Lateral intraparietal area activity as a temporal production signal during precise timing

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Dedication

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Abstract

We often perform movements without external cues telling us when to move. However, the way our brains time self-initiated movements is still unclear. For example, while temporal modulations in neuronal activity have been observed in a variety of timing tasks, it is not clear if these modulations are strictly related to the timing of movements or instead reflect timing measurements of external events such as sensory cues and rewards. To isolate the temporal production signals of movement initiation, we devised a self-timed task that requires non-human primates to saccade between two fixed targets at regular intervals in the absence of external cueing and without an immediate expectation of reward. To examine the potential neural basis of this temporally dependent behavior, we recorded from single neurons in the lateral intraparietal area (LIP), which has been implicated in the cognitive planning and execution of eye movements. In contrast to previous studies that observed a build-up of activity associated with the passage of time, we found that LIP activity decreased at a constant rate over the inter-saccadic interval. Moreover, this falling activity was found to be significantly predictive of inter-saccadic interval duration on an interval by interval basis. Interestingly, the relationship of this falling activity to the actual duration of the timed interval depended on eye movement direction: it was negatively correlated when the upcoming saccade was toward the neuron's response field, and positively correlated when the upcoming saccade was directed away from the response field. This suggests that LIP activity encodes timed movements in a push-pull manner by signaling for both saccade initiation towards one target and prolonged fixation for the other target. Thus timed movements in this task appear to reflect the competition between local populations of task relevant neurons, rather than a global timing signal. Additionally, microstimulation was delivered during separate experiments to determine if a causal relationship existed between LIP activity and motor production. Stimulation affected the animals perception of time in a manner consistent with the correlation results, suggesting that LIP activity provides a motor timing signal that is utilized in the initiation of precisely timed behaviors.

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Part I

Background and Introduction

Time and Behavior

One of the more interesting, and basic questions concerning time is where do the temporal signals originate that allow us to utilize and interact with time? We do not possess dedicated sensory receptors for timing as we do for vision, for example. Yet, we are quite capable of determining how long something lasted, reporting which event in a sequence occurred first, or comparing interval durations. This ability to perceive time is essential not only for temporal discrimination, but also for everyday behaviors and survival in humans and other organisms [Buhusi and Meck (2005); Wittmann (2009)].

Behaviors and internal processes can occur over multiple time scales [Buhusi and Meck (2005); Mauk and Buonomano (2004); Buonomano and Karmarkar (2002)]. Events that take place over long time scales, on the order of days, are classified as circadian rhythms. Sleep-wake cycles, hormone regulation, thermoregulation, appetite cycles, and reproductive fitness are all examples of circadian rhythms. To produce these events, the suprachiasmatic nuclei of the hypothalamus is thought to provide a timing signal that drives these rhythms by utilizing external cues, such as light-input [Kowalska and Brown (2007); Reppert and Weaver (2002, 2001)].

Other behaviors take place over much shorter time scales. Events that occur over milliseconds to minutes are collectively known as interval timing [Meck (2005)]. Complex cognitive tasks (e.g. decision making), breathing, and locomotion happen over intervals that range from seconds to minutes. Complex tasks that occur over millisecond time scales include speech generation, vocalization discrimination, motion detection, and motor coordination. It is also the range over which we process the spatial and temporal patterns of activity that our sensory neurons receive in order to provide sensory information [Buhusi and Meck (2005); Buonomano and Karmarkar (2002)].

Not only do behaviors occur over multiple time scales, but our ability to perceive time also occurs over multiple time scales. We are capable of estimating when events took place (5 minutes ago, 5 hours ago, or event 5 years ago) or how long events lasted (a 2 hour baseball game versus a 4 hour baseball game, for example). External stimuli can be utilized when judging these durations. For instance, when we are approaching a stoplight while driving, seeing it change from green to yellow allows us to anticipate the change to red. Our perceived estimation of time between the change from yellow to red allows us to decide if we can continue safely through the intersection or if we need to brake.

Although we are able to utilize external sensory cues to judge time, our sense of time cannot be a complete reflection of external events. For example, our ability to walk, talk, type and play a musical instrument are all examples of temporally dependent movements that can occur without external cueing. Yet the way in which our brains utilize time in order to produce internally driven movements is not understood. By investigating movements that happen in the absence of external cues, we hope to learn where these signals exist and what signals the brain utilized in order to generate temporally precise movements.

With a wide array of time dependent behaviors, it is not surprising that multiple brain areas have displayed time dependent activity. However, at shorter time scales (interval timing), it is still unclear where timing signals originate. Areas, including the cerebellum and basal ganglia, as well as cortical areas, such as the dorsolateral prefrontal cortex and parietal cortex, have shown temporal activity related to the more complex behaviors of the shorter time scales [Mauk and Buonomano (2004); Wittmann (2009)]. However, these findings do not necessarily mean that these areas are a source of the temporal signals required for these behaviors. They may simply reflect temporal signals generated elsewhere. Efference copy signals provide a nice

example of this. These signals are generated when our bodies produce movements to inform the brain of those commands. Yet, even though these signals are correlated to the movement (in that they occur at the same time as the movement), they do not cause the movement.

A number of experiments have been used to study timing. Many of the basic timing tasks often involve duration discrimination, duration estimation, duration reproduction, rhythm comparison, or even finger tapping [Meck et al. (2008)]. Most of these experiments involve some sort of cueing (typically visual or auditory) in order to provide the interval that is to be discriminated, estimated, reproduced or compared. The purpose of these experiments are to investigate time by observing how subjects perform during temporally dependent tasks. The subject's behavior during these tasks, combined with various techniques, can provide information on how organisms generate, process, and use time. A few examples of studies that have shed light on the mechanisms of timing are provided below.

One way researchers have studies timing is by investigating the performance of human subjects during psychophysical tasks. In these studies, it has been found that cognitive functions such as short-term memory, long-term memory, and attention, which are all dependent on time, may be used in determining temporal judgements [Brown (1997); Zakay and Block (2004); Taatgen et al. (2007)]. Additionally, they have found time estimation can be greatly affected by moods, emotions, and subjective well-being [Wittmann et al. (2006); Droit-Volet and Meck (2007); Noulhiane et al. (2007)]. For instance, time seems to speed up when we are having fun, but it can seem to slow down during times of restlessness. Essentially, temporal estimates are found to be longer when subjects pay greater attention to them [Wittmann (2009)]. Although these studies provide details on how cognitive states affect time perception, they tell us little about the neural mechanism behind timing.

Pharmacology studies provide a means for the reversible manipulation of neural activity. By altering the activity of various brain regions, effects on timing can be investigated. Some of the primary pharmacology findings on timing have come from systemic injections of dopamine agonists and antagonists in rats [Buhusi and Meck (2002); Cheng et al. (2006); MacDonald and Meck (2005); Maricq and Church (1983); Matell et al. (2006)]. When dopamine agonists, such as methamphetamine or cocaine, were administered it was found that the animals underestimated durations. When dopamine antagonists were delivered, animals overestimated durations. These findings implicated brain regions with dopamine dependence, such as the basal ganglia, in time perception. Similar effects were observed following chronic administration of acetylcholine [Meck (1983); Meck and Church (1987)], which suggests a frontal cortex involvement in timing [Meck (1996)]. However, the localization of the delivery and the potential side-effects of the drugs and their interactions within the brain, make these experiments far from perfect.

More recently, researchers have begun using fMRI, PET, and transcranial magnetic stimulation to investigate timing mechanisms. These studies can provide insight into the areas that are activated in awake behaving humans and animals [Rao et al. (1997, 2001); Schubotz et al. (2000); Lewis and Miall (2002); Basso et al. (2003); Rubia and Smith (2004)]. For instance, the dorsolateral prefrontal cortex and the inferior prefrontal cortex have been found to be active during time estimation, time discrimination, and sensorimotor synchronization tasks. Similar tasks have also shown that the supplementary motor area, anterior cingulate gyrus, basal ganglia, parietal cortex, and the cerebellum also display selective excitation during timing dependent tasks. However, the anatomical precision, causality of the behavioral output, and the relationship between neural activity and these techniques are still in question.

Diseased patients and lesion studies also allow researchers to see what happens

to timing capabilities when different brain regions are dysfunctional. By observing timing deficits, they can draw conclusions about what brain regions are valuable to different components of timing. Early lesion studies displayed the importance of the cerebellum in timing. Patients and animals with cerebellar lesions have shown dysfunctions in the precise timing of movements [Ivry et al. (1988)], in sensory duration discrimination [Ivry and Keele (1989)], and eyelid conditioning [Perrett et al. (1993); Garcia et al. (1999)], to name a few. Diseased patients, such as those with Parkinson's disease also have shown deficits in temporal behaviors [Koch et al. (2008); Malapani et al. (1998b, 2002, 1998a)], suggesting that the striatum is essential for interval timing [Buhusi and Meck (2005)]. Although these studies provide insights into abnormal timing as a result of brain damage, the extent of the damage and its effects are often unknown, making comparisons to normal subjects difficult.

Electrophysiological recordings using single electrodes, tetrodes, and arrays, provide great detail about neural activity that occurs during time related behaviors. Neural activity from a number of different brain areas have been recorded as animals performed duration discrimination and duration estimation tasks, for example. Cortical regions including the premotor, motor, parietal, prefrontal, and frontal areas, along with the striatum and cerebellum, have displayed time-dependent, single cell activity as durations were being monitored [e.g. Lucchetti and Bon (2001); Leon and Shadlen (2003a); Roux et al. (2003); Janssen and Shadlen (2005); Genovesio et al. (2006); Renoult et al. (2006); Genovesio et al. (2009); Chiba et al. (2008); Meck et al. (2008); Prsa et al. (2009)]. These, and many other electrophysiological studies, have shown that numerous areas display time-varying signals on the level of single cells. Yet, these signals may simply be a reflection of external events or activity generated in a different region, making distinctions between areas that utilized temporal activity and areas that produce temporal activity difficult.

While all of these techniques do provide some important information about temporal mechanisms [Rubia and Smith (2004); Ivry and Spencer (2004); Buhusi and Meck (2005); Meck (2005); Meck et al. (2008); Wittmann (2009); Coull et al. (2011)], each have their weaknesses. Most of the studies above investigated timing using external cueing (usually visual or auditory) to direct behavior. Therefore, most of these insights are based off of external events rather than an internally generated timing signal. Additionally, most of these techniques tell us little about causation (i.e. whether activity in these regions is sufficient to alter the timing of the behavior at hand).

In order to provide novel and insightful information to the neural timing field, we utilized a self-paced timing task, absent from any external timing cues, while recording single cell neuronal activity. Because no external cues were provided to our subjects, the observed activity should reflect timing signals based on internal estimates of time. Additionally, we introduced microstimulation as animals performed this task. This allowed us to determine if the observed activity contributed to the timing of behavior. For a clear understanding of how neural timing is accomplished, discoveries from multiple fields and methodologies must be required and analyzed collectively. Our goal was to provide new insights on where timing signals originate, what these internally driven signals look like, and how they are used to time behaviors.

Timing Models

There are currently two lines of thought pertaining to where temporal signals originate to affect neural processing and behavior on the millisecond to minute time scale. Some believe that there is a centralized timing mechanism [Gibbon (1977); Matell and Meck (2000)], while others believe in a distributed timing mechanism [Rao et al. (1997); Buonomano and Karmarkar (2002); Staddon (2005)]. In the central timing model a specific portion of the brain produces a timing signal that is utilized for

all timing related events for all modalities. Other brain regions that are required for the movement, simply tap into this timer in order to produce timed behaviors. The prominent model for this mechanism is the internal clock model. In this model, a pacemaker emits pulses at a regular interval. These pulses are then counted by an accumulator and compared to previously reinforced counts stored in memory. Support for this model developed due to the successful predictions produced by the model [Gibbon et al. (1984)] as well as neural and pharmacological based evidence [Plenz and Kital (1999); Meck (1996, 1983); Maricq and Church (1983); Matell et al. (2004); Buhusi and Meck (2007)]. Yet, due to inconsistencies with other studies [Malapani et al. (1998b); Holson et al. (1996); Buhusi and Meck (2002)], the relevance of this model to interval timing mechanisms within the brain remains unclear.

The distributed timing model suggests that there is no dedicated timing system but that the ability to represent time is an intrinsic property of distributed cell populations that are required for a given task [Ivry and Spencer (2004); Buonomano and Karmarkar (2002)]. Instead of all regions tapping into a central clock for all modalities, neurons concerned with a given modality and task produce timing information based on population interactions. There are two prominent models in the distributed scheme, labeled lines and population clocks.

In the labeled lines model, different neurons within a population respond at different interval lengths. For example, one neuron may respond at 100 ms while a second neuron responds at 200 ms. The labeled line population can be used to determine time by which neurons are active. In order to time an interval using this model, a regular temporal signal must exist that allows neurons to respond incrementally until the interval is reached. Because a variety of intervals must be accounted for in a timing model, regular temporal signals may be provided by various substrates that offer different signal frequencies. Labeled line models have utilized oscillators [Maill

(1989)], slow biochemical reactions [Fiala et al. (1996); Jaffe (1992)], intrinsic currents [Beggs et al. (2000)], and cell thresholds [Antón et al. (1991)] to time a range of intervals [Buonomano and Karmarkar (2002)]. Although effective in timing tasks such as interval discrimination, difficulties arise with this model when the temporal demands of tasks are increased (e.g. musical structure, speech) [Buonomano and Karmarkar (2002)].

In the population clock model, time is encoded through the network activity of a population of neurons. There is no specific time at which neurons are active, instead dynamic interactions or time-dependent changes between neurons within the network provide information about lapsed time. For instance, short-term synaptic plasticity [Buonomano (2000)], or inhibitory feedback signals [Medina et al. (2000)] between neurons may be used in millisecond to second timing. In this model, no temporal constants exist, yet a variety of intervals can be encoded. This type of activity has been observed to account for interval discrimination and temporal sequences [Buonomano and Karmarkar (2002)].

Although important for countless behaviors and cognitive processes, it is still not clear what mechanisms provide timing signals to drive internally generated movements or where in the brain time perception occurs. Multiple timing models, using various brain regions, have tried to account for interval timing behaviors. Yet answers to these questions about timing are still not fully developed. This gap in understanding provides the basis for my research. A greater knowledge of neural timing will not only help use to better understand normal motor and cognitive functions but it will also provide insights into numerous disease states that display timing deficits [Lebedev et al. (2008)].

LIP and Motor Production

One of the ways to study the neural basis of time and time perception, is through the use of a motor task that is highly dependent on timing. Animals can be trained to perform precision timing tasks and their behavior, along with neural activity, can be monitored in order to observe task related activity. Analyses concerning the timing of the behavior(s) and neural activity can then be made in order to answer questions about neural timing. To examine temporal signals, a temporally concise and trainable behavior must be used and the circuitry that controls this behavior must be known.

The ability of animals to perform timed ocular-motor movements suggests that the visual-motor pathway utilizes temporal signals that affect behavior and neural activity. Employing ocular-motor movements to investigate the origin of neural timing and time perception has many advantages. The circuitry that controls saccades, the rapid jerk-like eye movements that direct gaze to new locations either in response to a stimulus or in visual search, is quite well known. The motor output used to produce saccades is very reproducible and few muscles are involved in their generation. Also, the fact that saccades have very fast responses and can be easily and efficiently monitored makes them an ideal movement for study in cases where precise timing is a concern.

The lateral intraparietal area (LIP) of the posterior parietal cortex (PPC) has traditionally been seen as an integration center between visual input and motor output [Andersen (1995); Gottlieb (2007)]. It has also been shown to be part of the saccade generating pathway [Thier and Andersen (1998)]. LIP is located on the lateral wall of the intraparietal sulcus. It is highly inter-connected with the frontal eye fields and the superior colliculus, as well as a variety of other largely visual areas such as V3, V4, the middle temporal area (MT), and many others, making LIP a unique area in the PPC [Blatt et al. (1990); Cavada and Goldman-Rakic (1991); Ferraina et al. (2002);

Lewis and Van Essen (2000)]. LIP's location between visual and saccade related areas is evident in its activity. LIP neurons contain receptive fields; a representation of visual space for which the presence of a stimulus within that area causes increased activity [Blatt et al. (1990); Andersen et al. (1990a)]. LIP neurons also contain response fields; an area in space to which a movement occurs when that neuron is activated [Gnadt and Andersen (1988); Platt and Glimcher (1998)]. The combination of these fields, which are often similar in space for nearby neurons, suggests that this area is involved in visuomotor transformations.

