Object-based attention describes the brain’s capacity to prioritize one set of stimuli while ignoring others. Human research suggests that the binding of diverse stimuli into one attended percept requires phase-locked oscillatory activity in the brain. Even insects display oscillatory brain activity during visual attention tasks, but it is unclear if neural oscillations in insects are selectively correlated to different features of attended objects. We addressed this question by recording local field potentials in the Drosophila central complex, a brain structure involved in visual navigation and decision making. We found that attention selectively increased the neural gain of visual features associated with attended objects and that attention could be redirected to unattended objects by activation of a reward circuit. Attention was associated with increased beta (20- to 30-Hz) oscillations that selectively locked onto temporal features of the attended visual objects. Our results suggest a conserved function for the beta frequency range in regulating selective attention to salient visual features.

Selective attention refers to the brain’s capacity to focus on a subset of stimuli while ignoring others (1). While subjectively intuitive in humans (2), selective attention has also been documented in a wide variety of animals, such as other primates (3), birds (4), and even insects (5). What is attended to depends on stimulus salience (e.g., loudness or brightness), as well as on the perceived value of a stimulus and the motivational state of the animal (1, 6). What is attended to also depends on what is perceived as a singular object. Object-based attention (7, 8) refers to the capacity to direct attention to a conjunction of different features linked as part of the same object. Attending to one feature of a given object would thus enhance not only the neural representation of that particular feature, but also other features that are associated with the object (9). How this form of generalization works is not entirely understood (10) but seems to require some form of feature binding (11) to first determine which stimuli belong together as a unified object (6, 12, 13) and then to link the object to some inherent value, or valence (14). Hence, feature binding appears to be essential for object-based attention (15–17), as neural gain has to be allocated to specific features first in order to perceive an object as a whole. At the same time, distinct stimulus features can become unbound from an attended object if they are selectively ignored (18).

In the mammalian brain, feature binding and object-based attention have been proposed to be associated mechanisms (19), both of which seem to be facilitated by synchronized activity of neuronal assemblies, which can be detected as phase-locked neural oscillations (16, 20–23). In particular, oscillations in the range of 13 to 30 Hz (beta) and 30 to 80 Hz (gamma) seem to reflect this form of binding based on their strong synchronization at various time points following visual or auditory stimulation, with distinct oscillatory processes potentially reflecting different levels of perception. For example, early (<100-ms) stimulus-evoked synchronization in the gamma range has been suggested to represent rapid integration of unconscious sensory processes, whereas later (200- to 400-ms) synchronization in both the beta and gamma range is hypothesized to reflect feature binding and conscious perception (20, 22, 24, 25). Stimulus-evoked beta and gamma oscillations would thus represent a phase reset of ongoing neuronal activity associated with enhancing attentional gain for specific features, by facilitating information transfer or binding among different brain regions (26).

While there is neural evidence for object-based attention in nonhuman primates (27), it is unknown if the smallest animal brains, such as those of insects, combine diverse sensory stimuli into unified percepts, or if they even have a subjective awareness (28). Behavioral studies in honeybees suggest that some insects can detect illusory contours as single objects (29) and can group distinct stimuli into abstract concepts such as “sameness” or “difference” (30), which could indicate a form of categorization through object-based attention. Similarly, visual learning paradigms for Drosophila melanogaster have uncovered a capacity for context generalization, where flies perceived visual objects as the same despite changes in color (31, 32), suggesting they were attending to the object shape feature and ignoring color cues. There is growing evidence for attention-like processes in insects, such as during visual fixation, decision making, and novelty detection in Drosophila flies (33–36), as well as multiple object tracking in dragonflies (37). The latter electrophysiological study uncovered motion-detecting neurons in dragonflies that selectively lock onto the timing or phase of salient objects, which was shown by “tagging” competing objects with distinct flicker.
behind by the fly. Randomly timed perturbations of the visual object in their frontal visual field (FVF), a behavior known as object as attractive when flies maneuver the ball to place the background (Fig. 1 (height: 26.5°, width: 15°, luminosity: 67 or 301 lx) on an unlit luminosity: 110 or 579 lx) and an aversive small green bar

50 air-supported ball under closed-loop feedback conditions (33, arena of light-emitting diodes (LEDs) (Fig. 1 trolled the angular position of a virtual object in a wraparound processes are controlled in the insect brain or whether these neural frequencies (37). However, it is unknown how such selective neural
oscillations might be more broadly involved in regulating attention-like processes (5), which could also reflect ring attractor dynamics within CX circuits (47, 48). While its role in visual perception is increas-ingly evident, whether the CX produces neural oscillations relevant to visual attention and feature binding is unknown. To address this question requires not only measuring electrical activity in the CX of behaving flies, but also correlating any endogenous brain activity to distinct neural signatures associated with competing visual stimuli or stimulus features.

