Intrinsic Activity in the Fly Brain Gates Visual Information during Behavioral Choices

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Abbreviations used in this article:
LFP, local field potential
LPTC, lobula plate tangential cell

The small insect brain is often described as an input/output system that executes reflex-like behaviors. It can also initiate neural activity and behaviors intrinsically, seen as spontaneous behaviors, different arousal states and sleep. However, little is known about how intrinsic activity in neural circuits affects sensory information processing in the insect brain and variability in behavior. Here, by simultaneously monitoring *Drosophila*'s behavioral choices and brain activity in a flight simulator system, we identify intrinsic activity that is associated with the act of selecting between visual stimuli. We recorded neural output (multiunit action potentials and local field potentials) in the left and right optic lobes of a tethered flying *Drosophila*, while its attempts to follow visual motion (yaw torque) were measured by a torque meter. We show that when facing competing motion stimuli on its left and right, *Drosophila* generate large torque responses that flip from side to side. The delayed onset (0.1-1 s) and spontaneous switch-like dynamics of these responses make this behavior different from the classic steering reflexes. *Drosophila*, thus, seem to *choose* one stimulus at a time and attempt to rotate toward its direction. With this behavior, the neural output of the optic lobes alternates; being augmented on the side chosen for body rotation and suppressed on the opposite side, even though the visual input to the fly eyes stays the same. Thus, the flow of information from the fly eyes seems gated intrinsically, with this process highlighting chosen information while ignoring the irrelevant. We propose that in small insect brains of limited capacity, intrinsic activity can play an important role in modulating neural information processing and behavior.

INTRODUCTION

By evolving elaborate patterns of behavior, insects have conquered myriads of terrains. Adaptations in the behaviors to ongoing environmental changes further contribute to their success. Perhaps not surprisingly, an insect can react to the same cue quite differently. Although the mechanisms of this behavioral variability are not understood, it is likely to denote variability in the neural information processing, from sensors to effectors, and any factors between them (Poulet and Hedwig 2002, 2006; Faisal et al. 2008). Such factors can be noise (Osborne et al. 2005), recall of previous encounters with similar cues (adaptation, learning or memory) (Neuser et al. 2008), fatigue or change in behavioral or arousal states (van Swinderen et al. 2004; Poulet and Hedwig 2007; Lebestky et al. 2009), or it can arise "spontaneously" from circuits' rhythmic or nonlinear dynamics (Krishnan et al. 1999; Krishnan et al. 2001; Hardin et al. 2003; Tanoue et al. 2004; Andretic et al. 2005; Maye et al. 2007), named as intrinsic activity in contrast to activity evoked by external stimuli. The problem is that by observing an insect's reactions alone, it is very difficult, if not impossible, to deduce the neural basis for the change in its behavior

Studying the role of intrinsic activity in information processing within the small insect brain is particularly interesting for two reasons. First, one needs to verify whether intrinsic activity can modulate transmission of sensory information. In the insect eyes, the neural activity is typically considered input-driven (Frye and Dickinson 2004; Srinivasan and Zhang 2004). More centrally, neural activity may become dominated by circuits' internal dynamics. For example, the projections from the central brain of flies (Otsuna and Ito 2006; Katsov and Clandinin 2008) suggest that excitability of their optic lobes could be modulated by factors other than sensory environment (Rajaram et al. 2005; Zheng et al. 2006; Neuser et al. 2008). Second, intrinsic activity is likely to be closely related to the sensory experience of an insect, which varies throughout its arousal states and behaviors (*cf.* resting *vs.* actively probing its environment) (van Swinderen et al. 2004; Andretic et al.

2005). Most notably, if internal dynamics of circuits were to gate the rooting and processing of sensory information, they could sharpen insects' discriminative capabilities for making behavioral choices (Poulet and Hedwig 2007). Efficient neural control over choices could then facilitate "intelligent behavior" by increasing fitness to solve problems, such as how to find food or sex, or whether to avoid or fight off conspecifics.

Everyday observations suggest that flying insects could use discriminative neural mechanisms to guide their behaviors. Bees navigate, flies chase flies; to name but two of their well-known abilities that involve choosing and responding to relevant information while ignoring the irrelevant. Behavioral experiments further imply that insects can selectively "attend" visual objects (Land and Collett 1974; Wolf and Heisenberg 1980; Heisenberg and Wolf 1984; Srinivasan et al. 2000; Tang et al. 2004) with recent experiments associating changes in local field potentials (LFPs) around the mushroom bodies with object saliency (van Swinderen and Greenspan 2003; van Swinderen 2007). Thus, intrinsic activity might help the insect brain to better employ its limited processing capacity for behaviorally relevant information, while disregarding redundant, irrelevant or confusing information from sensory environment when making behavioral choices.

Motivated by these possibilities, we set out to examine if intrinsic activity within the small brain of *Drosophila* could affect the flow of information from its eyes, when a fly makes a behavioral choice to follow visual motion. In a modified flight simulator system, a tethered flying fly sees two competing motion stimuli (monocular flow fields) of equal strength, one on its left and the other on its right. A fly attempts to follow motion but can only choose one stimulus at a time. This response (yaw torque toward left or right) is taken as a fly's ''report'' for the chosen stimulus, whereas two microelectrodes, implanted in its left and right optic lobes, are used to look for neural signatures (in multiunit action potentials and local field potentials) for this choice. In a sequence of experiments using tethered flies that either rested (to provide baseline signals) or flew, we show that when a *Drosophila* chooses one stimulus and attempts to follow it, the neural activity in the optic lobes is boosted on the chosen side and suppressed on the opposite side, although visual input to its eyes remains unchanged during this behavior. Our results, therefore, suggest that intrinsic neural mechanisms gate visual information processing within the optic lobes, revealing possible neural correlates for "intending" (increase in activity) and "ignoring" (reduction in activity) in the *Drosophila* brain.

Based on these results, we propose a new coding scheme in which intrinsic activity within the fly brain acts upon visual neurons to tune their output to chosen stimuli. In this scheme, information in the visual systems of *active* insects flows simultaneously in two ways: from the eyes to the brain and from the brain to the eyes. The dynamic properties of the neural network in the eyes may set the limits for the visual information that can reach the central nervous system and be perceived, but the processing and routing of that information are further refined by the insect's choices of visual stimuli. Thus, besides highlighting a functional significance of intrinsic brain activity in visual selection, these results may provide new mechanistic insight into the origin of variability in insect behavior.

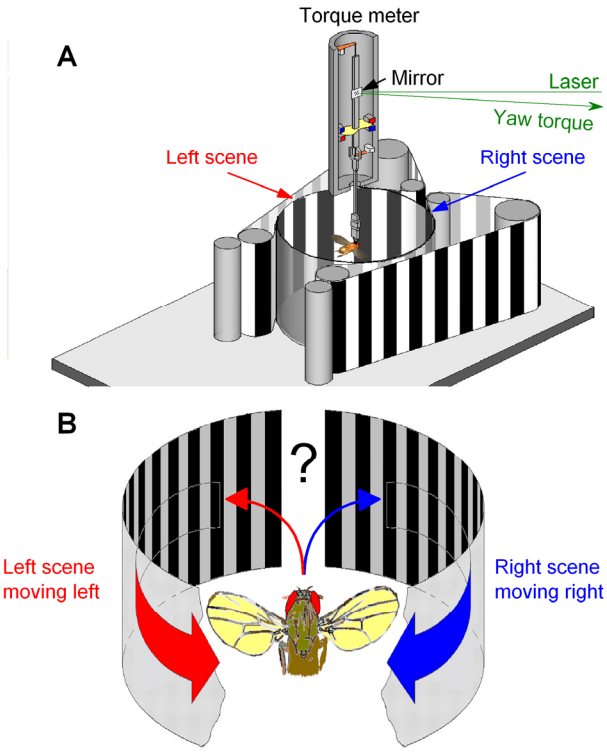

Figure 1. Open loop experiments for measuring a *Drosophila*'s behavior (torque responses) to competing stimuli. (A) Schematic drawing of the flight simulator system. Two identical paper strips, having the same black and white stripe pattern, curve along the surface of a transparent cylinder on the left (red) and right (blue) of a tethered fly, thus forming the left and right scenes, respectively. The scenes are moved by an electrical motor. The yaw torque of the fly, *i.e.* its responses toward the moving scenes, is measured by an opto-mechanical torque meter. A small mirror linearly reflects changes in the yaw torque; light-return of a laser beam over distance greatly amplifies this signal for an optical sensor. (B) Because the fly's head is clamped in a fixed position and orientation, preventing its movements, the fly should see two identical scenes, on its left and on its right, which simultaneously move to opposite directions without any overlapping visual fields. Thus, this stimulation generates two isolated monocular flow fields, one for each eye. The fly's torque response indicates which of the two stimuli (moving scenes) it has "chosen" to pursue at any one time.

RESULTS

Measuring Visual Behavior of *Drosophila* during Competing Motion Stimuli

To investigate how intrinsic activity in the fly brain could influence its visual information processing when making behavioral choices, we adapted a flight simulator system (Götz 1964; Wolf and Heisenberg 1980; Tang and Guo 2001; Tammero et al. 2004) for *Drosophila* to present competing visual motions (**Fig. 1A**). When a tethered flying fly sees a movement, it will orient (turn) toward it (Götz 1964;

Heisenberg and Wolf 1979). Although prevented from turning by a torque meter, the fly's efforts produce minute yaw torque signals, whose size and polarity give the strength and direction, respectively, of these attempts (Götz 1964; Heisenberg and Wolf 1979). When facing two moving objects, on its left and right, *Drosophila* can restrict its torque response to one of them (Wolf and Heisenberg 1980; Heisenberg and Wolf 1984). In attempt to *strengthen* this behavior, we expanded the size of the two moving objects to cover large sectors of the left and right hemifields, respectively (**Fig. 1B**).

Importantly, in our flight arena, both the left and the right eyes face 150º-wide moving scenes, *i.e.* two monocular flow fields; the frontal and caudal parts of the respective hemifields are blanked to eliminate binocular motion cues that can trigger landing or avoidance responses (Tammero and Dickinson 2002b). This motion stimulation, of using two isolated lateral flow fields, differs from the forward flight (Srinivasan and Zhang 2004) or frontal field expansion (Tammero et al. 2004), during which a fly would see a continuous flow field from left to right (for more details see Supporting Information).

Drosophila Generates Switch-like Torque Responses between Competing Motion Stimuli

In the competing stimuli paradigm, visual information in the left and right competes for the fly's torque responses (competitive selection). A tethered flying fly faces two symmetric scenes of visual patterns (*e.g.* black and white vertical stripes) running in opposite directions, to its left and right. Apart from the two opposing motion vectors, everything else in the two scenes remains equal. Most importantly, visual input to the fly's eyes remains equal even during large responses, because its head is immobilized by the torque meter (Götz 1964; Heisenberg and Wolf 1979). The fly cannot make two opposite responses at the same time. In this competitive case, it may choose to react to one direction, generating yaw torque toward (or against) this side, or by balancing its optomotor output, continue in a straight course (Götz 1964; Wolf and Heisenberg 1980; Srinivasan and Zhang 2004).

When the scenes were still, *Drosophila* generated small recurrent body saccades between left and right (**Fig. 2A**, stars; see also **Figs. S4 and S5**), similar to natural exploratory behavior (Heisenberg and Wolf 1979). However, once the scenes started moving (top, black arrow), the flies began to generate 2-10-times stronger yaw torque, *i.e.* intense attempts to rotate to right or left. These large torque responses flipped from side to side as if the right and the left movement were presented alternatingly to a fly, when instead both of the scenes were moving together. Because of the two-state nature of this behavior, *Drosophila* seemed to restrict their responses to one side at a time for 3-20 s, until reacting again to the other side. This periodicity varied considerably between individual flies (*cf.* **Fig. S2D**).

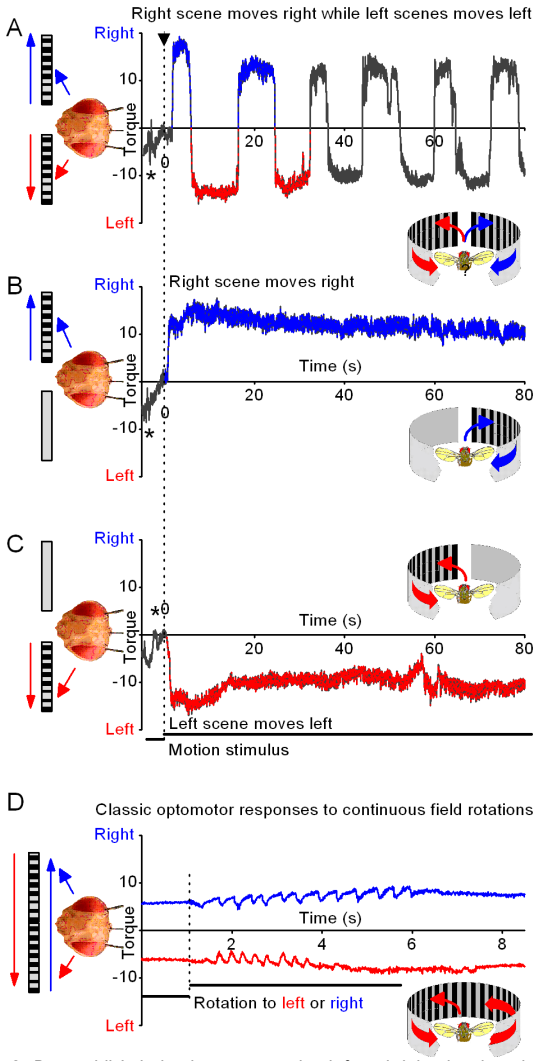

Figure 2. *Drosophila*'s behavior to competing left and right visual motion stimuli. (A) A flying tethered *Drosophila* faces competing stimuli: two identical scenes of black and white stripes, one on its left and the other on its right, in a flight simulator system. When the scenes are still, a fly often generates brief saccades (stars), characteristic of normal exploratory behavior (Heisenberg and Wolf 1979). When the scenes are set to sweep together to opposing directions (dotted line, at time zero), a fly's attempts to rotate (generating yaw torque) toward the left (red trace) or right (blue trace) stimulus begin to flip from side to side with switch-like dynamics, as measured by a torque meter. Throughout these strong responses, the visual input to the fly's eyes remains virtually unchanged, because the fly's head is firmly held by the torque meter in a fixed position. The behavior consists of stereotypical one-sided torque responses, which last 5-15 s, yet their duration and patterning varies greatly from fly to fly (*cf.* Figs. 6, S5 and S2D). The torque responses of a *Drosophila* to right (up) or left (down) during bilaterally moving scenes (A) are of similar strength to its orienting responses when the right (B) or left (C) scenes are moved separately. Thus, with competing stimuli (A), *Drosophila* appears to *choose* one scene at a time and exert its yaw torque according to it, before switching to the opposite stimulus. The general one-stimulus-at-a-time dynamics of the torque responses are comparable to the optomotor behavior evoked by unilaterally or bilaterally oscillating bars (Wolf and Heisenberg 1980). (D) The classical optomotor responses of a fly look different. Tethered to the same torque meter, a flying fly was exposed to 360º visual field (having similar black and white stripes, as above) that rotated left or right. A fly tries to stabilize its vision by attempting to turn into the same direction as the rotating stimulus. The resulting optomotor responses, which contain correction saccades, are typically evoked from the stimulus onset onwards, characteristic of steering reflexes. They are also much smaller than the torque responses to stimuli in A-C. Note the 10-times briefer time scale in D. The optomotor responses in D are shifted up and down to highlight their waveforms. Torque is in arbitrary units.

