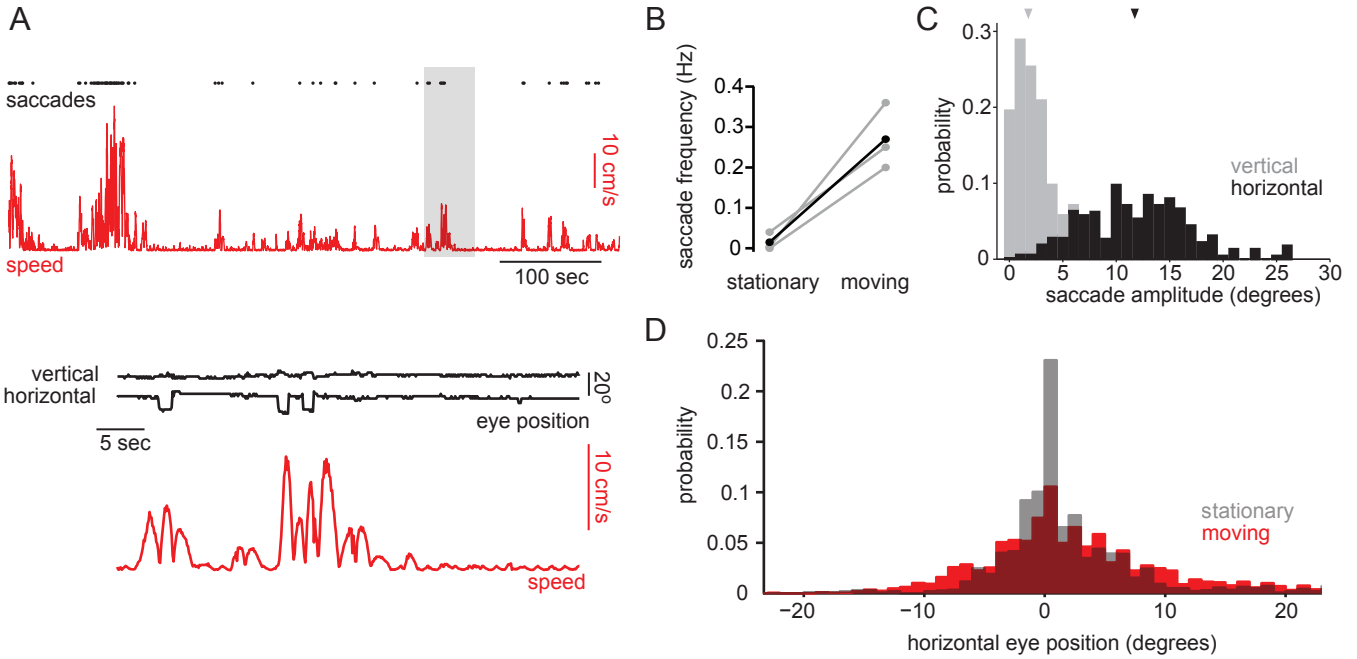