In tethered virtual reality experiments, flies tend to fixate on large objects and avoid small objects, whether they are flying (49) or walking (33). We exploited this innate visual dichotomy to examine mechanisms underlying visual selective attention in Drosophila. To disambiguate between the attractive and aversive stimuli in the fly brain, and to relate neural activity to ongoing behavioral choices, we recorded local field potentials (LFPs) from the CX and made the competing visual stimuli flicker at distinct frequencies, thereby evoking steady-state visually evoked potentials (SSVEPs) in the fly brain. We first showed that the SSVEPs varied in amplitude depending on the visual objects being fixated upon, allowing us to then investigate how attention guided the binding of different visual features, such as object size, brightness, and flicker frequency. By calculating phase-locking strength between the distinct SSVEPs and endogenous brain rhythms, we examined whether these oscillations in the CX interacted with one another. We found frequency-specific phase locking between endogenous oscillations in the 20- to 30-Hz frequency range and the object features that the fly paid attention to, suggesting that beta-like oscillations could be employed for object-based attention in the insect brain.

Results

Drosophila Flies Generalize Visual Object Preferences. To investigate visual attention in Drosophila, we exploited the flies’ innate attraction to large objects and aversion to small objects (33, 49). This innate preference (or valence) differential based on object size provided a well-grounded starting point to probe visual responsivity in our brain recording paradigm. To demonstrate visual responsivity behaviorally, tethered female flies con-trolled the angular position of a virtual object in a wraparound arena of light-emitting diodes (LEDs) (Fig. 1A) by walking on an air-supported ball under closed-loop feedback conditions (33, 50-52). The 360° visual scene consisted of two objects locked 180° apart, an attractive large green bar (height: 60°, width: 15°, luminosity: 110 or 579 lx) and an aversive small green bar (height: 26.5°, width: 15°, luminosity: 67 or 301 lx) on an unlit background (Fig. 1B). Under closed-loop conditions, we define an object as attractive when flies maneuver the ball to place the object in their frontal visual field (FVF), a behavior known as fixation (33). We define an object as aversive when it is placed behind by the fly. Randomly timed perturbations of the visual scene (60° to the left or to the right) (Fig. 1C) ensured that the flies actively attended to the virtual objects and thus recurrently displayed their fixation preference (33, 50). To track brain activity in this context, we recorded LFPs from the CX, a neuropil in the central brain that has been associated with visual processing (42-44, 53) (Fig. 1D, Materials and Methods, and SI Appendix, Fig. S1). Having previously shown that flies preferred to place the large bar in front and the small bar behind them [even when presented on their own (33)], we added additional visual features to these objects by changing their brightness (contrast) and making them flicker at distinct frequencies (5.9 or 6.6 Hz) (Materials and Methods has brightness and flicker char-acteristics). We presented all combinations of these three visual features (size, brightness, and flicker frequency) in competition, in a counterbalanced design (Fig. 1B and Materials and Methods).

This allowed us to determine if object size preferences persisted despite the layering of additional features or if these added visual features altered the innate preference assigned to these objects. We found that flies still fixated preferentially on the large bar, irrespective of brightness or flicker frequency (Fig. 1E and F and SI Appendix, Fig. S2). This suggests that the valence cue provided by the size of the object dominates over the other visual features or alternatively, that these other features become associated with the valence innately linked to object size. Together with our previous work showing that flies also fixate preferentially on large dark (unit) bars in a flicker background (33), this confirms that object size or shape is driving fixation choices in this para-digm. When object size is kept constant, flies prefer high-contrast objects in this paradigm (33). Here, a brighter, high-contrast small bar was still less attractive than a darker, low-contrast large bar (Fig. 1F and SI Appendix, Fig. S2).

Neural Gain Is Linked to the Valence of an Attended Visual Object. We next determined how the flies’ LFP activity in the CX covaried with their fixation behavior (Fig. 1C). We examined LFP power at the two distinct flicker frequencies (5.9 and 6.6 Hz), counterbalanced for all conditions. Visual flicker produces SSVEPs (or “frequency tags”) in the brains of insects as well as humans, and the amplitude of SSVEPs has been shown to be modulated by attention (36, 37, 54). The flicker frequencies we employed in this study were selected because they produce robust SSVEPs in the fly central brain (Fig. 1C and SI Appendix, Fig. S3A) and are in a range that evoked no innate behavioral preferences compared with other frequencies (Materials and Methods has a description of how frequency preferences were determined; SI Appendix, Fig. S3B). We found that the larger (attractive) bar evoked greater LFP power on average than the smaller (aversive) bar when either of these was in the FVF, irrespective of the brightness of the stimulus (high vs. low) (Fig. 1G, data pooled for both flicker frequencies; SI Appendix, Fig. S4A shows separated frequency data). As with the behav-ioral experiments, this suggests that object size determines the LFP response and that added visual features such as brightness or flicker are subsumed by this primary visual feature. When we compared LFP responses to identical objects of different brightness (e.g., two large bars, pooled for both flicker fre-quencies), we found no significant difference in power when either object was fixated upon in the FVF (Fig. 1H and SI Ap-pendix, Fig. S4B). However, when we examined the LFP re-sponses when the same objects were not being fixated upon (in the periphery, outside the FVF), we observed increased LFP power for the brighter and potentially more salient objects (Fig. 1I and SI Appendix, Fig. S4B) and no difference for the distinct frequencies (SI Appendix, Fig. S4A). These electrophys-iological findings support our behavioral data, showing that object cues dominate over brightness cues when flies are actively fixating by placing preferred objects in their FVF. Importantly, the fixated object determines which flicker frequency evokes a
greater SSVEP in the fly brain (Fig. 1G). These results suggest that neural gain in the CX is linked to the valence of an attended visual object.