Superimposed on the switch-like motif, the behavior often included smaller saccades (100-300 ms; *cf*. **Fig. S5**), possibly as attempts to stabilize, or enhance (Franceschini and Chagneux 1997), the flow of visual input from the same direction.

The switch-like torque responses between two motion scenes is a conspicuous behavior, and of course very different from the fast automatic steering reflexes that flying insects use to control their locomotion in changing environments (Tammero and Dickinson 2002a; Frye and Dickinson 2004; Srinivasan and Zhang 2004). We, therefore, need to test its generality in open loop settings by changing optic flow variables in the competing stimuli paradigm. We found that *Drosophila*'s torque responses flipped from side to side with different stimulus speeds (**Fig. S5**), and with patterns of different shapes and sizes (crosses or circles, **Fig. S2D**). Most flies displayed this behavior, sometimes for several minutes. Further experiments, in which a fly was slightly repositioned within the flight arena or in which we dephased the two stimuli, gave similar results, suggesting that the switch-like responses were unlikely to be evoked by visual asymmetry, or by certain pattern features. See Supporting Information for further details.

There is one important question, in particular, that is puzzling in the switch-like behavior. How could a fly initiate a torque response toward one stimulus when it faced an equal but opposite stimulus on the other side? For supposedly an input-driven brain, one would expect that the two opposing flow fields, by producing a seemingly translatory optic flow, would cancel out any turning attempts ("optomotor equilibrium") (Götz 1975; Srinivasan and Zhang 2004). Nonetheless, *Drosophila* was able to react to one stimulus at a time, engendering yaw torque toward it, as if there were no opposing stimulus on the other side. Consequently, it seemed that for executing this behavior, the fly brain would need to generate additional intrinsic activity.

There are two basic schemes how the fly brain could initiate the switch-like behavior during competing stimulation. It could either reduce - or increase - the flow of visual input from one eye, or reduce – or increase - the motor output of the flight control system to the opposing stimulus, thereby creating a neural imbalance to drive a torque response to one direction at a time.

Switch-like Responses Appear not Solely Input-Driven

To gain more insight on these hypotheses, we tested how a tethered flying *Drosophila* responds to a one-sided stimulus. In this monocular stimulus paradigm, the left or right moving scene was sweeping front-to-back, while the other side displayed a motionless blank screen. Interestingly, we found that the initial torque responses toward a single moving scene were of similar size and shape to the responses to the same moving scene in the competing stimuli paradigm (**Figs. 2B-C**). The level of reciprocal symmetry in these responses was analogous to that evoked by uni- or bilaterally oscillating bars (Wolf and Heisenberg 1980; Heisenberg and Wolf 1984), suggesting that both of these responses may share a common mechanism of initiation. Furthermore, classical steering reflexes (or optomotor responses) seemed very different (**Fig. 2D**). There, exposed to rotating visual stimuli, a fly tried to stabilize the visual scenery by turning (its head and body; eyes) into the same direction as the rotational stimulus (Götz 1964) (left or right), evoking "spiky" responses that were much smaller and briefer than the torque responses in the competing stimuli paradigm.

These results were important for two reasons. First, they implied that the conspicuous switch-like behavior might not be purely input-driven. Otherwise, the responses to a single stimulus would have been stronger without the competing stimulus than with it. Second, because these responses had stereotypical early waveforms in both uni- and bilater stimulus paradigms, neural activity that regulated them during competing stimulation must have originated before any motor commands were sent to the flight control system. This deduction, thus, further suggested that to initiate or facilitate switch-like behavior, the flow of visual input, from the eyes to the fly brain, might be modulated by endogenous processing; in other words, intrinsically.

There were other observations supporting these views. During the competing stimulus paradigm, the switch-like responses were infrequently interrupted with periods flying straight (zero torque sections in **Figs. S4** and **S5** indicated by small arrows). This aspect of the behavior implied that the responses might be in part voluntary, with the flies then seemingly "ignoring" the two stimuli. We also observed that in response to one-sided motion stimulus, the flies sometimes exerted yaw torque to the opposite direction, toward the blank motionless screen (**Figs. S6A-B**). This "rebel" behavior was interesting for its own right. It showed that even during torque responses evoked by a powerful monocular motion scene, the flies' reactions were not fully input-driven; a fly could attempt to readjust its body orientation at any stage of the stimulation.

Onset of Switch-like Responses Is Delayed and Variable

Thus, our findings increasingly suggested that *Drosophila*'s torque responses to competing motion stimuli could be initiated and modulated endogenously by a neural mechanism that constituted choice. To further examine this hypothesis, we next analyzed the initiation of the switch-like behavior from the stimulus onset. We were particularly interested in the variability of the first responses, because their early time course might give indications how the underlying neural dynamics leading to them differed from those of fast automatic steering reflexes.

For all the flies tested in the competing stimuli paradigm, we found the initiation of the first response highly variable from trial to trial (**Fig. 3A**). Once the scenes were set in motion (here 60°/s), a fly could wait sometimes for up to seconds (308 ± 196 ms, mean ± SD, n = 106 trials; 18 flies)

before exerting decisive yaw torque to its left or right. When the experiment was repeated (after 10-30 s), the same stimulus very often elicited a different response (**Figs. 3B**). The long-tailed distribution of the wait times (**Fig. 3C**), the varying side and dynamics of the first responses implied that these were not rigid steering reflexes toward visual motion or simple avoidance away from it (Tammero et al. 2004) but more complex actions (Maye et al. 2007). The reported minimal latency of the so-called "object response" to a single black bar, when moved front-to-back either at 110 or 300 °/s, is 35 ms (Heisenberg and Wolf 1984), whereas the latency of the collision-avoidance response to object expansion is typically around 50 ms (Tammero and Dickinson 2002b); also, the apparent delays in the steering reflexes to 360° field rotation in **Fig. 2D** seemed similar (53 ± 13 ms; mean ± SD; n = 6 trials). However, in our visual choice paradigm, it took at least another 30-45 ms for a fly to choose the direction of its response, as their shortest wait was 80 ms.

Taken together, the results from the behavioral experiments implied that during continuously moving competing scenes, a *Drosophila* chose one scene at a time and attempted to orient/turn toward it (or away from the other), *i.e. visual selection*. This view is again consistent with an earlier report of *Drosophila*'s switch-like torque responses between bilaterally oscillating bars (Wolf and Heisenberg 1980), albeit such stimuli moved differently and covered smaller sections of the eyes than the flow fields used here. However, it remained unclear how *Drosophila* used endogenous processing to decide upon which scene to choose. Without any neurophysiological evidence of the neural dynamics behind the switches, the behavioral evidence, as it stood here, could only be suggestive about the role of the intrinsic activity in decision making. To identify any signatures of relevant neural workings, we needed to compare the fly's behavior to the concurrent activity in the left and right optic lobes, picked up by the miniaturized electrodes.

Measuring Neural Activity in the Optic Lobes

In our experimental set-up, neural activity in the left and right optic lobes of *Drosophila* can be monitored simultaneously with its behavior (torque responses) using miniaturized electrodes (see Materials and Methods). These electrodes can pick-up both firing patterns of nearby neurons, and local field potentials (LFP) that, in case of the very small *Drosophila* brain, seem to signify more global information processing within each optic lobe. For examining how neural activity of the optic lobes might correlate with the fly's behavioral choices, we used the monocular stimulus paradigm for *resting* flies (non flying) and the competing stimuli paradigm for *flying* flies. This experimental design effectively explored encoding of visual motion stimuli in the optic lobes, enabling us to work out the general dynamics of possible intrinsic modulation during behavior.

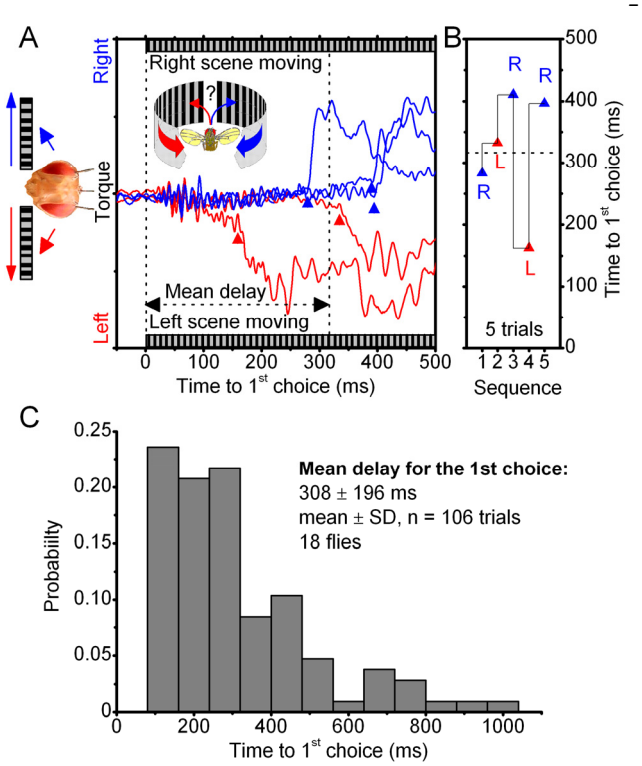

Figure 3. Time-to-choice varies greatly during competing stimulation. (A) A tethered fly is flying in the flight arena, when suddenly the identical scenes on its left and right, are made to move together at the moment of t = 0 (here 60°/s). It takes on average 316.6 ± 100.4 ms (mean ± SD, n = 5) before the fly begins to react either to the left (▲) or right (▲) scene, as measured by time-to-choice of its first switch-like torque responses. The scenes were stopped and started again with tens of seconds between the trials. The responses of flies are highly variable. The double-headed arrow (black) stretches out the mean delay for this fly. (B) Its first responses were either to left (▲) or right (▲), showing no side-preference and with time-to-onset, or wait-period, varying from one trial to another (14/18 flies behaved this way). Other flies preferred one stimulus over its counterpart, yet the wait-period for their first switch-like torque response changed greatly between the trials (4/18 flies behaved this way). The experimental settings were kept identical, but the flies "motivation" to perform varied greatly. In the worst case, we could only test this paradigm twice, before the fly lost "interest" and stopped flying. In the best case, the experiment was repeated 20 times. (*C*) Time-to-choice statistics of the flies are skewed with a heavy tail. As there was no real difference in the variable onset between left and right responses, these results are pooled. Notice, that sometimes it took a fly for over a second to initiate a switch-like torque response toward its chosen stimulus.

Resting Flies: More Activity in the Optic Lobe Facing Movement

Experiments with unilateral visual motion in *resting Drosophila* (**Figs. 4A-B**) showed that each optic lobe received and processed information from both eyes, but that the overall neural activity was always higher (boosted LFPs) within the lobe that faced the movement. During the experiments, the flies remained mostly still, as assessed by their zero torque signals, visual monitoring, and occasionally by video. Because the recordings contained relatively little spurious activity (see Discussion), they represented a reasonable account of how the outputs of the left and right optic lobes encoded monocular flow fields.

The finding that these neurons fired selectively to visual motion, suggested that the electrodes were either lodged within the neuropiles called the lobula plates or in their vicinity. The lobula plates contain an intricate web of large motion-sensitive neurons (Joesch et al. 2008), lobula plate tangential cells (LPTCs), many of which have binocular receptive fields and rapid adaptation dynamics (Hausen and Egelhaaf 1989; Haag and Borst 2004). The lobula plates are only a few synapses away from the photoreceptors and the flight muscles (Fischbach and Dittrich 1989), receive inputs from motion-sensitive elements in both the ipsi- and contralateral eyes (Hausen and Egelhaaf 1989) and from higher brain centers (Otsuna and Ito 2006; Katsov and Clandinin 2008), and participate in gaze control (Hausen and Egelhaaf 1989; Nordstrom and O'Carroll 2009). Based on their importance in visual behavior in dynamic environments, we reasoned that these neuropiles might play a role in intrinsic modulation of incoming visual information. However, given that our electrodes may also pick up activity outside the lobula plates, we call the recording sites more generally the optic lobes.

Flying Flies: Activity of the Optic Lobes Precedes Behavioral Choices

How does the neural activity of the optic lobes represent visual inputs in the competing stimuli paradigm? To begin distinguishing the relevant patters of neural activity involved, we first measured the time from the stimulus onset to the neural response and behavioral choice. Again, a tethered *flying Drosophila* (**Fig. 5A**) was stimulated by identical scenes (*e.g.* stripe patterns) on its left and right, moving to opposing directions. Each switch-like attempt of a fly to turn left or right was then taken as its momentary choice of the stimulus.

Neural activity picked up by the miniaturized electrodes from the optic lobes was typically low when the scenes remained still (**Fig. 5B).** Although the fly's flight muscles were in full action, the electrodes in the optic lobes recorded only sporadic spikes, few and far between, implying stable recording conditions. However, once the scenes were set in motion, it took approximately 15-20 ms (**Fig. 5C**, yellow section) until the electrodes picked up an obvious increase in activity (burst of spikes and hyperpolarizing LFPs), evoked by the visual motion. The delay in these neural responses is consistent with our intracellular measurements of the conduction delays in photoreceptors and primary visual interneurons (Juusola and Hardie 2001; Zheng et al. 2006; Zheng et al. 2009), and a time estimate of further processing stages leading to the visual motion information arriving to the circuitry in the lobula plate. The baseline activity of the optic lobes remained elevated throughout the competing motion stimulation, and showed little change when finally, after a further 190 ms, the fly chose the left stimulus (by beginning to restrict its torque response to left, **Fig. 5D**).

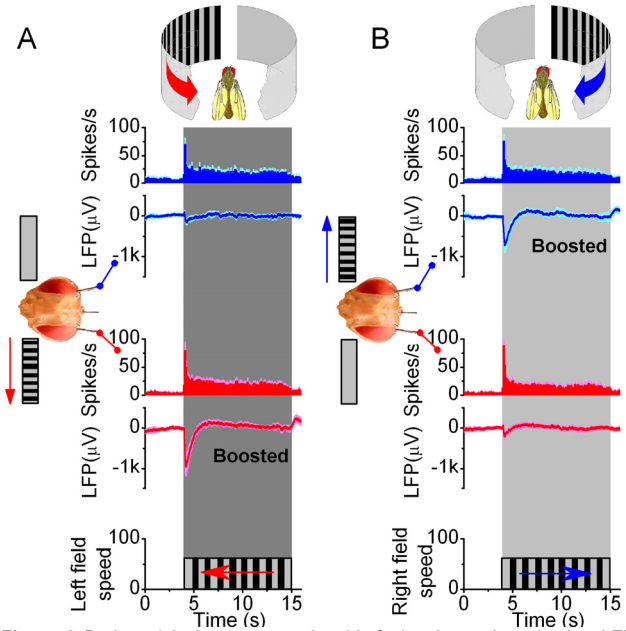

Figure 4. Brain activity increases on the side facing the motion stimulus. LFPs in the left and right optic lobes of *resting Drosophila* are boosted on the side of the moving scene (black and white stripes), whereas the firing patterns show that unilateral visual motion is processed bilaterally in the brain. (A) Neurons in both the right (blue traces) and left (red traces) optic lobes respond simultaneously and adapt rapidly to left motion; this transiently increases their firing rates, amplifying the LFPs. Peak rates: 69.6 ± 29.0 and 79.0 ± 38.0 spikes/s (mean ± SD; right and left electrode, respectively) show no statistical difference, whilst left LFPs are always larger ($p = 0.006$; ANOVA, one-way Bonferreoni test). (B) Similarly, neurons in both optic lobes respond to right motion. Peak rates: 75.3 ± 28.7 and 87.9 ± 40.4 spikes/s (mean ± SD; right and left electrode, respectively) do not differ statistically, but the right LFPs are always larger ($p = 0.012$, ANOVA, one-way Bonferreoni test). Without motion stimulus the activity is low: 5.2 ± 1.3 spikes/s (mean ± SD; $n = 12$). The strong motion-sensitivity suggests that the electrodes reside in the lobula plates. Scenes were separately moved for 6-20 times on either side with 5-10 s interstimulus periods; means ± SEMs shown, n = 6 flies.