However, determining exactly what this region does has proven to be complicated, in part because its response properties have been shown to be highly dependent on the task and task related experiences [Freedman and Assad (2006); Law and Gold (2008); Freedman and Assad (2009); Bennur and Gold (2011)]. LIP has been implicated in a wide variety of situations including the allocation of visual attention [Bisley and Goldberg (2006); Gottlieb et al. (2009); Bisley and Goldberg (2010)], perceptual decision making [Shadlen and Newsome (2001); Roitman and Shadlen (2002); Hanks et al. (2006); Gottlieb and Balan (2010)], goal selection during visual search [Ipata et al. (2006); Thomas and Paré (2007)], remapping of visual stimuli during eye movements [Duhamel et al. (1992); Kusunoki and Goldberg (2003); Heiser and Colby (2006)], as well as the planning of eye movements [Platt and Glimcher (1997); Bracewell et al. (1996); Mazzoni et al. (1996); Gnadt and Andersen (1988)]. Importantly, it has also been shown that LIP activity is modulated during the anticipation of a visual cue [Colby et al. (1996)] and also during times when the animal can anticipate a reward [Sugrue et al. (2004); Yang and Shadlen (2007); Bendiksby and Platt (2006); Seo et al. (2009)].

Recently, investigators have reported that explicit representations of the passage of time have been observed in LIP [Leon and Shadlen (2003b); Janssen and Shadlen

(2005); Maimon and Assad (2006)]. During these tasks, neural activity varied over time in a task-dependent manner. However, since visual cues were utilized in each of these tasks, it is unclear if the time-dependent activity was related to temporal production, the execution of a behavior at a specific moment, or temporal measurement, the time related to external cueing. For instance, in the study by Leon and Shadlen, monkeys were required to report the difference in cue duration. Animals were required to fixate a central dot as two potential saccade targets appeared on the monitor. The central dot then changed from blue to white for a fixed duration. The dot changed back to blue briefly prior to turing white again for a variable amount of time. The fixation point subsequently turned off, indicating to the animal to make a saccade to one of the two targets depending on which duration (indicated by the white fixation points) lasted longer [Leon and Shadlen (2003b)]. In this case, time related activity almost assuredly represents temporal measurement signals since the movement was not timed.

For the Janssen and Shadlen study, animals were again required to fixate a central target. Once the central target dimmed, animals were required to saccade to a second peripheral target. The "go" cue (dimming of the central fixation point) was programmed to occur during regular intervals that were drawn from pre-determined probability density functions [Janssen and Shadlen (2005)]. Animals were able to learn and anticipate the times at which the go cue was likely to happen. Increases in LIP activity were observed during times at which it was likely that the go cue would occur. However, because the go cue always preceded a movement, it is uncertain if the consistent variations in neural activity represented the time-dependent probability of cueing, or the initiation of the movement.

Similarly, in the Maimon and Assad task, animals were again cued with a visual stimulus to make a movement. Monkeys were required to fixate a target on a monitor

while two parallel bars appear in the periphery. A dot located between the bars appeared at the same time and, after a slight delay, the dot moved toward one of the bars at a constant speed. The animals were required to press a lever just before the moving dot would hit the bar to cause the dot to turn [Maimon and Assad (2006)]. Although this task also included trials where a delay was imposed between the lever press and the dot change and trials where the lever was pressed in response to the computer turning the dot, variations in activity may yet reflect a decision process based on stimulus dynamics rather than the movement itself.

In addition to visual cueing, temporally related activity may also reflect other events that are closely linked to the movement, such as reward. In most timing studies, rewards are delivered following a single movement that is cued at a consistent time. This allows that animal to not only anticipate the movement, but also to anticipate the sensory cue and reward delivery. This is indeed problematic when investigating temporal productions signals since LIP neurons have been shown to modulate their activity during both sensory [Colby et al. (1996)] and reward anticipation [Platt and Glimcher (1999); Sugrue et al. (2004); Yang and Shadlen (2007); Bendiksby and Platt (2006); Dorris and Glimcher (2004); Seo et al. (2009)]. Therefore, in order to investigate motor production signals within LIP, a timing task must be designed that avoids regularly occurring external events such as cues, stimulus changes, or rewards.

Overview of Analyses: Part 1

To accomplish this end, we devised a task termed the self-timed rhythmic saccade task (Figure 1). During this task, animals were required to perform saccades back and forth between two fixed targets at a consistent interval such that saccades occurred each second (0.5 Hz). One target was located near the center of the monitor while the other target was peripherally positioned within the RF of the neuron being recorded

(relative to fixation of the central target). Trial lengths were randomized and no external, movement related cues were provided to the animals. Trials were immediately aborted if animals produced an interval between saccades that varied by more than 200 ms from the trained 1 second interval. The lack of dynamic external cues served to control for sensory anticipation while randomizing trial lengths served to control for reward anticipation. Additionally, trials were allowed to end at any time within an interval, not only at times following saccades. This was done to further dissociate reward from motor production. Saccades made toward the central target are termed central saccades while saccades made toward the peripheral target are termed peripheral saccades.

Various analyses from this experiment provided insights into the timing mechanisms within the monkey brain. By performing a correlation analysis between the inter-saccade times produced by the animals and the neural activity that occurred during that same interval, we were able to determine that LIP activity does reflect a temporal production signal during this task. For this signal to be used in the timing of saccades, activity should be correlated with inter-saccade times throughout inter-saccadic intervals, which is what we found. Because the timing of eye movements was precisely defined in our design, if activity had only depended on the potential of immediately making a saccade, correlations between activity and behavior would have been restricted to very brief periods (100-150 ms) preceding the saccades. This motor "preparation" is different from motor production in that motor preparation is only concerned with initiating an imminent saccade while motor production is the timing of a future saccade. Thus, this experimental design allowed us to distinguish the neuronal activity associated with motor production from activity associated with motor preparation.

Additionally, by comparing saccadic activity between the two directions of move-

ment (central saccade vs. peripheral saccade), information on the distribution of temporal strategies within the brain was also obtained. Because activity during central and peripheral saccades was significantly different from one another, that suggests that temporal signals are distributed amongst the neurons concerned with a given movement. Had activity between saccade directions been similar, it is possible that temporal signals would have been obtained from a centralized timer since different movements (with respect to the RF) would have been timed in the same manner. Also, had individual neurons within the population appeared to be correlated with the behavior at different points within the interval, LIP could have been utilizing a labeled line mechanism to time behavior. However, we found little evidence for a labeled line method. Instead, very few individual cells displayed significant correlations between activity and duration. Yet, the population activity was significantly correlated. These findings suggest that a population clock mechanism potentially accounts for motor production. By comparing neuronal activity between saccade directions and by investigating individual neuronal responses, we are able to suggest how timing signals are distributed within the brain and how these signals were produced.

Another issue that remains unexplored is how the complexity of motor plans affects their representation. All existing data concerning the delay period responses of LIP neurons comes from tasks in which the behavioral requirement is limited to very small numbers of movements (usually one). Psychophysical evidence suggests that, when confronted with an array of saccade targets, subjects naturally plan entire saccade sequences [Zingale and Kowler (1987)]. The planning of entire sequences has been observed in a number of brain regions and for various tasks [Baldauf et al. (2008); Mushiake et al. (2006); Histed and Miller (2006); Lu and Ashe (2005); Ohbayashi et al. (2003); Fujii and Graybiel (2003); Tanji and Shima (1994)]. Therefore, motor neurons that control saccadic movements may also display the planning

of an entire sequence of movements. Because two saccade targets were stationary throughout the trial in our design, it may have encouraged the planning of an entire sequence of saccades. By requiring multiple saccades, the potential existed to see different phenomena than those reported by previous studies in which the vast majority required only a single motor event. Although our task provided an opportunity for sequence planning to occur, we did not observe such activity within LIP.

Overview of Analyses: Part 2

Even though temporal signals were observed within LIP during this task, those signals say nothing about the causality between neural activity and the behavior. LIP activity may simply reflect activity from another brain region and not directly influence behavior. In order to determine if the observed activity does influence behavior, we performed a second set of experiments during which brief pulses of sub-threshold microstimulation were delivered to LIP as each animal performed the self-timed delayed saccade task.

Stimulation was delivered during random intervals of random trials in order to prevent the animals from anticipating the potential effects. Two separate stimulation experiments were performed. In one experiment, consistent stimulation parameters were delivered (250 Hz, 150-180μA, 16 ms) at various times within the interval between saccades. This was done to see if the time at which stimulation was delivered affected the timing of the behavior. In the second experiment, stimulation was delivered at a consistent time (250 Hz, 150-180μA, 450 ms following the execution of a saccade) but the duration varied (16, 32, 48, 64, and 80 ms). This was done in order to see if duration had an effect on the extent to which the behavior was affected.

The application of stimulation allowed us to determine if LIP activity was involved in the perception of time. Since intervals during which stimulation was de-

livered were significantly different from "no-stim" intervals, it suggests that LIP is involved in the perception of time. Also, by comparing stimulation results between movement directions (central vs. peripheral), this experiment also provided further insight into the distribution of temporal strategies within the brain.

Because the inter-saccadic duration prior to a central saccade was altered by stimulation while the interval associated with a peripheral saccade was unaffected, that suggests that temporal strategies are distributed amongst the neurons associated with the RF. Had inter-saccade times for both movement directions been affected by the stimulation, it would have suggested that LIP was a component of the centralized timer since movements both towards and away from the RF would have been affected similarly by the stimulation. Also, had stimulation produced no effect on either movement, that could have indicated that the timing of the behavior was determined by a centralized pacemaker located elsewhere within the brain or, more simply, that we were not in an area that influenced the timing of behavioral events. In this way, delivering localized stimulation provided evidence for temporal perception within LIP and distributed timing mechanisms within the brain.

Summary

Accurate muscle timing is crucial for coordinated behavior. Movements can be triggered internally through self-initiated activity or externally by sensory signals. Our goal was to discern the neural signal responsible for the internal initiation of timed saccades and to determine if altering this signal affects the perception of time.

Previous studies have found evidence of temporal signals within LIP [Leon and Shadlen (2003b); Janssen and Shadlen (2005); Maimon and Assad (2006)]. However, these studies utilized sensory signals in order to cue movement onset. Additionally, the temporally related activity observed in these studies may have reflected other

events that were closely linked to the movement, such as reward. Therefore, the observed timing signals could be related to the external events associated with temporal measurement rather than temporal production of the behavior.

The focus of this work was to investigate the internal timing signals in LIP used to produce temporally precise behaviors. To do this, animals were trained to perform a task that was highly dependent on their perception of time. Because LIP has been shown to modulate its activity during periods where sensory events and reward can be anticipated [Colby et al. (1996); Platt and Glimcher (1999); Sugrue et al. (2004); Yang and Shadlen (2007); Bendiksby and Platt (2006); Dorris and Glimcher (2004); Seo et al. (2009)], efforts were made to dissociate the timed movement from both sensory cueing and reward delivery. This task was termed the self-timed rhythmic-saccade task.

By recording from LIP as each animal performed this task, analysis of the neuronal firing rates allowed us to determine that previous patterns of activity were due to the timing of external events, such as cueing or reward, instead of reflecting the upcoming initiation of behavior. Since a temporal signal was observed after dissociating sensory cueing and reward from the behavior, we then wanted to determine if the signal was directly linked to the production of the behavior, or if the signal simply reflected a timing signal produced in some other region.

Introducing stimulation to LIP as animals performed the self-timed rhythmic-saccade task allowed us to determine that the observed temporal production signal could affected behavior. Since behavioral timing was altered, it suggests that LIP was involved in time perception as the animal performed rhythmic saccades. Additionally, by investigating both movements toward and away from the RF in each experiment, we were able to provide support for a distributed (vs. centralized) neural timing mechanism.

Part II

Temporal Production Signals in Parietal Cortex

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Introduction

Whether we are walking, talking, typing, or simply reaching to grasp an object, movements must be executed at the correct time. The natural timing of these movements does not necessarily require an external cue. For instance, a musician is capable of keeping a beat while playing an instrument without any external cues telling the musician when to play each note. Rather, an internal representation of time is used to keep a steady tempo.

In order to plan for an upcoming movement (such as the next note to be played), the brain must be able to represent the passage of time. However, the nature of signals that encode time (measurement), and the way in which these signals are utilized in order to produce movement (production), are unclear [Genovesio et al. (2009); Mauk and Buonomano (2004); Buonomano and Karmarkar (2002)]. In particular, signals associated with temporal measurement, the representation of the passage of time between external events, need not reflect temporal production, the execution of a behavior at a specific time [Leon and Shadlen (2003a); Wencil et al. (2010); Genovesio et al. (2009); Chiba et al. (2008); Renoult et al. (2006); Harrington et al. (2004)]. Although the passage of time must be monitored during both temporal measurement and temporal production, temporal measurement is defined as timing associated with external cues while temporal production is defined as timing solely associated with movement initiation.

Recently, investigators have suggested that explicit representations of the passage of time can be found in the responses of lateral intraparietal (LIP) neurons of the posterior parietal cortex [Leon and Shadlen (2003a); Janssen and Shadlen (2005); Maimon and Assad (2006)]. The neuronal activity observed in these studies systematically varied over specific temporal intervals prior to the execution of a movement. However, these variations do not necessarily reflect temporal production. For instance, in

the study by Leon and Shadlen, animals were required to measure the duration that visual stimuli were displayed [Leon and Shadlen (2003a)] but the required behavior itself was not timed.

Consistent variations in activity over time may also reflect task parameters, such as stimulus events or probabilities, that systematically vary over time, rather than representing time itself [Janssen and Shadlen (2005); Hwang and Andersen (2009); Cui and Andersen (2007); Scherberger and Andersen (2007); Gail and Andersen (2006); Genovesio et al. (2006); Quian Quiroga et al. (2006); Scherberger et al. (2005); Coe et al. (2002); Lucchetti and Bon (2001); Snyder et al. (1998, 1997)]. For example, if the animal is explicitly cued when to make a movement and that cue tends to happen at certain times, then activity may represent the time-dependent probability of cueing rather than temporal production per se [Janssen and Shadlen (2005)]. Similarly, if movements are linked to sensory events, such as the approach of a moving target, temporal variations in activity may reflect stimulus dynamics rather than the movement itself [Maimon and Assad (2006)].

Activity could also reflect external events such as reward that, by virtue of being tightly coupled to the upcoming movement, can be readily anticipated. In most timing studies, rewards are contingent on a single specific eye movement [Janssen and Shadlen (2005); Leon and Shadlen (2003a); Cui and Andersen (2007); Quian Quiroga et al. (2006); Coe et al. (2002); Snyder et al. (1997)] that is cued at a particular time. Therefore, both the sensory cue instructing the movement, and the reward that is linked to the production of that movement, can be readily anticipated. This is of particular interest because LIP neurons have been shown to modulate their activity during visual anticipation [Colby et al. (1996)], and reward anticipation [Platt and Glimcher (1999); Sugrue et al. (2004); Yang and Shadlen (2007); Bendiksby and Platt (2006); Dorris and Glimcher (2004); Seo et al. (2009)], as well as during movement plan-

ning [Bracewell et al. (1996); Mazzoni et al. (1996); Andersen et al. (1997); Platt and Glimcher (1997); Snyder et al. (1997); Gnadt and Andersen (1988); Andersen and Buneo (2002)]. Therefore, a measurement of temporal production signals requires a timed behavior which occurs in the absence of regularly occurring external events such as cues, stimulus changes, or rewards.

To accomplish such measurements, we designed a self-timed task that requires animals to move consistently at regular time intervals without any external or environmental cues (Figure 1). Specifically, the task requires the animals to make rapid eye movements (saccades) back and forth between two fixed targets every second. Trials were immediately aborted if any inter-saccadic interval, the time between subsequent saccades, differed by more than 200 ms from the 1 second standard. The lack of any external, timing related visual cues serves to control for sensory anticipation and temporal measurement. Trial length and reward amount were randomized on a trial by trial basis to minimize reward anticipation. We further dissociated reward from the saccadic movement by allowing trials to end at any time within an interval, not just immediately following the completion of a saccade. Finally, by utilizing saccades instead of other movements (such as reaching), we minimized any variability in motor output since saccade metrics between two fixed locations are highly consistent.

After animals were trained consistently to saccade at one second intervals, we recorded from individual neurons in LIP. Our recordings confirm previous suggestions of temporal representations within the area, but suggest that the nature of these representations is far different from prior reports. First, we found that, unlike the activity observed in previous studies, activity within LIP was characterized by a constant decrease in activity throughout the timed interval prior to movement initiation. Second, activity throughout inter-saccadic intervals was significantly predictive of interval duration on an interval by interval basis. Lastly, the sign of activity's correlation with the

upcoming inter-saccadic interval reversed after saccades towards the response field. Therefore, it appears that localized LIP activity contributes to both saccade initiation and fixation, and temporal production in our task reflects the balance between these two signals.

