Selective attention in the honeybee optic lobes precedes behavioral choices

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Attention allows animals to respond selectively to competing stimuli, enabling some stimuli to evoke a behavioral response while others are ignored. How the brain does this remains mysterious, although it is increasingly evident that even animals with the smallest brains display this capacity. For example, insects respond selectively to salient visual stimuli, but it is unknown where such selectivity occurs in the insect brain, or whether neural correlates of attention might predict the visual choices made by an insect. Here, we investigate neural correlates of visual attention in behaving honeybees (*Apis mellifera*). Using a closed-loop paradigm that allows tethered, walking bees to actively control visual objects in a virtual reality arena, we show that behavioral fixation increases neuronal responses to flickering, frequency-tagged stimuli. Attention-like effects were reduced in the optic lobes during replay of the same visual sequences, when bees were not able to control the visual displays. When bees were presented with competing frequency-tagged visual stimuli, selectivity in the medulla (an optic ganglion) preceded behavioral selection of a stimulus, suggesting that modulation of early visual processing centers precedes eventual behavioral choices made by these insects.

invertebrate | vision | electrophysiology | local field potential

Attention allows animals to respond selectively to competing stimuli (1, 2). Stimulus-selective responses in the human brain can be endogenously driven, and this volitional form of attention has been referred to as a "top-down" process, to distinguish it from salience-driven or "bottom-up" attention (3). Although even insects display bottom-up attention (4–10), it is unclear whether attention-like selection in the insect brain might also precede or predict behavioral choices. The case for top-down attention is especially compelling for honeybees, which have well-demonstrated visual discrimination and cognitive capabilities (11–14). To effectively relate attention processes to behavior, however, requires sophisticated behavioral tracking or recording brain activity from behaving insects selecting distinct objects (15). Previous psychophysical studies in insects have measured whole body movements using tethered, closed-loop flight paradigms (4–8, 15). However, most studies of visual perception and memory in the bee have involved free flight (11, 13, 14; but see ref. 16). To address the neural mechanisms subserving these behaviors, researchers have traditionally recorded brain activity from immobilized bees performing elemental associative learning (e.g., refs. 17 and 18). Animal immobilization, however, is not ideal for gaining a better understanding of the relationship between the complex cognitive behaviors seen in freely moving bees and the underlying neural activity (13, 14). To this end, we developed a closed-loop paradigm for walking honey bees (19), allowing them to select and fixate visual cues by rotating an air-supported ball. To simultaneously examine attention dynamics in the bee brain, we tagged visual stimuli, such as bright green bars (20, 21), with distinct flicker frequencies (7, 22) and tracked the tags in brain recordings in behaving animals selecting visual objects. We found that honeybee fixation behavior increases selective responses in the brain, particularly in early visual processing centers, the medulla and lobula. When bees are faced with competing visual objects, attention-like responses in the optic lobes are object-specific and precede behavioral selection of one object. Our brain recording experiments in behaving honeybees suggest top-down modulation of neural responses early in the bee visual processing pathway, only one to two synapses away from primary sensory input (23).

Results

Walking Honeybees Fixate on Brightly Lit Vertical Bars. Walking bees actively oriented themselves toward a bright green vertical bar (presented against a dark background) by rotating the ball to bring the bar into their frontal visual field in the closed-loop arena, a behavior we call fixation (Fig. 1 *A–C* and Movie S1). Fixation on a single bar was quantified as a vector of defined length and angular orientation (Fig. 1*C*, blue arrow). Fixation was significantly greater for a single green bar than for an unlit control stimulus (i.e., in the absence of the bar, $n = 61$, mean vector: 0.2641 ± 0.1731; green bar, $n = 74$, mean vector: 0.4296 ± 0.2046; Mann–Whitney U test, rank sum: 3,111, $P < 0.00001$). Freely walking (untethered) bees also tracked rotating bright green bars ($n = 9$) (Fig. S1 *A–C*) (21). In contrast, tethered bees did not fixate on dark bars on a green background (dark bar mean vector: 0.1921 ± 0.1088; Mann–Whitney U test, rank sum: 99, $P = 0.0015$, compared with lit bar) (Fig. S1 *D–F*). However, we found that the fixation behavior was not only phototactic, but also depended on the features of the stimulus, such as high-contrast edges (Fig. S1*G*). For this study, we focused on using a bright green bar to induce fixation behavior (Fig. 1*C* and Fig. S1*D*).

