European Journal of Neuroscience, Vol. 27, pp. 1441–1451, 2008 doi:10.1111/j.1460-9568.2008.06114.x

Mushroom bodies modulate salience-based selective fixation behavior in *Drosophila*

Wang Xi,[1,2] Yueqing Peng,[1,2] Jianzeng Guo,[1] Yizhou Ye,[1] Ke Zhang,[1,2] Feng Yu[1,2] and Aike Guo[1,3]
[1]Institute of Neuroscience, State Key Laboratory of Neuroscience, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (CAS), 320 Yueyang Road, Shanghai 200031, China
[2]Graduate School of Chinese Academy of Sciences, Beijing 100049, China
[3]State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, CAS, 15 Datun Road, Chaoyang Dist., Beijing 100101, China

Keywords: behavioral neuroscience, *Drosophila melanogaster*, fixation, flight simulator, mushroom bodies

Abstract

Mushroom bodies (MBs), one of the central brain structures in *Drosophila melanogaster*, are involved in several cognitive behaviors, such as olfactory learning and memory, visual context generalization, choice behavior facing conflicting cues. Attention is a cognitive behavior, and it facilitates a focus on the attended event while filtering out irrelevant events, thereby allowing more rapid and accurate reactions at a lower threshold in primates. Using the visual orientation paradigm in a flight simulator, we observed that MBs modulate salience-based selective fixation behavior, which resembles attention in primates to a certain degree. We found that the fixation ability of MB-deficient flies was significantly reduced when the contrast levels were lowered as well as when a certain amount of background noise was applied. Moreover, MB-deficient flies exhibited poor object fixation ability in the presence of an olfactory ‘distracter’. Furthermore, during visual selection among multiple objects of different contrast, flies with MBs were able to ‘pop-out’ of the most salient object in a three-object selection paradigm. Finally, we determined that flies exhibited cross-modal synergistic integration between olfactory and visual signals during object-fixation behavior, which was independent of MBs. Taken together, our findings suggest that MBs do not contribute to cross-modal synergetic integration between olfactory and visual signals; instead, they confer sensory gain control and inhibitory gating in flies, this property allows entry of the salient signal as well as filters out background noise and irrelevant signals.

Introduction

Natural scenes contain more information than the sensory system can efficiently process at any given time. Attention allows an organism to focus on a very small proportion of the incoming information (Desimone & Duncan, 1995; Rensink *et al.*, 1997; Kanwisher & Wojciulik, 2000; Treue, 2001; Reynolds & Chelazzi, 2004). Fruit flies (*Drosophila melanogaster*), with their tiny brains, also display attention-like object fixation behavior in the flight simulator, and this behavior contains some features (orienting, expectancy, stimulus differentiation and sustainability) that are common to the attention behavior in mammals (van Swinderen & Greenspan, 2003; Greenspan & van Swinderen, 2004; Swinderen, 2005).

In the equivalent free-flight situation, flies demonstrate spontaneous orientation towards a black object that is either standing or moving relative to the room coordinate system. In the first instance this orientation behavior is called ‘fixation’, in the second termed ‘tracking’ (Poggio & Reichardt, 1976). Behavioral evidences for selective fixation have been reported during walking (Horn, 1978; Goetz, 1994) and tethered flight in the flight simulator (Heisenberg & Wolf, 1984; Wu *et al.*, 2000) when competing visual stimuli are present. During tethered ‘close-loop’ flight in the flight simulator, flies demonstrate their ‘perception’ of objects by using body torques to hold a dynamic object still in the front of their visual field (Heisenberg & Wolf, 1984). In *Drosophila,* this object-induced fixation behavior has been referred to as ‘selective attention’ (Heisenberg *et al.*, 2001; Swinderen, 2005). Recently, a novel paradigm of the optomotor maze was also used to study the attention-like process towards competing visual objects in *Drosophila* (van Swinderen & Flores, 2007).

