Brain state and cortical layer-specific mechanisms underlying perception at threshold

Mitchell P. Morton[1,2], Sachira Denagamage[1,2], Isabel J. Blume[1], John H. Reynolds[4], Monika P. Jadi[1,2,3] and Anirvan S. Nandy[1,2]
[1]Department of Neuroscience, Yale University, New Haven, CT 06511
[2]Interdepartmental Neuroscience Program, Yale University, New Haven, CT 06511
[3]Department of Psychiatry, Yale University, New Haven, CT 06511
[4]Systems Neurobiology Laboratories, The Salk Institute for Biological Studies, La Jolla, CA 92037
Correspondence: anirvan.nandy@yale.edu

ABSTRACT

Identical stimuli can be perceived or go unnoticed across successive presentations, producing divergent behavioral outcomes despite similarities in sensory input. We sought to understand how fluctuations in behavioral state and in cortical layer and cell-class specific neural activity underlie this perceptual variability. We analyzed physiological measurements of state and laminar electrophysiological activity in visual area V4 during a cued attention task. We find that hit trials are characterized by a larger pupil diameter and lower incidence of microsaccades, indicative of a behavioral state with increased arousal and improved visual sensitivity due to stable retinal images. Target stimuli presented at perceptual threshold evoke elevated multi-unit activity in V4 neurons in hit trials compared to miss trials, across all cortical layers. Putative excitatory and inhibitory neurons are strongly positively modulated in the input (IV) and deep (V & VI) layers of the cortex during hit trials. Excitatory neurons in the superficial cortical layers exhibit lower variability in hit trials. Deep layer neurons are less phase-locked to low frequency rhythms in hits. Hits are also characterized by greater interlaminar coherence between the superficial and deep layers in the pre-stimulus period, and a complementary pattern between the input layer and both the superficial and deep layers in the stimulus-evoked period. Taken together, these results suggest that a state of elevated arousal and stable

retinal images allow enhanced processing of sensory stimuli, which contributes to hits at perceptual threshold.

INTRODUCTION

Physical properties of stimuli strongly influence perception. Low intensity stimuli are detected infrequently. As intensity increases, detection probability remains low until some perceptual threshold is crossed, after which stimuli are perceived robustly. A psychometric function (Prins and Kingdom, 2018; Watson, 1979; Wichmann and Hill, 2001) mathematically describes this property of perception. Only within a narrow range around the perceptual threshold do stimuli lead to significant trial-to-trial perceptual variance. While many studies present stimuli at threshold (Herman et al., 2017; Levitt, 1971; Pins and ffytche, 2003; Ress and Heeger, 2003), few have probed the laminar cortical microcircuit mechanisms that underlie successful or unsuccessful perception under these conditions (McCormick et al., 2020; van Vugt et al., 2018).

Prior studies have characterized how perceived stimuli trigger stronger information propagation from earlier visual areas to higher order visual and frontal regions (Herman et al., 2017; van Vugt et al., 2018). Information propagation and sensory processing are strongly influenced by brain states such as arousal and attention (Harris and Thiele, 2011; McCormick et al., 2020). Arousal has long been known to modulate cortical activity (Livingstone and Hubel, 1981; McCormick and Bal, 1997; Vinck et al., 2015) and impact behavioral performance on a variety of sensory tasks (Aston-Jones and Cohen, 2005; McGinley et al., 2015; Yerkes and Dodson, 1908). Activity in visual area V4, a critical intermediate region in the ventral visual processing stream (Goodale and Milner, 1992; Mountcastle, 1997; Roe et al., 2012) is known to be strongly modulated by attention (Desimone and Duncan, 1995; McAdams and Maunsell, 1999; Moran and Desimone, 1985; Reynolds et al., 2000). Attention enhances the firing rates of V4 neurons, increases

54 the reliability in firing of individual neurons, and decreases correlated fluctuations among
55 pairs of neurons (Cohen and Maunsell, 2009; McAdams and Maunsell, 1999; Mitchell et
56 al., 2007, 2009). Brain state dynamics influence both cortical and subcortical structures
57 while contributing to behavior (Ghosh and Maunsell, 2021; Zénon and Krauzlis, 2012).
58 On and off state dynamics in V4 ensembles have been hypothesized to reflect fluctuations
59 in attention and have been shown to correlate with behavioral performance (Engel et al.,
60 2016; van Kempen et al., 2021).

61 The visual cortex has a columnar architecture in which multiple cell classes
62 (Connors and Gutnick, 1990; Markram et al., 2004; Migliore and Shepherd, 2005;
63 Wonders and Anderson, 2006; Zeng and Sanes, 2017) across the cortical layers
64 (Douglas and Martin, 2004; Mountcastle, 1997) form distinct sub-populations. These sub-
65 populations have unique and stereotyped patterns of connectivity, thus forming a
66 canonical microcircuit that orchestrates the encoding and flow of information (Douglas
67 and Martin, 2007; Hirsch and Martinez, 2006). Moreover, these sub-populations
68 contribute uniquely to sensory processing and are differentially modulated by brain states
69 (McCormick et al., 1985; Mitchell et al., 2007; Nandy et al., 2017; Pettine et al., 2019).
70 While it has been shown that attentional modulation varies across cortical layers (Buffalo
71 et al., 2011; Ferro et al., 2021; Mehta et al., 2000a, b; Nandy et al., 2017; Pettine et al.,
72 2019; Westerberg et al., 2022; Westerberg et al., 2021), the role of these sub-populations
73 in attentive perception at threshold remains poorly understood. Moreover, the influence
74 of physiological states, that may be responsible for different outcomes at threshold, on
75 these sub-populations has not been studied in detail.

76 Here we examine the neural mechanisms that regulate perception at threshold.

We specifically focus on the columnar microcircuit mechanisms within area V4. We hypothesized that minor fluctuations in behavioral state, such as arousal and visual sensitivity, and in the activity of neural sub-populations across the layers of the visual cortex, result in different perceptual outcomes at threshold. Specifically, we hypothesized the output layers (II-III, V-VI) that project to higher cortical areas and subcortical structures would show signs of improved capacity for stimulus representation. We also hypothesized that improved information propagation throughout a cortical column would characterize successful perception at threshold. We find that differences in behavioral and lamina-specific neural states characterize correct and incorrect trials at threshold and explain perceptual variability.

RESULTS

To study the neural dynamics responsible for determining whether a stimulus presented at perceptual threshold is perceived, we analyzed behavioral and cortical layer-specific neural data from area V4, collected while monkeys performed a cued attention task (Nandy et al., 2017). Monkeys were trained to detect an orientation change in one of two Gabor stimuli that were presented concurrently at two spatial locations, and to report having seen the change by making an eye movement to the changed stimulus. Prior to a block of trials, monkeys were cued as to which of the two spatial locations was likely to undergo the orientation change (95% valid cue; presented at the start of each block). During a trial, “non-target” stimuli at a fixed reference orientation were repeatedly presented. Non-targets were turned on for 200ms at the two spatial locations, and then turned off for a variable interval (200-400ms). At a random time (1-5s, mean 3s) a “target”

stimulus, differing in orientation from the non-targets, was presented at one of the locations. If the monkey reported having detected the orientation change by making an eye movement to the location of the target stimulus, it received a juice reward (Figure 1A, “hit” trial). If the monkey failed to detect the orientation change and instead continued to maintain fixation on the center of the monitor, it was not rewarded (Figure 1A, “miss” trial). In this study, we focused exclusively on trials in which the target stimulus was presented at the cued location (95% of trials). All figures relate exclusively to trials in which the change occurred at the cued location.

On each trial, the magnitude of the orientation change was drawn from a distribution that spanned multiple levels of difficulty. We fit the behavioral data with a logistic function (Prins and Kingdom, 2018) and defined the threshold condition as the orientation change that was closest to the 50% threshold of the fitted psychometric function for that session (Figure 1B, Experimental Procedures). We selected this subset of trials for further analysis, since the constant target stimuli in these trials were equally likely to be perceived or not perceived.

While monkeys performed this task, single- and multi-unit activity and local field potentials (LFPs) were recorded in area V4 using 16-channel linear array electrodes (Plexon inc., Figure S1A-E). The array was inserted perpendicular to the cortical surface and spanned the cortical layers. We used current source density (CSD) analysis (Mitzdorf, 1985) to estimate the boundaries between the superficial (I-III), input (IV), and deep (V-VI) cortical layers (Figure S1E-F), and assign individual neurons their layer identity (Nandy et al., 2017). Single units were classified as either broad-spiking (putative excitatory neurons) or narrow-spiking (putative inhibitory neurons) on the basis of their

waveform width using previously published techniques (peak to trough duration; Figure S1D; see Experimental Procedures; Connors and Gutnick, 1990; Kawaguchi, 1993; McCormick et al., 1985; Nandy et al., 2017; Nowak et al., 2003). Eye position and pupil diameter were also recorded (ISCAN ETL-200). When analyzing pupil diameter and eye position data, we considered all trials in the threshold condition in which the change occurred at the cued location, regardless of whether the cued location was within the receptive field of the recorded neurons. For all electrophysiological analyses, we only considered trials in which the cued stimulus was within the receptive field of the recorded neurons, and the stimulus change occurred at the cued location.

To assess the behavioral impact of variations in arousal and retinal image stability across trials at the threshold condition, we compared pupil diameter and microsaccade incidence across trail outcomes. Larger pupil diameter is thought to be a proxy for elevated alertness and arousal (Aston-Jones and Cohen, 2005; Beatty and Lucero-Wagoner, 2000; Hess and Polt, 1964; McCormick et al., 2020; McGinley et al., 2015; Reimer et al., 2014; Tang and Higley, 2020). We found that hit trials were associated with larger pupil diameters compared to miss trials, both before and during non-target and target stimulus presentations (Figure 2A). We quantified this difference in the estimation statistics framework (Calin-Jageman and Cumming, 2019; Ho et al., 2019) by comparing effect sizes and using bootstrapping to estimate uncertainty in the differences. We found that the mean of the distribution of pupil diameters associated with hit trials is greater than that associated with misses (Figure 2B; complementary null hypothesis testing results in Table S1). Prior work has shown that the optimal state for sensory performance occurs at intermediate levels of arousal, with states of low and hyper arousal associated with

decreased performance (Aston-Jones and Cohen, 2005; Cools and D'Esposito, 2011; McGinley et al., 2015; Murphy et al., 2011; Rajagovindan and Ding, 2011; Vijayraghavan et al., 2007; Yerkes and Dodson, 1908). In both hit and miss trials, the mean pupil diameter was close to the optimal arousal state for perceptual performance (Figure 2C; McGinley et al., 2015). The average differences in pupil diameter across hit and miss trials reflect differences within the optimal state of intermediate arousal. Our results thus suggest that hits are more likely to occur during periods of greater arousal.

Microsaccades, small fixational eye movements of <1° in amplitude that occur during normal fixation, are associated with periods of decreased visual sensitivity due to unstable retinal images (Dicke et al., 2008; Zuber and Stark, 1966). Microsaccades have been linked to suppressed neural responses in visual areas during perceptual tasks, impairing fine visual discrimination and behavioral performance (Beeler, 1967; Hafed and Krauzlis, 2010). We grouped trials in the threshold condition based on whether a microsaccade occurred in a 400ms window preceding the onset of the target stimulus. Most trials with a pre-target microsaccade were misses, whereas the majority of trials without a microsaccade in this window were hits (Figure 2D; X^2 proportion test, $p < 0.001$). There is a strong link between microsaccade direction and attention deployment (Engbert and Kliegl, 2003; Galfano et al., 2004; Gowen et al., 2007; Hafed and Clark, 2002; Lowet et al., 2018; Pastukhov and Braun, 2010; Yu et al., 2022). Consistent with previous reports we also find that microsaccades toward the attended stimulus were overrepresented in correct trials (Figure S2A, upper left). Conversely, microsaccades towards the attended stimulus were underrepresented in incorrect trials (Figure S2A, lower left). There was a very low but statistically significant negative correlation between

pupil diameter and microsaccade rate (Figure S2B, $r^2 = 0.006$, $p < 0.001$). Overall, these results suggest that successful trials at threshold are significantly more likely to occur during a state of greater arousal and improved visual sensitivity.

Having established that hit trials are more likely to occur in states of elevated arousal and visual sensitivity, we investigated whether hits are characterized by differential information processing in V4. Elevated stimulus-evoked firing rates in hits would indicate a stronger representation of the stimulus that could be necessary for accurate discrimination. We compared the firing rates of all neurons (single and multi-units) recorded in each cortical layer across hit and miss trials. For non-target stimuli, firing rates were equivalent for hits and misses in both the pre-stimulus (0-200ms before stimulus onset) and stimulus-evoked (60-260ms following stimulus onset) periods (Figure 3A). For the target stimulus, firing rates were once again equivalent in the pre-stimulus period, but hit trials were characterized by elevated firing across cortical layers in the stimulus-evoked period (Figure 3B-C). Broad and narrow-spiking neurons in both the input and deep layers respond more to target stimuli in hit trials, and trend towards elevated firing rates in the superficial layers during hits (Figure 3D). The average firing rate in response to target stimuli for each neuron is shown in Figure S3A for both hit and miss trials. It is important to note that the stimuli presented to the animals were identical for both hits and misses. Moreover, the responses to the target stimuli occur early, and elevated firing in hits emerges at the time of expected V4 response latencies (70-100 ms; Figure S3C), and thus cannot be attributed to elevated levels of firing due to subsequent saccade planning in these trials (Figure S3B; expected >200ms; Steinmetz and Moore, 2014).

192 Variability in response reflects how reliably information is encoded by a neural
193 population. Lower baseline variability can enhance the ability of neurons to encode
194 stimulus differences. We calculated the Fano factor, a mean-normalized measure of trial-
195 to-trial variability in firing, for single-units in our population (Figure 4A). We find that broad-
196 spiking units in the superficial layer exhibited lower Fano factor during the pre-stimulus
197 period in hit trials (0-60ms before non-target stimulus onset, Figure 4B), indicating this
198 population of neurons fired more reliably when the animal correctly detected the
199 orientation change. This was not the case for broad-spiking neurons in other layers
200 (Figure 4B) or narrow-spiking neurons (Figure S4).

201 We next wanted to test how the relationship between spiking activity and LFPs
202 may differ across hits and misses. Spike-LFP synchrony can reflect cortical processing
203 and both within- and inter-areal coordination (Fries, 2009; Fries et al., 2008; Siapas et al.,
204 2005). We calculated the PPC (Vinck et al., 2010), a frequency resolved measure of
205 spike-LFP phase-locking, for single and multi-units relative to their local LFP signal during
206 the pre-stimulus period (0-200ms before non-target stimulus onset, Figure 5A). We
207 averaged PPC values at low (3-12Hz), medium (15-25Hz), and high (30-80Hz) frequency
208 bands (superficial & Input: Figure S5A-B; deep: Figure 5B & S5C). Deep layer neurons
209 exhibit reduced low-frequency phase-locking in hit trials than in misses (Figure 5B),
210 suggesting an improvement in pooled signal-to-noise among this neural population.

211 Our results at the individual neuron or neural-sub-population levels suggest
212 enhanced processing of perceived stimuli. However, it is the concerted activity among
213 neural sub-populations that ultimately determine information flow through the laminar
214 cortical circuit. We therefore examined differences in interlaminar synchrony as

signatures of differential information flow between hit and miss trials. Spike-spike coherence (SSC) is a frequency resolved measure of the degree to which two spike trains fluctuate together (Mitchell et al., 2009; Mitra and Pesaran, 1999). We measured interlaminar SSC for spike trains from pairs of cortical layers, each spike train being comprised of all recorded action potentials in a given layer (See Experimental Procedures). We computed interlaminar SSC separately for hit and mis trials in both the pre-stimulus (0-200ms before non-target stimulus onset, Figure 6A) and non-target stimulus-evoked (60-260ms after non-target stimulus onset, Figure 7A) periods, matching the firing rates across hit and miss trials separately for the pre-stimulus and non-target stimulus-evoked conditions. We averaged SSC for each pair of layers across three frequency bands, 3-12Hz, 15-25Hz, and 30-80Hz (Figure 6B and 7B).

Overall, hit trials have greater interlaminar SSC compared to misses at almost all frequencies (Figure 6B and 7B). In the pre-stimulus period, the strongest SSC difference between hits and misses was observed between the superficial and deep layers across all frequencies (Figure 6B, middle panel). This implies greater synchrony of the output layers of the cortex during hit trials. In contrast, this pattern was directionally the same during the non-target stimulus-evoked period, but stronger in the other layer pairs, with greater SSC differences being found in pairs that involve the input layer (Figure 7B, top and bottom). This may reflect a higher degree of stimulus-driven feed-forward information propagation during hit trials. When comparing across time (pre-stimulus vs non-target stimulus-evoked), layers, and frequency band, there was a significant interaction effect of layer pair and time window (three-way ANOVA, $p = 0.0075$).

Finally, we sought to compare the predictive power of our results on the monkey's perceptual performance. We created a generalized linear model (GLM) to regress behavioral outcome from the pupil diameter, number of microsaccades in the pre-target window, and average target-evoked multi-unit firing rate in each of the three layers (see Experimental Procedures; Davis et al., 2020). Other reported measures (Fano factor, PPC, interlaminar SSC) that we could not estimate reliably on a single trial basis were not considered in the GLM analysis. Pre-target microsaccades were by far the strongest predictor of performance ($weight = -1.3116$; $p = 6.0757e-08$). Input layer firing rate also significantly predicted perception ($weight = 0.3276$; $p = 0.020068$). Superficial firing rate, deep firing rate, and pupil diameter were not significant predictors (Table S2, all $p > 0.5$). This indicates that, among the variables that we could estimate reliably on a single trial basis, stable retinal images in the pre-target window are critical for behavioral performance, and elevated firing in the input layer is the most reliable physiological signature of a perceived stimulus.

