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The Timing of Reward-Seeking Action Tracks Visually-Cued Theta Oscillations in Primary Visual Cortex

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Abstract

An emerging body of work challenges the view that primary visual cortex (V1) faithfully represents the visual world. Along this line, theta oscillations in the local field potential (LFP) of V1 have been found to convey temporal expectations and, specifically, express the delay between a visual stimulus and the reward it portends. We extend this work by showing how these oscillatory states in male, wild-type rats can even relate to the timing of a visually-cued, reward-seeking behavior. In particular, we show that with training, high precision and accuracy in behavioral timing tracks the power of these oscillations, and that the time of action execution covaries with their duration. These LFP oscillations are also intimately related to spiking responses at the single unit level, which themselves carry predictive timing information. Together, these observations extend our understanding of the role of cortical oscillations in timing, generally, and V1's role in the timing of visually-cued behaviors, specifically.

Significance Statement

Traditionally, Primary Visual Cortex (V1) has been regarded as playing a purely perceptual role in stimulus-driven behaviors. Recent work has challenged that view by showing that theta oscillations in rodent V1 may come to convey timed expectations. Here, we show that these theta oscillations carry predictive information about timed reward-seeking actions, thus elucidating a behavioral role for theta oscillations in V1 and extending our understanding of V1's role in decision-making.

Introduction

Timed responses to environmental stimuli are crucial for survival. Such stimulus-driven behaviors require knowledge of both what to expect and when, and many high-level brain areas have been shown to report this information. Neurons in the striatum (Hikosaka et al., 1989; Apicella et al., 1992; Shidara et al., 1998; Tremblay et al., 1998), orbitofrontal cortex (Schoenbaum et al., 1998; Tremblay and Schultz, 1999; Hikosaka and Watanabe, 2000), and amygdala (Schoenbaum et al., 1998) have been found to express temporal predictions about outcomes, while dorsolateral premotor cortex (Okano and Tanji, 1987; Romo and Schultz, 1987; Kurata and Wise, 1988), prefrontal cortex (Watanabe, 1996), and distinct regions of the striatum (Schultz and Romo, 1988) have been implicated in translating this temporal information into action. Sensory regions like primary visual cortex (V1)—the earliest stage of cortical visual processing—are typically regarded as contributing only to the first phase of such behaviors: perception (Hubel and Wiesel, 1962, 1965). Recent work suggests, however, that experience-dependent plasticity in V1 can also give rise to information about when to expect an outcome (Shuler and Bear, 2006; Sharma et al., 2015). It has even been shown that such sustained modulations in firing rate in V1 may be involved in visually-timed behaviors (Namboodiri et al., 2015).

Oscillations in V1 local field potentials (LFPs) have also generally been interpreted as relaying perceptual information. One of the key roles for oscillations, particularly in the gamma range, may be to enhance binding of visual features to create a complete visual percept (Eckhorn et al., 1988, 1990). Another crucial function of oscillations is to facilitate anticipation of upcoming

stimuli, though this type of predictive information is often reported as lasting on the order of only tens or hundreds of milliseconds (Engel et al., 2001; Arnal and Giraud, 2012; Gavornik and Bear, 2014). But recent observations have also pointed to a role for oscillations in stimulus prediction on the order of seconds (Lima et al., 2011; Sharma et al., 2015)—the temporal range that is crucial in most cognitive tasks. Moreover, it has been found that theta oscillations in the LFP of well-trained rodents predict the expected delay to reward (Zold and Hussain Shuler, 2015). While it is of interest that this LFP signal expresses temporal information, it is not known how it relates to interval timing activity expressed by V1 neurons, nor to the performance of interval timing behavior.

To address this, we analyzed data from a task (Namboodiri et al., 2015) in which rodents with chronic electrode implants in V1 execute a timed action in response to a visual cue in order to achieve reward. Surprisingly, we found that these visual cues evoked theta oscillations in V1, whose presence corresponded to improvement in timing accuracy and precision in the task. Further, the degree of this improvement was largest when the spatial extent of these oscillations was greatest. Importantly, we found that the duration of these oscillations covaried with the time of action on a per-trial basis, and that this relationship evolved with training. This theta oscillatory activity in the LFP was also found to be intimately related to the activity of single units, which were observed to spike at the frequency of the LFP oscillation and were themselves found to carry predictive information about the timing of the action. Interestingly, the likelihood of evoking these oscillatory states was found to depend on the rate of experienced reward, thus linking them to motivation and the balance between exploration and exploitation. Thus, these findings further our understanding of sensory cortex's involvement during stimulus-driven

behaviors, provide evidence for theoretical accounts of timing which implicate neuronal oscillators (Miall, 1989; Church and Broadbent, 1990; Buhusi and Meck, 2005), and extend our knowledge of the role for theta oscillations.

Materials and Methods

Behavioral task and neural recordings

Experimental procedures were as previously described (Namboodiri et al., 2015). Briefly, water-deprived, adult, male, wild-type, Long-Evans rats were trained to perform a visually-cued timing task, in which they entered a nosepoke, waited a random delay without licking, received a 100 ms full-field, monocular visual stimulus, executed a lick, and obtained a water reward (on 5/6 visually-cued trials). The amount of reward available upon licking post-stimulus increased linearly up to 1.5 seconds, after which no reward was available (Figure 1a). After animals were sufficiently trained (average wait times exceeded one second for three consecutive days), they were stereotaxically implanted bilaterally with 2x8 electrode arrays (2.5mm length; 0.5mm width) targeted to the binocular zone of primary visual cortex (V1) (1.5 mm anterior and 4.2 mm lateral from lambda, at a depth of 1.0 mm). Following recovery and water deprivation, animals performed the task while neural recordings were collected, amplified, and filtered by Neurlanyx (Bozeman, MT) hardware. For a different cohort of animals, referred to here as naïve, implantation occurred prior to training (and the ramp of available reward extended only to 1, instead of 1.5, seconds). All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by The Johns Hopkins University

Institutional Animal Care and Use Committee. Six spatially-distant electrodes (3 per hemisphere) were selected for local field potential analysis, to reduce redundancy in the signals.

