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Motion Processing Streams in *Drosophila* Are Behaviorally Specialized

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SUMMARY

Motion vision is an ancient faculty, critical to many animals in a range of ethological contexts, the underlying algorithms of which provide central insights into neural computation. However, how motion cues guide behavior is poorly understood, as the neural circuits that implement these computations are largely unknown in any organism. We develop a systematic, forward genetic approach using high-throughput, quantitative behavioral analyses to identify the neural substrates of motion vision in *Drosophila* in an unbiased fashion. We then delimit the behavioral contributions of both known and novel circuit elements. Contrary to expectation from previous studies, we find that orienting responses to motion are shaped by at least two neural pathways. These pathways are sensitive to different visual features, diverge immediately postsynaptic to photoreceptors, and are coupled to distinct behavioral outputs. Thus, behavioral responses to complex stimuli can rely on surprising neural specialization from even the earliest sensory processing stages.

INTRODUCTION

A central challenge in neuroscience is to link sensory experience to motor output at the level of identified cells and circuits. In the context of vision, electrophysiological and psychophysical studies have provided considerable insight into the neural computations that underlie motion, color, and form vision (Gegenfurtner and Kiper, 2003; Orban, 2008). However, the neural substrate that transforms retinal signals into changes in animal behavior remains poorly defined. Recent advances in the development of genetic tools for rapidly and reversibly manipulating neuronal activity in the fruit fly open the possibility of using these techniques to identify and define the neural circuits that underlie complex behaviors. To do so, however, requires the development of high-throughput, quantitative behavioral analyses that can adequately explore the stimulus-response relationship, and can be used to conduct forward genetic screens to identify functionally critical neurons in an unbiased fashion. Here we develop such an approach to dissect the neural circuits underlying motion vision.

Motion vision has long served as a well-defined, robust paradigm in which to study neural processing. What functional principles guide how visual circuits in general, and motion circuits in particular, are organized with respect to behavior? On the one hand, it is well established that simple visual cues can guide particular behavioral responses via specialized neural pathways in cases where a broader visual context is unnecessary to interpret the signal. For example, luminance signals received by melanopsin-expressing retinal ganglion cells control the pupillary light response and circadian entrainment through targeted axonal projections to distinct brain regions (reviewed in Peirson and Foster, 2006). On the other hand, it is thought that visual features perceived by image-forming eyes are, generally, first processed by neurons tuned to detect specific visual features without regard to their relevance to behavior; behavioral specialization is thought to arise in later processing stages where signals from local feature detectors are pooled (Orban, 2008).

In the context of motion vision, distinct global patterns of motion, such as optic flow, carry information that guides different behavioral decisions (Gibson, 1950). The effects of optic flow on insect behavior have been extensively examined (Hecht and Wald, 1934; Kalmus, 1949; Hassenstein, 1951; Gotz, 1964; Buchner, 1976; reviewed in Egelhaaf and Borst, 1993). By determining the strength of the observed behavioral response as a function of pattern contrast, velocity, and frequency, these studies derived a mathematical model the Hassenstein-Reichardt correlator, linking stimulus to behavior (Hassenstein and Reichardt, 1956; Reichardt, 1961; Reichardt and Poggio, 1975, 1976; Buchner, 1984). This framework posits a two-step computation. First, elementary motion detectors (EMDs) determine the strength of the motion signal in a limited number of directions, in each of many small regions of space. Second, by integrating the outputs of subsets of these detectors, downstream circuits compute global motion signals along a limited set of cardinal axes, and use this information to guide behavior (Egelhaaf et al., 2002). Thus, in this view, a uniform processing step first extracts global motion cues from a visual scene, irrespective of behavioral context. Then, multiple, specialized circuits interpret this common signal to guide different aspects of behavior. Here we examine this conceptual framework in the context of behavioral responses of freely walking fruit flies to visual motion.

Orienting responses to visual motion are found in every animal group with nonprimitive eyes, and are thought to be essential to course course control and navigation (Srinivasan et al., 1999). Indeed, arthropods, mollusks, and vertebrates spontaneously follow, or turn against, the direction of motion cues, depending

on their mode of locomotion and on how the stimulus is presented (Hecht and Wald, 1934; Kalmus, 1949; Wells, 1962; Gotz, 1970; David, 1979; Miles and Wallman, 1993). The robustness, ubiquity, and apparent simplicity of these optomotor responses makes them powerful paradigms to be examined for the neural mechanisms of motion detection. Consistent with the notion that a single motion estimate might guide different direction-selective (DS) responses to visual motion in both walking and flying fruit flies, similar relationships between turning reactions and motion parameters have been observed across a range of stimulus configurations (Buchner, 1976, 1984; Heisenberg and Buchner, 1977; Gotz, 1964; Gotz and Wenking, 1973; reviewed in Egelhaaf and Borst, 1993). However, fruit flies also appear to process motion signals differently under different light adaptation states (Heisenberg and Buchner, 1977; Pick and Buchner, 1979), and anatomical considerations suggest that multiple motion-sensitive retinotopic pathways segregate by the second or third stage of visual processing (Sinakevitch et al., 2003; Bausenwein et al., 1992; Bausenwein and Fischbach, 1992). Because the electrophysiological properties and anatomical connections of many interneurons linking the retinal input to deeper brain layers are unknown, much of the neural substrate of motion estimation remains to be defined. Thus, the relative contributions of a single motion estimator toward different behaviors, or the roles of potentially parallel pathways, are unclear.

For an animal to respond to motion in a particular direction, DS neurons must guide orienting behavior. Such neurons have been described in vertebrates and invertebrates (Clifford and Ibbotson, 2002; Egelhaaf et al., 2002). In vertebrates, DS responses emerge in retinal ganglion cells (Barlow et al., 1964; Kim et al., 2008; reviewed in Demb, 2007), and are common in primary visual cortex (Livingstone and Hubel, 1988; Gur and Snodderly, 2007). These cells provide motion-sensitive signals to area MT, in extrastriate cortex, through at least three parallel pathways (Gur and Snodderly, 2007). In primates, signals from DS neurons in extrastriate cortex can drive perceptual decisions about motion direction, and ablations of brain regions containing these cells disrupt responses to motion (Salzman et al., 1992; Celebrini and Newsome, 1995; Newsome et al., 1985). Intriguingly, aspects of human psychophysical performance can be described by an elaborated Hassenstein-Reichardt correlator model (van Santen and Sperling, 1984). In insects, DS neurons in the lobula plate are tuned to global patterns of motion and have been examined extensively (reviewed in Borst and Haag, 2002). These lobula plate tangential cells (LPTCs) resemble vertebrate neurons in their tuning properties and can alter DS behavior when microstimulated (Egelhaaf et al., 2002; Blondeau, 1981). As in primates, ablations of brain regions where LPTCs are found, or project, disrupt at least some responses to motion (Heisenberg et al., 1978; Geiger and Nassel, 1981; Hausen and Wehrhahn, 1990). Thus, while the full extent of the neural pathways that give rise to DS responses is unknown in both vertebrates and invertebrates, these studies suggest significant similarities between the properties of identified neurons and psychophysical performance in both animal groups.

To begin a systematic dissection of these pathways, we combined the forward genetic techniques available in the fruit fly with a quantitative analysis of its robust DS behaviors to identify motion-sensitive neurons required for specific behaviors in an unbiased fashion. This required development of a sensitive, high-throughput, quantitative assay of motion-evoked behavior. Using this approach, we define two distinct behavioral responses to motion, dominated by DS changes either in the rotation of the fly's body, or in its translatory movement. Most moving stimuli modulate both responses, but do so to differing extents. That is, changes in rotation and translation are sensitive to different combinations of visual stimulus parameters. We then demonstrate, using a forward genetic screen combined with a candidate-neuron approach, that these behavioral responses are at least partially genetically separable. We find that specific neural populations are differentially coupled to each behavioral response. Taken together, these results demonstrate that most motion stimuli simultaneously activate multiple motion processing streams, corresponding to at least partially distinct neural circuits. Remarkably, these circuits separate early in visual processing, in the neurons immediately postsynaptic to photoreceptors. In this way, an initial genetic dissection has shed light on how the pathways of motion processing are organized to inform behavior, and provides a quantitative paradigm for applying similar approaches to other sensorimotor integrations.