It is clear from these and other similar recordings that there was neither strong time-dependency nor correlations between the first neural responses of the optic lobes to motion stimuli and a fly's choice of the stimulus. The first neural responses appeared between 12 to 39 ms (1st spike: 20.63 ± 5.14 ms; mean ± SD; 42 optic lobes, 238 trials) from the stimulus onset, while the flies always "reported" their first choice of stimulus much later, typically after hundreds of ms had passed (*cf.* **Fig. 3C**). Because the exact recording locations of the electrodes within the optic lobes inevitably varied slightly from one lobe to another, so did their sensitivity to pick up neural activity. An observation that one electrode picked up more spikes to the competing stimuli than the other, had obviously nothing to do with a fly's choice of stimulus; thus, neural output of each optic lobe was compared to the torque output separately. However, in a fine time resolution of tens of ms, we failed to find general or consistent interdependencies between the neural outputs and the microstructure of a fly's first switch-like torque response (*cf.* **Fig. 5D**).

The lack of interdependence between the time of the first neural activity and the time of the first torque response means that (**1**) the processes, which initiate the motor output for choice, require a long integration period, and

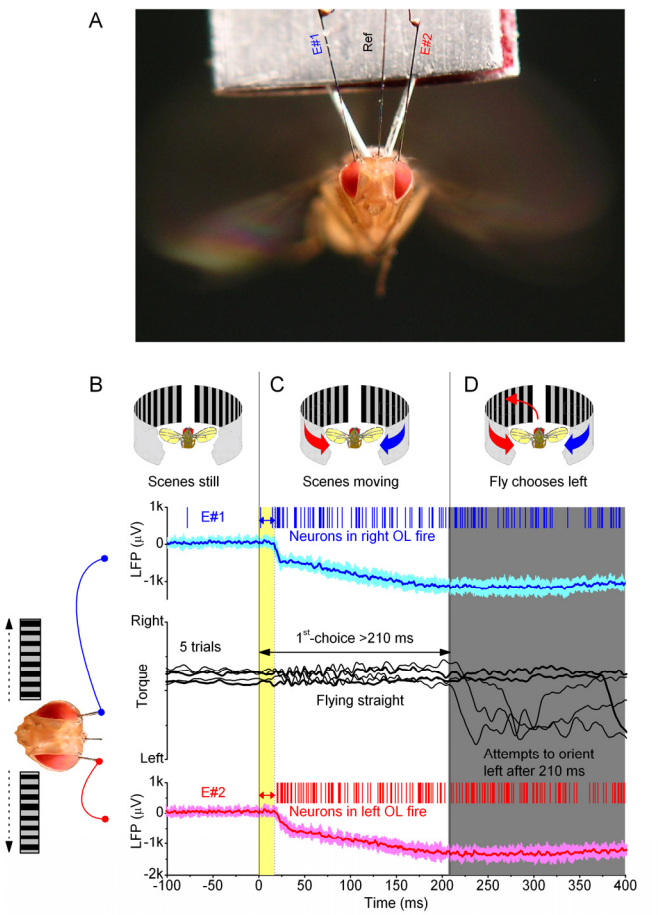

Figure 5. Neural output of the optic lobes to moving stimuli precedes behavioral choices. This figure shows five trials of a single fly in the competing stimuli paradigm. (A) A flying tethered *Drosophila* has three electrodes inserted into its brain: right (E#1) and left (E#2) optic lobes (OL) and reference (Ref). It flies in a flight simulator seeing identical scenes of black and white stripes on its left and right. (B) When the scenes are still, the fly continues flying strength, and the right and left optic lobes show little activity; only a sporadic spike and the local field potentials (LFPs) are flat (E#2, blue traces; E#1 red traces). (C) When the scenes start to sweep to the opposing directions (ft=0), it takes about 20 ms (yellow) for the optic lobes to respond to these visual stimuli (first spikes, and dips in LFPs). However, the fly still only makes little adjustments in its flight path, *i.e.* the yaw torque remains flat. (D) After minimum of 210 ms of competing stimulation, the fly finally chooses the left stimulus by attempting to turn left (gray area), seen as intensifying yaw torque (downward). The fly's choice of stimulus (left) is taken from the point where a new clear trajectory starts in the torque response, crossing the midline. The time to 1st-choice varies greatly; thick black traces show trials where the fly took 375 and 700 ms to choose the stimulus. In the presented fast time scale, the changes in the yaw torque show no obvious influence on the neural outputs of the optic lobes. Recordings like this imply that the early neural activity in the optic lobes is predominantly evoked by visual motion. Thus, here it appears neither induced by, nor corresponds to, stimulus artifacts or flight muscle activity. LFPs show means ± SDs.

that (**2**) while gathering more information, these processes seem to exert little impact on the neural outputs of the optic lobes, which thus appear predominantly vision driven.

Flying Flies: Activity of the Optic Lobes Changes with Behavior

As it could take hundreds of ms for the fly brain to gather enough information to choose between the two stimuli (*cf.* **Figs. 3C** and **5D**), we expected that possible correlations between neural activity and a fly's choices might emerge gradually or periodically over behaviorally relevant integration times. We therefore looked for such signatures of intrinsic activity, which could signal changes in the accumulation and interpretation of visual information within the fly brain, over prolonged time scales (**Fig. 6**). Owing to the slight sensitivity differences between the electrodes to pick up neural activity, the analysis was naturally done for each optic lobe (*i.e.* electrode) separately.

Crucially, we found that neural outputs of the optic lobes showed consistent periodic activity that appeared to correlate with a fly's choices over time scale of seconds (**Fig. 6A)**. When a fly was choosing between the stimuli, *i.e.* generating switch-like torque responses (centre, black), LFPs ("global activity") and firing of neurons ("local activity") in its left (below, red) and right optic lobes (above, blue) waxed and waned, seemingly matching some slower trends in its behavior. For assisting comparisons between these responses, we use a color code in the figures. When a fly exerted torque response to right (chose the right stimulus), the activity of the optic lobes is shown on light gray background; when it exerted torque response to left (chose the left stimulus), the background is dark gray.

Because the fly's eyes were immobilized by the torque meter (Götz 1964; Heisenberg and Wolf 1979), their visual input was the same. Therefore, for purely input-driven activity, adaptation within the eyes should have been equal and the outputs of the optic lobes regular and decaying over time, as happens in surgically-manipulated, fully-immobilized flies (Haag et al. 1999). Instead, as their activity varied when the visual input did not, this modulation was not by adaptation. Nor it was caused by stimulus-related features, such as the inter-pattern (stripe) interval or spatial contrast, because the modulation in the neural outputs of the optic lobes appeared similar for different stimulus patterns (**Fig. S2D**, circles and crosses).

Three observations further strongly argued against neck or head muscle activity (Lowne 1890), so called "clock-spikes" (Hengstenberg 1971; Patterson 1973; Franceschini and Chagneux 1997), as the source for the modulation. First, clear action potentials could only be picked up from a small area in the left and right brain that both in resting and flying tethered *Drosophila* fired to visual motion (**Figs. 4** and **5**). Moreover, the recordings showed only little or no activity, even in flying flies, without visual motion. Second, if the electrodes were placed elsewhere in the fly brain, they typically failed to pick up action potentials both from the resting or flying flies; LFPs were then also much reduced. Finally, in these sites, firing to visual motion (**Fig. 5C**) preceded large torque responses (**Fig. 5D**).

These observations also made it clear that was quite improbable that the electrodes moved within the brain during the torque responses, or that some hypothetical

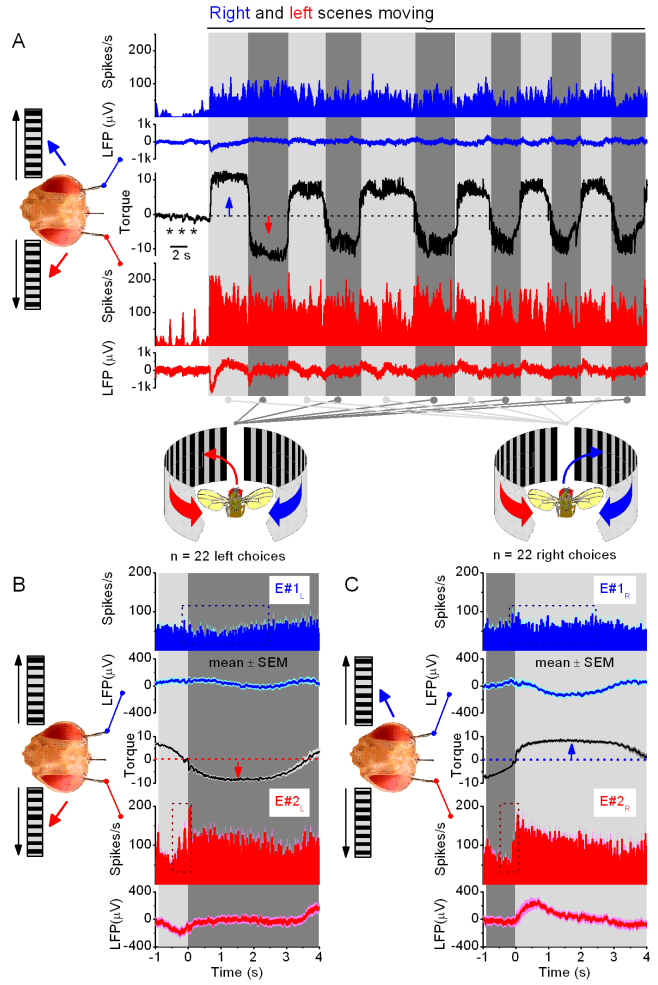

Figure 6. Neural output of the optic lobes is modulated with behavioral choices. (A) A flying tethered *Drosophila* faces identical scenes of black and white stripes on its left and right. When the scenes are still, the fly generates exploratory saccades (stars). When the scenes are set to move to opposing directions, the fly's yaw torque (black) begins eventually to flip between right and left. These behavioral choices of the fly are accompanied with an increased oscillating neural activity both sides of its brain (firing rates and LFPs; blue traces: right electrode, E#1 and red traces: left electrode, E#2). Each choice (or switch-like torque response) can be separated from its neighbors by its clean zero-crossings. Torque responses to right are shown in light gray, and those to left in dark gray. (B-C) show statistics of the neural activity in the left and right LPs for left and right torque responses, respectively (mean ± SEM, n = 22 choices to both directions). The traces were aligned in respect to the corresponding zero-crossings (dotted lines) in the torque signals (black traces). This data was then used for estimating intrinsic modulation (Figs. 7A-B) as the change in the activity of the right optic lobe: E#1 (right torque) – E#1 (left torque); and for the left optic lobe: E#2 (left torque) – E#2 (right torque). For firing rates, the bin-size is 100 ms; torque is in arbitrary units. The dotted boxes in B and C focus on the largest differences in the firing rate in each optic lope for left and right choices.

movements of the electrodes were the source for the modulation. Even if a fly's head was somehow able to move in the torque meter (which it did not in all practical purposes; see **Video S1**), these ultra-light and free-standing electrodes would move with it, not against it (**Fig. 5A**). Thus, for any motion artifacts to modulate the output of two electrodes, so as to match their separate patterns of activity, the left and right brain hemispheres would have needed to be moving in relative independence inside the head capsule, yet in still synchrony with a fly's torque responses. The difficulty of these conjectures, and the finding that the mean firing rate of the neurons often remained stable throughout minutes-long recordings, makes it unlikely that the changes in the neural activity were caused by moving electrodes. While fast rhythmic muscle contractions ("clock-spikes") within the head capsule may participate in refining the light input (Lowne 1890; Hengstenberg 1971; Patterson 1973) when a fly orients between the opposing stimuli, particularly in 5-6 Hz range as reported for *Musca* in a near free-flight conditions (Franceschini and Chagneux 1997), such actions must happen under neural control. Here, they would need to be driven in conjunction with *Drosophila*'s choices that have a 20-100-times slower periodicity (**Figs. 6A** and **2A**; more below).

Therefore, the observed modulation in the neural activity was almost certainly generated intrinsically, either within the optic lobes or within the brain proper that links the two eyes.

Correlating Behavior to Neural Activity

Because the fine structure of neural activity correlated weakly with the fine behavior in 1-100 ms time scales (*cf*. **Fig. 5C-D**), fast efferent flight control affected only marginally the neural responses of the optic lobes. This is not surprising, as one would not expect visual neurons to encode complex behaviors literally; particularly when visual inputs to the eyes are not affected by the behavior. Instead, their activity may *reflect certain aspects of ongoing behavior*. Therefore, we felt well justified to consider torque responses to left or right (over the whole duration of each response) as if these were two binary "choice states". The activity of each optic lobe could be then time-locked by these left and right choices for comparisons.

For correlating the behavioral choices to simultaneous neural activity (**Fig. 6A**), the prolonged torque responses to left (**Fig. 6B**; dark gray background) or right (**Figs. 6C**; light gray background) were aligned by their first zero-crossings and averaged (black traces in the middle). Such estimation was reasonable as the neural activity remained vigorous throughout the selected experiments and a fly's left or right choices often lasted quite similar periods. At zero-crossings, the polarity of the torque responses flipped between left and right, having the fastest rate of change in a fly's torque response. Consequently, time-locking the responses by zero-crossings minimized jitter. The activity in the right (E#1, blue) and left optic lobes (E#2, red) was then time-locked for each behavioral choice and averaged accordingly, making their mean estimates the most reliable.

The recordings, which had many torque responses of similar time course, presented in reliable average left and right "choice states". Although not prerequisite for bilateral comparisons of neural activity to binary choices, nonetheless, the first 3-4 s of the averaged torque responses often had very small SEMs. In such cases, the waveforms of a fly's left (**Fig. 6C**, downward) and right choices (**Fig. 6D**, upward) were similar but of opposite polarity. Whilst more importantly, the average neural activity, as pooled for the left or right choices, respectively, varied relatively little. That is, the corresponding outputs of the right (top, blue) and left (bottom, red) optic lobes were consistent (small SEMs) for each choice. However, their outputs differed for left or right choices. For example, compare the average LFPs and firing rates of the right optic lobe (E#1, blue traces) during left (**Fig. 6C**, E#1_L) and right (**Fig. 6D**, E#1_R) choices. The right optic lobe showed more activity during right choices than left ones, as its firing rate rose and LFP hyperpolarized more then. Clearly, some intrinsic process was exerting its dynamics at the optic lobes in a consistent and choice-dependent manner.

Neural Activity is Boosted on the Chosen Side

How does this intrinsic modulation affect the flow of neural information from the eyes? To answer this question, we subtracted the mean firing rates and field potentials of each optic lobe for a fly's left and right choices. In addition, the choice-dependent differences in the firing rates of local neurons were displayed as relative changes, for instance in the left optic lobe: 100*[E#2_L(spikes/s)-E#2_R(spikes/s)]/E#2_R(spikes/s). Such formulation provides an easy way to assess the relative strength of intrinsic modulation on the neural output of each optic lobe.