Local field potential processing

Neural signals were continuously sampled at 32kHz, downsampled to 2.2kHz, and bandpass filtered (1-400Hz). This filtered LFP signal was then converted to concentrated energy scores by applying the methodology in (Zold and Hussain Shuler, 2015), which was chosen in that study because it provided a better agreement between quantitative analysis of signal duration and visual inspection than using energy alone. Here, concentrated energy is defined as the mean energy divided by the purity. To calculate the mean energy, we first generate a time-frequency representation from the filtered LFP by applying Gabor filters with frequencies from 4 to 9 Hz in .5 Hz steps (standard deviation of Gaussian kernel=.5). The mean of this time-frequency representation across all frequency values for each point in time is defined as the mean energy. Purity, a measure of how concentrated the energy was among particular frequencies, was calculated as:

$$purity = \Sigma f^{2} * e_{norm} - (f * e_{norm})^{2}$$

where f is the frequency values and e_{norm} is the energy at each frequency at every point in time normalized to the total energy at that time. Importantly, to minimize the opportunity for bias, the parameters for this study were taken exactly from the prior study and were not adjusted across sessions or animals.

Oscillation detection and duration

As done previously (Zold and Hussain Shuler, 2015), the concentrated energy scores during a session were used to detect the presence of an oscillation and duration. To categorize trials into oscillatory and non-oscillatory groups, we first created a threshold according to the formula:

$$threshold = (CE_{max}\text{-}CE_{min})/c$$

where CE_{max} and CE_{min} are the maximum and minimum mean concentrated energy scores (taken from a 200-700ms window following a visual stimulus) for any visually-cued trial across the session, respectively, and *c* is a constant equal to 2.5. An oscillation trial is then defined as any trial where the concentrated energy value crosses this threshold at any point in the 200-700ms post-stimulus window. For trials with an oscillation, the duration of the oscillation was the amount of time between when the concentrated energy exceeded this threshold to when it subsequently fell below the threshold.

Oscillation states

In order to establish whether it is appropriate to treat trials as belonging to one of two classes (oscillatory or non-oscillatory), we modeled the post-stimulus responses across trials. To do this, we took the mean concentrated energy from a 200-700ms window post-stimulus on each trial and attempted to find a good fit to this distribution. We started with the most straightforward hypothesis that the concentrated energy values across trials arose from a Gaussian proces

, $\mathcal{N}(\mu, \sigma)$which would result in a unimodal distribution. This was tested against a mixed model in which two Gaussian processes are linearly combined, $p*\mathcal{N}(\mu 1, \sigma 1) + (1-p)*\mathcal{N}(\mu 2, \sigma 2)$, which would result in a bimodal distribution. To compare these "1-gaussian" and "2-gaussian" models, we calculated the Akaike information criterion (AICc) values for each. The AIC takes into account the likelihood (derived from maximum likelihood estimation) and also the model complexity, such that models with more parameters are penalized. In this case, the 2-gaussian model has 5 parameters whereas the 1-gaussian model has only 2 parameters. AICc is a correction for small samples and is calculated as $AICc = AIC + 2k(k+1)/(n\text{-}k\text{-}1)$. The difference in AICc values (or, more specifically, $\exp((AICc1 - AICc2)/2)$) provides a measure, then, of the relative likelihoods of the models.

Because a unimodal Gaussian model is a simplistic alternative, we also tested against a variety of more plausible models. Specifically, we tried to find the best alternative to the 2-gaussian model among 17 continuous distributions implemented in a custom MATLAB script by Mike Sheppard (and includes, among others, the following distributions: Beta, Exponential, Gamma, Generalized extreme value, Inverse Gaussian, Logistic, Log-logistic, Lognormal, Normal, Rayleigh, and Weibull). Of these, ten provided reasonable fits to the data in less than 30% of cases and, thus, were excluded from the data. Of the remaining seven candidates (which provided reasonable fits in 100% of cases), the Generalized extreme value distribution had the lowest overall AICc value across sessions and, therefore, was chosen as the best alternative to the 2-gaussian model. Unlike the unimodal Gaussian model, this model has skew and, thus, can fit the distribution of concentrated energies across trials better.

Sheppard, Mike (2012). Fit all valid parametric probability distributions to data, MATLAB Central File Exchange. Retrieved November 17, 2015.

Visually-evoked potential correlation

The acute response to the visual stimulus, termed the visually-evoked potential (VEP), is defined here as the voltage modulation in the local field potential during the first 200ms post-stimulus. To assess whether the correlation between the timed lick and oscillation can be explained by an earlier physiological event, we assessed whether the magnitude of the visually evoked potential (that is, the absolute difference between the peak and the trough in the voltage trace during this 200ms period) might be predictive of wait time. Specifically, we calculated the percent of variance explained by a single variable (either oscillation duration or VEP amplitude) compared to a linear regression with both variables, across all sessions and channels.

Spike-LFP phase locking

Spiking data was manually sorted using Offline Sorter software from Plexon (Dallas, TX). Finding the phase of the oscillation at which these spikes occurred required converting the filtered LFP signal into a phase position at each time point. This was achieved, as previously described (24, 54, 55), by decomposing the signal with a discrete, Meyer-type wavelet transform into its 3.9 to 7.9Hz components, applying a Hilbert transform on the reconstituted signal, and computing the angle of this result, z, with the following equation: $angle(z) = imag(\log(z))$.

Rayleigh's test for circular uniform distributions was then used to determine whether the phase angles at which the spikes occurred was isotropic.

Spike train analysis

In order to compare the degree of rhythmic activity on oscillatory and non-oscillatory trials, we created the Autocorrelation Difference Index (ADI). The ADI is the difference in the autocorrelation scores on oscillation and non-oscillation trials, which are defined as the sum of the sample autocorrelation function from 100 to 300ms (which encompasses the range of the oscillatory periods) derived from the peristimulus time histogram (PSTH). Note that this range is distinct from the 200-700ms range to determine the energy of the oscillation, which is a fixed window. This 100 to 300 ms range is not a fixed observation window, but rather a span over which the autocorrelation function is evaluated.