RESULTS

A High-Throughput Assay for Motion-Evoked Behavior

Visual behaviors observed in both flying and walking *Drosophila* are likely to be ethologically relevant (Carey et al., 2006). Therefore, to apply a forward genetic screen to the dissection of motion processing, we constructed an apparatus that allowed presentation of arbitrary visual stimuli to large populations of walking flies, while capturing the behavioral responses of each individual (Figures 1A–1C and S1, available online). This system enabled us to monitor the immediate responses of single animals to dynamic stimuli with the statistical power necessary to analyze subtle changes in behavior. Briefly, populations of flies walking in glass test tubes were presented with a visual stimulus from below, and filmed from above (Figure 1A). Individual fly trajectories were tracked in real time (Figure 1B); all data sets represent 3,000–30,000 responses of single flies at each experimental condition. In this system, animals react to stimuli independently: isolated flies produce responses to motion similar to those of flies tracked within a population, and crosscorrelation of responses in the population revealed no stimulus-specific interactions between animals (Figure S2). While flies were free to move within the test tubes, we examined only those flies that were upside-down, observing the stimulus from a nearly constant distance, using the same dorsal portion of the eye. For these flies, optical distortion along the axis of stimulus motion caused by the curvature of the test tube is negligible.

As a visual stimulus, we employed variants of the dynamic random dot stimulus (Newsome and Pare, 1988), adapted to the relatively poor spatial resolution of the fly (Figure 1D and Movies S1 and S2, available online). In these stimuli, each dot moves only a short distance before disappearing (after which it reappears at random on the screen), thus minimizing opportunities for flies to track single visual features without computing overall motion. Such stimuli allowed presentations of spatiotemporal frequency

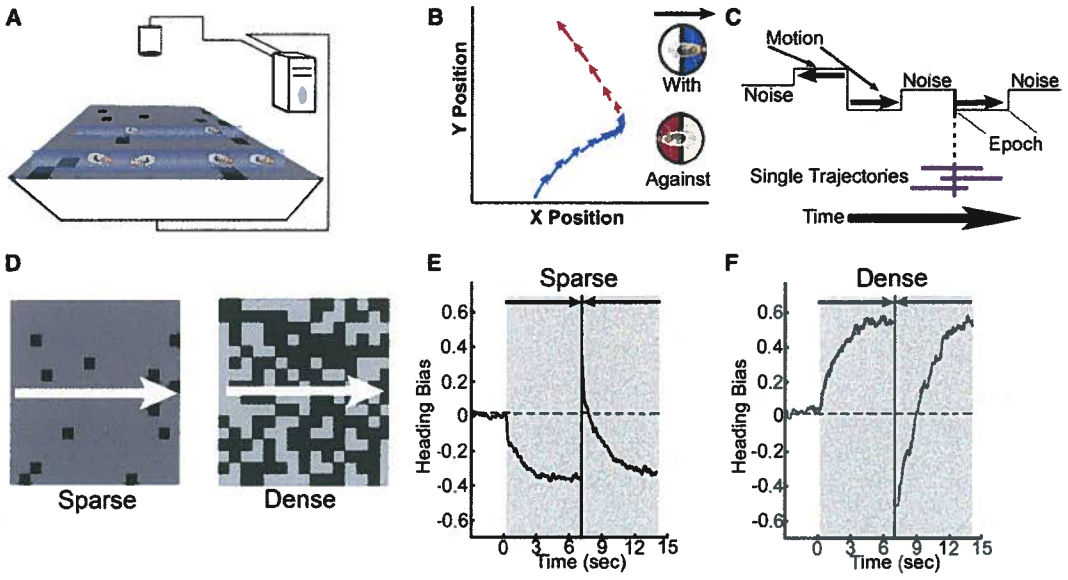

Figure 1. Flies Exhibit Two Distinct Responses to Motion

(A) Schematic illustration of the experimental apparatus. Flies in test tubes view motion underneath, and are filmed from above.

(B) A single fly trajectory. Fly heading is coded as either With (blue) or Against (red) the axis of visual motion.

(C) Trial structure. Motion cues in alternating directions are presented in short epochs, interspersed with random dot movement (noise). Trajectories are then aligned precisely in time to stimulus transitions.

(D) Schematic representations of a sparse stimulus and a dense stimulus, moving from left to right (arrows).

(E) Changes in heading bias elicited by long presentations of a Sparse stimulus (black line). Noise (white area) was followed by two periods of coherent motion in alternating directions (arrows in shaded areas).

(F) Changes in heading bias elicited by long presentations of a Dense stimulus (gray line). Trial structure as in (E).

patterns different from those possible using periodic stimuli, permitting a wide range of conditions to be systematically explored. To verify that all stimuli we used were sampled appropriately by the fly eye, we modeled the first stage of the fly visual system. Because our interest lay in DS behaviors, we confirmed that the particulars of how the fly eye registers these stimuli cannot reverse the perceived direction of visual motion relative to its true direction (Figure S3 and Supplemental Discussion). Finally, we designed our experiments such that the behavioral responses to a test stimulus could be directly compared with a behavioral baseline identical in all ways except for the presence of coherent motion. Each experiment alternated epochs in which all dots were displaced in the same direction (designated "motion") with epochs in which dots were displaced in random directions (designated "noise"; Figure 1C). Thus, the only change in the stimulus between baseline and experiment was the coherence of dot movement, and hence the motion signal; contrast and average luminance were held constant. In all subsequent analyses, we focus on changes in behavior caused by motion, relative to a baseline during the noise period.

Flies Exhibit Two Distinct Responses to Visual Motion

We reasoned that if multiple motion processing channels contribute to the fly's optomotor response, we should be able to excite them differentially under some stimulus conditions. We began by systematically varying the velocity, contrast, luminance, spatial density, and coherence of the visual stimulus. The sparsest or slowest stimuli caused flies to move against (opposite to) the direction of stimulus motion (Figures 1E and 2A). This response direction was previously observed by moving stripe patterns underneath flies or surrounding them with such patterns on all sides (Hecht and Wald, 1934; Kalmus, 1949; Gotz, 1970). Moreover, the response we observed was also consistent with previous studies in its tuning to critical motion stimulus parameters, and peaked at stimulus velocities the fly would experience itself while turning through a stationary world (Figure S4 and 2C), suggesting that our stimuli activated neurons within a physiologically relevant range. For brevity, we refer to this behavior as the Against response, and denote stimuli that cause it Sparse. Surprisingly, we also found stimuli that evoked movement in the same direction as the stimulus (a With response), marked by distinct kinetics and tuning properties (Figures 1F and 2B). This response was strongest on dense, relatively fast stimuli (Dense for brevity), and has not been previously reported in freely walking flies. These observations raised the possibility that a With response reflected the activity of a motion processing stream tuned differently than the stream underlying an Against response.

Changes in Heading Bias Emerge through Distinct Changes in Fly Translation and Rotation

To distinguish contributions of potentially different motion processing streams, we asked what immediate changes in flies' movements lead to either response over time. Fly movement can be completely described by the translational and rotational velocities of each individual (Figure 2C). One might imagine

