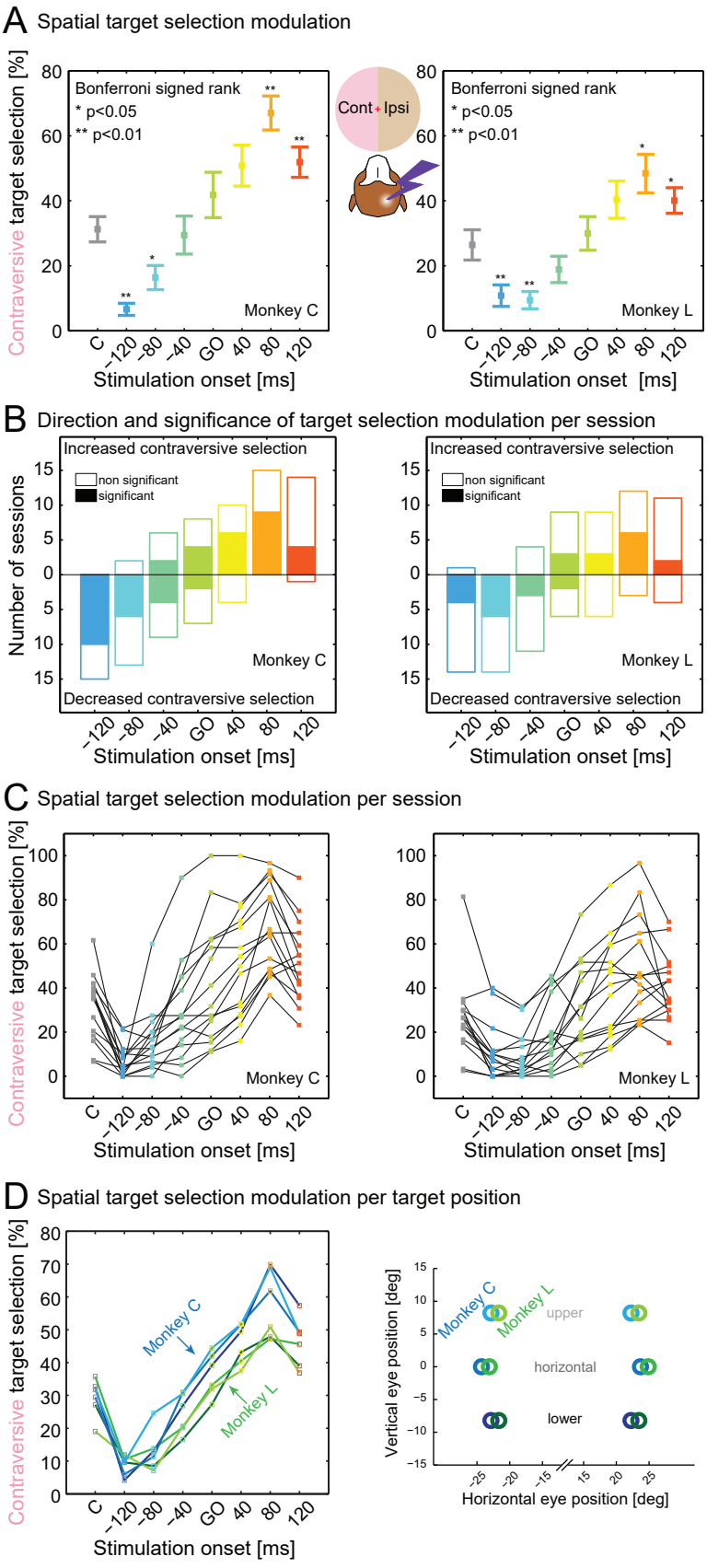

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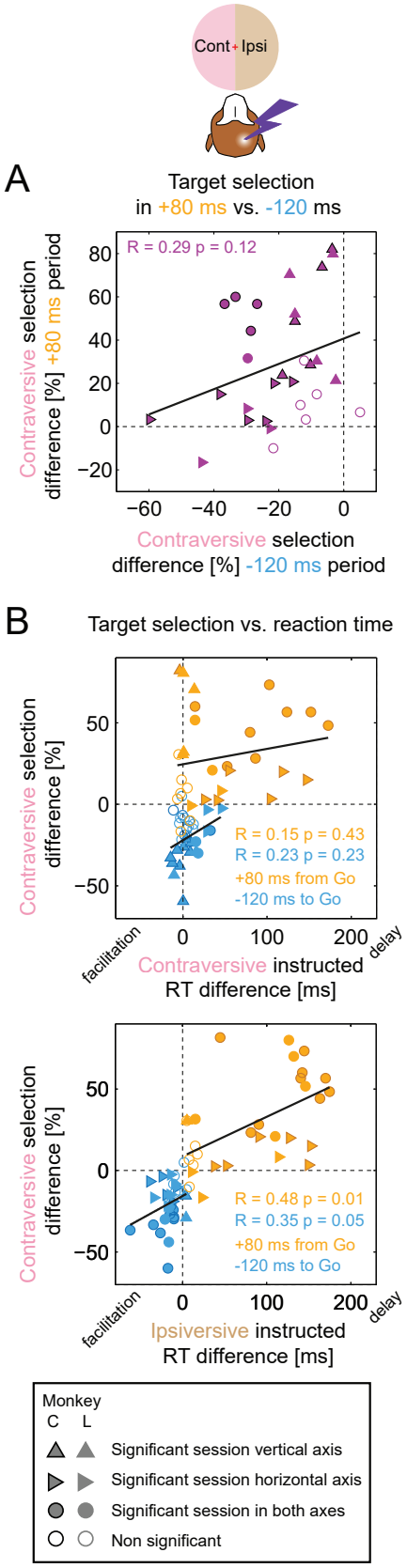

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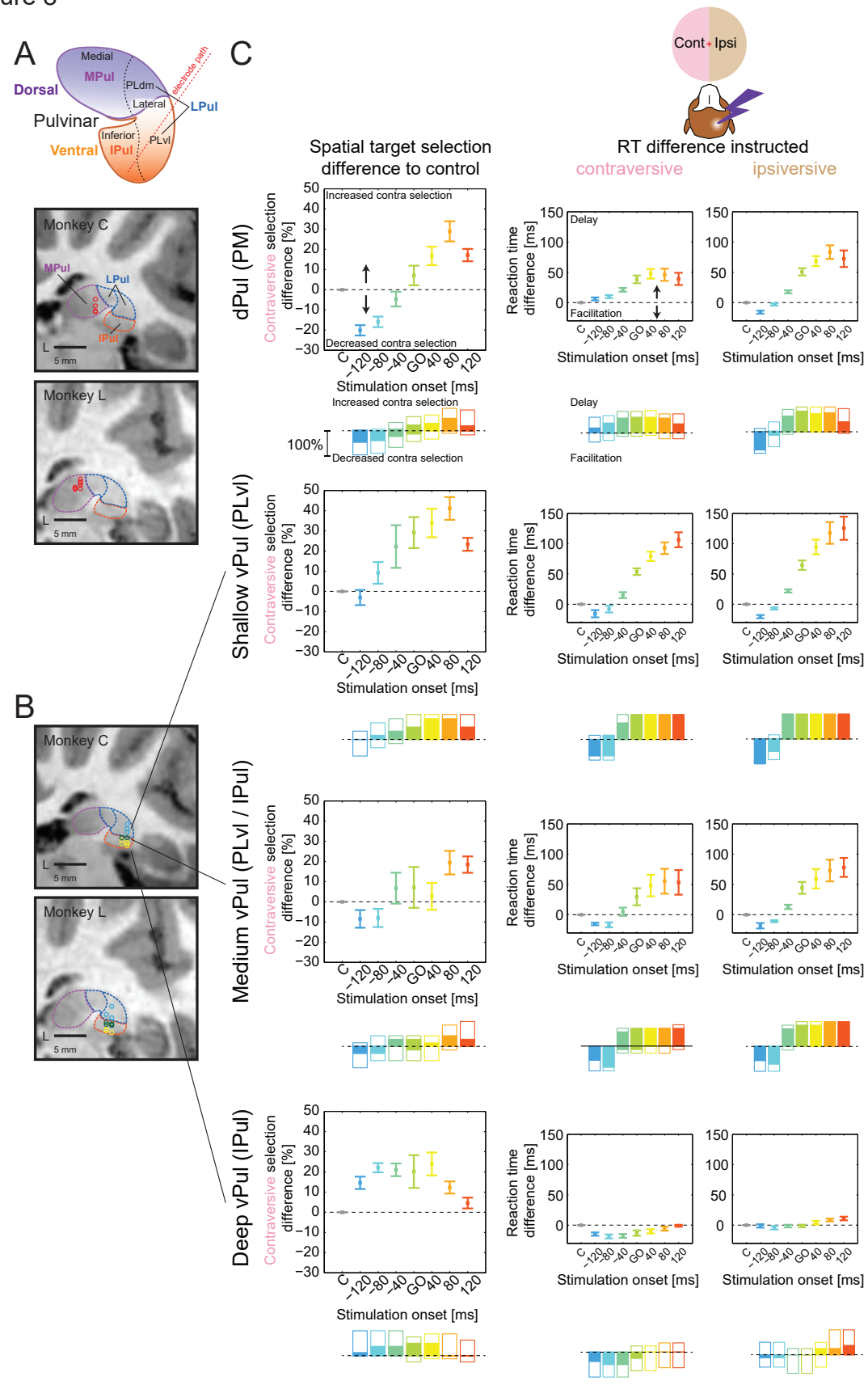

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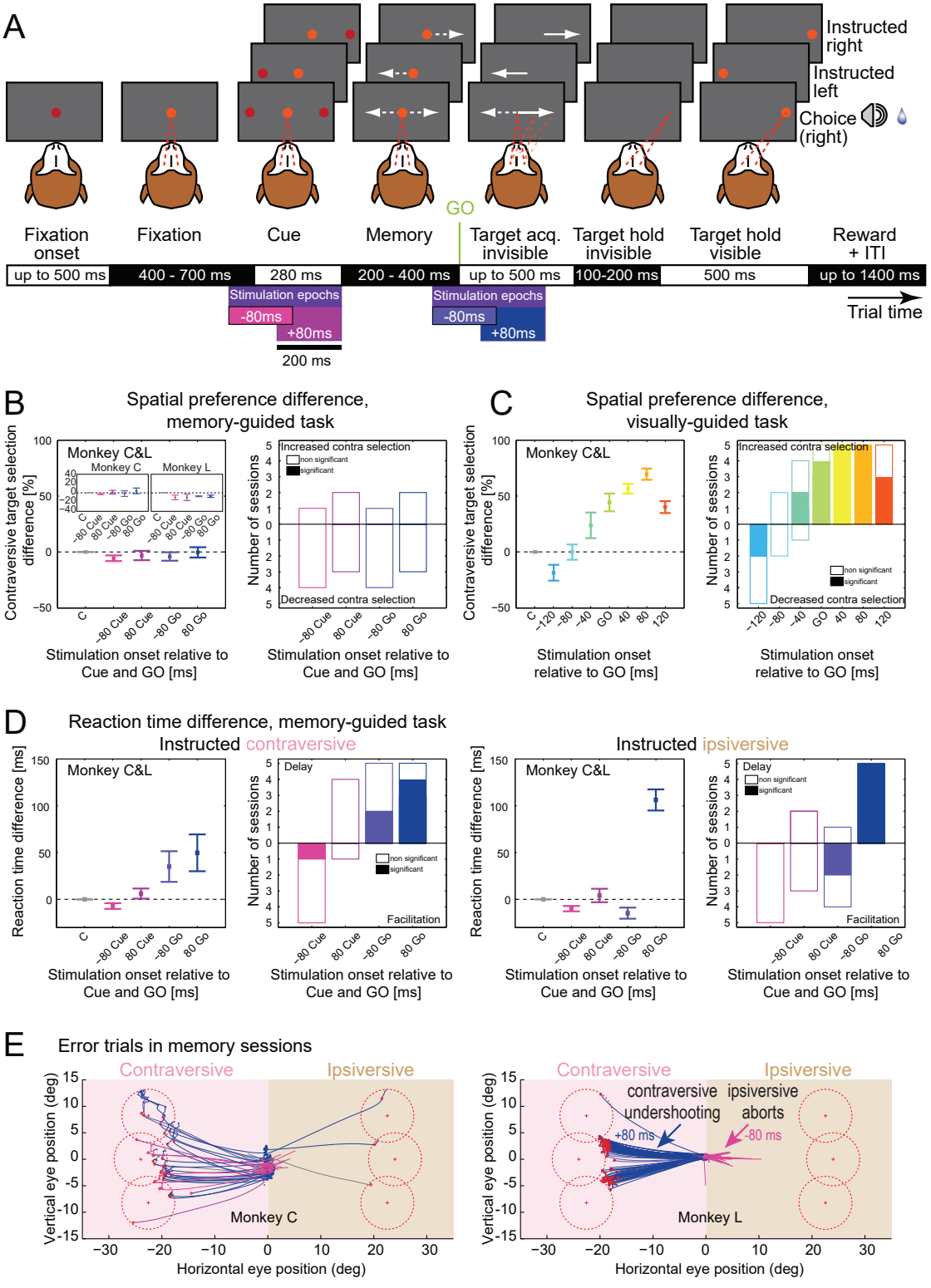

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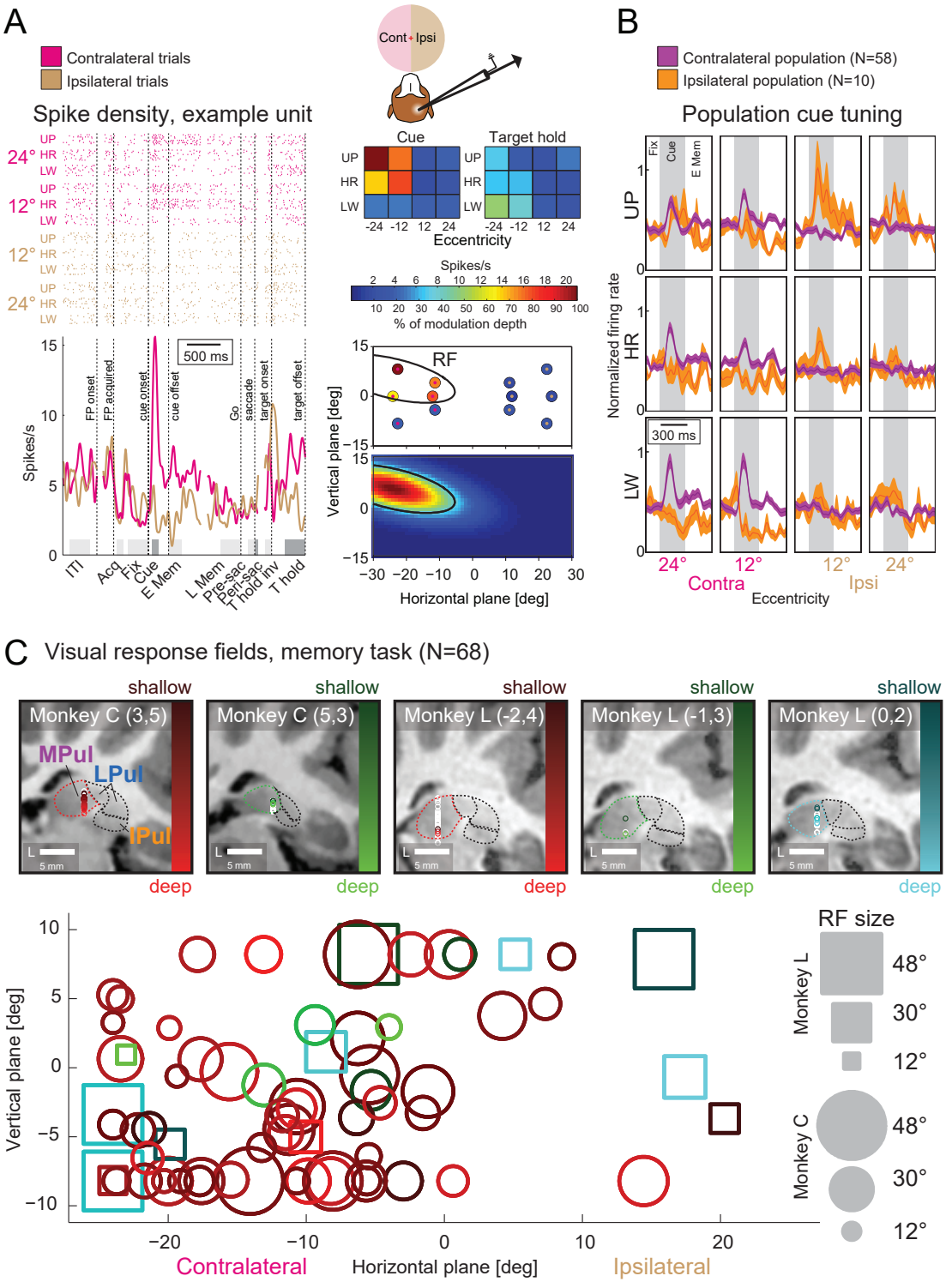