Significance

Attention, observed in a wide variety of animals from insects to humans, involves selectively attending to behaviorally relevant stimuli while filtering out other stimuli. We designed a paradigm that allowed us to record brain activity in tethered, walking bees selecting virtual visual objects. We found that stimulus-specific brain activity increased when the bees controlled the position of the visual objects, and that activity decreased when bees were not in control. When bees were presented with competing objects, brain activity in the optic lobes preceded behavioral choices; this suggests that in animals with tiny brains, such as bees, attention-like processes are pushed far out into the sensory periphery. This trait is likely important for efficiently navigating complex visual environments.

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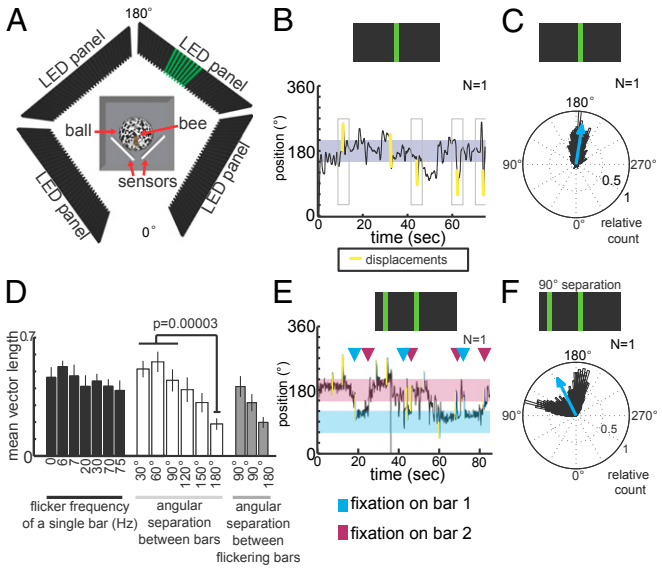

Fig. 1. Honeybees actively fixate on bright green bars in a closed-loop walking paradigm. (*A*) Top view of the schematized set-up. (*B*) A sample trace of angular position in the arena when a bee was controlling a single green bar. Blue shading: bar lying between 150° and 210° in front of the bee. Boxes indicate the periods when a random displacement occurred (yellow line). (*C*) Polar plot of the bar angular distribution data from the same sample experiment. Histograms indicate the mean relative count of positions of the bar throughout the experiment. Blue arrow indicates the mean vector length and direction for the data. (*D*) Mean vector lengths (± SEM) for different green bar flicker frequencies (black bar plot), for different angular separations between two steady green bars (white bar plot), and for different angular combinations of two bars flickering at 20 and 30 Hz (gray bar plot). For the 90° separation, left gray bar includes a left bar flickering at 20 Hz with the right bar flickering at 30 Hz if the bee is facing both bars simultaneously; the right gray bar plot is the reverse condition. $n > 9$ bees for all conditions, statistical comparisons via Wilcoxon rank-sum test. All other statistical comparisons between experiments were not significantly different (Kruskal–Wallis multiple-comparisons test). (*E*) An individual bee alternates fixation on one or the other of two identical, steady green bars (0-Hz flicker). Maroon shading: bar 1 in the fixation window; blue shading: bar 2 in the fixation window. Arrowheads indicate the instances when fixation switches to a different bar: maroon arrowheads, switch to bar 1; blue arrowheads, switch to bar 2. (*F*) Orientation histogram of the data from *E*. Blue arrow, mean vector length and direction. Displacements were included throughout all experiments, but removed during analysis to calculate the orientation histograms.

To test whether fixation behavior may involve active attention-like tracking of the stimulus, we embedded randomly timed "displacements" throughout the closed-loop experiments, wherein the bar briefly moved in open loop for 300 ms either 90° to the left or right at random times (Fig. 1*B*, yellow traces, and Fig. S1 *H* and *I*) (5). We found that bees responded to these salient displacements by returning the bar to the front (i.e., actively fixating it) if the bar had been rotated away (Fig. S1*H* and Movie S1). These behavioral corrections were rapid if the bees were already fixating the bar before a displacement (0.77 ± 0.076 s; bees returned the bar to the front in 75.9 ± 0.06% of the displacements). If bees were not originally fixating the bar when a displacement occurred, significantly more time was required for the bee to bring the bar to the front (1.27 ± 0.057 s; rank sum: 8137.5, $P = 0.00009$). Flickering the bar at different frequencies (6, 7, 20, 30, 70, and 75 Hz) did not significantly alter fixation behavior (Fig. 1*D*, black bar plot) ($n > 9$ for each experiment; Kruskal–Wallis multiple comparison test, $\chi^2 = 7.1$, $P = 0.3121$; displacements were embedded in all experiments).