DISCUSSION

We investigated the physiological processes responsible for variable behavioral outcomes at perceptual threshold. Controlling for *both* the attentive instruction (thus minimizing large-scale attentional effects) and the stimulus condition that elicited performance at a threshold level allowed us to examine the physiological and neural correlates that underlie correct versus incorrect behavioral outcomes. While this study cannot disentangle the independent roles of behavioral state fluctuations and neural fluctuations in determining behavioral outcomes, evidence suggests that differences in both are associated with hits. We found multiple lines of evidence which suggest that a

260 state of higher arousal and stable retinal images and the accompanying enhanced
261 processing of visual stimuli contributes to accurate perception in hit trials.

262 Pupil diameter is elevated in hit trials (Figure 2A-C), and prior studies have shown
263 that pupil diameter is strongly linked to arousal and alertness (Beatty and Lucero-
264 Wagoner, 2000; Hess and Polt, 1964; Tang and Higley, 2020). This provides evidence
265 that a state of higher arousal may contribute to improved sensory processing. The much
266 lower hit rate in trials with a microsaccade preceding the target (Figure 2D) and our GLM
267 analysis shows that stability of retinal images is critical for accurate discrimination at
268 threshold. It is unlikely that these two measures are reflecting the same phenomenon, as
269 there is a very weak correlation between them over the course of a trial (Figure S2B).

270 There is a strong link between oculomotor control and attentional deployment
271 (Moore and Fallah, 2001; Moore and Zirnsak, 2017; Schafer and Moore, 2011). In this
272 study, hits and misses differ in their behavioral responses, with hit trials being
273 characterized by a saccade to the target stimulus. Almost all of our neural results reflect
274 differences around the time of non-target stimulus presentations during which the
275 monkeys maintained fixation at the center of the screen and, therefore, were hundreds of
276 milliseconds prior to saccade planning and execution in the case of hit trials. Trials in
277 which saccades were made to non-target stimuli were excluded from analysis, as were
278 trials in which the monkey made a saccade to the target too soon after its presentation to
279 have been a behavioral response to stimulus perception (see Experimental Procedures).
280 The analysis of microsaccade occurrence focused on the window just before target
281 stimulus presentation and before monkeys could begin oculomotor planning. Only the
282 analysis of neural responses to target stimuli appears in conjunction with divergent

283 oculomotor behavior between the hit (saccade) and miss (no saccade) trials. However,
284 here too firing rates diverge much earlier, particularly in the input layer, than would be
285 consistent with the effects of saccade planning (Figure S3B; Steinmetz and Moore, 2014).
286 A body of evidence (See Martinez-Conde et al., 2013 for review), suggests that
287 microsaccades directed towards a target stimulus reflect attention-related processing and
288 performance (Engbert and Kliegl, 2003; Galfano et al., 2004; Gowen et al., 2007; Hafed
289 and Clark, 2002; Lowet et al., 2018; Pastukhov and Braun, 2010; Yu et al., 2022). In our
290 dataset, during the pre-target period, microsaccades towards the attended stimulus were
291 overrepresented in correct trials (Figure S2A, upper left). Conversely, microsaccades
292 towards the attended stimulus were underrepresented in incorrect trials (Figure S2A,
293 lower left). Microsaccades directed towards the location of the eventual target may reflect
294 elevated attentional deployment that can compensate for the reduced sensitivity due to
295 higher incidence of microsaccades.
296 Our electrophysiological findings and their laminar patterns associated with hit
297 trials *within* a cued attention state mirror several previous findings that are associated
298 with the deployment of covert spatial attention. Attention has long been known to increase
299 firing rates in V4 (McAdams and Maunsell, 1999; Mitchell et al., 2007; Spitzer et al., 1988),
300 and there is evidence that this increase occurs in all cortical layers in V4 (Nandy et al.,
301 2017). We find that elevated firing rates in hits occur across all layers in conjunction with
302 elevated arousal (Figure 3). Attention reduces the variability in the firing of V4 neurons,
303 and this reduction is thought to contribute to the improved information coding capacity of
304 a population of neurons (Cohen and Maunsell, 2009; Mitchell et al., 2007, 2009; Moreno-
305 Bote et al., 2014; Nandy et al., 2017). The reduction in Fano factor among broad-spiking

306 superficial-layer neurons in hit trials mirrors the effects of attention. Multiple lines of
307 evidence suggest broad- and narrow-spiking correspond to putative excitatory and
308 inhibitory neurons respectively. Narrow-spiking neurons exhibit higher firing rates, which
309 corresponds well with inhibitory interneuron (Connors and Gutnick, 1990; Contreras and
310 Palmer, 2003; Foehring et al., 1991; McCormick et al., 1985; Nandy et al., 2017,
311 Supplementary Table 1; Povysheva et al., 2006). Repolarization times in broad-spiking
312 neurons are also longer, as they are in excitatory pyramidal neurons (Hasenstaub et al.,
313 2005; McCormick et al., 1985; Nowak et al., 2003). Since these neurons are putative
314 projection neurons to downstream cortical areas, this reduction in Fano factor may
315 indicate increased reliability in stimulus encoding that could contribute to hits. Our finding
316 is also in agreement with previous reports of higher variability in representations of
317 unperceived stimuli in humans (Schurger et al., 2010). Synchronous neural activity
318 appears to modulate perceptual and cognitive ability in a variety of contexts (Abbas et al.,
319 2018; Fries et al., 2001; Rohenkohl et al., 2018; Worden et al., 2000). We found that
320 deep-layer neurons exhibit less low-frequency phase-locking in hit trials (Figure 5). This
321 is consistent with prior studies that find an attention-mediated reduction in the power
322 spectrum of the spike-triggered-averaged LFP (Fries et al., 2001).

323 Our examination of inter-laminar synchrony revealed two interesting and
324 complementary patterns: hits were associated with greater coherence between the
325 superficial and deep layers during spontaneous activity in the pre-stimulus period (Figure
326 6); in contrast, we found enhanced coherence between the input layer and both the output
327 layers (superficial and deep) in the stimulus-evoked period during hits (Figure 7).
328 Increased superficial-deep coherence in the pre-stimulus period could be the result of

329 neuromodulatory or top-down processes that maintain the cortex in a state of sustained
330 depolarization corresponding to a state of higher arousal during hits (McCormick et al.,
331 2020; McGinley et al., 2015). Increased synchrony between the input layer and the output
332 layers during the stimulus-evoked period could then reflect stronger information
333 propagation through the cortical circuit, and hence with improved stimulus detection
334 (Marshel et al., 2019). In contrast to broad global synchrony or local correlated
335 fluctuations, which may signal a default state of minimal processing or decreased
336 information coding capacity (Mitchell et al., 2009; Steriade et al., 1993; von Krosigk et al.,
337 1993; Zohary et al., 1994), these patterns of interlaminar coherence that we found
338 suggest that successful perception at threshold is mediated by pathway specific
339 modulation of information flow through the laminar cortical circuit.

340 Taken together, our results provide insight about how information about a
341 threshold stimulus may successfully propagate through a cortical column and influence
342 sensory perception. Lower baseline variability among broad-spiking superficial layer
343 neurons and decreased low-frequency synchronous activity in the deep layers could be
344 indicative of improved capacity to encode sensory information. Higher target-evoked firing
345 rates and elevated interlaminar synchrony could enhance the propagation of this encoded
346 signal. These results associate pre-stimulus baseline state differences with enhanced
347 cortical processing in the stimulus-evoked period.

348 Several studies have examined how information flow differs for perceived and
349 unperceived stimuli at a more macroscopic scale (Herman et al., 2017; van Vugt et al.,
350 2018). van Vugt et al. (2018) recorded from three brain regions, V1, V4, and dorsolateral
351 prefrontal cortex (dlPFC), while a monkey performed a stimulus detection task at

threshold. Their work supports the model that feedforward propagation of sensory information from the visual cortex to the PFC causes a non-linear “ignition” of association areas resulting in conscious perception (Dehaene and Changeux, 2011). Herman et al. (Herman et al., 2017) found that conscious human perception triggers a wave of activity propagation from occipital to frontal cortex while switching off default mode and other networks. Our study provides insight into the functions of the cortical microcircuit at the columnar level that could reflect these large-scale sweeping activity changes in perception.

Overall, we identified substantial layer-specific differences in cortical activity between hits and misses at perceptual threshold, during both the pre-stimulus and stimulus-evoked periods (Figure 8). These differences are indicative of greater fidelity of stimulus processing in hits, along with elevated arousal and visual sensitivity due to stable retinal images. Synchrony analysis reveals a potential higher state of anticipatory engagement in the pre-stimulus period followed by improved signal propagation after stimulus presentation. These physiological differences in the laminar microcircuit likely contribute to successful perceptual discrimination at threshold.

EXPERIMENTAL PROCEDURES

The surgical procedures, behavioral task, and electrophysiological recordings used in this study have been described previously (Nandy et al., 2017), and are also described in Supplementary Information. For each recording session we fit a psychometric function to the monkey’s behavioral performance at each orientation change condition. We then calculated the threshold of the fitted smooth logistic function (Prins and Kingdom, 2018) and selected this subset of trials for further study. We compared z-score normalized pupil

diameter traces around the time of target and non-target stimulus onset across hit and miss trials. Microsaccades were detected using ClusterFix (König and Buffalo, 2014) and their direction and presence across trial types was measured up to 400ms before target stimulus onset. Firing rates for all neurons were normalized by their maximum firing rate for each presented stimulus contrast and compared across hits and misses. We measured trial-to-trial variability in firing by Fano factor and compared the Fano factor modulation index (difference in Fano factor divided by the sum) across trial types for broad and narrow-spiking single units. PPC was calculated in the 200ms prior to non-target stimulus onset for single units with at least 50 spikes in three frequency bins (3-12 Hz, 15-25 Hz, and 30-80 Hz). To calculate interlaminar SSC we combined all the spikes from a layer into a multi-unit from each layer and calculated interlaminar SSC before or after non-target stimulus onset. To compare how our results predict behavioral performance, we fit a GLM with five regresssors: (1) pupil diameter, (2) number of microsaccades in the pre-target window, and target-evoked firing rates in the (3) superficial, (4) input, and (5) deep layers.

FIGURE LEGENDS

Figure 1. Orientation change detection task at perceptual threshold

(**A**) Schematic of task structure. The monkey initiated a trial by fixating on the center of the screen. Two Gabor stimuli (represented by oriented lines) were presented for 200 ms and then turned off for 200-400ms. This was repeated until, at an unpredictable time, one of the stimuli changed orientation. The monkey could report having seen the change by making an eye movement to the location of the target stimulus to receive a reward (hit trials). If the monkey failed to report the orientation change and maintained fixation on the center of the screen it was not rewarded (miss trials). Before a block of trials, the monkey was cued as to which stimulus was likely to undergo the change (95% valid cue). In 5% of trials the orientation change occurred at the other location (foil trials). Circles indicating the cued location and receptive field are drawn for figure reference only and were not presented during the task. (**B**) Example behavioral psychometric function from one recording session and attention condition. Behavioral performance (hit rate, circles) is presented as a function of orientation change. Data was fitted with a logistic function. The threshold condition, trials with performance halfway between the upper and lower asymptotes of the logistic function, is indicated by the orange box. Error bars represent standard deviation calculated with a jackknife procedure (20 jackknives). The square symbol indicates foil trial performance.

Figure 2. Hit trials have larger pupil diameter whereas microsaccades more often precede misses

(**A**) Normalized pupil diameter for hit and miss trails in the threshold condition. 0 ms corresponds to non-target and target stimulus onset. Mean +/- s.e.m. (**B**) Distribution of pupil diameter values associated with hit and miss trials. Pupil diameter was averaged from 100ms before to 100ms after non-target and target stimulus onset. Violin plots were generated using kernel smoothing (See Experimental Procedures). Error bars represent 95% confidence intervals for the mean of each distribution, and the mean difference (blue, right axes). *Inset*: zoomed in view of the mean difference between hit and miss trials. Black bar represents a 95% confidence interval of the mean difference. Shaded region reflects the distribution of the bootstrapped estimation of the mean difference. (**C**) Histogram of mean pupil diameter around the time of non-target and target stimulus onset (calculated as in **B**). Orange and gray lines represent the mean pupil diameter for hit and miss trials respectively. (**D**) Hit rate for trials with (*left*, 387 trials) and without (*right*, 1336 trials) a microsaccade detected in the time window 0-400ms before target onset. There is a significantly lower hit rate in trials with a microsaccade ($p < 0.0001$, X^2-test).

Figure 3. Target stimuli evoke higher firing rates in hit trials

Rows correspond to different layers (top=superficial, middle=input, bottom=deep). (**A**) Population (visually responsive single and multi-unit) PSTH for non-target stimuli during hit (orange) and miss (dark-gray) trials in the threshold condition (mean +/- s.e.m). The horizontal black bar indicates the time and duration of stimulus presentation. (**B**) As in **A** but for target stimuli. The star indicates the time at which firing rates in the input layer first differ significantly between hit and miss trials. Vertical lines represent the mean time firing rates for each neuron rise above the 95% confidence interval of their baseline activity (see also Figure S3C). (**C**) Bootstrapped estimation of the paired mean difference in target stimulus-evoked firing rate across hit and miss trials in the time window 60-260ms (red dotted box in **B**) after target stimulus onset. Shaded regions represent the bootstrapped estimation of the paired mean difference in firing rate (hit - miss), and black lines are 95% confidence intervals. Plots include data from both single and multi-units, separated by layer (top=superficial, middle=input, bottom=deep). (**D**) As in **C**, bootstrapped estimation of the paired mean difference in firing rate for hit trials compared to miss trials in the target stimulus-evoked period, but only for single-units broken up by cell class (gold=broad, teal=narrow).

Figure 4. Broad-spiking neurons in the superficial layer have decreased variability in hit trials

(**A**) Rows correspond to different layers (top=superficial, middle=input, bottom=deep). The Fano factor of broad-spiking putative excitatory neurons for the hit and miss trials in the threshold condition (mean +/- s.e.m). There is a significant decrease in variability for the hit trials prior to stimulus onset only in the superficial layer. 0 ms corresponds to non-target stimulus onset. The average Fano factor within a 60ms time-window (red dashed box) prior to non-target stimulus onset is plotted in **B**. (**B**) *Top:* Fano factor modulation index for each broad-spiking neurons recorded in each layer, averaged in the 60ms preceding non-target stimulus onset. *Bottom:* Bootstrapped estimation of the mean difference of the Fano factor modulation index from zero in each of the three layers. Colored curves represent the estimated

bootstrapped distribution. Black dots and lines reflect the mean and 95% confidence intervals of the distributions.

Figure 5. Deep layer neurons are phase-locked to low-frequency rhythms in miss trials
(**A**) Pairwise phase consistency (PPC) of single and multi-units in each layer to the local field potential (LFP) signal recorded from the same channel in hit and miss trials at threshold. PPC was calculated in the pre-stimulus period (0-200 ms before stimulus onset). Dashed red line indicates a PPC of 0, below which there is no consistent relationship between spikes and LFP phase. (**B**) Bootstrapped estimation plot for the paired mean difference in PPC for deep layer neurons over three frequency bands: 3-12Hz, 15-25Hz, 30-80Hz. Curves represent the bootstrapped distribution for the paired difference, and black dots and vertical lines represent the mean and 95% confidence intervals for the paired mean difference

Figure 6. Greater interlaminar coherence in hit trials in the pre-stimulus period
Interlaminar spike-spike coherence in the pre-stimulus period (0-200ms prior to stimulus onset). Rows correspond to different pairs of layers (*top*=superficial-input, *middle*=superficial-deep, *bottom*=input-deep). (**A**) Multi-unit interlaminar spike-spike coherence (SSC) calculated in the 200ms before non-target stimulus onset in hit and miss trials (solid lines, mean +/- s.e.m). Firing rates were matched across hit and miss trials. Dashed lines represent coherence calculated with shuffled trial identities (mean +/- s.e.m). (**B**) Bootstrapped estimation plot for the paired mean difference in SSC for each pair of layers averaged over three frequency bands : 3-12Hz, 15-25Hz, 30-80Hz. Curves represent the bootstrapped distribution for the paired difference, and black dots and vertical lines represent the mean and 95% confidence intervals for the paired mean difference.

Figure 7. Greater interlaminar coherence in hit trials in the stimulus-evoked period
Interlaminar spike-spike coherence in the stimulus evoked period (60-260ms after stimulus onset). Same conventions as in Figure 6.

Figure 8. Summary of results
Hit trials have a larger pupil diameter in both the pre-stimulus and stimulus-evoked time periods. In the pre-stimulus period, hits are characterized by decreased variability in superficial layer broad-spiking neurons, less phase-locking of deep layer neurons to low-frequency LFPs, and greater interlaminar spike-spike coherence between the superficial and deep layers. Microsaccades in the pre-stimulus period are associated with a much lower hit rate. Stimuli evoke higher firing rates across all three layers in hits. The stimulus-evoked period is associated with greater interlaminar spike-spike coherence between the input layer and the superficial and deep layers.