**Increased Neural Gain for the Preferred Object Persists under Passive Viewing Conditions.** The above results demonstrate that an innately attractive object (a large bar) evokes a greater LFP response in the fly central brain than an aversive object (a small bar). However, these experiments were done under closed-loop conditions, where the fly was in control of the object on which it decides to fixate (flies also fixate on the aversive object some of the time) (Fig. 1C). We therefore next asked if the differential neural responses assigned to the attractive and aversive bar persisted under “open-loop” conditions, when the fly was not in control. To test this, we placed both objects (large and small) side by side in the FVF for the flies to observe passively while we recorded their brain activity (Fig. 2A). As before, we tagged both objects with distinct flicker frequencies (5.9 or 6.6 Hz) and counterbalanced these for brightness (Materials and Methods) and position (left vs. right) (Fig. 2B). As both objects remained in the FVF, these evoked continuously robust SSVEPs in the central brain (Fig. 2C: in this example, f2 = frequency tag associated with the large bar). Under these passive viewing conditions, we found that the attractive object (the large bar) still evoked a stronger neural response than the aversive object (the small bar), irrespective of left or right position (Fig. 2D), flicker frequency (Fig. 2E), or stimulus brightness (Fig. 2F). This shows that the valence differential based on object size that we observed in closed loop persists in open loop (Fig. 2G). One interpretation of these results is that flies in this open-loop context are attending more to the larger attractive bar, which is reflected in its greater frequency tag, even if they cannot control the bar’s position in the arena.

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Optogenetic Activation of a Reward Circuit Modulates Visual Attention in Closed-Loop Experiments. A question that arises from the preceding results is whether the neural response to the large bar is greater simply because it is a larger object, which may also explain why larger bars evoke more fixation behavior than smaller bars. To disambiguate innate preferences from simple size effects would require identifying a small object that flies find attractive or a large object that they find aversive. Alternatively, the attractiveness of either bar could be altered by changing the motivational state of the flies when they fixated on either stimulus. We decided on an optogenetic strategy to increase the attractiveness of the small bar by activating a putative reward circuit in the fly brain (33, 55, 56) whenever the small bar was fixated upon. In a previous behavioral study in Drosophila, we have shown that an aversive object (a small bar) could be rendered more attractive by optogenetic activation of neurons that express Drosophila neuropeptide F (dNPF) in the fly brain (33). dNPF is the homolog of mammalian neuropeptide Y (57), which is involved in the regulation of emotional responses (58). In flies, dNPF function has been associated with aggression (59), reward (55), and arousal (60). dNPF-expressing neurons provide neuromodulatory input to various regions of the fly brain, including the CX (56), which has been proposed to influence value-based decision making (55). A notable target of dNPF modulation in the CX is the fan-shaped body (FB) (55, 56), which was made clearly visible with mVenus expression in dNPF neurons and was used as a signal to guide the positioning of our recording electrode (Fig. 3A, Materials and Methods, and SI Appendix, Fig. S1). We hypothesized that rendering the small bar more attractive via dNPF circuit activation should increase neural responsiveness to it, which would manifest in the amplitude of SSVEPs. To activate the dNPF circuit, we expressed a red-shifted channel rhodopsin (CsChrimson) (61) in flies that had been fed 0.2 mM all trans-Retinal (ATR+). Acute activation was achieved with red light emitting diodes (LEDs), which illuminated the tethered fly in
the arena (Fig. 3B). To test that the optogenetic manipulation was working, we performed closed-loop fixation experiments in our brain recording preparation, where we acutely activated dNPF circuits whenever the fly fixated on the small bar (Fig. 3 C and D and Materials and Methods). We confirmed that dNPF activation eliminated innate aversion to the small bar (Fig. 3 E and F), as shown previously (33). Interestingly, this induced change in behavior also eliminated any significant differences in neural responses between the fixated objects under closed-loop conditions (Fig. 3G), showing that object size alone did not determine SSVEP amplitudes.

**Activation of the dNPF Circuit Redirects Salience to an Aversive Object in Open-Loop Experiments.** We next investigated how dNPF circuit activation affects brain responses when flies are not able to control the position of the visual objects. We recorded LFPs in flies that were presented with both objects fixed in their VFV (in open loop), counterbalanced for flicker frequency, contrast, and position (Fig. 4A), as before (Fig. 2B). Following our closed-loop results above, we expected the frequency tags associated with either object to be similar in amplitude when the dNPF circuit was activated. However, we found that in the open-loop context, the smaller bar evoked a stronger neural response than the larger bar, irrespective of the frequency tag employed, the brightness, or the object position (Fig. 4B). This is the opposite of what we saw in the control (ATR−) condition (Fig. 4C) and shows that dNPF circuit activation can completely overturn innate salience assignations. More generally, these results confirm that larger objects do not necessarily evoke greater responses in the fly brain. Rather, as in the human brain (62), attention probably regulates SSVEP responses in the fly central brain, with neuromodulatory circuits playing an important role in determining associated neural gain.