Walking Honeybees Alternate Between Single Objects When Presented with Two Bars. When a competing visual cue in the form of a second green bar was added (30°, 60°, 90°, 120°, 150°, or 180° away), the bees changed their behavior significantly. Notably, instead of fixating on a single bar for the duration of the experiment, bees performed either one of two distinct behaviors, depending on the angle of separation between the bars (Fig. 1 *D*–*F*). When the bars were closer together (30° or 60° apart), bees tended to fixate between the competing bars, whereas when the bars were further apart, bees tended to alternate fixation on either bar. Although all experiments evoked either behavior, these tendencies resulted in significantly higher mean vector lengths for 30° or 60° compared with 180° separation ($n > 9$ for each angular separation; Kruskal–Wallis multiple comparison test, $\chi^2 = 34.42$, $P = 0.00003$) (Fig. 1*D*, white bar plots). We chose an intermediate separation (90°) for subsequent visual competition experiments because this separation resulted in significantly more time fixating on either bar in alternation (26% of the time) than in between the bars (12% of the time; $n = 10$; rank sum: 2,969, $P = 2.3489e-08$), while also ensuring that the competing bar remained in the bee's visual field (24). At this angular separation, random displacements of the panorama resulted in bees bringing the original fixated bar back to the front 32.1 ± 3.24% of the time, even if the competing bar was displaced directly ahead of the bee. This result indicates that both bars are visible to the bee at this separation, even if one is more peripheral.

To provide unique tags that could be simultaneously tracked in brain recordings, we flickered the competing bars at different frequencies (7, 22). We chose 20- and 30-Hz flicker because these represented a well-separated frequency pair within a neighboring range that evoked equally strong fixation behavior (Fig. 1*D*, black bar plots). When the two competing bars (20 Hz and 30 Hz) were separated by 90°, the bees demonstrated behavioral alternations between the two stimuli (Fig. S1 *J*–*L*) and there was no significant difference in the proportion of time spent fixating on either stimulus ($n = 18$; Mann-Whitney *U* test, rank sum: 700, $P = 0.4673$) (Fig. 1*D*, gray bar plots). We therefore focused on a 90° separation for these frequency-tagged stimuli (20 and 30 Hz) to study neural correlates associated with visual selection and alternation behavior.

Flickering Stimuli Induce Oscillations in the Bee Brain. We performed multisite local field potential (LFP) recordings to examine neural correlates associated with closed-loop visual fixation onto a single flickering bar (Fig. 2). The bee brain has been shown to produce LFP oscillations during olfactory learning that correlate with spiking activity (17), and these oscillations are thought to represent synchronous activity from populations of neurons (25). To record LFPs in the bee, glass electrode tips filled with saline were inserted into the animal's head capsule and secured in place, up to three per bee (Fig. 2*A*). Fine wires threaded into the electrodes recorded brain activity (6). Multiple electrodes per bee allowed us to record from different brain regions simultaneously, and the locations of the electrode tips were identified with local dye released after the experiment (Fig. 2 *B* and *C* and *Materials and Methods*). In this way, we recorded from 56 sites in the brain, distributed among 26 bees. These recording sites covered different regions, including the mushroom bodies, optic lobe structures, and the antennal lobes (Fig. 2*D*). We noted no significant difference in fixation behavior between bees with and without electrodes (Fig. S2 *A*–*D*), although average walking speed was affected (Fig. S2 *E* and *F*).

Our recordings revealed visual responses to the flickering stimulus in various brain regions (Fig. 2*E*), and the responses varied with brain region, as expected (26–29). In general, flicker responses were of greater amplitude in the optic lobes than in the central brain (e.g., mushroom body) (Fig. 2*E*, orange vs. red trace). These neural responses to visual flicker, also called steady-state visually evoked potentials (SSVEPs), can be characterized in the frequency domain as "frequency tags" (22). Because the flickering visual stimuli could be detected in the brain activity, we could link this brain signal to the behavior of the walking bee interfacing with the visual stimuli. We calculated the amplitude of the frequency tag (also called the SSVEP amplitude) by measuring the Morlet