Figure S1. Laminar recordings in V4
(**A**) An artificial dura (AD) chamber is shown over dorsal V4 in the right hemisphere of Monkey A. The native dura mater was resected and replaced with a silicone based artificial dura, thereby providing an optically clear window into the cortex. Scale bar = 5mm. (**B**) An enlarged view of the boxed region in **A** clearly shows the sulci and the microvasculature. sts = superior temporal sulcus, lu = lunate sulcus, io = inferior occipital sulcus. Area V4 lies on the pre-lunate gyrus between the superior temporal and lunate sulci. Scale bar = 2mm. (**C**) Electrophysiology setup: a plastic stabilizer with a circular aperture is secured in place inside the chamber such that the aperture is centered over the pre-lunate gryus. A 16-channel linear array electrode (electrode spacing 150 μm) is positioned over the center of the gyrus and lowered into the cortex under microscopic guidance. The microvasculature pattern was used as a reference to target different cortical sites across recording sessions. (**D**) Example recording session in monkey C depicting 12 single unit waveforms (mean +/- s.e.m.) isolated along the cortical column. Teal waveforms correspond to narrow-spiking putative interneurons and gold waveforms correspond to broad-spiking putative excitatory units. (**E**) Stimulus triggered local field potentials (LFPs) obtained by flashing 30ms high contrast ring stimuli in the receptive field of a V4 cortical column. LFP traces averaged across all stimulus repeats are shown color-coded as being part of either the superficial (green), input (gray) or deep (pink) layers. Layer assignment was done after current source-density analysis. (**F**) Current source-density (CSD) calculated as the second spatial derivative of the stimulus triggered LFPs and displayed as a colored map. The x-axis represents time from stimulus onset; the y-axis represents cortical depth oriented such that the pial surface is at the top and the white matter is at the bottom. Red hues represent current sink, blue hues represent current

source. The input layer is identified as the first current sink followed by a reversal to current source. The superficial and deep layers have the opposite sink-source pattern. The CSD map has been spatially smoothed for visualization. (**G**) Stacked contour plots show spatial receptive fields (RFs) mapped along each contact point in the laminar probe. The spatial receptive fields were obtained by applying reverse correlation to the LFP power evoked by sparse pseudo-random sequences of Gabor stimuli. The RFs are well aligned, indicating perpendicular penetration down a cortical column. Zero depth represents the center of the input layer as estimated from the CSD. (**H**) Contrast response functions – spikes rate as a function of stimulus contrast – are shown for 2 example units identified in a single recording session in Monkey A. Red and blue traces correspond to the attend-in to RF and attend-away from RF conditions respectively. The dotted lines represent the corresponding background firing-rates. The dashed lines are 4 standard deviations above baseline. A unit was considered as visually responsive, if the contrast response functions exceeded this threshold in both the attention conditions. Mean +/- s.e.m. Panels are reproduced from Nandy et al. (2017).

Figure S2. Microsaccades are preferentially directed towards the target in correct trials and have a slight correlation with pupil diameter

Data is presented for all trials, regardless of orientation change (not just the threshold condition). (**A**) The histograms represent the direction of microsaccades relative to the attended stimulus (left column) or unattended stimulus (right column) in correct (top row) and incorrect (bottom row) trials. Black lines represent the mean (solid) and 99.5% confidence interval (dashed) of the bootstrapped null distribution estimated by pooling correct and incorrect microsaccades. $* \mathrm{p} < 0.005$. *Inset:* Schematic for calculation of relative microsaccade direction. Microsaccade is represented by the gray arrow (**B**) Scatterplot of microsaccade rate versus mean normalized pupil diameter, shows a small but statistically significant relationship between the two quantities ($r^2 = 0.006$, $p < 0.001$). Each dot is color-coded by trial length. 4% of trials with mean pupil diameter >2 or <-2 not shown.

Figure S3. Firing rates for individual neurons and reaction time in threshold condition

(**A**) Target stimulus-evoked normalized firing rates in hit and miss trials for each recorded single and multi-unit cluster in hit and miss trials. Clusters are divided by layer: left=superficial, middle=input, right=deep. Related to Figure 3B. Each line represents the mean firing rate in response to target stimuli in hit and miss trials for a given unit. Data is color coded by unit type (gold=broad, teal=narrow, gray=multi-unit). See Experimental Procedures for normalization method. (**B**) Mean reaction time (time from target stimulus onset to saccade onset) for hit trials in the threshold condition in both attend-in (red) and attend-away (blue) trials for each recording session. (**C**) Mean time at which single- and multi-unit firing rates rise significantly above pre-stimulus baseline in response to target stimuli in hit and miss trials. Mean +/- s.e.m.

Figure S4. Narrow-spiking neurons do not have decreased variability in hit trials

Top: Fano factor modulation index for each narrow-spiking neurons recorded in each layer, averaged in the 60ms preceding non-target stimulus onset. *Bottom:* Bootstrapped estimation of the mean difference of the Fano factor modulation index from zero in each of the three layers. Colored curves represent the estimated bootstrapped distribution. Black dots and lines reflect the mean and 95% confidence intervals of the distributions.

Figure S5. Additional PPC data

(**A-B**) *Top:* Raw PPC values calculated for clusters recorded in the superficial (**A**) and (**B**) input layers in hit and miss trials, averaged into three frequency bands, 3-12 Hz, 15-25 Hz, and 30-80 Hz. PPC was calculated using the LFP recorded on the same channel as the spikes. *Bottom:* Bootstrapped estimation of the paired mean difference in PPC across hit and miss trials for each frequency band. Note that although there appears to be a difference in high-frequency PPC in the superficial layer, this population does not have significantly positive PPC in either condition, indicating that there is no phase-locking in either hits or misses. (**C**) Raw PPC values for neurons recorded in the deep layer in hit and miss trials, averaged into the same 3 frequency bands. Related to Figure 5B.

AUTHOR CONTRIBUTIONS

MPJ and ASN conceptualized the project. ASN collected the data and supervised the project. MPM analyzed the data, with assistance from SD and IB. MPM, SD, ASN, MPJ, and JHR wrote the manuscript.

ACKNOWLEDGEMENTS

This research was supported by NIH R01 EY021827 to JHR and ASN, NARSAD Young Investigator Grant, Ziegler Foundation Grant, Yale Orthwein Scholar Funds & Lawrence Family Young Investigator Funds to ASN, NIH R00 EY025026 to MPJ, and by NEI core grants for vision research P30 EY019005 to the Salk Institute and P30 EY026878 to Yale University. MM was supported by training grants T32-NS007224 and T32-NS041228 to Yale University. We would like to thank Catherine Williams and Mat LeBlanc for excellent animal care.

REFERENCES

Abbas, A.I., Sundiang, M.J.M., Henoch, B., Morton, M.P., Bolkan, S.S., Park, A.J., Harris, A.Z., Kellendonk, C., and Gordon, J.A. (2018). Somatostatin Interneurons Facilitate Hippocampal-Prefrontal Synchrony and Prefrontal Spatial Encoding. Neuron 100, 926-939.e923.

Aston-Jones, G., and Cohen, J.D. (2005). An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. Annu Rev Neurosci 28, 403-450.

Beatty, J., and Lucero-Wagoner, B. (2000). The pupillary system. Handbook of psychophysiology 2.

Beeler, G.W. (1967). Visual threshold changes resulting from spontaneous saccadic eye movements. Vision Research 7, 769-775.

Buffalo, E.A., Fries, P., Landman, R., Buschman, T.J., and Desimone, R. (2011). Laminar differences in gamma and alpha coherence in the ventral stream. Proc Natl Acad Sci U S A 108, 11262-11267.

Calin-Jageman, R.J., and Cumming, G. (2019). Estimation for Better Inference in Neuroscience. eneuro 6, ENEURO.0205-0219.2019.

Cohen, M.R., and Maunsell, J.H. (2009). Attention improves performance primarily by reducing interneuronal correlations. Nat Neurosci 12, 1594-1600.

Connors, B.W., and Gutnick, M.J. (1990). Intrinsic firing patterns of diverse neocortical neurons. Trends Neurosci 13, 99-104.

Contreras, D., and Palmer, L. (2003). Response to contrast of electrophysiologically defined cell classes in primary visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 23, 6936-6945.

Cools, R., and D'Esposito, M. (2011). Inverted-U-shaped dopamine actions on human working memory and cognitive control. Biol Psychiatry 69, e113-125.

Davis, Z.W., Muller, L., Martinez-Trujillo, J., Sejnowski, T., and Reynolds, J.H. (2020). Spontaneous travelling cortical waves gate perception in behaving primates. Nature 587, 432-436.

Dehaene, S., and Changeux, J.-P. (2011). Experimental and Theoretical Approaches to Conscious Processing. Neuron 70, 200-227.

Desimone, R., and Duncan, J. (1995). Neural mechanisms of selective visual attention. Annu Rev Neurosci 18, 193-222.

Dicke, P.W., Chakraborty, S., and Thier, P. (2008). Neuronal correlates of perceptual stability during eye movements. European Journal of Neuroscience 27, 991-1002.

Douglas, R.J., and Martin, K.A.C. (2004). NEURONAL CIRCUITS OF THE NEOCORTEX. Annual Review of Neuroscience 27, 419-451.

Douglas, R.J., and Martin, K.A.C. (2007). Mapping the Matrix: The Ways of Neocortex. Neuron 56, 226-238.

Engbert, R., and Kliegl, R. (2003). Microsaccades uncover the orientation of covert attention. Vision Research 43, 1035-1045.

Engel, T.A., Steinmetz, N.A., Gieselmann, M.A., Thiele, A., Moore, T., and Boahen, K. (2016). Selective modulation of cortical state during spatial attention. Science 354, 1140-1144.

Ferro, D., van Kempen, J., Boyd, M., Panzeri, S., and Thiele, A. (2021). Directed information exchange between cortical layers in macaque V1 and V4 and its modulation by selective attention. Proc Natl Acad Sci U S A 118.

Foehring, R.C., Lorenzon, N.M., Herron, P., and Wilson, C.J. (1991). Correlation of physiologically and morphologically identified neuronal types in human association cortex in vitro. J Neurophysiol 66, 1825-1837.

Fries, P. (2009). Neuronal Gamma-Band Synchronization as a Fundamental Process in Cortical Computation. Annual Review of Neuroscience 32, 209-224.

Fries, P., Reynolds, J.H., Rorie, A.E., and Desimone, R. (2001). Modulation of Oscillatory Neuronal Synchronization by Selective Visual Attention. Science 291, 1560.

Fries, P., Womelsdorf, T., Oostenveld, R., and Desimone, R. (2008). The effects of visual stimulation and selective visual attention on rhythmic neuronal synchronization in macaque area V4. The Journal of neuroscience : the official journal of the Society for Neuroscience 28, 4823-4835.

Galfano, G., Betta, E., and Turatto, M. (2004). Inhibition of return in microsaccades. Experimental brain research 159, 400-404.

Ghosh, S., and Maunsell, J.H.R. (2021). Single trial neuronal activity dynamics of attentional intensity in monkey visual area V4. Nature Communications 12, 2003.

Goodale, M.A., and Milner, A.D. (1992). Separate visual pathways for perception and action. Trends in Neurosciences 15, 20-25.

Gowen, E., Abadi, R.V., Poliakoff, E., Hansen, P.C., and Miall, R.C. (2007). Modulation of saccadic intrusions by exogenous and endogenous attention. Brain Res 1141, 154-167.

Hafed, Z.M., and Clark, J.J. (2002). Microsaccades as an overt measure of covert attention shifts. Vision Research 42, 2533-2545.

Hafed, Z.M., and Krauzlis, R.J. (2010). Microsaccadic suppression of visual bursts in the primate superior colliculus. The Journal of neuroscience : the official journal of the Society for Neuroscience 30, 9542-9547.

Harris, K.D., and Thiele, A. (2011). Cortical state and attention. Nature Reviews Neuroscience 12, 509-523.

Hasenstaub, A., Shu, Y., Haider, B., Kraushaar, U., Duque, A., and McCormick, D.A. (2005). Inhibitory postsynaptic potentials carry synchronized frequency information in active cortical networks. Neuron 47, 423-435.

Herman, W.X., Smith, R.E., Kronemer, S.I., Watsky, R.E., Chen, W.C., Gober, L.M., Touloumes, G.J., Khosla, M., Raja, A., Horien, C.L.*, et al.* (2017). A Switch and Wave of Neuronal Activity in the Cerebral Cortex During the First Second of Conscious Perception. Cerebral Cortex 29, 461-474.

Hess, E.H., and Polt, J.M. (1964). Pupil size in relation to mental activity during simple problem-solving. Science 143, 1190-1192.

Hirsch, J.A., and Martinez, L.M. (2006). Laminar processing in the visual cortical column. Curr Opin Neurobiol 16, 377-384.

Ho, J., Tumkaya, T., Aryal, S., Choi, H., and Claridge-Chang, A. (2019). Moving beyond P values: data analysis with estimation graphics. Nature Methods 16, 565-566.

Kawaguchi, Y. (1993). Groupings of nonpyramidal and pyramidal cells with specific physiological and morphological characteristics in rat frontal cortex. Journal of Neurophysiology 69, 416-431.

König, S.D., and Buffalo, E.A. (2014). A nonparametric method for detecting fixations and saccades using cluster analysis: removing the need for arbitrary thresholds. Journal of neuroscience methods 227, 121-131.

Levitt, H. (1971). Transformed Up - Down Methods in Psychoacoustics. The Journal of the Acoustical Society of America 49, 467-477.

Livingstone, M.S., and Hubel, D.H. (1981). Effects of sleep and arousal on the processing of visual information in the cat. Nature 291, 554-561.

Lowet, E., Gomes, B., Srinivasan, K., Zhou, H., Schafer, R.J., and Desimone, R. (2018). Enhanced Neural Processing by Covert Attention only during Microsaccades Directed toward the Attended Stimulus. Neuron 99, 207-214.e203.

Markram, H., Toledo-Rodriguez, M., Wang, Y., Gupta, A., Silberberg, G., and Wu, C. (2004). Interneurons of the neocortical inhibitory system. Nature reviews Neuroscience 5, 793-807.

Marshel, J.H., Kim, Y.S., Machado, T.A., Quirin, S., Benson, B., Kadmon, J., Raja, C., Chibukhchyan, A., Ramakrishnan, C., Inoue, M.*, et al.* (2019). Cortical layer–specific critical dynamics triggering perception. Science 365, eaaw5202.

Martinez-Conde, S., Otero-Millan, J., and Macknik, S.L. (2013). The impact of microsaccades on vision: towards a unified theory of saccadic function. Nature Reviews Neuroscience 14, 83-96.

McAdams, C.J., and Maunsell, J.H. (1999). Effects of attention on orientation-tuning functions of single neurons in macaque cortical area V4. The Journal of neuroscience : the official journal of the Society for Neuroscience 19, 431-441.

McCormick, D.A., and Bal, T. (1997). SLEEP AND AROUSAL: Thalamocortical Mechanisms. Annual Review of Neuroscience 20, 185-215.

McCormick, D.A., Connors, B.W., Lighthall, J.W., and Prince, D.A. (1985). Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex. J Neurophysiol 54, 782-806.

McCormick, D.A., Nestvogel, D.B., and He, B.J. (2020). Neuromodulation of Brain State and Behavior. Annual Review of Neuroscience 43, null.

McGinley, M.J., David, S.V., and McCormick, D.A. (2015). Cortical Membrane Potential Signature of Optimal States for Sensory Signal Detection. Neuron 87, 179-192.

Mehta, A.D., Ulbert, I., and Schroeder, C.E. (2000a). Intermodal selective attention in monkeys. I: distribution and timing of effects across visual areas. Cerebral cortex (New York, NY : 1991) 10, 343-358.

Mehta, A.D., Ulbert, I., and Schroeder, C.E. (2000b). Intermodal selective attention in monkeys. II: physiological mechanisms of modulation. Cerebral cortex (New York, NY : 1991) 10, 359-370.

Migliore, M., and Shepherd, G.M. (2005). An integrated approach to classifying neuronal phenotypes. Nature Reviews Neuroscience 6, 810-818.

Mitchell, J.F., Sundberg, K.A., and Reynolds, J.H. (2007). Differential attention-dependent response modulation across cell classes in macaque visual area V4. Neuron 55, 131-141.

Mitchell, J.F., Sundberg, K.A., and Reynolds, J.H. (2009). Spatial attention decorrelates intrinsic activity fluctuations in macaque area V4. Neuron 63, 879-888.

Mitra, P.P., and Pesaran, B. (1999). Analysis of dynamic brain imaging data. Biophys J 76, 691-708.

Mitzdorf, U. (1985). Current source-density method and application in cat cerebral cortex: investigation of evoked potentials and EEG phenomena. Physiol Rev 65, 37-100.

Moore, T., and Fallah, M. (2001). Control of eye movements and spatial attention. Proc Natl Acad Sci U S A 98, 1273-1276.

Moore, T., and Zirnsak, M. (2017). Neural Mechanisms of Selective Visual Attention. Annual Review of Psychology 68, 47-72.

Moran, J., and Desimone, R. (1985). Selective attention gates visual processing in the extrastriate cortex. Science 229, 782-784.

Moreno-Bote, R., Beck, J., Kanitscheider, I., Pitkow, X., Latham, P., and Pouget, A. (2014). Information-limiting correlations. Nat Neurosci 17, 1410-1417.

Mountcastle, V.B. (1997). The columnar organization of the neocortex. Brain 120 (Pt 4), 701-722.

Murphy, P.R., Robertson, I.H., Balsters, J.H., and O'Connell R, G. (2011). Pupillometry and P3 index the locus coeruleus-noradrenergic arousal function in humans. Psychophysiology 48, 1532-1543.

Nandy, A.S., Nassi, J.J., and Reynolds, J.H. (2017). Laminar Organization of Attentional Modulation in Macaque Visual Area V4. Neuron 93, 235-246.

Nowak, L.G., Azouz, R., Sanchez-Vives, M.V., Gray, C.M., and McCormick, D.A. (2003). Electrophysiological classes of cat primary visual cortical neurons in vivo as revealed by quantitative analyses. J Neurophysiol 89, 1541-1566.