To separate trials based on their spike trains alone, we assessed whether the autocorrelation score defined above increased or decreased as each trial was removed from a session's overall PSTH. If removing a trial decreased the overall autocorrelation, it was considered an oscillatory trial and vice versa. For the ensemble analysis, each neuron in the ensemble (that is, the group of neurons recorded simultaneously during a session) was given a vote based on the aforementioned criterion, and the majority vote determined whether a particular trial was labeled as oscillatory.

Oscillation prevalence modeling

As stated, a 200-700ms window post-stimulus was used to determine whether an oscillation was present on each trial. To dissociate the contributions of various behavioral rates (reward, trial, and photic) to the likelihood of evoking an oscillation, we systematically swept through a parameter space of integration filters that incorporated past behavioral statistics. For completeness, we used both a uniform and exponential distribution as filters. The distribution of means tested for each filter type were identical, and were 2^x seconds, where x took on all integer values from 0 to 11, inclusive. The differentiability between the rates computed for all these parameters on oscillation and non-oscillation trials was measured on each session using the receiver operating characteristic (ROC). The mean ROC for a particular filter, mean parameter, and rate type was the average ROC value computed in this way across sessions and channels. We define the maximal mean ROC as the highest mean ROC for a given filter type (across all mean parameters and rate types).

Assessing the acute effect of licking

We examined the possibility that the lick itself could affect an ongoing oscillation, thereby artificially creating a distinction between oscillatory and non-oscillatory states. Three analysis were brought to bear on this question. First, we asked whether licking acutely suppresses an oscillation. To address this, we calculated the average difference in concentrated energy between a 50ms window before and after a lick and compared it to the null distribution of concentrated energy differences obtained by repeatedly (n = 1000) shuffling the relationship between the wait times and trial number. Second, we investigated whether there was a phase relationship between licking and oscillations, in a manner similar to that described above in *Spike-LFP Phase Locking*, but for licks. Third, we asked whether there was a discernable

difference in oscillatory power even prior to licking. To address this, we calculated the distribution of concentrated energy scores in 50ms windows prior to the first lick on a given trial for oscillation and non-oscillation trials separately.

Experimental Design and Statistical Analysis

All of the above analyses were performed using MATLAB_R2015b. Experimental procedures were as previously described (Namboodiri et al., 2015) and were performed with eight wild-type adult male Long-Evans rats. In total, 150 experimental sessions, each consisting of 360 trials, were run (69 trained, 81 naive). Statistical tests and results are as reported in the Results section.

Results

Oscillatory states appear in V1 during a visually-cued timing task

Eight wildtype rats were trained on a timing task (Namboodiri et al., 2015). In this task, the animal enters a nosepoke to initiate a trial, waits a random delay without licking, receives a full-field, monocular visual stimulus (100ms, green LED, delivered through head-mounted goggles), and then licks at a chosen time. The time that the animal chooses to lick post-stimulus determines the amount of reward it obtains on a given trial. Specifically, the amount of water reward available rises linearly with time up until 1.5 seconds, at which point it drops to and

remains at zero (Figure 1a). In this way, animals are encouraged to time their licks from the visual stimulus so that they fall near, but not past, the peak of the reward ramp.

Animals trained in this task exhibit cue-evoked theta oscillations in the local field potential recordings from the primary visual cortex. This theta oscillation can be seen in the average voltage trace across trials of a session when aligned to stimulus onset, as in Figure 1b. In this example, the average voltage trace exhibits appreciable oscillatory strength for about one second following visual stimulation. Separating the responses per trial (Figure 1c) reveals differences in the presence, amplitude, and duration, of theta oscillations across trials (Figure 1c, inset).

In order to quantify these across-trial differences in the presence, amplitude, and duration of oscillations, we transform this raw voltage signal into a metric of oscillation strength. We focus our analysis within a 4 to 9 Hz frequency range as the preponderance of the signal power falls within this range (Figure 1d). Using this range, we generate a "concentrated energy" score—a measure of the power and purity of the oscillation (methods)—for every time point within each trial, as done previously (Zold and Hussain Shuler, 2015) (Figure 1e). (Note that, unlike for the raw voltage signal in Figure 1c, the concentrated energy scores rise before stimulus onset due to the blurring in time that occurs when translating to a time-frequency representation). Qualitatively, trials with large oscillations in voltage (as in Figure 1c) have high concentrated energy scores (as in Figure 1e). Using these concentrated energy scores, we can investigate how the oscillation strength— defined as the mean concentrated energy within a 200-700ms time window—varies across trials. By inspection, the probability density function (Figure 1f) of the oscillatory strength (Methods) is well described for this session by a bimodal fit (bottom), but not

297 a unimodal fit (top), suggesting that there are distinct oscillatory states across trials. Therefore,
298 we compared the quality of each fit by calculating the difference in the Akaike information
299 criterion (AIC) scores (Methods). For this example, the ΔAIC is large and negative (~-99.76)
300 which indicates that the bimodal model is heavily favored over the unimodal model. Applying
301 this process across all sessions, we found that the bimodal model is overwhelmingly preferred
302 ($p=6.87e-66$, $W_{414}=1186$, $z=-17.14$; Figure 1g, histogram), for a variety of metrics (including the
303 median concentrated energy (ΔAIC=-27.78), mean concentrated energy in a later window from
304 .5-1s (ΔAIC=-49.33), and using raw energy scores (ΔAIC=-120.99)) and when compared to a
305 number of alternative models ($p=4.20e-33$, $W_{414}=13753$, $z=-11.99$ for best alternative,
306 Generalized extreme value distribution; Materials and Methods). Given that trials appear to have
307 either a high or low-power oscillation, a threshold (Figure 1h) for sorting trials into "oscillation"
308 and "non-oscillation" trials was lawfully applied (Zold and Hussain Shuler, 2015). Ordering the
309 trials from Figure 1c by the strength of their oscillation makes the difference in oscillatory power
310 across trials visually apparent (Figure 1i). Finally, we define an oscillation's duration as the
311 interval between the first moment post-cue that the concentrated energy score surpasses this
312 threshold for detection and the first moment it falls below it.
313
314 **Lick timing precision and accuracy improve during theta oscillation states**
315
316 Having defined cue-evoked oscillations and their duration, we next addressed whether across-
317 trial differences in the performance of the visually-cued timing behavior tracks changes in the
318 oscillatory state. To visualize whether performance is related to the presence/absence of cue-
319 evoked theta oscillations, we plot, per trial, the time of the first lick post-stimulus (the behavioral