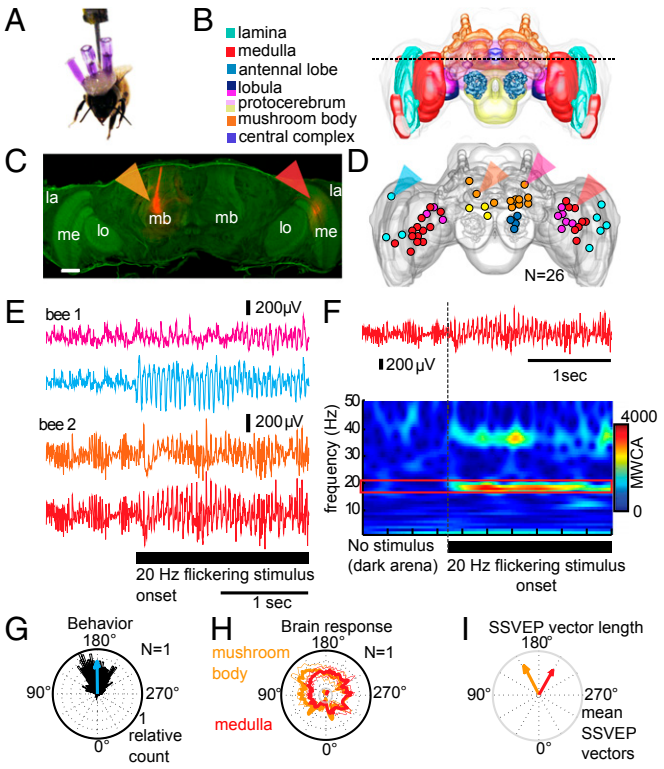

Fig. 2. Mapping frequency tags in the bee brain to fixation behavior. (*A*) Sharp glass electrodes filled with saline and fluorescent dye implanted into the head. (*B*) A 3D bee brain model to map the recording sites. (*C*) Fluorescent dye staining in sectioned bee brains (arrowheads) made it possible to map brain recordings sites to the lamina (la), medulla (me), lobula (lo), mushroom bodies (mb), or antennal lobe (not shown in this section). The level of the horizontal section is indicated by a dashed line in *B*. (Scale bar, 100 µm.) (*D*) Recording sites from 26 bees mapped onto the model brain, color-coded as in *B*. (*E*) LFP responses to a single green bar flickering at 20 Hz. Data for two bees with recording sites are indicated by the color-coded arrowheads in *D* (arrowheads). (*F*) 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 from *E*). A walking bee is presented with no visual stimulus until the dashed line, when a flicker response is present in the medulla LFP. MWCA, Morlet wavelet coefficient amplitude. (*G*) Histograms of locations of the bar around the arena during the bee recording indicated in *F*. (*H*) Corresponding flicker frequency amplitudes in medulla (red) and mushroom body (orange) recordings. Thick lines indicate mean MWCAs during behavior and thin lines indicate SE. (*I*) The distribution of flicker frequency amplitudes around the arena results in two separate MWCA mean vectors with different magnitudes and directions for the medulla (red arrow) and the mushroom bodies (orange).

wavelet coefficient amplitude (Fig. 2*F*) (30). We then determined whether the wavelet coefficient amplitudes at the flicker frequency varied with the angular position of the bar (Fig. 2 *G* and *H* and Fig. S2 *G*–*L*). Because the SSVEP amplitude was mapped onto the 360° visual field, which correlated with the location of the bar throughout the experiment, we could define a circular distribution of SSVEP values around the arena. Averaging both the direction and amplitude of this distribution results in a mean frequency tag (SSVEP) vector for any recording site relative to the bar positions around the arena (Fig. 2*I*), characterized by SSVEP vector length and direction (or angle).

Closed-loop behavior increased the frequency-specific response to the flickering bar (the SSVEP vector length) across all recorded brain regions except the olfactory regions, the antennal lobes (Fig. 3 *A*–*C*; see Table S1 for statistical comparisons). This increased SSVEP response was not a consequence of increased running speed, because SSVEP amplitude did not correlate strongly with running speed ($r = -0.0545$). Because recordings were taken from both brain hemispheres (Fig. 2*D*), there were significant differences in the SSVEP vector angle between most recordings from opposing hemispheres in the brain, indicating that the SSVEP responses are directional (e.g., electrodes in the left hemisphere respond more strongly when the bar is on the left side; Watson–Williams two-sample test: lamina: $P = 0.0017$; medulla: $P = 0.00002$; lobula: $P = 0.4020$; central brain: $P = 0.0063$). To be able to compare SSVEP effects across brain regions, we therefore analyzed SSVEP vector length data, which is not directional, to combine data from both hemispheres (as analyzed in Fig. 3*C*).