Materials and Methods

Task Training and Design

Two male monkeys (*Macaca mulatta*) (8.3 & 9.3 Kg) were seated in a darkened room in front of a computer monitor. Training began by learning to maintain fixation (within 3º) of a single target in order to receive a juice reward. The length of the fixation requirement was gradually increased to 1000 ms. Once the animal could fixate for a full second, a second target appeared and the initial target was extinguished. Animals then had to fixate one of the two targets as each was displayed for 1000 ms. Each target was identical in size (0.15 deg) and color (white). The number of fixations (and therefore the number of timed delayed-saccades) required was gradually increased and randomized (2-10 saccades). To this point, we have somewhat mimicked previous designs, in that temporal measurement (the regularity of fixation disappearance and cue appearance) and temporal production (the regularity of saccades) were both likely to be occurring. To encourage the animal to rely solely upon temporal production signals, the luminance of the non-fixated target was gradually increased until both targets remained on constantly and with equal luminance throughout the trial.

For physiological recordings, the animals performed this self-timed rhythmic-saccade task. Trials began with the monkey fixating the first of two targets to appear on the monitor (Fig. 1A). One of the targets was positioned near the center of the screen while the other target was positioned peripherally within the response field (RF) [Gnadt and Andersen (1988); Barash et al. (1991b); Colby et al. (1995, 1996);

Platt and Glimcher (1997, 1998); Andersen and Buneo (2002)] of the neuron being recorded (dashed box in Fig. 1A). Immediately following fixation of the first target ("Initial Target"), the second target appeared on the monitor ("Subsequent Target"). Once fixation of the initial target occurred, the monkey was required to perform saccades back and forth between the two targets so that a saccade occurred each second ("Rhythmic Saccades") (+/- 200 ms) (0.5 Hz). After both targets had appeared, no further changes in visual stimuli occurred. The monkey was required to continue making saccades (2-10) between the two targets at the 1 second interval for a randomized trial length before receiving a juice reward (Fig 1). Trials randomly alternated between having the initial target appear at the central and peripheral locations to ensure that we observed both saccadic directions for each interval within the sequence.

Saccade targets were constantly displayed following the first interval of the trial in order to minimize sensory anticipation [Colby et al. (1996)]. To help minimize reward expectations, the hazard function, which represents the instantaneous probability of the trial ending given that it has not yet ended, was flat throughout each trial [Ghose (2006); Janssen and Shadlen (2005)]. Therefore, the instantaneous probability that the animal received a reward at any given instant was kept constant throughout each trial. Trial times were exponentially distributed toward shorter trial times (decay constant = -1000 ms). Trials could end at any point, including the middle of an interval, in order to further dissociate saccadic movements from reward. Average trial length for both animals equaled 3.96 sec (sd = 1.14 sec). A minimum of 50 saccades was required from each cell while the animal performed the rhythmic-saccade task.

Eye movements made to the peripheral target (in the direction of the RF) are termed "peripheral" saccades while eye movements made to the central target (or away from the RF) are termed "central" saccades (Fig. 1A). For clarity, during this task, when the animal was fixating the central target, the response field was located at

the peripheral target. The animal's next saccade would then be made to the peripheral target, toward the RF. However, once the animal fixated the peripheral target, the RF was not located at either target and the next move (central saccade) would be made away from the direction of the RF.

In addition to monitoring the activity during the self-timed rhythmic-saccade task, the activity for all cells in our population were also monitored during a memory-guided delayed-saccade task [Hikosaka and Wurtz (1983); Colby et al. (1993)]. The memory-guided task was used to determine which cells displayed stereotyped LIP firing activity. Trials began by requiring the monkey to fixate a target near the center of the monitor (Figure 1C). Following fixation, a target within the RF was flashed for a brief period of time (200 ms) and then extinguished. The monkey was required to remember the location of the flashed target, while maintaining fixation at the center target. The monkey was then required to make a single saccade to the remembered location following extinction of the central target 1000 ms after the trial began to receive a juice reward. In order to be selected for further analysis, neurons had to display a light sensitive response to the target flashed in the RF [Gnadt and Andersen (1988); Andersen et al. (1990b); Barash et al. (1991b,a)]. Neurons also had to show maintained activity during the memory, or delay, portion of the task (time between the RF target being extinguished and the fixation point being extinguished). Only cells that displayed both light sensitive and memory activity were investigated further in this study. A minimum of 30 trials were required from this task, yet 40 trials were typically recorded.

Although the delay timing between the two tasks remained the same (1000 ms), the memory-guided delayed-saccade task differed from the self-timed rhythmic-saccade task in a number of ways (Figure 1A&C). First, in the memory task, the peripheral target was extinguished after its initial appearance within the neuron's response field

instead of remaining throughout the entire trial. Thus, the animal was required to remember the target location following the disappearance of the peripheral target. Second, the memory task always required just a single saccadic movement and rewards were always delivered immediately following a correct saccade. Lastly, the animal was cued when to make the movement by the extinction of the central fixation point during the memory task.

Electrophysiology

Prior to training animals were chronically implanted with titanium head posts in order to stabilize head position. Animals were also implanted with scleral eye coils in order to monitor eye position (sampling rate of 200 Hz), although an infrared eye tracking system (iView X Hi-Speed Primate camera system, SensoMotoric Instruments) was used most often to track eye position. Following training, animals were implanted with chronic stainless steel or customized PEEK (polyether ether ketone) recording cylinders. Cylinders were placed, stereotactically, in a manner that allowed electrode penetration of the lateral bank of the intraparietal sulcus (area LIP). Area LIP was identified anatomically using MRI prior to recording cylinder implantation. All surgeries were done in accordance with animal care guidelines of the University of Minnesota and the National Institutes of Health. Surgeries were performed under aseptic conditions with full anesthesia.

Single-cell recordings were done from 175 well-isolated neurons using standard extracellular recording techniques. Action potentials were sampled (1000 Hz) and digitized for on and off-line analysis. Mapping of each neuron's RF was done by randomly shifting the non-central target about the computer display, while the animal performed single-saccade trials of the rhythmic-saccade task, where the initial fixation point was always the central target. On the basis of neural responses to various

non-central target locations (usually 8), the location of the RF (and the peripheral target), relative to the central fixation point, was concluded. Five to ten correct saccades to each location were typically sufficient to determine a cell's RF. After mapping, we recorded from neurons while the animals performed the memory-guided delayed saccade task. One hundred of the 175 neurons sampled displayed both the light sensitive and sustained memory activity during the memory task and they are analyzed here. Recordings were typically taken from the right hemisphere (26/50 cells for animal 1 and 50/50 cells for animal 2).

Data Analysis

Visual stimulation, behavioral control, and data acquisition were controlled using customized computer software:

(http://www.ghoselab.cmrr.umn.edu/software.html)

Online analyses of average firing rate were used to determine RF locations. Offline analyses of firing rates relative to the events of the saccade tasks were done using Matlab (R) (MathWorks). Average firing rates were smoothed by convolving with a Gaussian kernel (s.d. = 35 ms). However, no smoothing was done for any correlation analysis. Saccade onset was defined according to eye velocity (> 85º/sec) in conjunction with the computer software's recognition of the eye position's arrival within the fixation window. The first saccadic intervals within a trial were analyzed separately from all subsequent intervals since, for initial saccades to the central position, the appearance of the subsequent target in neuron's RF elicited activity. Significant increases in pre-saccadic activity, were calculated by comparing firing activity within 150 ms of saccade initiation (-150 to 0) with the activity 250 ms prior to that interval (-400 to -150) (t-test, $p < 0.05$).

In order to determine what factors are associated with firing rate changes, we gen-

erated a prediction of neural activity by convolving observed neural activity aligned with one saccade direction with the inter-saccade distribution times. For example, if we convolve the inter-saccade distribution times aligned to central saccades with the actual firing rate aligned to peripheral saccades, we get a prediction for central saccade aligned activity (see Figure 5A for example) (convolution: $(f*\mathrm{d})(\mathrm{t}) = \int \mathrm{f}(\tau)\mathrm{d}(\mathrm{t}\text{-}\tau)\mathrm{d}\tau$). The difference between this prediction and the observed firing rate for activity aligned to central saccades indicates how well activity associated with peripheral saccades can completely explain task related modulations in activity. The same analysis is then repeated using activity aligned to central saccades. Fit is given by: $\%Fit = (1 - NMSE) * 100$ where NMSE is the normalized mean squared error. The NMSE is calculated by dividing the mean squared error by the explainable variance of the actual unsmoothed firing rate (variance of firing rate over the interval − average variance of all time points of the rate).

Results

We trained two monkeys to perform a variant of the delayed-saccade task [Hikosaka and Wurtz (1983); Colby et al. (1993)] called the self-timed rhythmic-saccade task (Figure 1 A&B). The task was designed to focus on temporal motor production and avoid any regular pattern of sensory stimulation or reward that might lead to temporal measurements within the task. In this paradigm, the monkey must rhythmically saccade back and forth between two static targets at a defined interval (0.5 Hz). Because there are no external cues regarding this interval, the monkey must form and follow an internally and explicit temporal representation to successfully perform the task [Colby et al. (1996)].

To verify that the animals learned the trained interval of one second, we examined the produced inter-saccadic intervals as a function of saccade direction and serial

order (Figure 2). Seventy eight thousand fifty nine saccades (average of 781 saccades/cell, with a standard deviation of 235 saccades/cell) were analyzed over 19,177 trials. We found that both animals displayed highly consistent behavior, with standard deviations much smaller than the allowable behavior window of +/- 200 ms. Interval production depended neither on saccade direction nor serial order. Animal 1 produced average inter-saccadic intervals of 1022 ms (standard deviation of 118 ms), 985 (101), and 1003 (111) prior to peripheral, central, and all saccadic movements, respectively. Animal 2 produced those same average inter-saccadic intervals at 974 ms (103 ms), 972 (100), and 973 (101), respectively. Average inter-saccade times for the first 5 intervals serially for Animal 1 are 991 ms (104 ms), 988 (102), 1028 (118), 1041 (129), 1024 (126). Animal 2 produced average intervals of 984 ms (94 ms), 971 (101), 948 (99), 976 (110), 978 (105). Because the behavioral comparisons within each animal and between animals are very similar, all future analyses will combine data for all saccade intervals (except first intervals) and both animals.

We also compared successive inter-saccadic intervals produced by the animals during the timed rhythmic-saccade task. Previous studies have noted that a negative correlation exists between subsequent repetitive behaviors, such as finger taps or saccades [Wing and Kristofferson (1973b,a); Collins et al. (1998)]. However, these studies focused on tempo reproduction rather than a self-timed behavior. An investigation of successive inter-saccade times in our self-timed task revealed that the combined behavioral data displayed a small but significantly positive correlation ($r = 0.05$, $p < 0.0001$). The lack of consistency with previous studies is likely due to task differences. Unlike tempo reproduction, our task resets the required timed interval after each movement. Thus, there is no behavioral advantage to compensating for a short interval with a long one, as in a tempo reproduction task.

In order to investigate the neural basis of this temporal precision, we examined

the activity of individual neurons within lateral intraparietal (LIP), which has been implicated in the cognitive control of eye movements. Neurons within LIP exhibit stereotyped delay period activity during tasks in which the the location of a transiently presented saccade target must be remembered [Andersen et al. (1990b); Barash et al. (1991a,b)]. 100/175 neurons in our sample exhibited activity consistent with previous reports of LIP memory guided activity [Gnadt and Andersen (1988); Andersen et al. (1990b); Barash et al. (1991b,a)]. As seen for both an example neuron (Figure 3A) and our population (Figure 3B), the flashed target within the RF (black bar along x-axis) elicited a transient increase in activity followed by sustained activity during the delay period (the time between the RF target being extinguished and the fixation point being extinguished). This sustained activity remains above activity levels that were observed prior to the start of the trial (not shown). Additionally, many neurons in our population displayed pre-saccade related activity (59/100 showed this trend while in 25/100 this increase was significant (t-test, $p < 0.05$)). This steady increase in activity is evident before saccade onset and can be seen in both the example cell and population activity. Such pre-saccadic activity has previously been attributed to to saccade planning [Gnadt and Andersen (1988); Andersen et al. (1990b); Barash et al. (1991b,a)]. However, this increase could also be due to reward expectations or changes in attention closely linked to the required movement [Platt and Glimcher (1999); Sugrue et al. (2004); Yang and Shadlen (2007); Bendiksby and Platt (2006); Dorris and Glimcher (2004); Seo et al. (2009)].

After this memory-guided task, we recorded from the same neurons while the animals performed the self-timed rhythmic-saccade task. The self-timed rhythmic-saccade task is designed to eliminate temporal measurement signals and minimize sensory and reward anticipation in order to better understand temporal production signals. In the following analyses concerning the rhythmic task, the first interval

was excluded because of the predictable onset of the subsequent target, which distinguishes the first interval from all subsequent intervals. As was done with the behavior, firing activity was segregated based on direction and the interval number within a trial (data not shown). The firing rate for each direction and interval (first intervals done separately) was then analyzed in order to determine if the activity varied from interval to interval within a trial. No significant correlation was found between neuronal activity and interval number for either monkey ($p < 0.05$). Therefore, in addition to the behavioral data, neural activity will also be combined for all intervals (aside from first intervals). Because the firing activity between both monkeys was very similar, data from both animals will be combined in all future analyses as well.

Figure 4A shows the neural activity of an example cell as the monkey performed the rhythmic saccade task. The dashed vertical line represents saccade initiation. Red traces are aligned to peripheral saccades (saccades to the peripheral or RF target) while blue lines are aligned to central saccades (saccades to the central target). The average population activity for all 100 neurons is shown in Figure 4B. For the example cell and the majority of the population (81/100), it is evident that significant increases in activity prior to saccades are not a prominent feature of the response (19/100 showed significant increases while 31/100 displayed this trend), as it is near saccade onset in the memory task (t-test, $p < 0.05$). Instead, activity decreases prior to saccade initiation for both directions of movement.

Although the predominant feature of activity modulation is a near linear decline in firing rate over time, other modulations are clearly present. Around the time of saccade onset (+/- 100 ms), the activity displays distinct modulations. Brief increases in activity just prior to saccade onset are followed by short intervals of decreased activity at the time of saccades. These peri-saccadic modulations in activity are similar between saccade directions and are consistent with previous studies as being signals

of a global remapping of the RF [Duhamel et al. (1992); Heiser and Colby (2006); Kusunoki and Goldberg (2003); Bisley and Goldberg (2003b)].

The largest deviation from the overall decline in activity is the sudden increase in activity immediately following central saccades (blue: 0 to 250 ms) (see Figure 4B). The increase in activity immediately following a central saccade is consistent with bottom-up sensory stimulation because, as an immediate consequence of the central saccade, the peripheral target is moved into the neuron's RF. A large increase in activity is also visible early within the interval prior to peripheral saccades (blue: -1000 to -700). Since saccades are performed back and forth between the two targets and since all trials outside of the first intervals are analyzed together, this increase may also represent sensory stimulation of the RF.

To isolate the factors that could cause temporal modulation of activity prior to saccades, we compared the activity observed in the memory-guided saccade task with activity observed in the rhythmic task prior to the same movement (a peripheral saccade) (Figure 4C). The left portion of the plot is aligned following a central saccade (blue) and to target onset (black), while the right portion of the plot is aligned to peripheral saccade onset (red and black). In both of these tasks, the actual upcoming movement (a peripheral saccade) and the timing (a one second interval) are consistent. However, the stimulus events and rewards associated with this planned movement are different. Early in the interval, a sensory response to the peripheral target was apparent (transient increase in activity) during the memory task (black, left) where the peripheral target was presented and subsequently extinguished. A similar response is also evident during the rhythmic saccade activity (blue, left), although the cause is likely due to a central saccade brining the RF to encompass the peripheral target instead of a flashed target. Sensory effects are also likely to explain the differences in activity between the two sets of traces (300-700 ms on left and -300 to -200 on the

right). During the rhythmic task, the target remained on, and thus the activity level during this period is higher than for the memory guided task in which there is no RF target present during this interval. Finally, immediately prior to the saccade, activity rises much more in the memory guided task (black) when the animal knows that a reward is imminent than in the rhythmic task (red). This difference suggests that the pre-saccadic activity reported in previous studies may reflect specifics of the task (such as reward anticipation), rather than generalized patterns underlying saccadic timing.

In the rhythmic task, the highest levels of activity were seen immediately after central saccades. Since the task requires saccades to be performed back and forth between the two targets and since we analyze all intervals subsequent to the first interval together, this increase can also be seen in activity aligned to peripheral saccades in the interval from -700 to -1000 ms. To explicitly test when the peripheral saccade aligned activity can be solely explained by activity locked to central saccades, we generated firing rate predictions of each saccadic alignment on the basis of the other (Figure 5). Assessing the fit of the predicted rate (green trace) to the observed firing rate (red and blue traces), shows what modulations in activity can be largely explained by saccade-related behavior, since the distribution times of the behavior were used to generate the predicted rate.