Mushroom bodies (MBs) are central brain structures that are believed to play an important role in olfactory learning and memory in *Drosophila* (Davis, 1993; Heisenberg, 1998, 2003; Zars, 2000). However, recent evidences have revealed that flies’ MBs are also involved in some visual cognitive tasks, such as context generalization in altered environmental conditions (Liu *et al.*, 1999) and choice behavior while facing conflicting visual cues (Tang & Guo, 2001; Zhang *et al.*, 2007). Electrophysiological recording took from the medial protocerebrum, a brain region bracketed by the MBs, demonstrated that the activity of 20–30 Hz local field potential (LFP) correlated with the salience of visual objects during visual fixation behavior (van Swinderen & Greenspan, 2003). However, there is no direct evidence to support that MBs are required for selective fixation tasks. Here, we use genetic approaches in *Drosophila* to investigate the roles of MBs in salience-based object fixation. When we tested *Drosophila* in a competing visual stimuli task, we observed that MBs modulate fixation behavior in one of three

---

Correspondence: Dr A. Guo, [1]Institute of Neuroscience, as above.
E-mail: akguo@ion.ac.cn

Received 20 August 2007, revised 23 January 2008, accepted 27 January 2008

ways: by inhibiting background noise; enhancing contrast discrimination; or facilitating visual selection. Taken together, we propose that MBs functions are to filter out unrelated signals and provide relevant information that can be used to guide related behaviors.

Materials and methods

Drosophila strains

Flies were cultured on standard medium as previously described (Guo *et al.*, 1996) at 25 °C with 60% relative humidity and a 12 h light : dark cycle. Wild-type Berlin (WTB) and mutant *mushroom body miniature*^{1} (*mbm*^{1}) flies with a WTB genetic background were used. Hydroxyurea (HU) flies were obtained from WTB flies by following a procedure described previously (de Belle & Heisenberg, 1994). The ablation of the MB structure was assessed by imaging the UAS-GFP/247 flies accompanied by the same procedure used of the WTB flies. Only the groups of flies in which the MBs were completely ablated were used in the behavior test. UAS-*shi*^{ts1}/247 flies were generated by crossing UAS-*shi*^{ts1} flies (Kitamoto, 2001; provided by Dr Tully, Cold Spring Harbor Laboratory, USA) with 247-GAL4 flies (Strausfeld *et al.*, 2003; provided by Dr Buchner, University of Wuerzburg, Germany). The 247-GAL4 enhancer-trap line drives preferential expression in the MBs. Wild-type CantonS (CS) flies served as the control for UAS-*shi*^{ts1}/247 flies. The mutant *ebo*^{678} flies (Strauss & Heisenberg, 1993; provided by Dr Liu, Institute of Biophysics, Chinese Academy of Sciences, China) are deficient in the ellipsoid body of the central complex.

We briefly immobilized 3–4-day-old female flies by placing them in vials on ice for several minutes in order to induce cold-anesthesia, and then glued (Locktite UV glass glue) their heads and thoraxes together via a triangular hooks (isosceles triangle composed of 0.05 mm metal wire, which one side is 1.5 mm and the other two sides are 2.36 mm each) by exposing them to the UV light (GEMINI-M, Dental light cure unit, JIMSON Dental) for 20 s on a cold iron plate 1 day before the experiment. They were kept individually in small chambers and fed 4% sucrose solution overnight.

Apparatus

The *Drosophila* flight simulator (Heisenberg & Wolf, 1984; Heisenberg *et al.*, 2001) is a computer-controlled feedback system in which the fly uses its yaw torque to control the rotations of a circular panorama. The core device is the torque meter (Goetz, 1964) that measures angular momentum of a fly around its vertical body axis. Individual flies with hooks were attached to the torque meter at the center of the panorama (44 mm diameter) that could be rotated using a fast electric motor. Flight orientation (panorama position) and yaw torque were recorded continuously and stored in the computer (sampling frequency, 20 Hz) for further analysis. All the experiments were performed in a close-loop condition in the flight simulator, and the positions are therefore given as relative positions from an arbitrary zero point in the arena. The temperature in the arena was increased with a hot plate underneath the flies and controlled by a feedback from a thermocouple in close proximity to the flies **(**Fig. 1A**)**.