Closed-Loop Control Increases SSVEP Responses to Visual Stimuli. The brain response to the frequency tag tended to be greatest when the flickering stimulus was positioned in front of the bee, hence the forward-facing vectors (Fig. 2*I* and Fig. S2 *G*–*L*). We questioned whether this positional specificity of the SSVEP simply reflected the fact that the flickering bar was often positioned in front of the bees because of fixation behavior, or if the population of neurons around most recording sites could have a forward-facing receptive field (24). To separate the contribution of closed-loop control from receptive field sensitivity, we presented the bees with "open-loop replay" stimuli, where animals experienced exactly the same visual sequences as in the previous closed-loop experiments, but in the absence of active control of bar position (*Materials and Methods*). Interestingly, even though the position of the bar around the arena was identical between the experiments (Fig. 3*A*), the LFP response to visual flicker during closed-loop experiments was generally larger than during the replay experiments (Fig. 3*B*). Open-loop replay abolished the significant differences between the SSVEP and other frequencies in all recordings except those taken from the central brain (Fig. 3*D* and Table S1). Thus, in the optic lobes, the position specificity of the SSVEP was greater when the bee was able to control the position of the bar, than when it was unable to do so. In contrast, the central brain remains similarly responsive with or without active control.

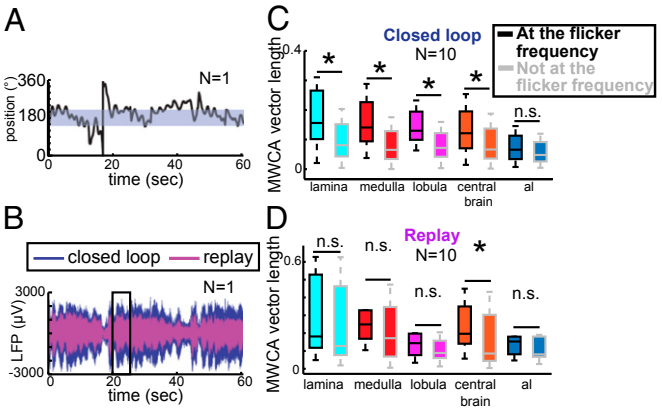

Fig. 3. Closed-loop control increases the response to the frequency tag. (*A*) The angular position of the green bar in an experiment. Blue shading indicates the front of the bee. (*B*) Raw LFP from a medulla recording for the closed-loop experiment in *A* (blue trace), overlaid with raw LFP from a replay experiment where exactly the same bar position sequence as in *A* was displayed (purple trace). (*C*) The mean vector length for the mapped MCWA (Fig. 2*I*) was higher for the frequency tag (black boxes) compared with other frequencies outside the flicker tag (including frequencies outside the 6-, 7-, 20-, and 30-Hz flicker tags specific to the experiment; gray boxes) (Table S1). (*D*) During open-loop replay, the mean vector length for the mapped MCWA was not significantly different from frequencies outside the tag in all brain regions, except for the central brain recordings. *$P < 0.05$, Kruskal–Wallis multiple-comparisons test. Data in *C* and *D* were pooled from bars flickering at 6, 7, 20, and 30 Hz. al, antennal lobe; n.s., not significant.

To further investigate whether closed-loop behavior modulates the brain response, we rotated the flickering bar evenly around the bee, thereby impacting all receptive fields in uniform open-loop bar movements rather than replay (Fig. S3 *A–D*). We found that uniform open-loop exposure broadened the SSVEP amplitude around the arena (Fig. S3 *C* and *D*), supporting the view that the SSVEP effects we observe during closed loop involve some fine-tuning of the visual response to flicker, rather than a receptive field effect. However, to further understand the potential contribution of receptive fields, we subdivided the arena into four quadrants (front, left, right, and back) (Fig. S3*E*). Mapping the SSVEP onto these four quadrants showed that the SSVEP is not significantly different when the stimulus is positioned in front or to either side of the bee for both closed-loop and open-loop experiments (Fig. S3*E*). Instead, SSVEP amplitude in the optic lobes during closed-loop experiments was significantly larger compared with SSVEP during open-loop experiments (Fig. S3*F*), indicating that closed-loop control modulated the optic lobe response to flicker more than the frontal position of the bar.

Stimulus-Specific Brain Activity Increases When the Bees Track Open-Loop Stimuli. It is possible that during open-loop experiments bees might still be tracking the bar around the arena behaviorally, and this might presumably require similar attention-like processes to when the bees can control the stimuli in closed-loop experiments. To address this possibility, we examined SSVEP data for open-loop experiments, comparing when bees were actively tracking the stimulus versus when they were not (*Materials and Methods*). For the uniformly rotating bar, the bees could track the bar by orienting their walking toward the slowly moving bar (5.6° per second), turning left when the bar was to the left, walking forward when it was in front, and turning right when the bar was on the right (Fig. S4*A*). Alternatively, they could ignore the rotation of the bar. We found that the SSVEP amplitude was higher in the optic lobes when the bees were tracking the rotating bar than when they did not track the bar (Fig. S4*B*).