This simple analysis exposes the powerful and dynamic nature of intrinsic modulation. In general, the activity in the left optic lobe (**Fig. 7A**, red) was boosted when a fly chose the left scene (black); and quite similarly, its right optic lobe (**Fig. 7B**, blue) was most active when a fly chose the right scene. During the rapid side-switching, the firing rates (centre) could increase over twofold. For some local neurons, the firing rates could in fact peak before a fly "had declared its choice"; before its torque responses crossed the zero mid-line (*cf.* **Fig. 7A**). Nonetheless, we found that for both left and right LFPs (bottom), the largest changes typically occurred slightly later, but still within the early phase of the behavioral choice (note, LFPs increase downwards). Significantly, the LFPs ("global activity") were always boosted on the chosen side (n = 25/25 flies), but the firing dynamics ("local activity") varied with the recording sites (**Figs. 7A-B**, centre).

The inspection of the relative changes in the firing rates across all the experiments reveals a large diversity among the responses (**Fig. S9A**). We expected to see variations in the "local activity" from one recording site to another, because we had little control over which microcircuit each recording electrode ended up touching. As the neurons in the optic lobes are oriented retinotopically, and at the level of the lobula plates, have many cross-connections with the other eye, each receives and processes information differently (Hausen and Egelhaaf 1989; Haag and Borst

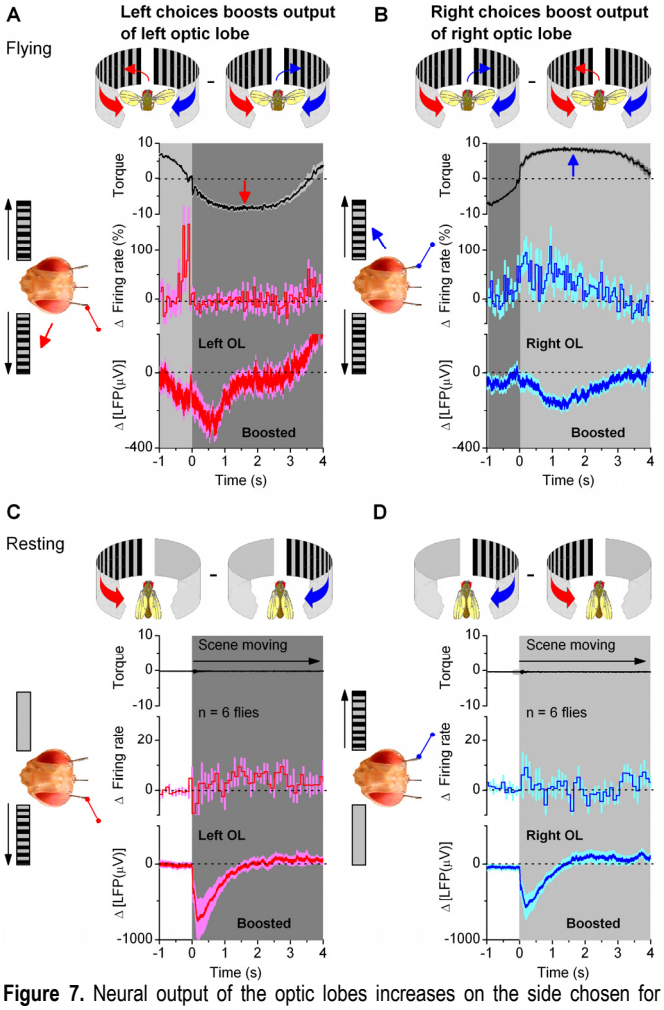

Figure 7. Neural output of the optic lobes increases on the side chosen for torque response. Changes (Δ) in the average transmission of visual motion information are shown for opposing choices (A, B; relative change for firing rates) in flying flies, and for opposing visual stimuli (C, D) in resting flies. Despite seeing equal but opposite motion stimuli (moving scenes of black and white stripes) on its left and right, the activity of the optic lobes changes when a fly chooses the stimulus for its torque response to (A, B) as if the left and right scenes were presented alternatingly to the fly at rest (C, D). (A) Choosing the left stimulus (torque down) boosts the output of the left optic lobe; (B) choosing the right stimulus (up) boosts the output of the right. This data, aligned by the zero-crossings (dotted) in the torque (top) with left/right division (dark/light grey), is from an experiment containing 22 symmetrical choices (switch-like torque responses) to left and right in Figs. 6B-C. Changes in firing rates and LFPs in the left (red) and right (blue) optic lobes, shown when a fly chooses ipsi- and contralateral sides, respectively. (C, D) At rest (zero-torque): left stimulus boosts LFP of the left optic lobe more than right stimulus (C, bottom); the right optic lobe also prefers ipsilateral stimulation (D, bottom). Due to the one-sided stimulation of step-like movements, these differences are larger and more transient than when a fly's chooses between the stimuli (A, B). Mean firing (C, D, middle) shows less modulation as averaging cancels out ipsi/contralateral preferences of individual sites (*cf.* Fig. S7A). The data in (C, D) is from 6 flies in Fig. 4. Torque, arbitrary units; means ± SEMs shown.

2004; Bolzon et al. 2009). However, our data also implies something even more fundamental in this layout. The firing patterns of neurons showed variable ipsi- or contralateral preference with variable tuning. Because of its possible

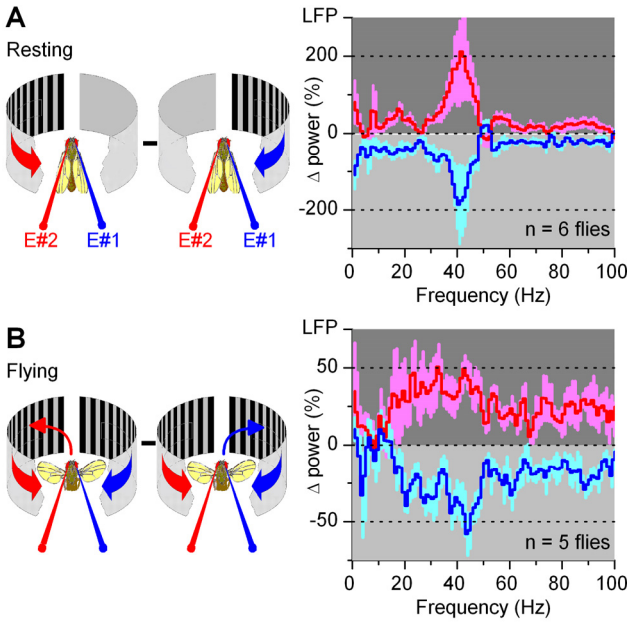

Figure 8. Neural activity (LFPs) increases at gamma-frequencies. Relative changes (Δ) in power spectra of neural activity in the optic lobes, when: (A) a moving screen of back and white stripes is presented on a resting fly or (B) when a fly chooses it (torque response toward it). Traces show mean ± SEM for the relative changes in LFPs pooled from experiments in different flies; E#1 and E#2 are the right and left electrodes. When presented with, or choosing, ipsilateral motion stimulus, the power spectrum of LFP in one LP increases by 20-200% between 20-100 Hz over its corresponding power spectrum for contralateral stimulus; maxima between 20-50 Hz (*i.e.* gamma-band). For details of the calculations and individual experiments, see Fig. S8.

evolutionary and cognitive advantages (Barlow 1961; Atick 1992; Li and Atick 1994), binocular comparison through close arrangement of neurons preferred by different eyes (Hubel and Wiesel 1962; Rossel 1983, 1996; Kara and Boyd 2009) might reflect a general wiring plan of animals with two eyes (Hubel and Wiesel 1965; Barlow et al. 1967; Goodhill 2007). Thus, segregation of neurons into monocular regions within a optic lobe might advocate efficient usage of constrained neural hardware (Li and Atick 1994; Chklovskii 2000) and improve discriminative capabilities (Barlow et al. 1967; Rossel 1983; Kara and Boyd 2009). Despite this potential organization, the firing of their neurons was always intrinsically modulated; when a fly chose the stimulus on a neuron's preferred side, its firing rate increased by 83 ± 16 % (mean ± SEM, n = 17).

Nonetheless, perhaps most remarkably, the increase in "global activity" in one optic lobe, when a fly chose the ipsilateral stimulus, resembled the increase in activity when this stimulus was presented alone to a resting fly (**Figs. 7C-D** and **S6B**). Thus, when a fly chose one of the two competing scenes, the intrinsic modulation made the LFPs look quite as if the fly was seeing only one scene; seemingly, "intending" the relevant and "ignoring" the irrelevant. Naturally, in this comparison, the LFPs to the step-like one-sided stimulation (*cf.* **Fig. 4**) showed larger and more transient differences; after all, one eye did not receive any motion stimulation then. However, it is a striking finding that during competing stimulation (*cf.* **Fig. 6**) neural activity in the chosen side, as defined by torque responses, was boosted with such comparable dynamics. Furthermore, since this modulation was somewhat similar when *Drosophila* were flying (**Figs. 7A-B**) or at rest (**Figs. 7C-D**), it was unlikely to be evoked by steering reflexes (Tammero et al. 2004); *i.e.* it should not have ascended from the haltere system in the thoracic ganglia (see Discussion and Supporting Information).

Intrinsic Modulation Gates the Flow of Visual Information from the Eyes

Having shown that LFPs presented a consistent "global" measure of how intrinsic neural activity modulated the flow of visual information in the optic lobes, we dedicate the rest of the results for analyzing these data further. We next probe whether intrinsic modulation gates the flow of visual information from the eyes in a uniform manner, as one would predict for the rationale of it neurally highlighting the chosen stimulus.

We compare the relative changes in signaling frequencies of the LFPs when the left or right stimulus was presented to a *resting* fly (**Fig. 8A**) or when a *flying* fly chose a stimulus during visual competition (**Figs. 8B** and **S8**). The differences in the LFPs' power spectra between left or right stimulation (monocular stimulus paradigm) or a fly's left or right choices (competing stimuli paradigm) were averaged for each optic lobe across different trials (see Materials and Methods). These dynamics were reproducible for individual optic lobes, but their strength and fine features varied from fly to fly (**Fig. S8**), suggesting again that the recording location influenced how activity from multitude of neural pathways was registered. Nonetheless, because the overall dynamics in each paradigm appear similar, we consider here the mean differences of the recordings. As expected from their bigger responses (*cf.* **Figs. 4** and **6**), the changes in the power spectra were the greatest for monocular stimulation at rest. Yet crucially, the increased activity during resting or choosing occurred predominantly upon similar frequencies. In both cases, neural activity increased (20-400%) in the ipsilateral optic lobe at 10-100 Hz; peaking at 20-50 Hz, at gamma-band. This band of frequencies is often associated with synchronized oscillations of synaptic networks and cognitive processes in higher animals (Jefferys et al. 1996; Varela et al. 2001).

Such modulation could result from a neural mechanism, which excited one optic lobe or, in addition, inhibited the opposite. Here the modulation appeared excitation-inhibition-coupled, as identified by calculating a continuous power-index for the relevant 20-100 Hz band of neural activity (= filtered variance in the time domain) in the left or right optic lobe (**Figs. 9A**; red and blue traces, respectively). When normalized, these simple metrics - for tracking the bilateral neural outputs over time - make it easy to see how the activity of the optic lobes changed during an

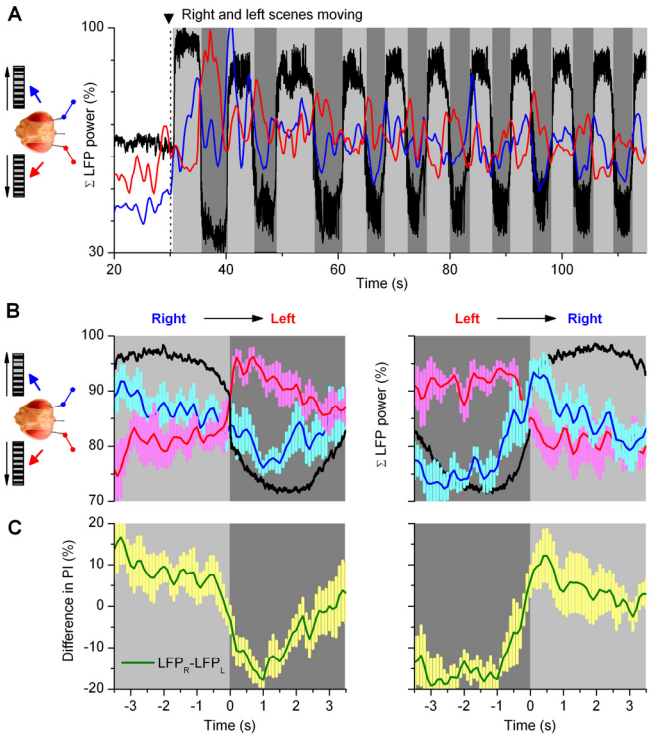

Figure 9. *Drosophila* brain gates the flow of visual information from the eyes. (A) A fly faces identical screens of back and white stripes on its left and right, and we measure the outputs of its left (red) and right (blue) optic lobes, as power-indexes (20-100 Hz frequencies) of their LFPs. When the scenes are set to motion, the left and right power indexes oppose each other (*i.e.* these are 180º phase shifted), alternating in synchrony with the behavior (black). Light grey sections highlight switch-like torque responses to right; dark grey sections to left. Notice also the effect of saliency in the power index; the overall neural activity settles down from the initial maxima during the competitive stimulation as the fly continues choosing between the stimuli. (B) The behavior-triggered average of the right (blue) and left (red) optic lobe's power-indexes during right-to-left and left-to-right torque responses (black); torque, arbitrary units. (C) The difference in power-indexes (green) correctly predicts the behavior in (B). Mean ± SEM of 5 flies. For details of the calculations and individual experiments, see Fig. S9.

experiment. Although modulated in synchrony with the fly's behavioral choices, *i.e.* torque responses (black), the left (red) and right (blue) indexes mostly opposed each other (180º phase shift). Thus, when a fly chose one stimulus, the optic lobe on this side was excited and the opposite optic lobe was inhibited. Hence, intrinsic modulation - either from the higher brain centers or within the optic lobes - gated the flow of visual information from the left and right eyes in a coordinated manner.

In a purely mechanistic view, the intrinsic modulation resembled activity, associated with discriminative information processing in higher animals (Desimone and Duncan 1995; Knudsen 2007): it increased activity in the chosen ("intended") side and decreased distracting activity in the ignored ("unintended") side. Interestingly, the effect of saliency was also clear in LFPs (van Swinderen 2007); as the scenes started to move, the indexes could jump up ~50% before stabilizing to a lower baseline.

To reveal the relative strength and time-course for these opposing signals in the optic lobes, we further calculated behavior-triggered averages of the power-indexes when the fly's behavioral choices of stimuli shifted from right-to-left or left-to-right (**Fig. 9B** and **S9**). Because the average power-indexes (**Fig. 9B**) of left and right optic lobes (n = 5 flies) are similar to each other and to those of individual optic lobes (**Figs. S9**), makes a powerful case that (**1**) gamma-band changes in the LFPs, irrespective of their exact recording location, are real, reproducible and general, and that (**2**) the neural output of each optic lobe is modulated with similar 2-phase dynamics during selective torque responses. The excitatory modulation to optic lobes (left, red; right, blue) seemed in part anticipatory, as the indexes typically rose before the flies settled pursuing the ipsilateral stimuli, peaking at the times (or before) the behavioral choices (black) switched sides, whereas the inhibition was weaker and slower (left, blue; right, red). Thus, the distinctive but coupled excitatory and inhibitory modulations make it unlikely that the left and right eye were rhythmically inhibiting each other and the motor system was simply steering to the side where from most information flows. Instead, this modulation was more likely to arise, and to be coordinated, by the brain proper.