Pastukhov, A., and Braun, J. (2010). Rare but precious: microsaccades are highly informative about attentional allocation. Vision Res 50, 1173-1184.

Pettine, W.W., Steinmetz, N.A., and Moore, T. (2019). Laminar segregation of sensory coding and behavioral readout in macaque V4. Proceedings of the National Academy of Sciences 116, 14749-14754.

Pins, D., and ffytche, D. (2003). The Neural Correlates of Conscious Vision. Cerebral Cortex 13, 461-474.

Povysheva, N.V., Gonzalez-Burgos, G., Zaitsev, A.V., Kröner, S., Barrionuevo, G., Lewis, D.A., and Krimer, L.S. (2006). Properties of excitatory synaptic responses in fast-spiking interneurons and pyramidal cells from monkey and rat prefrontal cortex. Cerebral cortex (New York, NY : 1991) 16, 541-552.

Prins, N., and Kingdom, F.A.A. (2018). Applying the Model-Comparison Approach to Test Specific Research Hypotheses in Psychophysical Research Using the Palamedes Toolbox. Frontiers in Psychology 9.

Rajagovindan, R., and Ding, M. (2011). From prestimulus alpha oscillation to visual-evoked response: an inverted-U function and its attentional modulation. J Cogn Neurosci 23, 1379-1394.

Reimer, J., Froudarakis, E., Cadwell, Cathryn R., Yatsenko, D., Denfield, George H., and Tolias, Andreas S. (2014). Pupil Fluctuations Track Fast Switching of Cortical States during Quiet Wakefulness. Neuron 84, 355-362.

Ress, D., and Heeger, D.J. (2003). Neuronal correlates of perception in early visual cortex. Nat Neurosci 6, 414-420.

Reynolds, J.H., Pasternak, T., and Desimone, R. (2000). Attention increases sensitivity of V4 neurons. Neuron 26, 703-714.

Roe, Anna W., Chelazzi, L., Connor, Charles E., Conway, Bevil R., Fujita, I., Gallant, Jack L., Lu, H., and Vanduffel, W. (2012). Toward a Unified Theory of Visual Area V4. Neuron 74, 12-29.

Rohenkohl, G., Bosman, C.A., and Fries, P. (2018). Gamma Synchronization between V1 and V4 Improves Behavioral Performance. Neuron 100, 953-963.e953.

Schafer, R.J., and Moore, T. (2011). Selective attention from voluntary control of neurons in prefrontal cortex. Science 332, 1568-1571.

Schurger, A., Pereira, F., Treisman, A., and Cohen, J.D. (2010). Reproducibility distinguishes conscious from nonconscious neural representations. Science 327, 97-99.

Siapas, A.G., Lubenov, E.V., and Wilson, M.A. (2005). Prefrontal Phase Locking to Hippocampal Theta Oscillations. Neuron 46, 141-151.

Spitzer, H., Desimone, R., and Moran, J. (1988). Increased attention enhances both behavioral and neuronal performance. Science 240, 338-340.

Steinmetz, N.A., and Moore, T. (2014). Eye movement preparation modulates neuronal responses in area V4 when dissociated from attentional demands. Neuron 83, 496-506.

Steriade, M., Nuñez, A., and Amzica, F. (1993). A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. The Journal of neuroscience : the official journal of the Society for Neuroscience 13, 3252-3265.

Tang, L., and Higley, M.J. (2020). Layer 5 Circuits in V1 Differentially Control Visuomotor Behavior. Neuron 105, 346-354.e345.

van Kempen, J., Gieselmann, M.A., Boyd, M., Steinmetz, N.A., Moore, T., Engel, T.A., and Thiele, A. (2021). Top-down coordination of local cortical state during selective attention. Neuron 109, 894-904.e898.

van Vugt, B., Dagnino, B., Vartak, D., Safaai, H., Panzeri, S., Dehaene, S., and Roelfsema, P.R. (2018). The threshold for conscious report: Signal loss and response bias in visual and frontal cortex. Science 360, 537.

Vijayraghavan, S., Wang, M., Birnbaum, S.G., Williams, G.V., and Arnsten, A.F. (2007). Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. Nat Neurosci 10, 376-384.

Vinck, M., Batista-Brito, R., Knoblich, U., and Cardin, J.A. (2015). Arousal and locomotion make distinct contributions to cortical activity patterns and visual encoding. Neuron 86, 740-754.

Vinck, M., van Wingerden, M., Womelsdorf, T., Fries, P., and Pennartz, C.M. (2010). The pairwise phase consistency: a bias-free measure of rhythmic neuronal synchronization. NeuroImage 51, 112-122.

von Krosigk, M., Bal, T., and McCormick, D. (1993). Cellular mechanisms of a synchronized oscillation in the thalamus. Science 261, 361-364.

Watson, A.B. (1979). Probability summation over time. Vision Research 19, 515-522.

Westerberg, J.A., Schall, M.S., Maier, A., Woodman, G.F., and Schall, J.D. (2022). Laminar microcircuitry of visual cortex producing attention-associated electric fields. Elife 11.

Westerberg, J.A., Sigworth, E.A., Schall, J.D., and Maier, A. (2021). Pop-out search instigates beta-gated feature selectivity enhancement across V4 layers. Proc Natl Acad Sci U S A 118.

Wichmann, F.A., and Hill, N.J. (2001). The psychometric function: I. Fitting, sampling, and goodness of fit. Perception & Psychophysics 63, 1293-1313.

Wonders, C.P., and Anderson, S.A. (2006). The origin and specification of cortical interneurons. Nature reviews Neuroscience 7, 687-696.

Worden, M.S., Foxe, J.J., Wang, N., and Simpson, G.V. (2000). Anticipatory biasing of visuospatial attention indexed by retinotopically specific alpha-band electroencephalography increases over occipital cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 20, Rc63.

Yerkes, R.M., and Dodson, J.D. (1908). The relation of strength of stimulus to rapidity of habit-formation. Journal of Comparative Neurology and Psychology 18, 459-482.

Yu, G., Herman, J.P., Katz, L.N., and Krauzlis, R.J. (2022). Microsaccades as a marker not a cause for attention-related modulation. Elife 11.

Zeng, H., and Sanes, J.R. (2017). Neuronal cell-type classification: challenges, opportunities and the path forward. Nature Reviews Neuroscience 18, 530-546.

Zénon, A., and Krauzlis, R.J. (2012). Attention deficits without cortical neuronal deficits. Nature 489, 434-437.

Zohary, E., Shadlen, M.N., and Newsome, W.T. (1994). Correlated neuronal discharge rate and its implications for psychophysical performance. Nature 370, 140-143.

Zuber, B.L., and Stark, L. (1966). Saccadic suppression: elevation of visual threshold associated with saccadic eye movements. Exp Neurol 16, 65-79.

Figure 1

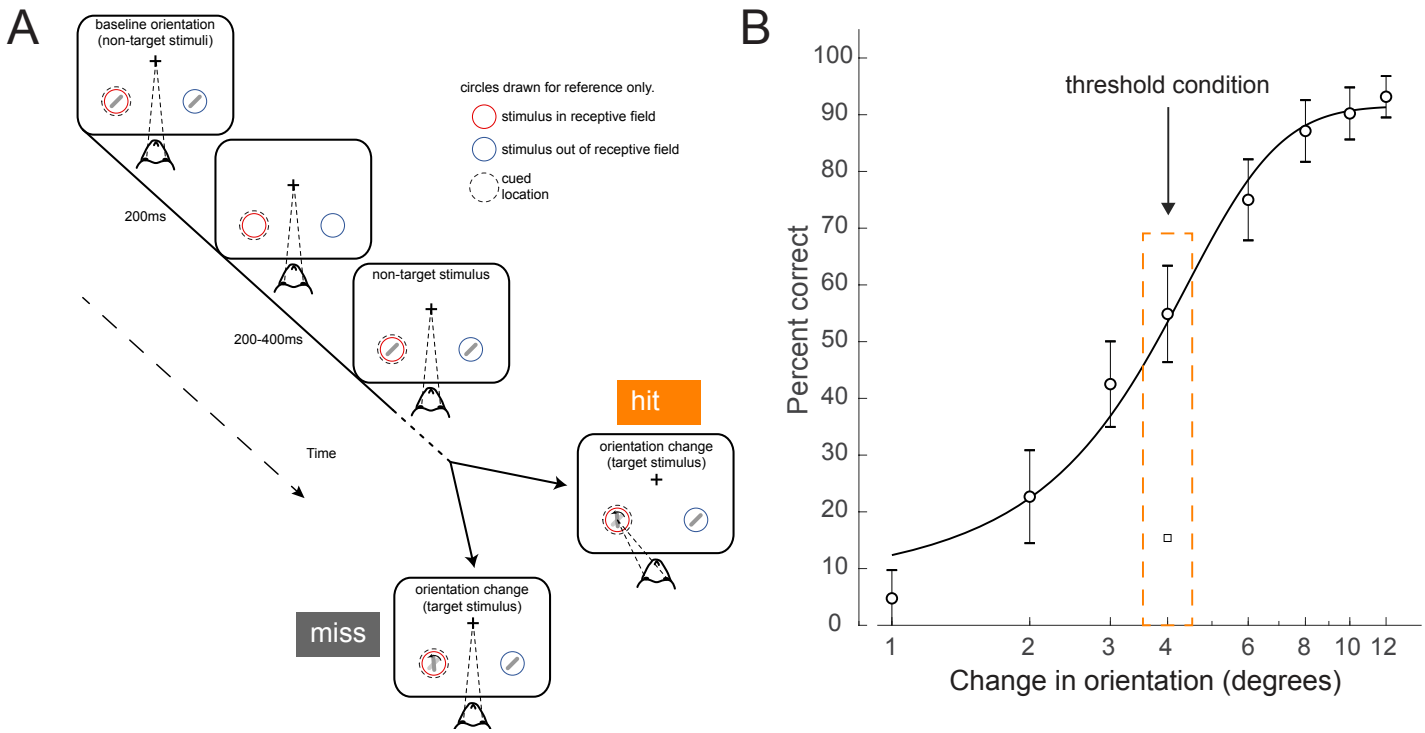

Figure 1. Orientation change detection task at perceptual threshold
(**A**) Schematic of task structure. The monkey initiated a trial by fixating on the center of the screen. Two Gabor stimuli (represented by oriented lines) were presented for 200 ms and then turned off for 200-400ms. This was repeated until, at an unpredictable time, one of the stimuli changed orientation. The monkey could report having seen the change by making an eye movement to the location of the target stimulus to receive a reward (hit trials). If the monkey failed to report the orientation change and maintained fixation on the center of the screen it was not rewarded (miss trials). Before a block of trials, the monkey was cued as to which stimulus was likely to undergo the change (95% valid cue). In 5% of trials the orientation change occurred at the other location (foil trials). Circles indicating the cued location and receptive field are drawn for figure reference only and were not presented during the task. (**B**) Example behavioral psychometric function from one recording session and attention condition. Behavioral performance (hit rate, circles) is presented as a function of orientation change. Data was fitted with a logistic function. The threshold condition, trials with performance halfway between the upper and lower asymptotes of the logistic function, is indicated by the orange box. Error bars represent standard deviation calculated with a jackknife procedure (20 jackknives). The square symbol indicates foil trial performance.

Figure 2

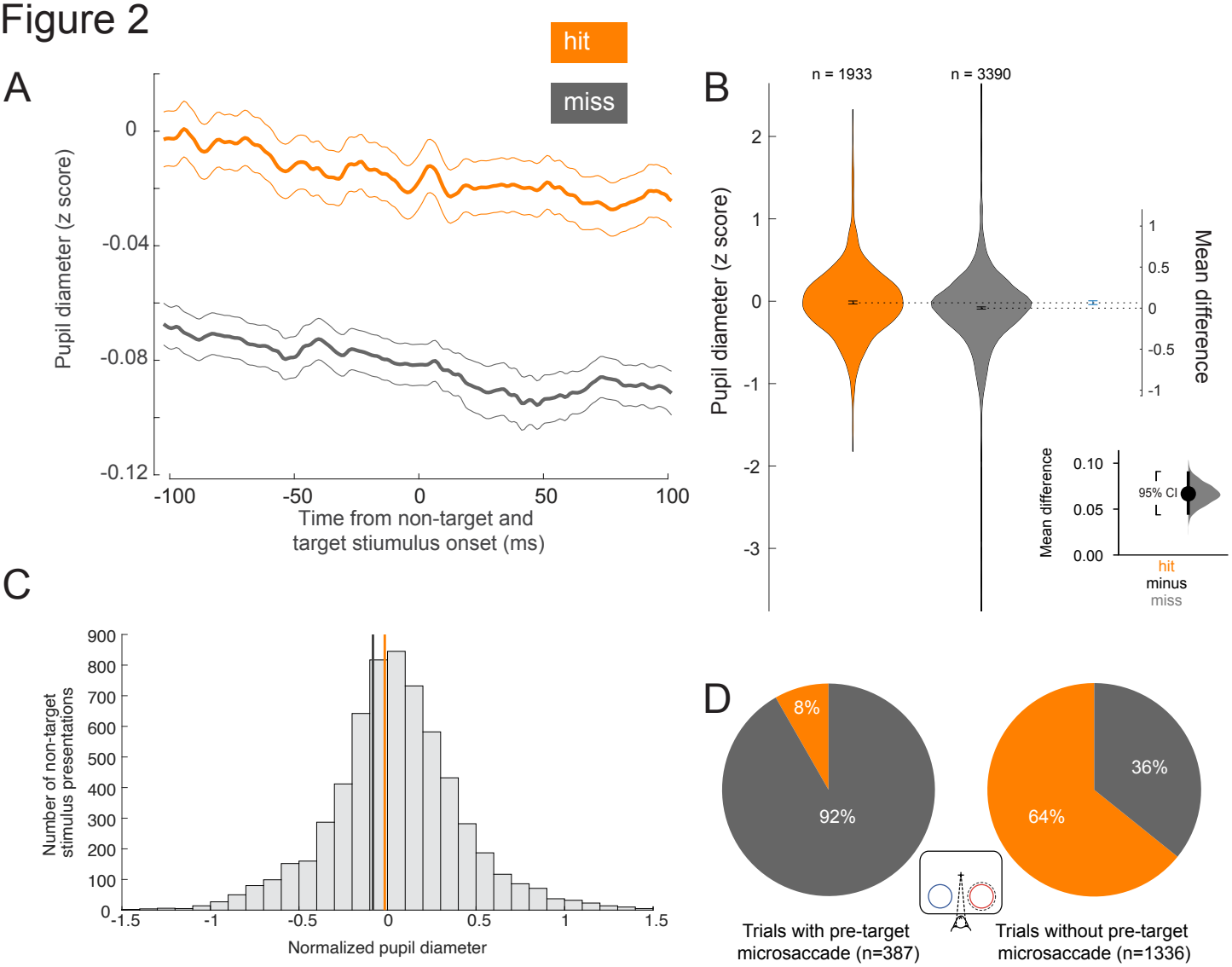

Figure 2. Hit trials have larger pupil diameter whereas microsaccades more often precede misses
(**A**) Normalized pupil diameter for hit and miss trails in the threshold condition. 0 ms corresponds to non-target and target stimulus onset. Mean +/- s.e.m. (**B**) Distribution of pupil diameter values associated with hit and miss trials. Pupil diameter was averaged from 100ms before to 100ms after non-target and target stimulus onset. Violin plots were generated using kernel smoothing (See Experimental Procedures). Error bars represent 95% confidence intervals for the mean of each distribution, and the mean difference (blue, right axes). Inset: zoomed in view of the mean difference between hit and miss trials. Black bar represents a 95% confidence interval of the mean difference. Shaded region reflects the distribution of the bootstrapped estimation of the mean difference. (**C**) Histogram of mean pupil diameter around the time of non-target and target stimulus onset (calculated as in **B**). Orange and gray lines represent the mean pupil diameter for hit and miss trials respectively. (**D**) Hit rate for trials with (left, 387 trials) and without (right, 1336 trials) a microsaccade detected in the time window 0-400ms before target onset. There is a significantly lower hit rate in trials with a microsaccade ($p<0.0001$, X2-test).