Similarly, we found that bees would choose to either actively track or to ignore open-loop replayed visual sequences of closed-loop experiments (Fig. S4 *C* and *D*). To test whether such tracking behavior was associated with different SSVEP effects, we divided the data into two categories: when the bees tracked the replay stimuli versus not, where tracking during replay was determined by correlation between the bees' turning behavior and the bar position (*Materials and Methods* and Fig. S4 *C* and *D*). We found that active tracking of the replayed sequences increased SSVEP amplitude in the lobula, compared with when bees ignored the replayed stimuli (Fig. S4*E*) ($n = 10$; Kruskal–Wallis multiple-comparisons test, $\chi^2 = 5.05$; $P = 0.0246$). In summary, we found that active tracking of the visual stimuli increases the flicker response (SSVEP) in the optic lobes of the bee brain, whether this is during closed-loop fixation or during open-loop tracking.

Selective Responses in the Optic Lobes Precede Behavioral Selection. To test whether the closed-loop SSVEP effects we see might also be selective in the bee brain, we next presented two competing flickering bars separated by 90° (as in Fig. 1 *E* and *F*). Presenting one bar flickering at 20 Hz and the other at 30 Hz (as in Fig. S1 *K* and *L*) allowed us to determine whether variations in the flicker response at the two frequencies reflected behavioral choices made by the bee (Fig. 4*A*). If attentional resources are limited to single percepts at a time and this phenomenon is reflected in the neural activity, then the brain responses to competing flicker should be anticorrelated in brain regions relevant to visual attention. This result is indeed what we found: the SSVEP amplitudes at the two frequency tags (20 and 30 Hz) alternated in their relative amplitude, depending on whether the bees were fixating on one bar or the other (Fig. 4 *A* and *B*). This alternation behavior resulted in significant negative correlations for the SSVEPs in the medulla and lobula ($n = 10$; Kruskal–Wallis multiple-comparisons test, $\chi^2 = 24.25$; $P = 0.00007$) (Fig. 4*C*). We questioned whether the negative correlation in the optic lobes was modulated by fixation behavior. To test this theory, we compared brain responses during closed-loop control to responses during open-loop displacements, focusing on epochs when one of the bars was moved to the front of the bee while the other bar was simultaneously rotated away (Fig. 4*D*, *Upper*). We found that, when the bees fixated on a stimulus (moved a bar to the front), the correlation between 20-Hz and 30-Hz SSVEP values was still significantly more negative in the medulla compared with other brain regions (Kruskal–Wallis multiple-comparisons test; $\chi^2 = 220.4365$; $P < 0.00001$) (Fig. 4*D*). Interestingly, identical movements resulting from open-loop displacements also produced anticorrelated SSVEPs in the medulla (Fig. 4*D*). This finding suggests that an open-loop salient event (e.g., a displacement to the front) and active fixation both evoke attention-like responses in the medulla, as measured by anticorrelated SSVEPs. This result is consistent with our previous observations comparing closed-loop and active tracking during open loop (Fig. S4).

Even though salient open-loop displacements and closed-loop fixation might yield similar attention-like responses in the bee optic lobes, the genesis for each response is clearly different: one follows a randomly timed event and the other is motivated by the bee. These differences should be detectable in the SSVEP time domain. Indeed, we found that recordings in the medulla and lobula displayed SSVEP selectivity before a behavioral switch, which is when the bee actively fixates one of the two competing bars (Fig. 4*E*, Fig. S5, and Table S1). The change in brain activity is consistent with this being a goal-directed behavior (note that the selected object is not yet in front during the time of the measurement) (Fig. S5). In contrast, random yet identical open-loop displacements of the two bars only increased the SSVEP amplitude in the optic lobes after the event, as would be expected from a salience-driven process (Fig. 4*F*, Fig. S5, and Table S1). Actively fixating on one bar is thus associated with a stimulus-selective response in the medulla and lobula up to 2 s before the behavior occurs (i.e., before the bee moves the selected bar to the front) (Fig. 4*F*). We propose that SSVEP selectivity before a closed-loop behavioral switch may reflect an ongoing, top-down attention process, whereas SSVEP selectivity during or after an open-loop displacement reflects bottom-up attention.