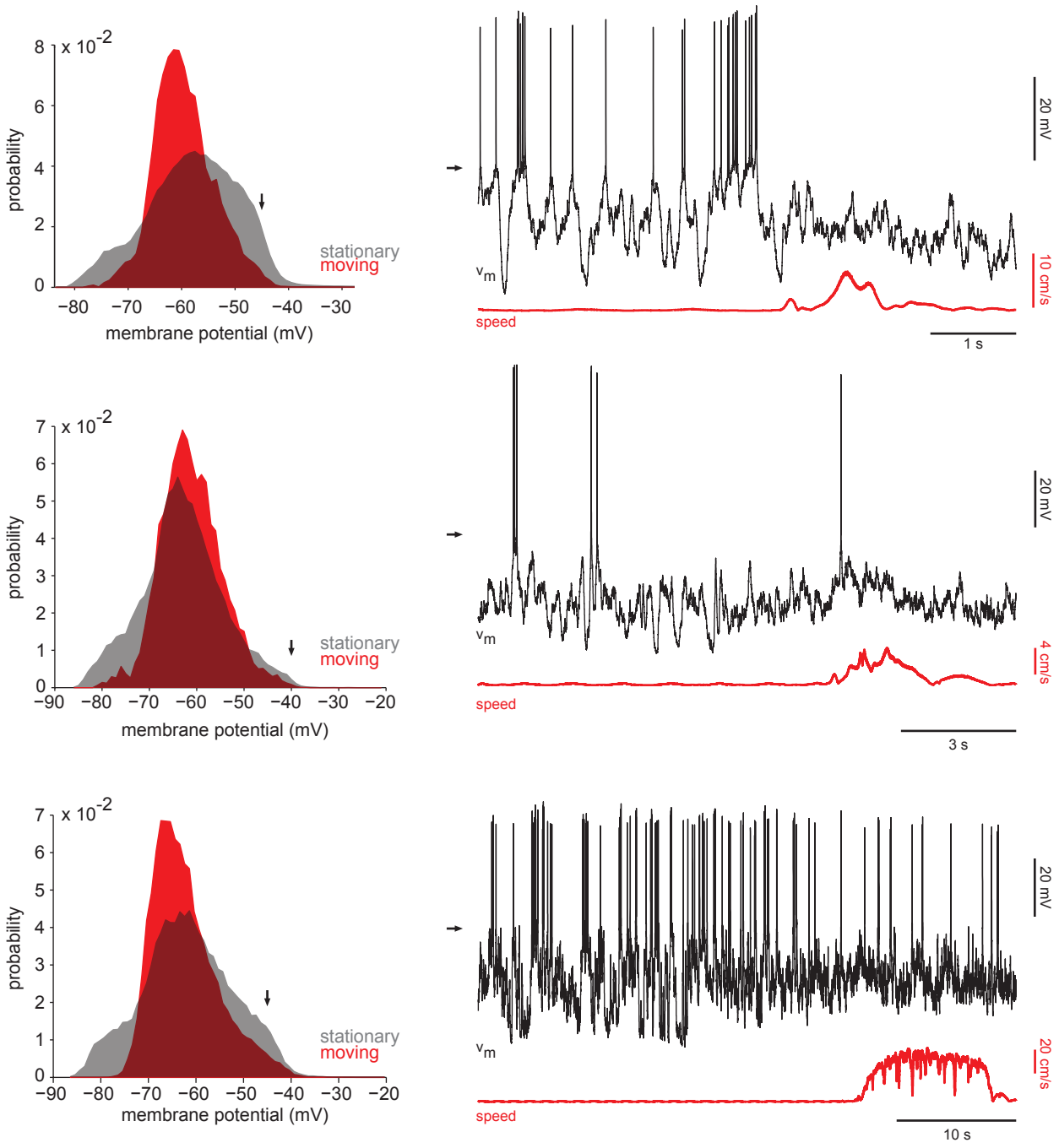


