Supporting Information

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SI Materials and Methods

Animal Preparation. All honeybees were captured leaving the hive entrance in a rooftop honeybee facility at The University of Queensland in Brisbane, Australia. After being briefly cold anesthetized at 4 °C (for around 5–10 min), bees were tethered to a paper clip via melted wax with a high melting point cautery tool (Bovie), with their wing joints waxed down to prevent flight. Unless specified for certain experiments, the heads of all bees were fixed to the thorax with blue light cured dental cement (Coltene Whaledent synergy D6 FLOW A3.5/B3) to prevent movement. All bees were fed 1 M sucrose (UNIVAR, Ajax Finechem) before being placed in a warmed, humidified chamber (≈35 °C) for approximately 1 h to allow for recovery and adaptation to the tether.

A total of 26 bees were tested for behavior in the closed loop and during brain recordings. Ten of these bees were tested both with closed-loop and open-loop replay and with competing visual flicker experiments. The other 16 bees were tested with closed-loop and open-loop rotating bar experiments. The majority of bees walked and fixated on the bar for nearly 2 h during brain recordings, and only three bees would stop walking before 2 h, therefore limiting the length of the behavioral experiments. Separate behavioral experiments were carried out on a total of 98 bees, all of which experienced multiple behavioral experiments each. By and large, bees were tested for up to 2 h across multiple behavioral tests. Of the 98 bees tested behaviorally, we found that 10% of bees would stop walking on the ball.

Arena. A diamond-shaped light emitting diode (LED) arena was assembled from four panels (Shenzhen Sinorad Medical Electronics), allowing display of stimuli of various shapes and colors (Fig. 1) (1). Each panel contained 32 x 32 pixels, each consisting of one blue, one green, and one red LED. The peak wavelength and luminance for a single green bar was measured as 518 nm and 168 lx, respectively. To determine what photoreceptors this wavelength stimulated in the bee, we calculated the brightness contrast and the green contrast values for the short, medium, and long wavelength-sensitive photoreceptors based on previous data from honeybee photoreceptor sensitivities (2, 3). The green contrast values are 0.2168 (short wavelength photoreceptors), 0.2510 (medium wavelength photoreceptors), and 0.4303 (long wavelength photoreceptors), with the brightness contrast value at 0.8980. These values were calculated by comparing the spectral curves of the green LEDs to the background lighting, which were halogen lights providing diffuse light from above. A Styrofoam ball (Sullivans International) was placed in a holder designed in Solid Edge and printed using 3D printing technology (Plastic Ink). After the holder was placed in the center of the arena, the ball was suspended on air blown from beneath, allowing the bee to walk freely over its surface while being suspended above it (Fig. 1).

Tracking Freely Walking Bees. Movements of a honeybee walking freely within a Petri dish lined with filter paper were captured by an overhead camera (Logitech 9000) and experiments were analyzed off-line. Pixels corresponding to the bee were identified using an adaptive thresholding technique, which involved labeling as background pixels with intensities corresponding to the central 20% of the expected distribution of filter-paper intensities calculated from all frames in the recording. After labeling as background similar neighboring pixels using a flood-filling technique, remaining darker pixels were then labeled as candidate bee pixels. These candidate pixels were attributed a weight (w) according to 

$$w = I_b - I_c$$

where $I_b$ was the median intensity of the background pixels and $I_c$ was intensity of the candidate pixel. The position of the bee within the image of weighted candidate pixels was localized by searching for the window with the highest total weight. Hysteresis was applied to the location of the highest scoring window to prevent the best estimate of the bee’s position from flickering between possibly several similar candidate window locations. The exact position of the bee was estimated by computing the weighted center of mass of the pixels within the best candidate tracking window. Finally, the bee’s orientation was determined by computing the principal axis of the blob of candidate bee pixels from the weighted singular value decomposition of the blob distribution within the tracking window. To overcome the 180° heading ambiguity introduced by this approach, two heuristics were used. First, the measured orientation was prevented from randomly flipping by choosing the solution closest to the solution for the previous frame. Second, it was assumed that on average the bee would face forward. Hence, if the estimated heading varied from the measured direction of motion (computed from the difference between estimated position between frames) for a significant distance, it was flipped 180°.

