The Role of the Pulvinar in Selective Visual Attention

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Abstract

Our visual system has a limited capacity to process the complex, cluttered environment around us. Information relevant to guiding our current behavior needs to be selectively enhanced. This process is known as selective visual attention. Lesion studies and electrophysiological recordings reveal a network of brain areas, including the pulvinar nucleus of the thalamus, that is important for visual attention. However, the role of the pulvinar in visual attention and how it interacts with other areas is poorly understood. To approach this problem, we first developed novel methods to record neuronal activity from multiple subdivisions of the pulvinar in macaque monkeys using multi-electrode arrays while they performed a spatial attention task. During the period of the task when attention was maintained, spiking activity of putative inhibitory neurons in the pulvinar was suppressed, suggesting that attention operates through selective disinhibition of pulvinar relay cells. In addition, spikes from dorsal pulvinar neurons synchronized with the local field potential in the ventral pulvinar during attention, which suggests that the dorsal pulvinar and connected attentional control areas may bias early visual areas by modulating ventral pulvinar activity.

Secondly, we identified low-dimensional structure in pulvinar population spiking activity that suggests oscillatory-like dynamics in spiking during sustained attention. Finally, we recorded neuronal activity simultaneously from the lateral pulvinar and two interconnected cortical areas and examined network interactions during attention. We found evidence suggesting that the pulvinar synchronizes oscillatory activity between cortical areas during attention and may also support sustained, elevated spiking activity in these areas. Together, these results provide a more comprehensive understanding of the thalamic and thalamo-cortical networks underlying selective visual attention.
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Chapter 1: General Introduction

Neural Mechanisms of Visual Attention

Every second that we are awake, we receive massive amounts of visual input from the environment around us. Our brains have a limited capacity to process all of this input at once. As a result, information that is relevant to guiding our current behavior needs to be selectively enhanced and processed in detail, at the expense of ignoring most other inputs. This process is known as selective visual attention.

How does the brain implement selective visual attention? Decades of research have identified several neural mechanisms that could underlie visual attention, as well as a broad network of brain areas involved. In macaques, attention to a visual stimulus has been shown to increase the firing rates of neurons in visual areas, such as cortical areas V1, V2, V4, and MT; the lateral geniculate nucleus (LGN); the pulvinar; the thalamic reticular nucleus (TRN); and the superior colliculus (Luck et al., 1997; Treue and Maunsell, 1999; McAlonan et al., 2006; McAlonan et al., 2008; Zenon and Krauzlis, 2012). Attention has also been shown to reduce the variability in spiking responses across stimulus repetitions (Mitchell et al., 2007; Cohen and Maunsell, 2009) and to modulate noise correlations in spike counts across neurons (Cohen and Maunsell, 2009; Mitchell et al., 2009; Ruff and Cohen, 2014). Finally, attention has been shown to increase both local synchrony within a brain area and long-distance synchrony across brain areas (Fries et al., 2001; Gregoriou et al., 2009; Buffalo et al., 2011; Bastos et al., 2015).

While the first three mentioned neural correlates of attention – increases in firing rate, reductions in response variability, and modulation of spike count correlations – all serve to increase the signal-to-noise ratio of neuronal populations, how might increasing neuronal synchrony subserve selective attention? Slice recordings have demonstrated that oscillations in a neuron’s membrane potential create temporal windows of high and low excitability (Volgushev et al., 1998; Burchell et al., 1998), during which inputs are more and less likely to generate spike output. Further, in vivo recordings have shown that the amplitude of a neuron’s evoked response to a sensory input depends on the timing of that input relative to the phase of an ongoing
oscillation (Cardin et al., 2009). One can extrapolate these findings to the level of neuronal populations: oscillations in the local field potential (LFP) thus reflect rhythmic fluctuations in the excitability of the local population. Fries (2005, 2015) hypothesized that increasing synchrony between groups of neurons facilitates their communication, a theory termed “Communication by Coherence.” When two neuronal groups are synchronized, their windows of high excitability are aligned, allowing spikes to propagate efficiently between them (Fries, 2005; Fries, 2015; Briggs and Usrey, 2007). Changing the synchronization patterns within a large-scale network could change which groups of neurons are effectively communicating without altering the network structure. This dynamic synchronization could bias information processing according to task demands and therefore serve as a neural mechanism of selective attention. Indeed, it has been shown that, when multiple stimuli compete for representation by higher-order areas, attention selectively synchronizes neuronal populations between lower-order and higher-order areas according to which stimulus is attended (Bosma et al., 2012; Grothe et al., 2012).

How might synchronization of neuronal groups across the brain occur? Most thalamic nuclei have widespread reciprocal connectivity with cortical and subcortical areas and are in a prime position to broadcast rhythms to selected neuronal populations across brain areas. This synchronization of multiple brain areas at the same oscillation frequency could lead to efficient information transmission of selected representations throughout the cortical visual processing hierarchy.

Most studies of selective visual attention and inter-areal synchrony have focused on the cerebral cortex, in part because the cortex is easier to access electrophysiologically in primates and much more is known about its circuitry. However, there is increasing evidence that the thalamus plays an important role in attention and other cognitive functions (Saalmann and Kastner, 2009; Halassa and Kastner, 2017). In particular, the pulvinar nucleus of the thalamus has long been thought to be critically important in visual attention, based on studies of pulvinar lesions and its widespread connectivity with visual areas (Chalupa, 1977; Petersen et al., 1987). However, few studies have directly explored the role of the pulvinar in selective visual attention and its interactions with the cortex.
Anatomy of the Pulvinar

The pulvinar is the largest nucleus of the primate thalamus. In an early anatomical study, it was referred to as the “terra incognita” of the thalamus, because of how understudied and poorly understood it was compared to the cortex and other thalamic nuclei (Walker, 1966). Since then, much more has been learned about the pulvinar, but it remains a mysterious structure in both anatomy and function.

The pulvinar primarily receives inputs from and sends output to nearly all visual areas, as well as some frontal and subcortical areas. Unlike the LGN, which receives direct input from the retina, the pulvinar does not receive any direct input from the retina or other sensory organs and instead interfaces only with cortical and subcortical areas, not including the LGN. Based on anatomical tracing studies, it is commonly thought that the pulvinar contains a topographic map of visual cortical areas; that is, different visual cortical areas, such as V1, V2, V4, TEO, and TE are reciprocally connected with different subregions of the pulvinar (Shipp, 2003). In addition, these connection zones in the pulvinar are thought to overlap such that, as a general rule, any two cortical areas that are directly connected are also indirectly connected through an overlapping region within the pulvinar (Shipp, 2003). For example, V1 and V2 have strong feedforward and feedback connections between them and are also both strongly connected with the pulvinar, such that there is a region of the pulvinar that projects to both V1 and V2 (Adams et al., 2000).

Although relatively few pulvinar neurons have been found to project to multiple cortical areas, close proximity of neurons projecting to different areas suggests that these neurons serve a shared function and interact with each other at minimal wiring costs. These interactions within overlapping cortical connection zones in the pulvinar could facilitate or gate the transfer of feedforward or feedback information between cortical areas, or they could modulate the excitability of multiple connected areas in tandem. Indeed, evidence suggests that the pulvinar synchronizes connected cortical areas during attention (Saalmann et al., 2012) and further evidence is presented in this dissertation.
Subdivisions of the Pulvinar

The pulvinar in humans and Old World monkeys has historically been subdivided according to several different parcellation schemes, based on cytoarchitecture, myeloarchitecture, neurochemistry, connectivity, and retinotopy (Stepniewska, 2004). Traditionally, the pulvinar has been parcellated based on cytoarchitecture into inferior, lateral, medial, and oral subdivisions (Olszewski, 1952), and this parcellation is commonly used in modern atlases (Paxinos et al., 2000; Saleem and Logothetis, 2012). As a result, many studies reference these traditional subdivisions. However, because the traditional parcellation is based on cell and axon density, including of axons passing through the pulvinar that are assumed not to be important to pulvinar function, it does not reflect well the finer-grained functional and connectional organization of the pulvinar. Newer parcellation schemes using different methods are able to capture this more detailed organization but have yet to agree in their details. Nevertheless, they largely agree on broadly splitting the pulvinar into dorsal and ventral subdivisions, demarcated approximately by the brachium of the superior colliculus. In neurophysiological experiments, this simple parcellation is especially useful because the brachium of the superior colliculus is readily visible in T2-weighted MR images. Thus, in the experiments described in Chapters 2, 3, and 4, we subdivide the pulvinar into dorsal and ventral subdivisions. Separately, in chapter 5, we analyze data from recordings across the lateral pulvinar.

The dorsal and ventral pulvinar differ substantially in their response properties and anatomical connectivity. The ventral pulvinar contains two well-defined retinotopic maps of the contralateral visual field: a more medial map, termed PI, which corresponds to much of the traditional inferior pulvinar plus the ventromedial part of the traditional lateral pulvinar; and a more lateral map, termed PL, which corresponds to the ventrolateral part of the traditional lateral pulvinar (Bender, 1981). The PI and PL maps also correspond to two maps termed P1 and P2, which are defined based on the organization of retinotopic inputs from V1 (Ungerleider et al., 1983), V2 (Ungerleider et al., 2014), V4 (Gattass et al., 2014), and MT (Ungerleider et al., 1984). The PI and PL maps share a representation of the vertical meridian and are arranged such that the PL map partially surrounds the PI map and the PL map has a split representation of the more
eccentric part of the horizontal meridian. This arrangement is similar to how areas V1 and V2 share a representation of the vertical meridian and V2 has a split representation of the horizontal meridian; thus, dorsal V2 represents the lower visual field and ventral V2 represents the upper visual field. In addition, receptive field sizes are larger in PL than in PI. Together these findings suggest the PI map is a first-order map like V1, and PL map is a second-order map like V2 (Bender, 1981). However, several different visual areas, including both V1 and V2, project to both pulvinar maps, in a topographic manner along an orthogonal anterior-posterior axis. It remains unclear what information is represented in each map and how the maps differ functionally from each other.

The dorsal pulvinar corresponds approximately to the medial pulvinar plus the dorsomedial portion of the lateral pulvinar. Bender (1981) and Petersen et al. (1985) identified a scattering of visually responsive neurons within the dorsomedial portion of the lateral pulvinar that may be coarsely retinotopically organized and labeled this area as Pdm. This region may be the same as the coarse retinotopic map defined based on retinotopic connectivity with the cortex, termed P4 by Ungerleider and colleagues (Ungerleider et al., 2014; Gattass et al., 2014). No other retinotopy has been identified in the dorsal pulvinar.

Neurons in the ventral pulvinar have reciprocal connections with areas V1, V2, V4, TEO, TE, MT, MST, and the superficial layers of the superior colliculus. Neurons in the dorsal pulvinar do not connect with V1, V2, or the superficial layers of the superior colliculus, but do connect with higher order areas of the visual cortical hierarchy, e.g. areas V4, TEO, TE, 7a, lateral intraparietal area (LIP), frontal eye fields (FEF), insula, dorsolateral prefrontal cortex, orbital frontal cortex (OFC), cingulate cortex, the amygdala, the hippocampus, and the intermediate and deep layers of the superior colliculus (Shipp, 2015). Given these anatomical connections, it is commonly thought that the ventral pulvinar is involved in low-level visual processing, and the dorsal pulvinar performs a more abstract function that may involve high-level visual processing, oculomotor integration, memory, emotion, planning, and cognitive control.
Lesions of the Pulvinar

Studies of pulvinar lesions have strongly suggested the involvement of the pulvinar in visual attention and visually guided movements. Lesions of the pulvinar in humans lead to deficits in directing spatial attention and filtering out visual distractors specific to the contralesional visual field (Arend et al., 2008; Rafal and Posner, 1987; Snow et al., 2009; Ward et al., 2002; Ward and Arend, 2007; Danziger et al., 2002; Karnath et al., 2002). Though human pulvinar lesions are often poorly localized and likely include damage to the corticotectal tract passing through the pulvinar, making these studies difficult to interpret, targeted reversible inactivation of the pulvinar in macaques leads to similar attentional dysfunction (Wilke et al., 2010, Petersen et al., 1987; Desimone et al., 1990). Further, inactivation of the dorsal pulvinar in macaques results in biases of saccade activity and decision making (Wilke et al., 2010; Wilke et al., 2013), suggesting different functions for different subdivisions of the pulvinar. Another study showed that inactivation of the lateral pulvinar dramatically suppressed both baseline firing and evoked firing of supragranular neurons in V1, suggesting active inputs from the pulvinar are necessary for the propagation of information through the cortex (Purushothaman et al., 2012).

The Pulvinar and Attention

How might the pulvinar be involved in selective visual attention? That is, what are the neural mechanisms underlying attention in the pulvinar? As described earlier, there are several neural correlates of attention observed across the brain, including increasing firing rates, reducing spiking response variability, modulating noise correlations, and increasing local and inter-areal synchrony. Petersen et al. (1985) found that half (8/16) of neurons in macaque Pdm were modulated by selective attention in a simple task where monkeys had to detect when a dimming of a stimulus. Most of these modulated neurons having increased firing rate with attention, with a small proportion having reduced firing rate (Fig. 14 in Petersen et al., 1985). Bender and Youakim (2001) found that 26% (61/237) of neurons across the pulvinar modulated their firing rates during attentive fixation to a stimulus in the receptive field, though it is difficult to dissociate effects of attention and of fixation in this study.
Given the pulvinar’s extensive connectivity with the rest of the brain, it is well positioned to synchronize multiple brain areas at once and enhance selected neuronal representations across brain areas according to attentional demands. Two recent studies investigated how the pulvinar interacts with ventral visual stream areas during selective visual attention. Saalmann and colleagues (2012) recorded single units and local field potentials from the macaque ventrolateral pulvinar and two interconnected cortical sites, areas V4 and TEO, simultaneously while monkeys performed an attention task. They found that during attention, ventrolateral pulvinar neurons increased their firing rates with attention. In addition, there was increased phase synchrony in the alpha frequency range (8 to 12 Hz) between local field potentials in the pulvinar, V4, and TEO during attention, suggesting functional coupling between the three sites and the relevance of alpha oscillations during attention. In addition, pulvinar neurons showed increased coupling between spikes and the phase of ongoing alpha oscillations in the pulvinar, V4, and TEO during attention, and there was increased Granger-causal influence of the pulvinar on each cortical area, after accounting for the influence of the third area. Together, these results corroborate previous findings that neurons in the ventrolateral pulvinar modulate their firing rate during visual attention and further suggest the pulvinar drives alpha oscillations in V4 and TEO and synchronizes those oscillations in order to facilitate information transmission.

Zhou and colleagues (2016) performed a similar experiment to Saalmann et al. (2012), except they recorded from the ventrolateral pulvinar, V4, and IT, simultaneously and looked at how attention modulated firing rates and synchrony within and between areas when monkeys directed attention to a cued object, waiting for a color change. They found that pulvinar neurons increased their firing rates during attention to a stimulus (as opposed to a cued, empty location as in Saalmann et al. (2012)) and that attention increased the synchrony between pulvinar spikes and V4 LFPs and between V4 spikes and pulvinar LFPs, both in the gamma frequency band. Reversible inactivation of the pulvinar reduced both the sensory response and attentional modulation of gamma spike-field coherence within V4. In addition, pulvinar inactivation increased low-frequency power in V4, which is commonly associated with an idling state of the cortex. Together with the inactivation results from Purushothaman and colleagues (2012), these results
suggest the pulvinar synchronizes with the cortex during attention and maintains the cortex in an active state to process incoming visual information.

These studies demonstrate clearly the importance of the pulvinar in selective visual attention. However, much remains to be learned regarding the specific neural mechanisms involving the pulvinar that underlie attention. In this dissertation, I first describe novel methods that we have developed to record from the pulvinar in awake, behaving macaques using multielectrode arrays and to identify different pulvinar subdivisions functionally. Using these methods, I then characterize attention-related neuronal activity within the pulvinar with respect to different subdivisions and cell types. Next, I describe how we use a novel analytical method to infer “de-noised” pulvinar firing rates and observe oscillatory-like dynamics in population spiking. And finally, I describe how attention modulates interactions between the pulvinar, the dorsal visual cortical stream, and the ventral cortical stream.

Together, the studies presented in this dissertation characterize how neuronal signals both within the pulvinar and between the pulvinar and cortex relate to selective visual attention. Furthermore, these studies provide evidence for several mechanisms by which the pulvinar could modulate the gain of information transmission across brain areas according to attentional demands, via disinhibition of relay cells within the pulvinar and modulation of synchrony between cortical areas.
Chapter 2: Overall Materials and Methods

Experimental subjects

The studies described in Chapters 3 and 4 used one male *Macaca fascicularis* monkey (animal M; 11-14 years old) and one male *Macaca mulatta* monkey (animal F; 3-5 years old). The Princeton University Animal Care and Use Committee approved all procedures, which conformed to the National Institutes of Health guidelines for the humane care and use of laboratory animals.