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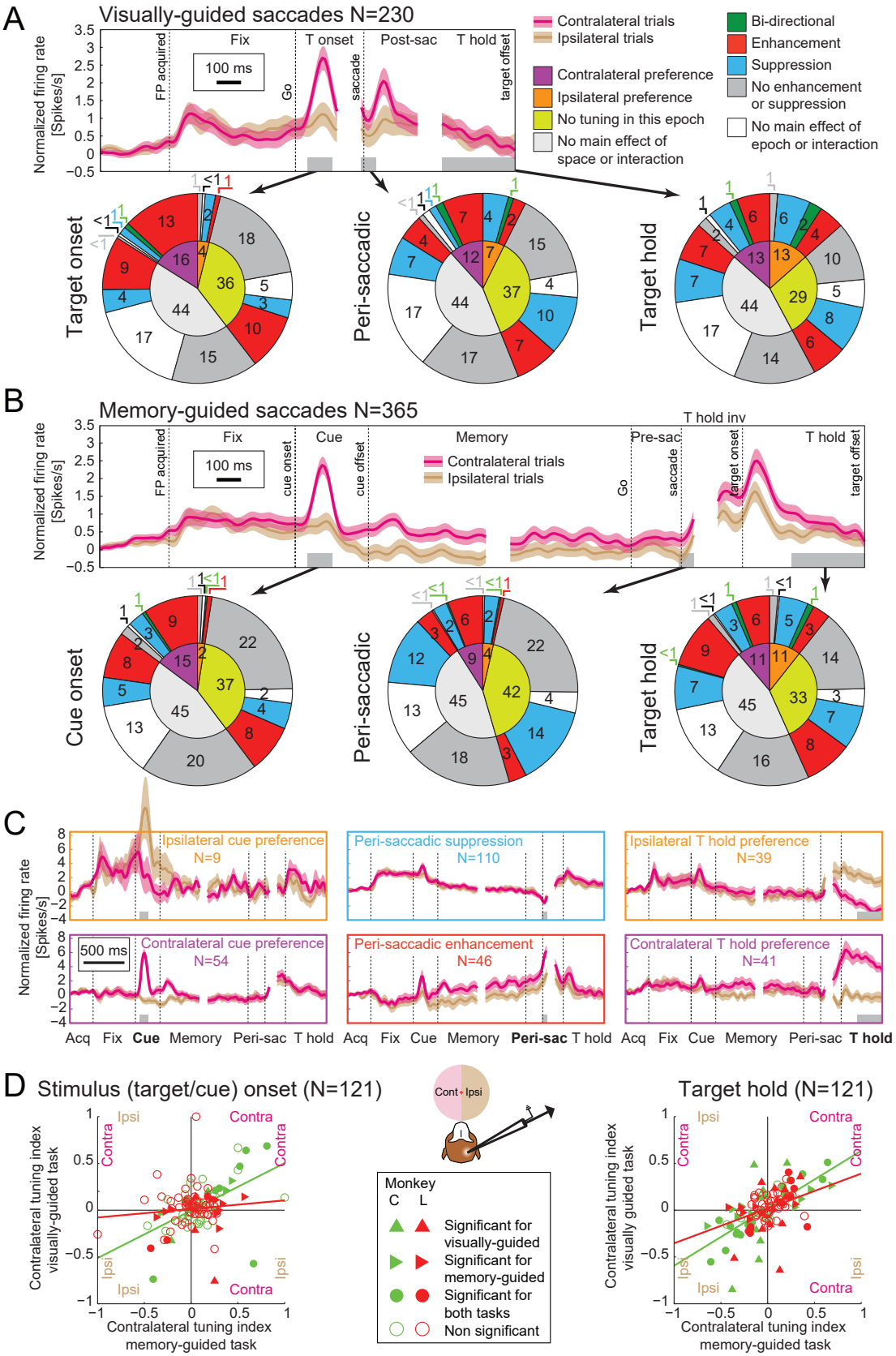

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L-dPul-V-4	696	6	22	250	-	50
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L-dPul-V-6	1895	0, 3	65	250	250	50
L-dPul-V-7	960	0	21	250	700*	29
L-dPul-V-8	960	5	24	250	19	14
L-dPul-V-9	960	5	21	250	160	32