variable relevant for reward acquisition) atop the concentrated energy values (see Figure 2a, top for an example session). Viewed in this way, it is apparent that there is considerable variability in the time of the first lick (white squares), but challenging to see what, if any, relationship there is between concentrated energy and the delay to the first lick (the "wait time"). However, sorting trials by the strength of the oscillation (Figure 2a, bottom) reveals that there is considerably greater precision in time to initiate licking on trials with higher oscillatory power. To quantify this difference, we compared the temporal distribution of wait times (under five seconds post-stimulus (>95%) to avoid outliers) on oscillation and non-oscillation trials (Figure 2b; threshold shown by black dotted line). Wait times on oscillation trials tend to be more tightly packed (purple line) than on non-oscillation trials (green line). Indeed, this tends to be the case across all sessions recorded on this channel (Figure 2c; p= 4.05e-11, W_{66}= 2139, z= 6.60, two-tailed Wilcoxon signed rank test against median=0) and all channels (p=4.8e-54, W_{408}=78618, z=15.48). Moreover, the difference in variability across sessions from this channel tends to be more pronounced in well-trained animals (i.e. rats performing at least three consecutive sessions with a median wait time of one second or greater), compared to naïve, on this channel (methods; p= 0.01, U=5274, z= 2.43, n_1=66, n_2=75, two-sided Mann-Whitney U-test) and across all channels (Figure 2d; p= 1.4062e-15, U=205955, z=7.98, n_1=408, n_2=457). Since this increased variability on non-oscillation trials predominantly comes from a higher fraction of early licks, the central tendency of the wait times across sessions is significantly lower on non-oscillation trials (median of ~1006ms) than oscillation trials (median of ~1103ms) (p=1.51e-14, U=193710, z=7.69, n_1=409, n_2=410, two-sided Mann-Whitney U-test). This means that, on average, licks on oscillation trials occur farther along the ramp, where more water is available and, thus, are more

342 accurate. Therefore, the precision and accuracy of timed licks are considerably higher on trials
343 with strong oscillations.
344
345 Since the presence of an oscillation detected at a given electrode covaries with behavioral
346 improvements, we hypothesized that there would be larger behavioral improvements during trials
347 with more spatially widespread oscillations in V1. Because we analyzed LFP recordings from
348 six channels (3 per hemisphere) per session, we can assess how the timed lick behavior varies
349 with the number of electrodes reporting an oscillation on a given trial. Variability systematically
350 decreases (Figure 2e, top; p=8.27e-05, slope=-1.91e+04, r=.98) and the central tendency
351 systematically increases (p=0.020, slope=23.78ms, r= 0.83) as the number of electrodes
352 reporting oscillations grows. These effects translate into a systematic increase in the amount of
353 water obtained per trial (Figure 2e, bottom; p=4.9e-04, slope=3.43, r=0.96). Thus, the greater
354 the spatial extent of cue-evoked oscillations within V1, the greater the precision and accuracy of
355 timed reward-seeking actions, and the greater the obtained reward.
356
357 These observations suggest that cue-evoked theta oscillatory states observed in V1 may be
358 effectors of timed behavior, but this relationship might arise from other sources. Because there
359 is a random delay period between nosepoke entry and visual stimulus onset, this higher
360 variability in lick precision on non-oscillation trials might arise from higher variability in time
361 waited prior to the stimulus (pre-stimulus wait time). Countering this hypothesis, we find 1) that
362 the difference in lick variability between oscillation and non-oscillation trials is considerably
363 higher than the difference in pre-stimulus wait time variability (Figure 3a; p=3.94e-30, z=11.4,
364 U=201251, n_1=414, n_2=414, two-sided Mann-Whitney U-test), and that 2) the lick variability is

consistently higher on non-oscillation trials when holding the time waited since nosepoke entry constant (Figure 3c). The same is true when controlling for inter-trial interval duration and trial number within session (Figures 3d and e respectively), indicating that these variables do not account for differences in timed licking. While the distribution of oscillation strength scores from a given electrode are best described by a bimodal fit, oscillation and non-oscillation classified trials do not form fully separable distributions within a session. The distribution for oscillation strength scores for non-oscillation and oscillation classified trials are given in Figure 3b, showing the degree of overlap when collapsing across all recordings. Given this overlap, it is not surprising that the median difference in lick variance between non-oscillation and oscillation trials across sessions consistently increases as trials with more extreme strength scores are selected (p-.0021, r=.9625).