Finally, when confronted with such attractive findings, one likes to speculate that this type of intrinsic mechanism for "gating sensory information" could be used in many predictive coding functions. For example, by subtracting (green) the low-frequency components of the opposing optic lobes (*i.e.* balancing excitatory and inhibitory loads; **Fig. 9C**), a post-synaptic mechanism could predict the fly's choices (**Fig. 9B**, black) reasonably well. Thus, such signals might be useful for matching the intended visual input (gaze-control system) to the intended actions (flight-control system), as the fly chooses "what" to look at and "where" to steer, respectively.

DISCUSSION

We investigated the role of intrinsic brain activity in visual information processing in *Drosophila* that faced two competing stimuli (monocular flow fields) in a customized flight arena (**Fig. 1**). A tethered flying fly exerted switch-like torque responses between the stimuli, as if it saw only one stimulus flipping side-to-side. Thus, it reacted by choosing, one stimulus at a time (stimulus selection) (**Fig. 2**). In repeated trials, a fly took a highly variable time of hundreds of ms to make its first choice (**Figs. 3A, C**), which often varied haphazardly between the stimuli (**Fig. 3B**). Such great variability makes this behavior very different from the classic optomotor steering reflexes. By using miniaturized electrodes lodged in a fly's left and right optic lobes (**Fig. 4**), we explored how their neural activity correlated with the visual motion stimuli and with the fly's behavioral choices (**Figs. 5-9**). We first showed that neural activity was predominantly stimulus-driven and that it always preceded the behavior (**Fig. 5**). Through using both monocular stimulus and competing stimuli paradigms for resting and flying flies, we then identified additional periodic activity, which was neither

set off by the visual stimuli nor a recording artifact, but occurred when a fly was making choices (**Figs. 6-7**). This modulation, which is likely to arise from circuits' internal dynamics, resulted in a gating-process that boosted the overall output (LFP) of the optic lobe facing the chosen stimulus while the output of the opposite side was suppressed (**Figs. 8-9**). The difference between these signals, distributed around gamma-frequencies (20-100 Hz), could in part predict a fly's orienting behavior (**Fig. 9C**).

Together these results present compelling evidence that when a fly makes a behavioral choice (for what ever cause), intrinsic activity acts upon the optic lobes to modulate visual input from the eyes, neurally highlighting the relevant - chosen information - while ignoring the irrelevant.

This study also revealed a somewhat surprising strength of reciprocal interactions between the opposing eyes on responses of individual neurons (**Figs. 5-6** and **S7**). While there has been a variety of electrophysiological approaches for studying signaling in the visual pathways of flies in various passive preparations, *e.g.* (Egelhaaf and Borst 1993; Borst and Haag 2002), our study is perhaps one of the first ones to simultaneously look at neural responses to visual motion at the left and right optic lobes during active behavior[1]. As is shown in the cricket auditory system that uses corollary discharge to reduce the effect of self-generated signals (Poulet and Hedwig 2002), analysis of sensory pathways in passive preparations might not provide a full view of their dynamics in active animals (Poulet and Hedwig 2007).

In the following discussion, we first make clear the reasons for adopting this experimental approach, emphasizing some of its benefits over other possible methods for studying intrinsic activity in *Drosophila*. We then briefly consider different neural mechanisms that could be responsible for our findings. We also comment on the limitations of our findings and our conclusions, and propose some future avenues of research.

About Using the Modified Flight Simulator System

It was fundamental for our experimental aims to have a device that could reliably and repeatedly present visual stimuli to a fly while measuring its behavioral responses. Here, an adaptation of the *Drosophila* flight simulator system served this purpose, as it has for many other vision related questions (Götz 1964, 1975; Heisenberg and Wolf 1979; Wolf and Heisenberg 1980; Heisenberg and Wolf 1984, 1993; Tang and Guo 2001; Tammero and Dickinson 2002a; Frye and Dickinson 2004; Tang et al. 2004; Dickinson 2006; Duistermars et al. 2007b; Duistermars et al. 2007a). The adapted design (**Fig. 1A**) reflected the need to present alike competing stimuli to the left and right eyes while measuring neural responses of the optic lobes in low noise conditions. Whilst these alterations were suited for our specific experiments, the general concept of measuring yaw torque as a fly's behavioral response to visual stimuli did not change. Thus, the large square-waved torque responses of flies to competing stimuli (**Fig. 2**) are not anomalies of the optical torque-meter used in this study, but they look similar in the classic electromagnetic torque-meter. The optical torque-meter (Materials and Methods) was used because of its smaller size and superior signal-to-noise ratio, frequency range and electrical noise properties (*cf*. **Fig. S1**) that made the simultaneous electrophysiological experiments feasible.

In the context of the behavioral assay, we would like to emphasize two important points about the results. First, whilst activity differences between the left and right optic lobes clearly seem to be established intrinsically, besides them *reflecting* different behavioral choices, we make no claim that this visual imbalance would *directly* cause the fly's torque responses. Presumably, the underlying neural mechanisms are more sophisticated, and may involve intricately coordinated parallel processing between multiple sites, similar to species with bigger brains (Knudsen 2007). Second, there remains some degree of ambiguity in the interpretation of flies choosing between the left and right stimuli by "attraction". For example, a fly takes variable time to exhibit switch-like torque responses, but once it starts, it only sporadically flies straight again (**Fig. S4**). Intuitively, its excessive side-to-side flipping may seem reflex-like. Moreover, as a fly tries to turn toward one side and cannot succeed because its head is fixed, it might experience "stress", which could influence the results. Because the competitive stimuli paradigm, by its design, quantifies only choices, not their causes, the exact levels of voluntariness and willingness of these behaviors remain debatable.

About Using Miniature Electrodes

In larger animals, with bigger brains, field potentials recorded with sharp tungsten needles are truly local. However, in the small *Drosophila* brain, the field potentials seem more global than local. Thus, we can only crudely discern from electrophysiological cues and the brain morphology in which circuits the activity arises. For example, we cannot assess contributions of small or large field neurons (Egelhaaf 1985c; Egelhaaf 1985b, 1985a; Egelhaaf et al. 1988; Kimmerle and Egelhaaf 2000; Duistermars et al. 2007a; Bolzon et al. 2009) that should participate in object tracking or flow processing, respectively, on these results. Nonetheless, the miniature electrodes are more robust and less damaging to the circuitry than alternative approaches, such as calcium-imaging or patch-clamping that require tissue-free recording paths. But most importantly, these electrodes can provide long-lasting stable recordings. Our results make it clear that their spatio-temporal properties are sufficient to characterize general

[1]Blondeau (1981) implanted electrodes in the optic lobes of blowflies and by current injection could influence their active behavior, whereas ipsilateral and contralateral optic lobe activity necessary for appropriate behavioral reactions to motion has been examined using lesion experiments (Heisenberg et al., 1978; Geiger and Nässel, 1981,1982).

changes in activity of the optic lobes during the stimulus selection behavior.

In the *Drosophila* brain, we could not find other sites - outside the possible lobula plates - that responded reliably with action potentials (spikes) to visual motion. It was also difficult to find other sites that generated reasonably large spikes; the sites were typically silent, or they fired sparsely with small spikes, uncorrelated to motion stimuli. When the electrode rested favorably within the optic lobe, we carefully tested that the reference electrode (of low impedance) did not influence the recorded activity. Once suitable spikes to a test stimulus (motion field) were picked up, we moved the location and depth of the reference electrode. Because this had no effect on the responses and the output of the two recording electrodes differed from each other, we knew that the spikes came were intended. If instead the spikes had been picked up by the reference electrode, the recording electrodes should have showed similar patterning. This we never saw.

Neural Mechanisms of Intrinsic Modulation?

While our results encapsulate how intrinsic modulation leads to periodic changes in the responsiveness of optic lobes during behavioral choices, one would also like to understand its mechanisms. In particular, the variable dynamics for excitation in the optic lobe of the chosen side, and for inhibition in the opposite side, suggest interlinked chains of events between peripheral visual processing and central initiation of actions. To what extent these dynamics could reflect noise, proprioceptive feedbacks or higher-order processing?

Could intrinsic modulation be neural noise? It is easy to imagine a toy-model for unstable flight motor equilibrium. Two neurons (L and R) receive visual inputs, integrating slowly. Both neurons are noisy and have variable thresholds. If L's threshold is reached first, a left turn is triggered; similarly, crossing R's threshold triggers a right turn. A turn then offsets both neurons, resets their thresholds and the integration starts again. But it is difficult to imagine how simple noisy circuits, during equal left and right stimulation, could generate and couple *faster increase* in the ipsilateral output to that of *slower decrease* in the contralateral output, before triggering the choices (**Fig. 9**). This is because such dynamics signify an ordered gating process, rather than a noisy process for random choices.

Could intrinsic modulation arise from signals ascending from the thoracic ganglia? The haltere sensory fields send anterior projections into the central brain, converging upon pre-motor centers housing lobula plate tangential cell axons (Sandeman and Markl 1980; Strausfeld and Seyan 1985). There is ample electrophysiological evidence that the haltere sensory system directly activates steering motoneurons of the wings (Frye and Dickinson 2004), and receives direct visual input (Chan et al. 1998). Thus, a reflex arc between visual input, the halteres, and the wings might explain how a fly commands a turn (Dickinson 2006). Since the haltere input also projects to the brain (Strausfeld and Seyan 1985; Chan and Dickinson 1996), it may modify network activity there. Perhaps this could manifest as changes in the field potentials?

Whilst these factors might be important in flight control, their feed upon LFP dynamics is probably small for two reasons. First, the visual motion input and the neural responses of the optic lobes precede the switch-like torque responses (**Figs. 5, 9, S9**). As the visual motion input for the eyes remains the same throughout the experiment, it seems unlikely that within these stationary conditions (for both visual and mechanosensory inputs) the haltere output would start to modify the neural activity of the optic lobes before the fly actually executes its major steering response. Second, when a fly rests, its halteres should not modulate neural activity of the brain in the same way as they do when the fly is flying. However, the intrinsic modulation in the neural activity of the optic lobes is quite similar in resting and flying flies; for one-sided visual motion (**Figs. 7C-D**), and when exerting torque responses toward the same-sided visual motion (**Figs. 7A-B**), respectively.

Could intrinsic modulation reflect higher-order processing? Single neurons in the auditory system of crickets provide corollary discharge information (efference copy) to maintain auditory functions, while generating loud courtship songs during vocalization (Poulet and Hedwig 2002, 2006). Could, similarly, gating of visual information by intrinsic modulation reflect efference copy of the orienting behavior?

When a fly selectively orients toward a stimulus, such efference copy could be used to predict the location of the expected visual input in respect to its eyes. In such scheme, the expected outcomes of the orienting would be fed to the neural circuits of the optic lobes that encode visual motion inputs. This efference copy would then converge with real visual inputs resulting from the ongoing orienting behavior. The difference or deviations between the expected and real visual inputs would be used to rectify the fly's orienting. While the fly brain perhaps sends a real-time copy of the initiated motion innervation to the optic lobes, our data gives no clear evidence for this proposition. The main intrinsic modulation seems too slow; it occurs in a prolonged time scale (*cf.* **Fig. 9**). The fast transient spike patterns may in part reflect correction signals (for instance some exploratory saccades show correlated activity; Fig. 6A, stars) but the general intrinsic biphasic modulation of the opposing optic lobes works more likely to enhance the fly's discriminative capacities.

Such modulation could be attributed to rivalry (crossing signals from optic lobes interact with the brain centers between) or to more central top-down activity (descending inputs from the higher brain centers). For rivalry,

both the left and right eye view different objects but their information is processed using the same overlapping visual field (stereopsis) (Blake and Logothetis 2002). In our paradigm, this seems unlikely. As the left and right eye of the fly saw separate scenes with visual fields that do not overlap (monocular flow fields), their perception should be stable. Thus, reasoning by elimination suggests that the intrinsic modulation might have a central top-down origin.

Efficient Utilization of Limited Resources

Drosophila has a small brain of ~100,000 neurons (Truman et al. 1993). The ability to enhance neural representations of chosen objects enables *Drosophila* to use its limited neural resources efficiently, facilitating successful behavior in complex environments. When tracking a moving object, various visual stimuli from the background may "swarm" into its eyes (Land and Collett 1974; Heisenberg and Wolf 1984). In light of our results, it is tempting to speculate that by using such discriminative mechanisms, the fly might be able ignore the distractors while orienting toward the target, and so increase the precision of the neural representation of the relevant information (Knudsen 2007). After separating the competing objects at the level of optic lobes, further processing might then let these pass the relevant brain centers one-by-one and then learn to distinguish them without confusion. Such a strategy for a one-object-at-a-time passing mechanism would reserve limited neural resources to access information on the interesting object effectively and simultaneously, and would give the fly a chance to bind all the necessary features of the interesting object together, including the "what" and "where" integration in the working memory (Baddeley 2003). Importantly, visual invariance relies on the "where" information. For a replaced object, the fly might then selectively switch on the match template in "where" location to recognize the object in this new location (Tang et al. 2004). Identification of specific structures for such complex processing, which should involve regulatory pathways interlinking sensory (Krapp and Hengstenberg 1996; Haag and Borst 2004; Bender and Dickinson 2006; Zheng et al. 2006; Rister et al. 2007; Joesch et al. 2008), motor (Strauss and Heisenberg 1993; Fotowat et al. 2009; Fry et al. 2009), storage and retrieval (Joiner and Griffith 1997; Dubnau et al. 2001; van Swinderen 2007; Neuser et al. 2008), and initiation or planning (van Swinderen et al. 2004; Pick and Strauss 2005; Card and Dickinson 2008; Maimon et al. 2008; Branson et al. 2009; Lebestky et al. 2009) facilities, is beyond the scope of this study. Nonetheless, new methods for selectively controlling neural activity in *Drosophila* (Miesenbock and Kevrekidis 2005; Schroll et al. 2006) may turn out to be useful for testing the workings and predictions of these models (but see also: Gonzalez-Bellido et al. 2009).

Neural selection and representations of visual objects of interest require top-down feedbacks for tracking their travel across the neural landscapes, and "automatic" bottom-up filtering to make them more recognizable against the background (Gilbert and Sigman 2007; Knudsen 2007). Future experiments therefore need also to address, how deep-down into the neural networks of the eyes the selective intrinsic modulation can reach. It is known that the more central processing centers in the fly brain connect to the optic lobes by parallel ascending and descending pathways (Strausfeld 1984; Otsuna and Ito 2006; Katsov and Clandinin 2008). How the eyes are wired suggests that retinotopical bottom-up and top-down information could meet already at the level of the first visual interneurons. In these confined networks, synaptic operations between the pathways (Tang et al. 2004; Rajaram et al. 2005; Zheng et al. 2006; Zheng et al. 2009) could cooperate in forming a local order for identifying and emphasizing relevant patterns in the visual world.

MATERIALS AND METHODS

Flies.

Wild-type *Drosophila melanogaster* ("Cantonese") were raised on standard medium at 22°C and 60% relative humidity under a 12/12-h light/dark cycle. Three to four day-old females were immobilized by cooling (<3 min) and small copper-wire harnesses (hooks) were glued between their head and thorax, using UV-sensitive glue (Loctite). Flies then rested overnight in single vials having sucrose and water.

Flight Simulator System and Behavioral Experiments

A tethered fly was connected to the torque-meter by a small clamp holding the copper-wire harness. Suspended between two taut wires, which acted as torsion springs, and damped by magnets, the torque meter's centre-axis supports a miniature mirror that linearly reflects changes in the yaw torque of the flying fly (**Fig. 1A**). By pointing a laser-beam to the mirror, its light-return over distance amplifies the yaw torque signal, which was then transduced to voltage by an optical sensor. The measured light-return was calibrated and found to be linear with respect to applied torque. The system has a fast rise-time and high signal-to-noise ratio (**Fig. S1**).