Behavioral task

Monkeys were trained to perform a variant of the Eriksen flanker task (Eriksen, 1995; Fig. 1). They initiated trials by depressing a response lever after an auditory "go" tone. At trial onset, a small white square fixation point (1.3° length) appeared at the center of the monitor. The monkey was required to fixate on the square to continue. After a variable delay of 350 to 450 ms, a circular spatial cue randomly appeared (6° radius, 100 ms duration) at one of four possible stimulus locations evenly distributed around the fixation point (8.75° eccentricity). After the offset of the cue and a variable delay period of 500 to 800 ms, an array of four circular stimuli (9.82° radius) - two grating and two plaid patterns - appeared around the fixation point at the four possible stimulus locations. The grating pattern was a circular, static, black and white, square wave pattern. The plaid pattern was a circular crop of a static, yellow and blue, square wave superimposed on a rotated copy of itself. This 500 to 800 ms delay period between cue offset and array onset is referred to as the "cue-target delay period."

On approximately 80% of trials, the stimulus that appeared at the cued location ("the target") was a grating. The grating dimmed after 475 to 1070 ms, with a bias toward longer durations (the strength of the bias was manually adjusted based on the animal's performance). After the grating dimmed, the monkey needed to release the lever immediately for juice or water reward (280-730 ms after target dimming). This 475 to 1050 ms delay period between array onset and target dimming is referred to as the “target-dim delay period.” On the remaining 20% of trials,
Figure 1. Behavioral task design. Animals press the lever and fixate the central fixation point (white square, back panel) to initiate the trial. Then a cue (white circle, second panel) appears in one of four locations, selected randomly, and disappears after 100 ms (third panel). After a variable delay period, an array of four shapes appears at the same four locations. On 80% of trials, a grating appears at the previously cued location. On these trials, this grating (the target) dims after another variable delay period. Animals must release the lever immediately after target dimming to receive a juice reward. On 20% of trials, a plaid appears at the cued location in the array. On these trials, animals must release the lever immediately to receive a juice reward. Trials are classified as Attend-RF if the cue appeared in the receptive field of the neuron (orange dashed circle) and Attend-Away if the cue appeared in the location opposite of the RF.
the target was a plaid, and the monkey needed to release the lever immediately for juice or water reward (280 to 730 ms after array onset).

We monitored eye position using an infrared eye tracker, and trials were aborted if the monkey broke fixation, that is, if eye position deviated by more than two degrees from fixation, up until the time at which the monkey could make a correct response (280 ms after array onset on plaid-target trials and 280 ms after target dimming on grating-target trials).

We controlled stimuli, response monitoring, and rewards using Presentation software (Neurobehavioral Systems, Berkeley, CA). Visual stimuli were presented at 100% contrast (white on gray background) on a 21-inch CRT monitor set at a refresh rate of 85 Hz. We verified visual stimulus timing using a photodiode. Monkeys reported decisions using a manual response lever and received juice (preferred by monkey M) or water (preferred by monkey F) reward delivered by an infusion pump. We monitored eye position using a stationary eye tracking system. We fed the video signal from an infrared camera (operating at 120 Hz) into an ASL eye-tracker (ASL Eye-trac 6; Applied Science Laboratories, Bedford, MA), which interfaced with the experimental control computer running Presentation, in order to allow eye position feedback to influence trial outcomes.

Acquisition of structural MR images for electrode positioning

Animals were sedated with ketamine (1-10 mg/kg i.m.) and xylazine (1-2 mg/kg i.m.), and provided with atropine (0.04 mg/kg i.m.). Sedation was maintained with tiletamine/zolazepam (1-5 mg/kg i.m.). We then placed the animals in an MR-compatible stereotaxic frame (1530M; David Kopf Instruments, Tujunga, CA) and monitored vital signs with wireless ECG and respiration sensors (Siemens AG, Berlin), and a fiber optic temperature probe (FOTS100; Biopac Systems Inc, Goleta, CA). Body temperature was maintained with blankets and a warm water re-circulating pump (TP600; Stryker Corp, Kalamazoo, MI).

We collected structural MRI data for the whole brain on a Siemens 3T MAGNETOM Skyra or Prisma using a Siemens 11-cm loop coil placed above the head. T2-weighted images were acquired with a 3-dimensional turbo spin echo with variable flip-angle echo trains (3D T2-
SPACE) sequence (voxel size: 0.5 mm, slice orientation: sagittal, slice thickness: 0.5 mm, field of view (FoV): 128 mm, FoV phase: 79.7%, repetition time (TR): 3390 ms, echo time (TE): 386 ms, base resolution: 256, acquisition time (TA): 17 min 51 sec). We used these images both to select coordinates for chamber placements and to position electrodes for recordings.

Tungsten electrodes create a clearly identifiable, susceptibility-induced signal void along the length of the electrodes in structural MRI images (Fig. 2c). This “shadow” has a width of approximately one voxel (0.5 mm$^3$ on either side of the electrode), allowing us to visualize electrode placement. Prior to recordings, we inserted one to three tungsten electrodes through the implanted customized grid of guide tubes to just dorsal of the pulvinar and acquired T2-weighted images. Because the pulvinar is a deep structure - approximately 20 to 30 mm from the dorsal surface of the brain, depending on the angle of approach - slight inaccuracy in the angle of the grid can lead to inserted electrodes missing the pulvinar. If the grid angle needed to be adjusted, we removed the electrodes, adjusted the grid, reinserted the electrodes, and acquired new images. The grid was positioned and angled to maximize the number of grid locations that could target the pulvinar. After we began recordings, whenever the grid needed to be replaced or adjusted, we first acquired new images with electrodes inserted before moving the grid. We used these before and after images, as well as daily microdrive measurements, to reconstruct electrode tracks.

**Electrophysiology setup**

All surgical procedures were performed under general anesthesia with isoflurane (induction 2-5%, maintenance 0.5-2.5%) under strictly aseptic conditions. We used titanium and/or ceramic skull screws, Metabond (Parkell, Edgewood, USA), and bone cement (Cobalt HV; DJO, Vista, CA) to affix head implants and customized plastic recording chambers (Cilux material; Crist Instruments, Hagerstown, MD) to the animals. One 4.5 mm craniotomy was drilled within a recording chamber to provide access to the left pulvinar. One to two other craniotomies were also drilled in other recording chambers in each monkey for a separate experiment. A customized grid of guide tubes, through which electrodes later traversed, was placed within the
craniotomy, and held in place semi-chronically at the desired angle with bone wax (Ethicon, Somerville, NJ) and dental acrylic.

**Grid of guide tubes**

The grid of guide tubes is a novel innovation for allowing multiple, densely packed (0.635mm center to center separation), reproducible penetrations, in a semi-chronic, adjustable manner. The grid is MR-compatible and can be filled with sterile saline for visualization of projected electrode tracts down the grid.

The grid was made in the following way:

1) We carefully cut two 200 ul standard pipette tips (Fisher Scientific, Hampton, NH) to have an inner diameter of approximately 4.292 mm or slightly larger (less than 0.01 mm larger) as measured repeatedly using calipers, and we discarded the smaller, tapered parts of the tips

2) We cut 37 polyimide micro-tubes (483 um inner diameter, 635 um outer diameter; Nordson, Westlake, OH) to 60 mm length and pushed them together through the cut pipette tips, testing for a snug fit within the pipette tips with the ability to slide the tubes in and out with moderate friction

3) We spread heavy duty epoxy (Loctite, Dusseldorf) around the tubes to completely cover their outer surfaces except for the ends, and quickly, before the epoxy set, we pushed the bundle of tubes through the two cut pipette tips, such that the wider side of the tips were arranged away from each other

4) We slowly pushed the tips away from each other along the bundle of tubes, without twisting the bundle, until the tips were at opposite ends of the bundle

5) We affixed the bundle to a hard surface with wax and let the epoxy cure for at least 12 hours

6) We used a sharp razor blade to cut the bundle adjacent to the junction between the bundle of tubes and the pipette tips such that we were left with a straight cylinder of tubes glued together. Gluing them together and forcing them through an opening of that diameter creates a dense, hexagonally symmetric packing without allowing for loose tubes (Fig. 2b). The densest
packing of 37 circles within a circle has the diameter of the larger circle being approximately 6.75877 times the diameter of the individual circles (Graham et al., 1998).

7) We used a sharp razor blade to cut down the bundle length to 15 mm long segments, and we visually inspected each guide tube to make sure there were no blockages. Blockages were removed using a 30-gauge needle.

Once the grid was placed in its final position after MR visualization, a light layer of dental acrylic was applied to the outside of the grid and its adhering bone wax to solidly adjoin the grid to the implant (Fig. 2a). If the grid became blocked by regrowth of skull or granulation tissue, this layer of dental acrylic could easily be drilled off and the grid replaced.

Electrophysiology

During recordings, we stabilized the animal's head using four thin metal rods that slid into hollows in the side of the cement implant. We independently micropositioned electrodes with NAN microdrives (NAN Instruments, Nazaret Illit) coupled to an adapter that fit around the top of the recording chamber. We inserted one to two 32-channel linear multi-electrode arrays (Plexon V-probe; Plexon, Dallas, TX) targeting the left pulvinar in acute penetrations in each recording session. Electrode signals were amplified 20X and filtered (4-pole Bessel high-pass 300 Hz for spikes, 4-pole Bessel low-pass 200 Hz for LFPs) using a Plexon preamplifier and using the Plexon Omniplex A data acquisition system (Plexon, Dallas, TX). We sorted spikes online to gauge the stability of the recording and assess whether the electrode had reached the target region. We re-sorted spikes for offline analyses using Plexon Offline Sorter software and custom scripts written in Matlab (Mathworks, Natick, USA). Multi-unit activity (MUA) spikes were detected for each recording channel using thresholding of the high-pass filtered signals. The threshold was set adaptively at 4 standard deviations less than the mean in 250 s windows in order to account for drift in signal quality and properties. MUA spike times were realigned to the lowest point between the time of threshold crossing and 10 samples (0.025 ms) later. There were 32 recording sessions in total (20 sessions with monkey M, 12 sessions with monkey F), with 1-2 electrodes
Figure 2. Novel electrophysiology setup allowing for dense, reproducible, MRI-guided recordings from linear multi-electrode arrays. (a) A 4.5 mm craniotomy is made in the skull, and a grid of polyimide tubes (yellow bars) is embedded in the craniotomy and temporarily secured with bone wax (a, light gray). (b) Top view of the grid of polyimide tubes. (c) Top-left inset: coronal MRI slice around the pulvinar. Bottom-left inset: sagittal MRI around the pulvinar. Main view: coronal slice zoomed in on the left pulvinar with dorsal pulvinar (dPul) and ventral pulvinar (vPul) demarcated. After MRI-guided alignment of the grid (b) to target the region of interest, tungsten electrodes are inserted through select tubes (b, white circles) and imaged around the region of interest to confirm their trajectories (c, white lines). The grid is secured in place with cement or acrylic (a, pink), and the top of the grid is sealed with silicone gel (a, dark gray).
inserted into the pulvinar per session (35 penetrations in monkey M, 20 penetrations with monkey F). Data from the two animals were qualitatively similar, so we combined them for all analyses.

**Spike rate analysis**

We calculated spike density functions, convolving each spike with a 10 ms Gaussian and averaging across trials. Next, we subtracted baseline activity (-200 to 0 ms from cue onset) from the response for each condition. Finally, to normalize responses, we divided the response by the maximum firing rate of any condition. For statistical analysis of delay period activity, we computed the mean normalized spike rates over time in the -175 to 0 ms window from array onset. For statistical analysis of the response to the array, we computed the mean normalized spike rates over time in the 25 to 200 ms window from array onset, only for trials where the grating (hold stimulus) was presented at both the RF location and the opposite location in order to ensure the stimuli presented in the attend-RF and attend-away conditions were identical. To contrast attention to the neuronal RF versus attention away, the neuronal RF is defined as the cue location that evoked the largest firing response 25 to 200 ms after cue onset; the attention away location is defined as the cue location that evoked the smallest firing response 25 to 200 ms after cue onset. Cue locations with insufficient trials or inconsistent firing to the cue were excluded from consideration.

To estimate changes in spike rate over time, time-locked to either the cue or the target, we convolved spikes from each trial with a Gaussian filter ($\sigma = 10$ ms) and averaged the resulting functions across trials. For each neuron, we determined whether there was a statistically significant increase in spike rate in response to the cue or the target (i.e., in the 25 to 200 ms window after cue or array onset) by using a non-parametric randomization procedure.

We randomly selected one response value from the pre-cue period (-175 to 0 ms) of each trial, averaging those values across trials. We repeated this procedure 1000 times to generate a reference distribution (for the baseline spike rate). The p-value for a non-parametric test is the proportion of values in the reference distribution that exceeds the test statistic (i.e., the observed value from collected data). For all statistical comparisons, unless otherwise specified,
we adopted an alpha criterion of 0.05, and used the Holm’s sequential Bonferroni correction to control for multiple comparisons.

To create population peri-stimulus time histograms, we normalized the spike rate for each neuron by its maximum response during trials in any condition, and then grand-averaged the normalized rates across neurons. To test whether between-condition comparisons (i.e., cued vs. uncued) were statistically significant (i.e., to establish significant attention effects), we used a Wilcoxon rank-sum test.

Spike-field coherence analysis

We used the coherence measure to study the temporal relationship between spikes and LFPs from either the same brain area or a different area. The coherency is given by $C(f) = \frac{S_{12}(f)}{\sqrt{(S_{11}(f)S_{22}(f))}}$, where $S(f)$ is the spectrum with subscripts 1 and 2 referring to the simultaneously recorded spike train and LFP. For each paired spike-LFP recording, we calculated the spike-field coherence in 300 ms sliding windows (50 ms steps across the trial) for each attention condition: attention at the RF location or attention away from the RF. Attention at the RF condition reflected the RF of the spiking neuron; however, there was overlap between this RF and the LFP response field. The random location of stimuli from trial-to-trial result in attention conditions from any one recording session having an unequal number of trials. Because the number of trials affects the coherence estimate, we bias-corrected/transformed coherence values (Bokil et al., 2007). The transformed spike-field coherence, $T(f)$, is given by $T(f) = \tanh^{-1}(C(f)) - 1/(v_0-2)$, where $v_0$ is the degrees of freedom. For our multi-taper estimates, $v_0 = 2*K*N$, where $K$ is the number of tapers (3) and $N$ is the number of trials. To obtain population values, we averaged the transformed coherence estimates. To control for spikes affecting the LFP, we excised 2 ms around each spike time from the raw data trace and linearly interpolated these segments of the data trace. Because the results of LFP analyses were the same regardless of whether spikes were excised or not (in the frequency range of interest), we reported LFP data without spike excision. For all spectral analyses, we mainly focused on the delay period after the evoked response until the array onset, because during this period the monkey maintained spatial
attention and the data in each session generally satisfied methodological assumptions of stationarity. To compare attention conditions across the population, we used Wilcoxon signed-rank tests to determine whether there was significantly greater coherence in particular frequency bands (e.g., alpha, beta, and gamma) when attention was at the RF location compared to when attention was away from the RF. For this and all other spike/LFP analyses, we controlled the experiment-wise error rate ($p < 0.05$) using the Holm’s sequential Bonferroni procedure, and reported $p$ values that survived this correction for multiple comparisons, unless otherwise specified.

**Receptive field mapping**

Receptive field locations (RFs) were estimated for single units, multi units, and LFPs separately (Fig. 3). Circular black and white square-wave grating stimuli were flashed in rapid succession around the visual field opposite of the recording hemisphere. Stimuli were on screen for 100 ms. There was a 50 ms inter-stimulus interval, and the next stimulus appeared at least 90 degrees away from the last stimulus. This scheme ensured that sites with RFs smaller than a quadrant would have at least 200 ms without stimulation before another stimulus might appear in the RF. Six to ten stimuli were presented on each trial, and animals had to maintain fixation throughout the trial. As with the primary behavioral task, animals had to press a lever and fixate centrally at the fixation point to initiate a trial, and only after the last stimulus disappeared, the luminance of the fixation point was reduced, and animals had to release the lever within a 175 to 600 ms time window after fixation dimming in order to receive a juice reward. Stimuli were presented at each location (3 to 4 eccentricities, 15 to 19 polar angles; Fig. 3, black dots) between 8 and 12 times. Stimulus evoked responses were averaged across presentations. For single and multi-unit data, mean firing rate per location were averaged in the time window 25 to 200 ms after stimulus onset, smoothed with a two-dimensional Gaussian, and plotted as a heatmap. The location on the extrapolated location map with the strongest response was deemed the RF center. For LFP data, the stimulus evoked response was defined as the difference between the maximum and minimum voltage values of the 10 Hz low-pass-filtered evoked
potential in the time window 25 to 200 ms after stimulus onset. If the largest response was not three standard deviations above baseline, the unit or channel was deemed to not have a RF within our mapping range.