In general, the predicted rate was similar to the actual firing rates, consistent with an overall decrease in firing rate being the dominant activity modulation during inter-saccade intervals (Figure 5A & B). However, there are a few instances for which the predicted activity poorly fits the observed activity. The first of these is the time period just before and just after both directions of saccade initiation (+/- 100 ms) which, as mentioned previously, is consistent with previous reports of RF remapping signals in LIP [Heiser and Colby (2006)]. The second significant discrepancy is during the

250 ms immediately following central saccades (Figure 5B) . By contrast, peripheral saccade aligned activity during the corresponding period of time (-1000 to -700 ms) is well fit. This suggests that the sudden increase in activity immediately following central saccades, which is likely explained by the saccade related movement of the stationary peripheral target into the RF, largely explains the gradual increase seen approximately one second prior to peripheral saccades.

Thus the activity modulation seen in our task can be explained by a gradual decrease in activity following the appearance of target in the RF and remapping signals. However, this modulation need not have any relationship to timed behavior. For example, although activity decays at a constant rate following the introduction of a stimulus into the RF, this decay might not have anything to do with how the animals actually timed their behaviors, and may simply reflect some intrinsic decay constant. In such a situation, activity fluctuations in LIP that are due to noise or some uncontrolled variable such as attention, would have no correspondence with fluctuations in the timed behavior. To examine this possibility, we studied whether LIP activity fluctuations during inter-saccadic intervals were predictive of the animals' actual saccadic interval. Figure 6A & B show the firing activity as a function of interval length for each saccade direction. Each trace is an average rate of one fifth of the trials and interval lengths are sorted based on current intervals both prior and subsequent to the saccade displayed at time point 0 (activity prior to the saccade is sorted based on the interval that ends at 0 and activity following the saccade is sorted based on the interval that begins at 0). The red traces represents the shortest fifth of intervals produced by the animals while the purple traces represent the longest fifth of intervals (as indicated by the inter-saccade distribution times shown at the beginning and end of each interval). The green and orange bars at the bottom of each figure indicate fixation location during each interval (green = fixation of peripheral target, orange = fixation of central

target). The consistent ordering of firing rates with respect to interval length suggests that activity is consistently predictive of the behavioral interval.

To further examine the relationship between activity and behavior, we computed the correlation between spike counts across all the neurons in our sample and the inter-saccadic period. We investigated this relationship over the 800 ms before and after saccade initiation by looking at correlations over 100 ms bins (Figures 6C & D). Correlations were calculated on an interval by interval basis across all trials of all cells. For each bin, the action potentials were summed and analyzed with respect to interval length. Correlations prior to saccades were analyzed using intervals that ended at time 0 while correlations following saccades were analyzed using intervals that began at time 0. In this way, all correlations are concerned with current intervals associated with upcoming saccades. This analysis allows us to examine whether time related signals are consistently present throughout inter-saccadic intervals or more prevalent immediately before or after saccades.

Activity aligned to precede central saccades and activity aligned to follow peripheral saccades was consistently predictive of current interval duration (Figures 6C & D) (correlation analysis, $p < 0.005$). These correlations are notable in two respects. First, they occurred during a period of time (fixation at the peripheral target, green shading) when there is no sensory stimulation in the RF and the RF is not a potential target. Second, and consistent with the orderly segregation of the traces in Figure 6A & B (green shaded intervals), the correlations were positive, meaning that increases in activity were associated with increases in the inter-saccadic interval. This is the opposite relationship to what would be expected by previously proposed threshold models of timing and sensory integration, in which increases in activity are associated with reaching a threshold earlier.

Activity aligned to precede peripheral saccades also displayed significant corre-

lations to interval length throughout the entire inter-saccade period (Figure 6D) ($p < 0.005$). However, in this case, the correlations are primarily negative. Surprisingly, the activity aligned to follow central saccades corresponding to the same fixation location does not show significant correlations ($p < 0.005$). This difference suggests that a saccade specific event, such as the sensory driven response transient following central saccades, can mask temporal production signals.

Correlations between firing rate and saccade metrics (saccade velocity) were also calculated in order to determine if LIP activity was related to saccadic motor output. We found that the overall population activity was not significantly correlated to saccadic velocity ($p < 0.005$). Therefore, LIP activity is likely related to motor planning rather than saccade metrics. Further support for motor planning is provided by the difference in the correlations between the rhythmic task (red) and the memory task (black) (Figure 6D). Although these tasks are similar in that the same movement is required at the same time, significant correlations only exist throughout the interval for the rhythmic task, where timing must be done internally by each animal.

Since firing rates throughout the delay periods of these intervals are largely predictive of the interval length, this suggests that activity within LIP is a temporal production signal that can be utilized in order to time saccade initiation. While only a minority of single cells displayed activity that was significantly correlated to each overall interval (10/100 prior to central, 8/100 following central, 11/100 prior to peripheral, 3/100 following peripheral, $p < 0.005$), the sign of the cells' significant correlations typically had the same sign as population correlations. The low number of neurons displaying significant correlations between rate and interval length suggests that the timing signals in LIP are most prominent at the population level, but too weak to be observed in the activity of most individual neurons over the time period of our recording sessions.

When the activity of each individual neuron was normalized (z-score) prior to calculating the correlations, the correlation values throughout the interval generally maintained the same sign (not shown). However, the r-values were consistently dampened. This suggests that neurons with greater modulations in activity contribute more to behavioral timing. When we looked at neurons with higher degrees of rate changes, we found that activity displayed stronger correlations while the animals were fixated at the peripheral target (Figure 6, green bars). Overall correlations were significant for pre-central and post-peripheral saccade aligned activity and increased from 0.050 and 0.076 to 0.145 and 0.157, respectively. Yet, correlations were not significant for either saccade alignment as animals fixated the central target in the high modulation cells. Cells with lower modulations in activity still displayed significantly correlated activity both prior to (r = -0.042) and following (r = 0.039) peripheral saccades, although the correlations following peripheral saccades were reduced compared to the combined population. These differences between cells with high and low modulations may support the idea that there are two populations of neurons within LIP [Maimon and Assad (2006)], each of which contributes to timing differently. However, since both populations of cells do contribute to the timing of saccades, we will continue to discuss temporal production for the entire combined population.

Although we observed that LIP activity is significantly predictive of current interval length, other studies have shown that parietal activity can also represent past and future events [Seo et al. (2009); Baldauf et al. (2008)]. To determine if LIP activity is also related to past and future intervals in our task, we performed a regression analysis where we examined the relationship between neural activity with past, current, and future interval lengths. This was done for both fixation targets (central and peripheral). Firing rates for this analysis were obtain from the 800 ms adjacent to saccade onset for the intervals with the highest overall correlation values. We found the

population activity to be significantly related to the current intervals at both locations (linear regression coefficients: at center = -9.4 spikes/s/s, at RF = 7.9 spikes/s/s), but not with past or future intervals ($p < 0.005$). Very few individual cells were significantly related to any intervals. Only 0, 1, and 0 cells were significant for past, current, and future intervals, respectively, while the animals were fixated at the central target. Similarly, no cells were significant while fixated at the peripheral target (regression analysis, $p < 0.005$). Therefore, it appears that neither future interval planning nor past interval production significantly contributed to LIP activity, and the relationship with current intervals is due to population activity.

The change in the sign of the correlation between activity and timed saccades suggests that a push-pull mechanism may underlie temporal production in our task. In this case, activity prior to a RF (peripheral) saccade pushes for saccade initiation in that more activity during this time leads to a faster onset of the behavior (negative correlation). By contrast, activity prior to a central saccade pulls for maintaining the peripheral position. In our task, the hemisphere containing response fields corresponding to the impending saccadic target would be pushing for a saccade, while the opposite hemisphere would be pulling or delaying a saccade.

The simplest form of a push-pull model is a linear differencing of activity. Our model can be simplified even more with a parameterization of the peripheral saccade aligned activity that is most predictive of behavior in which a linear decrease in activity over the inter-saccadic interval is paired with a linear increase around the central saccade. This is done with a triangle waveform (Figure 7A) which consists of a gradual decrease in activity corresponding to our observations of falling activity prior to saccades and a rapid increase in activity corresponding to a reset signal around the time of saccades. This reset signal is consistent with typical latencies between LIP activity and saccades (~100 ms) [(Cutrell and Marrocco, 2002)] and our observations

of a change in firing rates immediately prior to saccades. Although we used a waveform that is based on actual activity, part of the robustness of this model is that the exact waveform is not critical to the model as long as it is cyclical. To instantiate the push-pull model, we simply subtract the waveforms corresponding activity in the two hemispheres, which by virtue of the rhythmic nature of the task, are 180 deg out of phase from one another (Figure 7B). Higher activity in one hemisphere indicates a command signal to maintain eye position at one location, and saccades are initiated when the rapid reset signal occurs (i.e. the difference between the signals begins to change).

In the absence of any activity fluctuations, such a model produces a completely regular inter-saccade interval of one second (Figure 7C, dashed black trace). However, in the presence of activity fluctuations in one hemisphere, either due to noise or changes in an uncontrolled variable such as attention, this regularity changes, such that more than one second is spent at one location and less than one second at the other (Figure 7C). This would lead to a positive correlation between activity and interval duration while the animal was fixated on one location and a negative correlation while that animal was fixated on the other location, as observed in our data. The model predicts that the positive and negative interval changes should be equal and linearly related to changes in activity (Figure 7D). Using our simplified description of LIP activity, the model predicts slopes (+ or - 9.4 spk/s/s) which are similar to the regression coefficients seen in our observations (+7.4 spk/s/s and - 9.4 spk/s/s).

Discussion

In order to investigate neural activity related to temporal production, we devised a self-timed rhythmic-saccade task that controlled for temporal measurements while minimizing sensory and reward anticipation. Animals were required to make sac-

cades back and forth between two fixed targets at a fixed interval so that saccades occur each second (Figure 1). We found a systematic decrease, rather than an increase, in activity within LIP prior to saccades. The systematic decrease in activity was significantly predictive of inter-saccadic interval length (correlation analysis, $p < 0.005$). The relation to interval length was found to only be significant for current (not past or future) intervals (regression analysis, $p < 0.005$)

The animals in our study displayed the ability to precisely and consistently produce a rhythmic behavior very near the trained interval (Figure 2). However, aspects of our results were not consistent with previous studies that investigated motor timing in repetitive behaviors. A traditional timing model used to describe rhythmic movements was developed by Wing and Kristofferson from studies utilizing finger tapping [Wing and Kristofferson (1973b,a)]. This study has also been used to describe saccades [Collins et al. (1998)]. A primary finding of this study is that a negative correlation exists between subsequent timed repetitive movements. For example, if a saccade is longer than the trained interval then the following saccade is likely to be shorter than the trained interval. In order to be most accurate during tempo replication, it is advantageous to produce a negative correlation between successive intervals where a short interval can be compensated by a longer interval (and vice-versa) in order to stay on beat. This negative relationship has been attributed to the variability of motor output delay and the idea that chance variations about the mean delay will tend to produce a negative correlation between adjacent intervals [Wing et al. (1984)].

We found a small (r-value = 0.05), but significantly positive correlation ($p < 0.0001$) between subsequent saccades for our combined behavioral data. The fact that our results do not display the negative correlation described by the Wing and Kristofferson model likely reflect task differences. Unlike our task, these other studies did not require their subjects to precisely execute a trained interval. Instead, the

subjects first followed along with a cued motor sequence before the cue is turned off and then continued the motor task in a self-paced manner. There were no repercussions for imprecise timing as in our task (trial ends, no reward). Additionally, our task resets the trained interval following each saccade. This means that the better the animal is at precisely producing the trained interval on each saccade, the better chance he has of receiving a reward. In these other studies, the subjects are trying to replicate a tempo that is not reset with their behavior. Therefore, the Wing and Kristofferson model may only be useful in describing tempo replication and may not reflect general mechanisms governing temporal production.

One way in which the temporal production signal may be initiated is by being reset during each saccade. The notion of saccades effectively reseting time keeping in our rhythmic task is consistent with our physiological observations in several respects. First, as evidenced by saccade aligned firing rates, a similar up-down peri-saccadic modulation in firing is present irrespective of saccade direction. These modulations in activity occur approximately 100 ms prior to saccade initiation, similar to saccadic latency times within LIP [(Cutrell and Marrocco, 2002)]. Therefore, remapping may serve as a reset signal. Second, as would be expected by a reset, activity in LIP was only correlated with the current behavioral interval within a sequence, and not with past or future temporal production. Third, the relationship between activity and behavior flips after a peripheral saccade from negative to positive..

We have found significant correlations on an interval-by-interval basis between neuronal activity in LIP and the timed interval between saccades. Since an internal sense of time is the only cue available to the monkey with which to initiate saccades, we have interpreted this correlation, which has never been previously reported, as reflective of a temporal production signal. However, it is also possible that LIP, instead of representing information relevant to a decision to saccade, namely the passage of

time, instead represents a motor plan whose execution after a decision has been made can be delayed. We consider this explanation unlikely for several reasons. First, there is no evidence to suggest that increases in activity in LIP would be associated with delays in the execution of motor plan. On the contrary, many experiments have demonstrated that LIP activity appears to associated with pre-decision information, whether that information be stimulus related or time related. Second, we found no evidence that actual saccade metrics (e.g. velocity) depended on LIP activity. Third, because LIP activity was correlated with timing even when the response field location was not a potential target (e.g. when the monkey was fixating at the peripheral location), our results are not consistent with changes in a motor plan strictly associated with a particular retinotopic location. Fourth, if LIP activity solely reflected a motor plan, than activity fluctuations near the time of the saccade should be have particularly strong correlations with behavior. By contrast, we find that behavioral correlations are relative constant throughout the entire inter-saccadic interval, even when the saccade is going to occur 800 ms in the future. Finally, since the motor plan for the memory-guided and rhythmic saccades are identical, one would expect little difference in firing rates or behavioral correlations, in contrast to our observations (Figures 3 and 6).

Neuronal representations of time within LIP have previously been described by climbing activity, a steady increase in neuronal activity over time to a threshold level, at which time an action ensues [Leon and Shadlen (2003a); Janssen and Shadlen (2005); Maimon and Assad (2006)]. A higher rate of activity (or a faster rise to threshold) produces a shorter interval and therefore, a negative correlation between rate and time. Although brief periods of increased activity can be seen in our population activity (Figure 4B), these increases can be explained by RF remapping [Heiser and Colby (2006)] and sensory responses to the peripheral target being moved in and out of the RF as the animal produces saccades. These brief periods of increases in

activity do not fit the parameters of climbing activity as a timing signal [Durstewitz (2003, 2004)]. Instead, the prominent pattern of activity is a steady decrease in neural activity over the delay period.

One possibility for why we observed falling, as opposed to climbing, activity prior to saccades is differences between our task and those employed previously to study timing. Because of the close associations of sensory cues and reward that occur near the time of the behavior in previous studies, which are absent by design in our task, it is possible that climbing activity is more related to sensory and/or reward anticipation (time measurement) than motor planning. This notion is consistent with our observations of neuronal activity during a task that is much more analogous to previous studies. The same neurons that displayed falling activity during the self-timed rhythmic-saccade task displayed very different activity during the memory-guided delayed-saccade task. In the memory task, the basic behavior, namely waiting one second prior to making a saccade, is similar to the rhythmic task. However, in terms of temporal measurement, the tasks are quite different. Specifically, in the memory task (and unlike the rhythmic task), the timing of the cue to make a saccade and reward can be readily anticipated. Consistent with previous observations, a pre-saccadic rise in activity was observed in the memory-guided task. However, this rise is largely absent in the rhythmic task, suggesting that climbing activity may reflect reward anticipation rather than a motor plan.

Given these task differences, it is also possible that distinct timing systems are responsible for tasks which require temporal measurement and those that do not. Lewis and Miall have proposed that there are two distinct timing systems: an automatic system responsible for predictable intervals defined by movements and a cognitively controlled system involved in temporal measurements that direct attention [Lewis and Miall (2003)]. Since LIP is involved in both motor production and attentional alloca-

tion [Robinson et al. (1995); Colby et al. (1996); Gottlieb et al. (1998); Gottlieb and Goldberg (1999); Powell and Goldberg (2000); Bisley and Goldberg (2003a, 2006)], it may be that this area is a part of both timing systems and that the task determines which timing system is utilized. For instance, when the animal is performing an interval duration comparison task or a task for which movement is cued or immediately rewarded [Leon and Shadlen (2003a); Janssen and Shadlen (2005); Maimon and Assad (2006)], the cognitive timing system would be engaged since these tasks require the timing of discrete epochs and do not control for the attentional effects that sensory and reward anticipation can have [Kusunoki et al. (2000); Maunsell (2004)]. The cognitive system would employ climbing activity as its timing signal in order to time the temporal measurement related events of the task. However, when those forms of anticipation are minimized (as in our self-timed delayed-saccade task), the automatic timing system may be engaged since the task requires saccades be made at regular intervals. This would then allow falling activity, the signal responsible for the production of the timed interval, to emerge as the temporal production signal.