The preceding results could suggest that dNPF activation causes flies to redirect their attention from the large bar to the small bar when they cannot control the angular position of the objects. We wondered if this potential switch in attention could nevertheless be verified by tracking walking behavior. To address this, we conducted open-loop behavioral experiments to determine if dNPF activation increased turning bias in the direction of the more salient small bar (Fig. 4D and SI Appendix, Fig. S5). Since the two competing objects were in the VFV and therefore, unlikely to evoke a strong left–right bias, we also tested a second open-loop configuration where the competing objects were farther apart, and we counterbalanced for all object size, brightness, and flicker frequency combinations (SI Appendix, Fig. S5A). Determining a fictive track per fly allowed us to assess their left/right walking preferences for each condition (Fig. 4D). As found previously (33), dNPF activation makes flies walk more slowly (SI Appendix, Fig. S5B). However, dNPF activation during open loop did not bias flies to turn toward either bar, on average (Fig. 4E and SI Appendix, Fig. S5C). Instead, flies increased their walking speed specifically when they were confronted with competing small and large bars (Fig. 4F), a behavior that was not observed in control animals (SI Appendix, Fig. S5D). This suggests that the increased SSVEP assigned to the small bar during dNPF activation in open loop is associated with increased walking speed rather than altered turning behavior.

**Activation of the dNPF Circuit Promotes Selective Endogenous Oscillations.** We observed that dNPF activation appeared to increase overall LFP power in the fly brain, in addition to redirecting salience to the competing smaller object (Fig. 4C). We confirmed this by examining SSVEP power for each object separately, compared with nonactivated controls (SI Appendix, Fig. S5).

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**Fig. 3.** Optogenetic activation of a dNPF circuit abolishes aversion to the small bar. (A) dNPF-Gal4 circuit. dNPF neurons project to the fan-shaped body (FB). LFPs are recorded from the vicinity of the FB. (Scale bar: 50 μm.) (B) Closed-loop setup. dNPF circuit activation is achieved with red LEDs surrounding the arena. Black arrows indicate fly ball and bar movements. (C) Experimental paradigm. Triggering of red LEDs is achieved when the small bar enters the FVF. (D) Example for one fly. Fixation on the small bar in the FVF triggers dNPF circuit activation. A small bar in the FVF is associated with an increase in LFP power for the corresponding input frequency, 6.6 Hz. (E) Mean stimulus position of the large and small visual stimuli for pooled trials of all animals (Rayleigh test for directionality, n = 9, ATR+); 0.2 = axis for mean vector length r. (F) Average stimulus choice for large vs. small bar, pooled for both contrasts and frequencies (Wilcoxon rank sum test, n = 9, ATR+. error bars = SEM). (G) Normalized LFP power for each bar size (Wilcoxon rank sum test on averaged LFP power for corresponding stimulus positions of each animal, n = 9, ATR+. error bars = SEM, data points represent individual instances of averaged LFP power for the corresponding stimulus within the highlighted positions in the LED arena). Red color indicates red LED activation. All animals are dNPF-Gal4; UAS-CSChrimson(x)::mVenus that have been fed all trans-Retinal (ATR+). n.s., not significant.
The overall increase in LFP activity suggested a broadly deployed gain-control mechanism regulated by the dNPF circuit. To further investigate this potential mechanism, we examined the effect of dNPF circuit activation under baseline conditions, when no visual stimuli were present. Under baseline conditions, dNPF circuit activation resulted in an overall increase in LFP power (0.2 to 100 Hz) in the CX (Fig. 5A). This suggested a broad effect on neural gain across a wide range of endogenous LFP frequencies; an increase in overall endogenous LFP activity may explain why SSVEP power is increased for both frequency tags simultaneously. It does not explain, however, why SSVEPs are increased proportionally more for the smaller bar, under dNPF circuit activation. Given that dNPF circuit activation powerfully modulated visual responsiveness, we pursued this controlled optogenetic approach to arrive at a better understanding of how object-based attention operates in the fly brain and what role endogenous oscillations might have in selecting one stimulus vs. another. In humans, endogenous oscillations have been proposed to act as a perceptual binding mechanism in the brain for a variety of frequency ranges such as alpha (7 to 14 Hz) (63), beta (15 to 30 Hz) (64), and gamma (30 to 80 Hz) (65). We, therefore, examined more closely the effect of dNPF circuit activation on endogenous oscillations in the fly brain, under the open-loop conditions that produced such strong selective responsiveness to the small bar (Fig. 4B and C). We partitioned baseline endogenous brain activity recorded from the CX into five frequency ranges (10 to 20, 20 to 30, 30 to 40, 40 to 50, and 50 to 100 Hz) and found a significant increase for all frequencies between 10 and 50 Hz during dNPF activation (Fig. 5B–E) but not 50 to 100 Hz (Fig. 5F). We then asked whether adding visual stimuli (during dNPF activation) evoked any additional LFP effects within these endogenous frequencies and found a further increase specifically in the beta range (20 to 30 Hz) (Fig. 5C). As endogenous beta-like oscillations have previously been associated with visual salience in Drosophila (39, 40), this suggested that the dNPF circuit might be regulating visual attention by controlling 20- to 30-Hz activity in the CX.

To investigate the interplay between endogenous and evoked LFP activity in the CX, we performed phase–amplitude correlation analyses between all endogenous frequency amplitudes (0.2 to 100 Hz) and the phases of the competing frequency tags (5.9 and 6.6 Hz) to measure the envelope to signal correlation (ESC) (66–68) (Materials and Methods and SI Appendix, Fig. S7). A positive correlation in this analysis indicates a modulatory link between the endogenous and evoked oscillations. We found a
significant correlation between endogenous LFP activity (at ~30 Hz) and the phase of the visually evoked oscillations during visual stimulation (Fig. 6A and SI Appendix, Fig. S8). This suggested that the increased endogenous 20- to 30-Hz activity seen during visual stimulation (Fig. 5C) might be involved in selecting which visually evoked tag was bound to the attended percept.