Data Collection. To measure the walking response on the ball, two laser computer mice (boards from Logitech MX400 mice, incorporating ADNS7050 chips) were positioned orthogonally to measure the three axes of rotation of the ball: x (forward motion of the top of the ball), y (sidelip motion of the top of the ball), and z (rotation of the ball around a vertical axis) of the ball’s movement (Fig. 1). The mice had the following properties: counts per millimeter, 31; maximum speed (mm/s), 508; maximum acceleration (g), 8; focal distance (mm), 2.18–2.62. A custom CPNMouse driver was used to interface with both computer mice, and data were recorded using a custom application in C++ at 100 Hz. The sensors were positioned as shown in Fig. 1. The data pertaining to the rotation of the ball were read by the Python program Vision Egg (4), which, in turn, updated the position of the stimuli displayed on the LED screen at 200 frames per second. To close the loop between the bees’ movements and the visual stimulus, data were saved to a text file by the custom CPNMouse driver, which could be sampled by the Vision Egg program at a rate of 200 Hz. This system, as measured by photodiodes at 25 KHz, resulted in a delay of ~46 ms. Therefore, although Vision Egg updated the screen image at 200 Hz, the movement of the bee was recorded at 100 Hz.

Stimulus. Honeybees were exposed to green bars, 54° high and 20° wide. The movement of the bar was set at a gain of 1, wherein a 1° rotation of the ball will result in a 1° rotation of the bar in the same direction around the bee. The bees exhibited three general behavioral types in response to these green bars: (i) active and fixating, (ii) active but not fixating, and (iii) not active. In an attempt to reduce the error induced by the second behavioral pattern (active but not fixating), open-loop displacements in the position of the bar were used to add exogenous variance to the position data. These displacements involved shifting the stimulus by 90° to either the left, right, or not at all. The time between bar displacements was randomly generated between 10 and 60 s. After the bees had adapted to the tether they were suspended on the Styrofoam ball and exposed to stimuli in a pseudorandom order for 2 to 3 min at a time. If bees
were still active after completing every stimulus once, then multiple trials were undertaken. In addition, the bright green bars were flickered at 6, 7, 20, 30, 70, and 75 Hz; the bees controlled the stimuli in closed loop. These frequencies were chosen to sample a wide range of visual flicker for the brain recording experiments. For open-loop experiments involving rotating the bar around the bees, visual stimuli were moved clockwise and then counter-clockwise at a constant speed of 5.6° per second. During open-loop replay experiments, we allowed the bees to perform closed-loop behaviors for 3 min and saved the angular bar positions during these experiments. We then took those positions and replayed them back to the bees ∼1 min after they had completed the closed-loop behavior. Therefore, for each experiment the bees were exposed to the same visual stimulus sequences they previously experienced during closed loop.

Data Analysis. Data analysis was conducted through custom programs written in Matlab (Mathworks). The angular direction of the bee at each sampling point was determined from the rotation around the vertical axis of the ball, 0°. This value was then transformed from the 128 pixels of the display to the 360° around the bee. The probability of the angular position of the bar was computed in the form of a radial histogram, which specified the dwell time. For display purposes, the histogram was normalized to a peak value of 1. Note that this method of normalization results in histograms that do not necessarily sum to one; thus they represent an indication of the strength of the bee’s fixation rather than a probability distribution in a strict sense. Separate from the binned data shown in polar plots, a mean vector was determined using the circular statistical toolbox in Matlab (5). This vector is a representation of the sharpness of the distribution of the data (vector length) and the mean direction in which the bar is viewed (vector direction, or angle). We also calculated the proportion of time that the bees spent fixating each bar. Fixation on a particular bar was defined as the bee holding the center of the stimulus within ±30° from the straight-ahead direction (the “fixation window”). This window was determined after examining the distributions of bar locations for 74 bees (Fig. S1D). We also examined how long the bees took to return the stimuli to the fixation window after a displacement (0.77 ± 0.076 s). We excluded open-loop displacements that moved the bar into the fixation window, and those in which the bee did not refixate within 1.2 s.

Bee Preparation for Electrophysiology. Bees were collected as previously described and tethered as per the “closed-loop tethered” preparation with the exception of using dental wax (Carmel Wax), melted with the cautery tool in place of dental cement. The bees were then fed to satiation with 1 M sucrose solution and left previously experienced during closed loop.