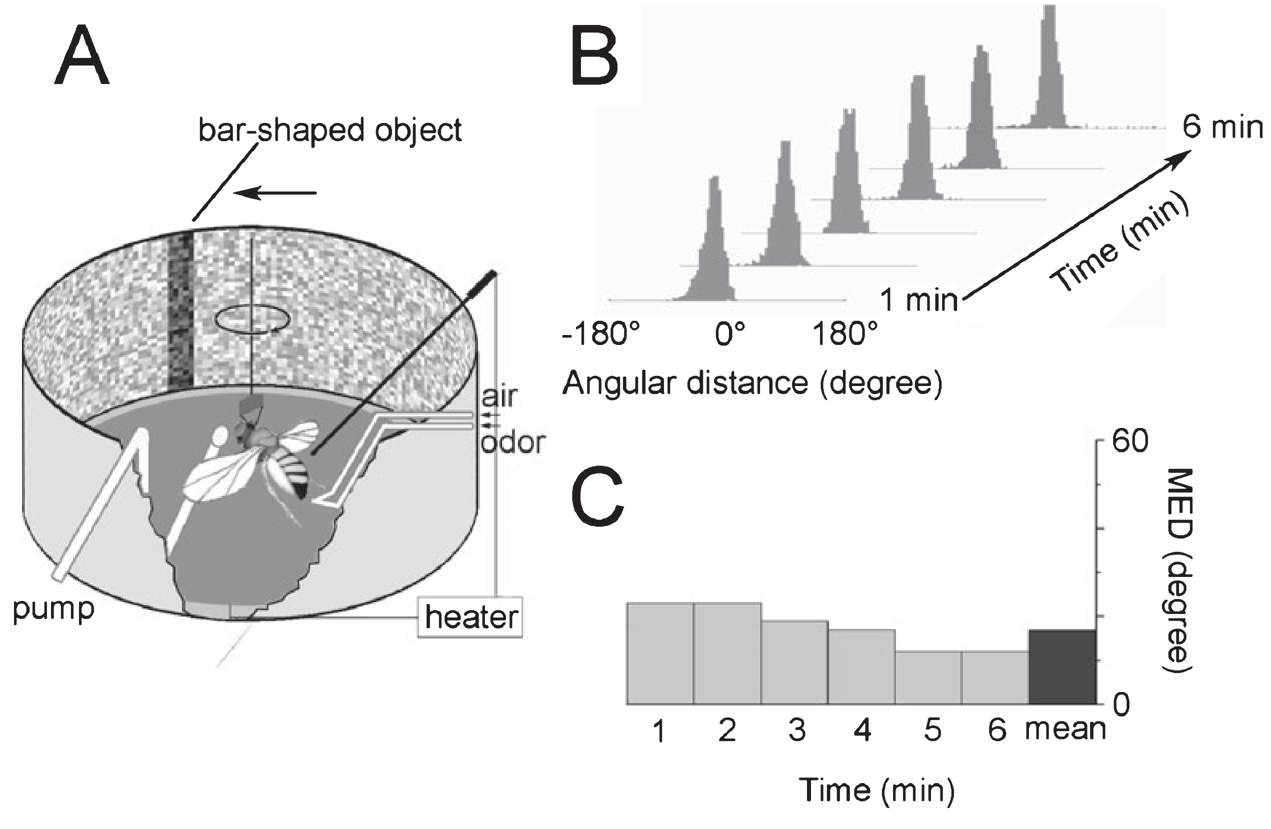

Fig. 1. Experimental setup and protocol of visual fixation behavior in the flight simulator. (A) A tethered fly orientated to a stimulus in the visual background noise (a vertical stripe at a contrast-defined noise level of 0.5). In some experiments, the flow of odor served as a distracter toward the back of the fly or a hint toward the head of the fly. The heater beneath the fly was used to raise the temperature in the arena. (B) Fixation performance of an individual WTB fly in the 0.5 contrast-defined noise level condition within a 6-min flight period. (C) The mean error distance (MED) was used to quantify fixation performance.

Visual stimulus

A vertical stripe (12° width and 107° length) was used as the visual target. Visual stimuli, including visual background noise, were printed on white paper using a color printer (Epson Photo 925). The printed papers were placed on the arena in the flight stimulator, which was uniformly illuminated by a circular fluorescent tube (168 mm diameter, 22 W). The contrast, which was defined as the luminance difference between the object and background, was gradually changed from 1.0 to 0. Contrast-defined noise in this case indicated that the contrast of every dot in the background (approximately 2.5°) randomly varied from 0 to a particular value, while the contrast of dots in the object varied from 1 to the same value. Spatial noise was generated by adding random dots (approximately 2.5°) at a particular space density in the background and white random dots of the same density on the object. In the visual selection paradigm, two objects of different contrast were positioned at $\psi = \pm 60°$, respectively (angular interval: 120°, measured from the center of a test fly; ψ denotes the horizontal angular position with respect to the frontal midline of the test fly), or three objects with different contrast were symmetrically positioned at $\psi = 0°, \pm 120°$ in the circular panorama, respectively.