Discussion

The honey bee has a long history as a model system for investigating the cognitive capabilities of insects (11–14, 31). However, it has been difficult to combine electrophysiology with behavior in this insect to study the neural underpinnings of such capabilities (13, 14). Our paradigm combines operant visual behavior and electrophysiology in bees. Although we deliberately only used competing objects of similar salience in this study, future studies using this paradigm could uncover whether SSVEP selectivity in the optic lobes is indeed predictive of any visual goal-directed behavior by, for example, determining whether it can be directed to an inherently less salient object (e.g., following a training session). This approach has recently been used to demonstrate plasticity in medulla cells in crabs following training (32) and could be the key to unraveling the predictive qualities of population-level responses of neurons in the medulla. In particular, because we know that attention in bees may be modulated by experience and different types of training (33–35), as has been observed in humans (36), in-depth studies of brain activity in walking bees navigating more complex environments or training regimes built into this paradigm could allow us to unravel the relationship between SSVEP activity in the optic lobes and learning and memory.

Our results suggest that attention-like mechanisms in the bee brain might guide behavioral choices, rather than merely following salient sensory events (such as sudden displacements). We find that active tracking behavior increases responses to visual stimuli, and that visual choices in walking honey bees are associated with stimulus selectivity in the optic lobes, even when the

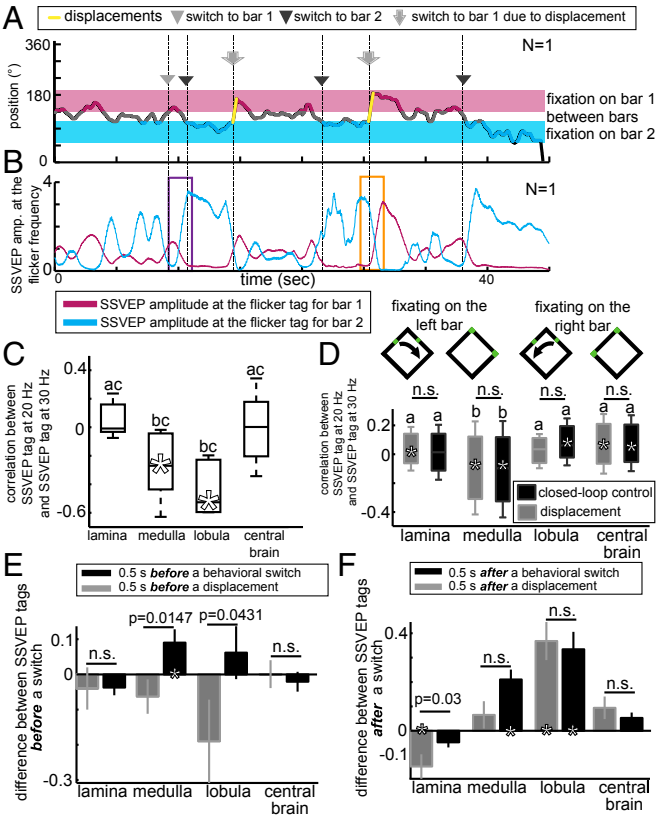

Fig. 4. Frequency tag modulation in the medulla and lobula precedes behavioral choices. (*A*) A sample bee exhibited behavioral switches to bar 1 (dark gray arrowheads), behavioral switches to bar 2 (light gray arrowheads) or switches to bar 1 because of an open-loop displacement (gray arrow indicating yellow trace). (*B*) Medulla recording matching the behavior in *A*. Purple box, behavioral switch from one bar to the next. Orange box, switch from one bar to the next because of a displacement. (*C*) Correlation values between SSVEP amplitude at 20 Hz versus SSVEP amplitude at 30 Hz across different brain structures for competition experiments. Significant differences, Kruskal–Wallis multiple-comparisons test, $\chi^2 = 24.25$; $P = 0.00007$. (*D*) Correlation values were examined only for fixation epochs where one of two objects was moved to the front (*Upper*), and the effect of closed-loop behavior was compared with open-loop displacements. Correlation values were significantly more negative between the 20- and 30-Hz SSVEP amplitudes in medulla compared with other structures (Kruskal–Wallis multiple-comparisons test; $\chi^2 = 220.4365$; $P < 0.00001$), although there was no significant difference between closed-loop and displacement data during these epochs. (*E*) The same epochs as in *D* were examined for changes in the SSVEP before and after fixation. The SSVEP amplitude for the selected bar was significantly higher in the medulla and lobula before a behavioral switch (black bars) than before an open-loop displacement (gray bars). SSVEPs were averaged across a 1.5-s window 0.5 s before a switch (*Materials and Methods* and Table S1) ($n = 10$; $P < 0.05$; Wilcoxon rank-sum test). (*F*) 0.5 s after a switch to fixation, there were no significant differences between open- and closed-loop SSVEPs in the medulla and lobula, although the tag in these optic lobes was significantly higher overall for the bar positioned in front (asterisks) ($n = 10$; $P < 0.05$; Wilcoxon rank-sum test) (Table S1). In *C* and *D*, the letters "a–c" denote a statistically separable group. White asterisks represent values that are significantly different from zero ($P < 0.001$). 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.