Knowing that dNPF circuit activation dramatically increased neural responsiveness to the smaller bar in this experiment (Fig. 4 B and C), we then examined if 20- to 30-Hz oscillations were specifically associated with this effect. Indeed, we found that optogenetic activation of the dNPF circuit during visual stimulus presentation specifically increased the mean ESC between endogenous 20- to 30-Hz activity and the visually evoked tags (Fig. 6B). Significant ESC effects for higher gamma-like frequencies (30 to 50 Hz) were also observed when the visual stimuli were present (Fig. 6B), but only 20 to 30 Hz showed an increased correlation to the evoked frequency tags upon dNPF circuit activation. This suggests a specific role for endogenous beta-like (20- to 30-Hz) oscillations in valence-driven stimulus selection.

How might endogenous beta-like activity be selecting one evoked tag over another, when these are associated with a more salient object? Although our ESC calculations highlighted endogenous 20- to 30-Hz activity as a potential driver of the frequency tags during dNPF circuit activation, this did not discriminate between the two different tags. Given that dNPF circuit activation increased neural responses to the smaller bar (Fig. 4 B and C) and that this was associated with increased 20- to 30-Hz activity (Fig. 6B), we hypothesized that frequency tags associated with the smaller bar should be more correlated to endogenous 20- to 30-Hz activity. To investigate this, we performed phase-locking analyses between endogenous 20- to 30-Hz activity and the evoked frequency tags assigned to either object to determine a phase-locking value (PLV) (Materials and Methods and SI Appendix, Fig. S7). We found that dNPF activation specifically increased the PLV between endogenous 20- to 30-Hz activity and the tag associated with the smaller bar, compared with the larger bar (Fig. 6C), irrespective of the stimulus frequency. This suggests that under passive viewing conditions, increased neural responsiveness to the small bar is associated with endogenous beta-like activity phase locking specifically to the visual flicker associated with the small bar. dNPF circuit activation appears to drive this effect, as increased phase locking was not observed under control conditions in open loop (Fig. 6C).

Endogenous 20- to 30-Hz Oscillations Lock onto the Visual Features of an Attended Object. Our results suggest that 20- to 30-Hz oscillations in the CX of Drosophila might be employed to drive visual salience effects by increasing the SSVEP gain for the small bar. To determine if this was indeed an attentional mechanism modulated by endogenous beta-like activity, we examined dNPF activation effects under closed-loop conditions, when the fly could demonstrate its visual choices behaviorally. Specifically, we examined brain activity during fixation events when flies returned the smaller bar to the FVF after a perturbation (Figs. 1C and 6D), which was promoted by dNPF circuit activation (Fig. 3). Consistent with an attentional effect, we detected increased 20- to 30-Hz activity when dNPF-activated flies returned the small bar to the FVF (Fig. 6E). Significantly increased 20- to 30-Hz activity was already evident ~250 ms after a perturbation (Fig. 6E; SI Appendix, Fig. S9 shows other frequency domains). Transiently increased 20- to 30-Hz activity was associated with increased power in the evoked 5.9- or 6.6-Hz frequency tags when either of these was associated with the small object (Fig. 6F). Interestingly, significance for either tag power was only evident ~1,000 ms after the perturbation (Fig. 6F), suggesting
Fig. 6. Endogenous 20- to 30-Hz oscillations are phase locked to selected visual objects. (A) Comodulation maps of ESCs during open-loop experiments. The panels show significant positive correlations between endogenous amplitude frequencies (20 to 50 Hz) and the phase of induced frequencies (4 to 10 Hz) for baseline and visual stimulation conditions. Control: ATR−, red LED light, n = 6. dNPF activation: ATR+, red LED light, n = 7 (Pearson’s correlation).
(B) Mean ESC amplitude between 20 to 30 Hz (Left), 30 to 50 Hz (Right), and phase of frequency tags (4 to 10 Hz) during baseline and visual stimulation conditions. Control: ATR−, red LED light, black, n = 6, dNPF activation: ATR+, red LED light, red, n = 7. Repeated measures one-way ANOVA: 20 to 30 Hz: P < 0.0001, degrees of freedom = 3, F(1.863, 190.1) = 15.98; 30 to 50 Hz: P < 0.0001, degrees of freedom = 3, F(2.413, 251.0) = 11.39; both: comparisons for differences between individual groups were analyzed using the false discovery method of Benjamini and Hochberg.
(C) Mean PLVs of evoked frequencies of visual objects with endogenous 20- to 30-Hz activity. Baseline PLV values were subtracted from values during visual stimulation. Control (Left): ATR−, red light, black, n = 6, trials = 80 (P = 0.3307, effect size = 0.15). dNPF activation: ATR+, red light, red, n = 7, trials = 109 (P = 0.009, effect size = 0.51; paired t test [two tailed], bootstrapped data showing means with 95% CIs). (D) Behavioral fixation on small bar after perturbation in closed loop, causing dNPF activation.
(E) Spectrogram showing mean LFP power over time for endogenous 20- to 30-Hz activity, following a perturbation. (F) Spectrogram showing mean LFP power over time for evoked frequencies during visual stimulation. (G) Mean PLV of evoked frequencies of visual objects and endogenous 20 to 30 Hz. Baseline PLV values were subtracted from values during visual stimulation. Control (Left): ATR+, no red light; flies return the large visual object to the FVF. n = 9, perturbation events = 19 (P = 0.004, effect size = 1.03), successful perturbations. dNPF activation (Right): ATR−, red light, flies return the small visual object to the FVF, n = 7, perturbation events = 32 (P = 0.009, effect size = 0.7667), successful perturbations (paired t test [two tailed], bootstrapped data showing means with 95% CIs, data were tested for skewness: 0.95 and showed a log-normal distribution [Shapiro–Wilk test, P = 0.06, α = 0.05, resampling data with 1,000 permutations showed no bimodal distribution]). n = 9. All animals are dNPF-Gal4; UAS-CSChrimson(x)::mVenus flies that were either fed all trans-Retinal (ATR+) or not (ATR−). BL, baseline; n.s., not significant; Stim, stimulus. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.
these followed the 20- to 30-Hz response. We next examined if 20- to 30-Hz activity might be selecting the tag specifically bound to the attended small object. To determine this, we again analyzed phase locking between endogenous 20- to 30-Hz and the evoked frequency tags to calculate a PLV for either visual object. Under control conditions, when dNPF is not activated, flies return the preferred large bar to the FVF following a perturbation (Fig. 1) (33). We found that this behavior was associated with a significant increase in phase locking between endogenous 20- to 30-Hz oscillations and the evoked frequency tags bound specifically to the large bar (Fig. 6G and SI Appendix, Fig. S10). In contrast, when flies returned the small bar to the FVF (induced by dNPF circuit activation) (Fig. 3), the PLV between 20- to 30-Hz oscillations and the evoked frequency tags bound to the small bar increased significantly (Fig. 6G). This effect was not observed in control animals where the optogenetic mechanism was not activated (SI Appendix, Fig. S11). These results suggest that endogenous 20- to 30-Hz activity locks onto the precise temporal features (i.e., the flicker frequencies) associated with an attended object and that this capacity for object-based attention is promoted by dNPF circuits in the fly brain.