Alignment of electrodes across penetrations

Electrode depth measurements based on the microdrive were often unreliable because our guide tube approach precluded observing when the electrode penetrated the dura and dimpling of the brain during electrode insertion sometimes distorted our measurements. Thus, it was important to develop additional, and more precise, verifications that our electrode was in the pulvinar and assignments of which channels correspond to which subdivisions of the pulvinar.

**Figure 3.** Example LFP response fields of ventral pulvinar electrodes. Gratings were flashed one at a time at locations around the screen (black dots), mostly contralateral to the recording site. Response fields of channels were mapped (colored contours) based on the magnitude of evoked responses. Response fields progressed from the lower visual field on more dorsal channels (red) toward the upper field on more ventral channels (purple).
Because the pulvinar shows strong visual responses, we adapted a method from Schroeder et al. (1991) to create a reproducible, neural flash-evoked response profile for aligning electrodes across penetrations by repeatedly and briefly flashing a full-field visual white stimulus to the animal while he fixated centrally. Most electrodes showed a strong visually evoked potential in the LFP (Fig. 4a) and/or a strong flash-evoked increase or decrease in firing rate (Fig. 4b), and these electrodes were thus highly likely to be localized to the pulvinar.

In addition, on each penetration, we consistently found a contiguous group of electrodes showing clear retinotopy with RF centers moving from the lower visual field to the upper visual field with electrode depth. This pattern is consistent with reported retinotopy in the ventrolateral pulvinar and the inferior pulvinar (Bender, 1981). It was difficult to distinguish the ventrolateral pulvinar with the inferior pulvinar functionally and anatomically, so they are referred to collectively as the ventral pulvinar. There are also no known functional markers distinguishing the dorsal lateral pulvinar with the medial pulvinar, so the pulvinar electrodes dorsal of the ventral pulvinar are referred to as the dorsal pulvinar. The electrodes with retinotopy also showed a strong negativity in the flash-evoked LFP relative to the common average reference, which corroborated our assignment of those electrodes as corresponding to the ventral pulvinar.
Figure 4. Example functional alignment of multi-electrode arrays across sessions using LFP and MUA flash-evoked response profiles across electrodes. (a) Mean Z-scored evoked LFP responses to a 100 ms full-field white flash. Each row represents a different channel on the multielectrode array. Alignment reveals a reproducible evoked negativity spanning about 1 mm, which support the classification of those electrodes as being in the ventral pulvinar. The 10 channels above the top of the ventral pulvinar are classified as the dorsal pulvinar. (b) Same as (a) but plotting z-scored MUA firing rate instead of LFP voltage, revealing probable extent of units and white matter around the pulvinar.
Chapter 3: Mechanisms of Attention within the Pulvinar

Introduction

The pulvinar has long been considered to be important for visual attention. Lesions of the primate pulvinar lead to attentional deficits and spatial neglect, and neurons in the pulvinar respond to visual stimuli and modulate their spiking during visual attention. Few studies have investigated the role of the pulvinar in attention, and their findings have not converged. This is in part because the pulvinar is a large, heterogeneous nucleus, and it has been difficult to identify and target the same subregions electrophysiologically across studies. In addition, past studies have used several different behavioral tasks that are not straightforwardly comparable. In order to resolve these differences and expand upon our understanding of the pulvinar, in the current study, we systematically characterize neuronal activity in the pulvinar, with respect to its different subdivisions and cell types, during two types of selective visual attention.

As the largest nucleus in the primate thalamus, the pulvinar is composed of multiple subregions which communicate with different cortical and subcortical areas and possess diverse electrophysiological response properties (Shipp, 2003; Shipp, 2015). For example, the ventral pulvinar is reciprocally connected primarily with early visual areas and contains two well-defined retinotopic maps, whereas the dorsal pulvinar is reciprocally connected primarily with the frontal eye fields (FEF), the lateral intraparietal area (LIP), and other higher-order visual areas, and it does not contain clear retinotopy (Bender, 1981; Stepniewska, 2004). While the dorsal and ventral pulvinar could be further subdivided, these differences in anatomical connectivity and functional response properties between the dorsal and ventral pulvinar already suggest that these broad subdivisions of the pulvinar play different roles in visual processing and behavior and that attentional modulation may vary between them.

An early study by Petersen and colleagues (1985) characterized visual and attention-related response properties of neurons across three pulvinar subdivisions. The authors found that half of neurons (8 of 16) in the dorsolateral pulvinar (the “Pdm” region or “P4” retinotopic map), very few (1 of 14) in the ventrolateral pulvinar (the “PL” or “P2” retinotopic map), and none (0 in
14) in the inferior pulvinar (the “PI” or “P1” retinotopic map) modulated their firing rate during covert spatial attention. Although this study found that attention modulated only the dorsolateral pulvinar out of the three subdivisions, it suffered from low sample sizes and trial counts. Using stronger experimental designs, recent studies have found that, at the population level, neurons in the lateral pulvinar (not distinguishing between ventrolateral and dorsolateral) increased their firing rates during attention to a cued location, prior to visual stimulation (Saalmann et al., 2012), and neurons in the ventrolateral pulvinar increased their firing rates during attention to a cued stimulus (Zhou et al., 2016), which contradicts the findings from Petersen and colleagues (1985) regarding the ventrolateral pulvinar. Another recent study found that, at the population level, neurons in the dorsomedial pulvinar increased their firing rates during attention to a cued location, with visual stimulation (Fiebelkorn et al., 2019). While these recent studies characterized attentional modulation in the lateral, ventrolateral, and dorsomedial pulvinar and provide converging evidence that the pulvinar displays elevated spiking activity during attention, they assessed each subdivision in isolation and used different behavioral task designs (training animals to attend with and without visual stimulation). It would thus be useful to reproduce these findings and directly compare the neural correlates of attention across pulvinar subdivisions under a unified experimental design. Thus, using microelectrode arrays, we recorded neuronal activity from the dorsal and ventral pulvinar simultaneously, and we used an experimental design composed of both a task period where animals had to direct attention to a cued location, prior to visual stimulation, and a task period where animals had to direct attention to a cued stimulus, thereby combining components emphasized in the studies used by Saalmann and colleagues (2012), Zhou and colleagues (2016), and Fiebelkorn and colleagues (2019). We hypothesized that because the dorsal pulvinar is connected with attentional control areas, such as LIP and FEF, neurons in the dorsal pulvinar would be more strongly modulated by attention than neurons in the ventral pulvinar, both as an increase in firing rate and a decrease in response variability, and specifically during a delay period when attention is directed to a cued location without visual stimulation. In contrast, when attention is directed to a visual object, we hypothesized that both the dorsal and ventral pulvinar would be modulated by attention.
Due to limitations in recording technology, past electrophysiologic studies of the pulvinar have recorded only single neurons or local field potentials (LFPs), one at a time, using single electrodes. However, recording from a larger population of neurons simultaneously could reveal population-level dynamics (see Chapter 4) and interactions across spatially separated subpopulations, e.g. different subdivisions of the pulvinar. While studies of thalamus anatomy have overall found little evidence of local circuit neurons with dendritic arbors or axonal branches that cross nucleus boundaries, Imura and Rockland (2006) identified interneurons in the posterior medial pulvinar that may cross traditional subdivisions. These interneurons projected up to 2 mm away from the cell body, possibly crossing into the posterior lateral pulvinar. No other studies have looked at local circuit neurons within the macaque pulvinar, and it remains unknown whether there exist any direct anatomical connections between pulvinar subdivisions. Such connections could provide a pathway to link disjoint brain networks, for example, brain areas that communicate primarily with the dorsal pulvinar would be indirectly connected with brain areas that communicate primarily with the ventral pulvinar, through local inhibitory interneurons that cross pulvinar subdivisions. For example, higher-order areas related to attentional control, such as the frontal eye fields (FEF) and the lateral intraparietal area (LIP) communicate with the dorsal pulvinar, while the ventral pulvinar projects to early visual areas, such as V1 and the superficial layers of the superior colliculus. If the dorsal pulvinar has a causal influence on the ventral pulvinar, then attentional control signals carried between FEF, LIP, and the dorsal pulvinar could bias activity in early visual areas through local interactions between the dorsal and ventral pulvinar. The ventrolateral pulvinar has been shown to synchronize areas V4 and TEO according to attentional demands (Saalmann et al., 2012), and the dorsal pulvinar is in a prime position to efficiently signal to the ventrolateral pulvinar the locus of attention at minimal wiring costs and to guide which subpopulations it should synchronize. We therefore hypothesize that the dorsal pulvinar influences the ventral pulvinar during selective attention to both a cued location without visual stimulation and a cued visual object. Since we cannot detect direct causal influences of one area on another using only neuronal recordings, we thus use a measure that infers causal influence, cross-area spike-field coherence, and we hypothesize that spikes from the dorsal
pulvinar selectively synchronize with the local field potential in the ventral pulvinar during attention, and not in the reverse direction.

Studies on the effects of selective attention in the cortex have found that the strength of attentional modulation depends on cell type. Two different types of neurons can be broadly distinguished based on electrophysiological properties, such as spike waveform duration and firing rate: narrow-spiking (NS) putative inhibitory interneurons have sharper spike waveforms and tend to fire more frequently than the more common broad-spiking (BS) putative pyramidal cells (McCormick et al., 1985; Mitchell et al., 2007). Putative inhibitory cells in V4 and FEF are more strongly modulated by attention, reflected as both a greater enhancement of firing rate and a greater reduction of response variability across stimulus repetitions (Mitchell et al., 2007; Thiele et al., 2016). In addition, putative inhibitory cells in V4 showed stronger phase synchronization with the local field potential in the gamma frequency range (Vinck et al., 2013). These findings suggest that attention selectively targets inhibitory networks. Importantly, such cell-type specific effects of attention can inform and constrain neural models of attention, for example, those emphasizing response normalization and competitive interactions (Desimone and Duncan, 1995; Reynolds and Heeger, 2009; Itti and Koch, 2001). However, these cell-type specific effects have as of yet been studied only in cortex. In the lateral pulvinar, attention increases firing rates (Petersen et al., 1985; Saalmann et al., 2012; Zhou et al., 2016), but it is unknown how attentional modulation of pulvinar neurons may vary with respect to cell types.

Little is known about the microcircuitry within the pulvinar. Like other thalamic nuclei, the macaque pulvinar consists of approximately 20-30% local GABA-ergic interneurons; the remainder are excitatory thalamocortical relay cells (Arcelli et al., 1997; Hunt et al., 1991). Across the thalamus, a specific triadic circuitry involving inputs, interneurons, and relay cells is widespread: afferent corticothalamic axons synapse onto both a thalamocortical relay cell dendrite and an interneuron dendrite, and this interneuron forms a dendro-dendritic synapse with the same relay cell dendrite (Sherman, 2004). This arrangement of three synapses between the corticothalamic axon, the interneuron dendrite, and the relay cell dendrite is enclosed with a glomerulus. Based on studies of this triadic circuitry in the cat LGN, the interneuron is thought to
mediate gain control and adaptation of the relay cells (Sherman and Friedlander, 1988; Sherman, 2004). For example, if the corticothalamic neuron sends a burst of activity to signal the appearance of a high-contrast stimulus, the relay cell would fire strongly but then be suppressed due to strong activation of the interneuron (Blitz and Regehr, 2005; Kasten and Anderson, 2015). This gain control would ensure that the relay cell can respond to intensity changes within the dynamic range of its current inputs, and such modulation may be a primary mechanism mediating sensory adaptation.

This triadic circuitry could also be used to support selective attention through selective control of the interneuron. If pulvinar interneurons representing an attended location or stimulus are selective suppressed, then the activity of their connected relay cells would not be dampened, which would allow those relay cells to fire more faithfully to their corticothalamic input. In contrast, interneurons representing non-attended locations or stimuli would not be suppressed, which would inhibit their corresponding relay cells and prevent or weaken the transmission of distractor information back to the cortex. Reduced spiking from the pulvinar to the cortex could shut down spiking in superficial layers of the cortex (Purushothaman et al., 2012), preventing cortico-cortical transfer of distractor information to the input layer of a higher-order cortical area. Therefore, we hypothesize that, unlike in the cortex, which uses fundamentally different circuits, inhibitory interneurons in the pulvinar are selectively suppressed during selective visual attention to their receptive fields in order to allow for the efficient relay of behaviorally relevant information back to the cortex.

While it has been shown that selective visual attention modulates the activity of pulvinar neurons, a systematic investigation of attentional modulation throughout the pulvinar as a function of subdivision and cell type has not yet been undertaken. Using linear microelectrode arrays, we recorded from dozens of neurons in the dorsal and ventral pulvinar simultaneously in monkeys trained to perform a spatial attention task. We compared attentional modulation of neurons, split into dorsal and ventral pulvinar and into putative relay cells and interneurons. We also investigated functional interactions between pulvinar subdivisions, thereby probing population dynamics of the pulvinar during spatial attention for the first time.
Materials and Methods

Data Analysis

Results using SUA and MUA differed in several regards, which may be due to the inclusion in the MUA of spikes from passing axons or from the MUA sampling from a larger population of neurons. Because the MUA potentially included axonal spikes that did not originate from the pulvinar, we focused our analysis on pulvinar SUA, as well as its relationship with pulvinar LFP.

Visual neurons were defined as single units that significantly increased their firing rate in the cue response period (25 to 200 ms after cue onset) compared to baseline (-175 to 0 ms from cue onset) and had a baseline firing rate of at least 1.5 Hz. A small proportion of single units significantly decreased their firing rate and were not included in the analyses. In addition, only visual neurons that could be classified as putative relay cells or interneurons (see later text) were included.

Attentional Modulation of Spiking Response

In order to assess the degree of attentional modulation on neuronal measures, we computed attentional modulation indices for firing rate (AMIfr) and spiking response variability across stimulus repetitions, as measured by Fano factor (AMIf), of single-unit spiking activity. AMIfr is defined as \( \frac{\text{FR}_\text{att_RF} - \text{FR}_\text{att_away}}{\text{FR}_\text{att_RF} + \text{FR}_\text{att_away}} \), where \( \text{FR}_\text{att_RF} \) is the firing rate of a neuron when attention is directed to its RF, and \( \text{FR}_\text{att_away} \) is the firing rate of the neuron when attention is directed to the opposite location of the RF, during a specified time window. AMIf is defined analogously as AMIf, replacing firing rate with Fano factor.

Classification of Putative Relay Cells and Interneurons

Single units were classified as either putative relay cells or putative interneurons based on the shape of their average extracellular waveform (Fig. 5; McCormick et al., 1985; Pape and McCormick, 1995; Mitchell et al., 2007). In order to handle edge cases, raw waveforms were interpolated from 40 kHz to 200 kHz using piecewise cubic spline interpolation, and peaks and
troughs were identified in the interpolated waveform. Units with a trough-to-peak duration, e.g. time from first trough to first peak after trough, less than or equal to 0.375 ms were considered narrow-spiking, putative interneurons. Units with a trough-to-peak duration greater than 0.400 ms were considered broad-spiking, putative relay cells. Units with a trough-to-peak duration between 0.375 and 0.400 ms were considered ambiguous. These boundaries were selected based on the distribution of trough-to-peak durations, which was approximately, though not statistically, bimodal, with the trough between the two peaks of the distribution lying between 0.375 and 0.400 ms. In the cortex, inhibitory neurons tend to have greater spontaneous and stimulus-driven firing rates than excitatory neurons (Connors and Gutnick, 1990), and in the thalamus, the same level of injected current results in higher-frequency firing in interneurons than relay cells. However, whether inhibitory interneurons in the thalamus have greater spontaneous or stimulus-driven firing rates than relay cells has not yet been directly tested. Although our sample size of interneurons was small, we found no evidence of a difference in spontaneous or stimulus-driven firing rates in pulvinar putative interneurons compared to relay cells, as defined by waveform duration, and no evidence of a correlation between spontaneous firing rate and waveform duration.

Recordings from near a cell soma have a stereotyped extracellular waveform shape that is either a large, sharp (short-duration) trough followed by a broad peak, or a low-amplitude, fast, sharp peak, followed by a large-amplitude, sharp trough, followed by and a broad peak. Recordings from near a cell axon or neurite have a stereotyped waveform shapes that are either large-amplitude peak followed by a trough or a sharp, triphasic pattern of peak-trough-peak, with a relatively large-amplitude first peak. Neurons that had a stereotypical somatic waveform inflection pattern (i.e. neither trough-peak nor peak-trough-peak) were excluded from all analyses. In addition, if the waveform inflection pattern was peak-trough-peak, and if the amplitude of the first peak was greater than half of the amplitude of either the trough or the second peak, then the neuron was also excluded.
Since spike-field coherence is a particularly noisy measure in cases of low baseline firing rates, multi-unit activity was used instead of single-unit activity to compute spike-field coherence. This had the added benefit of increasing statistical power by increasing the number of pairs of units and LFPs. Spike-field coherence across subdivisions was computed as described in Chapter 2, using spikes of a unit and the LFP of the channel nearest to the unit in the other subdivision, which could be between 0.15 mm and 3 mm away. Spike-field coherence within a subdivision was computed similarly, using spikes of a unit and the LFP of a channel adjacent to the channel of the unit within the same subdivision (0.15 mm away. Spike-field coherence was

Figure 5. Classification of Putative Pulvinar Relay Cells and Interneurons. (a) Example of mean spike waveform with long peak-to-trough duration that was classified as a putative relay cell. (b) Example of mean spike waveform with short peak-to-trough duration that was classified as a putative interneuron. (c) Distribution of trough-to-peak durations of pulvinar spike waveform in 0.05 ms bins. Cells with trough-to-peak durations equal to and under 0.375 ms were classified as putative interneurons. Cells with trough-to-peak durations between 0.375 ms and 0.4 ms were classified as ambiguous. Remaining cells with trough-to-peak durations greater than 0.4 ms were classified as putative relay cells.