Timed reward-seeking action tracks oscillation duration on a per-trial basis

Given these differences in behavior with respect to the presence and spatial extent of cue-evoked theta oscillations within V1, we next assessed whether the duration of these oscillations is directly related to the timing of the reward-seeking action (lick initiation). Figure 4a shows the first lick time (wait time) per trial (pink squares) plotted over the concentrated energy values for an example session, sorted by oscillation duration. Lick initiation tends to follow the edge of the oscillations' termination (black circles). By transforming this data into a scatter plot (Figure 4b), it appears that there is a positive relationship between wait time and oscillation duration (slope=.236, p=1.49e-04, r=.240). Indeed, across all sessions from this electrode, the distribution of regression slopes is significantly right-shifted (Figure 4c, histogram; p=3.29e-05, W_{69}=1902,

388 z=4.15, two-tailed Wilcoxon signed rank test against median=0), meaning that there tends to be a
389 positive linear relationship between wait time and oscillation duration. This relationship holds
390 across all sessions and channels (Figure 4d, blue line; p=3.43e-25, W_{414}=65641, z=10.28), and is
391 more pronounced in well-trained compared to naïve animals (Figure 4d, blue vs red line;
392 p=3.72e-10, U=196120, z=6.27, n_1=414, n_2=486; two-sided Mann-Whitney U-test). The same is
393 also true when collapsing across channels per session (p=.0029, U=5999, z=2.98, n_1=69, n_2=81),
394 and using a variety of other metrics/filters (using unrewarded trials only (p=6.61e-08, U=174568,
395 z=5.40, n_1=414, n_2=486) and using the correlation coefficient instead (p=5.65e-07, U=191575,
396 z=5.00, n_1=414, n_2=486)). Moreover, the mean slope across sessions is significantly higher
397 ($p<<.05$) than the distribution of mean slopes for shuffled wait time data (Figure 4e; black dotted
398 line is actual mean slope). Finally, as described previously (Zold and Hussain Shuler, 2015), the
399 amplitude of the visually evoked potential (VEP) (Figure 5a)—the acute response to the visual
400 stimulus—is also related to the duration of the oscillation, but is a considerably worse predictor
401 of wait time than oscillation duration (Figure 5b).
402
403 Given these observations, we investigated whether the strength of the oscillation influences the
404 relationship between wait time and oscillation duration. Since the oscillation would likely exert
405 less influence over behavior the weaker it is, we hypothesize that the relationship between wait
406 time and oscillation duration would degrade with oscillation strength (as appears to be the case in
407 Figure 4a). Indeed, filtering by trials with the strongest oscillations (that is, taking the x percent
408 strongest oscillations, as defined by the mean concentrated energy in a 200-700ms window post-
409 stimulus, in a given session) yields the strongest correlations (Figure 4f). Note that, while the
410 largest drop occurs from the top 5% to top 10% strongest oscillations (which may be due to non-

linear control over behavior by these strongest oscillations or to relatively low statistical power inherent in selecting a small sub-group), there is a consistent downward trend. Coupled with the observations above, this indicates that the duration of cue-evoked oscillations relates to the timing of reward-seeking actions.

Cue-evoked single unit oscillations are predictive of timing performance

Having observed this timing-related activity at the level of the local field potential, we sought to investigate the response patterns of single neurons recorded during this task. An example response is shown in Figure 6a. The spike raster (top) and peristimulus time histogram (bottom) across the whole session (i.e. all trials with a stimulus) suggest that this neuron primarily responds acutely to the visual stimulus (presented at time zero). However, separating each trial by whether an oscillation was detected in the local field potential (for a given electrode within the same hemisphere) reveals that there are, in fact, quite different response patterns during oscillation and non-oscillation trials (Figure 6b and c). In particular, there is a long-duration oscillatory firing pattern on the oscillation trials, whereas there is predominantly an acute stimulus response on non-oscillation trials. Indeed, many neurons (~66%) show a significant difference in their responses on oscillation and non-oscillation trials (Figure 7a; methods). This difference is quantified as the Autocorrelation Difference Index (ADI; methods) (Figure 7b), for which positive scores indicate more oscillatory spiking activity on LFP-identified oscillation trials. The ADI for this example neuron is ~1.46, and the distribution of ADI's across all neurons is positively-shifted (Figure 7c, histogram; p =5.27e-34, W_{263}=32152, z=12.16, two-tailed Wilcoxon signed rank test against median=0).

Given this rhythmic discharge pattern, we characterized how oscillatory single unit activity was synchronized with the local field potential signal. To assess this, we converted the local field potential voltage into a phase angle at every point in time and asked how well the spikes aligned to a particular phase of the signal (methods). For this example, the spikes (white squares) appear to be concentrated before the peak of the oscillatory envelope (Figure 7d; Figure 7e, left; p=1.50e-88, z=182.33, Rayleigh's test for non-uniformity). Indeed, the spikes from most neurons across the population cluster around this phase (Figure 7e, right), indicating that these single units tend to be part of ensembles of neurons which are locked with one another.

Given that the LFP oscillations are related to timing behavior and that single unit activity is related to the LFP signal, we next assessed whether, and in what way, single unit oscillatory activity could be related to timing behavior. We addressed this issue by restricting our analysis to the spiking activity for each recorded neuron, setting the categorization of trials on the basis of the LFP aside. For each neuron in a recording session, we categorized each trial as oscillatory or non-oscillatory on the basis of its spike train (methods), and then quantified the difference in first lick variance between these categories. As with categorizing trials on the basis of oscillations detected in the LFP, we found that sessions tended to have higher lick variance on non-oscillatory trials, which in this case corresponds to leftward-shifted scores (Figure 8a, blue line; p=6.49e-05, W_{257}=11812, z=-3.99). Further, given that neurons tended to be phase-locked to a particular phase of the LFP theta oscillation, we assessed whether aggregating evidence from multiple spike trains recorded simultaneously might boost the signal, improve classification, and consequently accentuate these behavioral differences. Indeed, by categorizing a trial based on

the activity of multiple units, we found an even greater average difference in lick variance on oscillation and non-oscillation trials (Figure 8b, red line; p=4.06e-05, W_{63}=416, z=-4.05). In addition, the performance of timed reward-seeking behavior on oscillating trials improves with the size of the ensemble, as indexed by an increase in the difference of lick variance between oscillation and non-oscillation trials (Figure 8b, sessions in gray, session averages in pink; p=.03, slope=-276, r=.28)