Discussion

The brain’s ability to link complex patterns of sensory input into coherent objects has been termed the binding problem (13), with the “problem” being that it remains unclear how diverse sensory streams are unified into a single conscious percept. Our subjective experience of the world is of discretely bound units rather than segregated sensory streams, and this capacity of the human brain is probably adaptive as sensory cues are often correlated, such as voices with faces (69) or fruits with colors (70). The adaptive advantage of perceiving the world in such a unitary fashion raises the question of whether other brains do this (28) and if so, whether the selective attention mechanisms observed in simpler animals such as insects facilitate a form of feature binding.

In humans, the modulation of endogenous beta (15- to 30-Hz) oscillations is associated with the perception and integration of visual stimuli (71), as well as decision making (72), among other cognitive functions (73). Intriguingly, beta oscillations have also been associated with task-related engagement and reward processing as well as stimulus-locked attentional load effects (20, 74–75). However, a full understanding of how beta oscillations are deployed to achieve these functions is lacking, and there remains debate regarding their functional role (75). Our finding beta-like oscillations involved in object-based attention in the insect brain lends support to the view that these oscillations perform a conserved function relevant to perception, as it seems unlikely that a completely different neuroanatomy (an insect brain) would have preserved a neural epiphenomenon. Consistent with a causal role for oscillations in the insect brain, we found that dNPF circuit activation in Drosophila increased 20- to 30-Hz activity in the CX, which promoted phase locking to attended visual stimuli. Interestingly, in open-loop conditions dNPF activation seemed to produce a valence reversal, suggesting that attention was redirected covertly to the smaller, aversive object. Why the smaller object should have higher value in this specific open-loop context remains unclear. An alternative interpretation of this result is that salience for the smaller object was increased, rather than it having been rendered more attractive. Thus, the dNPF circuit might be more involved with regulating salience rather than valence (76), and the salience of the aversive small bar could thus have been magnified by dNPF activation in open-loop conditions, when the fly is not in control. Interestingly, the increased salience assigned to the competing small bar was associated with increased walking speed, suggesting a motivation to respond behaviorally. Electroencephalography (EEG) studies have found that when humans have no control over an array of emotionally laden visual images, these images evoke a higher SSVEP response compared with emotionally neutral images; however, strongly aversive images evoked the greatest SSVEP responses of all (62). Perhaps similarly in the fly brain, an un-controllable aversive object becomes much more salient upon dNPF activation. This highlights the importance of accounting for behavioral control in any understanding of brain functions underlying perception, including flies in open- vs. closed-loop experiments (52).