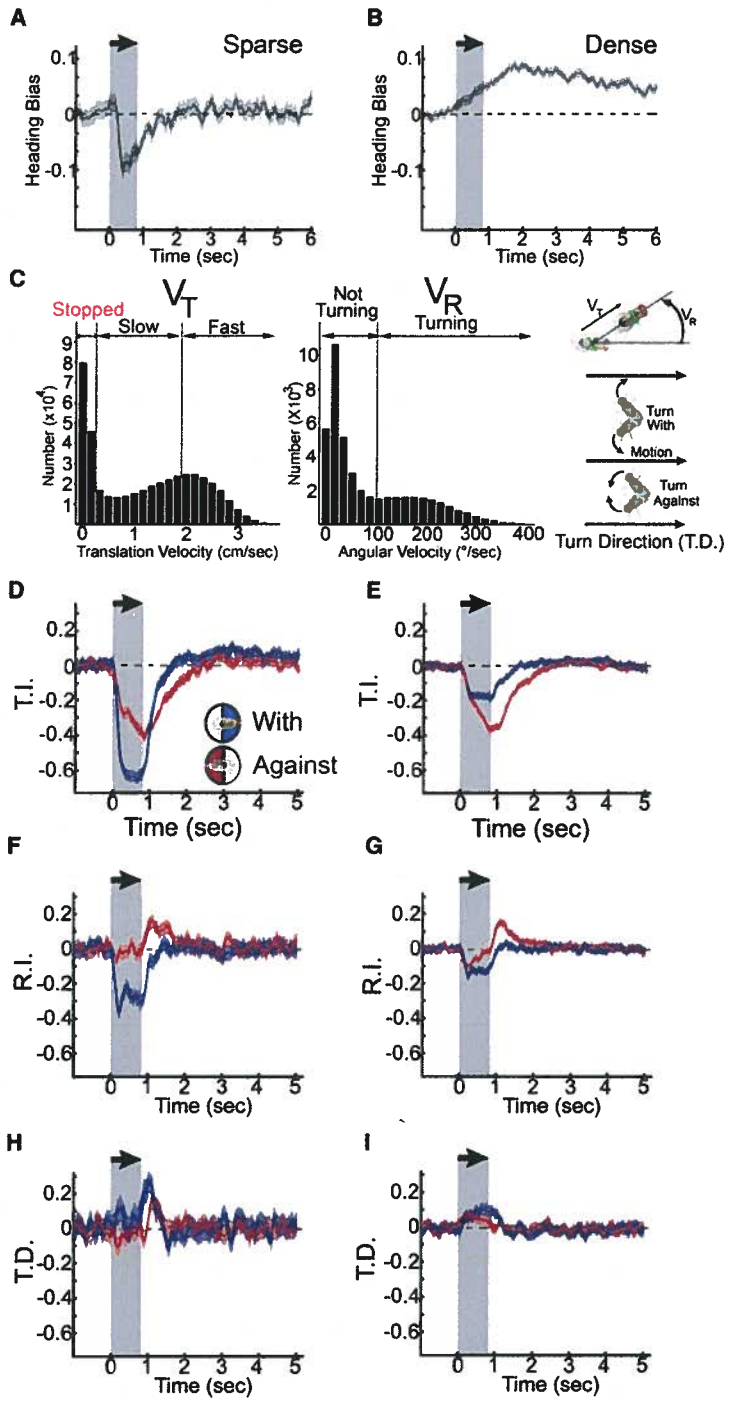

Figure 2. Different Changes in Translation and Rotation Underlie Each Behavioral Response

(A and B) Heading bias ±2×standard error (SE) elicited by a short (800 ms) presentation of either a Sparse stimulus ([A], gray bar) or a Dense stimulus ([B], gray bar), moving from left to right (arrows). (C) Histogram of the full distributions of translational and angular velocities observed in the population. From this distribution of wild-type flies, two thresholds in translational velocity were set. The lower threshold defined stopped flies as those that move slower than 0.26 cm/s. Another threshold is set at the mode of walking speeds (1.9 cm/s), defining the boundary between fast and slow walking speeds. Turning was defined as angular velocities above 100°/s. (D) A translation index (T.I.) reports change in the fraction of flies moving at fast walking speeds. A rotation index (R.I.) reports change in the fraction of turning flies. A turn direction index (T.D.) was computed from the fraction of flies turning with, relative to those turning against, the direction of motion (arrows). (D and E) T.I. ±2×SE for the Sparse (D) and Dense (E) stimulus. Motion was presented as a movement from left to right (arrow), flies were categorized as facing either With (blue lines) or Against (red lines), and the stimulus was either Sparse (D, F, and H) or Dense (E, G, and I). (F and G) R.I. ±2×SE in time. (H and I) T.D. ±2×SE in time.

that opposite responses to different stimuli simply result from reciprocal changes in movement. For example, flies could turn to follow one stimulus or turn to move against the other. Alternatively, opposite responses could emerge due to different effects of the two stimuli on translation, rotation, or both. To capture these effects, we defined three metrics that measured changes in these velocities across the population. As flies turned and walked forward at stereotyped angular and linear velocities, respectively, we defined a range in each velocity representing "turning" versus "not turning" and "fast" versus "slow" forward movement. Motionless flies were excluded from analysis because they contributed no directional signal. To calculate indices from these distributions, we measured the stimulus-induced change in the frequencies of flies above and below each threshold, in each distribution, independently (see Experimental Procedures). The translation index (T.I.) captured the relative increase or decrease in the speed of forward movement, with a T.I. of −0.2 indicating, for example, that the number of flies walking fast (as defined in Figure 2C) decreased by 20% after stimulus onset. Similarly, the rotation index (R.I.) measured whether turning increased or decreased relative to the prestimulus baseline. By analyzing our data using different thresholds to define high versus low velocities, we found that our description of behavior using these metrics was not sensitive to precisely where the thresholds were defined. Moreover, analysis of the stimulus-induced changes in the full translational and angular velocity distributions (see Experimental Procedures) produced identical conclusions. Lastly, since flies could turn in one of two directions relative to the direction of stimulus motion, we also computed a turn direction index (T.D.) that measured the prevalence of turns into alignment with the stimulus direction versus out of it, also as a fractional change from baseline (Figure 2C).

To test whether With and Against responses were generated by changes in different aspects of movement, we asked how Sparse and Dense stimuli affected translation and rotation over time. In order to produce a DS response, the behavior of flies oriented in the same and opposite directions as stimulus motion

must be affected in different ways. Hence, we analyzed the translation and rotation indices at With and Against orientations separately. This classification of flies into just two orientations relative to stimulus motion captured as much directional information as the flies themselves exhibited in their response (Figure S5). That is, flies did not align themselves precisely with or against either stimulus in the course of response, but only roughly biased their heading direction.

Sparse and dense stimuli, which biased headings in opposite directions, decreased forward movement at both orientations, although to different extents (Figures 2D and 2E). Translation of flies oriented in the With direction decreased three times as much on a sparse stimulus as it did on a dense one, while translation at opposite orientations was affected almost identically on both stimuli. As a result, With flies slowed more than Against flies on the stimulus that caused an Against response, while With flies slowed less than Against flies on a stimulus that caused a With response. Turning was also suppressed by both stimuli, causing flies to move in straighter paths (Figures 2F and 2G). On both stimuli, turn suppression was greatest at With orientations. Compared with a 3-fold difference in T.I., however, turns at With orientations were suppressed only about two times as strongly on the sparse stimulus as on the dense one. R.I. also followed a somewhat different time course: on the sparse stimulus, following an initial peak of turn suppression, turning rebounded slightly and remained at the new level for the duration of the stimulus. At the Against orientation, only relatively weak stimulus onset and offset responses were observed in R.I. (Figures 2F and 2G). Finally, when flies did turn, they tended to turn into alignment with the stimulus direction on both stimuli (T.D. > 0, Figures 2H and 2I).

Hence, between two stimuli that cause opposite responses over time, only flies at the With orientation differed in their behavior. Notably, contrary to the models of response to motion in flies that assume optomotor response is aimed at matching stimulus velocity (Götz, 1975), the strongest DS changes on either stimulus were inhibitions of movement—that is, inhibition of forward movement or of turning. At the same, With orientation, these two changes in behavior must have opposite consequences for the longer-term direction of migration. That is, preferential slowing in one direction will lead to the diffusion of flies in the other direction over time, while preferential straightening of trajectories at one orientation will cause flies to move longer in the same direction by preventing them from turning into other directions. Other aspects of behavior being equal, the balance of T.I. and R.I. inhibition at the With orientation would determine in which direction flies displace over time. Indeed, the effects on T.I. and R.I. at the Against orientation were nearly constant between the two stimuli, even though these two stimuli cause very different heading biases. In addition, while turning (T.D.) on the dense stimulus favored movement with the stimulus direction regardless of orientation, turn bias did not explain why movement reverses direction on the sparse stimulus, because T.D. was either positive or only weakly negative, depending on orientation, on the sparse stimulus (Figures 2H and 2I). Hence, the overall response direction must be a consequence of how much flies slowed down at the With orientation relative to the stimulus-invariant slowing at the Against orientation; a consequence of how much flies slowed relative to how much they suppressed turning at the same, With orientation; or both. That is, response direction is determined by relative changes in translation of With- versus Against-facing flies, by relative changes in translation versus rotation among With flies, or a combination of the two effects. In any case, the two stimuli had different effects on short-term changes in fly movement.