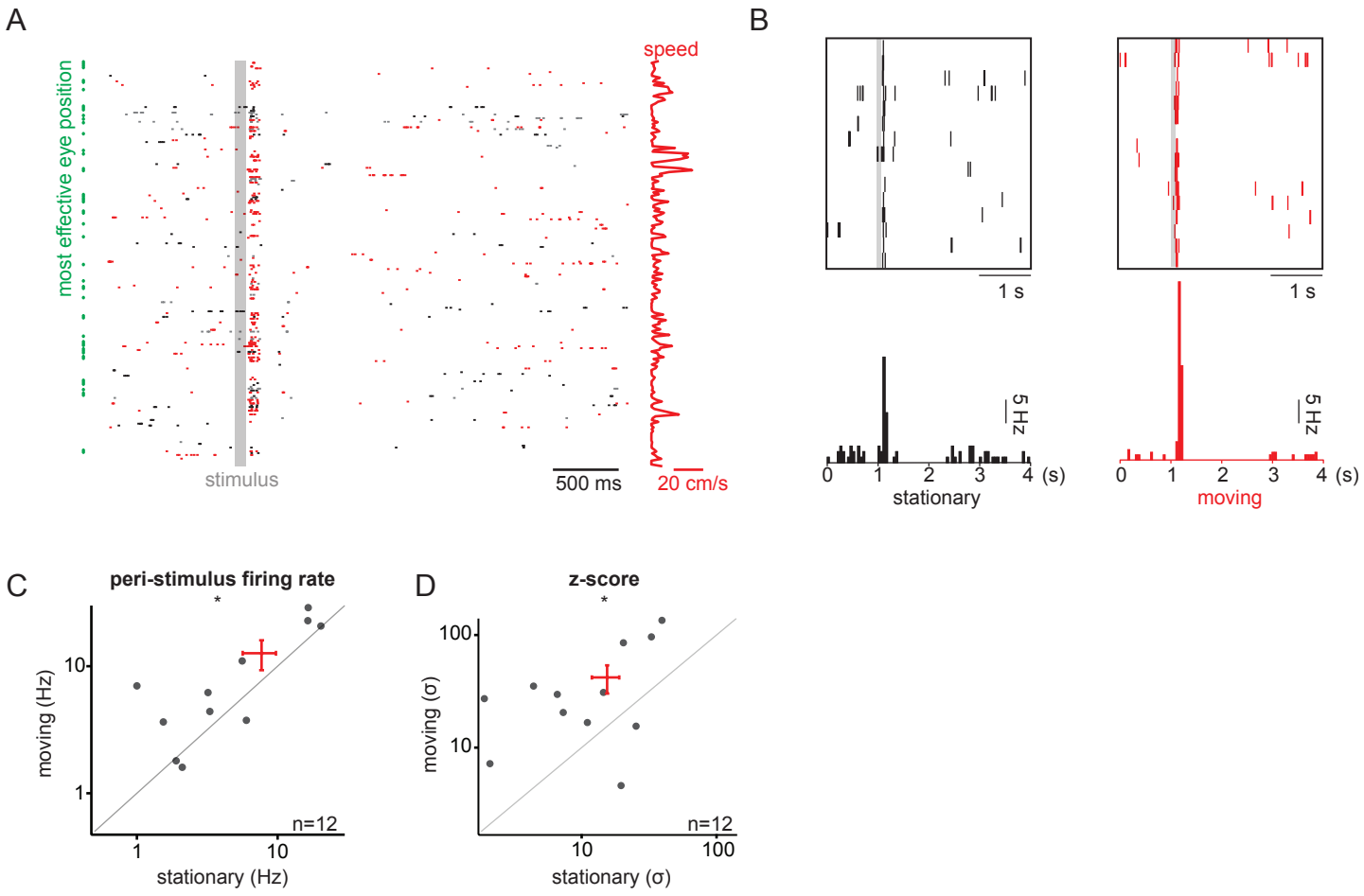
Supplemental Figure 1 (corresponds to Figure 1): Eye position during quiet wakefulness and locomotion