selected object is in the periphery (before fixation behavior is initiated). Our results are reminiscent of data from lateral geniculate nucleus recordings in primates, where the effects of top-down attention have been found to alter neural activity at this visual processing stage (37–39). In addition, the activity of single neurons in the superior colliculus have been shown to predict top-down inhibition of eye saccades in monkeys (40), suggesting that the effects of top-down control are observed early on in the visual processing pathway in primates. Along similar lines, the increased stimulus-specific neural activity in early visual processing centers before behavioral fixation in bees could reflect a convergent, and analogous, mechanism for attention: filtering the visual surround by altering neural activity early along the visual processing pathways could enable more efficient processing of the visual world. However, one of the major questions in the field is localizing the brain regions that could be playing a role in modulating early visual processing centers (40, 41).

A role for the medulla or lobula in insect visual selective attention is compelling, particularly in light of a number of recent studies on neurons connecting contralateral optic lobes with the central brain in insects (42–44) have shown that centrifugal feedback neurons also display attention-like properties at a single-cell level (44). Similar long-ranging neurons may be key for inducing the coherent population activity we observe in the medulla and lobula. This model suggests, however, that the locus for top-down control of attention involves feedback from the central brain, or at least coordination between opposing optic lobes. One of the striking results from our study is the absence of attention-like effects for SSVEPs in the central brain, compared with the inner optic lobes, even though the central brain of the bee seems broadly responsive to visual flicker (Fig. 3 *C* and *D*). One simple explanation for this may lie in how data were pooled in our study: the insect central brain is comprised of multiple distinct structures (e.g., mushroom bodies, central complex, protocerebrum) that may play different roles in visual attention, and a future strategy focused on specific structures might reveal distinct attention-like effects in the central brain. An additional consideration is whether color could alter the behavior and the correlated brain activity of the bees. Although we used green bars to elicit behavior, there is the possibility that the use of UV or blue stimuli could trigger a different set of behaviors, particularly because color sensitivity could be distributed into different anatomical structures, such as the mushroom bodies versus the lateral protocerebrum (45). An important test would be to determine whether bees can fixate on single bars that contrast with the background based on chromatic cues, although this behavior, and the subsequent neural activity, could be along achromatic visual pathways (45).

However, our finding that SSVEP responses in the central brain, broadly sampled, are not anticorrelated (Fig. 4*C*) and do not precede behavioral choices (Fig. 4*E*) may also indicate that these central decision-making processes are not necessarily coherent at the population level, but could instead be reflected at the single-cell level (44), particularly among the complex optic glomeruli of the central brain in insects (46, 47). This idea is also supported by data from cockroaches, where spiking activity in large mushroom body extrinsic neurons have been shown to precede locomotor activity (48). In addition, mushroom body neurons are known to exhibit sparse responses to olfactory stimuli in other insects (49), supporting the idea that recording the activity of large single neurons in the central brain may better reflect the participation of the mushroom bodies and central complex in these attention-like tasks. A future challenge will be to identify the decision-making circuits in the central brain that might govern the population-level selective responses we have found in the optic lobes by simultaneously recording single-neuron activity along with the local field potential.

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 and prepared for experiments. Details are provided in *SI Materials and Methods*.

Arena. A diamond-shaped light-emitting diode (LED) arena was assembled from four panels (Shenzchen Sinorad Medical Electronics), allowing display of stimuli of various shapes and colors. Details are provided in *SI Materials and Methods*.

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. Details are provided in *SI Materials and Methods*.

Behavioral Data Collection. To measure the walking response of the bees on an air-supported ball, we used two computer mice. Details are provided in *SI Materials and Methods*.

Visual 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. Details are provided in *SI Materials and Methods*.

Data Analysis and Statistics. Data analysis was conducted through custom programs written in Matlab (Mathworks). Details are provided in *SI Materials and Methods*.

Recording Brain Activity and Brain Histology. Neural activity in the form of LFPs was recorded. Details are provided in *SI Materials and Methods*.

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