Rotational and Translational Responses Are Differentially Sensitive to Stimulus Parameters

To determine which aspects of behavior are modulated systematically by stimulus parameters, leading to one or another response direction, we examined the transition from an Against to a With behavior. As described above, changes in stimulus speed and density alone can determine the direction of response (Figures 3A and 3B). Broadly speaking, heading was biased most toward the Against response at the slowest or sparsest stimuli, while reactions to denser or faster stimuli were biased more toward a With response (Figure S6). Consistent with the fact that these stimuli affect different aspects of fly movement, Against and With responses occur with distinct kinetics and are not mutually exclusive, because intermediate speed and density stimuli elicit both an early Against phase as well as a later With phase (Figure 3B). Quantitatively, though, modulation of heading bias by stimulus parameters was not systematic (Figure S6), reflecting the fact that an aggregate of changes in different aspects of behavior contribute to overall heading bias (Figure 2). Rather, it was modulation of fly rotation and translation that did vary systematically with stimulus parameters, although these two aspects of movement were not sensitive to the same parameters, and their modulation was orientation dependent. In particular, motion speed, but not stimulus density, strongly modulated translational movement (Figure 3C), while both stimulus parameters in combination modulated rotation in flies oriented in the same direction as stimulus motion (Figure 3D). By contrast, flies that experienced motion in the opposite direction did not modulate relative amounts of translation or rotation consistently with changing stimulus parameters, although some speed tuning is apparent in R.I. (Figures 3F and 3G). The monotonic, orientation-specific changes in two aspects of movement represent the effects of a wide range of stimulus conditions, demonstrating that neither change is specific to stimuli that evoke the strongest With or Against responses. Unlike measures of the degree of translation and rotation, turn direction varied with stimulus density, but not stimulus speed, at Against orientations, and showed a similar pattern at With orientations (Figures 3E and 3H). Interestingly, turn direction at either orientation did not predict heading bias consistently, arguing that turn direction bias represents an additional aspect of behavior that contributes to, but does not determine, the response direction. On sparser stimuli, flies may orient to individual dots or dot clusters, including those that require turning against the stimulus direction. Such a response does not necessarily require the computation of stimulus motion. We therefore used stimuli of moderate density as the Sparse condition in subsequent experiments to minimize this effect.

In sum, different stimulus parameters systematically modulate forward movement and turning at With orientations, while at opposite orientations, they do so inconsistently, weakly, or both.

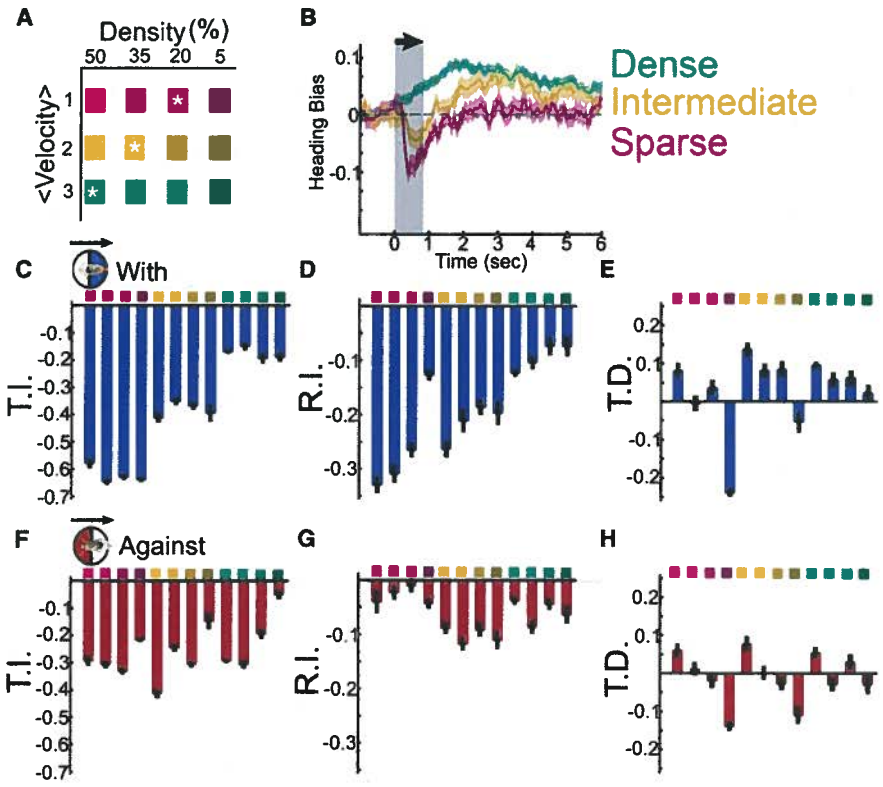

Figure 3. Different Behavioral Responses Are Sensitive to Distinct Stimulus Parameters

(A) Stimuli spanning a range of speeds and densities, at constant contrast and luminance, between those optimal for Against and With responses. Speed ranges as seen throughout the behaviorally relevant vision field of a fly: magenta (420°–920°/s), yellow (700°–1540°/s), and green (980°–2150°/s). Density is expressed as a percentage of the screen covered by moving dots. Lighter, higher density; darker, lower density. (B) Heading biases ±2×SE evoked by 800 ms of three stimuli (gray bar), separated by zero coherence noise of equivalent contrast and density (white regions). (C–E) Flies oriented With the stimulus direction (blue). (F–H) Flies oriented Against the stimulus direction (red). (C and F), T.I.; (D and G), R.I.; (E and H), T.D. Error bars: 2×SE.

Therefore, while changes in behavior at both orientations contribute to the heading bias, we exclude Against-oriented flies from subsequent analysis. The systematic modulation of movement at With orientations by motion stimulus parameters that modulate overall behavior suggests that response at these orientations is the most sensitive readout of DS motion processing circuits. The measure of heading bias, on the other hand, while qualitatively informative, was at best a secondary readout of systematic stimulus effects on different aspects of movement. Taken together, these results show that the different components of the fly's DS response to motion, namely the translational and rotational responses of With-facing flies, are sensitive to distinct stimulus features, raising the possibility that they are differentially controlled by the underlying neural circuitry.

Neural Contributions to Specific Behavioral Responses Diverge Early in Visual Processing

To identify circuits that may contribute selectively to different aspects of DS behavior, we first asked whether inactivation of neurons at the earliest anatomical stages of the visual system affects all aspects of the behavioral response equally. We took a candidate-neuron approach, using available Gal4 drivers specific to single cell types to express the temperature-sensitive synaptic silencer *shibire*^{TS} (Kitamoto, 2001). As a control, we used a well-characterized Rh1-Gal4 driver to inactivate R1–R6 photoreceptors (Mismer and Rubin, 1987). Consistent with previous studies (Heisenberg and Buchner, 1977), R1–R6 provide critical signals for responses to motion; silencing these cells severely compromised behavior on both sparse and dense stimuli (Figure 4). Indeed, all measurable aspects of DS response to sparse (Figures 4A, 4C, and 4E) and dense (Figures 4B, 4D, and 4F) stimuli in translation and rotation indices were eliminated by R1–R6 silencing.

Next, we silenced one of the immediate postsynaptic targets of R1–R6 photoreceptors, the L2 lamina monopolar cell (LMC), using a driver expressed specifically in those cells (Mollereau et al., 2000; Górska-Andrzejak et al., 2005; Figure 6A). To our surprise, L2-silenced flies moved robustly With the direction of all stimuli tested, including sparse stimuli that normally cause an Against response (data not shown). L2 silencing compromised translational response consistently on all conditions, but preserved different aspects of rotational responses depending on the specific stimulus used (Figures 4, 7, and S7). On the sparse and dense stimuli, L2 silencing eliminated suppression of both forward movement and turning (Figures 4A–4D), but did not eliminate a directional bias in turns (Figures 4E and 4F).