(A) Top, saccades (black dots) and speed plotted together for a 10 minute interval of one recording session. Grey box expanded below. Bottom, vertical and horizontal eye position plotted with speed on an expanded timescale. (B) Saccade frequency during stationary and moving epochs for three mice. (C) Distribution of vertical and horizontal saccade amplitudes for three mice in (B). (D) Distribution of horizontal eye position for stationary and moving epochs (n = 4 mice; 11 recording sessions).



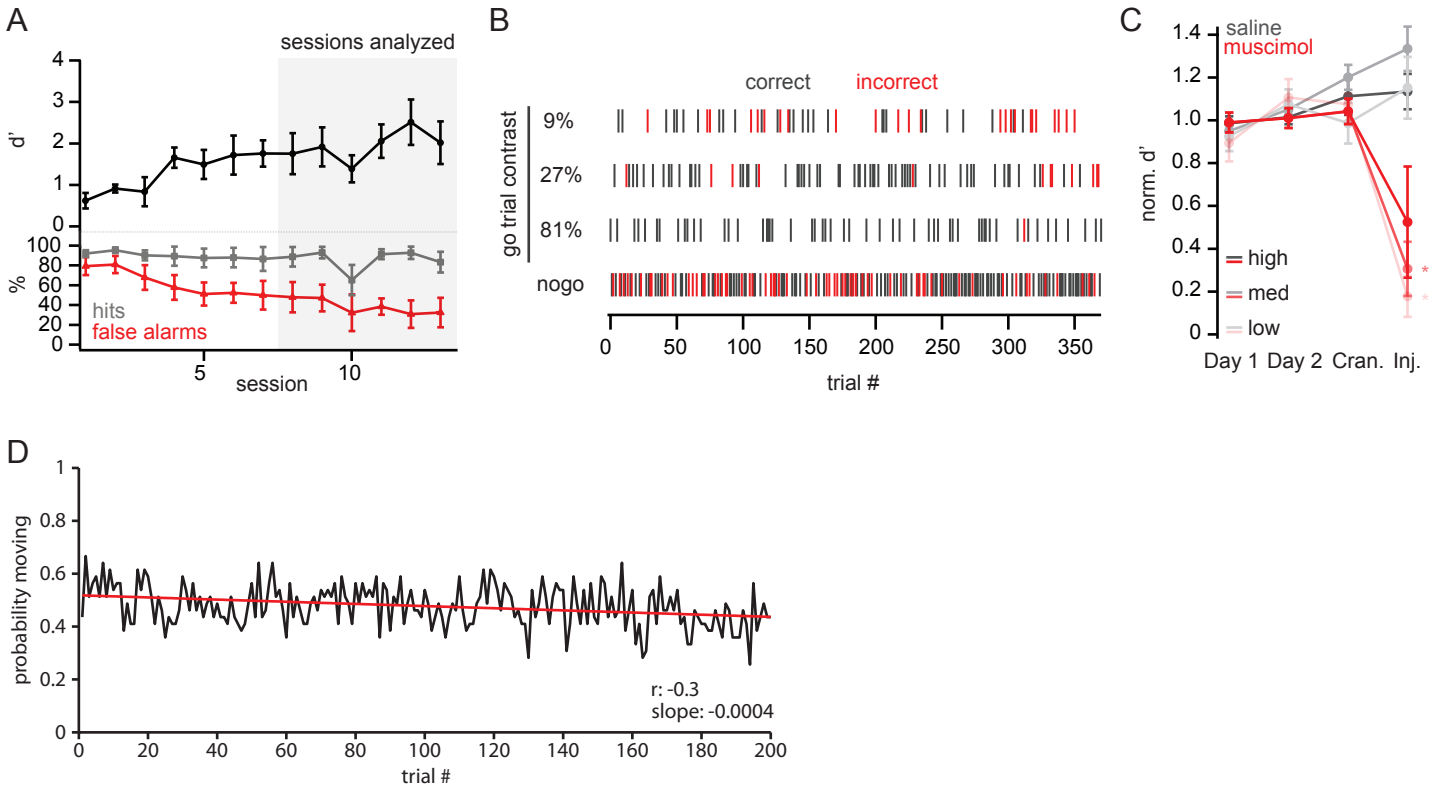
Supplemental Figure 2 (corresponds to Figure 2)

All-point histogram (left) and example Vm and speed traces (right) for three neurons. The top example is taken from the same cell used in Figure 2F. Arrows indicate spike threshold.



Supplemental Figure 3 (corresponds to Figure 3): Single-unit responses to visual stimulation during quiet wakefulness and locomotion

(A) Raster plot of spikes elicited by flashed gratings. Red ticks represent spikes during moving epochs, black ticks represent spikes during stationary epochs, and grey ticks represent intermediate speeds. Green dots represent trials for most effective eye position. Speed trace on the right represents mean speed during the stimulus presentation only. Grey box indicates the timing of the visual stimulus. (B) Raster plots and peri-stimulus time histograms (PSTHs) during stationary (black) and moving (red) states for trials marked by green circles in (A). (C, D) Peri-stimulus firing rate and z-score for moving vs. stationary states (n=12 cells). Red symbols represent mean ± s.e.m *, $p < 0.05$, Wilcoxon signed-rank test.