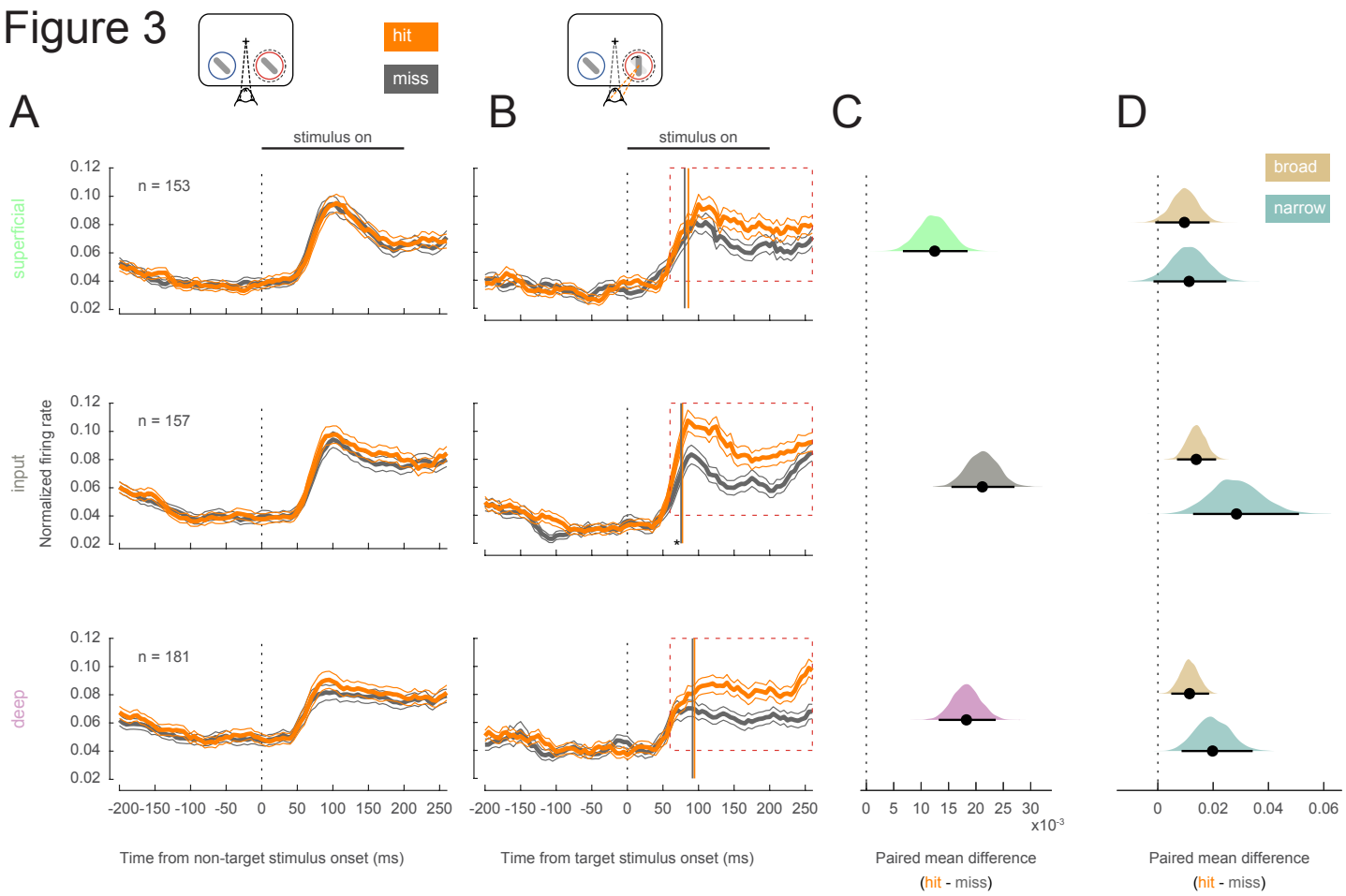

Figure 3. Target stimuli evoke higher firing rates in hit trials
Rows correspond to different layers (top=superficial, middle=input, bottom=deep). (**A**) Non-target population (single and multi-unit) non-target PSTH of visually responsive neurons for the hit (orange) and miss (dark-gray) trials in the threshold condition (mean +/- s.e.m). The horizontal black bar indicates the time and duration of stimulus presentation. (**B**) As in **A** but for target stimuli. The star indicates the time at which firing rates in the input layer first differ significantly between hit and miss trials. Vertical lines represent the mean time firing rates for each neuron rise above the 95% confidence interval of their baseline activity (see also Figure S3C). (**C**) Bootstrapped estimation of the paired mean difference in target stimulus-evoked firing rate across hit and miss trials in the time window 60-260ms (red dotted box in **B**) after target stimulus onset. Shaded regions represent the bootstrapped estimation of the paired mean difference in firing rate (hit - miss), and black lines are 95% confidence intervals. Plots include data from both single and multi-units, separated by layer (top= superficial, middle=input, bottom=deep). (**D**) As in **C**, bootstrapped estimation of the paired mean difference in firing rate for hit trials compared to miss trials in the target stimulus-evoked period, but only for single-units broken up by cell class (gold=broad, teal=narrow).

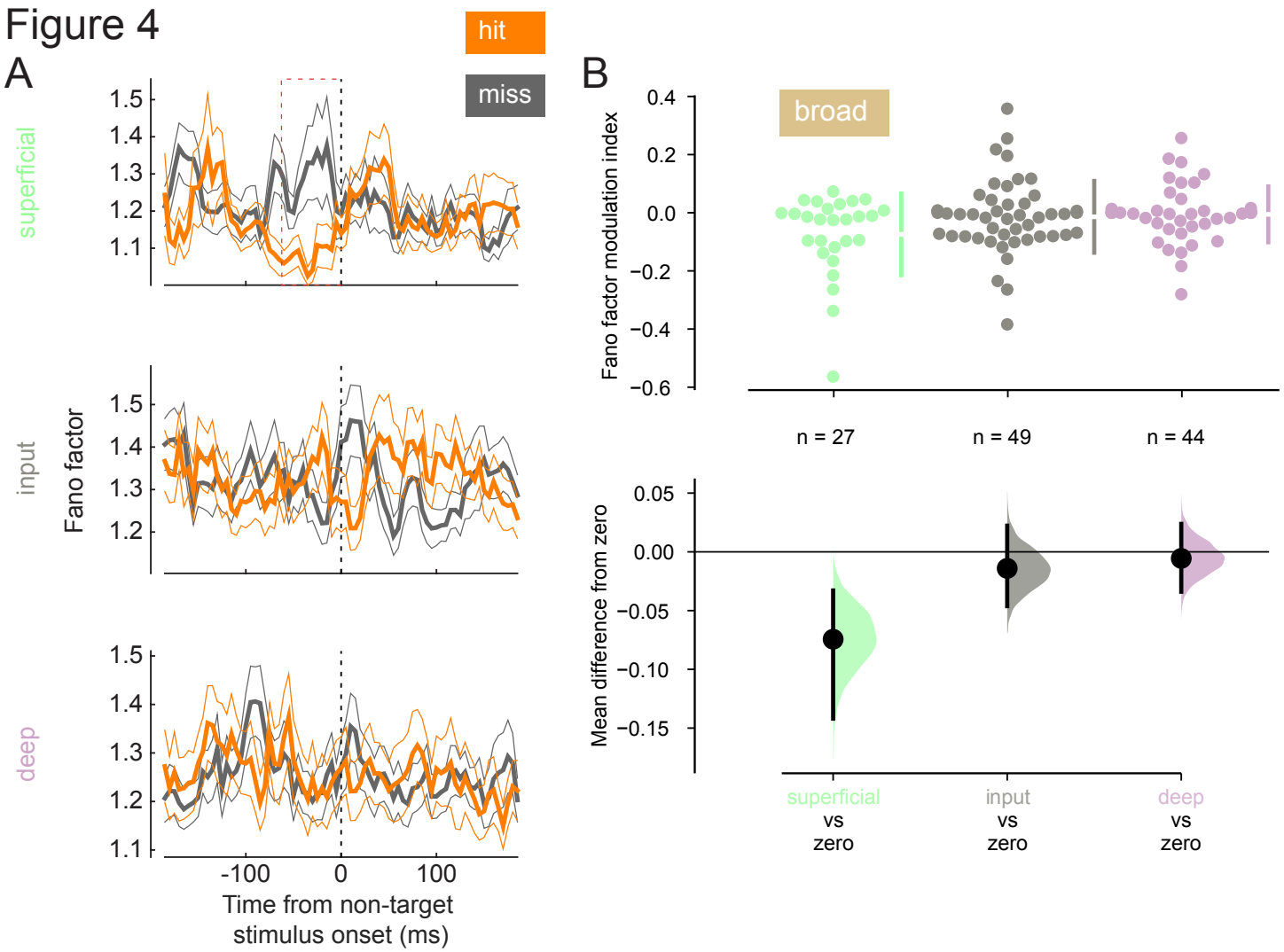

Figure 4. Broad-spiking neurons in the superficial layer have decreased variability in hit trials
(**A**) Rows correspond to different layers (top=superficial, middle=input, bottom=deep). The Fano factor of broad-spiking putative excitatory neurons for the hit and miss trials in the threshold condition (mean +/- s.e.m). There is a significant decrease in variability for the hit trials prior to stimulus onset only in the superficial layer. 0 ms corresponds to non-target stimulus onset. The average Fano factor within a 60ms time-window (red dashed box) prior to stimulus onset is plotted in **B**. (**B**) *Top:* Fano factor modulation index for each broad-spiking neurons recorded in each layer, averaged in the 60ms preceding non-target stimulus onset. *Bottom:* Bootstrapped estimation of the mean difference of the Fano factor modulation index from zero in each of the three layers. Colored curves represent the estimated bootstrapped distribution. Black dots and lines reflect the mean and 95% confidence intervals of the distributions.

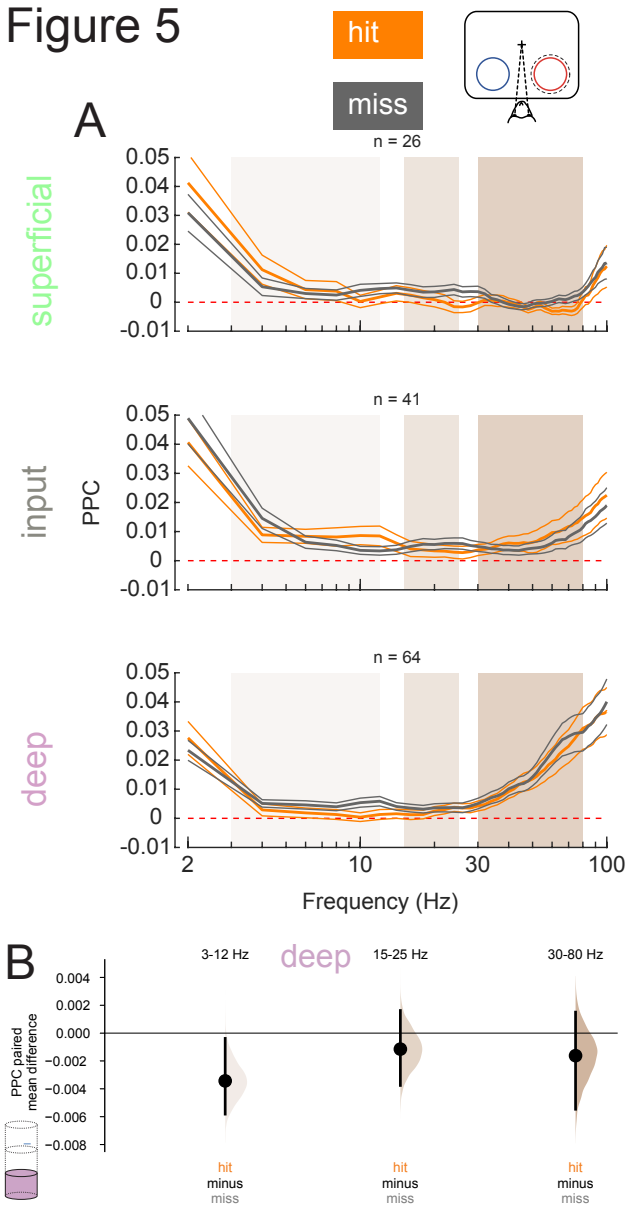

Figure 5. Deep layer neurons are phase-locked to low-frequency rhythms in miss trials
(**A**) Pairwise phase consistency (PPC) of single and multi-units in each layer to the local field potential (LFP) signal recorded from the same channel in hit and miss trials at threshold. PPC was calculated in the pre-stimulus period (0-200 ms before stimulus onset). Dashed red line indicates a PPC of 0, below which there is no consistent relationship between spikes and LFP phase. (**B**) Bootstrapped estimation plot for the paired mean difference in PPC for deep layer neurons over three frequency bands: 3-12Hz, 15-25Hz, 30-80Hz. Curves represent the bootstrapped distribution for the paired difference, and black dots and vertical lines represent the mean and 95% confidence intervals for the paired mean difference

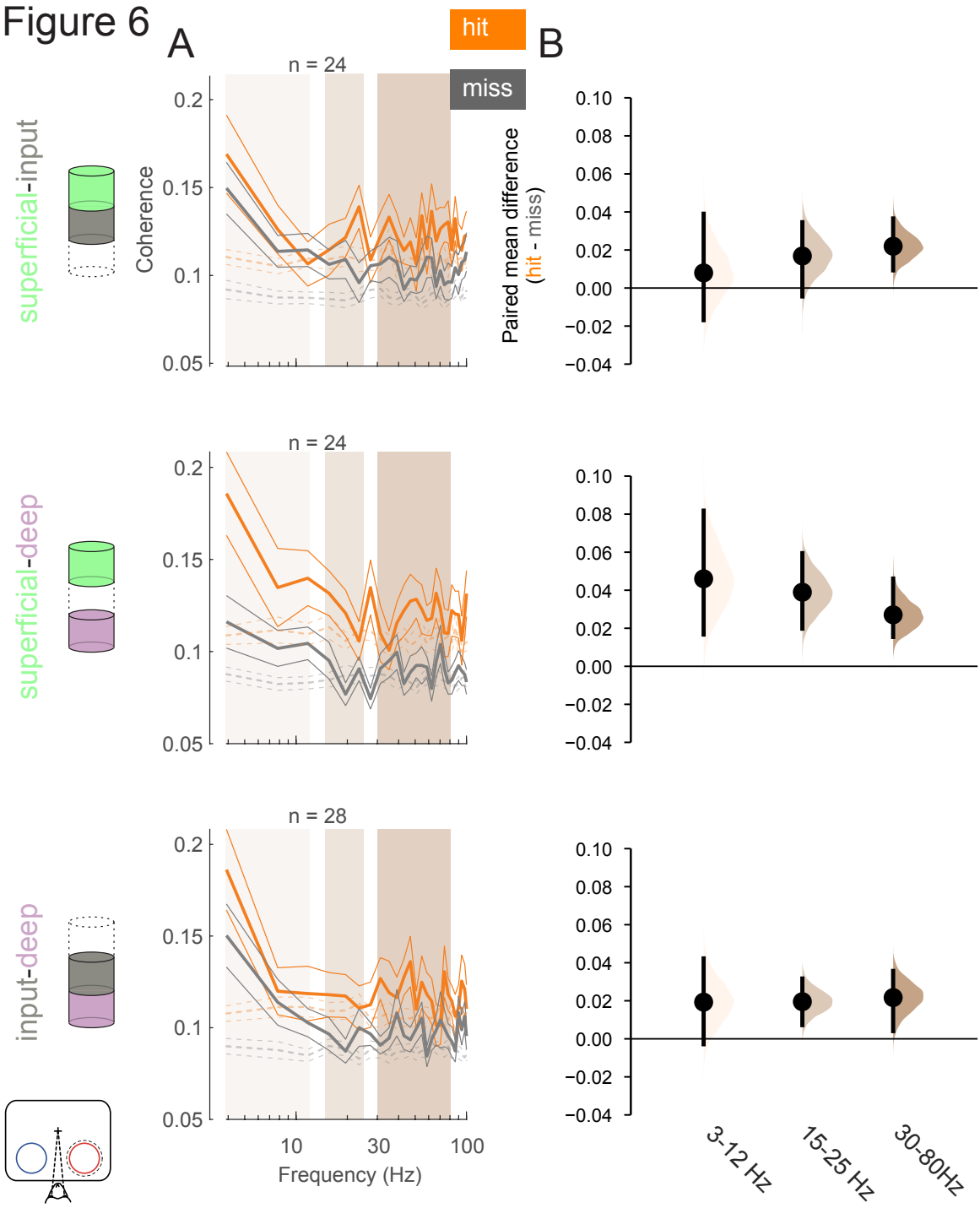

Figure 6. Greater interlaminar coherence in hit trials in the pre-stimulus period
Interlaminar spike-spike coherence in the pre-stimulus period (0-200ms prior to stimulus onset). Rows correspond to different pairs of layers (*top*=superficial-input, *middle*=superficial-deep, *bottom*=input-deep). (**A**) Multi-unit interlaminar spike-spike coherence (SSC) calculated in the 200ms before non-target stimulus onset in hit and miss trials (solid lines, mean +/- s.e.m). Firing rates were matched across hit and miss trials. Dashed lines represent coherence calculated with shuffled trial identities (mean +/- s.e.m). (**B**) Bootstrapped estimation plot for the paired mean difference in SSC for each pair of layers averaged over three frequency bands : 3-12Hz, 15-25Hz, 30-80Hz. Curves represent the bootstrapped distribution for the paired difference, and black dots and vertical lines represent the mean and 95% confidence intervals for the paired mean difference.