If the primary role of the cognitive timing system is to direct attention, it may be particularly unlikely to play a role in our task given that the spatial positions and direction of the impending saccades are never ambiguous or subject to cognitive choice. Although spatial attention may not be required, this does not mean that attention is not allocated to the targets at some point prior to saccade initiation. However, the activity we observe during the rhythmic-saccade task displays a decrease in rate prior to saccade initiation, not an increase as might be associated with the increasing priority of making a saccade as time elapses [Bisley and Goldberg (2010)].

Another theory concerning LIP is that its activity represents the accumulation of evidence toward a threshold at which point a decision is reached. During two choice motion discrimination tasks, climbing activity has been observed as animals moni-

tored a motion patch. When the activity reaches a threshold, the animal subsequently saccades. Therefore, a threshold may represent a decision [Shadlen and Newsome (1996, 2001); Roitman and Shadlen (2002); Huk and Shadlen (2005); Hanks et al. (2011)]. Thresholds have also been observed in an LIP timing study [Maimon and Assad (2006)]. Perhaps the temporal activity observed within this study also represents sensory evidence accumulation to a threshold. During the task, temporal evidence based on the sensory cue provides the evidence. As time passes, the evidence related to the stimulus accumulates until a threshold is reached. This again may mean that timing signals were related to sensory events instead of motor planning. If true, climbing activity to a common level could represent perceptual events instead of decisions or motor triggers. We do not observe a common threshold prior to action in our task (Figure 6), even though motor events and decisions about when to move do occur. This suggests that thresholds in LIP may simply reflect external visual cues instead of decisions or motor cues and that the activity we observe may be a timer for other areas to initiate saccades.

Another difference between our task and previous single-saccade tasks is the potential for sequence planning in our task. Psychophysical evidence suggests that, when confronted with an array of saccade targets, subjects naturally plan entire saccade sequences [Zingale and Kowler (1987)]. The planning of entire sequences has been shown to take place in a number of brain regions and for a number of tasks [Baldauf et al. (2008); Mushiake et al. (2006); Histed and Miller (2006); Lu and Ashe (2005); Ohbayashi et al. (2003); Fujii and Graybiel (2003); Tanji and Shima (1994)]. Additionally, a study by Seo et al. (2009) showed that LIP activity contains information about past events [Seo et al. (2009)]. However, we found that neither future nor previous temporal production significantly contributed to the activity of our population during this task (regression analysis, $p < 0.005$). Also, our observation of near

independence between adjacent intervals (correlation value of 0.05) is not consistent with sequence planning. These data suggest that, presumably because we reset the behavioral requirement after each saccade, both the animals and our neural population are concerned solely with timing single intervals within the rhythmic sequence.

In any case, the observation that firing rates in LIP are so dependent on task design, as evidenced by the difference in our population between memory-guided and rhythmic saccades (Figure 4C), demonstrates LIP activity can only be interpreted with knowledge of the behavioral context. For example, LIP activity can not be strictly interpreted as reflecting an evidence signal whose magnitude is associated with increasing likelihood of reaching a decision to saccade, since in our experiments activity decreases with the passage of time, which is the sole evidence the animal can use to make a saccade. Similarly, our data is not consistent with LIP solely representing an attention signal, since there are no stimulus cues present or relevant for saccades, and the observation of positive correlations between activity and interval means more activity can actually delay a saccade.

Our results constrain the spatial distribution of timing signals within the brain. Two traditional theories concerning where timing signals originate are the central and distributed timing mechanisms [Buonomano and Karmarkar (2002); Ivry and Spencer (2004)]. In the central timing model a specific brain region produces a timing signal that is utilized for all timing related events for all modalities. The distributed timing model suggests that there is no dedicated timing system but that the ability to represent time is an intrinsic property of distributed cell populations that are required for a given task. If LIP activity strictly reflected a broad timing system (like those described by centralized timing models), its activity would have a consistent relationship with time irrespective of saccade direction

Because activity patterns and behavioral correlations depend in a number of re-

spects on the particular planned saccade, our results support the notion that local neuronal populations are responsible for temporal production. First, the activity immediately preceding central and peripheral saccades is different when sorted by intersaccadic interval. Prior to central saccades, there is no evidence for a response threshold because different rates are seen at saccade onset (Figure 6A). By contrast, a common activity level is observed at peripheral saccade onset. Second, although activity was consistently predictive of saccadic interval, the exact relationship was significantly different for peripheral and central saccades. Activity prior to saccades made to the peripheral target was negatively correlated with interval production while activity prior to saccades to the central target had a positive correlation.

Our results also provide insight concerning the neural mechanisms underlying timing. Multiple mechanisms have been proposed to underlie behavioral timing. Three mechanisms include the clock (pacemaker/accumulator) model, labeled lines, and population clocks [Buonomano and Karmarkar (2002)]. In the clock model, a neural pacemaker produces rhythmic pulses. These pulses are then counted (or accumulated) in order to time an event. Clock models are generally classified as centralized systems, as this one clock is used in all timed events [Buonomano and Karmarkar (2002)]. Because the relationship between LIP activity and behavior varies depending on the impending saccadic target, it is not consistent with a single universal representation of time. Moreover, because activity was observed to decrease rather than increase over time, accumulation is ruled out.

In the labeled line model, different neurons within a population respond at different interval lengths. For example, one neuron may respond at 100 ms while a second neuron responds at 200 ms. The labeled line population can be used to determine time by which neurons are active. Labeled line models could work in a distributed timing system. However, our data did not show strong evidence of individual neurons being

significantly correlated to specific intervals.

The population clock model encodes time through the population activity of a network of neurons. There is no specific time at which neurons are active, instead dynamic interactions or time-dependent changes between neurons within the network provide information about lapsed time (e.g. short-term synaptic plasticity, inhibitory feedback, etc.). Such a model could account, in part, for the small correlation values we observed between neuronal activity and interval length if the neuronal populations underlying timed behavior were much larger than our sample. In this model, individual neurons will not contain large amounts of temporal information, consistent with the low number of individual cells that display significance in the regression and correlation analyses. From this model, we would predict that as we sampled more neurons from this population, the population correlation values would increase.

The correlation of LIP activity prior to central saccades to timing is particularly notable because it is inconsistent with the belief that LIP RFs are exclusively tuned for contralateral visuomovement space [Blatt et al. (1990)]. In a purely retinotopic framework, activity prior to a central saccade should be minimal and irrelevant given that there is not stimulus in the RF nor is that location a potential saccadic target (Figure 1). However, a study by Dickinson et al. (2003) found that neurons in LIP can be activated by the instruction to perform a saccade, in the absence of any spatial information [Dickinson et al. (2003)]. Similarly, Bennur and Gold found information on perceptual decisions within neurons whose RF's did not correspond with the upcoming movement [Bennur and Gold (2011)]. Freedman and Assad have shown that LIP activity contains information regarding categories of stimuli, even when the stimulus is presented outside of the RF [Freedman and Assad (2009)]. Additional studies have suggested that LIP activity is highly dependent on the task and task related experiences that can shape response properties [Bennur and Gold (2011); Freedman and

Assad (2006); Law and Gold (2008)]. Therefore, it is possible that neurons with visual response fields may be used in planning eye movements to other locations.

The fact that activity fluctuations are correlated to timed interval production for both saccades toward the RF and away from the RF, but those correlations are opposite in sign, suggests that activity differences between the two hemispheres may drive temporal production in our task via a push-pull mechanism. The success of the simplest version of such a model, a linear differencing of activity, in explaining the quantitative relationship between activity and interval supports such a proposal. In our differencing model, we described LIP activity with a two parameter model of linear increases and decreases in activity over the saccadic cycle. The slope of the gradual activity decrease primarily determines the mean inter-saccadic interval. By contrast, the increasing activity slope largely determines how much intervals will change with activity fluctuations.

Although the model makes a number of simplifying assumptions, especially with regard to linearity of activity changes over time and the linearity of the differencing operation, it both explains our present observations and generates easily testable predictions. One such prediction is that when animals are trained for inter-saccadic intervals other than one second, the duration of falling activity should vary. In our model, a decision to saccade is made when the falling activity reaches a minimum. Thus longer intervals should be associated with either more gradual decreases in firing rate over time, or a lower minimum firing rate. Although this is a clear violation of the scaling law of timing [Gibbon (1977, 1991)], there are examples in which timing variance does not scale with timing interval [Lejeune and Wearden (2006)]. Finally, the model predicts that changes in LIP activity, irrespective of their origin, should evoke specific changes in temporal production. This could be examined by applying brief micro-stimulation within LIP, which, the model predicts, should alter the timed

interval irrespective of when within the interval the stimulation is applied.

Figures

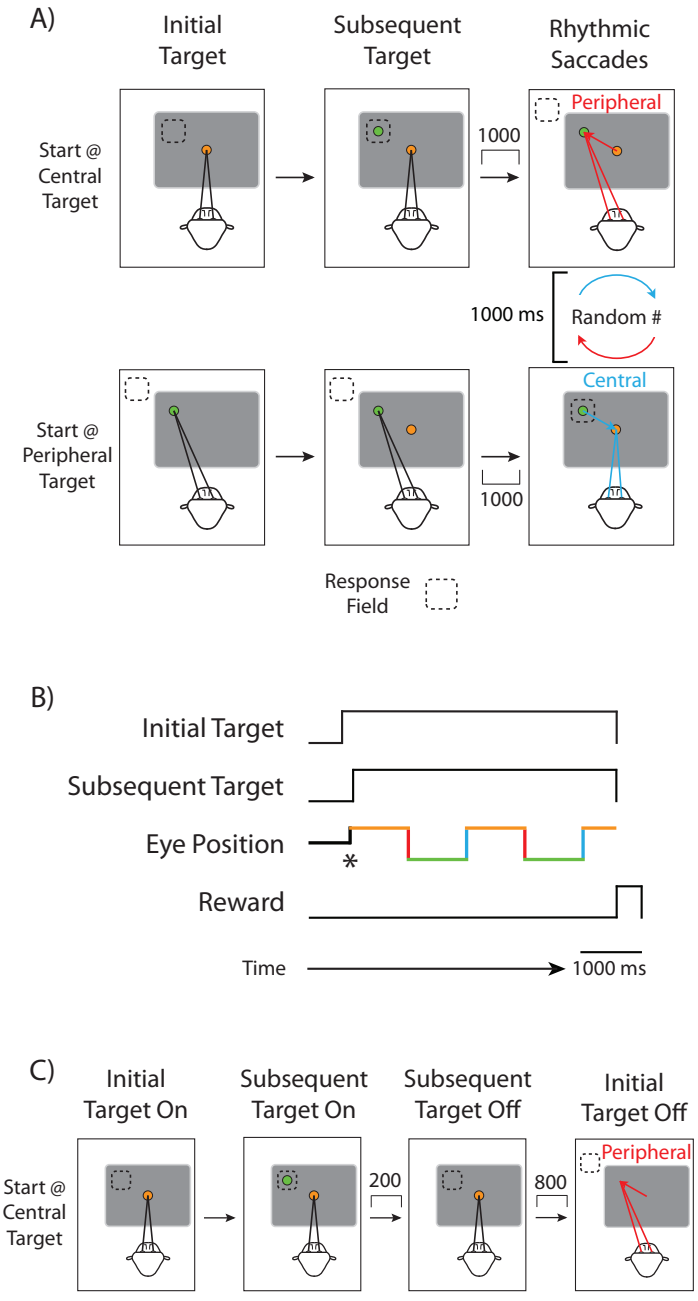

Figure 1: Self-timed rhythmic-saccade task

Self-timed rhythmic-saccade task (A) with example trial (B) and the memory-guided delayed-saccade task (C). A) For the self-timed rhythmic-saccade task, animals were required to fixate on the first of two targets to appear (randomly) on a computer monitor. One target was located near the center of the screen while the other target was located peripherally. When the animal fixates the central target, the response field (RF) of the neuron being recorded is located at the peripheral target. Immediately following fixation, the second target appeared. The animal was then required to saccade between the two targets at a 1 second interval (0.5 Hz; allowable error window = +/- 200 ms) for a random number of saccades in order to receive a juice reward. B) In this example trial, following initial fixation (*), the animal was required to produce 4 saccades prior to receiving reward. Notice that once they appeared, both targets remained constantly displayed so no visual cues were provided. C) For the memory-guided delayed-saccade task, animals first fixate the central target. Following fixation, the peripheral target appears in the RF for 200 ms. The peripheral target then turns off and the animal is required to remember the peripheral target location. 800 ms after the peripheral target is extinguished, the central fixation point is also extinguished cueing the animal to make a saccade to the remembered location in order to receive a reward. Red lines indicate saccades to the peripheral target. Blue lines indicate saccades to the central target. Colored targets correspond to target location (orange = central target, green = peripheral target), but the actual targets were identical in size and color.

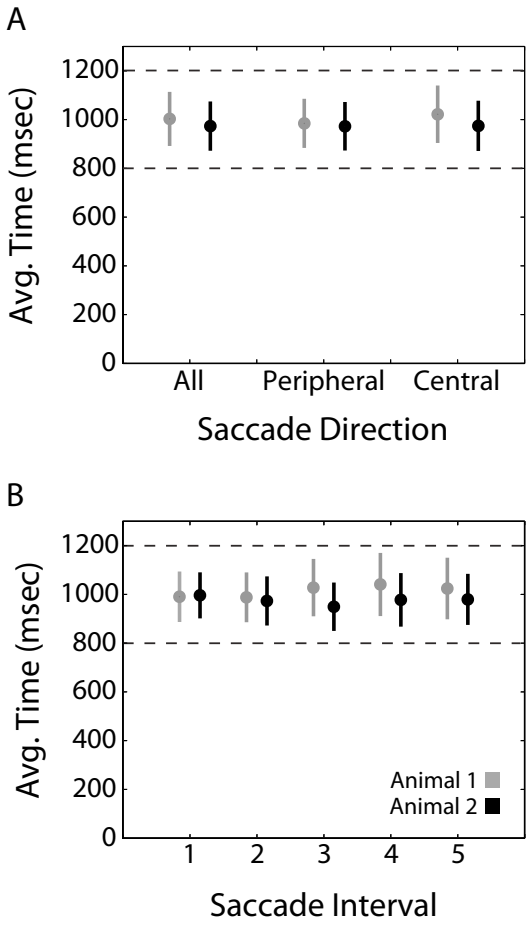

Figure 2: Rhythmic Saccade Behavior

Inter-saccade durations by direction (A) and sequence number (B) for both animals. A) Average inter-saccade durations (dots) by direction of movement with standard deviations (bars). B) Same as in (A) showing inter-saccade durations by sequence number for first 5 saccadic intervals. Inter-saccadic intervals were tightly distributed around the trained interval of 1000 ms with standard deviations less than the allowable error (+/- 200 ms,dashed lines in A & B). Interval durations are similar between directions and intervals within each animal and between animals. Gray color indicates Animal 1. Black color indicates Animal 2.

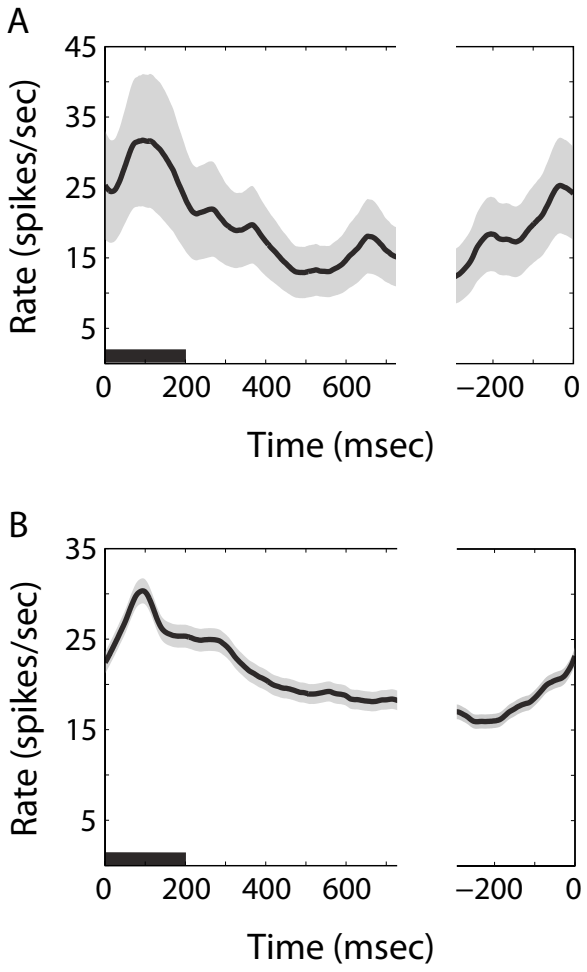

Figure 3: Memory-Guided Saccade Activity

Average neuronal responses of an example cell (A) and population (B) during a memory-guided delayed-saccade task. A) Average response of an example cell during the memory-guided delayed-saccade task. B) Average population response for Animal 1 and Animal 2 combined for the same task in (A). Plots are aligned to two events in the trial: left, target onset; right, saccade onset. Black bar along x-axis represents the time that the peripheral "non-fix" target is displayed. Gray shading indicates standard deviation of the mean.