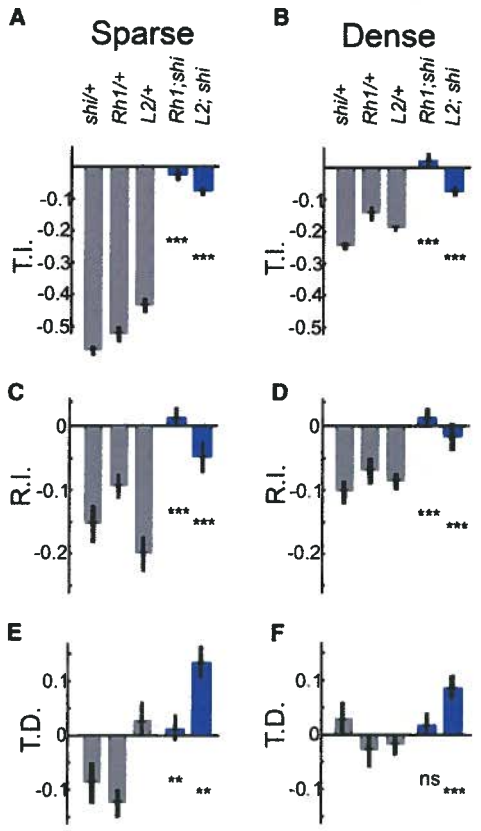

Figure 4. Genetic Dissection of Visual Motion Processing

(A and B), T.I.; (C and D), R.I.; (E and F), T.D. An 800 ms presentation of either a Sparse (A, C, and E) or a Dense (B, D, and F) stimulus was used. Control genotypes (*Gal4/+* and *shibire^{TS}/+*; gray bars) and experimental genotypes (*Gal4/+; UAS shibire^{TS}/+*; blue bars) are shown. ***$p < 0.001$; **$0.01 > p > 0.001$. All significance comparisons were against the least favorable control. All experiments were done at the restrictive temperature (34°C). Error bars: 2×SE.

In particular, L2-silenced flies displayed a robust, aberrant turn bias into alignment with stimulus motion, irrespective of response direction in controls (Figures 4E and 4F). This remaining directional aspect of behavior explained the long-term heading bias of L2-silenced flies. However, this finding failed to elaborate on the contribution of L2 to translation and turn suppression, because both were equally compromised. To explore this issue further, we reasoned that while overall responses to sparse or dense stimuli may be dominated by either translation or rotation responses, the contribution of neurons toward one or the other behavior response might be detected more sensitively when the magnitudes of rotation and translation responses induced by the stimulus are approximately balanced. Hence, instead of comparing two conditions in which both the stimuli and aspects of behavior differ, we tested L2-silenced flies on a stimulus that evoked roughly balanced With and Against responses, separated in time (Figures 3A and 3B). On such an "intermediate" stimulus, L2 silencing eliminated all response in translation as on other conditions, but preserved almost half of the normal turn suppression and retained a robust turning bias, as before (Figure S7). Hence, while L2 was strictly required for translation response under all conditions, it was not absolutely required to suppress rotation under some conditions, or to turn directionally under any conditions. These findings imply that there must be at least one motion processing pathway downstream of R1–R6 that can provide directional signals to turning behaviors in the absence of L2's activity. Conversely, DS translational responses to motion stimuli are completely dependent on L2 function.

Forward Genetic Analysis Identifies Neurons with Highly Specific Motion Deficits

To identify neurons required for motion processing, we conducted a forward genetic screen of 400 novel enhancer trap lines using the GAL4/UAS system to express *shibire^{TS}* in randomly selected subpopulations of neurons. Flies were raised at permissive temperatures and shifted to restrictive temperatures as adults immediately prior to behavioral testing. By presenting visual stimuli to populations of flies bearing different enhancer trap lines, we identified lines that displayed behavioral deficits only when specific motion stimuli were presented, but which otherwise moved and behaved normally when presented with stationary visual stimuli. This behavioral selection enriched significantly for enhancer trap lines with optic lobe expression (data not shown). However, many such lines were broadly expressed and were excluded from further analysis. One line, designated *Failed optomotor assay-1* (*Foma-1*), both had a specific behavioral deficit, and was expressed in a very small number of neurons in the optic lobe.

At the restrictive temperature, *Foma-1* flies expressing *shibire^{TS}* moved normally in the absence of motion stimuli and responded robustly to a sparse motion stimulus, but were significantly impaired in their response to the dense stimulus. In particular, *Foma-1*-silenced flies failed to move With the dense stimulus (data not shown). Inactivation of neurons in *Foma-1* preserved 80% of the response in translation on the sparse stimulus, and did not affect translation response on the dense stirnulus (Figures 5A and 5B). By contrast, turns were suppressed more strongly on the sparse stimulus, and only weakly, if at all, on the dense stimulus relative to either control (Figures 5C and 5D). Finally, *Foma1/+; shibire^{TS}/+* flies lost almost all normally elicited directionality in turning, reversing the remaining small tum bias relative to that of their controls on both sparse and dense stimuli (Figures 5E and 5F).

Thus, *Foma-1* had a stronger effect on rotation compared with translation on the dense stimulus, and modest effects on both movement aspects on the sparse stimulus. This stimulus-specific, differential effect argues that the *Foma-1* deficits do not reflect nonspecific impairments in visual function, such as contrast sensitivity, because stimulus contrast modulates translation and rotation proportionally in control flies (data not shown). In addition, because the turning response was greater in *Foma-1* than that of controls on one stimulus condition and smaller on another, the behavioral deficit cannot reflect a stimulus-independent impairment in the ability of flies to turn. Thus, *Foma-1* causes specific deficits in a DS response.

Foma-1 was expressed in two dominant groups of neurons (Figure 6). In the visual system, the driver labeled a small cluster of three neurons per optic lobe, each with a broad arbor confined

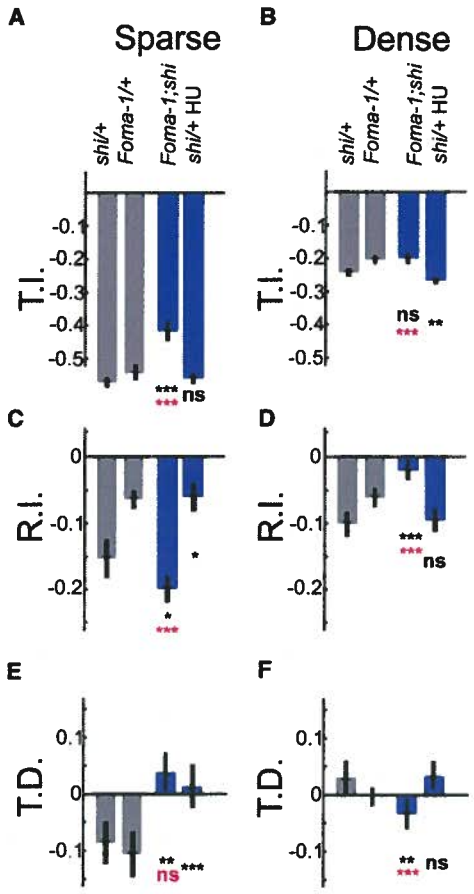

Figure 5. Disrupting a Small Group of Interneurons Causes a Specific Deficit in Behavioral Responses to Visual Motion

(A, C, and E), Sparse stimulus; (B, D, and F), Dense stimulus. (A and B), T.I.; (C and D), R.I.; (E and F), T.D. Control genotypes (*Foma1/+* and *shibire^{TS}/+*; gray bars) and experimental genotypes (*Gal4/+; UAS shibire^{TS}/+*; blue bars) are shown. An additional experimental genotype was *UAS shibire^{TS}/+* treated with hydroxyurea (HU). ***$p < 0.001$; **$0.01 > p > 0.001$; *$0.05 > p > 0.01$. n.s., not significant. All comparisons were against the least favorable control. Red asterisks/lettering denote comparisons between *Foma1/+; UAS shibire^{TS}/+* and *shibire^{TS}/+* HU. All experiments were done at the restrictive temperature (34°C). Error bars: 2×SE.

to specific layers within the lobula plate (Figures 6A–6D), with at least one neuron extending a process to the contralateral optic lobe. Intriguingly, morphologically similar neurons known in larger Diptera are thought to be critical to visual motion processing (Egelhaaf et al., 2002), and have been activity labeled in *Drosophila* using optomotor stimuli (Bausenwein et al., 1990). In addition to this specific expression in the visual system, *Foma-1* also labeled two clusters of mushroom body (MB) neurons comprising mostly the alpha'/beta' cell types (Figure 6B). However, inactivation of these neurons is unlikely to contribute to the stimulus-specific *Foma-1* deficits, because chemical ablation of the entire MB had only a weak effect on translational responses (Figure 5B), and no effect on turning responses (Figures 5D and 5F), evoked by the dense stimulus. It also produced a distinct phenotype unlike that of *Foma-1* on the sparse stimulus (Figures 5A, 5C, and 5E). By expressing lacZ from the *Foma-1* Gal4 driver in flies used for behavior, we confirmed that our hydroxyurea (HU) treatment eliminated most of the driver's expression in the MBs while preserving expression in the lobula plate (Figure 6K). Thus, HU treatment selectively eliminated most of the MB neurons that would be silenced in the *Foma-1* line, without reproducing the *Foma-1* phenotype. Thus, inactivating the three lobula plate neurons in *Foma-1* likely causes its characteristic visual behavior deficits.