At the torque-meter, a *Drosophila* was fixed in a rigid position and orientation, flying stationarily (Götz 1964; Heisenberg and Wolf 1979). Here its eyes/head could only move $<0.03^{o}$. Because this is $<1/160^{th}$ of the inter-ommatidial angle ($\sim 5^{o}$) that defines its eyes' spatial resolution (Heisenberg and Buchner 1977; Stavenga 2003), the fly's body movements were not expected to affect the stream of images it saw during the experiments.

Perpendicular to the fly's long axis, facing its left and right eyes, were two semi-circular screens presenting competing visual stimuli. They displayed printed patterns (stripes, crosses or circles) on two identical looped paper-strips. The strips were spun by a stepping motor, generating two equal scenes that swept to the opposite directions (left and right) synchronously. This simple mechanism made the scenes continuous; it was free of artificial motion, flashing and aliasing. Typical stimulus parameters for moving stripe

scenes were: azimuth ± 150°; elevation ± 40°; wavelength, 20°; velocity, 60 °/s; contrast, 1.0, as seen by the fly. These values represent the maxima (or minima) also for the crosses and circles (**Fig. S2D**) that were smaller and more separated. The scenes were illuminated by day-light and/or by a cold-light-source via fiber optics.

In a competing stimuli paradigm, a tethered flying fly is suddenly presented with two motion stimuli (monocular flow fields) of equal strength (**Fig. 1B**), one on its left and the other on its right. After a neural processing delay, a fly intends to turn either to left or right, as seen by yaw torque (**Video S1**). This response is taken as a fly's ''report'' for the chosen stimulus, whereas two microelectrodes, implanted in its left and right optic lobes (below), are used to look for neural signatures (in multiunit action potentials and local field potentials) for this choice. We call the resulting 3-20 s long, side-to-side slipping, square-waved responses simply as torque responses to distinguish them from the classical optomotor responses to continuous field rotation that are smaller and much briefer (**Fig. 2D**). Importantly for purposes of analysis (see below), each behavioral choice was considered a binary state (or "choice state"), which lasted the period of the torque response. For example, a "left choice" started when a torque signal crossed the zero-midline to left, and it ended when the signal crossed the zero-midline again (to point right)[2].

The behavioral results of this article were further confirmed by additional experiments in which the competing motion stimuli were delivered via fiber optic bundles on the two hemifields of a cylindrical arena that surrounded the tethered flying fly (see Supporting Information). The arena contained a dense grid of 128 x 4 optical slits (pixels), covering 360°; thus, each slit extended horizontally 2.81°, as seen by the fly. Light output from clusters of LEDs were channeled into columns of slits under user-control, generating moving stripe patterns, whose speed, intensity and horizontal width could be altered during the experiments. The competing motion scenes in both systems were efficient in evoking torque responses of similar general dynamics, as tested by different stimulus patterns, speeds and luminances (Supporting Information), making this paradigm robust.

Electrodes

We designed a miniature electrode with a soft connecting wire (**Fig. S2A**) that left the fly's visual behavior and torque measurements undisturbed. 20 µm (Ø) tungsten rods were thinned by gravitational pull and current injection before cutting them into 1 cm sections. A small (Ø 20 µm) insulated copper wire was welded to each rod 1.5 mm from its tip. The rods were sharpened with standard electrolytic procedures to taper 30°, insulated by polyimide resins (leaving the finest 30-50 µm tip exposed), and cut to 1.5 mm lengths with the wires at their end. Their impedance varied 1-1.5 MΩ.

Three miniature electrodes – the left, right and reference - were glued to the small clamp that attached the fly to the torque-meter (**Fig. 5A**). The fly was clamped and the electrodes were inserted by hand (**Video S2**) in the chosen brain areas in 100-150 µm depth. The electrodes were wired to a connector-block, taking their signals via shielded cables to high-impedance amplifiers (Cerebus-128, Cyberkinetics, USA).

In trials, we inserted up to six electrodes in the brain to find the best location to record neural activity to visual moving stimuli. Finding the recording sites was difficult and the rate of successful experiments low. Therefore, the electrophysiological results in this study were accumulated over months of experimentations. We tested micromanipulators to place the miniature electrodes, but given the small dimensions about the set-up, manual insertion under stereomicroscope was deemed to be the most efficient technique. We learned that LFP and action potentials can be picked up reliably when the electrodes reside at the distal region near the dorsal eye rim; where the last neuropile of the optic lobe, the lobula plate (http://flybrain.neurobio.arizona.edu/), is located. Placing an electrode in each of these sites, about a centrally positioned reference electrode, could give electrophysiological data for hours. Based on their motion-selectivity and rapid adaptation (**Fig. 4**), typical for large tangential cells (LPTCs) (Haag et al. 1999), we concluded that the electrodes probably were in the lobula plates. These neurons were insensitive to light intensity, sound or mechanical stimulation. Back-to-front motion also increased their activity, but since it evoked weaker and less clear torque responses (**Fig. S3**), this stimulation was not studied further.

During the experiments, the flies were monitored to ensure that their responses were not induced by, or related to, spurious muscle activity or self-induced visual motion stimuli, *i.e.* rubbing the eyes or lifting up the proboscis to the

[2]There are at least two different ways to interpret a fly's torque responses left or right in the competing stimuli paradigm. (**1**) A fly is inclined to follow motion but can only exert its response to one stimulus at a time (Heisenberg and Wolf, 1984; Wolf and Heisenberg, 1980), or (**2**) it processes the stimuli as an expanding flow-field and makes an unstable steering reflex to avoid collision (Duistermars et al., 2007; Duistermars et al., 2007; Tammero and Dickinson, 2002a; Tammero and Dickinson, 2002b; Tammero et al., 2004). The greatly delayed and variable latencies of the switch-like responses are more difficult to explain with simple collision avoidance reflexes (see Supplemental Material and Discussion); at least this explanation would require an additional "noisy integrate and trigger process". Overall, different aspects of experimental evidence seem to favor one view or the other, implying that the underlying processes are perhaps intermixed, and we feel that both interpretations could be true, but in different behavioral contexts. Here, our writing follows the logic of the first interpretation, but this should not deter the reader from acknowledging also the apparent merits of the other view. Nonetheless, irrespective of what might have caused a fly to attempt turning, the simple quantitative choice paradigm, by virtue of its design that reduces the process of decision making to a binary event (left vs. right), can be used to siphon new information about complex sensory-motor interactions in its visual behavior.

visual field. Although such activity can disrupt LFPs and spike rate measurements, being quite common with some resting flies, we did not see this with flying flies; the flies typically flew with their legs neatly dangling under the abdomen, even during switch-like torque responses (**Fig. 5A**; **Video S3**). For the resting flies, we eliminated the data sections in which the fly was 'active grooming and trumpeting' from the analysis, such as for **Figure 4**. However, when flying, considering the hours of recordings from successful experiments, even if there were few such events, these could affect the results only little. More details are in Supporting Information.

Data Analysis.

Signals were processed by a Cerebus-128 system (Cyberkinetics, USA). The spikes were amplified 5,000-fold; high-pass filtered at 0.5 kHz, low-pass filtered at 7.5 kHz; sampled at 30 kHz with 16-bit resolution. The LFPs were low-pass filtered at 0.25 kHz and high-pass filtered 0.3 Hz. Together with yaw torque and speed of the moving screen, LFPs and spikes were sampled at 1 kHz, monitored on-line and stored in a hard-drive. The spikes were detected using on a discriminative threshold; with a spike-sorting algorithm counting each spike only once (**Figs. S2B-C**). Their waveforms and patterns, and other signals were analyzed using custom-written software (Juusola and Hardie 2001).

There are three important points to consider when searching for correlations between neural activity of the left or right optic lobes and behavioral choices:

(**1**) Because of the sensitivity differences and variable recording locations of individual electrodes, one should not directly compare their unprocessed signals. However, for the same experiment, activity of each optic lobe can be analyzed separately. For example, one can compare the signals in the left electrode for left and right choices, given that its sensitivity does not deteriorate and the choices are clearly distinguishable.

(**2**) Because highly variable single trial behavior (*cf*. **Fig. 3**) correlates weakly with the fast neural activity of the optic lobes (*cf*. **Fig. 5**), one is justified to search for slower associations by linking neural activity to the binary "choice states", even though the fine structure of torque responses vary. Slow associations, as signs of intrinsic modulation, can be quantified consistently in a prolonged trial, where a fly's has made many left and right choices, by averaging neural responses for all left or right choices of similar duration.

(**3**) Once neural activity picked up by an electrode is analyzed for the choices, results from different trials or flies can be compared for similar trends. If such trends seem frequent, their generality can be established by pooling the representative results from different experiments, given that these show similarly patterned choices.

Therefore, the intrinsic modulation (Δ) of one optic lobe (or electrode) was estimated from the torque-trigged averages of the electrical activity (LFP or firing rate), as a difference between left and right choices. For the right lobe: E#1 (*right*) – E#1 (*left*) (**Fig. 7B**); in the left lobe: E#2 (*left*) – E#2 (*right*) (**Fig. 7A**). E#1 and E#2 are the right and left electrodes, respectively, as in **Fig. 5A;** *left* and *right* are respective torque responses (or choices: **Figs. 7A-B**) (moving scenes: **Figs. 7C-D**). In some of the plots, the intrinsic modulation was given as a relative changes in a percentage scale: 100*(E#2 (*left*) – E#2 (*right*))/ E#2 (*right*) or 100*(E#1 (*right*) – E#1 (*left*))/ E#2 (*left*), to highlight its dynamic strength changes (firing rates in **Figs. 7A-B**; power spectra of LFPs in **Figs. 8A-B**)

LFPs of the selected data sections were segmented into 50% overlapping stretches (1,000 points) and windowed with a Blackman-Harris 4th-term window (Harris 1978) before their spectra, LFP(f) were calculated with an FFT algorithm. The spectra were then averaged to improve the estimate. For power spectrum, $\left\langle \left| LFP(f) \right|^2 \right\rangle$, $\left| \; \right|$ denotes the norm and $\langle \; \rangle$ the average over the different stretches.

For an optic lobe's power-index, we calculated the power spectra of its LFP using 1,000 point window, moved in 100 or 200-point steps (**Figs. 9B** and **9A**, respectively). From each power spectrum a 20-100 Hz range was summed, giving us a continuous account of the dynamic changes in these frequencies at 100 or 200 ms time-resolution. For fair comparison of the activity in the left and right optic lobes, their power-indexes were normalized by maxima, and given in a percentage scale (**Figs. 9A-B**).

To emphasize the mean trends of the LFP power-indexes for both optic lobes (**Fig. 9A**), these signals were smoothed with Savizky-Golay 2nd order function using 5 points. This procedure eliminated extraneous "noise", attributable to the many data points used for each trace, but as executed, this had little effect on the shape and timing of the mean trends. No smoothing was used for **Figs. 9B** and **C**, which have twice as high data-point density than **Fig. 9A**, as averaging data from 5 flies smoothed the traces naturally. More details are in Supporting Information.

Limitations of Electrophysiological Analysis

One argument for the authenticity of "behavioral choices" is derived from the observation, that in the competing stimuli paradigm the neural output of the optic lobes is modulated with fluctuations, which are in coincidence with the yaw torque switches. We interpret the modulations as an effect of intrinsic gain control mechanism (gating) in the optic lobes, triggered by the fly's behavioral choices (**Fig.6**). Nonetheless, we have not failed to notice that in some recordings spontaneous torque correlates with the neural output of the optic lobes as well, even in the absence of any motion stimulus, as it happens from the three torque saccades indicated by the three stars in **Fig. 6A.** From this can be deduced that either the torque-switches toward the right and left would already by themselves modulate the signals from

the electrodes E#1 and E#2, or that E#1 and E#2 measure efferent copies of the torque-switches (see Discussion). Thus, LFPs might not be purely discriminative in what they measure. Furthermore, optomotor responses can be demonstrated by measuring the fly's head movements: the fly responds to rotating visual stimuli with rotation of its head into the direction of the motion stimulus (Rosner et al. 2009). Here, we emphasize again that we did not measure classic optomotor responses to field rotation, but torque responses to competing visual motion stimuli. Nonetheless, since the fly's head is fixed in our experiments, one may hypothesize that the fly tries to move its head in response to the stimulus and thus activates its muscles in the head, close to the electrodes.

Therefore, although we made efforts to ensure that the recordings were from the optic lobes, as indicated by the trends and delays that typify neural activity (see **Fig. 5**), this of course still cannot disprove the possibility that muscle activity from the head- and flight muscles might have polluted these signals. However, it seems to us that even if the LFPs were to contain also muscle activity, such effect on the overall results was probably small. This is because when testing resting flies with one-sided movement, our ultrasensitive torque-meter indicates virtually zero torque, *i.e.* no obvious head and body movements during stimulation (**Figs. 7C-D**), whereas the difference in LFPs to ipsi- or contralateral motion is considerable and follows the same adaptive trends and delays as neural firing (**Fig, 4**). Future experiments with fast live imaging technologies, which are currently under development, is expected to resolve this issue over time.

Online Supporting Information

Supporting Information for this paper consists of nine figures and detailed discussions of the possible causes, which might influence *Drosophila*'s stimulus choices, and of the applied methods. **Fig. S1** shows the characteristic output of the torque meter to an electro-magnetic pulse (input). **Fig. S2** shows how the spikes were detected from the electrical signals, and how flies respond to different competing stimuli. **Fig. S3** shows that a fly's torque responses between competing stimuli are evoked efficiently by front-to-back motion. **Fig. S4** shows that a fly's torque responses occur similarly for different sizes of motion scenes. **Fig. S5** shows how storque responses are switch-like at different speeds of competing stimuli. **Fig. S6** shows that a tethered flying *Drosophila* can react against unilateral motion field. **Fig. S7** shows observed differences in local and global neural activity across the experiments. **Fig. S8** shows how ipsilateral neural activity is boosted during visual choice paradigm, as measured from a single fly. **Fig. S9** shows intrinsic modulation of neural activity in the optic lobes during competing stimuli paradigm in single experiments, and illustrates how the data was analyzed.

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SUPPLEMENTAL MATERIAL

I. CONSIDERING DIFFERENT VIEWS

I.1. UNSTABLE FLIGHT MOTOR EQUILIBRIUM

Automatic steering reflexes help a fly to control its flight path in changing environment (Frye and Dickinson 2004). Some of these reflexes are protective, and thus may have the strength to override the behavior a fly is engaged in. In particular, it has been shown by different experimental assays that large-field expansion elicits high magnitude and *tightly phase-locked* optomotor responses away from the pole of expansion. Hence we ask: could the square-waved "torque-turns" and the intrinsic modulation, which we report here as signs of selective orientation, be outcomes of unstable steering reflexes, evoked by competitive stimuli? We next consider evidence for steering-reflex hypothesis, before presenting other evidence, which seems more difficult to explain by this tenet in our experimental conditions.

I.1.1. STEERING-REFLEX HYPOTHESIS: Behavior

In response to an expanding flow field, *Drosophila* show extremely robust optomotor steering reflexes (Tammero and Dickinson 2002b, 2002a; Tammero et al. 2004; Duistermars et al. 2007b; Duistermars et al. 2007a). An expanding flow field generates very strong optomotor responses. Centering the focus of expansion frontally, generates a highly unstable equilibrium - if the frontal pole moves slightly to one side, the steering response is immediately and powerfully in the other direction. Thus, when the fly is given control to steer the pole, it *always* turns to face the contracting pole.