Spike-Field Coherence

Since spike-field coherence is a particularly noisy measure in cases of low baseline firing rates, multi-unit activity was used instead of single-unit activity to compute spike-field coherence. This had the added benefit of increasing statistical power by increasing the number of pairs of units and LFPs. Spike-field coherence across subdivisions was computed as described in Chapter 2, using spikes of a unit and the LFP of the channel nearest to the unit in the other subdivision, which could be between 0.15 mm and 3 mm away. Spike-field coherence within a subdivision was computed similarly, using spikes of a unit and the LFP of a channel adjacent to the channel of the unit within the same subdivision (0.15 mm away. Spike-field coherence was
assessed in the alpha (8-15 Hz), beta (15-30 Hz), low gamma (30-50 Hz), and high gamma (50-70 Hz) frequency bands by averaging coherence values across frequencies.

**Statistics**

Paired differences in attention modulation indices and spike-field coherence were not assumed to be normally distributed, so non-parametric statistical tests (e.g. Wilcoxon signed-rank test) were used at an alpha of 0.05. For tests of spike-field coherence, alpha was adjusted for multiple comparisons across the four frequency bands of interest (alpha, beta, low gamma, high gamma) using the Holm’s sequential Bonferroni procedure.

**Results**

**Attention Effects on Pulvinar Population Spiking Activity**

At the population level, visual pulvinar neurons showed no significant attentional modulation of firing rate (Fig. 6a), as measured by a modulation index, during the cue-target delay period (-175 to 0 ms from array onset; Fig. 6a; n=95, signed-rank test) or the array response period (25 to 200 ms after array onset). In addition, at the population level, these neurons showed no significant attentional modulation of spiking response variability, as measured by a modulation index, during the cue-target delay period (n=95, signed-rank test) or the array response period. These population-level measures consider the sample of pulvinar neurons as homogeneous; however, there was considerable heterogeneity in the nature of attentional modulation of firing rates of individual neurons (Fig. 6b).

At the single unit level, 19% of neurons (18/95) showed significant attentional modulation of firing rate during the cue-target delay (p<0.01, permutation test, 7 increases, 11 decreases), and 19% (18/95) showed significant attentional modulation of firing rate during the array response (p<0.01, 6 increases, 12 decreases). This suggests that attention has a strong effect on a subset of pulvinar neurons, both increasing and decreasing firing rates.
Figure 6. Task-related population spiking in the pulvinar. (a) Mean normalized firing rates when attention was directed to neurons’ RFs (red) and away from neurons’ RFs (blue) (N=95), aligned to different events. (b) Individual neurons’ normalized firing rates aligned to events. Each row represents a different neuron. (c) Correlation between attentional modulation indices of firing rate during cue-target delay period (-175 to 0 ms from array onset) and array response period (25 to 200 ms from array onset). Black dots signify units that had a significant attentional modulation index in either period. The correlation between modulation indices was stronger (Pearson r = 0.84, N = 27) using only those units.
Half of the neurons that showed significant attentional modulation of firing rate in one period showed significant modulation in the other period, and the strength of these modulations strongly linearly correlated (Pearson rho=0.97, p<1e-4, n=9, Fig. 6c). This relationship was maintained when looking at the larger set of all visual pulvinar neurons (Pearson rho=0.46, p<1e-5, n=95). The strength of attentional modulation during the cue-target delay predicted modulation of the subsequent response to the array. These findings suggest not only that selective attention biases pre-stimulus spiking activity in the pulvinar, but also that the strength and direction of this attention-related bias is maintained while feedforward visual information drives spiking activity in the pulvinar. This finding is consistent with a gain model of attention that is invariant to visual stimulation.

Differences Between Attention Effects in Dorsal and Ventral Pulvinar

Splitting neurons by whether they are part of the dorsal pulvinar or ventral pulvinar revealed modest differences in attentional modulation between the two subdivisions (Fig. 7a). At the population level, during the cue-target delay, while attention did not modulate firing rates in the dorsal pulvinar (n=61, signed-rank test), attention slightly decreased firing rates in the ventral pulvinar, though this effect did not cross the significance threshold (p=0.051, n=37). During the array response period, while attention did not modulate firing rates in the dorsal pulvinar, attention significantly decreased firing rates in the ventral pulvinar (p<0.05).

At the population level, during the cue target delay, attention decreased spiking response variability across trials in the dorsal pulvinar (p<0.05) but did not modulate response variability in the ventral pulvinar (Fig. 7b). Neither dorsal pulvinar nor ventral pulvinar populations showed attentional modulation of response variability during the array response period (Fig. 7b).
Attention Modulates Synchrony Across Pulvinar Subdivisions

Although thalamic nuclei have been generally considered not to have long-range intrinsic connections, Imura and Rockland (2006) found long-range interneurons within the traditionally defined medial pulvinar, which have axonal arbors that cross the finer subdivisions of the medial pulvinar. The authors also found evidence that putative long-range interneurons reside in the inferior and dorsolateral pulvinar, though at considerably lower density. Although conclusive evidence of anatomical connections across pulvinar subdivisions is lacking, are these subdivisions functionally connected?

Spikes are the outputs of neurons, and the LFP is thought to be the summed post-synaptic potential of a neuronal population and reflect its synchronous input. If spikes from one area correlate with LFP activity in a second area, it can be inferred that the spikes from the first

Figure 7. Spike-field coherence across pulvinar subdivisions when attention was directed to neuronal RFs (red) and away from RFs (blue) during the cue-target delay and the response to the array.
Figure 8. Attentional modulation indices split by pulvinar subdivision and task period. (a) Histograms of attentional modulation indices of firing rates for the dorsal pulvinar (light blue) and ventral pulvinar (olive green) for the cue-target delay period (first row) and array response period (second row). At the population level, attention suppressed the firing rates of ventral pulvinar neurons during both task periods. (b) Histograms of attentional modulation indices of Fano factor, as in (a).
area drive LFP activity in the second area. By recording spikes and LFPs from both the dorsal and ventral pulvinar simultaneously, we tested whether the two subdivisions interacted during the task and could infer directionality between them. During the cue-target delay period, attention modestly modulated the phase coherence between dorsal pulvinar spikes and ventral pulvinar LFPs, specifically in the low gamma frequency band, though this effect did not cross our significance threshold (Fig. 8; 30-50 Hz, \( p=0.051, N=35, \) signed-rank test). There was no significant attentional modulation of phase coherence between ventral pulvinar spikes and dorsal pulvinar LFPs. Altogether, these findings suggest that the dorsal and ventral pulvinar interact during the delay period, and the dorsal pulvinar influences the ventral pulvinar. In addition, during the array response period, there was no significant attentional modulation of phase coherence between dorsal pulvinar spikes and ventral pulvinar LFPs or between ventral pulvinar spikes and dorsal pulvinar LFPs in any frequency band. There was also no significant attentional modulation of phase coherence locally using spikes and LFPs from the same subdivision.

**Attention Suppresses Firing of Pulvinar Interneurons**

Different classes of neurons may play different roles in the pulvinar during attention. We split the pulvinar population of single units into putative excitatory relay cells and inhibitory interneurons based on the shape of their spike waveform. This revealed a suppressive effect of attention specifically on interneurons and not relay cells (Fig. 9a). At the population level, putative interneurons fired significantly less when attention was directed to the receptive field compared to attention away, in the cue-target delay \( (p=0.057, n=14, \) signed-rank test) and did not modulate their firing rate with attention during the array response period. Putative relay cells did not modulate their firing rate with attention at the population level in either task period \( (n=68). \)

Neither class of neurons showed attentional modulation of spiking response variability across trials in either the cue-target delay or array response periods (Fig. 9b), in contrast to previous findings of strong modulation of response variability in putative inhibitory neurons in the cortex (Mitchell et al., 2007; Thiele et al., 2016).
Attention Effects in the Pulvinar during Sustained Visual Stimulation

The attention task was designed to instruct animals to attend to both a cued location in space without stimulation and to a visual object at that location in different task periods (the cue-target delay period and the target-dim delay period). During the target-dim delay period, when attention was directed to and sustained on a grating shape in anticipation of its dimming, there was no significant modulation of firing rates of pulvinar neurons as a population and split by subdivision and cell type. These findings are in contrast with recent findings showing increased firing rate in the ventrolateral pulvinar during sustained attention to a visual object (Zhou et al., 2016). There was also no significant modulation of spike-field coherence between pulvinar subdivisions.

Relationship between Pulvinar Activity and Task Performance

We tested whether neuronal activity in the pulvinar predicted task performance, as measured by response time on either the release trials, where animals had to make a response immediately after array onset, or hold trials, where animals had to make a response immediately after the target in the array dimmed. At the population level, firing rate during none of the cue-target delay period, the array response period, and the target-dim delay period significantly correlated with response time. This is not surprising, given that population-level attentional modulation of firing rates during all task periods was weak. Using only units that showed significant attentional modulation of firing rates at the neuron level, there was still no correlation between firing rates and response time. In addition, although phase coherence between dorsal pulvinar spikes and ventral pulvinar LFPs in the low-gamma frequency band increased during attention in the cue-target delay, this increase did not correlate with response time. These findings are in contrast with recent work showing that activity in the mediodorsal pulvinar correlates with behavioral performance in a visual target-detection task at perceptual threshold (Fiebelkorn et al., 2019). This difference may be due to the present task not being sufficiently difficult to engage attentional processes during the target-dim delay period, and/or that the
**Figure 9.** Attentional modulation indices split by pulvinar cell type and task period. (a) Histograms of attentional modulation indices of firing rates for broad-spiking putative relay cells (green) and narrow-spiking putative interneurons (red) for the cue-target delay period (first row) and array response period (second row). At the population level, attention suppressed the firing rates of putative interneurons during the cue-target delay period. (b) Histograms of attentional modulation indices of Fano factor, as in (a).
The pulvinar reliably signals detection of the target dimming in this task and variations in response time are due to decision-making or motor-related processes outside of the pulvinar.

Discussion

Anatomical and lesion studies of the primate pulvinar have long suggested that the pulvinar plays an important role in selective visual attention. The pulvinar is highly interconnected with nearly all visual areas of the brain, and lesions of the pulvinar in monkeys and humans lead to attentional dysfunction, such as spatial hemineglect, without affecting basic vision. However, few studies (fewer than ten) have yet recorded neuronal activity in the primate pulvinar during attention, and those studies primarily probed a specific subdivision of the pulvinar in isolation. In this study, we present the first systematic characterization of attention-related neuronal activity in the primate pulvinar, with respect to different subdivisions and cell types, and including analysis of cross-subdivision interactions.

About 20% of visual pulvinar neurons had significantly modulated firing rates during attention in the cue-target delay period, with about the same number of increases as decreases. This suggests that attention balances overall excitation and inhibition of pulvinar population activity, which would allow the pulvinar to encode information about the locus of attention while maintaining overall population firing at the same level. If instead, attention changed the overall population firing rate significantly during the delay period, downstream areas could mistake this change as signaling the onset of new visual input. Under Sherman and Guillery’s classification of neurons in the thalamus and cortex (Sherman and Guillery, 1998), the neurons involved in this balanced modulation of thalamocortical output to downstream cortical targets could be considered “modulators,” whereas neurons that responded only to visual activity, regardless of attention, which were the vast majority of pulvinar neurons, could be considered “drivers.” Such balanced modulation has been shown to be a possible mechanism for implementing gain control or divisive normalization in biophysically plausible models of neuronal networks (Abbott and Chance, 2005; Chance et al., 2002) and has recently been observed in population recordings in V4 during a pre-stimulus delay period (Snyder et al., 2018).
Interestingly, the population-level balance of increases and decreases in pulvinar firing rate in the cue-target delay period was maintained into the response to the visual array, in contrast with what has been observed in cortex, where attention to a stimulus in a neuron’s RF typically increases the neuron’s firing rate (Luck et al., 1997; Treue and Maunsell, 1999). This increase in firing rate is thought to amplify the neuronal representations of the attended information, which could result in behavioral improvements. That the pulvinar does not show such increases in firing rate at the population level suggests that different neuronal mechanisms are involved in to enhance attended representations in the pulvinar than in the cortex.

Attention specifically suppressed firing rates of pulvinar putative inhibitory neurons during the cue-target delay period, though more recordings from putative inhibitory neurons are needed to support this conclusion. If true, considering only the triadic circuits of the pulvinar, suppression of interneurons that represent the attended location would lead to disinhibition of the corresponding thalamocortical relay cell and thus increase the gain on those relay cells, thus facilitating information transmission from the cortex to the pulvinar and back to the cortex of only attended representations. However, if this mechanism is accurate, then we should also observe an increase in firing rates of relay cells representing the attended location, which we do not at the population level, though some excitatory neurons do significantly increase their firing rate at the neuronal level. It is likely that suppression of pulvinar interneurons plays a more complex role in attention, and it would be informative to record from a greater number of relay cells and interneurons simultaneously to try to capture evidence that suppression of interneurons results in disinhibition of relay cells during attention. In addition, it would be informative to probe other sources of inhibition of pulvinar activity. The thalamic reticular nucleus (TRN) provides inhibitory input into the pulvinar (Wang et al., 2001) that may be important for its role in visual attention (Wimmer et al., 2015). Simultaneous recordings of the pulvinar and TRN would provide a greater understanding of how thalamic circuitry could support attention and other complex behavior.

In the cortex, attention has a greater impact on putative inhibitory neurons: firing rates are more strongly increased, and response variability is more strongly reduced. However, in the pulvinar, although attention also had a greater impact on putative inhibitory neurons, the effects
were quite different: firing rates were suppressed (in contrast to relay cells not being modulated) and response variability was not affected. This finding suggests that the mechanisms that underlie attention in the cortex are fundamentally different than those in the thalamus, and circuit-level models of attention that include both the cortex and the thalamus (e.g. Jaramillo et al., 2019) need to account for these differences.

There was increased phase synchrony between dorsal pulvinar spikes and ventral pulvinar LFPs in the low-gamma frequency band during attention, specifically during the cue-target delay, which suggests that the dorsal pulvinar influences the ventral pulvinar during attention in the absence of visual stimulation. Further study is required to establish a direct causal influence of the dorsal pulvinar on the ventral pulvinar, and using the data at hand, an analysis of the Granger-causal influence of the dorsal pulvinar on the ventral pulvinar would corroborate the current finding. If the dorsal pulvinar indeed influences the ventral pulvinar during attention, such influence could prepare the pulvinar and connected areas to receive information about an upcoming stimulus at the attended location and process it more efficiently. Attentional control areas, such as FEF and LIP, do not have strong widespread connectivity with early visual areas; however, they are reciprocally connected with the dorsal pulvinar, and a recent study shows that the mediodorsal pulvinar interacts with these attentional control areas during attention in a rhythmic fashion (Fiebelkorn et al., 2019). Although the dorsal pulvinar is not directly connected with early visual areas, by modulating excitability in the neighboring ventral pulvinar, it could bias neuronal activity in early visual areas by increasing synchrony in the alpha/low-beta frequency band between subpopulations of cortical areas corresponding to the attended location (Saalmann et al., 2012). To confirm such a theory would require recordings from a more extensive network of brain areas, such as the pulvinar, FEF, LIP, and V1, as well as causal manipulation of one or several nodes of this network, during selective visual attention.
Chapter 4: Using Sequential Auto-Encoders to Characterize Population Activity in the Pulvinar

Introduction

Modern methods, such as multi-electrode array recordings and wide-field two-photon calcium imaging, now allow for simultaneous recordings of tens to thousands of neurons during behavior. However, these methods have been slow to be adopted by systems neuroscientists using macaques as their animal model. Prior to the last few years, electrophysiological investigations of the macaque pulvinar involved relatively limited recordings from single neurons, only one or a few at a time. As described in Chapter 2, using modern multi-electrode arrays, we recorded over 100 multi-units simultaneously from the macaque pulvinar while animals performed an attention task. With this substantially larger dataset, we can compute more accurate estimates of the average activity of a neuronal population as it relates to behavior, but more so, we can take advantage of the simultaneous nature of the recordings and apply novel analyses that account for correlations in neuronal activity and model the dynamics of population activity during behavior.