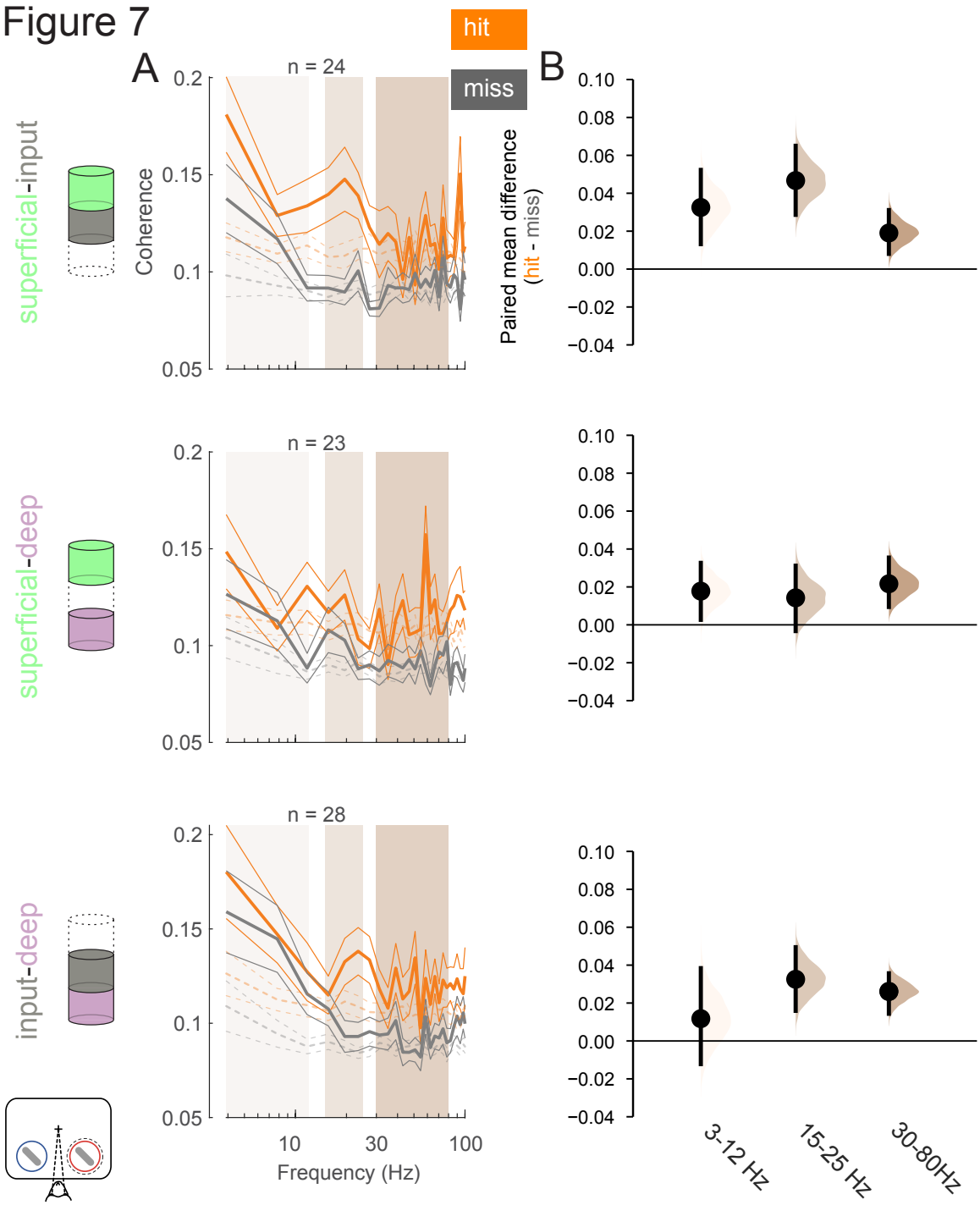

Figure 7. Greater interlaminar coherence in hit trials in the non-target stimulus-evoked period
Interlaminar spike-spike coherence in the non-target stimulus evoked period (60-260ms after stimulus onset). Same conventions as in Figure 6.

Figure 8

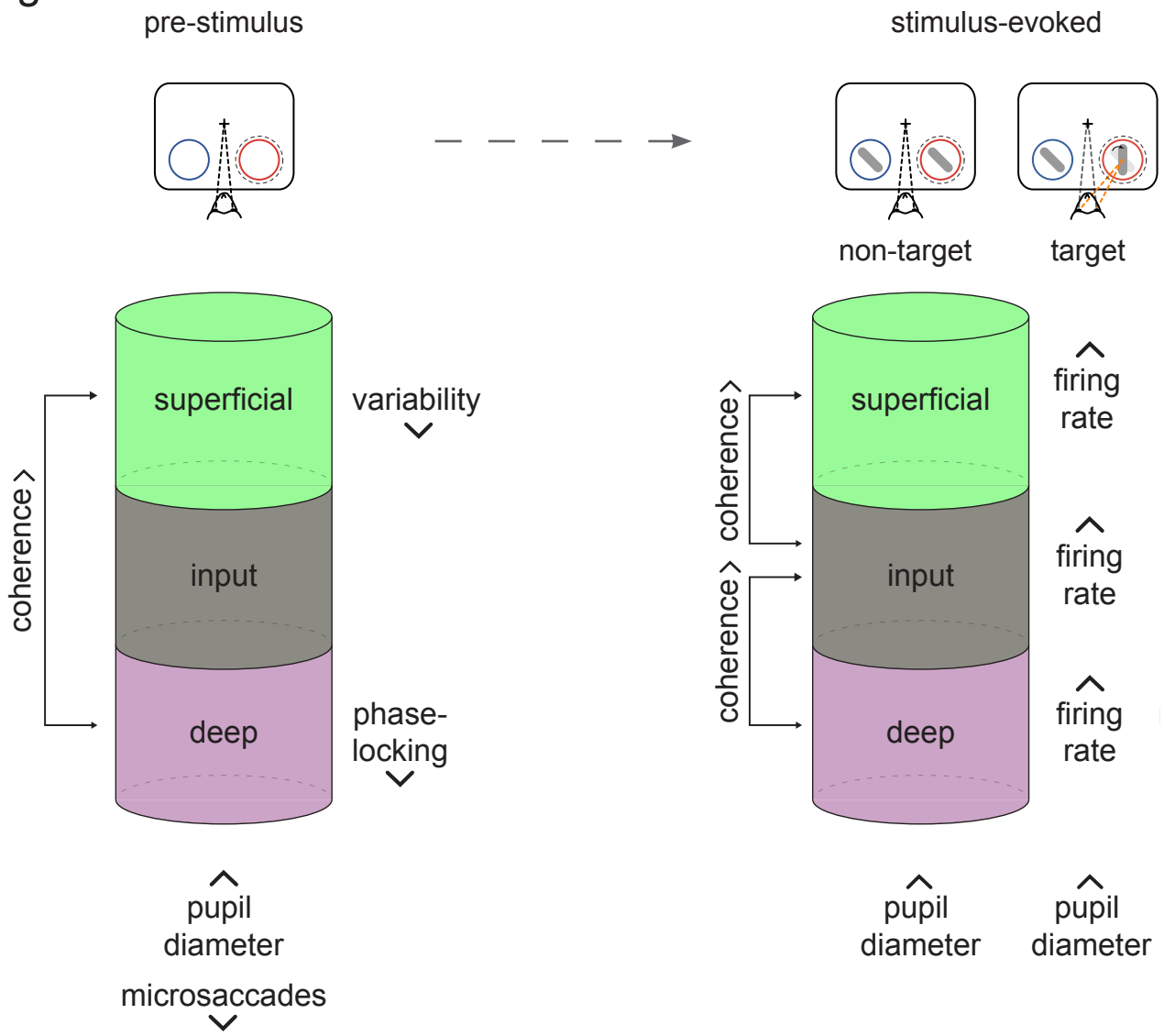

Figure 8. Summary of results
Hit trials have a larger pupil diameter in both the pre-stimulus and stimulus-evoked time periods. In the pre-stimulus period, hits are characterized by decreased variability in superficial layer broad-spiking neurons, less phase-locking of deep layer neurons to low-frequency LFPs, and greater interlaminar spike-spike coherence between the superficial and deep layers. Microsaccades in the pre-stimulus period are associated with a much lower hit rate. Stimuli evoke higher firing rates across all three layers in hits. The stimulus-evoked period is associated with greater interlaminar spike-spike coherence between the input layer and the superficial and deep layers.

Figure S1

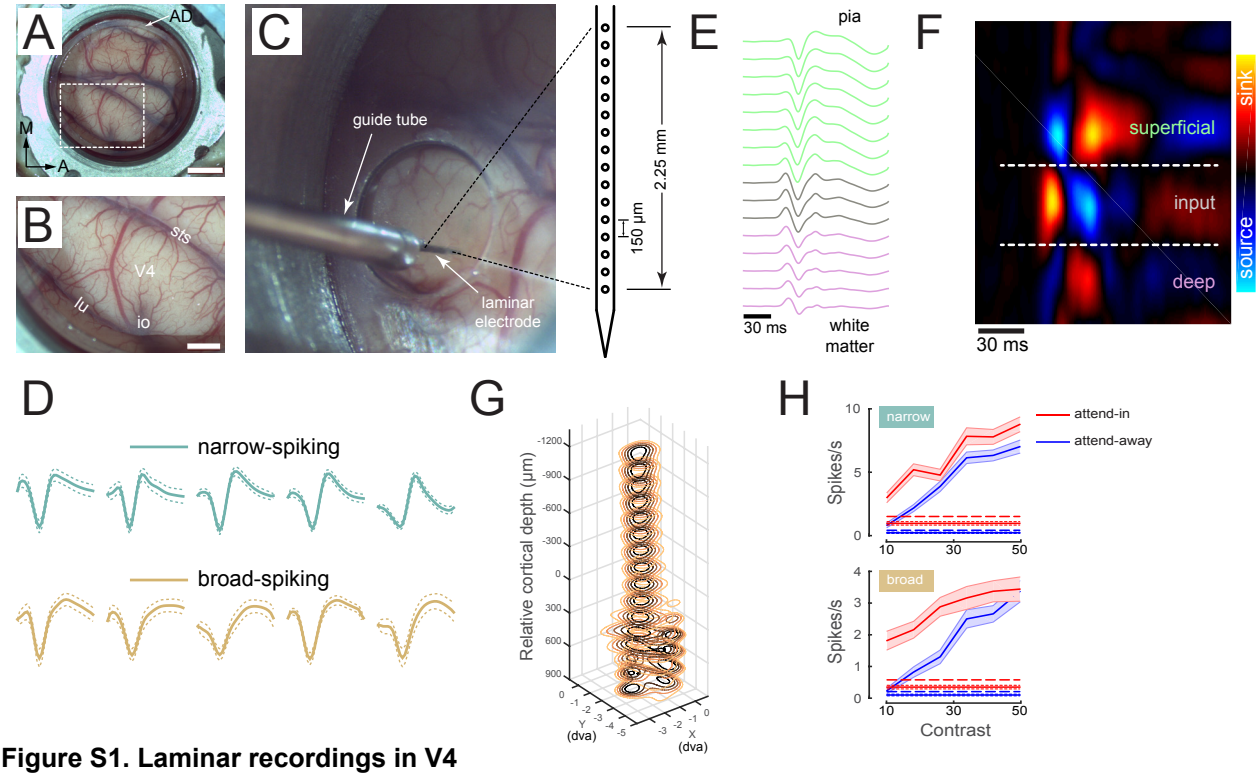

Figure S1. Laminar recordings in V4

(**A**) An artificial dura (AD) chamber is shown over dorsal V4 in the right hemisphere of Monkey A. The native dura mater was resected and replaced with a silicone based artificial dura, thereby providing an optically clear window into the cortex. Scale bar = 5mm. (**B**) An enlarged view of the boxed region in **A** clearly shows the sulci and the microvasculature. sts = superior temporal sulcus, lu = lunate sulcus, io = inferior occipital sulcus. Area V4 lies on the pre-lunate gyrus between the superior temporal and lunate sulci. Scale bar = 2mm. (**C**) Electrophysiology setup: a plastic stabilizer with a circular aperture is secured in place inside the chamber such that the aperture is centered over the pre-lunate gryus. A 16-channel linear array electrode (electrode spacing 150m) is positioned over the center of the gyrus and lowered into the cortex under microscopic guidance. The microvasculature pattern was used as a reference to target different cortical sites across recording sessions. (**D**) Example recording session in monkey C depicting 12 single unit waveforms (mean +/- s.e.m.) isolated along the cortical column. Teal waveforms correspond to narrow-spiking putative interneurons and gold waveforms correspond to broad-spiking putative excitatory units. (**E**) Stimulus triggered local field potentials (LFPs) obtained by flashing 30ms high contrast ring stimuli in the receptive field of a V4 cortical column. LFP traces averaged across all stimulus repeats are shown color-coded as being part of either the superficial (green), input (gray) or deep (pink) layers. Layer assignment was done after current source-density analysis. (**F**) Current source-density (CSD) calculated as the second spatial derivative of the stimulus triggered LFPs and displayed as a colored map. The x-axis represents time from stimulus onset; the y-axis represents cortical depth oriented such that the pial surface is at the top and the white matter is at the bottom. Red hues represent current sink, blue hues represent current source. The input layer is identified as the first current sink followed by a reversal to current source. The superficial and deep layers have the opposite sink-source pattern. The CSD map has been spatially smoothed for visualization. (**G**) Stacked contour plots show spatial receptive fields (RFs) mapped along each contact point in the laminar probe. The spatial receptive fields were obtained by applying reverse correlation to the LFP power evoked by sparse pseudo-random sequences of Gabor stimuli. The RFs are well aligned, indicating perpendicular penetration down a cortical column. Zero depth represents the center of the input layer as estimated from the CSD. (**H**) Contrast response functions – spikes rate as a function of stimulus contrast – are shown for 2 example units identified in a single recording session in Monkey A. Red and blue traces correspond to the attend-in to RF and attend-away from RF conditions respectively. The dotted lines represent the corresponding background firing-rates. The dashed lines are 4 standard deviations above baseline. A unit was considered as visually responsive, if the contrast response functions exceeded this threshold in both the attention conditions. Mean +/- s.e.m. Panels are reproduced from Nandy et al. (2017).

Figure S2

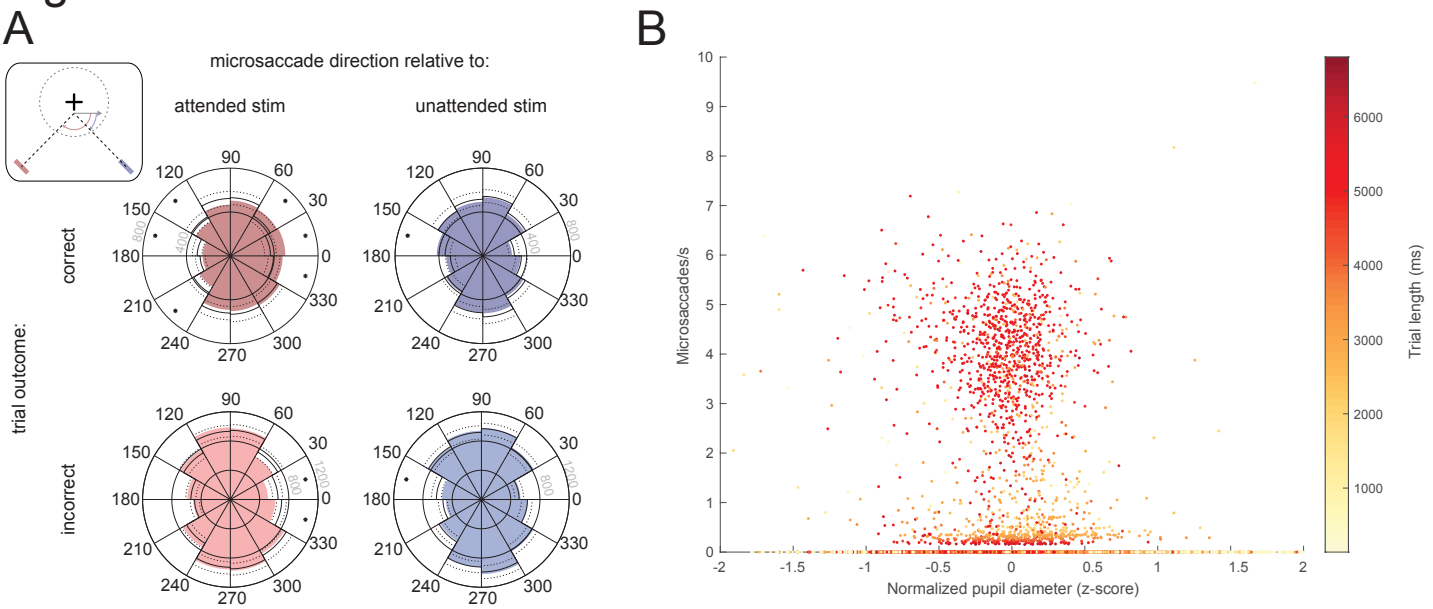

Figure S2. Microsaccades are preferentially directed towards the target in correct trials and have a slight correlation with pupil diameter

Data is presented for all trials, regardless of orientation change (not just the threshold condition). (**A**) The histograms represent the direction of microsaccades relative to the attended stimulus (left column) or unattended stimulus (right column) in correct (top row) and incorrect (bottom row) trials. Black lines represent the mean (solid) and 99.5% confidence interval (dashed) of the bootstrapped null distribution estimated by pooling correct and incorrect microsaccades. . *Inset:* Schematic for calculation of relative microsaccade direction. Microsaccade is represented by the gray arrow (**B**) Scatterplot of microsaccade rate versus mean normalized pupil diameter, shows a small but statistically significant relationship between the two quantities (r^2 = 0.006,). Each dot is color-coded by trial length. 4% of trials with mean pupil diameter >2 or <-2 not shown.