Odor distracter

The odor of 100-fold diluted 4-methylcyclohexanol (MCH) in mineral oil was delivered with pressurized air flowing horizontally at a rate of 200 mL/min through a tip near the tail of the fly (approximately 1 cm). A vacuum tube was placed under the fly and the airflow orifice was in front of the head of the fly to continuously draw off the remaining odor (Fig. 1A). The odor was applied randomly in the last 3 min (6-min procedure) at 0.3 probability, which means that the odor was present only 30% of the time, and the duration of each hint episode was 1 s. The switch between air and odor was controlled by a computer via fast electromagnetic valves.

Cross-modal hint paradigms

In the odor hint paradigm, the odor of 667-fold diluted 3-octanol (OCT) in mineral oil was delivered to the head of the fly in the front (approximately 5 mm) for 800 ms at 0.3 probability only when the flight orientation shifted to the frontal 30° ($\psi = \pm 15°$) sector of the fly's visual field. A visual stimulus at a contrast-defined noise level of 0.7 was selected because this object does not appear fuzzy. In the visual hint paradigm, an attractive odor of 1000-fold diluted ethanol (ETH) in mineral oil was delivered to the head of the fly when flight was orientated to the frontal 60° ($\psi = \pm 30°$) sector of the fly's visual field. In the remaining sectors, an aversive odor of 111-fold diluted OCT in mineral oil was delivered to the head of test fly in the dark. A stripe at a noise level of 0.7 was used as visual hint, which was flashed for 500 ms at 0.5 probability when the fly was orientated to the ETH sector. The flash was controlled by a computer through an array of LEDs (4 × 24 white light 5 mm LEDs symmetrically placed in the arena) surrounding the panorama (100 mm diameter). A noise level stimulus of 1.0 was used as the control.

Data analysis

The fixation performance of individual flies was calculated as an unsigned mean error distance (MED), which is the average error angle between the direction of the visual object and that of the fly's flight (Poggio & Reichardt, 1976; Ye *et al.*, 2004). The higher the value, the worse is the fixation performance. To quantify the performance of visual selection between two objects, we used the selective index (SI), $\mathrm{SI} = (t_A - t_B)/(t_A + t_B)$, where t_A represents the time that the fly headed towards the 40° ($\psi = \pm 20°$) sector of the salient stimulus, while t_B denotes the time that is headed towards the 40° sector of the weaker stimulus. The value of the MED during single stripe fixation was approximately 20°; therefore, we regarded the flight orientation in the ±20° sector surrounding the stimulus as valid fixation performance. In the three-object selection paradigm, $\mathrm{SI} = (2t_A - t_B - t_C)/(2t_A + t_B + t_C)$, where t_A represents the time that the fly headed towards the ±20° sector of the most salient stimulus, and t_B and t_C denote the time heading towards the ±20° sectors of the other two stimuli. The time of t_A is doubled to balance the different sectors so that SI = 0 corresponds to random selection and SI = 1 corresponds to full selection of the most salient object. Student's *t*-test was used for the MED test in the single object fixation paradigm ($^*P < 0.05$; $^{**}P < 0.01$; two-tailed *P*-value). Error bars in figures are SEMs. As the SI data in the multiobjects fixation paradigm were not normally distributed, the Kolmogorov–Smirnov *Z*-test was performed using SPSS 11.5 software ($^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; two-tailed *P*-value).

Results

Visual fixation performance towards luminance-contrasted objects

We first examined the visual fixation behavior using tethered flies in a flight simulator (Fig. 1A). The visual pattern was a single vertical black stripe at an angular position of $\psi = 0°$ (ψ denotes the horizontal angular position with respect to the frontal midline of the test fly) in the panorama of the flight simulator with different levels of visual background noise. The Gaussian forms of the flight distribution indicated stable fixation performance during a 6-min procedure (Fig. 1B). We used the MED (see Materials and methods) between the direction of the visual object and the fly's direction of flight as a quantitative index of fixation performance (Poggio & Reichardt, 1976; Ye *et al.*, 2004). Thus, the MED value increases as fixation performance worsens (Fig. 1C). Next, we examined the effects of altering luminance contrast, a parameter of visual stimulus that is widely used to study attention in primates (Desimone & Duncan, 1995; Reynolds & Chelazzi, 2004). We found that the distribution of MED depended on object salience, i.e. the histograms sharpened as the object contrast increased (Fig. 2A).