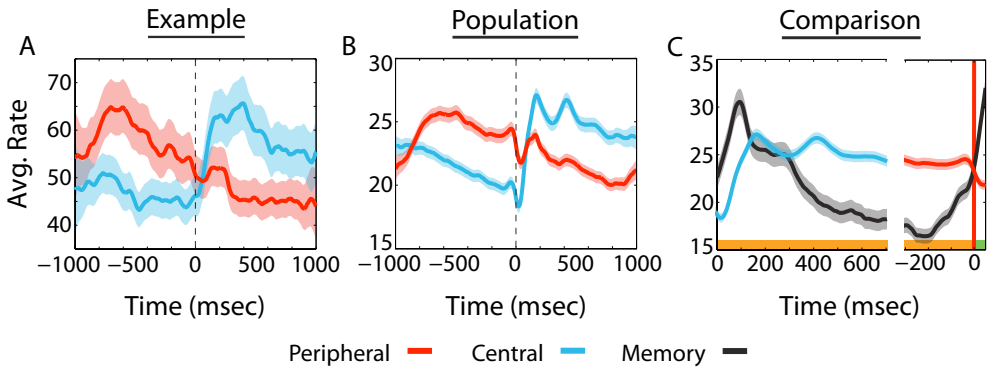

Figure 4: Rhythmic Saccade Firing Rates

Average neuronal response of an example cell (A) and the combined population (B) during the rhythmic-saccade task, and a comparison of "Peripheral", "Memory", and "First" firing rates (C). Zero time point (vertical dashed line) indicates time of saccade onset. Red lines indicate responses aligned to saccades to the peripheral target. Blue lines indicate responses aligned to saccades to the central target. Activity decreases at constant rate prior to both types of saccades. Activity during inter-saccadic intervals is different from what is observed from the same cells during a memory guided saccade task in which only a single saccade is required and the reward can be anticipated (C). Black line indicates response obtained during the memory-guided saccade task (aligned to peripheral target onset on left and peripheral saccade onset on the right). Red and blue lines are the same as in B, for periods of central fixation (orange shading) aligned to the end of central saccades (left, blue) and to the onset of peripheral saccades (right, red). Shading of firing activity represents standard deviation of the mean. Shading along x-axis represents fixation location (orange = central, green = peripheral).

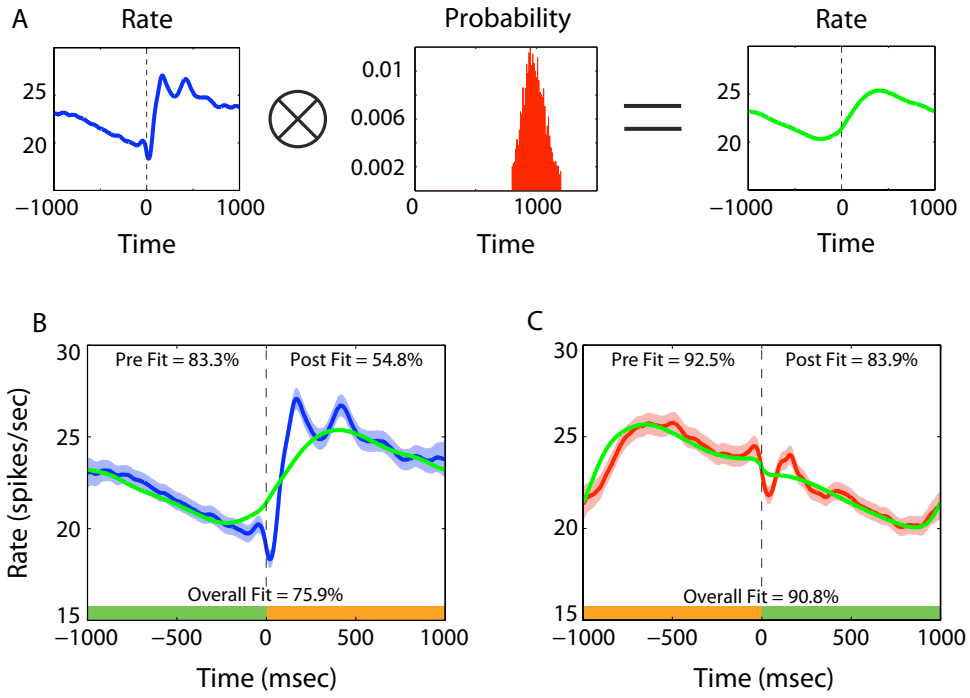

Figure 5: Inter-Saccade Distribution Weighted Rates

Neuronal activity predictions on the assumption that only one type of saccade modulates activity. A) Example calculation of predicted activity. The actual firing rate aligned to central saccades (blue) is convolved with the inter-saccade distribution times of peripheral saccades (red) in order to produce the predicted rate (green). B) Actual averaged neuronal activity (blue), predicted activity (green), with error measurements (% Fit) between actual and predicted activity, aligned to saccades moving away from the RF. C) Same as in (B) except activity (red) and prediction (green) are aligned to saccades moving into the RF. Error calculations represent differences between actual FR and predicted FR. Shading of firing activity shows standard deviation of actual firing rates. Shading along x-axis show fixation location and data sets that differ only in their alignment.

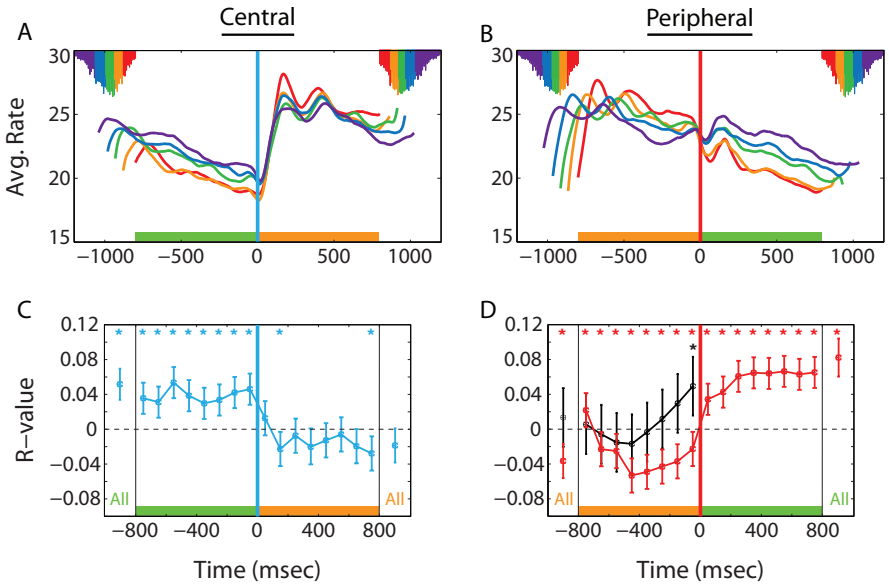

Figure 6: Correlations Between Rate and Duration

Peri-saccadic firing activity segregated by interval length (A, B) and corresponding saccade by saccade correlations between firing rate and interval length (C, D). A & B) Average combined population activity, grouped by interval length, aligned to central saccades (A) and peripheral saccades (B). Zero time points and vertical colored lines indicate saccade onset (blue: saccade to central aligned, red: saccade to peripheral aligned). Inter-saccade distribution times are shown at the beginning and end of each interval and the coloring corresponds to the firing rates. Orange bars represent time intervals during which animals were fixating the central target (peripheral target located in RF). Green bars represent time intervals during which animals were fixating the peripheral target (no stimuli in RF). Orange Intervals: Red = 0.08 - 0.89 ms, Orange = 0.90 - 0.94, Green = 0.95 - 0.99, Blue = 1.00 - 1.05, Purple = 1.06 - 1.20. Green Intervals: Red = 0.80 - 0.91 ms, Orange = 0.92 = 0.96, Green = 0.97 - 1.01, Blue = 1.02 - 1.07, Purple = 1.08 - 1.20. C &D) Binned correlations between firing rate and current interval lengths for all trials of the combined population, aligned to central saccades (C) and peripheral saccades (D). Last bins (C, D) display correlation of the 800 ms of the interval ("All", 0 - 800 ms, or -800 - 0 ms) about saccade onset. Black points in D correspond to correlations observed during the memory-guided saccade task. Horizontal colored bars along the x-axis represent the 800 ms of the intervals over which correlations were made. 800 ms intervals were investigated as that is the minimum time for correctly made saccades as defined by the error window (1000 ms +/- 200 ms). Asterisks indicate bins that are significantly correlated ($p < 0.005$). Bars represent the 99% confidence interval for each bin. The horizontal line represents an R-value of zero for reference. Time bins are correlated to the current intervals represented by the colored bars along the x-axis.

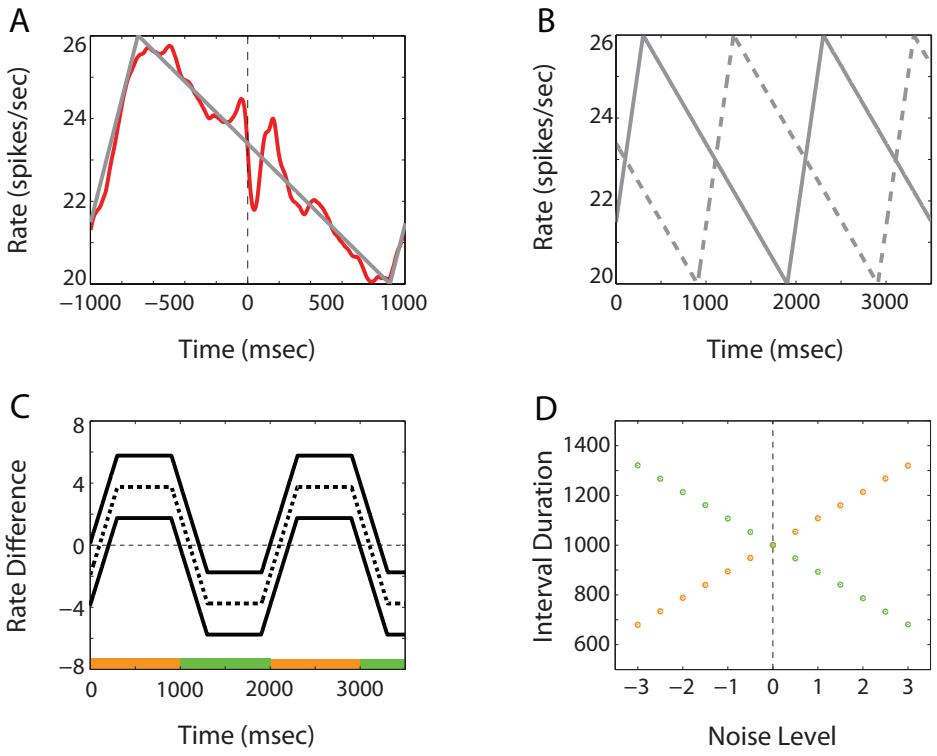

Figure 7: LIP Timing Model

Difference model of cross-hemispheric LIP activity driving temporal production. A) Actual average LIP population activity aligned to saccades to the periphery (red) along with a simple triangle waveform estimation of that population activity (gray). B) Neural activity estimate from (A) (gray) along with the same estimate of activity phase shifted to represent the same movement but in the opposite direction within the opposite hemisphere (dashed gray). C) Difference in firing rates between the two activity estimates shown in (B) (dashed black) and the difference between those same rates with added or reduced activity (solid black). The time points at which the difference traces begin changing toward 0 represents the times at which saccades are initiated. The dashed black line displays signaling for equal time to be spent at both target locations (i.e. the time between positive and negative location values are equal). An increase (upper solid black trace), in activity shifts the signaling for equal time being spent at both locations to more time being spent at the "positive" location versus the "negative" location. Conversely, a decrease (lower solid black trace), in activity signals for more time to be spent at the "negative" location. D) Interval durations change linearly with activity. Green dots represent the interval duration that is seen at the "negative" location as noise levels change. Orange dots represent the interval duration that is seen at the "positive" location as noise varies.

Part III

Microstimulation of LIP Affects Motor Timing

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Introduction

An awareness of the passage of time is fundamental to cognition and behavior. It allows us to store events that occurred in the past and make use of those memories to form plans for the future. The precision with which animals can anticipate predictable events and execute responses at appropriate times testifies to the importance of time perception. Despite many models, the physiological signals underlying time perception remain unclear. Part of the challenge is that every process, be it psychological, cellular, or molecular, has characteristic time courses and dynamics. Although such processes, like membrane conductance time constants, decay rates of short-term synaptic plasticity, and decay rates of inhibitory post-synaptic potentials can be utilized to measure time [Hooper et al. (2002); Fortune and Rose (2001); Buonomano (2000); Saitoh and Suga (1995)], they don't necessarily reflect the active process of timing and they may reflect the dynamics of external events rather than the internal sense of time. This problem is illustrated by recent studies which have suggested that a parietal cortical area is critically involved in the timing of eye movements.

The lateral intraparietal area (LIP) of the posterior parietal cortex, an area typically thought to integrate visual input and motor output [Andersen (1995)], has recently been shown to displays activity that consistently varies over time [Leon and Shadlen (2003a); Janssen and Shadlen (2005); Maimon and Assad (2006)]. Yet, it is not evident that these temporally modulated signals are related to the timing of motor production. Each of these studies investigated timing through tasks that utilized time-varying task-dependent stimuli, such as a predictable go cue or delivery of reward. Therefore, the temporal signals observed within these studies may reflect temporal measurement, the representation of time related to external events, rather than temporal production, the internally generated timing of movement initiation.

Previous work in our lab showed that when task execution was not dependent on

temporal measurement signals, LIP activity was predictive of inter-saccadic interval length (correlation analysis, $p < 0.01$). For this self-paced saccade task, animals were required to made saccades each second between two fixed targets. All external cueing was eliminated and trial length was randomized. Therefore, the temporal signals observed during our task are based on internal representations of time instead of sensory perceptions. However, correlations between firing rate and interval length do not necessarily support a causal relationship between neural activity and behavior. The observed correlation may simply be a coincidence. For example, efference copy signals that occur during motor movements are correlated with the movement, yet these signals do not cause the movement.

In order to determine if LIP activity is utilized in temporal production, we introduced altered local activity in LIP through microstimulation while animals performed a self-timed task (Fig. 8). We found that stimulation affected the perception of time. Brief trains of stimulation altered the timing of the animals' self-paced actions, even when those actions occurred 800 ms later. This suggests that LIP activity may contribute to the temporal production of saccades and that LIP could be a source of temporal information.

Self-timed rhythmic saccade task

In order to investigate temporal production signals, 2 animals were trained to perform a self-paced motor timing task that was dependent on an internal perception of time rather than on sensory cueing. This task, called the self-timed rhythmic-saccade task, began by requiring the animal to fixate the first of two targets to appear on a computer monitor (Fig. 8a). Following fixation of the first target, the second target immediately appeared. One target was positioned near the center of the screen ("central target") while the second target ("peripheral target") was placed within the response

field [Gnadt and Andersen (1988); Barash et al. (1991b); Colby et al. (1995, 1996); Platt and Glimcher (1997, 1998); Andersen and Buneo (2002)] (RF) of the neuron of interest (relative to central fixation). Trials were randomly selected to begin at either target. The animal was then required to make saccades back and forth between the two targets at a one second interval (+/- 200 ms) in order to receive a reward. Microstimulation was delivered randomly throughout this task as a means to investigate the link between activity and time perception.