In an effort to further characterize the lobula plate neurons labeled by *Foma-1*, we costained the *Foma-1* driver with dendritic and presynaptic markers (Figures 6E–6J). Both major and minor branches of the lobula plate arbors are labeled in a punctate pattern with the dendritic marker Dscam::GFP (Figures 6E–6G; Wang et al., 2004), while the presynaptic marker Snb::GFP could only be resolved in the major branches of these arbors (Figures 6H–6J; Estes et al., 2000). Although the morphology of lobula plate interneurons in *Drosophila* is not completely characterized, these findings are consistent with lobula plate interneuron properties in other Diptera, in which wide-field arborizations in the lobula plate can represent both dendritic and axonal processes, either of which can make electrical contacts with other neurons (Farrow et al., 2005).

On the whole, *Foma-1* deficits complement those of L2. Whereas L2 was strongly required for stimulus-specific slowing under all circumstances, *Foma-1* was weakly required for slowing on some conditions, but not necessary for slowing at all on others. Across a wide range of stimuli, L2 silencing preserved directional response only in aspects of rotation: turn bias, turn inhibition, or both. L2 is thus required less strictly for rotation than for translation. *Foma-1*, by contrast, is required more strictly for rotational aspects of response in a stimulus-specific manner. Because photoreceptors R1–R6 are required for all aspects of behavior under all stimulus conditions, these phenotypes argue that rotational and translational responses are at least partially separable by genetic criteria, diverging downstream of R1–R6. Most surprisingly, L2, a neuron type immediately postsynaptic to photoreceptors, already carries information more essential for some aspects of response to motion than for others.

Different Motion Processing Streams Respond to Different Visual Information

R1–R6 photoreceptors make synaptic connections with three LMCs, designated L1–L3 (Meinertzhagen and Hanson, 1993). Although L1 and L2 receive anatomically identical photoreceptor inputs, all three cell types differ in their postsynaptic partners. The stimulus dependence of the rotational response in L2-silenced flies could therefore reflect the differential processing of each stimulus toward different aspects of response either at the level of L2, or by downstream neurons along pathways that split in the lamina. Because LMCs are thought to extract information about stimulus contrast (Srinivasan et al., 1982), we tested the contributions of R1–R6 and L2 to the sparse stimulus response under different contrast conditions. We reasoned that if motion pathways diverged in the lamina and had different contrast sensitivities, they would contribute differentially toward translation and rotation under some contrast conditions. In this

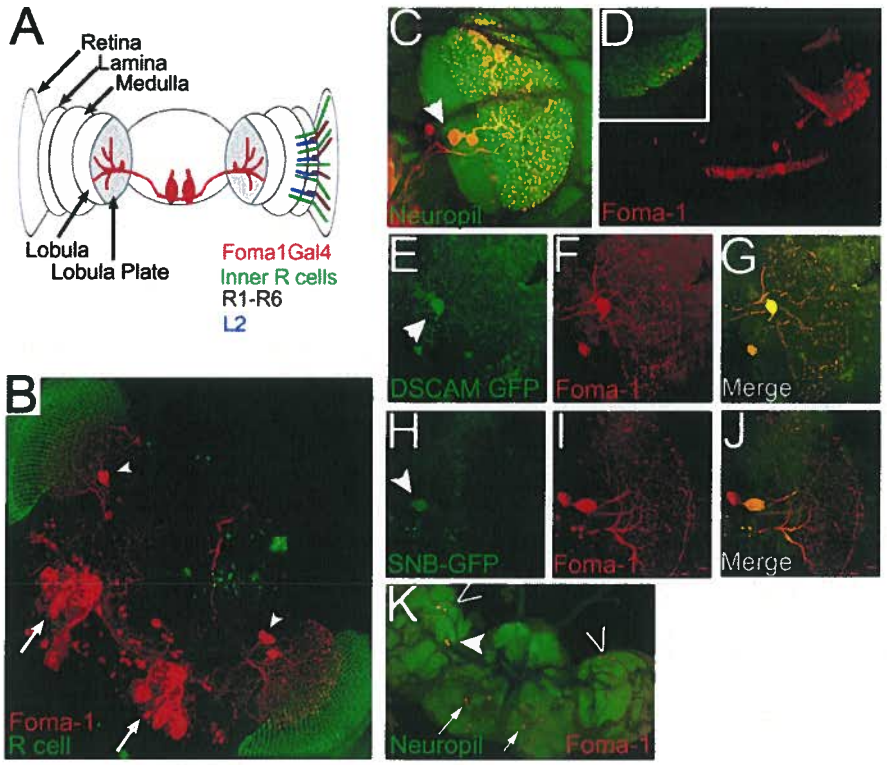

Figure 6. *Foma-1* Labels a Small, Specific Group of Neurons in the Optic Lobes and Mushroom Bodies

(A) Schematic depiction of the fly brain.

(B) Image of the neurons labeled by *Foma-1* Gal4 (red), and of photoreceptor terminals (green), in the medulla. Two groups of neurons are labeled in all *Foma-1* flies: a large cluster of neurons in the mushroom body (arrows), and two small clusters of three or four neurons in each optic lobe, with projections confined to the lobula plate (arrowheads).

(C and D) High-magnification views of the optic lobe neurons labeled by *Foma-1* (red), combined with a neuropil marker (green), from two orientations.

(E–G) Optic lobe neurons labeled by *Foma-1* (red), expressing the dendrite marker Dscam::GFP (green).

(H–J) Optic lobe neurons labeled by *Foma-1* (red), expressing the axon marker Snb::GFP (green).

(K) Image of a *Foma1/+*, *shibire*^{TS}/+ brain, treated with HU. *Foma-1* cell bodies in optic lobe (arrowhead) can be seen in one lobe (and are out of focus in the other); some of the processes (hash marks) can be seen in both lobes. This brain retains a few labeled cells in the central brain (arrows); many others had none.

case, inactivating L2 should have different effects on behavior under specific contrast conditions, using otherwise identical stimuli. If, on the other hand, contrast processing in the lamina does not contribute toward differentiating stimuli for one aspect of response versus another, inactivation of L2 under two different contrast conditions where the responses of control flies are comparable should affect both translational and turning behaviors, under both conditions, equally.

To do this experiment, we systematically varied the contrast of the visual stimulus to find two contrasts that produced responses of comparable magnitude in both translational and rotational aspects of response. We chose two identically Sparse stimuli, one of moving dots that were darker than background (contrast decrement), and the other, of dots that were lighter than the background (contrast increment). The magnitudes of both translational and rotational responses were roughly matched between the two stimuli (Figure 7). To ensure that potentially separable aspects of response were not confounded during the course of a longer stimulus presentation, we presented flies with brief motion pulses that revealed distinct phases in all response metrics (Figures 7A, 7C, 7E, 7G, 7I, and 7K). We found that L2's contribution was not specific to any one phase of response (data not shown), and therefore we present summary data from just the first, representative response phase.

As a control, we confirmed that inactivation of R1–R6 photoreceptors abolished nearly all response components under both stimulus conditions, leaving only a small, aberrant turning response on the decrement stimulus that may reflect incomplete inactivation (Figures 7B, 7D, 7F, 7H, 7J, and 7L). L2, however, was required differentially between the two contrast conditions for different aspects of response. The contrast decrement stimulus produced the stronger phenotype. Here, silencing L2 abolished almost all responses, producing a phenotype similar to that of R1–R6-silenced flies (Figures 7D, 7H, and 7L). However, on the contrast increment stimulus, silencing L2 abolished all translation response, but left almost half of the normal turn suppression intact (Figures 7B and 7F). Moreover, L2 silencing did not diminish directionality in the remaining turns, enhancing it somewhat instead (Figure 7J). Although the magnitudes of