Figure S3

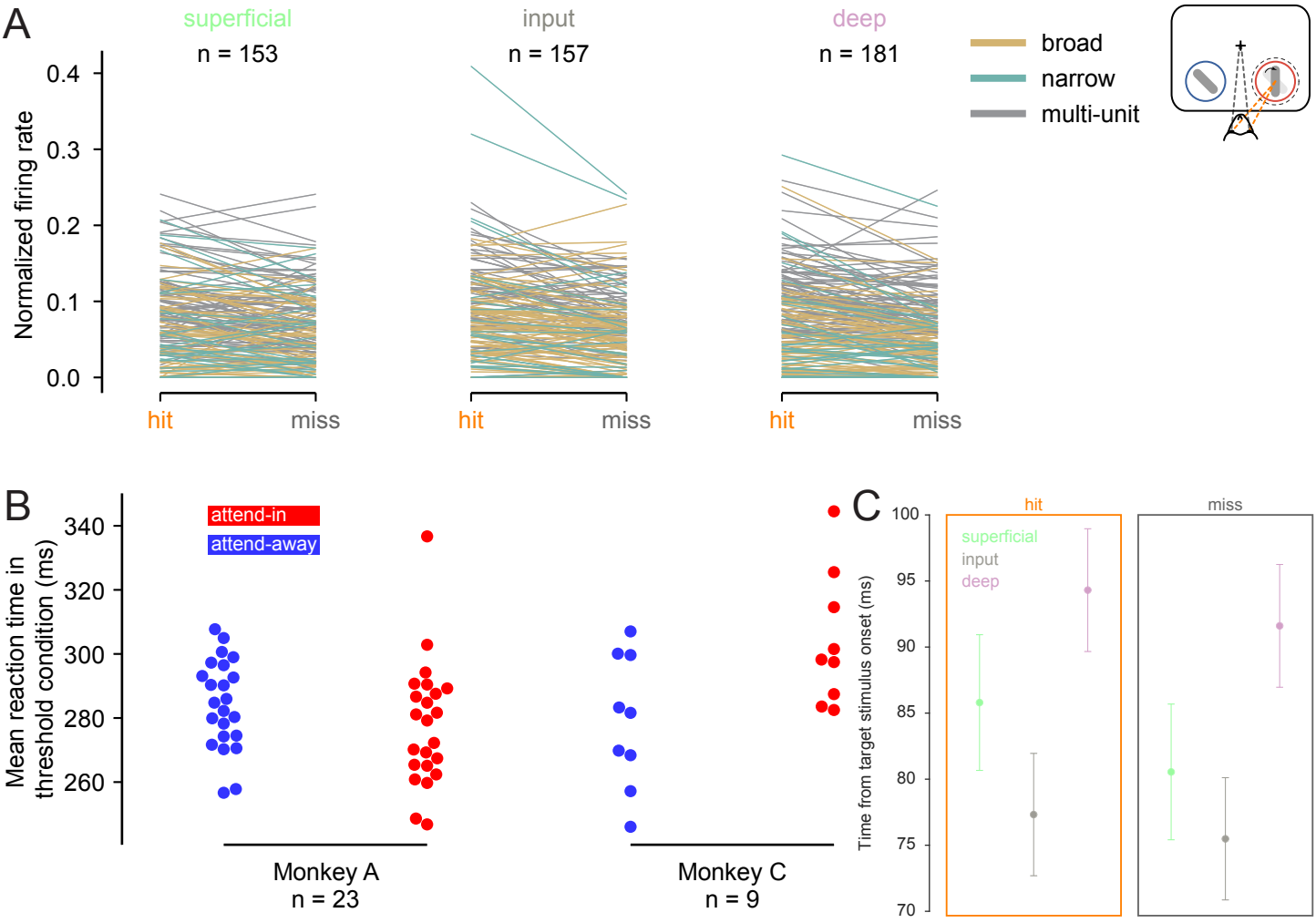

Figure S3. Firing rates for individual neurons and reaction time in threshold condition
(**A**) Target stimulus-evoked normalized firing rates in hit and miss trials for each recorded single and multi-unit cluster in hit and miss trials. Clusters are divided by layer: left=superficial, middle=input, right=deep. Related to Figure 3B. Each line represents the mean firing rate in response to target stimuli in hit and miss trials for a given unit. Data is color coded by unit type (gold=broad, teal=narrow, gray=multi-unit). See Experimental Procedures for normalization method. (**B**) Mean reaction time in response to the target stimulus in the threshold condition in both attend-in (red) and attend-away (blue) trials for each recording session. (**C**) Mean time at which single- and multi-unit firing rates rise significantly above pre-stimulus baseline in response to target stimuli in hit and miss trials. Mean +/- s.e.m.

Figure S4

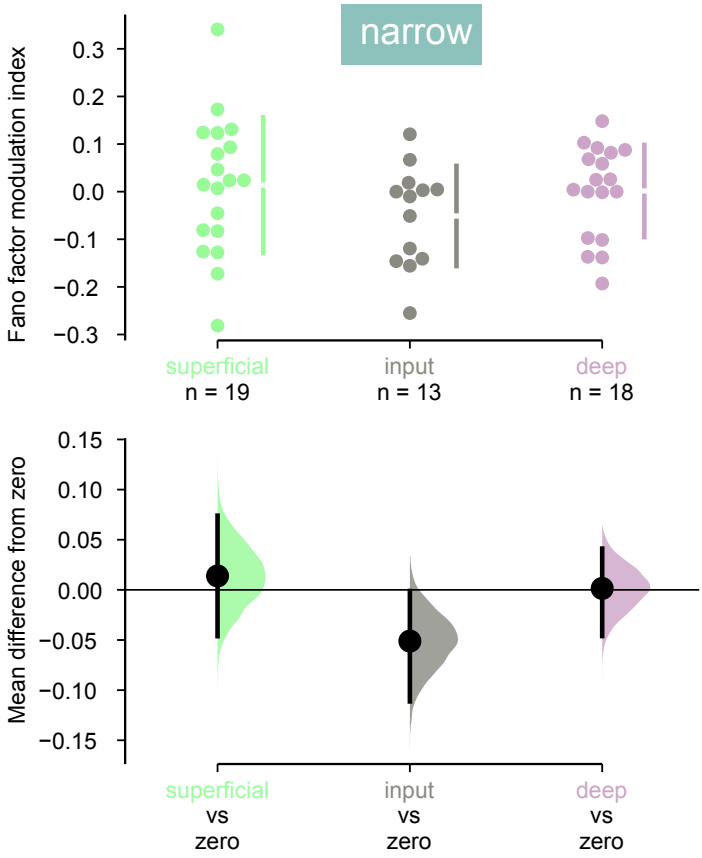

Figure S4. Narrow-spiking neurons do not have decreased variability in hit trials
Top: Fano factor modulation index for each narrow-spiking neurons recorded in each layer, averaged in the 60ms preceding non-target stimulus onset. *Bottom*: Bootstrapped estimation of the mean difference of the Fano factor modulation index from zero in each of the three layers. Colored curves represent the estimated bootstrapped distribution. Black dots and lines reflect the mean and 95% confidence intervals of the distributions.

Figure S5

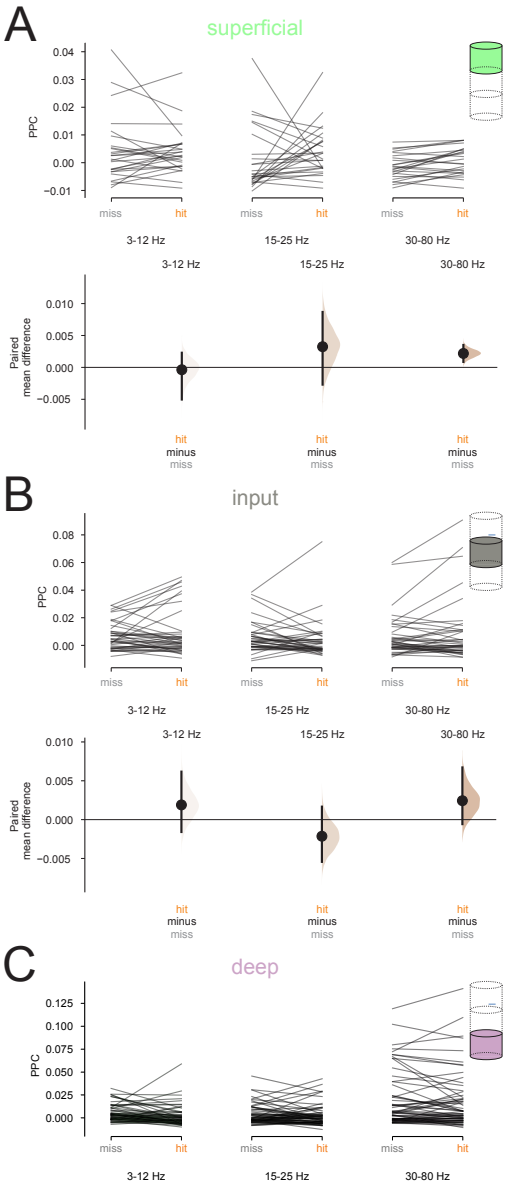

Figure S5. Additional PPC data

(**A-B**) *Top:* Raw PPC values calculated for clusters recorded in the superficial (**A**) and (**B**) input layers in hit and miss trials, averaged into three frequency bands, 3-12 Hz, 15-25 Hz, and 30-80 Hz. PPC was calculated using the LFP recorded on the same channel as the spikes. *Bottom:* Bootstrapped estimation of the paired mean difference in PPC across hit and miss trials for each frequency band. Note that although there appears to be a difference in high-frequency PPC in the superficial layer, this population does not have significantly positive PPC in either condition, indicating that there is no phase-locking in either hits or misses. (**C**) Raw PPC values for neurons recorded in the deep layer in hit and miss trials, averaged into the same 3 frequency bands. Related to Figure 5B.

Table S1: Corresponding Null-Hypothesis Testing Results

Figure	Null-Hypothesis Test	P-Value
2B (pupil diameter)	*t*-test (unpaired)	$p = 6.63624\mathrm{e} - 09$
2C (microsaccades)	X^2-test	$p \ll 0.0001$
3B (target-evoked firing rate, single and multi-units)	*t*-test (paired) Bonferoni Corrected for 3 comparisons, $\alpha = 0.0166$	Superficial: $p = 4.19108\mathrm{e} - 05$ Input: $p = 1.10838\mathrm{e} - 11$ Deep: $p = 1.75826\mathrm{e} - 11$
3C (target-evoked firing rate, broad-spiking)	*t*-test (paired) Bonferoni Corrected for 3 comparisons, $\alpha = 0.0166$	Superficial: $p = 0.0526902$ Input: $p = 0.000124947$ Deep: $p = 0.00119012$
3C (target-evoked firing rate, narrow-spiking)	*t*-test (paired) Bonferoni Corrected for 3 comparisons, $\alpha = 0.0166$	Superficial: $p = 0.103689$ Input: $p = 0.00570757$ Deep: $p = 0.00393437$
4B (Fano Factor modulation index, broad)	*t*-test (unpaired) Bonferoni Corrected for 3 comparisons, $\alpha = 0.0166$	Superficial: $p = 0.0102155$ Input: $p = 0.418197$ Deep: $p = 0.708115$
S4 (Fano factor modulation index, narrow)	*t*-test (unpaired) Bonferoni Corrected for 3 comparisons, $\alpha = 0.0166$	Superficial: $p = 0.676039$ Input: $p = 0.10532$ Deep: $p = 0.950487$
5B (deep PPC)	*t*-test (paired) Bonferoni Corrected for 3 comparisons, $\alpha = 0.0166$	3-12 Hz: $p = 0.0142$ 15-25 Hz: $p = 0.4064$ 30-80 Hz: $p = 0.3600$
6B (top, superficial-input SSC modulation index)	*t*-test Bonferoni Corrected for 3 comparisons, $\alpha = 0.0166$	3-12 Hz: $p = 0.925338$ 15-25 Hz: $p = 0.107304$ 30-80 Hz: $p = 0.00394525$
6B (middle, superficial-deep SSC modulation index)	*t*-test Bonferoni Corrected for 3 comparisons, $\alpha = 0.0166$	3-12 Hz: $p = 0.0125919$ 15-25 Hz: $p = 0.00116552$ 30-80 Hz: $p = 0.00142568$
6B (bottom, input-deep SSC modulation index)	*t*-test Bonferoni Corrected for 3 comparisons, $\alpha = 0.0166$	3-12 Hz: $p = 0.260698$ 15-25 Hz: $p = 0.0358178$ 30-80 Hz: $p = 0.0325516$
7B (top, superficial-input SSC modulation index)	*t*-test Bonferoni Corrected for 3 comparisons, $\alpha = 0.0166$	3-12 Hz: $p = 0.00344279$ 15-25 Hz: $p = 3.17111\mathrm{e} - 05$ 30-80 Hz: $p = 0.00396824$
7B (middle, superficial-deep SSC modulation index)	*t*-test Bonferoni Corrected for 3 comparisons, $\alpha = 0.0166$	3-12 Hz: $p = 0.0164846$ 15-25 Hz: $p = 0.0890114$ 30-80 Hz: $p = 0.00441928$
7B (bottom, input-deep SSC modulation index)	*t*-test Bonferoni Corrected for 3 comparisons, $\alpha = 0.0166$	3-12 Hz: $p = 0.241036$ 15-25 Hz: $p = 0.00140176$ 30-80 Hz: $p = 6.84271\mathrm{e} - 05$
S5B (superficial PPC)	*t*-test (paired) Bonferoni Corrected for 3 comparisons, $\alpha = 0.0166$	3-12 Hz: $p = 0.8307$ 15-25 Hz: $p = 0.2812$ 30-80 Hz: $p = 0.0037$*
S5C (input PPC)	*t*-test (paired) Bonferoni Corrected for 3 comparisons, $\alpha = 0.0166$	3-12 Hz: $p = 0.3440$ 15-25 Hz: $p = 0.2517$ 30-80 Hz: $p = 0.1881$

* Although this p-value is significant, the PPC in both conditions is below 0, indicating there is no phase-locking in either condition (Vinck et al., 2010).

Table S2: GLM Values

Variable (Z-Scored)	Estimated Coefficient	P-Value
Pupil Diameter	0.11754	$p = 0.32869$
Pretarget Microsaccades	-1.3116	$p = 6.0757e - 08$
Target Superficial FR	0.22414	$p = 0.091946$
Target Input FR	0.3276	$p = 0.020068$
Target Deep FR	0.11399	$p = 0.45762$

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Surgical Procedures

Surgical procedures have been described in detail previously (Nandy et al., 2017; Nassi et al., 2015; Ruiz et al., 2013). In brief, an MRI compatible low-profile titanium chamber was placed over the pre-lunate gyrus, on the basis of preoperative MRI in two rhesus macaques (right hemisphere in Monkey A, left hemisphere in Monkey C). The native dura mater was then removed, and a silicone based optically clear artificial dura (AD) was inserted, resulting in an optical window over dorsal V4 (Figure S1A, B). Antibiotic (amikacin or gentamicin) soaked gauzed was placed in the chamber between recording sessions to prevent bacterial growth. All procedures were approved by the Institutional Animal Care and Use Committee and conformed to NIH guidelines.

Electrophysiology

At the beginning of each recording session a plastic insert, with an opening for targeting electrodes, was lowered into the chamber and secured. This served to stabilize the recording site against cardiac pulsations. Neurons were recorded from cortical columns in dorsal V4 using 16-channel linear array electrodes (‘laminar probes’, Plexon Inc., Plexon V-probe). The laminar probes were mounted on adjustable X-Y stages attached to the recording chamber and positioned over the center of the pre-lunate gyrus under visual guidance through a microscope (Zeiss Inc., Figure S1C). This ensured that the probes were maximally perpendicular to the surface of the cortex and thus had the best possible trajectory to make a perpendicular penetration down a cortical column. Across recording sessions, the probes were positioned over different sites along the center of the

gyrus in the parafoveal region of V4 with receptive field (RF) eccentricities between 2 and 7 degrees of visual angle. Care was taken to target cortical sites with no surface micro-vasculature, with surface micro-vasculature used as reference so that the same cortical site was not targeted across recording sessions. The probes were advanced using a hydraulic microdrive (Narishige Inc.) to first penetrate the AD and then through the cortex under microscopic visual guidance. Probes were advanced until the point that the top-most electrode (toward the pial surface) registered local field potential (LFP) signals. At this point, the probe was retracted by about 100-200 μm to ease the dimpling of the cortex due to the penetration. This procedure greatly increased the stability of the recordings and increased the neuronal yield in the superficial electrodes.

The distance from the tip of the probes to the first electrode contact was either 300 μm or 700 μm. The inter-electrode distance was 150 μm, thus minimizing the possibility of recording the same neural spikes in adjacent recording channels. Electrical signals were recorded extracellularly from each channel. These were then amplified, digitized and filtered either between 0.5 Hz and 2.2 kHz (LFPs) or between 250 Hz and 8 kHz (spikes) and stored using the Multichannel Acquisition Processor system (MAP system, Plexon Inc.). Spikes and LFPs were sampled at 40 and 10 kHz respectively. LFP signals were further low-pass filtered with a 6th order Butterworth filter with 300Hz cut-off and down-sampled to 1 kHz for further analysis. Spikes were classified as either multi-unit clusters or isolated single units using the Plexon Offline Sorter software program. Single units were identified based on two criteria: (a) if they formed an identifiable cluster, separate from noise and other units, when projected into the principal components of waveforms recorded on that electrode and (b) if the inter-spike interval (ISI) distribution

had a well-defined refractory period. Single-units were classified as either narrow-spiking (putative interneurons) or broad-spiking (putative pyramidal cells) based on methods described in detail previously (Mitchell et al., 2007; Nandy et al., 2017). Specifically, only units with waveforms having a clearly defined peak *preceded* by a trough were potential candidates. The distribution of trough-to-peak duration was clearly bimodal (Hartigan's Dip Test, $p = 0.012$) (Hartigan and Hartigan, 1985). Units with trough-to-peak duration less than 225 μs were classified as narrow-spiking units; units with trough-to-peak duration greater than 225 μs were classified as broad-spiking units (Figure S1D; gray=narrow, black=broad).

Data was collected over 32 sessions (23 sessions in Monkey A, 9 in Monkey C), yielding a total of 413 single units (146 narrow-spiking, 267 broad-spiking) and 296 multi-unit clusters. Per session unit yield was considerably higher in Monkey C compared to Monkey A, resulting in a roughly equal contribution of both monkeys toward the population data.

Task, Stimuli and Inclusion Criteria

Stimuli were presented on a computer monitor placed 57 cm from the eye. Eye position was continuously monitored with an infrared eye tracking system (ISCAN ETL-200). Trials were aborted if eye position deviated more than 1° (degree of visual angle, 'dva') from fixation. Experimental control was handled by NIMH Cortex software (http://www.cortex.salk.edu/). Eye-position (all sessions) and pupil diameter (18/32 sessions) data were concurrently recorded and stored using the MAP system.