LIP neurons have been shown to modulate their activity not only during saccade planning [Bracewell et al. (1996); Mazzoni et al. (1996); Andersen et al. (1997); Platt and Glimcher (1997); Snyder et al. (1997); Gnadt and Andersen (1988); Andersen and Buneo (2002)], but also during times when sensory events [Colby et al. (1996)] and reward [Platt and Glimcher (1999); Sugrue et al. (2004); Yang and Shadlen (2007); Bendiksby and Platt (2006); Dorris and Glimcher (2004); Seo et al. (2009)] can be anticipated. Responses to these events can complicate the study of temporal production for tasks in which sensory events or reward occur at regular times and are tightly coupled to the movement. In order to minimize sensory and reward anticipation, animals were forced to utilize an internal clock (no external cueing) in order to accomplish this task. Therefore, the behavior should reflect internal timing mechanisms. To minimize temporal measurement signals, no changes in visual stimuli occurred during each trial following the appearance of both targets. This served as a means to reduce sensory anticipation. Additionally, trial length and reward amount were randomized on a trial by trial basis to minimize reward anticipation. By diminishing non-movement related temporal measurement signals, and by injecting microstimulation during a time-dependent task, we aim to discover if LIP activity can alter behavioral timing.

Mapping & memory task electrophysiology

In addition to the rhythmic saccade task, animals also performed a mapping task and a memory-guided delayed saccade task [Hikosaka and Wurtz (1983); Colby et al. (1993)] while neural activity from individual cells was recorded. The mapping task is simply a single trial of the rhythmic task where the initial fixation point always occurs at the central location and the target is moved about the periphery to determine the neuron's RF [Gnadt and Andersen (1988); Barash et al. (1991b); Colby et al. (1995, 1996); Platt and Glimcher (1997, 1998); Andersen and Buneo (2002)]. Mapping was required prior to stimulating in order to establish the spatial location for which stimulation is likely to have an effect (RF) [Mazzoni et al. (1996)].

The memory task (Fig. 9a) was used to verify the location of our stimulating electrode within LIP. Stereotyped activity has been reported in LIP during this task [Gnadt and Andersen (1988); Andersen et al. (1990b); Barash et al. (1991a,b)]. The stereotyped activity is characterized by transient increased response while the peripheral target is flashed within a neuron's RF. Activity is then maintained throughout the remainder of the trial while the animal remembers the target location. In order for the data obtained during the rhythmic task to be analyzed further, neurons must display these patterns of activity during the memory task.

Fifty six out of the 81 neurons we sampled displayed the prerequisite activity. The stereotyped activity is evident in Fig. 9 for both an example neuron (Fig. 9b) and the 56 cells of our population (Fig. 9c). Both showed a transient response to the flashed RF target (black bar along x-axis) and sustained memory related activity above baseline. Additionally, both the example neuron and the population displayed an increase in activity prior to saccade onset. This peri-saccadic increase is thought to be caused by reward anticipation [Platt and Glimcher (1999); Sugrue et al. (2004); Yang and Shadlen (2007); Bendiksby and Platt (2006); Dorris and Glimcher (2004);

Seo et al. (2009)] since reward is consistently provided following a single predictable saccade.

Stimulation, correlation, and timing

Previous reports of temporal activity within LIP were performed under conditions in which the sensory cues of the tasks varied in a time-dependent manner [Leon and Shadlen (2003a); Janssen and Shadlen (2005); Maimon and Assad (2006)]. Therefore, the temporal nature of these signals may be related to temporal measurement signals instead of temporal production. Since movements can be, and often are, generated internally without cues directing the timing of their initiation, we were interested to see if temporal production signals exist within LIP when sensory cueing was absent.

However, even if LIP activity appears to act as a temporal production signal, the activity does not necessarily represent causality with behavior. In order to determine if LIP activity does affect temporal production, stimulation was delivered to LIP while the animals performed the rhythmic task. Supra-threshold stimulation of LIP has been shown to directly illicit saccades [Thier and Andersen (1996, 1998); Mushiake et al. (1999); Cutrell and Marrocco (2002); Constantin et al. (2007)]. Yet, sub-threshold stimulation has been shown to influence a number of cognitive processes such as decision making and attention, as well as saccadic behavior [Hanks et al. (2006); Cutrell and Marrocco (2002); Mirpour et al. (2010)]. Therefore, the application of sub-threshold stimulation may allow us to investigate timing by influencing saccadic latencies without directly triggering a saccade.

Stimulation was delivered during random intervals of random trials as animals performed the self-timed rhythmic saccade task (Fig. 8a, b). Although stimulation was always applied during the last interval of a given trial, trial lengths varied so stimulation was delivered at different sequence lengths. Two different stimulation

experiments were carried out. In the "interval" experiment (27 sessions), a consistent train of stimulation (4 pulses/16 ms train duration) was delivered at various times throughout the inter-saccadic interval. If LIP activity does act as a motor timing signal then an injection of current should affect interval duration similarly throughout the interval. In the "duration" experiment (29 sessions), stimulation was delivered at a consistent time within the inter-saccadic interval (450 ms after a saccade), but the number of pulses delivered varied (4, 8, 12, 16, or 20) (also see Methods Summary) in order to determine how microstimulation duration affects motor timing.

Previous work from our lab has showed that temporal production signals do exist within LIP. These results were obtained as the animals performed the self-timed rhythmic-saccade task in the absence of stimulation (Fig. 8a). These findings are consistent with the "interval" experiment in that interval by interval correlations of 100 LIP neurons were calculated by summing the number of action potentials over each bin, throughout each interval, and comparing those values with the interval duration. Correlations were always based on current intervals prior to the upcoming saccade (Fig. 10a, b).

Interval correlation results showed that when inter-saccadic activity was aligned to begin at saccade initiation, only activity aligned to follow peripheral saccades (while the animal fixated the peripheral target prior to central saccades) consistently displayed bins of significant activity throughout the inter-saccadic interval (Fig. 10b) ($p < 0.005$). Because correlations existed throughout these intervals, it is possible that LIP activity could potentially be used as a motor timing signal. Timing signals should be evident throughout the period during which the movement is being anticipated, while something like a pre-motor signal would only be evident just prior to the initiation of the movement. Yet, when activity was aligned to follow central saccades (while the animal fixated the central target prior to peripheral saccades), significant

correlations were not consistently observed (Fig. 10a).

LIP activity may be a timing signal for saccade onset since significant correlations between rate and interval length were observed throughout the interval following peripheral saccades. In order to test this and to deliver stimulation at a consistent time during each interval, we have to align stimulation delivery to follow saccade onset. Therefore, based on the correlations from our previous work (Fig. 10a, b), we would predict that when stimulation is delivered following peripheral movements (central target fixation), initiation of the upcoming saccade will be delayed, while little to no effect will be seen when stimulation is delivered following central saccades (peripheral target fixation).

This is in fact what we found. When stimulation was delivered as the animal fixated the central target, no time points displayed a significant effect compared to the "no-stim" condition (Fig. 10c) (ANOVA, $p < 0.0001$). This finding is consistent with the correlation results in that increased activity while the animal is fixated at the central target (orange bar along x-axis) was not correlated with interval length (Fig. 10a). However, when stimulation was applied as the animal fixated the peripheral target, the upcoming saccades were significantly delayed (Fig. 10d). This again is consistent with the corresponding correlation results in that greater activity (stimulation) led to longer intervals (Fig. 10b).

Similar correlation results were also found during the duration experiment for various bin sizes about the 450 ms time points (Fig. 11a, b). Significantly positive correlations were observed for various bin sizes as animals fixated the peripheral target (Fig. 11b, green), while no significant effects were observed as animals fixated the central target (Fig. 11a) ($p < 0.005$). These r-values closely resembled the corresponding correlations observed in Fig. 10a and c at 450 ms. Again, based on these results, we predicted that when stimulation is delivered as the animal is fixated at the

peripheral target, the initiation of the upcoming saccade will be delayed. When stimulation is delivered as the animal is fixated at the central target, no significant effects will be observed.

Stimulation results during the duration experiment were also consistent with the correlation findings. When stimulation was delivered while the animals fixated the central target, no significant effects were observed for any stimulation duration (Fig. 11c) (ANOVA, $p < 0.0001$). When stimulation was delivered while the animals fixated the peripheral target, the upcoming movement was significantly delayed for all stimulation durations (Fig. 11d). These delays were similar between experiments. Stimulation results from both the interval and duration experiments are summarized in Table 1.

One interesting finding from the interval experiment is that brief trains of stimulation (16 ms) can produce an effect nearly 1 second later. This is consistent the idea that activity within LIP acts as a temporal production signal since activity early within (and throughout) the interval has behavioral consequences at the end of the interval. If this activity were a pre-motor signal, stimulation effects would only be expected to be evident just prior to saccade initiation, and not throughout the interval. Support for this idea has been provided by the correlation results (Fig. 10a,b) that demonstrated that LIP activity is predictive of interval length throughout the interval. The stimulation results, which are also consistent with the correlation findings, demonstrated that changes in LIP activity led to changes in saccadic behavior. Therefore, microstimulation within LIP appears to affect temporal production.

A potentially surprising result from the duration experiment is that all train durations had a similar effect following peripheral saccades. We expected that longer stimulation durations would inject more current into the area, which would then lead to greater delays in saccade initiation. Although all durations produced significant

increases in interval length, they were not statistically different from one another (ANOVA, $p < 0.0001$). The reason for this observation may have to do with how much a sub-population of neurons can contribute to a population timing mechanism. We find similar results between individual neurons in different locations. This suggests that neurons throughout LIP contribute to the timing of saccades. Since we are stimulating a relatively small area (~0.38 mm spherical radius, Stoney et al. (1968); Tehovnik et al. (2006)) compared to the size of LIP (~15 x 5 x 1.5 mm, Van Essen et al. (2001)), the effective contribution of the stimulated neurons to timing may be maximized for all train durations, suggesting that there is a limit to how much any local population can contribute.

Altering temporal production through microstimulation has not been directly shown before. Although, other stimulation and inactivation studies within LIP have hinted at this result, these studies were not specifically investigating motor timing. Instead, they observed changes in behaviors that did not require precise timing. For instance, a motion discrimination task found that when LIP was stimulated, a greater effect was observed on reaction times than on choices. The reaction times toward the RF were reduced while reaction times away from the RF were increased [Hanks et al. (2006)]. Similarly, when LIP was inactivated with muscimol, contralesional saccade latencies were increased [Li et al. (1999)] as were contralesional search times [Wardak et al. (2002)]. Yet, reaction times and search times are very distinct from motor timing. Reaction times simply occur as fast as possible, and not at a specific time while visual search tasks typically have no precise temporal requirements. Therefore, our results provide a unique look at timing not addressed by previous stimulation or inactivation studies.

Studies that have introduced microstimulation in V1 have reported changes in the timing of saccade onset when saccades are made toward the RF [Tehovnik et al.

(2004); Tehovnik and Slocum (2005); Tehovnik et al. (2005); Tehovnik and Slocum (2007)]. However, since their stimulation effects are only observed when current is delivered immediately before saccade onset in a cued task, it is possible that their delays in saccade initiation are either related to motor production of the saccade (instead of a motor planning component, such as timing) or to attentional effects of phosphene induction by stimulation. Since stimulation effects are seen throughout the interval without directly eliciting a saccade in our results, it is unlikely that our effects are related to motor production. Also, it is unlikely that phosphenes affect the behavior of our animals. First, the animal is trained to withhold saccades to visual targets until the trained interval. Second, we do not observe low latency saccades as would be expected when a novel target (such as a phosphene) is flashed.

However, other studies have suggested that LIP activity can be strongly modulated by spatial attention [Robinson et al. (1995); Gottlieb et al. (1998); Powell and Goldberg (2000); Bisley and Goldberg (2003a, 2006); Cutrell and Marrocco (2002); Wardak et al. (2004); Liu et al. (2010); Wardak et al. (2011)]. One model for attention within LIP is the priority map, where high neural activity draws attention which then guides eye movement [Bisley and Goldberg (2010)]. Although the spatial attention demands of our task are low (two fixed targets), perhaps by introducing stimulation we are briefly distracting the animal's allocation of attention to timing (attention to the passage of time) which simply delays him. This view could be consistent with our stimulation results. Yet, we do not believe our effects are a result of attention. Studies investigating timing and attention in both humans and animals suggest that when subjects are distracted, they underestimate temporal intervals [Buhusi and Meck (2009)]. In our study, stimulation increased saccadic latency, neural activity did not peak at the time of saccade onset (previously reported, not shown), and the attentional (spatial, sensory, and reward) demands of our task were low.

Discussion

Previous studies that have investigated timing within LIP have done so using time-varying, task-dependent cues [Leon and Shadlen (2003a); Janssen and Shadlen (2005); Maimon and Assad (2006)]. Because the movements were visually cued, we were curious if the temporal signals represented temporal production related to movement onset or the external probabilities of visual cueing or reward. Through the use of the self-timed rhythmic saccade task, we investigated temporal production of movement initiation within LIP by eliminating temporal measurement cueing. We found that LIP activity was predictive of interval length and that introducing microstimulation to this production signal did affect behavior.

Previous work from our lab (unpublished) has suggested that timing occurs as a result of population activity within a region associated with a given movement. The population activity within LIP may affect saccade output through a difference model where the difference in LIP activity from opposing hemispheres is used to determine the time of saccade onset. In this model, small changes in that difference (1 spike/sec) lead to large changes in interval duration (106 ms). This would mean that the average change of 45 ms that we observed in the interval stimulation experiment would have been caused by a change in that difference rate of only 0.42 spikes/sec. This suggests that a large population of neurons within LIP contributes to the timing of saccade initiation. Since relatively large sub-threshold stimulation currents only have a small effect on timing, it is likely that only a local population is being activated (stimulation volume ~0.23 mm^3[Stoney et al. (1968); Tehovnik et al. (2006)], LIP volume ~112.5 mm^3). Consistent with this, temporal signals within LIP may be defined by a distributed timing mechanism, such as the population clock model. In this model, single cells contain very little temporal information. Instead, it is the population activity that encodes time [Buonomano and Karmarkar (2002)]. This type of model, along with

the variable firing rates of our cells, may explain the small R-values observed in our correlations.

The behavioral effects produced by stimulation suggest that the internally generated perception of time within LIP can be utilized in temporal production. The decision to saccade is completely cognitive based, reflects training, and is not due to sensory perceptions. This explicit temporal representation of internally initiated behavior provides insights into general timing mechanisms within the brain that may be utilized in numerous applications.

Methods Summary

Response fields [Gnadt and Andersen (1988); Barash et al. (1991b); Colby et al. (1995, 1996); Platt and Glimcher (1997, 1998); Andersen and Buneo (2002)] were mapped for each recorded and stimulated neuron. Response field locations were determined by the spatial position that elicited the largest neuronal response as animals performed single saccade trials of the rhythmic task as the peripheral target was moved about the monitor. To determine which cells displayed stereotyped LIP activity, a minimum of 30 trials of the memory-guided delayed saccade task were also performed while recording neural activity (Fig. 9a). Neurons that displayed a response to the flashed target and maintained activity while the animal remembered the target location, were selected for further analysis (100/175 recordings and 56/81 stimulation sessions) (Fig. 9).

For physiological recordings and stimulation sessions, 2 animals were trained to perform a self-timed rhythmic saccade task. Briefly, animals were required to make a random number of saccades back and forth between two fixed targets so that saccades occurred each second (0.5 Hz) (Fig. 8a). For stimulation trials, current was delivered during random intervals of random trials to minimize stimulation expectations. 150-

180 μA biphasic current pulses were delivered at 250 Hz. During interval stimulation sessions, 4 pulses (16 ms train duration) were delivered at random times following saccade initiation. During duration stimulation sessions, 4, 8, 12, 16, or 20 pulses were delivered 450 ms after saccade onset (Fig. 8b). Control experiments were also conducted where the stimulation protocol was engaged, but no stimulation was delivered. No differences were observed between "sham-stimulation" and no-stimulation conditions.

Visual stimulation, behavioral control, and data acquisition for each experiment were controlled using customized computer software:

(http://www.ghoselab.cmrr.umn.edu/software.html)

All surgeries required were done in accordance with animal care guidelines of the University of Minnesota and the National Institutes of Health and were performed under aseptic conditions with full anesthesia.