Brain Histology. Following the experiments, alternating current (±10 mA in 200-ms pulses for several seconds) was passed through the electrode to eject the Texas red dye conjugated to dextran from the electrode tip. The front head capsule was then removed and the brains were fixed with 4% (wt/vol) paraformaldehyde, (Electron Microscopy Sciences) in PBS, while the electrodes were in place. The brains were then dissected out of the head capsule and left overnight in 4% (wt/vol) PFA in PBS, embedded in 5% (wt/vol) agarose (Sigma-Aldrich), sectioned horizontally at 100 μm using a sliding vibratome (Zeiss), and imaged using a slide scanning fluorescence microscope (Metasystems Slide Scanners), using a Zeiss 10× 0.45 NA objective to determine the location of the electrode. The location of the electrode site was identified as belonging to one of six main structures: lamina, medulla, lobula, protocerebrum, antennal lobe, and mushroom bodies. The electrode locations were generally consistent from bee to bee (Fig. 2), which meant that many brain regions were recorded from several times. These brain regions were easily identifiable from the autofluorescence of the tissue (Fig. 2C) relative to previously identified and mapped bee brain regions (8, 9). To be able to map these brain locations to a 3D model, we created a model by fixing a bee brain in 4% (wt/vol) glutaraldehyde in PBS (Electron Microscopy Services), embedding it in 5% (wt/vol) agarose, and sectioning it frontally with a vibratome at 100 μm. We then outlined and filled each brain region with assigned colors at 1-μm steps using the Art Studio application on an iPad 2 (Apple) (Fig. 2B). These regions were merged to form volumes of specific brain regions (Fig. 2B). The electrode locations were then pinpointed to this model based on two perspectives, dorsally and frontally, allowing us a rough estimate of the location of the sites across the bee brain (Fig. 2D).

Data Analysis of Electrophysiology. Both the LFP signal from each wire and the signal from each of five photodiodes around the diamond shaped arena were recorded and digitized on the same computer, with one photodiode placed at each arena apex. To match the brain recordings with the visual stimuli, an additional fifth photodiode was recorded to obtain the timing information. This fifth photodiode acted as a counter, recording a 2-Hz flicker from a single LED “counter pixel” whenever the main visual stimulus was displayed. A second computer controlling the LED arena recorded the angular position of the stimulus, the rotation of the ball and the on/off state of the counter pixel. Two recordings of the counter pixel were made: (i) from the photodiode concurrent with the LFP recording device and (ii) internally from the computer generating the visual signal. These two signals were matched to allow the LFP signal to be aligned with the angular position of the visual stimulus. To measure the amplitude of the different frequencies within the LFP signal, we performed continuous Morlet wavelet transforms for the flicker frequency and for other frequencies outside of the flicker frequency tag (which included 1–100 Hz, except for ±3 Hz around the flicker frequency) from...
the Fieldtrip toolbox (www.ru.nl/fcdonders/fieldtrip) (10). This approach involved a 1-Hz spectral resolution and a 30-ms window sliding every 5 ms (thereby giving the Morlet wavelet coefficients at each point of the visual stimulus presented at 200 Hz). The real value of the Morlet wavelet coefficient was taken to measure the magnitude of the oscillations as the Morlet wavelet coefficient amplitude (MWCA). For the frequencies at which the bars were flickered, we define the MWCA as the steady state visually evoked potential (SSVEP) amplitude.

The SSVEP amplitude during open-loop rotations of the bar around the bee was calculated by examining periods of time when the bees were both walking (had an average velocity above zero) and were turning toward the bar’s location, which was done by examining the bee’s turning rate as the bar rotated around the bee. For example, a positive turning rate while the bar was to the left indicated that the bee was turning toward the bar (to the left). Walking forward (when the bee was not turning), was classified as periods when the bee was tracking the bar to the front. We compared the SSVEP values during these periods when the bees were not turning toward the bar (Fig. S4).

We also compared the SSVEP values during closed loop versus replay, where the same visual sequence the bees experienced during closed loop was played back to the bees in open-loop replay. We focused on the periods of time when the bees corrected for displacements during the closed loop within 1.5 s, which included epochs of data 5 s before and after each 300-ms displacement. These epochs were periods of time when the bees were originally fixating on the bar and, following a displacement, would bring the bar back to the front. Because this same visual sequence was also present during replay, we calculated the SSVEP amplitudes during these visual sequences during open-loop replay. We subdivided the data further by examining when the bees could track the position of the bar versus when they were not tracking (Fig. S4), which was done by calculating the circular-circular correlation statistic values (5) for each displacement epoch between the bee’s behavior during replay and the bar’s location. Any correlation statistic above 0.5 was counted as epochs when the bees were tracking the stimuli.