Receptive Field Mapping: At the beginning of each recording session, neuronal RFs were mapped using subspace reverse correlation in which Gabor (eight orientations, 80% luminance contrast, spatial frequency 1.2 cycles/degree, Gaussian half-width 2°) or ring stimuli (80% luminance contrast) appeared at 60 Hz while the monkeys maintained fixation. Each stimulus appeared at a random location selected from an 11x11 grid with 1° spacing in the appropriate visual quadrant. Spatial receptive maps were obtained by applying reverse correlation to the evoked local field potential (LFP) signal at each recording site. For each spatial location in the 11x11 grid, we calculated the time-averaged power in the stimulus evoked LFP (0-200ms after each stimulus flash) at each recording site. The resulting spatial map of LFP power was taken as the spatial RF at the recording site. For the purpose of visualization, the spatial RF maps were smoothed using spline interpolation and displayed as stacked contours plots of the smoothed maps (Figure S1G). All RFs were in the lower visual quadrant (lower-left in Monkey A, lower-right in Monkey C) and with eccentricities between 2 and 7 dva.

Current Source Density Mapping: In order to estimate the laminar identity of each recording channel, we used a current source-density (CSD) mapping procedure (Mitzdorf, 1985). Monkeys maintained fixation while 100% luminance contrast ring stimuli were flashed (30ms) centered at the estimated RF overlap region across all channels. The size of the ring was scaled to about three-quarters of the estimated diameter of the RF. CSD was calculated as the second spatial derivative of the flash-triggered LFPs (Figure S1E). The resulting time-varying traces of current across the cortical layers can be visualized as CSD maps (Figure S1F; maps have been spatially smoothed with a Gaussian kernel for aid in visualization). Red regions depict current sinks in the corresponding region of

the cortical laminae; blue regions depict current sources. The input layer (Layer 4) was identified as the first current sink followed by a reversal to current source. The superficial (Layers 1-3) and deep (Layers 5-6) layers had opposite sink-source patterns. LFPs and spikes from the corresponding recording channels were then assigned to one of three layers: superficial, input or deep.

Attention task: In the main experiment, monkeys had to perform an attention-demanding orientation change-detection task (Figure 1A). While the monkey maintained fixation, two achromatic Gabor stimuli (orientation optimized per recording session, spatial frequency 1.2 cycles/degree, 6 contrasts randomly chosen from an uniform distribution of luminance contrasts, $c = [10, 18, 26, 34, 42, 50\%]$) were flashed on for 200 ms and off for a variable period chosen from a uniform distribution between 200-400 ms. One of the Gabors was flashed at the receptive field overlap region, the other at a location of equal eccentricity across the vertical meridian. At the beginning of a block of trials, the monkey was spatially cued ('instruction trials') to covertly attend to one of these two spatial locations. During these instruction trials, the stimuli were only flashed at the spatially cued location. No further spatial cue was presented during the rest of the trials in a block. At an unpredictable time drawn from a truncated exponential distribution (minimum 1 s, maximum 5 s, mean 3 s), one of the two stimuli changed in orientation. The monkey was rewarded for making a saccade to the location of orientation change. The monkey was rewarded for only those saccades where the saccade onset time was within a window of 100-400 ms after the onset of the orientation change. The orientation change occurred at the cued location with 95% probability and at the uncued location with 5% probability ('foil trials'). We controlled task difficulty by varying the degree of

orientation change (Δ_{ori}), which was randomly chosen from one of the following: 1, 2, 3, 4, 6, 8, 10 and 12°. The orientation change in the foil trials was fixed at 4°. These foil trials allowed us to assess the extent to which the monkey was using the spatial cue, with the expectation that there would be an impairment in performance and slower reaction times compared to the case in which the change occurred at the cued location. If no change occurred before 5s, the monkey was rewarded for maintaining fixation ('catch trials', 13% of trials). We refer to all stimuli at the baseline orientation as 'non-targets' and the stimulus flash with the orientation change as the 'target'.

Inclusion criteria: Of the 413 single units, we included only a subset of neurons that were visually responsive for further analysis. For each neuron we calculated its baseline firing-rate for each attention condition (attend into RF ['attend-in' or 'IN'], attend away from RF ['attend-away' or 'AWAY']) from a 200ms window before a stimulus flash. We also calculated the neuron's contrast response function for both attention conditions (Figure S1H). This was calculated as the firing rate over a window between 60-200 ms after stimulus onset and averaged across all stimulus flashes (restricted to non-targets) of a particular contrast separately for each attention condition. A neuron was considered visually responsive if any part of the contrast response curves exceeded the baseline rate by 4 standard deviations for both attention conditions. This left us with 274 single units (84 narrow-spiking, 190 broad-spiking) and 217 multi-unit clusters for further analysis.

Data analysis

Behavioral Analysis: For each orientation change condition Δ_{ori}, we calculated the hit rate as the ratio of the number of trials in which the monkey correctly identified the

target by making a saccadic eye-movement to the location of the target over the number of trials in which the target was presented. The hit rate as a function of Δ_{ori}, yields a behavioral psychometric function (Figure 1B). We performed this analysis independently for each recording day for each monkey, yielding a similar but distinct psychometric function for every session. Psychometric functions were fitted with a smooth logistic function (Prins and Kingdom, 2018). Error bars were obtained by a jackknife procedure (20 jackknives, 5% of trials left out for each jackknife). Performance for the foil trials were calculated similarly as the hit rate for trials in which the orientation change occurred at the un-cued location (Figure 1B, square symbol). For each fitted psychometric function in both the attend-in and attend-away conditions, we calculated the threshold of the fitted logistic function (i.e. the Δ_{ori} at which performance was mid-way between the lower and upper asymptotes). Because the threshold of the fitted function always lies somewhere on the axis of Δ_{ori}, but not exactly at an orientation change presented to the subject, we then defined the threshold condition as the subset of trials in which the orientation change of the target stimulus was closest to the threshold of the fitted function (Figure 1B). We restricted further analysis to this threshold condition. For this threshold condition we identified the trials in which the monkey correctly identified the target as 'hit' trials and those in which the monkey failed to identify the target as 'miss' trials. Analysis of behavior, pupil diameter, and microsaccades was conducted on both the attend-in and attend-away conditions; all electrophysiological analysis was applied only to the attend-in condition.

Pupil Diameter: The raw pupil diameter measurements from the infrared eye-tracking system could differ across days due to external factors such as display monitor illumination. To control for this, we normalized the raw data by a Z-score procedure

separately for each session (using the mean and standard deviation of all measurements during the session). We analyzed normalized pupil diameter traces for hit and miss trials in the threshold condition, over a time window from 100 ms before to 100 ms after all stimulus presentations (non-target and target), excluding the first stimulus presentation in a trial. The first stimulus was excluded to avoid pupil diameter changes due to the pupillary near response caused by acquiring fixation (McDougal and Gamlin, 2015). The pupil diameter was averaged over this time period and compared across conditions using bootstrap estimation and *t*-test. Distribution violin plots were generated using kernel density estimation (Hoffmann, 2015) (bandwidth(hit) = 0.0801, bandwidth(miss) = 0.0648).

Microsaccade Analysis: Saccadic eye-movements were detected using ClusterFix (König and Buffalo, 2014). We identified microsaccades by filtering for eye movements with amplitudes between 0.1 and 1 degree of visual angle. We then split all trials in the threshold condition into two groups: those in which a microsaccade was detected in the 400ms preceding the target stimulus presentation, and those without a detected microsaccade. We calculated the hit rate for trials within those two groups. For all trials in which a target stimulus was presented at the attended location, we determined the direction of all microsaccades in the 400ms period preceding target presentation, relative to both the attended and unattended stimuli. The relative microsaccade direction was defined as the angle between two vectors: the one defined by the eye positions at the beginning and end of the microsaccade, and the vector from the initial eye position to the center of the stimulus (calculated separately for attended and unattended stimuli). Relative microsaccade directions were grouped into 12 bins from 0-360°. The distribution

191 of relative microsaccade directions were calculated separately for correct and incorrect
192 trials, relative to both the attended and unattended stimuli (Figure S2A).

193 We next created a null distribution of relative microsaccade direction. This was
194 done by pooling together microsaccades from correct and incorrect trials and then
195 sampling with replacement from this pooled data (bootstrap procedure (Efrom and
196 Tibshirani, 1993); 1000 samples). The number of microsaccades chosen for each sample
197 was the same as the number in correct or incorrect trials respectively. These
198 bootstrapped samples were used to create 99.5% confidence intervals for the count of
199 microsaccades expected in each of the 12 bins. A bin was considered significantly
200 different from chance if it's true count fell outside this confidence interval.

201 We calculated microsaccade rate for an entire trial by dividing the total number of
202 detected microsaccades in the whole trial by the trial length (6995 total trials). The
203 Pearson correlation between microsaccade rate and mean normalized pupil diameter
204 (see above) for the trial was calculated for all trials with pupil diameter data, regardless
205 of trial type or outcome (Figure S2B). Not pictured in Figure S2B but included in
206 correlation analysis were trials with a mean normalized pupil diameter greater than 2 or
207 less than -2 (~4% of trials). Only 4 of these trials were longer than 1 s, out of which 2 trials
208 contained detected microsaccades.

209 *Firing Rate:* Firing rates were normalized per neuron to that neuron's maximum
210 stimulus-evoked response to each contrast before being combined across contrasts and
211 trial types. We averaged stimulus-evoked firing rates from 60-260 ms following non-target
212 or target stimulus presentations. We used bootstrapped estimation to compare firing rates
213 in hit and miss trials in a paired comparison. This was done for all single and multi-unit

clusters, as well as broad- and narrow-spiking single-units in each layer. Firing rates were also compared across hit and miss trials by paired *t*-test for each group. PSTH of firing rates were calculated in 30ms bins shifted in 5ms increments. To calculate the time of firing rate divergence, we compared the difference in each single neuron or multi-unit firing rate in the two conditions over time. At each time point, we performed a Wilcoxin rank-sum test comparing the firing rates across hits and misses, and defined the divergence point as the first time the firing rates were significantly different. Divergence was calculated separately for each layer. To determine the time at which firing rates rose above baseline levels, we used bootstrapping to estimate 95% confidence intervals for each neuron's pre-stimulus firing rate (0-100 ms before target or non-target stimulus onset). We then calculated the target PSTH for each neuron in 30ms bins shifted in 5ms increments. We defined the response latency as the first time bin in which the neuron's firing rate in the PSTH exceeded the upper limit of the 95% confidence interval of baseline firing. We calculated the response latency independently for hit and miss trials for each neuron.

Fano factor: Trial-to-trial variability was estimated by the Fano factor, which is the ratio of the variance of the spike counts across trials over the mean of the spike counts for each broad and narrow-spiking single unit. The Fano factor was calculated over non-overlapping 20ms time bins in a window from 200ms prior to each non-target flash onset to 200ms after each non-target flash onset for hit and miss trials in the threshold condition. To compare across conditions, we calculated the Fano factor modulation index (MI), defined as

$$\mathrm{MI} = \frac{FF_{hit} - FF_{miss}}{FF_{hit} + FF_{miss}}$$

where FF_{hit} and FF_{miss} represent the Fano factor for a given unit in hit and miss trials respectively at each point in time with respect to non-target stimulus onset. The Fano factor MI was averaged from 0-60ms prior to non-target stimulus onset and compared across trial types in the threshold condition for each sub-population.

Pairwise Phase Consistency (PPC): We calculated PPC (Vinck et al., 2010) for single and multi-units in the non-target pre-stimulus period (0-200ms preceding onset) in trials in the threshold condition. Although PPC is unbiased by spike count, we set a threshold of 50 spikes for analysis so that only units with enough spikes for a reliable estimate of PPC were included (superficial: $n = 26$, input: $n = 41$, deep: $n = 64$). LFP phase was calculated using Morlet wavelets. PPC for each unit was calculated for the phase of the LFP recorded on the same channel and averaged in three frequency bands (3-12 Hz, 15-25 Hz, and 30-80 Hz). PPC was calculated separately for hit and miss trials and compared across trial outcomes by t-test, corrected for multiple comparisons.

Spike-spike coherence (SSC): For each recording session, all spikes recorded from visually responsive single and multi-units in each layer were combined into a single spike train for that layer (layer multi-unit). Separately for both the pre-stimulus and non-target stimulus-evoked periods, we randomly deleted spikes from the layer multi-unit with a higher firing rate so that the firing rates were matched across hit and miss trials. SSC was calculated for each of the three possible pairs of layer multi-units in each session for both the pre-stimulus (0-200 ms preceding stimulus onset) and non-target stimulus evoked period (60-260 ms following non-target stimulus onset) separately for hit and miss trials using Chronux (NW = 1; K = 1; http://chronux.org) (Mitra, 2007; Mitra and Pesaran, 1999). To control for differences in firing rates across hit and miss trials we used a rate

matching procedure (Mitchell et al., 2009). For estimation statistics, interlaminar SSC values was calculated for each frequency and subsequently averaged across three frequency bands: 3-12 Hz, 5-15 Hz, and 30-80 Hz and compared across hit and miss trials for each pair of layers in each recording session. For null-hypothesis testing, we calculated the SSC modulation index, defined as

$$\mathrm{MI} = \frac{SSC_{hit} - SSC_{miss}}{SSC_{hit} + SSC_{miss}}$$

The SSC MI was calculated for each frequency and subsequently averaged across three frequency bands: 3-12 Hz, 5-15 Hz, and 30-80 Hz. MI values for each frequency band were compared to zero by t-test, Bonferroni corrected for multiple comparisons. We tested for interaction effects with a three-factor ANOVA, with frequency, pair of layers, and time window (pre or post stimulus) as factors. We calculated a shuffled distribution of SSC by shuffling the trial identities of the spikes in one of the layers in the pair. We then calculated SSC with the shuffled trial identities. This procedure was repeated 10 times to create the shuffled distribution.

GLM quantification: To compare how well our results can predict behavioral performance we fit a GLM to the response of the monkeys in trials in the threshold condition (Davis et al., 2020). We included five regressors in our analysis: (1) average pupil diameter during the trial, (2) number of microsaccades in the pre-target window (0-400ms before target stimulus onset), and average target-evoked multi-unit firing rate in the (3) superficial, (4) input, and (5) deep layers. We calculated the average target-evoked firing rate by averaging the firing rate of all single- and multi-units in a given layer 60-260ms after target stimulus onset in each trial. In order to be able to compare weights across regressors, each regressor was transformed into a z-score before being included

in the model. We fit the GLM using a logit link function, using the predictors to regress the categorical binary trial outcome (hit or miss). A total of 309 trials were included in the GLM.

Supplemental References

Davis, Z.W., Muller, L., Martinez-Trujillo, J., Sejnowski, T., and Reynolds, J.H. (2020). Spontaneous travelling cortical waves gate perception in behaving primates. Nature 587, 432-436.

Efrom, B., and Tibshirani, R.J. (1993). An introduction to the bootstrap. Champman and Hall/CRC.

Hartigan, J.A., and Hartigan, P.M. (1985). The dip test of unimodality. The annals of Statistics, 70-84.

Hoffmann, H. (2015). violin.m - Simple violin plot using matlab default kernel density estimation. (INRES (University of Bonn), Katzenburgweg 5, 53115 Germany.,).

König, S.D., and Buffalo, E.A. (2014). A nonparametric method for detecting fixations and saccades using cluster analysis: removing the need for arbitrary thresholds. Journal of neuroscience methods 227, 121-131.

McDougal, D.H., and Gamlin, P.D. (2015). Autonomic control of the eye. Compr Physiol 5, 439-473.

Mitchell, J.F., Sundberg, K.A., and Reynolds, J.H. (2007). Differential attention-dependent response modulation across cell classes in macaque visual area V4. Neuron 55, 131-141.

Mitchell, J.F., Sundberg, K.A., and Reynolds, J.H. (2009). Spatial attention decorrelates intrinsic activity fluctuations in macaque area V4. Neuron 63, 879-888.

Mitra, P. (2007). Observed brain dynamics (Oxford University Press).

Mitra, P.P., and Pesaran, B. (1999). Analysis of dynamic brain imaging data. Biophys J 76, 691-708.

Mitzdorf, U. (1985). Current source-density method and application in cat cerebral cortex: investigation of evoked potentials and EEG phenomena. Physiol Rev 65, 37-100.

Nandy, A.S., Nassi, J.J., and Reynolds, J.H. (2017). Laminar Organization of Attentional Modulation in Macaque Visual Area V4. Neuron 93, 235-246.

Nassi, J.J., Avery, M.C., Cetin, A.H., Roe, A.W., and Reynolds, J.H. (2015). Optogenetic Activation of Normalization in Alert Macaque Visual Cortex. Neuron 86, 1504-1517.

Prins, N., and Kingdom, F.A.A. (2018). Applying the Model-Comparison Approach to Test Specific Research Hypotheses in Psychophysical Research Using the Palamedes Toolbox. Frontiers in Psychology 9.

Ruiz, O., Lustig, B.R., Nassi, J.J., Cetin, A., Reynolds, J.H., Albright, T.D., Callaway, E.M., Stoner, G.R., and Roe, A.W. (2013). Optogenetics through windows on the brain in the nonhuman primate. J Neurophysiol 110, 1455-1467.

Vinck, M., van Wingerden, M., Womelsdorf, T., Fries, P., and Pennartz, C.M. (2010). The pairwise phase consistency: a bias-free measure of rhythmic neuronal synchronization. NeuroImage 51, 112-122.