One such novel analysis method, called Latent Factor Analysis via Dynamical Systems (LFADS), models simultaneously recorded population activity as the result of a dynamical system (Pandarinath et al., 2018). LFADS relies on the idea that population activity in a brain area can often be well described by low-dimensional dynamics, and by observing a limited subset of the population simultaneously, we can infer the relatively few latent factors that underlie the dynamics of the entire population. The LFADS model includes a sequence of nonlinear recurrent neural networks (akin to a variational autoencoder), which after training, results in a set of factors, substantially fewer than the input number of neurons, that capture the low-dimensional dynamics of the population. On a single trial, LFADS uses these factors and the observed firing rates of the population to estimate the initial state of the dynamical system and produce firing rates for each neuron for that trial. Compared with spike trains, which follow a Poisson random distribution, the inferred firing rate is a continuous measure of the likelihood of spiking and has substantially reduced trial-wise variability. This continuous firing rate can thus be considered “de-noised,” and
it can be more precisely correlated with behavioral measures, such as response time, and behavioral variables that vary continuously over time, such as movement. Indeed, LFADS has been successful in inferring single-trial population dynamics in macaque and human motor cortex and predicting reach movements (Pandarinath et al., 2018). Similar methods have been successful in inferring dynamics in posterior parietal cortex, prefrontal cortex, and other non-sensory areas (Harvey et al., 2012; Mante et al., 2013; Kobak et al., 2016). However, would methods like LFADS be able to model fast population dynamics in sensory areas, such as the pulvinar, or in behaviors that do not have clear, external behavioral correlates, such as attention?

In this chapter, I describe our initial attempts at using LFADS to model pulvinar population dynamics on single trials and the relationship between those dynamics and known features of pulvinar activity. This work is done in collaboration with Feng Zhu and Chethan Pandarinath, who performed the modeling work. We first show that pulvinar activity has low-dimensional structure and could thus be well modeled by methods like LFADS, which rely on a low-dimensional representation of population activity to infer de-noised single trial firing rates. We then show that a version of LFADS, augmented with discrete task inputs and with data stitched together across sessions, infers firing rates quite accurately, with a few exceptions. Finally, we show preliminary evidence that LFADS finds oscillatory-like structure during both delay periods of the task, which may reflect underlying rhythmic fluctuations of neuronal excitability or rhythmic sampling during attention.

Materials and Methods

The LFADS model is described in detail in Pandarinath et al., 2018. In brief, the LFADS model consists of an encoder and a generator, both recurrent neural networks, and a series of affine nonlinear transformations of the generator output to latent factors to firing rates. The encoder transforms data vectors, e.g. binned single trial spike trains, into a conditional distribution over latent variables, like a latent stochastic “code.” The generator, or decoder, maps this code to an approximation of the original data vectors. The approximation is then transformed, via multiplication by a weight matrix, into a set of latent factors, with dimensionality considerably
lower than the dimensionality of the original data. The latent factors are then multiplied by another weight matrix, and the result is passed through an exponential function (the inverse canonical link function for the Poisson distribution) to transform the factors back into the dimensionality of the original data and to infer a firing rate for each input neuron. The network is ultimately trained one session at a time using backpropagation, maximizing the likelihood of the data while minimizing the Kullback-Liebler divergence between the encoding distribution and the Gaussian prior distribution of the generator over all data points.

Task inputs were added to the LFADS model to represent the visual stimuli presented to the animal throughout the task, e.g. the cue, array configuration, and target dimming. These inputs were necessary for the model to capture sharp (in time) task-evoked increases or decreases in firing rate. The inputs consisted of six time-dependent variables that represented whether a cue was presented at one of the two possible cue locations and whether a shape was presented at each of the four array locations (Fig. 10). At baseline, the task input variables were all 0. Upon onset of a cue stimulus at a particular cue location, the task input for the corresponding location was 1. Upon cue offset, the corresponding task input returned to zero. When a grating stimulus appeared in the array, the task input for the corresponding location was 1, and when a plaid stimulus appeared, the corresponding task input was -1. When the grating stimulus that is the target in the array dimmed, the task input changed from 1 to ½. Finally, when the animal made a response, all stimuli disappeared, and all task inputs returned to 0.

Multi-unit data from the first six sessions of monkey M were included in the LFADS model, and these data were stitched together, as described for the stitched multi-session LFADS model by Pandarinath and colleagues (2018). 120 multi-units were used in total, after excluding units that were considered unstable over the course of the session. A unit was considered unstable if the spike rate, binned into overlapping 300 second windows, stepped by 150 seconds, showed a significantly positive or negative monotonic trend over time, against the null hypothesis of the absence of a trend, evaluated using the Mann-Kendall statistical test at alpha = 0.005. While this test does not capture more subtle or non-linear fluctuations in firing rate, it is a good first approximation test of a non-stationary firing rate, and it finds the most problematic neurons
which appear or disappear over the course of the session. The LFADS model was trained to infer 30 latent factors and used all data from correct trials from fixation onset to the break of fixation after the manual response.

Principal components analysis (PCA) was performed on a separate, but related, population of 217 well isolated single units identified to be in the pulvinar, recorded across 37 sessions from monkey M. Peristimulus spike density functions were computed for the -300 ms to +300 ms window from cue onset, -300 ms to +300 ms window from array onset, -300 ms to +150 ms window from target dimming, and -150 to 0 ms window from the animal breaking fixation. Spikes were convolved with a 10 ms Gaussian kernel, and spiking traces were averaged across trials. The resulting peristimulus spike density functions were concatenated in time by unit. Time

Figure 10. Schematic of time-varying external inputs to LFADS model. When the cue is on screen at either location 1 or 3, the corresponding vector has value 1. When a grating shape (hold shape) within the array is on screen at locations 1, 2, 3, or 4, the corresponding vector has value 1, and when the grating dims, the vector has value ½, e.g. fifth vector pictured. When a plaid shape (release shape) within the array is on screen, the corresponding vector has value -1.
was binned into 1 ms bins, resulting in PCA being performed on 217 observations (single units) of 1804 variables (time points).

**Results**

We first verified that population activity in the pulvinar, like in other brain areas, can be well represented in a lower dimensional space. One method to show this is to examine how much of the variance in activity from a population of N neurons can be well explained by P factors, where P<N. We used a linear dimensionality reduction technique, principal components analysis (PCA), on time-locked spiking activity concatenated across events from a population of 217 single units in the pulvinar, and we found that the first 31 principal components explain over 99% of the variance in the population. Even more strikingly, the top four principal components explain over 90% of the variance, and the first principal component itself explains 80% of the variance. These results strongly suggest that, despite the large heterogeneity in the firing rates of single units, pulvinar population activity during the attention task is highly redundant and can be represented in a much lower dimensional space without sacrificing much information in the overall population.

Next, we trained an LFADS model on pulvinar population spiking activity to model low-dimensional population dynamics. Our initial attempts generated spiking responses that failed to capture the sharp transient changes in firing rate that occurred in response to the presentation of visual stimuli (Fig. 11e-f). Since LFADS was designed for the motor system, where dynamics are considerably slower (on the order of hundreds of milliseconds) than dynamics in sensory systems (on the order of milliseconds), we decided to augment LFADS with discretely valued external task inputs that represented the visual stimuli given to the animal at each point in time. These task inputs consisted of step functions at key visual events, namely cue onset, cue offset, array onset, target dimming, and manual response, all of which could elicit sharp increases or decreases in firing rate.
Figure 11. Comparison of example neurons’ actual firing and LFADS-inferred firing. Top row, blue: peristimulus time histograms around cue onset using an 8 ms bin and no smoothing. Red line: mean LFADS-inferred firing rate across trials. Bottom row: raster image plot around cue onset. In actual data, yellow represents a spike and blue represents no spike. In LFADS-inferred firing, yellow represents a high inferred firing rate, and blue represents zero. (a-d) Example units where LFADS captured the actual spiking activity well. (e-f) Example units where LFADS appeared to capture noise and impose that noisy firing pattern on the units’ activity across all trials.
The augmented LFADS model captured multi-unit activity in the pulvinar quite well. Figure 11a-d shows the actual firing rates and the LFADS-generated firing rates of four representative multi-unit recordings, on individual trials and averaged across trials. However, there were several units where the LFADS model inferred task modulation when there was none that was obvious to the eye in individual or trial-averaged firing patterns, as shown, for example, in Figure 11e-f. While there may be true structure hidden in the sparse, seemingly random pattern of these units, it is more likely that because LFADS was trained to generate activity that explains the variance observed, it may be most accurate for units that have high variance over time but low variance across trials and least accurate for units like these that have low variance over time and high variance across trials. In addition, because LFADS considers the activity of all units, whether they are task-responsive or not, generated firing rates for a unit are likely to be sensitive to small, sharp changes in firing rate at time periods that have strong variance in firing rate within a unit and across units, even if the unit being modeled is not itself modulated by the task. It is as if those time periods are “marked” as important events across the population, and the smoothing kernel that LFADS applies to spikes is reduced to account for the increased variance during those periods. This can lead to accurate modeling of sharp transients for some units and overfitting of noise transients for other units.

We parameterized LFADS to fit 30 factors to pulvinar population activity, effectively reducing the dimensionality of the data from 120 (the number of multi-units used in the population) down to 30. In order to visualize population dynamics on single trials, we used PCA to reduce the dimensionality of the data. In Figure 12, each line represents a single trial activity trace plotted along the top three principal components, split up by time segment and cue location. After cue onset, there is a clear divergence of population activity between trials where the cue is presented at the receptive field location (red; attend-RF) versus at the opposite location (blue; attend-away). Interestingly, during both the cue-target delay period (Fig. 12a) and the time period immediately after array onset (Fig. 12b), there is no clear differentiation between activity in the two attention conditions. It is possible that 30 factors are too few to capture attention-related
differences in firing rate, which are subtle, but still observable at the individual unit level for some units.

Visualizing the top three principal components during the target-dim delay period revealed oscillation-like structure on single trials, as shown in Fig. 13a. Population activity was cyclical in this space, at a frequency of about 4 Hz, and consistent across trials. Further analysis did not reveal any clear differences between attention conditions or relationship between the phase of this oscillation-like activity and response time. Applying jPCA, another dimensionality reduction technique designed to pull out rotational structure, reveals the oscillations even more vividly (Fig. 13b). Visualizing the top three principal components during the cue-target delay period revealed weaker oscillation-like structure, which was cyclical at a frequency of about 10 Hz, in agreement with a previous study suggesting the pulvinar synchronizes connected cortical areas during visual attention within the alpha frequency band (Saalmann et al., 2012).
One of the key innovations of LFADS is its ability to infer population dynamics on single trials and effectively “de-noise” a unit’s firing rate response on a trial-by-trial basis. By doing so, trial-to-trial variability of firing rate and the randomness from the Poisson nature of spiking are reduced, so we may be able to more effectively correlate a unit’s firing rate with behavior on individual trials. In practice, however, there were no significant correlation between LFADS-inferred firing rate during both the cue-target delay period and the target-dim delay period and response time in the population. Further analysis needs to be done to examine whether LFADS-inferred firing rates correlate with response time for individual units and whether the relationship is stronger using LFADS-inferred firing rates compared to raw firing rates.

Figure 13. State-space trajectories of single trials during target-dim delay period, aligned to array onset (top row) and target dimming (bottom row). After the evoked response from the array onset, single-trial population trajectories stabilize and move in a 2-dimensional circular space. Traces in top row are formatted as in Fig. 12. This circular motion is more clearly visible in plots of single-trial population trajectories along jPC1 and jPC2 axes aligned to target dimming (bottom row).
Discussion

We first showed that pulvinar population activity can be well described in a low-dimensional space and then demonstrated that this activity can be well modeled as the result of a low-dimensional dynamical system using a new technique, LFADS. This model can infer and effectively de-noise single-trial firing rates of pulvinar units, particularly those that respond strongly to the task. Although these de-noised firing rates are not clearly correlated with task variables or behavioral measures, such as the locus of attention or response time, more refinement of these correlations needs to be done. Units that show strong task modulation and/or high trial-to-trial variability may be particularly correlated with response time at the unit level.

Synchrony, or correlation, between de-noised firing rates and the local field potential, akin to spike-field coherence, may also prove to be correlated with response time. In addition, it would be interesting to explore whether measures of population dynamics on single trials, such as deviations of a single-trial trajectory within a reduced dimensional space (e.g. via PCA) from the mean trajectory, are predictive of response time.

Visualizing a low-dimensional representation of the factors derived from LFADS over time revealed oscillatory-like behavior in population dynamics at single trials. Interestingly, during the cue-target delay period, this oscillatory-like behavior, though weak, was primarily restricted to the alpha frequency band, which suggests that pulvinar spiking activity fluctuates at approximately 10 Hz. A similar result was shown previously by Saalmann and colleagues (2012), in which pulvinar spikes synchronized with the alpha and beta frequency bands components of the pulvinar local field potential selectively during visual attention. Furthermore, this is the same frequency band that Saalmann and colleagues (2012) had suggested the pulvinar uses to synchronize connected cortical areas according to attentional demands. It would be interesting to see if the oscillatory-like behavior observed in population dynamics is modulated by attention, and how similar are these fluctuations to the actual observed local field potential measured simultaneously on individual trials.

Oscillatory-like behavior in population dynamics was also observed in the theta frequency band, but only during the target-dim delay period. A recent theory proposes that in the presence
of competing targets in the environment, attention may rhythmically sample between the targets at a theta rhythm, and recent evidence provides both behavioral and neural evidence supporting this theory (Fiebelkorn et al., 2018). Although in the current attention task, the cue was 100% valid, attention may still sample between shapes in the array due to a lack of confidence in the remembered cue location, and this rhythmic sampling may manifest itself as theta modulation of population dynamics in the pulvinar. Further work needs to be done to test this idea.

By inferring de-noised firing rates and compressing population dynamics to a low-dimensional space, LFADS provides novel, and perhaps more refined and powerful, ways of characterizing activity in the pulvinar. Using these measures, it would be interesting to test whether there are differences between dorsal and ventral pulvinar activity. Can an LFADS model be trained on activity from the dorsal pulvinar and accurately infer activity of a unit in the ventral pulvinar? If separate models are trained for the dorsal and ventral pulvinar, how would single-trial trajectories differ? Would there be stronger inferred correlates of behavioral measures or attentional state in the dorsal or the ventral pulvinar?

LFADS and related methods have proven useful in understanding the activity of large-scale neuronal recordings from motor, parietal, and prefrontal areas during motor, decision-making, and working memory tasks. We have shown here preliminary evidence that LFADS can infer single-trial population dynamics, including fast task-evoked transients, in a non-primary sensory brain area, the pulvinar. Lessons learned from applying LFADS to pulvinar data could transfer well to modeling the dynamics of primary sensory areas and interactions between sensory areas. It would be quite interesting to model the dynamics of cortical areas and their interactions, e.g. V4 and V2, with pulvinar activity as an external input, and create testable predictions of how inactivation of the pulvinar might interfere with local dynamics, e.g. within V4, or inter-areal interactions, e.g. between V4 and V2.

While dynamics in the pulvinar have not yet been linked to attention, given that neural correlates of attention have been observed across the pulvinar in this data (Chapter 3) and in previous studies (Saalmann et al., 2012; Zhou et al., 2016), it is likely that further refinement of LFADS and analyses of its inferred firing rates will reveal differences in population dynamics with
attention and possibly reveal single-trial correlates of behavioral measures that are currently difficult to observe. Such findings would provide another demonstration of the power of large-scale recordings and methods like LFADS in enriching our understanding of the neural mechanisms underlying cognition.
Chapter 5: Pulvinar Influences Information Transmission Between Dorsal and Ventral Visual Cortex and Contributes to Parietal Delay Activity

Introduction

Attention increases the signal-to-noise ratio of selected neural representations by increasing their gain (Reynolds and Chelazzi, 2004), reducing variability in responses across stimulus repetitions (Mitchell et al., 2007; Cohen and Maunsell, 2009), and modulating noise correlations in a neuronal population (Cohen and Maunsell, 2009; Mitchell et al., 2009; Ruff and Cohen, 2014). In addition, increasing evidence suggests that, at the network level, attention enhances selected neural representations by synchronizing activity between connected brain areas, thereby efficiently routing information across the brain (Buschman and Kastner, 2015; Fries et al., 2005; Fries et al., 2009; Bosman et al., 2012; Grothe et al., 2012; Roberts et al., 2013). How are these brain areas synchronized?