Oscillation prevalence covaries with reward rate

Since timing is more precise and accurate during oscillatory states in V1, we wondered what behavioral variable(s) might influence the likelihood of observing an oscillation on a given trial. To assess this, we created a logistic regression model with several candidate explanatory variables, in which the dependent variable was the fraction of channels detecting an oscillation (out of six). Of the variables tested, the inter-trial interval (that is, the time from nosepoke exit to subsequent trial initiation) was consistently the most informative (i.e. the distribution of its t-statistic across sessions was shifted farthest from zero) (Figure 9a). Because the regression statistics can be influenced by extreme values, we probed this relationship further by plotting the likelihood of oscillation with respect to inter-trial interval alone (Figure 9b). It can be seen from this plot that longer inter-trial intervals decrease the probability of evoking an oscillation. Such a relationship may arise if the cortical state was tracking some behavioral rate, such as the trial rate, photic rate (i.e. the rate of visual stimulation), or reward rate experienced by the animal. Therefore, we sought to dissociate these possibilities. Specifically, we compared the receiver operating characteristic (ROC) values—a measure of the discriminability between two

480 distributions, in this case the rates on oscillation vs non-oscillation trials—across all sessions.
481 For the filter parameter (which sets the integration dynamics for calculating the behavioral rates)
482 associated with the maximal mean ROC (methods), all three variables are good predictors of
483 oscillation likelihood, but the experienced reward rate is the best predictor of the three (Figure
484 9c). In fact, the reward rate was consistently the best predictor over the full range of time
485 windows analyzed (that is, the windows over which the rates were calculated) (Figure 9d). This
486 suggests that oscillations are most prevalent during periods of high experienced reward rate in
487 this behavioral timing task.
488

489 **Discussion**

490 Appropriately timing actions in response to sensory stimuli is necessary for survival. Here, we
491 show that oscillatory states evoked by reward-predicting cues in primary visual cortex may
492 contribute to this ability. Specifically, we show that there is an enhancement of precision and
493 accuracy of timed reward-seeking responses following a visual cue when that cue evokes theta
494 oscillations in V1. The more widespread this theta oscillation across V1, the greater the
495 improvement in timing performance. An appealing hypothesis to explain the difference in timed
496 lick behavior between oscillatory and non-oscillatory states is that an ongoing oscillation in V1
497 exerts an influence on the animal's decision to lick (perhaps via a downstream motor region) by
498 suppressing licking throughout its duration. Under this hypothesis, we would expect the time of
499 the first lick to covary with the duration of the oscillation. Indeed, this relationship was stronger
500 for well-trained compared to naïve animals, suggesting that the association between the
501 oscillatory state and the timed behavior is learned. Furthermore, we found evidence for this
502 oscillatory state in the spiking data of simultaneously recorded neurons. These oscillatory firing

503 signals are related to enhanced timing precision, apparently acting in concert to boost the
504 predictive signal. Together, these data suggest that there is a distinct oscillatory state in primary
505 visual cortex that is related to the performance of visually-timed actions.
506
507 An alternative to this interpretation is that lick initiation itself shuts down ongoing oscillations.
508 If this were the case, non-oscillation trials would appear to have earlier (and perhaps more
509 variable) wait times, as a lick during the scoring window would increase the likelihood of being
510 categorized as a non-oscillation trial. This explanation is not satisfactory for a number of reasons,
511 however. First, a prior study (Zold and Hussain Shuler, 2015) in which animals could lick freely
512 post-stimulus did not detect a suppression in ongoing oscillatory power. In line with this
513 observation, we find that the first lick following a visual stimulus does not acutely suppress an
514 ongoing oscillation ($p=.90$, by random shuffling; methods). Second, as shown previously (Zold
515 and Hussain Shuler, 2015), we did not find any phase relationship between licking and
516 oscillations, suggesting that the oscillation was not being driven by motor output ($p>.05$,
517 Rayleigh's test for non-uniformity; methods). Third, we found that the distribution of oscillation
518 strengths is already much lower for non-oscillation than oscillation trials prior to a lick (Figure
519 3b; $p<.001$, $U=2.89e09$, $z=-261.86$, $n_1=59466$, $n_2=143514$, two-sided Mann-Whitney U-test;
520 methods) indicating that these differences exist before the action. In sum, these observations
521 suggest that the timing activity in V1 is not merely a consequence of the behavioral action itself.
522
523 Another interpretation of this data is that the oscillatory state is driven by some non-specific
524 variable like arousal or motivation. While this is plausible, it seems that a) the duration of the
525 oscillation is specifically related to the wait times in the task and b) even when controlling for

variables related to motivation, we still observe wait time differences between oscillation and non-oscillation trials. Specifically, the wait time differences are maintained when controlling for the time waited since nosepoke entry, inter-trial interval duration, and trial number within the session (Figures 3d-f). Together, these results suggest that the theta oscillations in V1 carry timing information that is not explained by broad changes in behavioral state. Still, it is possible that this signal carries information about motivation or arousal (as addressed by the oscillation prevalence analysis and discussion below).

Our findings thus further our understanding of V1's involvement during stimulus-driven behaviors. Traditionally, V1 was thought to contribute only to the first stage of such behaviors: sensation. Along these lines, primary visual cortex has been regarded as a feature detector which relays faithful representations of the visual world to downstream regions. This view has been challenged by recent work suggesting that V1 can actively generate predictions about visual input (Murray et al., 2002; Summerfield et al., 2008; den Ouden et al., 2009; Alink et al., 2010; Kok et al., 2012) and can be influenced by behavioral variables such as attentional states (Ahissar and Hochstein, 1993; Roelfsema et al., 1998; Gandhi et al., 1999; Somers et al., 1999; Fahle, 2004) and reinforcement (Serences, 2008; Seitz et al., 2009; Stănişor et al., 2013) (e.g. water reward). Whereas these findings pertain to influences on perception, our findings provide evidence that V1 relates to the timing of behaviorally-relevant actions. Specifically, we find that following the acute visual response, V1 exhibits long-lasting theta oscillations that subtend the interval between stimulus and action during a timing task. Thus, these oscillations in primary visual cortex may be a signature of V1's involvement beyond perception and into the decision-making phase of a timed, stimulus-driven behavior.