Supplemental Figure 4 (corresponds to Figure 4)

(A) d', hits, and false alarms are plotted for seven mice over thirteen sessions. Grey box indicates sessions used for analysis in Figure 4 (six sessions). (B) Behavioral responses for one session separated by trial type. Grey tick marks represent correct responses (hits/correct rejections); red tick marks represent incorrect responses (misses/false alarms). (C) Normalized discriminability (d') for two cohorts of mice receiving saline (light, medium, and dark grey correspond to low, medium and high contrast conditions, respectively) or the GABAA agonist muscimol (light, medium, and dark red correspond to low, medium and high contrast conditions, respectively). Each trace represents the average performance of the cohort (muscimol: n=4; saline: n=4). Performance was normalized to mean d' over days 1 and 2. Cran.: day of craniotomy; inj.: day of injection. (D) Probability that a trial was classified as running over the course of an entire behavioral session (200 trials), averaged across mice and behavioral sessions (n=7 mice; 39 sessions). Red line represents the linear fit to the data.

Supplemental Experimental Procedures

Animals and surgery

All procedures were approved by the Administrative Panel on Laboratory Animal Care at Stanford University. 32 C57BL6J mice (4-8 weeks) were used in this study. To attach headplates, mice were anaesthetized with isoflurane and placed in a stereotaxic frame. Headplates were centered above V1 on the left hemisphere and fixed to the skull with C&B-METABOND® Quick! Cement (Parkell). After surgery, mice were injected with buprenorphine (0.05 mg/kg) and given at least 2 days to recover before habituation to the spherical treadmill. During habituation, mice were placed on the treadmill for at least 20 minutes per day. Habituation typically lasted for 3 days, after which mice were either used for recordings (22 mice) or trained on a visual discrimination task (10 mice).

In vivo recording

On the day of recording, mice were anaesthetized with isoflurane and a small (<200 μm) craniotomy was made over monocular V1 (2.5-3 mm lateral from lambda). After this procedure, mice were returned to their home cage and given at least two hours to recover before being placed on the treadmill. For whole cell recordings, glass electrodes (4–8 MΩ) were filled with an internal solution composed of the following (in mM): 2.7 KCl, 120 potassium methyl sulfate (KMS), 9 HEPES, 0.18 EGTA, 4 MgATP, 0.3 NaGTP, 20 sodium phosphocreatine, pH 7.3, 295 mOsm/L. For juxtacellular recordings, the same electrodes were filled with saline (0.9% sodium chloride). For voltage clamp recordings, KCl and KMS were exchanged for CsCl and CsMS. Standard blind patching techniques were used. Briefly, the pipette was lowered into the brain under visual guidance with high positive pressure (2.5 PSI). Once in the brain, positive pressure was reduced (0.4 PSI) and the pipette was advanced along a 60 degree axis in short 1-2 μm pulses. If the pipette resistance increased abruptly by 10-20%, positive pressure was released. For whole cell recordings, slight negative pressure was applied to facilitate the formation of a 2-3 GΩ seal. Only recordings made at a depth of less than 400 μm were included in this study. Recordings were performed with a Multiclamp 700B amplifier (Molecular Devices) and digitized by an ITC-18 (InstruTECH). Data acquisition was controlled by custom software written in Igor Pro (Wavemetrics). All recordings were corrected for a junction potential of -10 mV.

Visual Stimulation

Visual stimuli for both recording and behavior were presented on gamma-corrected, LED ASUS VK278Q monitors (60 Hz refresh rate, ~75 cd/m^2). Stimuli were generated by custom scripts written in Presentation (Neurobehavioral Systems). The monitor was placed 30 cm from the mouse and subtended ~90 degrees of visual space.

For whole-cell recordings, a uniform grey screen was presented for the duration of the experiment. The stimuli for all visual stimulation experiments were full-screen sinusoidal

gratings (spatial frequency: 0.05 cycles/degree). For each cell, the optimal orientation was first determined by presenting drifting gratings (velocity: 40^{o}/second, 8 directions), after which visual stimuli were presented as follows.