We used several approaches to impair *Drosophila*'s MBs in order to assess the roles of MBs on salience-based fixation behavior. Mutant *mbm*^{1} flies are female genetic mutants with miniature MBs (de Belle & Heisenberg, 1996), and UAS-*shi*^{ts1}/247 flies express a thermo labile *Dynamin* protein (Koenig *et al.*, 1983; Chen *et al.*, 1991; Kitamoto, 2001) in MBs, which can conditionally block synaptic transmission at the restrictive temperature (RT, 30 °C) and perform normally at the permissive temperature (PT, 25 °C). Finally, HU flies were obtained by applying HU to kill MB neuroblasts during the early first larval instar (de Belle & Heisenberg, 1994).

We observed that both WTB and MB-deficient flies exhibited similar fixation performance at luminance contrast levels ranging from 1.0 to 0.5, indicating that MB-deficient flies have normal flight control and basic visual pattern discrimination (Fig. 2B–D). At a very low contrast level (0.03), all flies demonstrated poor fixation performance that was close to random choice. However,

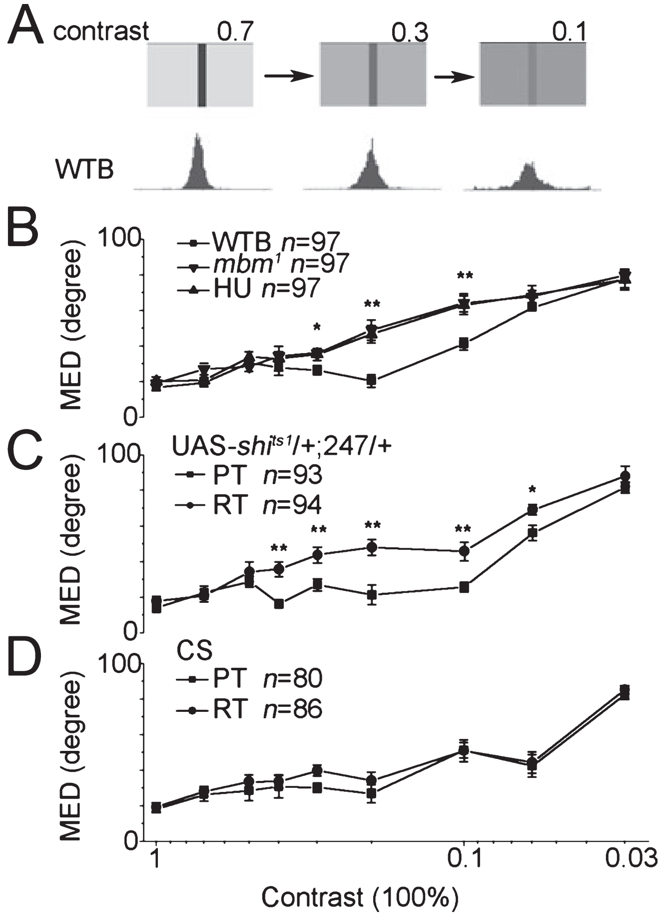

FIG. 2. MBs improve contrast discrimination. (A) The luminance contrast of the visual stimuli and histograms of time spent in various flight directions of an individual wild-type Berlin (WTB) fly are illustrated. (B) The fixation performance at several contrast levels in MB-deficient [*mbm*^{1} and hydroxyurea (HU)] flies was significantly reduced, as compared with WTB flies. (C and D) The performance of UAS-*shi*^{ts1}/247 flies exhibited a significant difference between permissive temperature (PT; 25 °C) and restrictive temperature (RT; 30 °C) at several contrast levels; however, CantonS (CS) flies demonstrated similar fixation performance at both temperatures. Approximately 10 flies of each mutant line were used for every visual stimulus; *n* indicates the total number of flies in all the tests. MED, mean error distance. $*P < 0.05$; $**P < 0.01$; Student's *t*-test.