549

550 Nevertheless, it is likely that V1 does not act in isolation. Indeed, several studies have pointed to
551 a top-down influence on intrinsic dynamics and expectancy signals in visual cortex (Engel et al.,
552 2001). Given the breadth of evidence suggesting that timing emerges from interaction across
553 multiple brain regions, it is likely that the contribution from V1 is part of a broader cortico-
554 thalamic-basal ganglia (CTBG) loop (Merchant et al., 2013). In this view, the core CTBG
555 timing circuit, which is engaged across a broad range of behavioral contexts, interacts with a
556 distributed network of local timing circuits which are involved in timing in a task and modality-
557 dependent manner. One influential model of timing in this vein, the Striatal Beat Frequency
558 model, posits that the striatum recognizes an interval of time by detecting that pattern of
559 activation from a bank of cortical oscillators (Matell and Meck, 2004). Besides top-down
560 influence, V1 may also receive bottom-up expectation signals. In this regard, non-primary
561 thalamic neurons have been implicated in reward expectation in a modality-specific manner
562 (Komura et al., 2001). In the future, it would be informative to make specific manipulations of
563 the oscillatory activity in V1 and other regions implicated in timing to observe their influence on
564 each other and their effect on timing behavior.

565

566 These observations also extend our knowledge about the role and behavioral significance of theta
567 oscillations. In the hippocampus, theta oscillations have been implicated in several cognitive
568 functions, including voluntary movement, learning, and memory processes (Hasselmo, 2005).
569 This rhythm is believed to contribute to these processes partly through facilitation of information
570 transfer with prefrontal cortex (Hyman et al., 2005; Siapas et al., 2005). Indeed, oscillatory
571 synchrony is a common mechanism for inter-regional communication which has been shown in a

number of circuits (Fries, 2005), including those involving visual cortex (Roelfsema et al., 1997; Bernasconi et al., 2000; von Stein et al., 2000; Siebenhuhner et al., 2016). In our visuomotor task, this mechanism may allow the output from primary visual cortex to be more effectively read out by a motor region that ultimately initiates the action. Within visual cortex itself, oscillations are often studied from a perceptual perspective and have been found to enhance responding to particular stimuli (Fries et al., 2001, 2002; Schroeder and Lakatos, 2009) and enable feature binding (Eckhorn et al., 1988, 1990). Yet, recent work has found that theta oscillations in V4 cortex may be important for maintenance of information during the delay period of a working memory task (Lee et al., 2005) and that in primary visual cortex LFP oscillations may be related to expectancy of future outcomes (Lima et al., 2011; Zold and Hussain Shuler, 2015). We extend these findings by showing that theta oscillations in V1 are related to the precise timing of visually-cued behaviors. Though theoretical accounts of timing often implicate oscillatory processes in such timed behaviors (Buhusi and Meck, 2005), evidence supporting these theories has been lacking (Kononowicz and Wassenhove, 2016). Finding this kind of signal as the earliest stage of cortical visual processing is particularly surprising and may suggest that such a mechanism is a common feature of local circuits. This view is supported by evidence that disruption of MT/V5 selectively impairs visual, but not auditory, timing (Bueti et al., 2008).

These findings also raise the question of why there are oscillatory and non-oscillatory states in V1, given that one appears superior, behaviorally, over the other. One straightforward possibility is that maintenance of an oscillatory response pattern is energetically taxing and, therefore, must be limited. Another, compatible possibility—given the relationship between

reward rate and oscillation prevalence (Figure 9)—is that animals performing the timing task are seeking to balance knowledge accumulation with reward accumulation (i.e. the exploration vs exploitation trade-off) (Cohen et al., 2007). Under this construction, it may be advantageous for an agent to exploit its knowledge of the environment by tracking a theta oscillation and waiting a precise amount of time when the reward rate is high, but explore the environment otherwise. In support of this hypothesis, a prior study found that experimentally increasing the reward rate increased the likelihood of evoking an oscillation (Zold and Hussain Shuler, 2015). Future studies that precisely manipulate reward rate during a behavioral timing task will help elucidate the role this factor plays in governing cortical state and temporal decision-making.

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Figure Legends

Figure 1: Oscillatory states are present in V1 during a visually-guided timing task. **a**) Schematic of the task reward structure, in which waiting longer to lick following a visual stimulus (time zero) results in a larger volume of water delivery at the lick tube. Maximum delivery occurs at 1.5 seconds, and drops to zero thereafter, so that animals must time their lick. **b**) The average voltage trace in the local field potential (LFP) taken from an electrode in an example session, with a green bar overlaid to indicate when the visual stimulus was on. The voltage values seem to oscillate for ~1 second post-stimulus. **c**) Voltage traces per trial for the example session. **d**) Average time-frequency representation of the trials in **c**. **e**) Concentrated

781 energy through time of the trials in **c**. f) The empirical probability density function (PDF) for the
782 log(mean concentrated energy) scores on each trial shown in **e** are shown in blue. The mean
783 concentrated energy is calculated in a 200-700ms window post-stimulus. A unimodal Gaussian
784 fit is shown in red (top) and a bimodal Gaussian fit is shown in green (bottom). **g**) The
785 distribution of the difference in Akaike Information Criterion (AIC) values for each model across
786 all sessions is left-shifted, indicating an overall preference for the bimodal model. The dotted
787 lines around zero are the bounds at which the relative likelihood of a model compared to another
788 model is 5%. **h**) Sorted concentrated energy scores for the example session with a dotted line
789 indicating the threshold used for determining whether a trial has an oscillation. If the
790 concentrated energy score crosses this threshold during the 200-700ms window post-stimulus, it
791 is considered to have an oscillation. **i**) The raw voltage trace in **c** sorted by the mean
792 concentrated energy in the analysis window on a given trial. Oscillations were detected for trials
793 above the dotted line.
794
795 *Figure 2: Wait time precision is higher during oscillatory states.* **a**) Concentrated energy values
796 with first wait times (white squares) post-stimulus overlaid for each trial of an example session
797 in chronological order (top) and sorted by oscillation duration (bottom). The dashed black line is
798 the threshold for being categorized as oscillatory. **b**) Empirical cumulative density functions for
799 the first lick times (wait times) post-stimulus on oscillation (black) and non-oscillation (green)
800 trials in **a**. **c**) Histogram of the difference in lick variability on oscillation and non-oscillation
801 trials for each session recorded on a given electrode. **d**) Differences in wait time variability on
802 oscillatory and non-oscillatory trials for all sessions and channels of trained (blue) and naïve
803 (red) animals. **e**) (top) Lick variability decreases as the number of electrodes on which an