Beta-like oscillations have been observed previously in the insect brain. For example, recordings in the locust have identified 20- to 30-Hz oscillations associated with processing of olfactory stimuli (77, 78), and comparable oscillations have also been associated with visual attention in flies (40, 79). Additionally, there is increasing evidence that insect brains employ a variety of oscillations, comparable in range with the mammalian brain. These include 7 to 12 Hz (alpha) (80, 81), 20 to 50 Hz (beta and gamma) (80, 82), and even 1 Hz (delta) (83). These oscillations have been shown to be involved in processes such as olfaction, vision, and sleep, suggesting conserved functions that might transcend the differences in brain architecture between insects and mammals. Whether any of these oscillations are functionally comparable remains to be seen. Nevertheless, our current findings suggest that beta-like oscillations might be employed by the insect brain to bind different stimulus features into unified percepts that guide the animal’s attention. Although we did not investigate nonvisual stimulus modalities in this study, previous work has demonstrated that odors modulate the amplitude of visually evoked 20- to 30-Hz activity (40), suggesting these oscillations might govern cross-modal binding as well. Whether endogenous 20- to 30-Hz activity in the fly brain is performing a similar function to beta oscillations in the human brain remains an open question. It is, however, possible that oscillatory processes are supported by different brain architectures that have conserved circuit timing relationships through evolution (84–86). Such conservation might be expected if these oscillations were performing a key function for a variety of adaptive behaviors, such as navigation, finding food, or avoiding predators (87, 88). Our study suggests that oscillations in the beta range (20 to 30 Hz) are indeed performing an important phase-locking function to choreograph meaningful information under real-world conditions, guide selective attention. Although mammalian and fly brains are obviously different, they share some organizational principles (28, 89) that could support the preservation of such oscillatory functions (84).

To determine whether the significant phase–amplitude correlations we observed were due to a physiologically relevant shift between SSVEP phases and endogenous 20- to 30-Hz oscillations, rather than just due to increases in stimulation frequency amplitudes, we performed a simulation where we artificially increased the amplitude of visually evoked 20- to 30-Hz activity (40), suggesting these oscillations might govern cross-modal binding as well. Whether endogenous 20- to 30-Hz activity in the fly brain is performing a similar function to beta oscillations in the human brain remains an open question. It is, however, possible that oscillatory processes are supported by different brain architectures that have conserved circuit timing relationships through evolution (84–86). Such conservation might be expected if these oscillations were performing a key function for a variety of adaptive behaviors, such as navigation, finding food, or avoiding predators (87, 88). Our study suggests that oscillations in the beta range (20 to 30 Hz) are indeed performing an important phase-locking function to choreograph meaningful information under real-world conditions, guide selective attention. Although mammalian and fly brains are obviously different, they share some organizational principles (28, 89) that could support the preservation of such oscillatory functions (84).

To determine whether the significant phase–amplitude correlations we observed were due to a physiologically relevant shift between SSVEP phases and endogenous 20- to 30-Hz oscillations, rather than just due to increases in stimulation frequency amplitudes, we performed a simulation where we artificially increased the amplitudes of only the SSVEPs while keeping other frequencies constant. In the simulation, we found no effect of LFP amplitudes on ESC (SI Appendix, Fig. S12; see SI Appendix, Supplementary Methods). We then repeated the simulation with a specific increase in 20- to 30-Hz and 30- to 40-Hz amplitudes and also saw no correlation to the SSVEPs. This indicates that the phase correlations observed in real fly brain activity are functionally relevant and not a by-product of multiple superimposed oscillations of varying amplitudes. Although 20- to 30-Hz activity stood out as relevant for phase locking to attended objects, other endogenous frequencies showed significant changes upon visual stimulation. In open-loop conditions, visual stimulation alone (without NPF activation) led to an increase in phase–amplitude coupling between SSVEPs and endogenous frequencies in the gamma range (30 to 50 Hz). In the mammalian brain, gamma oscillations have been proposed to provide different functions in sensory processing, depending on the frequency range and timing poststimulus induction. For
example, EEG activity in the lower gamma range (30 to 40 Hz) can be elicited by brief and steady visual stimuli, and an increase in oscillatory power for this frequency range can be observed up to 100 ms after stimulation (90). One idea is that these stimulus-locked gamma oscillations might be relevant for rapid (i.e., unconscious) integration processes that might not necessarily be stimulus relevant (91). Nevertheless, gamma oscillations in humans can also be significantly modulated by attention and stimulus saliency (22, 92, 93). In contrast, a non-stimulus-locked component in the gamma range, occurring around 250 to 350 ms after stimulus presentation, has been proposed to be more relevant for object representation (65). Intriguingly, we see a similar frequency shift in the fly brain. In our study, we observed an increase of 30- to 50-Hz phase locking when visual stimuli were presented, while 20- to 30-Hz phase locking predominated upon NPF circuit activation (Fig. 6B). In humans, it has been shown that synchronized oscillations in the gamma and beta ranges have a high degree of interdependence, showing a so-called “gamma-to-beta” transition in response to novel auditory stimuli, for example (24). Whether a gamma-to-beta transition is also occurring in the insect brain, associated with visual perception, remains difficult to address because any evidence for perception in flies must ultimately depend on behavior, which occurs on a slower timescale than stimulus-evoked neural oscillations.

By grounding our study on innate visual preferences, we could, however, infer how the flies were most likely paying attention to. We found that an innately attractive visual object evokes a greater response in the fly brain than an aversive object and that this effect is preserved even under open-loop conditions, when flies are not in control. This suggests a neural correlate of object-based attention or in other words, a brain signal that correctly identifies what a fly is paying attention to—in the absence of correlated behavior. Although this remains speculative, future experiments tapping directly from this brain signal in closed-loop paradigms should be able to test if it indeed provides a level of cognitive control.