Examination of SSVEP changes during image selection required identifying a baseline fixation condition, which was when the bee was facing exactly between the two competing bars 90° apart. This baseline position needed to be maintained for at least 1 s to justify subsequent selection analyses. A behavioral switch to either bar was then identified when one bar was moved to within 30° of the front of the bee, within 1 s from the baseline condition, and fixation on that bar needed to be maintained for at least 2 s for these data to be considered a behavioral switch. After identifying whether these switches were caused by the bee’s own closed-loop behavior or by random open-loop displacements, the brain activity was examined for a ±5-s window around the moment the bar passed the frontal 30° threshold. For the correlation measures in Fig. 4D, correlations between the 20-Hz and 30-Hz SSVEP values were calculated for the entire ±5-s window epochs of the bees fixating on one or the other bar. For the box plots in Fig. 4E, the differences between 20- and 30-Hz SSVEP amplitudes for the flicker frequencies were averaged across a 1.5-s window 0.5 s before the switch initiation (for open or closed loop) (Fig. S5). The same approach was used to calculate the SSVEP amplitude differences after the switch to fixation, in this case 0.5 s after the switch initiation across a 1.5-s window (Fig. 4E and Fig. S5).

Statistical Analyses. Statistical analysis of the power differences between the flickered stimulus and control frequencies, and between closed and open loop was done by first testing for normality using a Lilliefors test (11). As the data were not normally distributed, a Wilcoxon rank-sum test with a 95% confidence interval was used to test significance of the difference between two medians. MWCA was plotted against stimulus position in a polar plot. The vector length represents the width of the MWCA distribution around the arena (0–360°) and the direction represents the mean stimulus position, where the LFP MWCA for that frequency was maximum. A Kruskal–Wallis multiple-comparisons test was used to test differences across multiple groups. A false-discovery rate control test was included to control for multiple comparisons.