L-dPul-V-10	960	8	52	250	200	60
L-dPul-V-11	960	5	45	250	100	40
L-dPul-V-12	960	5	34	250	180	25
L-dPul-V-13	480	3	41	250	65	24
L-dPul-V-14	480	7	84	250	60	28
L-dPul-V-15	480	7	36	250	600*	22
C-dPul-V-1	818	0	49	250	105	60
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C-dPul-V-7	960	0	62	250	1100*	33
C-dPul-V-8	960	5	49	150	1100*	33
C-dPul-V-9	1920	0, 5	38	250	1300*	65
C-dPul-V-10	960	2	43	250	390*	32
C-dPul-V-11	960	0	37	250	65	28
C-dPul-V-12	480	-2	47	250	75	20
C-dPul-V-13	480	0	51	150	600*	19.5
C-dPul-V-14	480	10	64	250	170	-
C-dPul-V-15	864	6	61	200	440*	38
L-dPul-M-1	600	-5	22	250	60	28
L-dPul-M-2	600	5	54	250	600*	22
C-dPul-M-1	600	3	89	150	600*	19.5
C-dPul-M-2	600	6	53	200	440*	38
C-dPul-M-3	240	10	93	250	170	-
L-vsPul-V-1	960	0	36	250	700*	25
L-vsPul-V-2	960	2	19	200	850*	20
L-vsPul-V-3	960	3	19	250	33	25
C-vsPul-V-1	960	2	37	250	600*	200
C-vsPul-V-2	960	5	34	250	37	35
C-vsPul-V-3	960	0	71	150	41	31
L-vmPul-V-1	960	4	11	250	140	29
L-vmPul-V-2	1920	3	22	150, 200	850*	12
L-vmPul-V-3	960	2	19	250	25	20
L-vmPul-V-4	960	3	33	250	40	21
L-vmPul-V-5	960	3	19	250	19	11
C-vmPul-V-1	960	0	66	250	41	31
C-vmPul-V-2	960	0	50	250	600*	60
L-vdPul-V-1	960	3	32	200	40	21
L-vdPul-V-2	960	3	19	250	19	11
L-vdPul-V-3	960	3	19	250	33	25
C-vdPul-V-1	960	2	46	250	28	19
C-vdPul-V-2	960	0	44	250	37	35
C-vdPul-V-3	960	0	33	250	41	31
C-vdPul-V-4	960	0	59	250	600*	60
C-vdPul-V-5	960	0	46	200	200	19

Table 2. Hit rates in the visually-guided saccade task, instructed trials, dorsal pulvinar stimulation (mean ± SE across sessions).