Materials and Methods

Experimental Model and Subject Details.

Optogenetic activation of CsChrimson was achieved by feeding flies 0.2 mM Jayaraman, Janelia Research Campus, Ashburn, Virginia) for all experiments. Lines crossed to UAS-CsChrimson(x)::mVenus(attp40) (provided by Vivek (provided by Ulrike Heberlein, Janelia Research Campus, Ashburn, Virginia) for experiments, 3- to 10-d-old adult female flies were used. We used dNPF-Gal4 (provided by Erich Schwind, University of California, Berkeley) and dNPF-Gal4; UAS-BlueFlash (provided by Ulrike Heberlein, Janelia Research Campus, Ashburn, Virginia) for experiments. Optogenetic activation of Chrimson was achieved by feeding flies 0.2 mM ATX, 3- to 10-d-old adult female flies were used. For our experiments using the data acquisition software AxoGraph X 1.6.9 (Axon Instruments), preamplified via a field effect transistor (NB Labs), amplified (low: 0.1 Hz, high: 5 kHz; A-M Systems Differential AC Amplifier Model 1700), digitized (Axon Digidata 1440A Digitizer), and sampled at 25 kHz.

Visual Stimulation for Electrophysiology.

Closed loop. Flies were presented with two different bar sizes, large (8 x 32 px) and small (8 x 14 px), that could be displayed at two different contrasts, low (Red = 0, Green = 140, Blue = 0) and high (Red = 0, Green = 255, Blue = 0), and flicker at two different frequencies, 5.9 and 6.6 Hz, resulting in a combination of 12 conditions for the binary choice experiment (Fig. 1B). Electrical recording was achieved by inserting a condition depended on the choice behavior of the fly. If the orientation of the ball (turning angle \( \Theta \)) and calculated a fictive path of the fly movements, which was used to generate in a 1:1 translation between the movement of the ball and the rotation on the stimulus within the 360° arena (25-ms delay).

For the optogenetic control of the dNPF circuit, three orange–red LED lights (Luxeon Rebel; 617 nm, 700 mA, LXM2-PH01-00700) were mounted around the arena, focused on the center of the arena. The activation and inactivation of the red LED lights were linked to the position of the visual stimulus in the arena, which was determined by FicTrac and controlled by BlinkStick (Agile Innovative Ltd.), an LED controller board, driven by a custom-written Python (2.7) script. Chrimson was activated with a red light (570 nm, 6 mW/mm², 15 s, Start), and inactivation (25 s, Stop) was achieved by linking the output (movement of the ball) of FicTrac with the position of the stimulus (closed loop), the position of the stimulus on the LED panels was linked to the movements of the ball. This was achieved by linking the output (movement of the ball) of FicTrac with the position of the stimulus (closed loop), the position of the stimulus on the LED panels was linked to the movements of the ball. This was achieved by linking the output (movement of the ball) of FicTrac with the position of the stimulus (closed loop), the position of the stimulus on the LED panels was linked to the movements of the ball. This was achieved by linking the output (movement of the ball) of FicTrac with the position of the stimulus (closed loop), the position of the stimulus on the LED panels was linked to the movements of the ball.
analyze fly walking behavior, we extracted movements of the stimulus; the visual stimuli remained static. In order to every animal, half of the trials were illuminated with three red LEDs sur-

1 min) to ensure that all stimuli were presented with both frequencies. For Each trial was 2-min long. Flicker frequencies were swapped midtrial (after

brightness (bright vs. dark, same values as for all other experiment), size (large 60° bar and small 26.25° bar, 41.25° apart [configuration 1] and 105°

configuration) was randomized.

Open-loop behavior. Flies were presented with two competing visual stimuli (large 60° bar and small 26.25° bar, 41.25° apart) that were both placed in the FVF. We used four different conditions for this paradigm, where we changed the position of each stimulus (left and right) as well as the flicker frequency of the stimulus (5.9 and 6.6 Hz). In order to control for differences in object luminosity, we introduced variability in the contrast of each stimulus by choosing the previous two contrast/luminosity values and randomly assigning one of these values for every cycle of the stimulation, resulting in ~50% of the stimulation period being high contrast (Red = 0, Green = 255, Blue = 0) and ~50% of the stimulation period being low (Red = 0, Green = 140, Blue = 0) contrast for each stimulus. The paradigm consisted of a 10-s baseline where no visual stimulus was present (dark arena) fol-

lowed by a 20-s visual stimulation period and a 10-s baseline in the dark again. For optogenetic stimulation of the dNPF circuit, we activated the red LEDs that were mounted around the arena. The virtual setup for this paradigm was derived from our previous studies (33, 50). A custom-written Python 2.7 script driving a Blinkstick (Agile In-

novative Ltd.) activated the red LEDs that were connected to a photodiode that was connected to a photodiode. The LED activity recorded by the photodiode was digitized and recorded in Axograph in order to synchronize LFP recordings with behavioral data, recorded by FiTrac.

Data Availability. All of the datasets and code supporting the current study are publicly available from the University of Queensland Research Data Management, which is made available via UQ eSpace, the University of Queensland data storage repository https://doi.org/10.14264/43fb51f1.

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