Fig. S1. Honey bees actively fixate on bright green bars in a closed-loop walking paradigm. (A) To test whether untethered bees were also attracted to the brightly lit bar, we tracked freely walking bees in a Petri dish while presenting a rotating green bar on the arena. (B) The tracking program detected the relative orientation of the freely walking bee in the arena (blue line), which closely followed the movement of the bar (red line). (C) Multiple bees would track the bar moving in the arena (n = 9), with an r^2 correlation between the angular location of the bar relative to the bee = 0.2979, P < 0.00001. (D) A polar plot displaying the distribution of locations of a single green bar during fixation experiments for tethered bees. Black histograms indicate the binned normalized distribution of positions of the bar throughout the experiment. Blue arrow indicates the mean vector of the data. Bees fixate strongly on a single green bar, as indicated by this average distribution across 74 bees, which is not seen when there is no stimulus. (E) Of the total 61 and 74 bees in each experiment, 37 bees were tested with both a single green bar and with the no stimulus control. (F) Dark bars presented on a green background produced significantly lower mean vector values compared with a green bar on a dark background (Mann–Whitney U test, rank sum: 99, P = 0.0015, compared with lit bar). The blue arrow is the mean vector, n = 8. (G) When presented with an arena half lit in green (with a green “bar,” which was 180° wide), the bees oriented to the edge of the bar, which resulted in the bar being placed to the right of the bees, with the edge more often in front of the bees. (H) Average response to rightward (gray) or leftward (black) displacements with a single green bar. Red indicates significant differences between the gray and black lines. Significant differences were determined for pairwise comparisons between the black and gray curves over time with the Wilcoxon rank-sum test with the threshold for significance set at P < 0.001. The comparisons were corrected using false-discovery rate control. The dotted lines bracket the time during which the bar was displaced in open loop (SI Materials and Methods). n = 74 bees. (I) Average response to rightward (gray) or leftward (black) displacements with a nonvisible black bar on a black background. There were no significant differences between the points in the two curves, n = 61 bees. Shaded regions in H and I signify SE. (J–L) With two competing bars (90° apart), the bees would fixate on one or the other bar over time, resulting in a broad or multimodal distribution in the histogram plots. The bars were flickering at 20 Hz or 30 Hz in (K and L); random displacements were embedded in all experiments.
Fig. S2. Mapping frequency tags in the bee brain to fixation behavior. (A–D) Fixation behaviors are unaffected by electrode insertion. For A–D, the statistical tests and $P$ values for between bees with electrodes compared with bees without electrodes are as follows: (A) The ratio of instances the bees refixedated on the single bar following a displacement was not significantly different between bees with or without electrodes: rank-sum test statistic $= 120,099$; $P = 0.1425$. (B) In addition, the mean vector length was unaffected with electrode insertion for single green bars: rank-sum test statistic $= 12,259$; $P = 0.9983$. (C) In the presence of two bars 90° apart, there was no change in the ratio of instances when the bees refixedated on the originally fixated bar after a displacement in bees with electrodes: rank-sum test statistic $= 4,538$; $P = 0.4105$. (D) The mean direction of the fixated bars was the same whether the bees had electrodes or not: $P$ test statistic of the common median test $= 3.1369$; $P = 0.0765$. (E and F) Walking behaviors were affected by electrode insertion. (E) The mean velocity per experiment was significantly lower with electrode insertion with single bars: rank sum test statistic $= 10865$; $P = 0.0024$. (F) The ratio of time spent running was significantly lower in bees with electrodes: rank-sum test statistic $= 11,094$; $P = 0.0110$. $n = 26$ bees with inserted electrodes and $n = 10$ bees without inserted electrodes in A–F. (G) Position of the bar over time as the bee fixates and then refixates after a displacement (yellow line). (H) Polar plot of the angular distribution data from the entire sample experiment. Histograms indicate the mean relative count of positions of the bar throughout the experiment. Blue arrow indicates the mean vector length for the data. (I) LFP responses to the same green bar flickering at 20 Hz. Data for this recording site is indicated by the red arrowhead in Fig. 2D. (J) Polar plot for 20-Hz SSVEP amplitude mapped onto the arena, for recordings from the medulla (red line) and mushroom body (orange line), for a 2-min recording (recording sites as shown in Fig. 2D). The SSVEP amplitudes were averaged for each bar position around the arena. This circular distribution of SSVEP amplitudes per bar position had direction and amplitude information which could be averaged into a mean vector signifying the mean direction and amplitude of the SSVEP amplitudes and bar positions. Thick lines indicate mean wavelet coefficient magnitudes at each bar position and the thin lines indicate SE. (K) Continuous Morlet wavelet transform analysis shows increased oscillation amplitudes at 20 Hz and at its harmonic in this medulla recording (red trace is time-matched LFP). (L) Correspondingly, colored arrows indicate mean vector length and direction. The mean vector lengths for the SSVEP amplitude as mapped onto the arena is plotted as the flicker tag vector lengths. The mean vector lengths of MWCA values from frequencies outside the flicker tag as mapped to the arena are shown in Fig. 3. SSVEP is at the flicker frequency.