The pulvinar, like other higher-order thalamic nuclei, forms prevalent cortico-thalamo-cortical pathways and projects to most, if not all, visual cortical areas along the ventral and dorsal visual cortical pathways. It is therefore in a prime anatomical position to synchronize visual brain areas according to attentional demands. An earlier study by our group demonstrated that the pulvinar synchronizes neuronal activity between two ventral visual cortical areas, V4 and TEO, during selective visual attention (Saalmann et al., 2012), and other studies showed the pulvinar additionally regulates neural activity in V1 (Purushothaman et al., 2011) and between V4 and TE (Zhou et al., 2016). However, little is known about the pulvinar’s influence on neural activity in the dorsal visual cortical pathway and whether the pulvinar synchronizes activity between the dorsal and ventral visual cortical pathways.

Within the dorsal visual cortical pathway in macaques, the lateral intraparietal area (LIP) is involved in representing attentional priorities, oculomotor processing, decision-making, and categorization (Andersen and Cui, 2009; Bisley and Goldberg, 2010; Freedman and Assad, 2016;
LIP has been shown to provide attentional feedback to dorsal extrastriate cortex, in order to modify the gain of MT neurons (Herrington and Assad, 2009; Saalmann et al., 2007). In contrast, within the ventral visual pathway, area V4 is involved in representing the shape and surface features (e.g. color, texture) of visual objects, and numerous studies have focused on V4 for its role in visual attention (Roe et al., 2012; Reynolds et al., 2000). Areas LIP and V4 are reciprocally connected (Felleman and Van Essen, 1991; Blatt et al., 1990; Ungerleider et al., 2007), and LIP is hypothesized to be at a higher level in the visual hierarchy based on lamina-specific tracer studies (Felleman and Van Essen, 1991; Markov et al., 2012). Additionally, activity in LIP, but not V4, matches performance when attention is spread in a change detection task (Arcizet et al., 2017). However, no studies have examined the interactions between LIP and V4 during spatial attention. We hypothesized that attention increases synchrony between neural activity in LIP and V4 and that LIP influences V4 during spatial attention (Hypothesis 1). In light of the fact that the frontal cortex can influence V4 (Gregoriou et al., 2009; Moore et al., 2003), an additional parietal influence on V4 would allow for greater flexibility in the attentional modulation of V4 responses, e.g., LIP would provide information on stimulus salience and the frontal eye fields (FEF) would provide internally-generated goal-directed information (Buschman and Miller, 2007).

The lateral pulvinar has reciprocal connections with both the dorsal visual cortical pathway (Asanuma et al., 1985; Baizer et al., 1993; Hardy and Lynch., 1992) and the ventral visual cortical pathway (Benevento and Rezak, 1976; Baleydier and Morel, 1992; Webster et al., 1993). Because the lateral pulvinar has been shown to regulate interactions within the ventral pathway (Saalmann et al., 2012; Zhou et al., 2016), we hypothesized that the lateral pulvinar also regulates interactions between the dorsal and ventral visual cortical pathways (Hypothesis 2).

How might the pulvinar influence the cortex during spatial attention? Neurons in LIP show stimulus-evoked responses as well as characteristic delay period activity, i.e., increased spike rate during maintained attention, action planning or decision-making (in the absence of visual stimulation) relative to baseline (Andersen and Cui, 2009; Bisley and Goldberg, 2010). While feedforward inputs to LIP can account for early stimulus-evoked responses, it is not clear how LIP
activity is sustained during delay periods. Because the pulvinar has reciprocal connections with the dorsal pathway, it is well positioned to influence LIP activity. We hypothesized that the pulvinar influences delay period activity of LIP neurons (Hypothesis 3) based on three findings. First, studies in rodents suggest that two higher-order thalamic nuclei, the mediodorsal nucleus and the motor thalamus, are crucial for sustained neuronal firing in frontal cortex (Schmitt et al., 2017; Guo et al., 2017; Bolkan et al., 2017). Second, in macaques, there is strong pulvinar influence on the cortex during delay periods along the ventral visual cortical pathway (Saalmann et al., 2012). Finally, deactivation of the dorsal pulvinar, which is interconnected with LIP, produces severe deficits in visually-guided actions (Wilke et al., 2010), similar to damage to the posterior parietal cortex (Corbetta and Shulman, 2011).

To test our hypotheses, we simultaneously recorded from interconnected sites in the lateral pulvinar, LIP, and V4 while macaques performed a selective attention task. In support of Hypothesis 1, LIP and V4 synchronized during attention, based on spike-field coherence and Granger causality estimates between areas. In support of Hypothesis 2, the pulvinar influenced both LIP and V4 in overlapping frequency ranges, in which LIP and V4 also interacted. Finally, in support of Hypothesis 3, attentional modulation of pulvinar spike-LIP field coherence correlated with LIP delay period spiking activity and Granger-causal influence of the pulvinar on LIP depended on the strength of pulvinar-LIP anatomical connectivity, suggesting the pulvinar influences LIP delay activity.

**Materials and Methods**

**Behavioral task**

We trained two male monkeys (*Macaca fascicularis*, 4-8 years old) to perform a flanker task variant (Saalmann et al., 2012; Fig. 14). Monkeys initiated trials by depressing a response lever after an auditory “go” signal. This triggered the appearance of a 0.5° square fixation point at the center of the monitor (eye-monitor distance = 57 cm). After a variable delay of 300-700 ms, a 1.5° circular spatial cue randomly appeared for 100 ms duration, at one of six possible stimulus locations. After another variable delay period of 400-800 ms, six barrel- or bowtie-shaped stimuli,
each 4x2°, appeared equally-spaced in a circular array around the fixation point, for 700 ms duration or until the monkey released the response lever. We positioned the circular array such that at least one stimulus appeared in the receptive field (RF) of recorded neurons. On half the trials, the stimulus at the pre-cued location, the target, was congruent with its nearest neighboring stimuli, the distracters; i.e., each of these three stimuli was barrel-shaped, or each was bowtie-shaped. On the other half of trials, the target and its nearest distracters were incongruent; i.e., a barrel target was flanked by bowtie distracters, or vice versa. If the target was barrel-shaped, then the monkey needed to release the lever immediately for juice reward (150 to 650 ms after target onset). Conversely, if the target was bowtie-shaped, then the monkey needed to release the lever after the stimulus array disappeared (150 to 650 ms after array disappearance). Because the stimulus array contained equal numbers of barrels and bowties, the expected performance accuracy for random responses was 50%. In fact, the monkeys performed the task with greater than 80% accuracy overall, suggesting that they maintained attention at the cued location during
the delay period until target presentation. To ensure that the monkey maintained fixation throughout trials, 10% of all trials were ‘catch’ trials, in which the fixation point disappeared at a random time, requiring the monkey to immediately release the lever. Trials aborted if the monkey broke fixation, i.e., if eye position deviated by more than one degree from fixation.

We controlled stimuli, response monitoring and rewards using Presentation software. We presented visual stimuli at 50% contrast (light gray on darker gray background) on a 21-inch cathode ray tube monitor set at a 100 Hz refresh rate. A customized photodiode system affixed to a second monitor receiving identical input enabled verification of visual stimulus timing. Monkeys manipulated a lever with their hands to report decisions and received juice reward via a tube connected to an infusion pump. We monitored eye position using an infrared camera, operating at 120 Hz, with an ASL eye-tracking system.

Acquisition of Structural and Diffusion-Weighted Images

We anesthetized monkeys with Telazol (tiletamine/zolazepam, 10 mg/kg i.m.) and atropine (0.08 mg/kg i.m.) during scan sessions. We positioned monkeys in a customized MRI-compatible stereotaxic apparatus and monitored their respiration rate and pulse rate respectively using an MRI-compatible respiratory belt and pulse oximeter. We acquired images at a 3 T head-dedicated scanner using a 12-cm transmit-receive surface coil. Prior to the head implant surgery, we acquired diffusion-weighted images (DWI) using an eddy-current compensated double spin-echo, echo-planar pulse sequence (Croxson et al., 2005; Ramnani et al., 2006; Reese et al., 2003), with 1.0 mm2 in-plane resolution and 60 different isotropic diffusion directions (Jones et al., 1999) (field of view (FOV) = 128 x 96 mm; FOV phase = 75%; matrix = 128 x 96; phase partial fourier = 6/8; no. of slices = 47; slice thickness = 1.1 mm; repetition time (TR) = 10,000 ms; echo time (TE) = 145 ms; b-values = 0 and 1,000 s/mm2; slice orientation = transverse; 12:1 ratio of DWI to non-DWI) (Jones et al., 1999; Zhu et al., 2008). Data acquisition included twenty 60-direction sets of diffusion-weighted data for subsequent averaging, matching in-plane gradient echo field map and magnitude images to perform geometric unwarping of the diffusion-weighted data (TR = 500 ms, TE = 6.53/8.99 ms, flip angle = 55°), and T1-weighted structural images for
co-registration (Magnetization-Prepared RApid Gradient-Echo (MPRAGE); FOV = 128 mm²; matrix = 256 x 256; no. of slices = 128; slice thickness = 0.5 mm; TR = 2,500 ms; TE = 4.38 ms; flip angle = 8°; inversion time (TI) = 1,100 ms; in-plane resolution = 0.5 mm²). In a separate scan session, we acquired 12 T1-weighted structural images and calculated the average image for each monkey, to generate a higher-quality structural brain image.

**Electrophysiology**

We surgically implanted a customized plastic recording chamber, affixed to the skull with titanium screws and self-curing acrylic, in monkeys anesthetized with isoflurane (induction 2–4%, maintenance 0.5–2%). Four 2.5 mm craniotomies drilled within the recording chamber provided access to our pulvino-cortical regions of interest (ROIs) in the right hemisphere. We fitted each craniotomy with a conical plastic guide tube filled with bone wax (Saalmann et al., 2012; Saalmann et al., 2007), through which glass-coated platinum-iridium electrodes traversed. These guide tubes held electrodes in place between recording sessions. During recordings, we stabilized the animal’s head using four thin rods that slid into hollows in the side of the acrylic implant. We micropositioned electrodes in each ROI with electrode microdrives coupled to an adapter system, attached to the top of the recording chamber, allowing different approach angles for each ROI. We amplified and filtered (150-8,000 Hz for spikes; 3-300 Hz for LFPs) electrode signals (40,000 Hz sample rate for spikes; 1,000 Hz sample rate for local field potentials (LFPs)) using a preamplifier with a high input impedance headstage and Plexon Multichannel Acquisition Processor controlled by RASPUTIN software. Control recordings for LFP quality in each ROI with three different reference electrodes – either a skull screw, silver wire in contact with the dura, or electrode in the white matter just outside the ROI – yielded similar LFPs, so we used a skull screw as the reference electrode during recording sessions. We sorted spikes online to map the RF of isolated neurons, then re-sorted spikes offline using Plexon Offline Sorter software. We first plotted a neuron’s RF using hand-held stimuli, then confirmed the RF by systematically flashing visual stimuli around the RF location while the monkey fixated centrally. The reported cells and LFPs in each recording session had overlapping RFs.
Probabilistic tractography on diffusion MRI data

We used FSL software to analyze diffusion MRI data (Smith et al., 2004; Woolrich et al., 2009). We corrected DWI and non-DWI for eddy currents using affine registration (12 degrees of freedom (DOF), FMRIB’s Linear Registration Tool (FLIRT)) to a non-DWI reference volume, and averaged to improve the signal-to-noise ratio (Jenkinson and Smith, 2001). Next, we geometrically unwarped images using field map and magnitude images acquired in the same session (Jezzard and Balaban, 1995). That is, the magnitude image was skull-stripped using FMRIB’s Brain Extraction Tool (BET) (Smith, 2002), forward-warped using FMRIB’s Utility for Geometrically Unwarping EPIs (FUGUE), and registered (6 DOF) to an averaged, skull-stripped non-DWI reference volume. We applied the resulting transformation matrix to the field map image (scaled to rad/s and regularized by a 2-mm 3D Gaussian kernel), which was subsequently used to unwarps DWI and non-DWI with the FUGUE utility. We then skull-stripped the T1-weighted structural brain image and co-registered to the averaged, skull-stripped and geometrically unwarped non-DWI reference volume (12 DOF), to produce the transformation matrix between the two spaces.

For probabilistic diffusion tractography (PDT) analyses, we manually delineated LIP, V4 and pulvinar ROIs for the right and left hemisphere of each monkey. We used the individual monkey’s T1-weighted structural brain image, in conjunction with a stereotaxic atlas (Saleem and Logothetis, 2002), to guide the definition of the ROIs. We applied the transformation matrix, derived from the co-registration of the structural image to the reference non-DWI, to the ROI masks for PDT analyses.

We performed tractography analyses using FMRIB’s Diffusion Toolkit (FDT). The tractography algorithm modeled two fiber populations per voxel (Behrens et al., 2007), suited to the complex fiber architecture of the thalamus (Saalmann et al., 2012; Behrens et al., 2003). For each monkey, we calculated probability distributions of fiber direction at each voxel (Behrens et al., 2003a; Behrens et al., 2003b). To identify pulvinar voxels with a high probability of connection with V4 and LIP, we performed a PDT analysis to estimate pathways passing through any voxel.
in a pulvinar seed, and the probability such pathways will pass through a voxel in either of the two cortical targets, V4 and LIP (i.e., FDT's "single mask seed with classification targets" tractography). From each pulvinar seed voxel, 5000 samples were drawn from the probability distribution (0.2 curvature threshold, 0.25 mm step length), and the proportion of these samples passing through each cortical target equated to the probability of connection to that target. We applied a threshold removing voxels with a less than 5% of maximum connection probability with the target, then calculated the overlap between thresholded pulvinar volumes respectively connected to V4 and LIP.

**Imaging electrodes in situ**

To verify electrode locations in the pulvinar, V4 (prelunate gyrus) and LIP, we acquired T1-weighted structural brain images with platinum-iridium electrodes held in situ by the customized guide tubes. Although the electrode itself is not visible in the T1-weighted images, a susceptibility “shadow” artifact appears along the length of the electrode with a width of approximately one voxel (0.5 mm³, either side of the electrode). Our experimental approach was to position electrodes at the most dorsal point of an ROI (for a particular dorsal-ventral trajectory), then acquire structural brain images. During subsequent recording sessions, we used a microdrive to lower electrodes through ROIs to isolate neurons and logged all recording site coordinates from the microdrive system. At the end of an electrode track, i.e., at the most ventral point of our ROI, we acquired additional structural brain images, before starting a new track. We reconstructed the position of the electrode for each recording session, using the structural images of the start and end of each track as well as the daily microdrive coordinates.

**Spike rate analysis**

We calculated spike density functions, convolving each spike with a 10 ms Gaussian and averaging across trials. Next, we subtracted baseline activity (200 ms before cue onset) from the response for each condition. Finally, to normalize responses, we divided the response by the maximum firing rate of any condition. For statistical analysis of delay period activity, we computed
the mean across the 200 ms period before array onset. To contrast attention to the neuronal RF versus attention away, the neuronal RF is defined as the cue location that evoked the largest firing response 25-200 ms after cue onset; the attention away location is defined as the cue location that evoked the smallest firing response 25-200 ms after cue onset. Cue locations with insufficient trials or inconsistent firing to the cue were excluded from consideration.

Spike-field coherence analysis

We used the coherence measure to study the temporal relationship between all possible spike-LFP combinations involving LIP, V4 and pulvinar, as described in Chapter 2. To measure coherence in different frequency bands with sufficient frequency resolution, we first bandpass filtered data into alpha (8-15 Hz), beta (15-30 Hz), and gamma (30-50 Hz) bands using FIR filters (Kaiser window; model order 3960, transition bandwidth 1 Hz, stopband attenuation 60 dB, passband ripple 0.01 dB). We calculated spike-field coherence in the 300 ms period before array onset (with zero-padding to 1024 ms), using the Chronux toolbox for Matlab (http://chronux.org/; Bokil et al., 2010). For frequencies greater than 30 Hz, coherence was computed using 3 Slepian tapers (time-bandwidth product of 2). For frequencies less than 30 Hz, coherence was computed using a single Slepian taper (time-bandwidth product of 1). Coherence estimates obtained without the initial step of bandpass filtering data showed similar results.

To compute the correlation between attentional differences in pulvinar spike-cortical field coherence and attentional differences in cortical firing rate, the RFs of the pulvinar neurons were used. These RFs broadly overlapped with the RFs of the cortical neurons.

Conditional spectral Granger causality analysis

We bandpass-filtered (3-100 Hz) the LFP from each brain area, downsampled to 200 Hz, subtracted the mean, then divided by the standard deviation. For each recording session, we derived a multivariate autoregressive model for each attention condition (attention at the response field for LFPs corresponds to the location of the cue evoking the peak response; attention away from the response field corresponds to the location most far away in the opposite
visual hemifield). The autoregressive equation is given by \( \sum_{m=0}^{p} A_m X(t-m) = E(t) \), where \( A_m \) are the coefficient matrices, \( m \) is the lag, \( X(t) \) is the multidimensional process defined for a segment of the time series, and \( E(t) \) is the noise vector. The model order, \( p \), generally corresponded to the first minimum Akaike information criterion value. We used a model order of 10. To estimate \( A_m \) and \( V \), the covariance matrix of the noise vector, we used the Levinson, Wiggins, Robinson algorithm. To check autoregressive models, we tested the assumption of white model residuals, the stability of the model (i.e., stationary and convergent), and the consistency between the recorded and model-generated data (Ding et al., 2000). The spectral matrix of the time series is given by \( S(f) = H(f)VH^\ast (f) \), where \( H(f) = (\sum_{m=0}^{p} A_m e^{-im2\pi f})^{-1} \) is the transfer function, and \( ^\ast \) denotes the matrix transpose and complex conjugate.