Figures

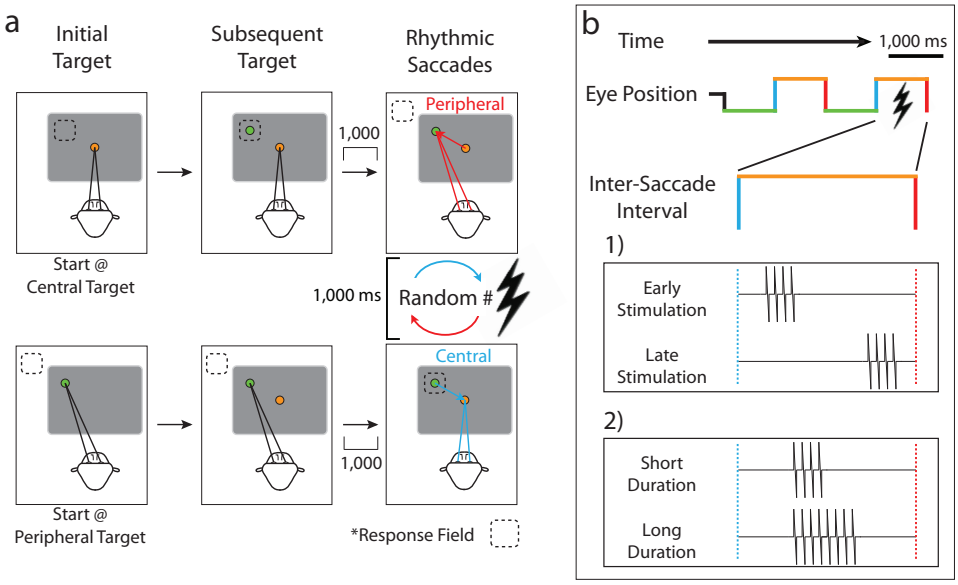

Figure 8: Rhythmic-Saccade Stimulation Task and Parameters

Task design for investigating temporal production signals within LIP. a, Self-timed rhythmic-saccade task performed by animals during electrophysiological recordings and stimulation sessions. Dashed box in each window (not visible to animals) depicts neuronal RF. Colored targets represent target locations (orange = central target, green = peripheral target). Both targets were white while animals performed the task but are colored in this figure for illustrative purposes. Red lines indicate "peripheral" saccades, while blue lines indicate"central" saccades. Black lightning bolt depicts delivery of microstimulation during random intervals of random trials. b, Example trial of the self-timed rhythmic saccade task (top) and magnification of the interval during which stimulation was delivered (bottom). 1 depicts the interval stimulation experiment. 2 depicts the duration stimulation experiment.

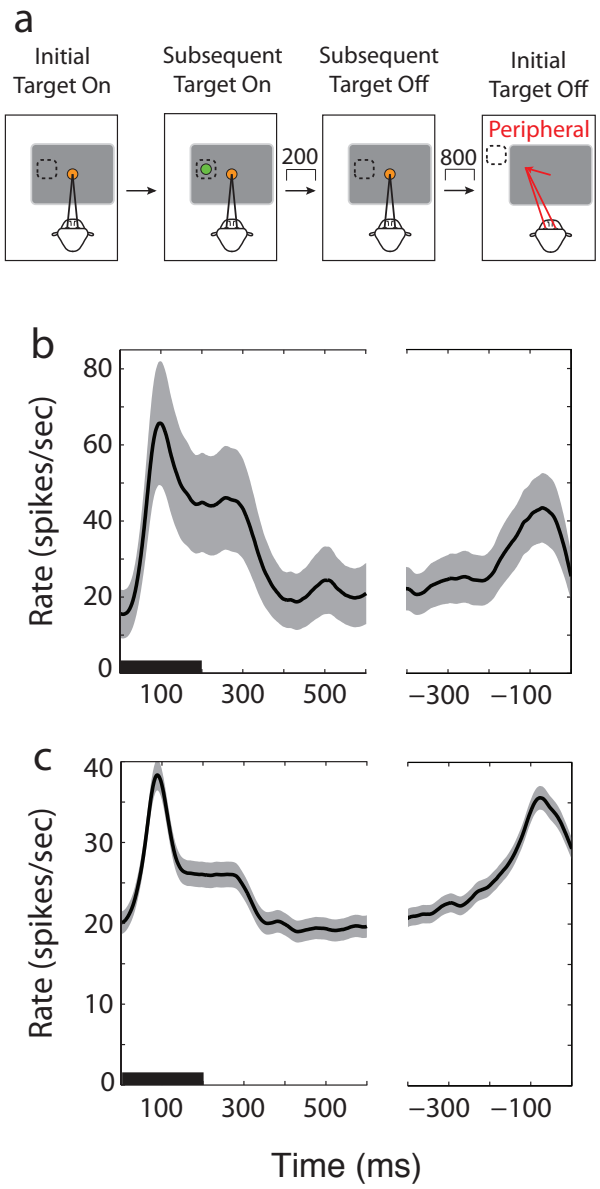

Figure 9: Memory-Guided Saccade Activity (Stimulation)

Neural activity displaying stereotyped LIP responses observed during the memory-guided delayed-saccade task. a, Memory-guided delayed-saccade task. b, c, Example cell response (b) and the population response (c) obtained while the animals performed the memory-task. Black bar along x-axis depicts time period when the peripheral target is flashed within the response field. Left portions of the plots are aligned to target onset. Right portions are aligned to saccade onset. Gray shading represents standard deviation of the mean.

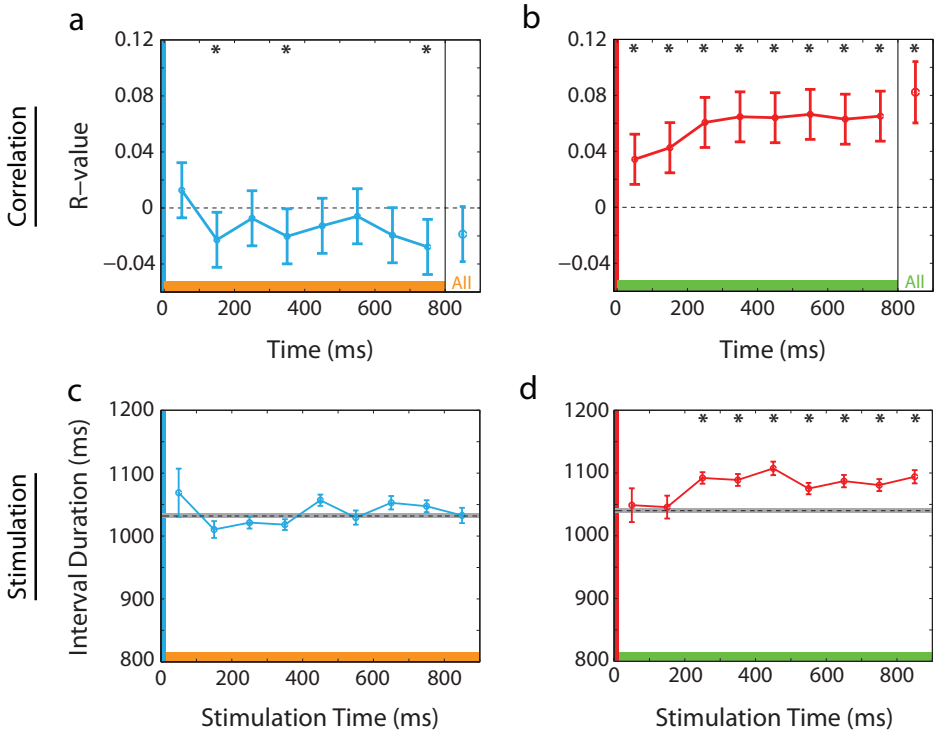

Figure 10: Interval Experiment Results

LIP activity is predictive of inter-saccadic interval length and affects behavior. a, b, Correlation analyses between spike rate and interval duration calculated over 100 ms bins for the first 800 ms of the interval ("All") ($p < 0.01$). Bars on the data points depict 99% confidence intervals. Correlations are aligned following central saccades (a) and peripheral saccades (b). Dashed line depicts an R-value of zero for reference. c, d, Temporal effects observed during the interval stimulation experiment following saccades aligned to the center target (c, blue) and the peripheral target (d, red). Bars on the data points represent the S.E.M. Dashed black line depicts the average inter-saccade interval length observed when no stimulation is applied. The gray shading represents the S.E.M. for the no-stim condition. Asterisks represent intervals that are significantly different from the no-stim condition (ANOVA, $p < 0.001$). Colored bars along the y-axis depict central (blue) and peripheral saccades (red). Colored bars along the x-axis depict fixation location (orange = fixated at central target, green = fixated at peripheral target).

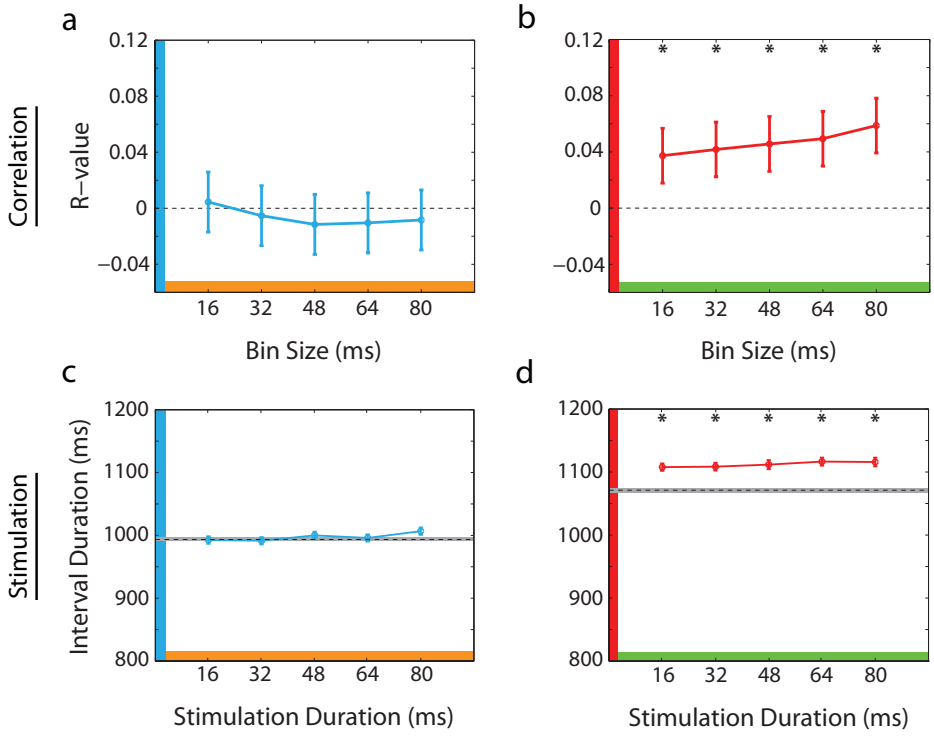

Figure 11: Duration Experiment Results

LIP activity and its effects on behavior are not largely affected by bin size or stimulation duration. a,b Correlation analyses between spike rate and interval duration calculated for various bin sizes at 450 ms following saccade initiation ($p < 0.01$). Bars on the data points depict 99% confidence intervals. Correlations are aligned following central saccades (a) and peripheral saccades (b). Dashed line depicts an R-value of zero for reference. c, d, Temporal effects observed during the duration stimulation experiment following saccades aligned to the center target (c, blue) and the peripheral target (d, red). Bars on the data points represent the S.E.M. Dashed black line depicts the average inter-saccade interval length observed when no stimulation is applied. The gray shading represents the S.E.M. for the no-stim condition. Asterisks represent intervals that are significantly different from the no-stim condition (ANOVA, $p < 0.001$). Colored bars along the y-axis depict central (blue) and peripheral saccades (red). Colored bars along the x-axis depict fixation location (orange = fixated at central target, green = fixated at peripheral target).

Table 1: Stimulation effects on interval duration

Time of Stimulation	50	150	250	350	450	550	650	750	850	Avg.	No
Post – Central	1069	1010	1021	1018	1057	1029	1053	1047	1033	1036	1032
Post – Peripheral	1049	1046	1092*	1089*	1107*	1075*	1087*	1081*	1094*	1085*	1040

Pulses	4	8	12	16	20	Avg.	No
Post – Central	993	991	1000	996	1007	998	993
Post – Peripheral	1108*	1108*	1112*	1117*	1116*	1112*	1071

* Significantly different from "No" stimulation conditions. ANOVA ($p < 0.0001$)

Table 1: Stimulation Times

Top: Interval durations (ms) based on the time stimulation was delivered within the interval (Time of Stimulation) following central (Post – Central) and peripheral (Post – Peripheral) saccades. Average interval durations (Avg.) are for all stimulations within each conditions. "No" represents conditions where no stimulation was delivered. Bottom: Interval durations (ms) based on the number of stimulation pulses delivered.

Part IV

Concluding Remarks

Internal Motor Production Signals are Predictive of Interval Length

In Chapter 2, it was evident that when sensory cueing was eliminated and reward was dissociated from movement in the self-timed rhythmic-saccade task, the timing signals we observed were drastically different from previous reports of time related activity in LIP [Leon and Shadlen (2003b); Janssen and Shadlen (2005); Maimon and Assad (2006)]. This suggests that the time-dependent climbing activity reported during these previous tasks were likely due to stimulus dynamics or reward anticipation rather than movement initiation. This does not mean that the activity observed during these studies is not related to timing. It simply means that the temporal signal was concerned with temporal measurement cueing rather than the temporal production of movement.

Significant correlations between inter-saccade duration and firing rate existed throughout inter-saccadic intervals. This suggests that falling activity may serve as a temporal production signal in the timing of rhythmic saccades as it is predictive of interval length. In addition to the correlation analysis, a few other interesting findings came to light that have not been seen in previous timing studies. First, the sign of the correlation (+ vs. -) depended on some combination of fixation location (central vs. peripheral) and the upcoming saccade direction (toward RF vs. away from RF). This means that LIP activity, contrary to previous views [Blatt et al. (1990)], reflects movement parameters to multiple visual locations, not just toward the RF. Another interesting finding is that the sign switch of the correlation occurs at the time of saccades. Therefore, saccades may serve to reset the timing signal. This notion is further supported by the fact that LIP activity was only concerned with the current (not past or future) interval.

It was also interesting that activity prior to a peripheral saccade (negative correlation) signals for saccade production while activity prior to a central saccade (positive

correlation) signals to maintain fixation. This discrepancy between correlation signs may provide insight into a new timing model. Since each hemisphere of LIP typically contains RFs in opposite hemi-fields from one another, it means that they encode an upcoming movement in different ways (positive correlation exists in one hemisphere while a negative correlation exists in the other hemisphere for the same movement). A difference model where the activity between the two hemispheres is subtracted from one another is simple in its implementation and does a good job of explaining our results.

These results also shed light on the mechanisms being used. The difference between timing a central saccade and a peripheral saccade may imply that a central timer is not the mechanism being used to time this behavior since each direction of movement is encoded differently. Additionally, the lack of significant temporal information in individual cells, along with low correlation values of the population suggests that our difference model may be the result of competing population clock mechanisms that are active in each hemisphere.

Together, our results, along with the results from previous LIP studies, suggest that LIP is utilized in the timing of both external sensory and internal motor events and that the task demands determine which timing system is utilized.

Time Perception Altered by Stimulation

Although Chapter 2 provided substantial evidence in favor of LIP activity acting as a temporal production signal, average activity does not imply causality. In Chapter 3, we investigated LIP's effect on behavior by stimulating the area as each animal performed the self-timed rhythmic saccade task. Results showed that stimulation of LIP altered the animal's sense of time. Stimulation delivered prior to central saccades delayed the onset of the behavior, in agreement with the correlation results of Chapter

2. This suggests that LIP is used in the production of saccades and that LIP activity is involved in the perception of time.

The stimulation results also lend credence to the population clock model as the mechanism of motor production. First, stimulation only had an effect in one direction. Since both directions of movement were not affected equally by the stimulation, it suggests that a centralized timer is not being used. Secondly, relatively large amounts of stimulation (150-180μA) only produced small effects in behavior (~ 40 ms). Therefore, a large population of neurons is likely to be used to time this behavior since stimulating a small region of neurons with a high level of current only produced small behavioral effects.

Broad Significance

Understanding how the brain functions to control the timing of our movements is one of the most compelling problems in the field of neuroscience. Insights into these issues have relevance to how we locomote, speak, attend to and grasp for objects, and to cognition and survival [Buhusi and Meck (2005); Wittmann (2009)]. Without proper timing, movement and many other functions are adversely affected. The number of neurological disorders that display time processing deficits are ample and include: aphasia, dyslexia, attention-deficit/hyperactivity disorder, Parkinson's disease, and schizophrenia [Lebedev et al. (2008)]. A more complete understanding of the timing mechanisms within the brain will better equip the scientific community to understand and treat these disorders.

The results of my research also have the potential to contribute to a diverse array of scientific areas. For example, although great technological advances have been made in the area of neural prosthetics and such devices are already improving the lives of many who have lost motor control of their limbs, the movements produced

by this technology remain quite crude and unrefined. The current technology is based purely on motor signals, making the movements of prosthetics slow and jerky. By knowing where motor planning signals exist and utilizing these signals, movements could be made faster, more fluid, and more life-like. These improvements would be a great benefit to the field of motor control and prosthetics, the field of robotics, and especially the patients.

Closing Statement

The results from our studies may someday have broad reaching effects outside the field of neuroscience. Not only did these experiments provide information on what internally generated motor production signals look like, and how these signals may be implemented to affect behavior, but it actually provides a location at which the perception of time can be altered. Subsequent research on timing may be able to build upon these findings and utilize them as the field pushes toward compiling a global view of neural timing mechanisms.

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