We first tested how flies responded to frontally expanding flow fields by using a fiber optic display (*i.e.* two panoramic hemifields of vertical stripes moving left and right). We found that frontal field expansion from a single central pole evoked left or right "torque-turns". But for thick stripes ($\lambda = 40^o$) these responses were intermixed with separate landing responses toward the pole of expansion (the flies extended their legs up front), whereas narrower stripes ($\lambda = 30^o$) evoked only turns. Thus, the behavior seemed to depend upon how close the fly "perceived" the centre of expansion to be. These findings differ from those of Tammero and Dickinson (2002a), who used an expanding object as a stimulus. They found that when an object expanded within the frontal field of view, *Drosophila* typically elicited leg extensions without a steering response. Nonetheless, these results could be interpreted as signs of unstable steering-reflex to avoid collision.

We next blocked both the frontal pole of expansion and the rear pole of contraction with black (or white) cardboard cuttings of various sizes, thereby optically isolating the left and right moving scenes. The results from these experiments present a bigger challenge to a simple steering-reflex explanation.

I.1.1.1. Behavioral Evidence Challenging the Steering-Reflex Hypothesis.

- **i.** Because both the frontal and ventral components of the visual motion scenes were missing (MATERIALS AND METHODS), it was clear that our flight simulator did not generate an obvious frontal field expansion; thus, the spatially isolated left and right moving scenes were very different from the spatially continuous visual input used to test frontal expansion in (Tammero and Dickinson, 2002a, b; Tammero et al., 2004; Duistermars et al., 2007a; Duistermars et al., 2007b). Hence the resulting torque responses could not have been evoked by expansion in the frontal pole because there really was no clear 'frontal pole' for the flies to see. Furthermore, regardless of the stimulus patterns used in this assay, bilateral stimuli never evoked landing responses; unlike the case when the frontally expanding pole was visible to the fly (see above).
- **ii.** We found that a fly could exert is torque responses between the left and right scenes even during back-to-front motion. These responses were much weaker, mostly containing saccade-like events (**Fig. S3**). Importantly, however, even though when a fly faced the contracting pole of the "flow field", this did not quench its selective (exploratory) visual behaviour.
- **iii.** Smaller bilateral stimuli did not alter the amplitudes of the torque responses nor their dynamics considerably (**Fig. S4**). This finding is consistent with the earlier study that used small objects (vertical bars) to investigate changes in yaw torque in relation to competing visual motion stimuli (Wolf and Heisenberg 1980).
- **iv.** Fruit flies sometimes mixed left and right torque responses with periods of flying straight (**Figs S4** and **S5**). This finding implies that - although the switch-like dynamics of torque responses seem suggestively reflect-like -, the flies can withhold from responding to the competitive stimuli at any one moment.
- **v.** We found that unsymmetrical input (i.e. moving the fly in respect to the left and right scenes or dephasing the movements of the scenes) did not change the dynamics

of the torque responses. This finding opposes directly the steering-reflex hypothesis in our stimulus paradigm, in which case the torque responses should have been strictly toward the side further away from the centre of the field expansion (*i.e.* the expanding pole).

vi. The finding that there was a long latency before a fly chooses the side from which to modulate its yaw torque, and the variability of these choices (**Fig. 3**), is against the torque responses resulting from the flies simply trying to avoid a head-on collision. We estimated that the fly's central brain obtains visual motion information within 30 ms from the stimulus onset (**Fig. 5**). If a fly waited further several hundreds of milliseconds, as happens here, before exerting a protective torque response, it may find its end with the rapidly approaching object/predator.

vii. The flies could also respond against to unilateral motion scenes (**Fig. S6**). This observation suggests that in our stimulus paradigm the flies indeed choose which motion scene to pursue.

viii. Visual motion input is the same for different flies, yet they responded with individually patterned torque responses (duration and intervals). Thus, the responses were far from simple reflex-like - all or nothing events -, but had highly variable time-courses and patterning (*cf.* **Figs. 2** and **S2D**).

ix. From the results shown in **Figs. 3** and **5,** we suggest that the prolonged delay is a typical attribute of the competing stimuli paradigm. However, the same delay can obviously also happen even without any competing motion stimulus, as seen from the red torque trace in **Fig. S6A** (delay ~110 ms), and from the blue trace of **Fig. S6B**, where the delay between onset of the (unilateral) motion amounts to about 700 ms. Such variability suggests that even during unilateral stimulation, the flies decide when they exert their torque response; this degree of spontaneity is very different from what we see in a classical reflex (**Fig. 2D**).

Summary

Based on these findings and observations, it appears that the torque responses in the competing stimuli paradigm may resist simple generalizing interpretations for their initiation. Here, our consensus estimate is that during this paradigm, different aspects of neural processing for both object tracking and avoidance is perhaps blended to generate these results.

Nonetheless, it is important to realize that whether the torque responses resulted from stimulus attraction, repulsion, or other cause, this has relatively little to do with the two main messages of this article, which are:

(1) By using the simple competing stimulus paradigm, we could specify torque responses as binary left or right choices, and correlate them to neural activity in the left and right optic lobes.

(2) Behavior and neural responses together made a highly suggestive case that intrinsic activity in the *Drosophila* brain modulates visual information processing during behavioral choices.

II. NEURAL ACTIVITY

II.1. DIFFERENCES BETWEEN FIRING PATTERNS AND LFPS

We pool data from experiments, which contained switch-like torque responses of roughly equal size and duration to left and right, to compare how *Drosophila*'s choice of left or right visual stimulus affects the neural activity in the optic lobes. Again, we remind the reader that because the tethered flies are firmly held to the torque-meter (Götz 1964), the visual input to their eyes during the torque responses remain the same (**Materials and Methods**).

Local information processing: By subtracting the neural activity during left and right torque responses (or stimulus choices) in one side (measured by a single electrode; left or right), we find that the neural firing patterns of local neurons come in two major classes (**Fig. S7A**). They show either ipsi- or contralateral ocular dominance to visual motion, in similar numbers (*i.e.* neurons that fire more during ipsi- [blue] or contralateral [red] torque responses). By their temporal activity patters, these neurons can be further sorted into two groups: transiently firing and more slowly firing neurons of ipsi- or contralateral preference.

Global information processing: On the other hand, similar comparison for the left and right LFPs during left or right torque responses shows that the global neural activity in the left side of the brain always increases during the left choices and the neural activity in the right optic lobe always increases during the right choices. This ipsilateral global preference of LFPs was true for all the analyzed experiments (**Fig. S7B**), suggesting that the global activity of the optic lobes is gated in respect to the fly's orientation choices.

II.2. CHANGES IN POWER SPECTRA OF LFPS DURING OPPOSITE "TORQUE-TURNS"

We estimated the relative changes in the frequency content of LFPs in this way:

i. LFP recordings from the right (blue) or left (red) optic lobes were aligned, cut and pooled for each switch-like torque response, using the zero-crossings in the yaw torque as start-points (**Fig. S8A**, the left edge of the gray and light gray bars).

ii. These LFP traces were then trimmed to a suitable size, typically lasting about 60% of the average torque response (**Fig. S8A**, black; area within the gray bars). With 1 kHz sampling, the traces had usually 3,000-5,000 points.

iii. As this experiment consisted of 14 torque responses to left and 14 responses to right (**Fig. S8A**), each optic lobe (or electrode) is represented with two matrixes: one containing traces for the left choices and the other for the right choices; thus we have four [14 x 4,096]

matrixes. The means of such matrixes, or average signals, are shown in blue, navy, red and wine (**A**).

iv. We calculate the power spectra for each 4,096 points-long LFP trace (n = 14), using 1024-point samples with 50% overlap. For each trace, this gives 7 spectral samples that are averaged. These average samples are then averaged across each matrix giving the mean power spectral estimate of LFP during one-sided torque response in one optic lobe (n = 7 x 14 = 98; **Fig. S8B**).

v. Thus, we obtain two mean power spectral estimates of LFPs for each optic lobe (or electrode). One for the right choice and the other for the left choice. To see if the neural activity in the right side of the brain was increased during the right torque responses, we subtract the spectra $E\#1_R$-$E\#1_L$. For the differences in the neural activity in the left side of the brain, we subtract the spectra $E\#2_L$-$E\#2_R$. These differences are then given as relative changes: $100*(E\#1_R-E\#1_L)/-E\#1_L$ and $100*(E\#1_L-E\#1_R)/-E\#1_R$ (**Fig. S7C**).

LFP recordings (**Figs. 8**, **9**, **S8** and **S9**) show that outputs of the left and right optic lobes are dynamically modulated with a fly's choices between the left and right moving scenes. To characterize this modulation further, we calculated the power-indexes for the LFPs in the left and right optic lobes during the visual choice paradigm. The rationale and details of such calculations are shown in **Fig. S9**. The results from single experiments (such as **D-E** and **F-G**) and those pooled from 5 different flies (**Figs. 9A-B**) show that the outputs of the optic lobes seem to be gated simultaneously by separate inhibitory and excitatory processes. The output of the left optic lobe is boosted during left choices (torque responses) and suppressed during right choices, whereas the output of the right optic lobe is boosted during right choices and suppressed during left choices. The dynamics for the ipsilateral boosting ("intending") are faster than for the contralateral suppression ("ignoring").

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SUPPLEMENTAL MATERIAL FIGURES

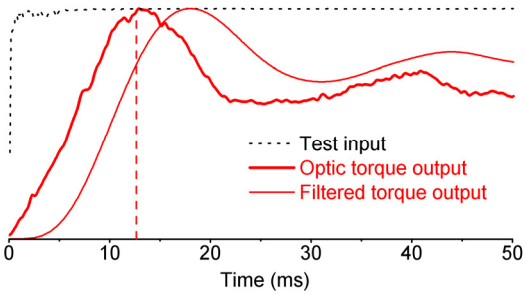

Figure S1. Output of the torque meter to an electro-magnetic pulse (input) shows a fast rise-time and signal-to-noise ratio. The rise time is 12.5 ms (red dotted line).

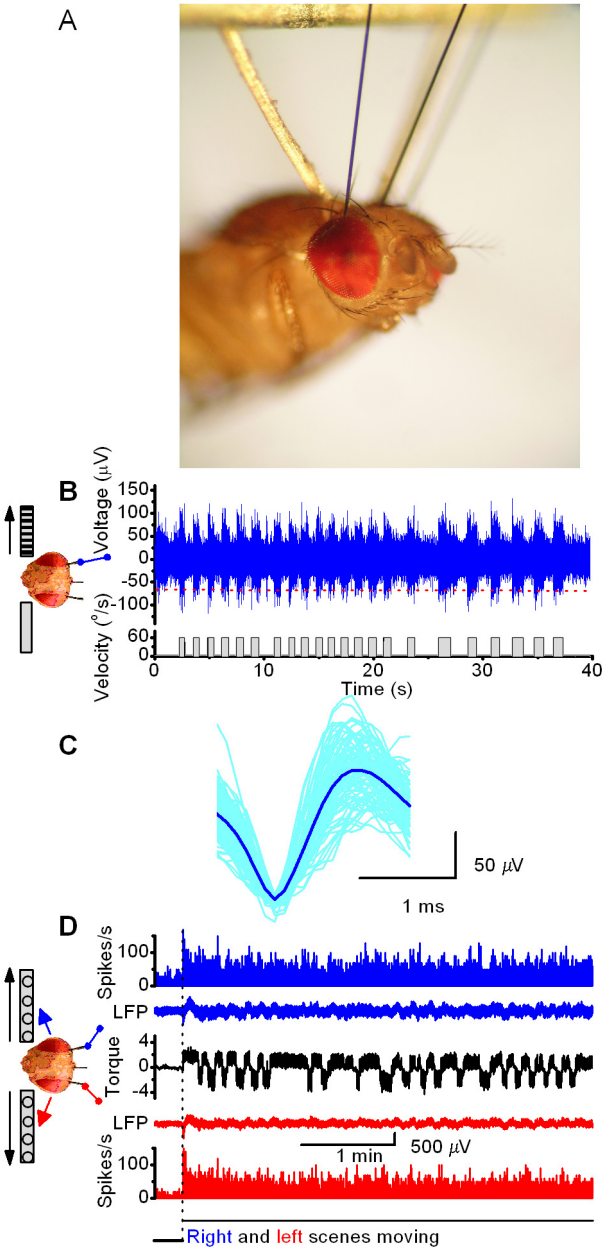

Figure S2. Spike detection, and orientation with different competing stimuli. (A) The standardized positions for the recording electrodes in the head of a flying *Drosophila*. The small harness, glued between the head and body (the thicker silver wires), was used to clamp the fly's head in a fixed position and orientation. The miniaturized electrodes are the black thin wires. Electrical responses from a fly's optic lobe (most likely from the lobula plate) to a single moving field, recorded with a miniaturized tungsten electrode. (B) Continuous

voltage signals (blue) during a constant velocity motion (stripes) stimulus on the right scene (light grey, below) recorded from the right optic lobe of a *resting* (non-flying) fly. The spikes are detected by a threshold (red dotted line). (C) Mean spike (blue) and individual spikes (light cyan). (D) Characteristic behavior (yaw torque, black) and neural responses in the OLs (firing rates, above; LFPs, below) to O-patterns that move to opposing directions at 60°/s; recorded form tethered *flying* female *Drosophila*. During the experiments both eyes receive continuous motion stimuli, as indicated by the fly-head cartoon. Similar to stripe-patterns (*cf*. Fig. 6), the fly attempts to follow either a left or right moving scene at any one time (*i.e*. generatinig torque responses to left or right); the neural output of its optic lobes (right, blue; left, red) are tuned with these responses. Notice the more variable time courses of the left and right torque responses of this fly, in comparison to the behavior of two other flies in experiments in Fig. 6 and S1.

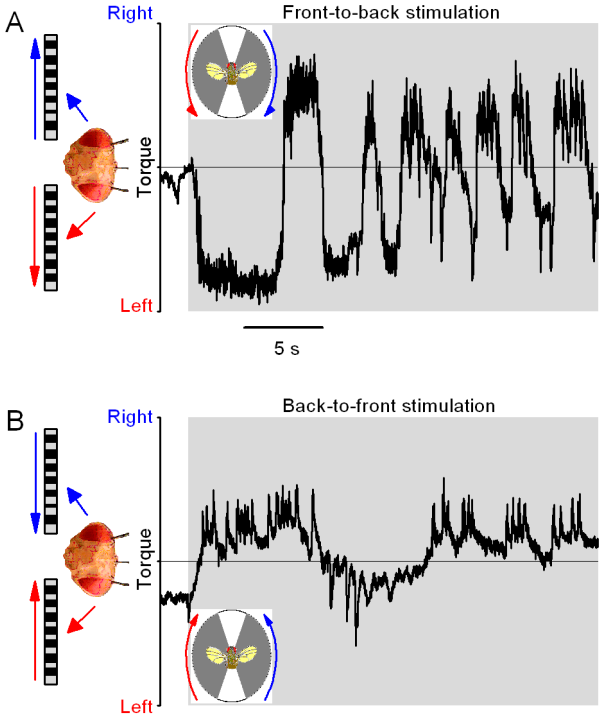

Figure S3. In competing stimuli paradigm, a fly's switch-like behavior between the left or right stimuli is evoked efficiently by front-to-back motion. The figure shows traces of behavior (yaw torque) of the same tethered flying *Drosophila* to (A) front-to-back and (B) back-to-front motion (bilateral stripe scenes: azimuth ± 150°, elevation ± 40°, velocity 60°/s). When the scenes are set in motion (light gray areas), the fly begins to exert torque responses between the left (down) and right scenes (up). These responses are much stronger for the front-to-back than for the back-to-front motion.