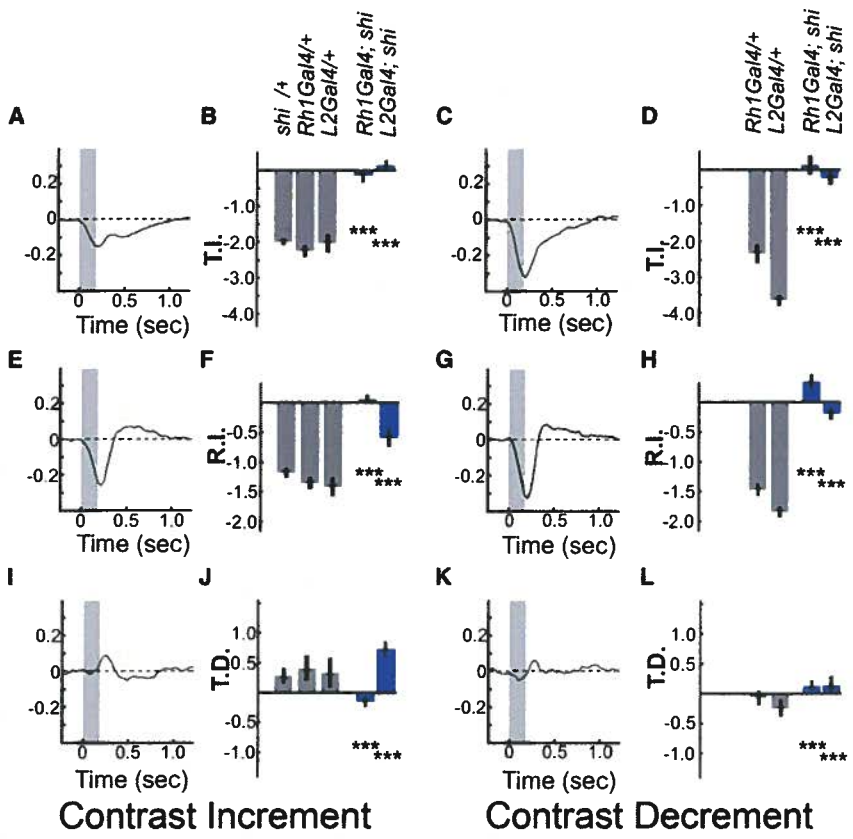

Figure 7. Visual Pathways Underlying Translational versus Rotational Behavioral Responses Diverge Early

(A–D) T.I. in time (A and C), or integrated (B and D), following a very brief (150 ms, gray bar) presentation of a sparse stimulus.

(E–H) Control R.I. in time (E and G) or integrated by genotype (F and H).

(I–L) Control T.D. in time (I and K) or integrated by genotype (J and L).

Contrast increments (A, B, E, F, I, and J) or contrast decrements (C, D, G, H, K, and L) are shown. Significance of effect was tested by bootstrap (see Experimental Procedures). Error bars: 2×SE.

control responses were not perfectly matched between increment and decrement stimuli, it was the stimulus that elicited a stronger response in controls that was most broadly compromised by L2 silencing. This demonstrates that the differential effects of L2 on translation versus rotation do not reflect quantitative differences in the strength of the response itself, but rather are a function of the specific stimulus conditions used. Thus, L2 participates in the motion processing stream that guides rotational responses to different extents depending on contrast polarity, but is required for translational responses under all circumstances. This finding suggests that visual contrast information already differentiates the contributions of LMCs to particular behavioral responses.

DISCUSSION

We have developed a high-throughput system that is amenable to forward genetics, provides a sensitive, quantitative analysis of behavior, and easily permits detection of very subtle behavioral changes. Using this system, we can detect small deficits in behavior due to neuronal disruption. Moreover, we can parameterize the stimulus-behavior relationship efficiently, allowing neural function to be described precisely in the context of a freely behaving animal. Combining a forward genetic approach, targeted neuronal disruption, and extensive stimulus parameterization, we have demonstrated by double-dissociation that at least partially nonoverlapping circuits diverge in the early stages of the visual system to affect translational or rotational responses to motion. This finding is incompatible with the notion that a common motion processing step guides all DS behaviors, and with the more general view that sensorimotor decisions are typically informed by sensory cues that are first generically processed without regard for their consequences to behavior. The fly visual system is likely to be one of the first contexts in which the organization of complete pathways, from sensory input to behavior, is understood at the level of identified cells and circuits. Our results provide a behavioral framework for constraining the neural computations that underlie motion vision, and raise the possibility that many, if not all, visual stimuli undergo behaviorally specialized processing early in the visual stream.

Understanding the Behavioral Mechanisms Underlying the Optomotor Response

The optomotor response has long been a paradigm used to examine the neural mechanisms underlying motion detection (Reichardt and Poggio, 1976; Buchner, 1984). It has also long been appreciated that this response varies substantially in different contexts, having opposite signs depending on precisely how the stimulus is presented and on how the animal is allowed to move (Hecht and Wald, 1934; Kalmus, 1949; Gotz, 1970; David, 1979). Here we uncover novel aspects of this classical behavior, synthesizing previously discordant observations in the context of a single behavioral paradigm. We find that in freely walking flies, the long-term direction of migration is a secondary consequence of rapid, DS changes in specific aspects of individual animal movements (Figure 8A). We find that motion seen in the same direction, back-to-front across the retina, effects two types of immediate, sustained changes in movement, depending on stimulus characteristics. In particular, sparse, relatively slow stimuli strongly suppress both forward movement and turning. The net effect of these changes is dominated by suppression of forward movement in one direction, as flies move in the opposite direction over time. Conversely, straightening of trajectories by

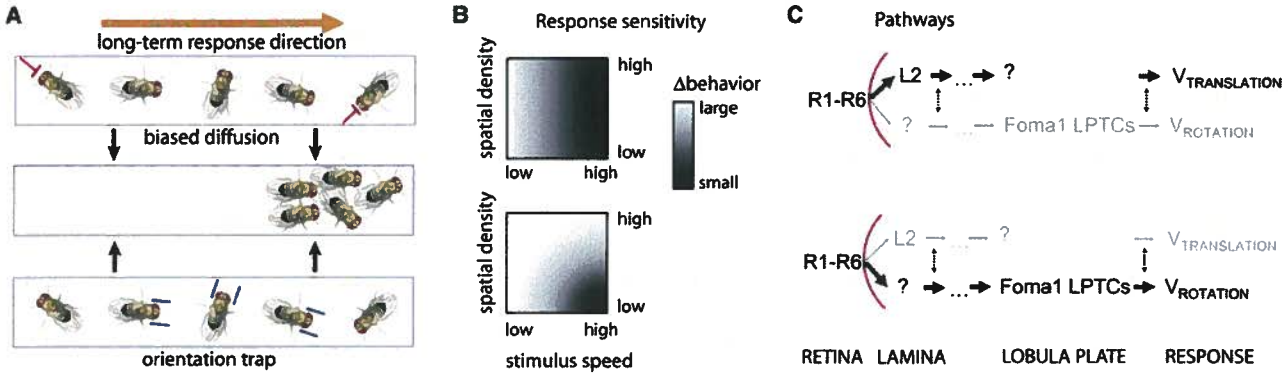

Figure 8. Summary and Model

(A) A directional response over time results from selective slowing of flies at one orientation (top), or selectively stabilized headings at a different orientation (bottom) with respect to the long-term response direction. Relative to the direction of stimulus motion, both changes take place at the same orientation, where back-to-front motion is seen.

(B) Translation and rotation changes in behavior are modulated differently by two stimulus parameters.

(C) Preferential activation of an L2-dependent pathway promotes translational changes in response to visual motion; activation of a *Foma-1*-dependent pathway promotes rotational changes preferentially. Both pathways depend on photoreceptor type R1–R6, but diverge immediately postsynaptic to these cells.

turn suppression dominates on different stimuli, stabilizing the headings of flies facing in the same direction as the stimulus. In addition, all but the sparsest stimuli bias remaining turns in the same direction as stimulus motion. All of these effects are elicited in different proportions by different stimuli, spanning a wide range of stimulus conditions, suggesting that most motion cues normally encountered by the fly will modulate multiple behavioral outputs simultaneously (Figure 8B).