We calculated conditional Granger causality (Geweke, 1984; Granger, 1980) as a measure of the influence one brain area (\( Y \)) has on another area (\( X \)), after taking into account additional areas (\( Z \)). The conditional Granger causality can be expressed as a function of frequency, to investigate the oscillatory nature of LFPs. In the frequency domain, the conditional Granger causality is given by \( I_{Y \rightarrow X|Z}(f) = \ln \left( \frac{\Sigma_{xx}(X,Z) / (|Q_{xx}(f) \Sigma_{xx}(X,Y,Z)Q_{xx}^\ast (f)|)}{\Sigma_{xx}(X,Z)} \right) \), where \( \Sigma_{xx}(X,Z) \) is the variance of the noise in the joint regression of \( X \) and \( Z \) (variance associated with \( X \)), and \( Q_{xx} \) and \( \Sigma_{xx}(X,Y,Z) \) are functions of the transfer function and noise covariance matrix (Dhamala et al., 2008; Ding et al., 2006). For each attention condition (attention at the response field, defined as the cue location evoking the peak response across areas, and attention away from the response field, defined as the location most far away in the opposite visual hemifield), we calculated the conditional spectral Granger causality in 300 ms sliding windows across the trial. To compare attention conditions across the population, we used t tests to determine whether there was significantly greater conditional Granger causality in particular frequency bands (i.e., alpha, beta and gamma) when attention was at the response field location compared to when attention was away from the response field. We controlled the experiment-wise error rate (\( p < 0.05 \)) using the Holm’s sequential Bonferroni procedure.
**Results**

We report simultaneous recordings of single-unit spiking activity and local field potentials (LFPs) from three areas: the pulvinar (n=51 cells and 56 LFPs), LIP (n=41 cells and 56 LFPs), and V4 (n=31 cells and 56 LFPs), in two macaques performing a flanker task. This task allowed us to manipulate the monkey’s spatial attention, because a spatial cue appeared randomly at one of six different locations, drawing the monkey’s attention to the upcoming target position in a circular array of barrel and bowtie shapes (both monkeys >80% correct performance overall; Fig. 14). For each macaque, we used anatomical connectivity maps derived from diffusion MRI, as well as overlapping receptive fields (RFs) for recording sites, to guide electrode placements in interconnected thalamo-cortical networks. Some, but not all, of the pulvinar and V4 data were included in an earlier analysis of simultaneous recordings from the pulvinar, V4, and TEO (Saalmann et al., 2012).

**Delay period spiking activity in LIP, V4, and pulvinar**

Delay period spiking activity is a signature of many neurons in LIP (Anderson and Cui, 2009; Bisley and Goldberg, 2010; Shadlen and Kiani, 2013) and has also been reported in other areas, including V4 (Hayden and Gallant, 2013; Luck et al., 1997; Mirabella et al., 2007) and pulvinar (Saalmann et al., 2012). In agreement with those studies, there was significantly increased delay period activity (200 ms period before array onset) observed in LIP neurons (n=41; p=0.00003, signed-rank test; Fig. 15a), pulvinar neurons (n=51; p=0.0046; Fig. 15b) and V4 neurons (n=31; p=0.00002; Fig. 15c) at the population level, when attention was directed at neuronal RFs as compared to when attention was directed away from the RF. This was due to both significantly enhanced delay period spiking when attention was directed at neuronal RFs as compared to baseline (100 ms period before cue onset) in all three areas (LIP: p=0.055; pulvinar: p=0.014; V4: p=0.0012; signed-rank test) and significantly suppressed delay period spiking when attention was directed away from the RF as compared to baseline in LIP and V4 (LIP: p=0.00001; V4 p=0.0062; signed-rank test). For an individual neuron, there did not appear to be any clear preference for spiking at one particular time during the delay period of individual trials; rather,
there was variable spike timing from trial-to-trial across the delay. To probe the underlying mechanisms contributing to this attention-enhanced delay period spiking in LIP, V4, and pulvinar, in the following sections we measured cortico-cortical and thalamo-cortical interactions across the delay period.

**Figure 15.** Delay period spiking activity in LIP, pulvinar, and V4. Population spike density functions for LIP (a), pulvinar (b), and V4 (c). LIP, pulvinar and V4 neurons show increased spike rate in the delay period between cue onset and array onset when attention was directed at the RF (red) compared to attention away from the RF (blue). All error bars are SEM.
LIP influence on V4

The putative salience map in LIP (Bisley and Goldberg, 2010) is well positioned to provide attentional feedback to V4. Spatial attention increased LIP influence on V4 during the delay period, but attention did not significantly change V4 influence on LIP. At the population level, there was significantly increased LIP spike-V4 field coherence with attention to the neuronal RF (versus attention away) in the alpha (8-15 Hz; n=38; p=0.015; signed-rank test), beta (15-30 Hz; p=0.006) and gamma frequency ranges (30-50 Hz; p=0.0005; Fig. 16a, 16d). This shows a linear dependency between LIP output (spikes) and V4 input (reflected in the LFP). We next calculated a statistical measure of causality between LIP and V4. Conditional Granger causal influence of LIP on V4 (accounting for pulvinar influence) also significantly increased with attention in the gamma range (n=56; p=0.013; t test) and trended in the alpha (p=0.096) and beta ranges (p=0.081; Fig. 16c, 16f). In comparison, there was no significant increase in V4 spike-LIP field coherence in these frequency ranges (n=28; 8-15 Hz: p>0.1; 15-30 Hz: p>0.1; 30-50 Hz: p>0.1) or conditional Granger causal influence of V4 on LIP (n=56; 8-15 Hz: p>0.1; 15-30 Hz: p>0.1; 30-50 Hz: p=0.017; Fig. 17).

Considering the delay period activity observed in V4 (Fig. 15c), these data suggest that the dorsal visual cortical pathway provides information about attentional priorities to the ventral visual cortical pathway, to selectively modulate V4 neuronal excitability (supporting hypothesis 1). Spatial attention also increased within-LIP spike-field coherence in the gamma range (n=40, p=0.002; Fig. 16b, 16e), consistent with attention adjusting the degree of synchrony between LIP cells. This suggests that LIP feedback to V4 can be modulated by adjusting LIP spike rate or LIP neural synchrony.

Pulvinar influence on information transmission between LIP and V4

The pulvinar has previously been shown to influence information transmission along the ventral visual cortical pathway, including V4 (Saalmann et al., 2012; Zhou et al., 2016). If the pulvinar regulates information transmission between the dorsal and ventral visual cortical pathways, then this might be done by the pulvinar influencing both LIP and V4 in an overlapping
At the population level, there was significant pulvinar spike-LIP field coherence in the alpha (n=32, p=0.005, signed-rank test) and beta (p=0.0009) ranges (Fig. 17a, 17d), as well as significant pulvinar spike-V4 field coherence in the beta range (n=36, p=0.006), and trending in the alpha range (p=0.054; Fig. 18a, 18c), with attention at neuronal RFs (versus attention away) during the delay period. Similarly, spatial attention increased conditional Granger causal influence of the pulvinar on LIP (accounting for V4) in the alpha (n=56, p=0.00039, t test) and beta (p=0.0063) ranges (Fig. 17c, 17f; consistent with hypothesis 1), as well as conditional Granger causal influence of the pulvinar on V4 (accounting for LIP) in the alpha (n=56, p=0.007) and beta (p=0.0117) ranges (Fig. 18b, 18d). The pulvinar influenced both LIP and V4 in the alpha
and beta ranges, which would allow for LIP-V4 communication through coherence at these frequencies (supporting hypothesis 2). This is consistent with the aforementioned LIP spike-V4 field coherence in the alpha and beta ranges. Within-pulvinar spike-field coherence also increased in the beta range (n=44, p=0.013, signed-rank test; Fig. 17b, 17e) with attention, and trended in the alpha range (p=0.08), suggesting that the pulvinar influenced the cortex by increasing the synchrony as well as spike rate of pulvinar neurons.

Tracer studies suggest that the dorsal pulvinar predominantly connects with the dorsal cortical pathway, whereas the ventral pulvinar connects with the ventral visual cortical pathway (Shipp, 2003; Kaas and Lyon, 2007). This prompts the question of what zone(s) of the pulvinar, if any, regulates communication between dorsal and ventral cortical areas? Although tracer studies of pulvinar connections with LIP and V4 have not been performed in the same animal to the best
of our knowledge, different studies show that both LIP and V4 connect with the lateral pulvinar (Shipp, 2003; Asanuma et al., 1985; Baizer et al., 1993; Hardy and Lynch, 1992; Baleydier and Morel, 1992). Considering the brachium of the superior colliculus as the demarcation between dorsal and ventral pulvinar, our probabilistic tractography on diffusion MRI data showed that dorsal pulvinar predominantly connected with LIP, whereas ventral pulvinar predominantly connected with V4, consistent with tracer studies. However, LIP and V4 projection zones did overlap in the intermediate region between dorsal and ventral pulvinar, particularly in lateral pulvinar (Fig. 19). In comparison, the projection zones of two ventral visual cortical areas, TEO and V4, showed greater overlap in ventral pulvinar (Shipp, 2003) (including in the same animals.

Figure 18. Pulvinar influence on V4. (a-b) Time-frequency plots of (a) Pulvinar spike-V4 field coherence, and (b) conditional Granger-causal influence of pulvinar LFP on V4 LFP (accounting for LIP). Plots are as in Fig. 16. (c-d) Spectra calculated in 0 to 300 ms window prior to array onset. (c) Pulvinar spike-V4 field coherence. (d) Conditional Granger-causal influence of pulvinar LFP on V4 LFP (accounting for LIP).
This suggests that the lateral pulvinar, an intermediate region between dorsal and ventral pulvinar, mediates interactions between dorsal and ventral visual cortical areas, which would likely offer reduced wiring costs within the pulvinar.

Figure 19. Overlapping projection zones of LIP and V4 in the pulvinar. (a) Pulvinar voxels connected with LIP (red), V4 (blue) or both (yellow) are shown overlaid on T1-weighted coronal slice at 4 mm anterior to interaural line. (b) Sequential slices (0.5 mm separation) zoomed in on pulvinar.

Pulvinar influence on cortical delay period spiking activity

If the pulvinar influences LIP delay period activity, then one might expect coherence between pulvinar output (spikes) and LIP input (reflected in the LFP) to correlate with LIP spike rate during the delay period. Indeed, there was a significant positive correlation between
attention-enhanced (i.e., difference between attention at RF and attention away) pulvinar spike-LIP field coherence in the alpha range and attention-enhanced LIP spike rate during the delay period (300 ms period before array onset; n=26; Spearman \( r=0.441, p=0.0128 \); Fig. 20a). This is consistent with hypothesis 3, i.e. that the pulvinar supports the maintenance of LIP delay period activity.

In this case, pulvinar influence on LIP should depend on the strength of anatomical connectivity between pulvinar and LIP. To test this, we first reconstructed all electrode tracks and recording sites. Next, we calculated the probability of paths, derived from diffusion MRI, between every pair of thalamic and cortical recording sites (each site localized to a 0.5mm\(^3\) voxel on the structural MRI). Finally, we compared the conditional Granger causality for the paired thalamo-cortical recording sites within the top 33% connection probability to that within the bottom 33% connection probability. There was a significantly higher conditional Granger causal influence of the pulvinar on LIP in the alpha range during the delay period, for highly anatomically connected recording sites (n=19, \( p=0.027 \), t test). This finding supports the spike-field coherence and Granger

**Figure 20.** Pulvinar contributes to cortical delay activity. Scatter plots showing relationship between attention-related change (difference between attention at RF and attention away) in coherence and spike rate. Coherence and spike rate calculated in 0 to 300 ms window prior to array onset. Each point corresponds to data from an individual recording session. (a) Attention-related increase in pulvinar spike – LIP field alpha (8-15 Hz) coherence correlates with increase in LIP delay firing rate. (b) Attention-related increase in pulvinar spike – V4 field alpha (8-15 Hz) coherence correlates with increase in V4 delay firing rate.
causality results, which suggest pulvinar influence on LIP in the alpha range contributes to LIP delay period activity.

Given that the pulvinar influenced LIP delay period activity, it prompts the question of whether the pulvinar influenced V4 delay period activity as well? There was a trending positive correlation between attention-enhanced pulvinar spike-V4 field coherence in the alpha range and V4 spike rate during the delay period (300 ms period before array onset; n=24; Spearman r=0.389, p=0.03329 but not significant after correcting for multiple comparisons; Fig. 20b). This suggests the possibility that pulvinar influence on cortical delay period activity is a mechanism operating in both parietal and visual cortex.

Recurrent interactions between pulvinar and cortex

The pulvinar and cortex are reciprocally connected, and considering that the pulvinar influences cortical activity, it is possible that the cortex also influences the pulvinar, setting up a recurrent feedback loop that sustains neural representations of attentional priorities throughout the delay period. Consistent with this, at the population level, both LIP spike-pulvinar field coherence (n=40, p=0.003, signed-rank test; Fig. 21a, 21c) and V4 spike-pulvinar field coherence (n=29, p=0.010; Fig. 21b, 21d), significantly increased during the delay period in the beta frequency range when attention was at neuronal RFs (versus attention away). In addition, spatial attention did not significantly increase conditional Granger causal influence of V4 on pulvinar (accounting for LIP) in the alpha (n=56, p=0.020, t test) or beta (p=0.044) ranges, though the effect is trending. And attention did not significantly increase conditional Granger causal influence of LIP on pulvinar (accounting for V4) in the alpha (n=56, p>0.1, t test) or beta (p=0.029) ranges. Although attention significantly increased phase-locking between cortical spikes and pulvinar beta oscillations, it did not significantly increase the conditional cortical Granger-causal influence on the pulvinar beta oscillations, which suggests that pulvinar LFPs are primarily driven by local processes or from unmeasured outside sources. In contrast, attention did significantly increase within-pulvinar spike-LFP coherence, pulvinar spike-cortex LFP coherence, and pulvinar
Granger-causal influence on the cortex, which suggests that cortical beta oscillations are driven by pulvinar spikes, which are time-locked to pulvinar beta oscillations.

Discussion

Interactions between dorsal and ventral visual cortical pathways (Hypothesis 1)

During the delay period, spatial attention modulated only LIP influence on V4 (in gamma and lower frequencies) and not V4 influence on LIP. These results suggest that LIP and V4 both represented visual stimulus information at a similar time after stimulus onset, with LIP subsequently providing information on spatial attention priorities to V4. In a recent study where monkeys were cued to categorize stimuli based on either their motion or color (Siegel et al.,
2015), early (bottom-up) representation of visual information about the cue in both V4 and LIP (with no significant latency difference) was followed shortly after by a transient representation of the task-relevant information in V4, and later by a sustained (top-down) representation of the task-relevant information first in LIP and then in V4. These temporal dynamics were similar to that in our study, except our study suggests that LIP may extract salient task information without early V4 input, depending on task requirements (i.e., especially if largely spatial).

Spatial attention modulates V4 spiking activity (McAdams and Maunsell, 1999; Moran and Desimone, 1985; Reynolds et al., 2000) and neural synchrony, e.g., increasing gamma activity (Fries et al., 2001, 2008). Previous work on attentional feedback to V4 has focused on frontal cortical sources (Gregoriou et al., 2009; Moore et al., 2003; Buschman and Miller, 2007), e.g., electrically stimulating FEF modulates V4 spiking, and attention increases FEF synchrony with V4. However, lesioning the entire lateral prefrontal cortex, including FEF, in one hemisphere reduced, but did not eliminate, attentional modulation of ipsilateral V4 activity; and monkeys showed little decrement in behavioral performance (Gregoriou et al., 2014). Our study shows that the posterior parietal cortex (PPC), particularly LIP, is another source of top-down attentional influence on V4. The LIP influence on V4 operated in the same frequency range, i.e. gamma, as FEF influence on V4 (Gregoriou et al., 2009). However, we also found significant LIP spike-V4 field coherence at alpha and beta frequencies. Different frequency bands have been proposed to predominantly contribute to different functions (e.g. Engel and Fries, 2010; Jensen and Mazaheri, 2010), and processing in different cortical layers (Buffalo et al., 2011; Spaak et al., 2012; van Kerkoerle et al., 2014, but see Haegens et al., 2015). Longer-distance feedback pathways tend to target superficial layers, and shorter-distance feedback pathways tend to target deep layers (Henry et al., 1991; Markov et al., 2014). As LIP feedback pathways to V4 might be considered of intermediate distance (targeting both superficial and deep layers), it is possible that LIP gamma-frequency influence represents feedback to superficial layers in V4, and LIP-V4 alpha/beta interaction represents feedback to deep layers in V4 (Mejias et al., 2016). This suggests a possible difference in the contributions of FEF and LIP to V4 processing, i.e., LIP may have greater influence over activity in deep layers of V4.
Pulvinar regulation of LIP and V4 interactions (Hypothesis 2)

The pulvinar influenced LIP and V4 activity at both alpha and beta frequencies, and LIP spikes synchronized with V4 LFPs at these frequencies. This is consistent with the pulvinar adjusting functional connectivity between LIP and V4. Previous work has shown that pulvinar influence on LIP and V4 extends to the gamma range, via alpha-gamma cross-frequency coupling (Saalmann et al., 2012; Wang et al., 2012). This suggests that the pulvinar may also regulate the LIP influence on V4 at gamma frequencies reported here. Such pulvinar influence over gamma-band cortico-cortical connectivity is supported by a recent macaque study showing that pulvinar deactivation with muscimol reduces gamma as well as beta coherence between LFPs in V4 and inferior temporal cortex (Zhou et al., 2016). Taken together, these results indicate an important role for the pulvinar in shaping the pattern of rhythmic activity across a range of frequencies within and between LIP and V4, thereby enabling coordinated processing and information transfer between both areas.