Stimulation period onset	Contraversive hit rate (%)			Ipsiversive hit rate (%)		
	Both monkeys	Monkey C	Monkey L	Both monkeys	Monkey C	Monkey L
Control (no stimulation)	99 ± 0	99 ± 1	100 ± 0	98 ± 1	100 ± 0	97 ± 1
-120 ms to Go	100 ± 0	99 ± 1	100 ± 0	98 ± 1	98 ± 1	98 ± 1
-80 ms to Go	99 ± 0	99 ± 1	100 ± 0	99 ± 0	99 ± 0	99 ± 1
-40 ms to Go	99 ± 0	99 ± 1	100 ± 0	99 ± 0	100 ± 0	99 ± 0
Go (target onset)	98 ± 1	98 ± 1	99 ± 0	97 ± 1	98 ± 1	96 ± 1
+40 ms from Go	97 ± 1	96 ± 1	99 ± 1	98 ± 0	98 ± 1	98 ± 1
+80 ms from Go	98 ± 1	97 ± 2	99 ± 1	97 ± 1	98 ± 1	97 ± 1
+120 ms from Go	97 ± 1	95 ± 2	100 ± 0	96 ± 1	97 ± 1	95 ± 2

Table 3. Hit rates in the memory-guided saccade task, instructed trials, dorsal pulvinar stimulation (mean ± SE across sessions). In two stimulation periods, -80 ms to ipsiversive cue onset, and +80 ms from Go in contraversive trials, there was a drop in performance <80% (shaded; in **bold** and with asterisk, significant in at least one session, Fisher's exact test, Bonferroni corrected, $p<0.05$). Hit rates drops <80% that did not reach significance are marked in *italics*.

Stimulation period onset	Contraversive hit rate (%)			Ipsiversive hit rate (%)		
	Both monkeys	Monkey C	Monkey L	Both monkeys	Monkey C	Monkey L
Control (no stimulation)	96 ± 2	93 ± 3	100 ± 0	89 ± 8.3	82 ± 13	100 ± 0
-80 ms to Cue onset	94 ± 5	90 ± 7	100 ± 0	**73 ± 9***	*74± 14*	**71 ± 11***
+80 ms from Cue onset	96 ± 2	94 ± 3	98 ± 2	91 ± 8	84 ± 13	100 ± 0
-80 ms to Go	89 ± 5	87 ± 7	92 ± 8	84 ± 15	*74 ± 25*	98 ± 2
+80 ms from Go	**53 ± 14**	**52 ± 22***	**54 ± 21***	89± 7	84± 12	97 ± 0

Table 4. Hit rates (a fraction of successfully completed trials, regardless of chosen hemifield) in the memory-guided saccade task, choice trials, dorsal pulvinar stimulation (mean ± SE across sessions). Similar to instructed trials, in two stimulation periods, -80 ms to cue onset, and +80 ms from Go, there was a drop in performance (shaded; in **bold** and with asterisk, significant in at least one session, Fisher's exact test, Bonferroni corrected, $p<0.05$). Note that since these were two-target free-choice trials, we did not assign aborted, incomplete trials to left or right choices; therefore, here the trials are not divided into contraversive and ipsiversive. However, the plot of eye position trajectories in error trials, similar to the **Figure 9E** for the instructed trials, demonstrated similar effects: saccades to the ipsiversive cue after the offset of early -80 ms stimulation, and contraversive undershooting in +80 ms from Go late stimulation period (plot not shown).

Stimulation period onset	Hit rate (%)		
	Both monkeys	Monkey C	Monkey L
Control (no stimulation)	92 ± 4	89 ± 6	97 ± 2
-80 ms to Cue onset	**76 ± 4**	**78 ± 7**	**72 ± 1**
+80 ms from Cue onset	93 ± 6	88 ± 10	99 ± 1
-80 ms to Go	89 ± 6	85 ± 9	95 ± 0
+80 ms from Go	**74 ± 15**	**67 ± 25**	**86 ± 4**