For the data in Supplemental Figure 2, static gratings at the preferred orientation were presented for 5 frames (~83 ms). Each trial was separated by 4 seconds of uniform grey screen. To characterize whether a cell was visually responsive, we binned activity into 100 ms bins and compared the maximum firing rate during the 300 ms following the onset of visual stimulation to the baseline firing during the second preceding visual stimulation. If a cell's visually evoked activity did not reach at least 2 standard deviations above baseline, it was excluded from further analysis (4/18 cells). To prevent the inclusion of interneurons in the analysis, we excluded cells with a peak to trough time of less than 0.5 ms (2/18 cells; 11%, consistent with proportion of FS cells in upper layers of visual cortex). For the data in Figure 3, visual stimuli were drifting gratings as above; 16% contrast gratings were used for current clamp experiments and 100% contrast gratings were used for voltage clamp experiments.

Behavioral data acquisition

To monitor speed, we placed an optical mouse on the anterior pole of the spherical treadmill (Fig. 1a). The output from the optical mouse was read with custom software written in Presentation (Neurobehavioral Systems) and converted to an analog signal by a National Instruments NI USB-6008 board.

For behavioral experiments, licks were registered as breaks in an infrared beam placed just below the mouth. The lick signal was passed through a custom built amplifier and read as analog inputs by an Arduino Mega 2560 board. Reward and punishment contingencies were handled by custom software written in the Arduino programming environment. To control rewards, the Arduino sent a TTL pulse to a custom-built current source driving a high speed solenoid valve (Cole-Parmer P/N 01540-01). Visual stimuli were controlled by serial signals sent from an Arduino RS232 shield (CuteDigi) to Presentation. Licks, speed, reward timing, and stimulus identity were digitized by an ITC-18 and recorded.

Data analysis

For speed analysis, the raw speed signal was first convolved with a 100 ms boxcar filter. Moving epochs were identified as periods during which the speed was greater than 1 cm/s, a threshold similar to that used elsewhere (Ayaz et al., 2013; Niell and Stryker, 2010). Stationary epochs were defined as periods during which the speed was less than 0.5 cm/s. For analysis of spontaneous activity (Figures 1 and 2), running and stationary epochs were required to span at least 500 ms; these epochs were then sub-divided into individual 500 ms segments prior to analysis. For visual stimulation experiments (Figure 3 and Supplemental Figure 3), running and stationary trials were classified by the mean speed over the visual presentation only.

Membrane potential power spectra, resting potential, spike rate, and membrane potential variance were calculated for each 500 ms segment and averaged to obtain stationary and moving values. To calculate the variance of the membrane potential, spikes were removed by linear interpolation. To determine spike threshold, only those spikes preceded by at least 200 ms without spikes were analyzed. Traces were aligned by the time point at which the membrane potential crossed -20 mV and averaged. Threshold was defined as the membrane potential when dV_m/dt reached 3% of its maximum (Azouz and Gray, 2000). The pre-spike interval (Figure 2) was defined as 150 to 100 ms before spike initiation. To calculate dV_m/dt, the mean derivative was taken over the 10 ms preceding each spike. For maximum rate of rise, the maximum dV_m/dt after threshold crossing and before the peak of the spike was taken.

During visual stimulation experiments for Supplemental Figure 3, pupil position was acquired with a high speed (200 Hz) Guppy Pro camera and analyzed offline with custom Matlab software. Pupil position was calculated by comparing the pupil center to a corneal reflection from an LED placed in the camera axis and assuming that the center of rotation for the eyeball was 1.09 mm behind the pupil (Stahl et al., 2000). Only trials in which the eye position differed by less than 0.5 pixels (corresponding to ~2 degrees) were used for comparisons of visual responses during moving and stationary conditions. For each cell, eye positions were ranked based on the number of spikes elicited by the visual stimulus (before dividing the data into moving and stationary conditions). The eye position yielding the most spikes and having at least 10 trials for both moving and stationary conditions was then selected for further analysis. To analyze the visual response, the data was first divided into 100 ms bins and averaged across trials. Baseline firing rates were calculated from the second preceding visual stimulation and included data from all eye positions. Stimulus evoked firing rates were taken from the 300 ms after the onset of visual stimulation and included only trials from the chosen eye position. Z-scores were calculated by comparing the maximum stimulus bin to the mean and standard deviation over the baseline bins.