The mechanisms of response we have described are different from mechanisms thought to guide the optomotor behavior of immobilized animals, either walking on a moving surface or flying in place, even though these behaviors follow the same relationships with stimulus parameters as in our paradigm (Buchner, 1984; Gotz and Wenking, 1973; Gotz, 1975). This discrepancy is significant; the dominant model of optomotor response in freely moving animals was extrapolated from responses to rotating visual patterns by immobilized animals. In particular, this model proposed that a goal of the fly motion system is to achieve "equilibrium" of rotational cues between the two eyes (Gotz, 1975). This model predicted that the only DS responses in freely walking flies would be limited to rotations aimed at balancing bilateral optic flow. Our findings do not bear out this prediction. We find that both rotational and translational aspects of movement are modulated in a DS way, and that this modulation is due to distinct neural processes, because inactivation of specific neurons leads to deficits that affect either translational or rotational responses to motion in different ways. We also find that while most stimuli bias the direction of turns into alignment with the stimulus, which is a change in behavior consistent with the equilibrium model, a prominent, independently tuned effect of all stimuli is to suppress turning in all directions. This response is not consistent with a control mechanism to achieve a bilateral equilibrium of velocities, because such a model would predict that turns that increase optic flow are suppressed, while those that decrease it are promoted. In sum, observing DS changes in all aspects of behavior of freely moving animals has revealed prominent features of response to motion that were not detected in earlier studies using immobilized animals. Moreover, while a different approach examined optomotor response in freely walking flies, DS changes in all degrees of freedom of movement were not examined; focus was placed only on the aspect of response we measured as turn direction bias (Strauss et al., 1997). Indeed, using this metric we find the same tendency of flies to turn with most stimuli as previously described. Our results are also consistent with earlier studies that found strong behavioral responses to back-to-front motion in freely walking and freely flying *Drosophila* (Hecht and Wald, 1934; Kalmus, 1949; David, 1979). Thus, our findings are not at odds with previous results, although they do not support the model derived from them.

Defining the Structure of the Motion Detection System

The precise nature of the circuit underlying motion detection is unknown. Electrophysiological and behavioral studies in immobilized animals have proposed a circuit in which a single, common elementary motion detection step early in the visual system extracts local motion cues, which are then assembled at later neural stages into complex, ethologically relevant patterns that control specific behavior outputs in different contexts (Hildreth and Koch, 1987; Reichardt and Poggio, 1976; Buchner, 1984). In their simplest forms, models that posit a single, common motion detector predict that distinct, DS motor outputs should be similarly sensitive to each parameter of motion stimuli, and that disrupting the function of a neuron involved in elementary motion processing should affect all motor outputs similarly for the same stimulus. Our observations suggest a more complex view. In particular, our results argue that multiple motion-sensitive pathways contribute to behavioral responses to motion in different contexts, informed by signals that segregate early in the visual system (Figure 8C). Intriguingly, anatomical evidence suggests that motion-sensitive behaviors may be executed in some animals independently of lobula plate neurons; activity labeling studies detect up to three retinotopically organized pathways

in the medulla, and electrophysiological evidence has suggested that luminance ON and OFF signals may contribute to motion pathways through separate DS channels (Franceschini et al., 1989; Sinakevitch et al., 2003; Bausenwein et al., 1992; Bausenwein and Fischbach, 1992). Our work provides the first evidence that this underlying complexity of the visual system subserves specific behavioral roles.

Our model advances understanding of the specific behavioral functions of LMCs and provides a context for considering their electrophysiological responses. By measuring all aspects of behavioral response in freely walking animals to a wide range of stimuli, we discovered that DS changes in translation depend more critically on contributions from one of these LMCs, L2, than do DS changes in rotation. This differential effect is profound, making the difference between following a stimulus and moving against it. Moreover, our finding that behavioral responses elicited by presentations of contrast increments and decrements are different, and are affected differently by disruption of L2, provides a context for understanding the specialization of LMC responses to different pattern contrast regimes. In particular, Dipteran LMCs respond proportionately to stimulus contrast (Srinivasan et al., 1982), display asymmetric ON and OFF transients, and are sensitive to the spatial structure of the stimulus (van Hateren, 1992). Because we find that translation and rotation are sensitive to different combinations of stimulus density and speed, the specialized behavioral contributions of L2 may reflect the differential sensitivities of individual LMC types to spatiotemporal structure.

Recent work has examined the effects of ablating L2 and other LMCs on optomotor behavior, analyzing only the turning responses to rotational motion in immobilized flies (Rister et al., 2007). This work found that ablation of L2 does not affect turning bias evoked by moving gratings, except at very low stimulus contrasts. This result is consistent with our data, because we see a strong With turning bias under all stimuli in L2 silenced flies, even when this response is aberrant. However, because these studies could not measure either DS changes in translation or turn suppression, the differential contributions of L2 to these processing streams were not detected.

Our behavioral data also complement electrophysiological studies of LPTCs. Detailed examination of connections among a subset of lobula plate neurons in large insects has defined microcircuitry capable of pooling DS signals, given inputs from motion detectors for small regions of visual space (Haag and Borst, 2004). However, the limitations of electrophysiology in this system have hampered our understanding of how DS signals arise in the lobula plate. Hence only indirect evidence suggested that the array of detectors estimating local motion does so uniformly for all motion-sensitive behaviors (Buchner, 1984). Moreover, the diverse response properties of LPTCs have led to a range of predictions about behavioral contexts where their signals may be required (van Hateren et al., 2005; de Ruyter van Steveninck et al., 2001). Using a forward genetic approach that began only with the requirement that motion-evoked behavioral responses be specifically altered, we identified a small group of cells in the lobula plate as playing critical roles. In our system, these cells adjust the extent of turning in response to different stimuli, with a weaker effect on translation on some stimuli. This observation suggests that different LPTCs participate in distinct microcircuits, each of which is tuned to particular stimulus parameters, and is coupled to particular behaviors. Thus, a complete description of any neuron's role in motion processing can only come through examining the effects of a diverse stimulus set on all of the DS behavioral responses of the animal.

Forward Genetic Dissection of Visual Circuitry

The growing set of genetic tools for manipulating neuronal activity has raised the possibility of dissecting neural processes using increasingly precise techniques. In flies, these tools have previously been applied to the examination of the behavioral roles of "candidate" neurons, preselected for analysis based on anatomical or molecular considerations, in combination with either high-resolution studies of single animals, or relatively low-resolution studies of populations (Suh et al., 2004; Demir and Dickson, 2005; Manoli et al., 2005; Rister et al., 2007). While powerful, such applications are limited in their ability to identify new circuit components and, in the case of richly structured sensory processes like vision, limited in their capacity to capture behavioral responses to diverse stimuli, one animal at a time. By developing a forward genetic approach combined with a high-throughput, quantitative behavioral analysis, we have overcome both limitations. Indeed, our study has opened the possibility that an unbiased, "saturation" genetic screen for neurons can now be conducted, resulting in an extensive description of many circuit elements in a particular neural pathway. In the context of motion vision, the unbiased approach is predicated on applying behavioral criteria to select lines in which motion processing has been compromised, and then identifying the sets of neurons that, when disrupted, are responsible for the defect. The choice of motion stimuli to elicit behavior thus sets the range of processing for which the visual system is probed; the resolution of behavioral observation limits the sensitivity to changes in processing. Our approach has extended the notion of a forward screen to specific circuit elements, in the context of a comprehensive, real-time description of the behavior of freely moving animals reacting to complex stimuli. We anticipate that the combination of sensitivity, temporal resolution, and parameterization we have introduced will be critical to identifying and characterizing the many different neuron types that make specific contributions to behavior, or alternatively, play complex roles in processing stimuli. Because many basic neural processes are widespread in the animal kingdom, and potentially evolutionarily ancient, an increased understanding of their circuit implementation in the fly will broadly inform our understanding of their functions in other organisms.

EXPERIMENTAL PROCEDURES

All strains and stocks were maintained under 12:12 light/dark cycles, at 25°C, 45%–60% humidity, and were tested from between 2 to 4 days of age. The following stocks were used: Oregon R, *UAS shibire^{TS}* (on III), *Foma1Gal4*, *D21Gal4*, *Rh1Gal4* (on X), *UAS synaptobrevin GFP* (on II), *UAS DSCAM-GFP* (on III or X) and *UASmCD8GFP* (on III). All wild-type experiments were carried out at the restrictive temperature for *shibire^{TS}*, 34°C. For each experiment, 100–233 flies were placed inside the behavioral apparatus. Each trial consisted of a motion epoch, in which most dots were displaced coherently in one of two opposite directions, and a noise epoch, in which each dot was

displaced independently of all others in a random direction. Motion and noise epochs alternated. Within each trial, stimulus luminance was clamped at a constant value. Stimuli in which dots were lighter than background were named contrast increment stimuli, while stimuli in which dots were darker than background were named contrast decrement. Brains were dissected from adult flies using standard methods (Lee et al., 2001), and were imaged using either a Leica TCS SP2 or SP5 confocal. For additional details, see Supplementary Materials and Methods.

SUPPLEMENTAL DATA

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/59/2/322/DC1/.

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