Fig. S3. Closed-loop control increases the response to the frequency tag. (A) A sample trace showing a bee responding behaviorally to a 20-Hz flickering bar in closed loop. (B) The correlated SSVEP amplitude at 20 Hz for the different positions around the arena in a medulla channel (as in Fig. 2I). (C) Locations of the bar rotating around the bee in open loop. (D) Polar plot for 20-Hz SSVEP amplitude mapped onto the arena during open-loop rotation. The brain recording in B is the same as in D. (E) MWCA values when the bar is in the front, to the right and left, and behind the bees. MWCA amplitudes at the flicker tag (colored lines, SSVEP) is significantly higher than the MWCA outside the flicker tag (black points and error bars) during closed-loop experiments (P < 0.000001). The MWCA at the flicker tag, or the SSVEP amplitude, is significantly higher in the front for the lamina, medulla, and central brain in during closed-loop experiments (P < 0.01) and in the medulla and central brain during open-loop experiments (P < 0.01; Kruskal–Wallis multiple-comparisons test). These differences are indicated by the colored asterisks, where each asterisk is color matched to the brain region indicating significant differences between the front and back bar positions. (F) When the data for the front and side quadrants are pooled, there is a significant increase in MWCA at the flicker tag during closed-loop experiments compared with open-loop experiments in the medulla (rank sum test statistic = 97578; P = 0.0053), but not if the bar is behind the bee (rank-sum test statistic = 11,468; P = 0.9969). This separation of the closed- and open-loop brain activity was not significant in any other brain region, although it was close to significance in the lobula (rank-sum test statistic = 14230; P = 0.0719). Open-loop experiments include both open-loop replay and rotation of the bar around the bee at 5.6° per second. Data in E and F were pooled from experiments were bars were flickering at 6, 7, 20, and 30 Hz. n = 26.
Fig. S4. Behavioral tracking increases SSVEP amplitude. (A) A bar was rotated around the bee at 5.6° per second (black line, with corresponding bar positions shown in the schemas above). The bee could respond to the rotating bar by orienting toward the bar at any point in time (red trace), or bees could choose not to track the bar. (B) When the bee oriented toward the position of the bar, SSVEP amplitude increased significantly in the optic lobes compared with when the bees did not track the bar (Kruskal–Wallis multiple comparison test, n = 10). (C and D) We examined the bees’ orientation behavior during open-loop replay experiments, where the bar locations the bees experienced in closed loop were replayed back to the bees. Orientation could be uncorrelated with bar position (C), or correlated (D). (E) The boxplots display SSVEP amplitudes during open-loop replay when the bees would track the stimulus versus when they did not track the stimulus (SI Materials and Methods). SSVEP amplitude values were significantly higher in the lobula (P < 0.01; Kruskal–Wallis multiple comparison test, n = 10). χ² = 5.05; *P = 0.0246.
Fig. S5. Frequency tag modulation in the medulla and lobula precedes behavioral choices. (A) Schema of the switches to fixate onto one bar (Left) or the other bar (Right). (B) Average angular position (± SEM) for closed-loop switches to bar 1 (Left) and bar 2 (Right). When the bees move one or the other bar into the frontal visual field, they could either fixate on one or the other bar, resulting in these mean distributions of bar locations present throughout the experiment. Black lines indicate which bar the bees are fixating on, and gray lines indicate the other bar (which the bees are not fixating on). These examples are to show how the data were subdivided for analysis. Dashed line: when the bar moves to within 30° of the front of the bee (n = 7, n = 212 behavioral switches). (C) The same movement to shift one or the other bar can be produced through open-loop displacements resulting in bar 1 being placed in front (Left) or bar 2 being in front (Right, n = 7, n = 111 switches because of displacements). Frontal fixation zones per bar: bar 1, maroon; bar 2, blue. (D) The switch described above as a result of active control by the bee (behavioral switch) versus as a result of random open-loop displacements (displacement) can induce differing brain responses. The difference for the SSVEP amplitude at the flicker tag for the selected bar versus that for the nonselected bar during a switch (SSVEP1-SSVEP2) is shown for different brain regions. Because the switch can be produced through active control (black line) or open-loop displacements (gray line), we compared the SSVEP differences through time before and after the switch. (n = 10; P < 0.05; Wilcoxon rank sum test) (Table S1). The green brackets indicate the 1.5-s time span 0.5 s before a switch used to calculate the histograms in Fig. 4E. The blue brackets in B and C are the time spans used to calculate the mean SSVEP 0.5 s after a switch, shown in Fig. 4F. n.s., not significant. At least two trials were performed per experiment with all experiments balanced for 20-Hz and 30-Hz spatial positions. SSVEP amp., SSVEP amplitude, or the wavelet coefficient magnitude at the flicker frequency. n = 10. Data were pooled from experiments where bar 1 was 20 Hz and bar 2 was 30 Hz, as well as the reverse condition. Red dots signify significant differences between SSVEP (bar 1)-SSVEP (bar 2) during a behavioral (active control) switch versus a displacement, P < 0.00001, Wilcoxon rank sum test, corrected with a false-discovery rate control.
Table S1. Statistical comparisons within brain structures

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Brain regions</th>
<th>Lamina</th>
<th>Medulla</th>
<th>Lobula</th>
<th>Central brain</th>
<th>Antennal lobe</th>
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<td></td>
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<td>Mean vector length for MWCA around the arena for the flicker tag versus outside the flicker tag</td>
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<td>Closed loop (n = 20)</td>
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<td>&lt;0.0001</td>
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<td>0.1587</td>
<td>2,442</td>
<td>0.0609</td>
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<td>SSVEP amplitude difference between flicker tags either before or after a behavioral switch versus before or after a displacement</td>
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<td>Before (n = 10)</td>
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<td>5,789</td>
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(Upper half) Mann-Whitney U test rank-sum statistical comparisons between the mean vector length for the wavelet coefficient amplitude around the arena for the flicker tag versus the same computation for outside the flicker tag during closed-loop and replay experiments. (Lower half) Mann-Whitney U test rank-sum statistical comparisons for the difference in SSVEP amplitude for the selected bar either before or after behavioral switches versus the same for displacements. n indicates the number of bees.

Movie S1. Fixation behavior.

Movie S1