For current clamp experiments in Figure 3, response amplitude was defined as the mean response over the stimulus window (~1.2 s duration). Trial-to-trial correlations were calculated over the same window. The coefficient of variation was calculated for a 100 ms window centered on the mean response peak after the initial visual transient. To better isolate subthreshold responses, hyperpolarizing current was injected (resulting V_m: -82.7 ± 4.1 mV) and traces were median filtered (5 ms window) to remove spikes.

For voltage clamp experiments in Figure 3, visual responses were recorded at +20 mV and -70 mV, and g_e and g_i were calculated over the stimulus window by the following equation:

$$I_{synaptic}(t) = g_e(t) * (V_{holding} - V_{exc\_reversal}) + g_i(t) * (V_{holding} - V_{inh\_reversal}),$$

where $I_{synaptic}$ was the current during the stimulus window after subtraction of the mean baseline current during the one second preceding stimulation.

For our internal solution, $V_{exc\_reversal}$ and $V_{inh\_reversal}$ were calculated to be 0 mV and -96 mV, respectively. Recordings were corrected for a junction potential of -10 mV. Before compensation, series resistance was 37 ± 3 MΩ and was compensated online by 50-80%, resulting in an effective series resistance of 13 ± 1 MΩ. $V_{holding}$ was adjusted to account for the error due to this residual series resistance ($V_{holding} = V_{command} - R_{series*}I(t)$). To calculate the reversal potential, g_e and g_i were averaged over a window of 100 ms around the maximum for g_e (after the initial visual transient). The values reported for g_e and g_i in Figure 3 and Table 1 reflect the mean conductance averaged over the same window. The conductance traces in Figure 3F were smoothed with a 50 ms boxcar filter for display purposes.

All paired statistical comparisons were performed with the non-parametric Wilcoxon signed-rank test. Non-paired comparisons were performed with the non-parametric Wilcoxon rank-sum test. All analysis was performed in Matlab.

Behavior

Mice for behavioral experiments were placed on a water restriction regimen (1 mL per day) for several days before training. Weight was monitored daily to ensure that none of the mice dropped below 80% of their baseline weight (taken as the average weight in the week before water restriction). Behavioral training occurred in two phases. First, mice were placed on the treadmill and rewarded automatically for go stimuli. During this phase, only full contrast gratings (go condition) or grey screens (nogo condition) were presented. Mice were not required to lick for reward, and water was dispensed automatically 500 ms after the onset of the visual stimulus. Licks during nogo trials were not punished during this initial training period. After one or two days, mice began training on the full behavioral paradigm. Mice were now required to lick within the response window (500 to 4000 ms after stimulus onset) to activate a solenoid and receive reward. After the response window, a 4-second grey screen was presented. Licks during the response window on nogo trials (false alarms) were punished by prolonging the inter-trial interval by four seconds (to a total of 8 seconds). At the end of each session, the amount of water consumed during the task was measured, and mice were provided with additional water up to 1 mL. For all 7 mice included in the behavioral analysis, the overall d' across all contrasts reached a criterion of at least one before data was collected.

To categorize behavioral trials as stationary or moving, we analyzed the 500 ms before stimulus onset. Trials for which the mean speed during this period exceeded 1 cm/s were categorized as moving trials, and trials for which the mean speed was less than 0.5 cm/s were categorized as stationary trials. For one animal, more than 50% of the stationary trials came from the first behavioral session. Data from this animal were excluded from further analysis. For the remaining mice, data from six behavioral sessions were combined and analyzed. We observed that behavioral performance was most variable at the beginning and end of sessions, perhaps due to anxiety and satiety. To account for these factors, d' was calculated for a rolling 200 trial window, and the block of trials that yielded the highest d' were selected from each session.

Sessions for which errors occurred in reading the optical mouse were excluded (3/74 total sessions) before analysis.

The cortical inactivation experiment was performed over four days. Baseline performance was measured over the first two days, a craniotomy was performed on the third day, and either muscimol (4.4 mM; ~400 nL) or saline was injected on the fourth day (saline cohort: n=4; muscimol cohort: n=4). Performance was normalized to the mean performance on days 1 and 2.

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