It has been proposed that directly connected cortical areas are indirectly connected via the pulvinar – called the “replication principle” (Shipp, 2003). Although there are direct connections between LIP and V4 (Blatt et al., 1990; Ungerleider et al., 2008) as well as between those areas and the pulvinar, evidence for indirect connections via the pulvinar, i.e. via an overlapping projection zone, is lacking. Our diffusion MRI data suggest that pulvino-LIP connections and pulvino-V4 connections partially overlap at an intermediate depth in the lateral pulvinar. Although diffusion MRI data does not have sufficient spatial resolution to identify individual neurons, the pulvinar control of LIP-V4 functional connectivity is consistent with a cortico-thalamo-cortical path carrying information from one cortical area to the pulvinar, in order to coordinate activity with the second cortical area.

The dorsal and ventral visual cortical pathways are commonly considered to preferentially represent spatial/intentional and object-based information, respectively. Previous attentional work has focused on pulvinar interactions with the ventral visual cortical pathway (Saalmann et al., 2012; Zhou et al., 2016). This study shows that the pulvinar not only interacts with the dorsal
cortical pathway as well but does so in a similar mechanistic manner as with the ventral cortical pathway. Current data support the pulvinar increasing the gain of cortical neurons and regulating cortico-cortical coherence across a range of frequencies, to increase the efficacy of cortical transmission of behaviorally relevant information. The pulvinar may also reduce the efficacy of cortical transmission of irrelevant information through inhibitory mechanisms, as evidenced by its modulation of cortical alpha oscillations (Jensen and Mazaheri, 2010; Haegens et al., 2011). The pulvinar may thus amplify relevant and filter out irrelevant information in the world around us.

**Pulvinar contributions to cortical delay period activity (Hypothesis 3)**

Spatial attention increased pulvinar Granger causal influence on LIP and V4 in the alpha and beta frequency ranges. Attention also increased coherence between pulvinar spike output and cortical LFPs (in LIP and V4), and this pulvino-cortical interaction in the alpha range correlated with cortical delay period spiking activity. These results suggest that the pulvinar played a role in shaping and maintaining delay period spiking and LFP activity in both parietal and visual cortex. There were at least three possible underlying mechanisms increasing the effect of pulvinar output on cortical excitability during spatial attention: first, pulvinar spike rate increased; second, pulvinar neurons synchronized, allowing for summation of post-synaptic responses in the cortex; and third, the pulvinar and LIP/V4 synchronized (within the 8-30 Hz range), increasing the likelihood that pulvinar spikes arrived in the cortex during periods of reduced inhibition.

Previous studies that pharmacologically manipulated the pulvinar in primates have shown that the pulvinar can strongly augment visual cortical activity during visual stimulation. In anesthetized prosimians (galago), stimulating the lateral pulvinar (with bicuculline) increased stimulus-evoked spiking activity of V1 neurons, when both the pulvinar and V1 RFs overlapped; and deactivating the pulvinar (with muscimol) had the opposite effect (Purushothaman et al., 2012). In behaving macaques, deactivating the ventro-lateral pulvinar (with muscimol) reduced both visually-evoked V4 spiking activity and later attention-enhanced V4 activity during visual stimulation (Zhou et al., 2016). Further evidence has also supported thalamic influence over cortical activity during the delay period in attentional and working memory tasks. Our previous
macaque work, using the same flanker task as in this study, has shown that the pulvinar influenced LFPs (at frequencies >8 Hz) in the ventral visual cortical pathway (V4 and TEO) during maintained attention across the delay period in the absence of visual stimulation (Saalmann et al., 2012). Recent mouse studies have shown that other higher-order thalamic areas, specifically the mediodorsal thalamus and motor thalamus, contribute to the sustained spiking activity of frontal cortical neurons (Schmitt et al., 2017; Guo et al., 2017; Bolkan et al., 2017). Beta synchrony between the mediodorsal thalamus and frontal cortex was important for maintaining information across the delay (Bolkan et al., 2017). We extend these findings to show that the pulvinar contributes to the sustained activity of parietal and visual cortical neurons in primates. The attention-mediated increases in spike-field coherence (within the 8-30 Hz range) between pulvinar and cortex in both directions suggest that a subset of cortical neurons may first activate a subset of pulvinar neurons, which in turn can either help activate additional cortical neurons, and so on, similar to the mechanism proposed for interactions between frontal cortex and mediodorsal thalamus (Schmitt et al., 2017; Bolkan et al., 2017) (although the preferential spiking of an individual neuron at a particular time during the delay in the mouse studies, giving rise to a “tiling” of responses from different neurons across the delay period, was not clear in our study and may be due to a species difference) or reciprocally excite the same cortical neurons. Such cortico-thalamo-cortical cycles could continue across the delay period until the appearance of a new visual stimulus. Future studies that inactivate the pulvinar while recording from the cortex while the animal attends are needed to more solidly test this mechanistic hypothesis and to what degree the pulvinar contributes to cortical delay activity.

LIP is part of the fronto-parietal attention network in macaques, and previous attention studies have shown that the response of LIP neurons to a visual stimulus and across a delay reflects attentional priorities (Bisley and Goldberg, 2003), as in our task. Accordingly, PPC lesions in humans (Nachev and Husain, 2006) and macaques (Wardak et al., 2004) can give rise to severe attention deficits, such as perturbed attentional orienting to contralesional space, including visuo-spatial hemineglect. Because our study shows that the pulvinar contributes to LIP delay period activity, and likely PPC excitability more broadly, considering the anatomical connectivity
between the pulvinar and PPC (Shipp, 2003; Kaas and Lyon, 2007), it should not be surprising that thalamic lesions involving the pulvinar in humans (Danziger et al., 2001; Karnath et al., 2002; Rafal and Posner, 1987) and macaques (Wilke et al., 2010; Petersen et al., 1987) can also produce deficits in directing attention to contralesional space. In addition to maintaining a representation of attentional priorities, PPC delay period spiking and LFP activity has been proposed to reflect working memory, accumulation of sensory evidence for decision-making and action planning (Anderson and Cui, 2009; Shadlen and Kiani, 2013; Curtis and Lee, 2010). The pulvinar is well positioned to engage in all these cognitive functions, via its influence on PPC delay period activity.
Chapter 6: General Discussion

In this dissertation, I characterize neuronal activity in the primate pulvinar as well as interactions between the pulvinar and cortex during selective visual attention. Few studies have recorded neuronal signals from the pulvinar or between the pulvinar and cortex simultaneously while animals were engaged in an attentionally demanding task. The studies presented here expand upon the current literature and demonstrate:

1) a new method for MR-guided linear array recordings of a deep brain structure (Chapter 2),
2) a new method to distinguish between dorsal and ventral pulvinar using functional responses (Chapter 2),
3) differences in attentional modulation of pulvinar spiking activity by subdivision and cell type, specifically that selective visual attention suppresses firing rates of neurons in the ventral pulvinar and of interneurons (Chapter 3),
4) attentional modulation of interactions between dorsal and ventral pulvinar, specifically that attention increases phase synchrony between dorsal pulvinar spikes and ventral pulvinar LFPs (Chapter 3),
5) that pulvinar population spiking activity during an attention task can be well described by trajectories in a low-dimensional state space, which have oscillatory-like structure (Chapter 4),
6) attentional modulation of interactions between nodes of the dorsal cortical visual stream and the ventral cortical visual stream, specifically that attention increases phase synchrony between LIP spikes and V4 LFPs and Granger-causal influence of LIP on V4 (Chapter 5), and
7) attentional modulation of interactions between the pulvinar and nodes of the dorsal cortical visual stream and the ventral cortical visual stream, specifically that attention increases Granger-causal influence of the pulvinar on the cortex and increases
phase synchrony between pulvinar spikes and cortical LFPs, where this synchrony correlates with elevated delay period spiking in the cortex (Chapter 5).

Early electrophysiological studies of the primate pulvinar provided valuable insights into pulvinar function; however, they were technologically limited to recording single unit activity from single sites in the pulvinar while animals performed relatively simple behavioral tasks. Recent studies (Saalmann et al., 2012; Zhou et al., 2016, Fiebelkorn et al., 2019) and the studies presented here take advantage of newer recording methods, richer behavioral tasks, and finer analytical tools to probe pulvinar function at the population level and network level and more systematically than before. Simultaneous recordings of the pulvinar and connected cortical areas during attention have helped to elucidate how, during attention, the pulvinar influences neural activity in the cortex and regulates information transmission between areas in the ventral visual cortical stream (Saalmann et al., 2012), in the frontoparietal attention network (Fiebelkorn et al., 2019), and between nodes of the ventral stream and dorsal stream (Chapter 5). These findings suggest that, as a general rule, the pulvinar coordinates activity between all visual areas and modulates their functional connectivity during attention; however, further studies are needed to test the extent of this hypothesis. Specifically, it would be informative to test whether the pulvinar also regulates information transmission within the dorsal visual cortical stream, e.g. between areas LIP, 7a, and DP. The pulvinar also has direct connections with several brain areas not primarily associated with vision, such as the amygdala, and a growing line of research has focused on a fast pathway from the superior colliculus to the amygdala via the pulvinar that is thought to rapidly signal threatening or emotional visual stimuli (Ward et al., 2007; Van Le et al., 2013; McFadyen et al., 2019). Besides serving as a relay between the superior colliculus and amygdala, the pulvinar may also coordinate activity between limbic areas and visual cortical areas that could be modulated by attention.

Causal manipulations of the pulvinar, such as pharmacological inactivation, electrical microstimulation, and optogenetic activation/inactivation, would more directly establish a causal role of the pulvinar in regulating cortical activity. Inactivation of the ventrolateral pulvinar using a
GABA-agonist muscimol resulted in a connected cortical area, V4, to enter an “inactive” state, where baseline firing rates increased, stimulus-evoked responses decreased, low-frequency LFP power increased, and coherence between V4 and IT decreased (Zhou et al., 2016). Pulvinar inactivation also resulted in reduced effects of attention, though attentional modulation was still observed in spiking, spike-phase coupling, and inter-areal coherence (Zhou et al., 2016).

Consistent with the idea that the pulvinar maintains the cortex in an active state, pharmacological inactivation of the pulvinar shunted nearly all visually evoked responses in the superficial layers of V1 in the anesthetized Galago (Purushothaman et al., 2012). Activity in the pulvinar, and specifically, temporal coordination between the pulvinar and connected cortical areas may be necessary for cortical activity, and visual attention may have a modulatory effect on top of that. In this thesis, we found that during attention, the pulvinar influenced the cortex and that attention-related increases in synchrony between pulvinar spiking and cortical LFPs correlated with attention-related increases in cortical spiking during a delay period (Chapter 5). These findings corroborate recent findings which suggest that spiking activity within higher-order thalamic nuclei and synchrony between these nuclei and cortex are necessary for sustained, elevated neural activity in the cortex during a delay period (Schmitt et al., 2017; Guo et al., 2017; Bolkan et al., 2017). Further causal study of the pulvinar would shed light on how the pulvinar influences connected cortical areas and how this influence may be modulated by attentional demands.

Recently, our lab proposed a Rhythmic Theory of Attention (Fiebelkorn and Kastner, 2019; Fiebelkorn et al., 2019), which included a hypothesis of how the pulvinar influences cortical areas and vice versa in a rhythmically alternating fashion. Specifically, the authors found that behavioral performance in a visual detection task oscillated at around 3-6 Hz, in the theta band, and so did neural dynamics across the frontoparietal attention network. When behavioral performance was good, the pulvinar influenced the frontal eye fields (FEF) and the lateral intraparietal area (LIP) in the alpha and low beta frequency range, and when behavioral performance was poor, LIP influenced the pulvinar and FEF in the alpha and low beta frequency range. The authors thus proposed first that attentional modulation of perception is rhythmic in the theta frequency band, and second that the pulvinar, LIP, and FEF dynamically re-weight their
functional interactions between an engaged mode associated with good behavioral performance and a disengaged mode associated with poor behavioral performance, but during which the brain can more easily covertly or overtly shift attention and explore the visual environment.

The findings presented in Chapter 5 are mostly consistent with this theory. Spikes from the pulvinar phase-locked with the LFP in LIP in the beta frequency range, and vice versa, though the task was not designed to identify periods of good and poor behavioral performance at high temperature resolution. Delay periods were too short and trials were too few to see whether the directionality of influence alternated at a theta frequency. If we assume that the cue resets the phase of ongoing theta oscillations across the brain, it may be interesting to split trials in the current dataset by the cue-target delay duration, in 100 or 125 ms bins (about half of a theta cycle), and test if there are differences in behavioral performance or directionality of influence between the pulvinar and cortex in adjacent bins. However, given the already low number of trials, this analysis may be grossly underpowered. It would also be worthwhile to do a similar analysis on the within-pulvinar data described in Chapter 3, where delay periods are longer and there are more trials, to see whether local theta oscillations are linked to behavior, and if so, in which subdivision are they strongest. Given that some pulvinar cells in the dorsal pulvinar display strong saccade-related activity, it would be interesting to see whether these more motor-related neurons are differentially affected by theta phase than more visual neurons. The present findings in Chapter 5 also add to the Rhythmic Sampling theory that the pulvinar influence on the cortex in the alpha/low-beta frequency band is associated with and may support sustained elevated delay activity in the cortex.

Thalamic neurons display two major modes of firing: burst firing and tonic firing. While few studies have characterized bursting properties of pulvinar neurons in any animal model (Ramcharan et al., 2005; Wei et al., 2011), it would be worthwhile to investigate how this prominent phenomenon observed in the pulvinar relates to its neural mechanisms of attention. The triadic circuitry (Sherman, 2004) between corticopulvinar cells, pulvinocortical cells, and pulvinar interneurons has the potential to keep pulvinocortical relay cells in a relatively hyperpolarized state, where inputs from corticopulvinar cells are automatically negated or at least
dampened by interneuron input. When thalamic neurons are in a hyperpolarized state, they tend to suppress interneurons, which may occur during selective attention (Chapter 3), would then result in disinhibition of the pulvinocortical relay cells, which would result in a calcium transient and a burst of T-channel-mediated, low-threshold calcium spikes. This initial burst of firing could be the fundamental cause behind the observed phase reset of theta rhythms in the pulvinar (and perhaps in the cortex as well). After this initial burst, pulvinar relay cells with RFs at the attended location would enter a tonic mode in order to more faithfully and linearly signal its inputs to downstream areas. Cells with RFs at other locations would stay hyperpolarized, and in burst mode, in order to signal any salient or surprising events that are behaviorally relevant. This theory could be tested using a larger data set of pulvinar responses to attentional cues or other salient stimuli.

Together, the studies presented in this dissertation provide a deeper understanding of the primate pulvinar and its role in selective visual attention. More research needs to be performed to better understand the function of the pulvinar and the mechanisms of its influence on other brain areas at both the local circuit level and the large-scale network level. Given the striking attentional deficits that result from pulvinar inactivation, the widespread connectivity of the pulvinar with other visual areas, and the neural correlates of attention observed within the pulvinar and between the pulvinar and cortical areas, understanding the pulvinar will continue to be important for understanding the neural mechanisms of visual attention across the brain.
Prior Presentation and Publication

Chapter 2:
Some of the work described was previously presented at the following conferences:


Chapter 3:
Some of the work described was previously presented at the following conferences:


Chapter 4:
Some of the work described was previously presented at the following conferences:


Chapter 5:
The chapter was adapted from a manuscript written in collaboration with Yuri Saalmann, Mark Pinsk, and Sabine Kastner. The manuscript has been submitted for publication, and a preprint was made available here: https://www.biorxiv.org/content/10.1101/405381v1
Bibliography