804 oscillation was detected increases for a given trial. Standard error bars shown in black, with
805 regression line in red. (bottom) The percent of water obtained over baseline (defined as trials in
806 which no oscillations were detected on any electrodes) increases as the number of electrodes
807 showing an oscillation increases. Standard error bars shown in black, with regression line in red.
808
809 *Figure 3: Trial and session statistics do not account for differences in lick precision between*
810 *oscillatory and non-oscillatory trials.* **a**) Differences in wait time variability (blue) are
811 considerably larger than differences in stimulus onset time (from nose-poke entry) variability
812 (red) on trials in which there was no licking pre-stimulus. **b**) The concentrated energy scores
813 taken from a 50ms window prior to the first lick on oscillation (blue) and non-oscillation
814 (yellow) trials from all sessions and channels. **c**) Differences in lick variability between
815 oscillatory and non-oscillatory trials for trials within a given range of times to stimulus onset
816 (from nose-poke entry) from 0 to 2.5s in 100ms steps, collapsed across all sessions and channels.
817 **d**) Differences in lick variability between oscillatory and non-oscillatory trials for trials within a
818 given range of inter-trial intervals from 0 to 10s in 100ms steps, collapsed across all sessions and
819 channels. **e**) Differences in lick variability between oscillatory and non-oscillatory trials for a
820 given trial number in a session, collapsed across all sessions and channels.
821
822 *Figure 4: Wait time correlates with oscillation duration in trained animals.* **a**) Concentrated
823 energy values with first lick times (wait times) overlaid (pink squares) on trials sorted by
824 oscillation duration. **b**) Scatter plot showing the relationship between oscillation duration and
825 wait times for the trials in **a** with a regression line shown in orange. **c**) The distribution of the
826 slopes of regression for each session recorded on a given channel. **d**) The empirical cumulative

distribution of the slopes of regression for all sessions and channels from naïve (red) and trained (blue) animals. **e**) The null distribution of slopes for the sessions taken from the trained cohort, calculated by randomly shuffling the relationship between the wait time and oscillation duration 1000 times. The actual mean slope across session is shown by the black dotted line. **f**) The slope of regression decreases as the percentage of trials with the strongest oscillations is systematically increased. To do this systematic sweep, we sorted trials recorded on a given session/electrode by their mean concentrated energy and took the top x percent of trials. Therefore, the x-axis ranges from 5% (in which only the trials in the top 5% of oscillation strength are included) to 100% (in which all trials are included).

Figure 5: Wait time correlates with oscillation duration across a wide range of metrics and parameters. **a**) Local field potential trace from a single trial with a 250ms gray bar overlaid to highlight the visually evoked potential [VEP]. **b)** The percent of variance explained by a regression of wait time against oscillation duration (brown) or VEP amplitude (green) relative to a model containing both variables.

Figure 6: Neural oscillations occur during LFP oscillations. ***a)*** Spike rasters (top) for an example neuron on all trials, **b)** oscillation trials, and **c**) non-oscillation trials of a session. The peristimulus time histogram for each group is shown below.

Figure 7: Neurons spike at a consistent phase of the oscillations in the local field potential. **a**) P-values for the null hypothesis that there is no difference in spike distributions between trials with and without an oscillation in the local field potential for each neuron. The dotted red line

indicates where p=.05. **b**) The Autocorrelation Difference Index (which is a measure of the difference in the level of autocorrelation between spike-separated oscillation and non-oscillation trials) is considerably higher in neurons for which the null hypothesis stated in **a** is rejected (blue) than in those for which it is not (red). **c**) Heat maps showing the filtered local field potential (top) and phase angle (bottom) on LFP oscillation trials, with spikes from the example neuron in Figure 7 overlaid (white squares). **d**) The distribution of the Autocorrelation Difference Index across all neurons is right-shifted, indicating that the spike train autocorrelation is higher on LFP oscillation trials than non-oscillation trials. **e**) Polar plots indicating the distribution of LFP oscillation phase angles at which spikes occur for the example neuron (left) and the mean phase angle for each neuron in the population (right).

Figure 8: Neural oscillations are predictive of timing performance. **a**) Empirical cumulative distribution functions for the difference in lick variance on spike-separated oscillation and non-oscillation trials (var[osc]-var[non-osc]) for individual neurons (blue) and neural ensembles (red). **b**) Relationship between neural ensemble size and difference in lick variance on each session (gray dots), shown with a regression line (dotted black line), and session means per ensemble size (pink dots).

Figure 9: Oscillation prevalence is related to experienced reward rate. **a**) Distributions of t-statistics across sessions for several variables in a logistic regression model in which the dependent variable is the fraction of electrodes displaying an oscillation on a given trial (out of six). Of the variables considered here, the distribution of t-statistics for the inter-trial interval (red line)—the time between exit on the previous trial to subsequent trial initiation—is the

farthest shifted from zero. **b**) Relationship between the probability of oscillation and the inter-trial interval (exit to poke time). Probabilities are calculated by taking the number of oscillations divided by the total number of observations (i.e. all analyzed channels and trials) falling within a range of inter-trial intervals 500ms wide, sweeping from .5s to 30s. **c**) Empirical cumulative distribution functions (CDFs) for the receiver operating characteristic (ROC) values, across sessions, associated with the difference in various behavioral rates (reward, trial, and photic) between oscillation and non-oscillation trials. These CDFs correspond to the exponential filter (used to calculate the rates) yielding the maximal mean ROC (methods). **d**) The mean ROC values for each rate variable across sessions, for each exponential filter size tested. Daggers denote where the mean ROC value associated with reward rate is significantly different from that associated with trial rate.