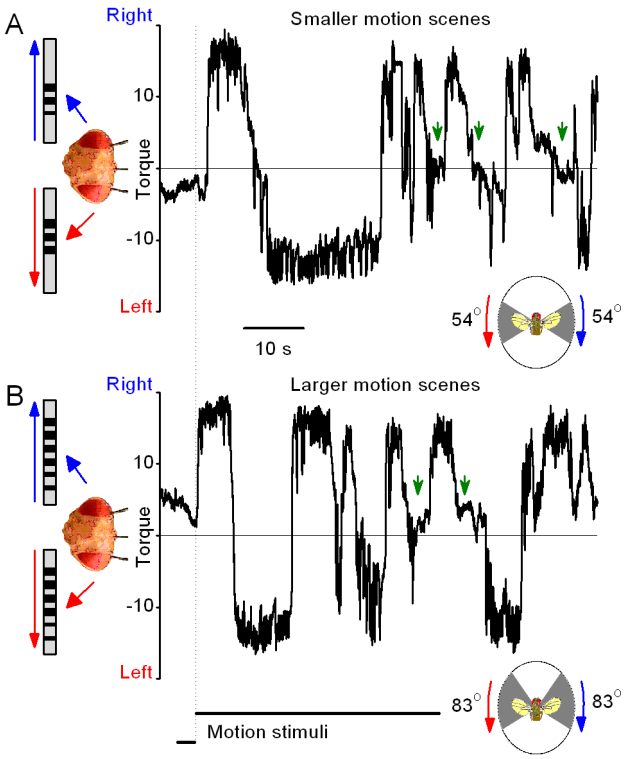

Figure S4. In competing stimuli paradigm, a fly's switch-like behavior occurs similarly for different sizes of motion scenes (*cf*. **Fig. S5**). The figure shows traces of behavior of the same tethered flying *Drosophila* to smaller and bigger stimuli (gray sectors in the circular inserts): (A) at 54° lateral scenes (126° in the front and back blacked out), and (B) 83° (69° blacked out frontally and 125° dorsally), respectively. In both cases, when the scenes are set in motion (dotted line), there is a variable delay after which the fly starts to generate side-to-side flipping torque responses, attempting to rotate toward the left (down) or right stimulus (up), one at a time. Intermixed with these responses are briefer periods when the fly flies straight (approximately zero torque; green arrow heads), implying that it can also "ignore" the stimuli. Here, the field size of stimulation seems to have little influence on the size of torque responses. These results imply that torque responses are not evoked by some specific features in the stimulus patterns, but that they define the fly's choices. The speed of the stimuli was 60°/s.

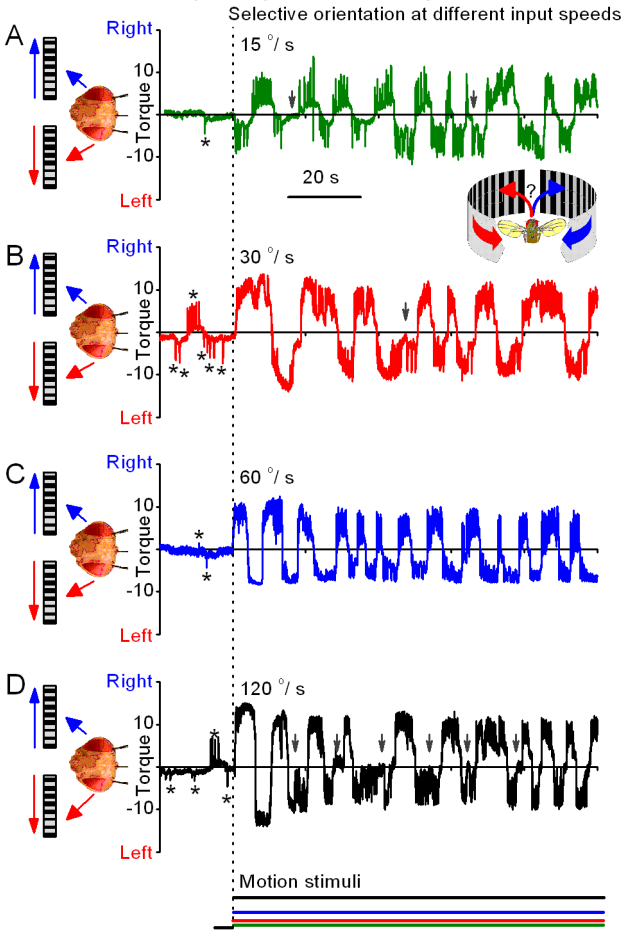

Figure S5. Visual selection occurs switch-like at different speeds of competing stimuli (150º left and right). The figure shows traces of behavior of the same tethered flying *Drosophila* during competing visual stimuli paradigm, delivered at different speeds: (A) at 15º/s, (B) 30º/s, (C) 60º/s, and (D) at 120º/s. At the beginning of each experiment, the fly faces motionless left and right scenes containing virtually identical stripe patterns. At this situation, it makes spontaneously characteristic exploratory saccades to left and right (stars). When the scenes are set in motion, the fly starts to generate larger side-to-side flipping torque responses,to the left (down) or right stimulus (up), one at a time. Intermixed with these responses, are brief periods of flying straight (small gray arrows). Although increasing the speed of stimulation appears to slightly amplify these responses (*cf.* A and D), this has no obvious effect of their duration and frequency. These results further imply that the switch-like behavior was not evoked by some specific features in the stimulus patterns, but that it defined the fly's stimulus choices.

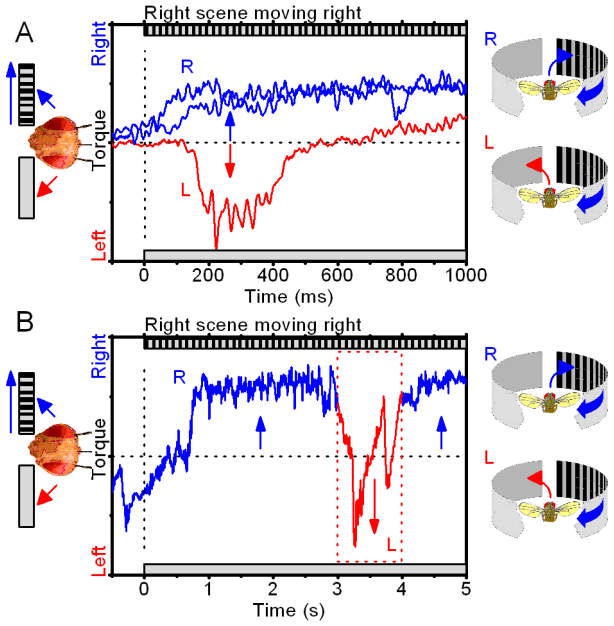

Figure S6. A tethered flying *Drosophila* can react against unilateral motion field. Although the right scene, R, moves, the fly exert its torque response to the opposite, unmoving side (the blank left scene, L). (A) Even at the onset of the unilateral motion, a fly may occasionally respond against it, by generating a torque response toward the opposing blank scene. (B) Also when engaged in pursuing the unilateral motion, a fly sometimes briefly interrupts its response as if to "check out" the unmoving blank scene. This "rebel" behavior highlights that even if its reactions are triggered by a strong moving field-stimulus, these can be modified at any one time by internal motor commands.

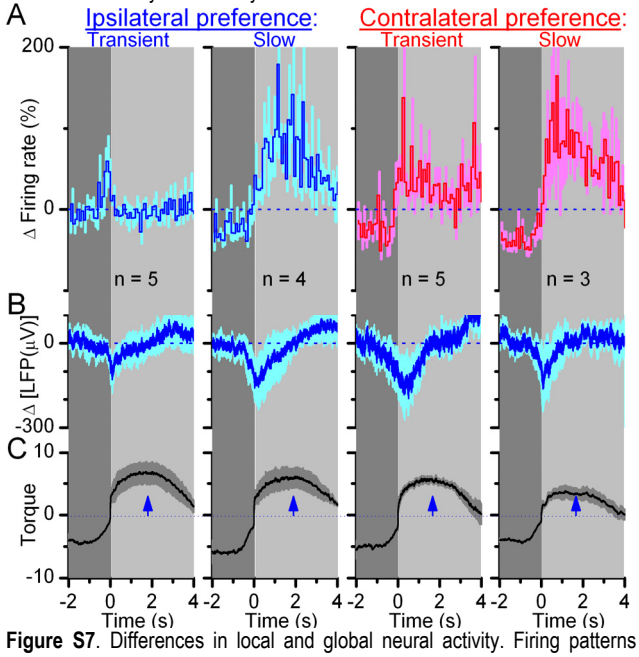

Figure S7. Differences in local and global neural activity. Firing patterns of individual neurons (A) in the OLs show variable tuning for ipsi- (blue traces) and contralateral (red traces) visual motion, (B) but the global activity of the OLs (LFPs) always increases when selecting (making torque responses toward) ipsilateral scenes. Except for torque (C), traces show the relative change in responses toward the preferred side; aligned by the torque responses. By their preference for ipsi- or contralateral stimuli, the neurons in the LPs can be sorted into four groups (data from 9 flies, from 17 neurons [thus from 17 different electrodes]; bin-size 100 ms; note, the base firing rate picked up by one electrode was too low for reliable calculation of the relative change in neural activity). "Transient increase" neurons fire the most at the early part of the fly's torque response to ipsilateral objects. "Slow increase" neurons steadily increase their firing rate, until the ipsilateral torque response starts to ease off. "Transient decrease" neurons respond best at the beginning of a shift to

contralateral side. "Slow decrease" neurons are most active when the torque response toward the contralateral stimulus peak. Unlike the firing rates, the absolute amplitudes of the corresponding LFPs always reach their maxima (i.e. points downwards) during ipsilateral viewing. Means ± SEMs shown.

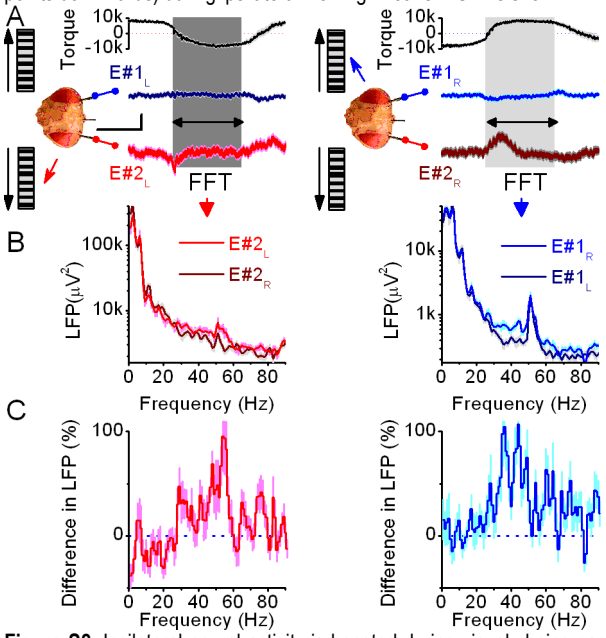

Figure S8. Ipsilateral neural activity is boosted during visual choice paradigm. This figure shows typical changes in the power spectra of LFPs, as measured from the optic lobes during left and right torque responses of a single fly. (A) The fly generated 14 switch-like torque responses to left and right during bilateral motion stimulus (stripe-bars). Mean torque responses (black) to left (down) and right (up) are aligned with the corresponding mean LFPs, measured by the right (E#1, blue) and left electrode (E#2, red). Scale: 2 s / 300 μV. Gray areas indicate the sections across LFP recordings that were used for calculating the power spectral estimates. (B) Power spectral estimates (mean ± SEM, n = 98 samples) for the left optic lobe (left) and the right optic lobe (right) during left (red and navy LFPs) and right (blue and wine LFPs) choices. (C) Relative differences in the neural activity in the left (red) and right (blue) optic lobes during the left and right choices (mean ± SEM, n = 14 torque responses). Similar to the pooled data across the flies (Fig. 8B), here ipsilateral boosting occurs ~20-90 Hz.

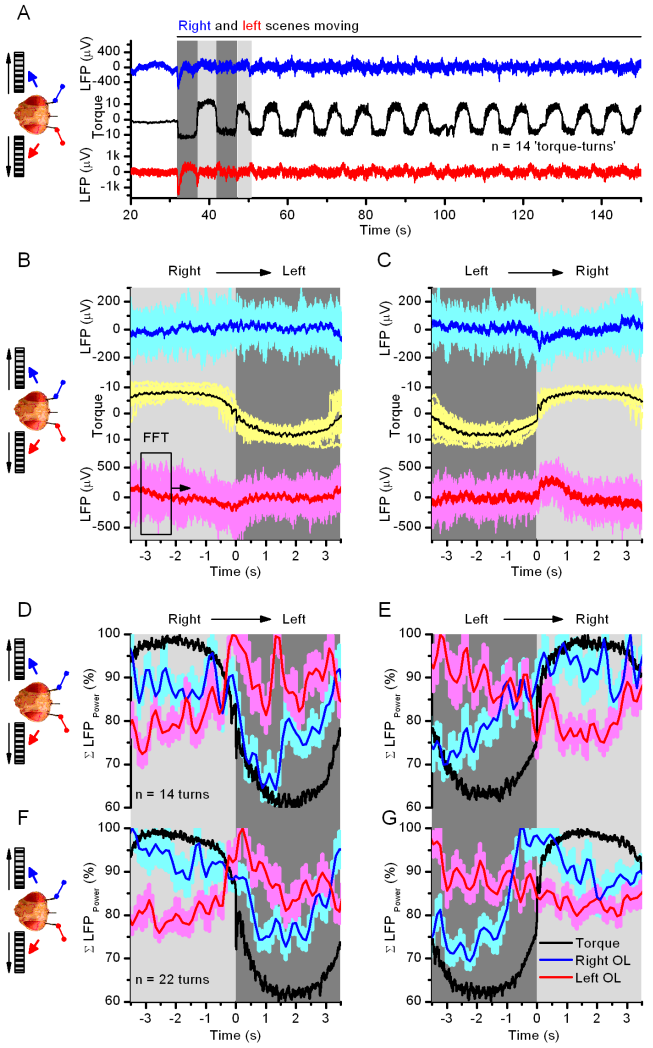

Figure S9. Intrinsic modulation of neural activity in the optic lobes during competing stimuli paradigm; visual motion input to the eyes remains unchanged, yet the neural outputs of the optic lobes oppose each other. Changes in the mean neural output of the left and right optic lobes (OL), monitored as the summed power (20-100 Hz) of their LFPs, coincide with the torque responses. (A) LFPs from the right (blue) and left (red) optic lobes; yaw torque (black) shows attempts to rotate to right (up) and left (down), highlighted by light-gray and gray bars, respectively; data from the same fly as in Fig. S7, again with 14 torque responses to left and right. (B-C) shows neural activity during right-to-left and left-to-right choices. 14 LFPs from the right (cyan) and left (magenta) optic lobes superimposed and aligned by the corresponding zero-crossings in the torque (yellow). The means are shown in blue, red and black, respectively. (D-E) Power indexes of LFPs are then calculated for each separate torque response; here, giving 14 power-indexes for left and 14 power-indexes for right choices. For, each torque response we used a 1,000-point sliding window with 100-point jumps (B) and their frequencies from 20-100 Hz are summed up for each data-point. The mean ± SEM of these 14 power-indexes are shown in (D) and (E) for right-to-left and left-to-right choices, respectively. Changes in the output of the left and right optic lobes precede the changes in the torque responses, oscillating with a 180° phase shift. (F-G) shows similar dynamics in the power-indexes of another experiment (Fig. 6). (F: mean ± SEM). These general dynamics in LFPs are also seen in the pooled data for five flies, as shown in Fig. 9B.