WTB flies demonstrated better fixation performance than MB-deficient flies (HU and *mbm*^{1}) in response to low-contrast objects (Fig. 2B; contrast levels 0.3, 0.2 and 0.1). Moreover, the fixation ability of UAS-*shi*^{ts1}/247 flies was significantly worse at RT than that at PT at contrast levels between 0.4 and 0.06 (Fig. 2C). On the contrary, wild-type CS flies exhibited similar fixation performance at both temperatures, indicating that temperature shift did not affect fixation behavior (Fig. 2D). Performance of the UAS-*shi*^{ts1}/247 flies at RT was similar to that of the HU and *mbm*^{1} flies, with slight exceptions that may be due to subtle modulation of the different environmental temperatures and genetic backgrounds. Together, these results indicate that MBs facilitate contrast discrimination by causing a rightward shift in the fixation response curve (Fig. 2). Further analysis of the flight histogram at the 0.2 contrast level demonstrates sharper tuning curves in WTB flies than those in MB-deficient flies (Supplementary material, Fig. S1), indicating that MBs may modulate the salience of an object.

Object fixation in visual background noise

In primates, attention not only improves contrast discrimination but also enhances the salience of stimuli and suppresses accompanying distracters (Everling *et al.*, 2002; Reynolds & Chelazzi, 2004). To further explore the roles of *Drosophila*'s MBs in fixation behavior, we used two types of visual noises. The first type was contrast-defined noise (Fig. 3A). We found that both WTB and MB-deficient flies showed similar fixation performance for objects at noise levels from 0 to 0.2 (Fig. 3B–D). However, WTB flies showed better fixation ability than MB-deficient flies at noise levels of 0.6 and 0.7 (Fig. 3B). The *mbm*^{1} flies performed worse than WTB flies at the 0.8 noise level. In addition, the UAS-*shi*^{ts1}/247 flies showed worse fixation performance at noise levels from 0.3 to 0.7 at RT than that at PT (Fig. 3C). The temperature shift from PT to RT had almost no effect on object fixation in CS flies except for a slight but significant difference at the 0.5 noise level (Fig. 3D). Our results also demonstrated that the transgenic heterozygous control lines (UAS-*shi*^{ts1}/+ and 247/+ flies) showed normal fixation performance at RT (supplementary Fig. S2). Tight stripe fixation is highly dependent upon intact optomotor reflex-circuits (Heisenberg & Wolf, 1984). In order to exclude the alternative explanation for the findings regarding the optomotor response in fixation behavior, we introduced an optomotor response paradigm (see supplementary Appendix S1). Our results indicated that the MB-deficient flies' optomotor response to the contrast noise 0.7 pattern was unaffected as compared with that of the wild-type flies (supplementary Fig. S3).

The second type of visual noise was spatial noise (Fig. 3E), which differs from contrast noise. The fixation ability of both *mbm*^{1} and HU flies was significantly worse than that of WTB flies at spatial noise levels of 0.3, 0.4 and 0.5 (Fig. 3F). The UAS-*shi*^{ts1}/247 flies also exhibited poor performance at noise levels of 0.5 and 0.6 (Fig. 3G and H). Thus, compared with the fixation response curves of WTB flies, those of MB-deficient flies were shifted to the left in both types of noise backgrounds, indicating that MBs may enhance object salience by inhibiting background noise.

For contrast noise, the contrast of the noise dots was changed; whereas for spatial noise, the intensity of the noise dots was changed. However, this leads us to question whether flies could visually discriminate the difference between noise background (approximate 2.5° for noise dots) and the contrast noise background. To address this issue, we set a visual stripe at the pixel resolution of 2.5°. Visual resolution of compound eyes in *Drosophila* is not only determined by interommatidial angles ($\Delta\Phi = 5°$), optical quality and rhabdom dimensions, but it is also affected by light levels and speed of movement (Goetz, 1964; Land, 1997). We discovered that the fly could track the 2.5° stripe in white or contrast noise backgrounds (supplementary Fig. S4). The results suggested that *Drosophila* can perceive moving objects with a vertical pixel resolution less than 2.5° in the flight simulator. Moreover, the random dots used can be composed into larger noise dots of greater than 5°. Therefore, our findings indicated that the noise background is considerably distinct from the uniformed contrast background to the flies.

Visual fixation in the presence of an olfactory 'distracter'

In humans, attention to a single sensory modality can impair the perception of information from other modalities (Strayer *et al.*, 2003). To test whether this also applies to flies, we added a second sensory modality, an odor 'distracter', to the visual object fixation task. Because MBs are not necessary for basic odor discrimination and perception in *Drosophila* (de Belle & Heisenberg, 1994), we used

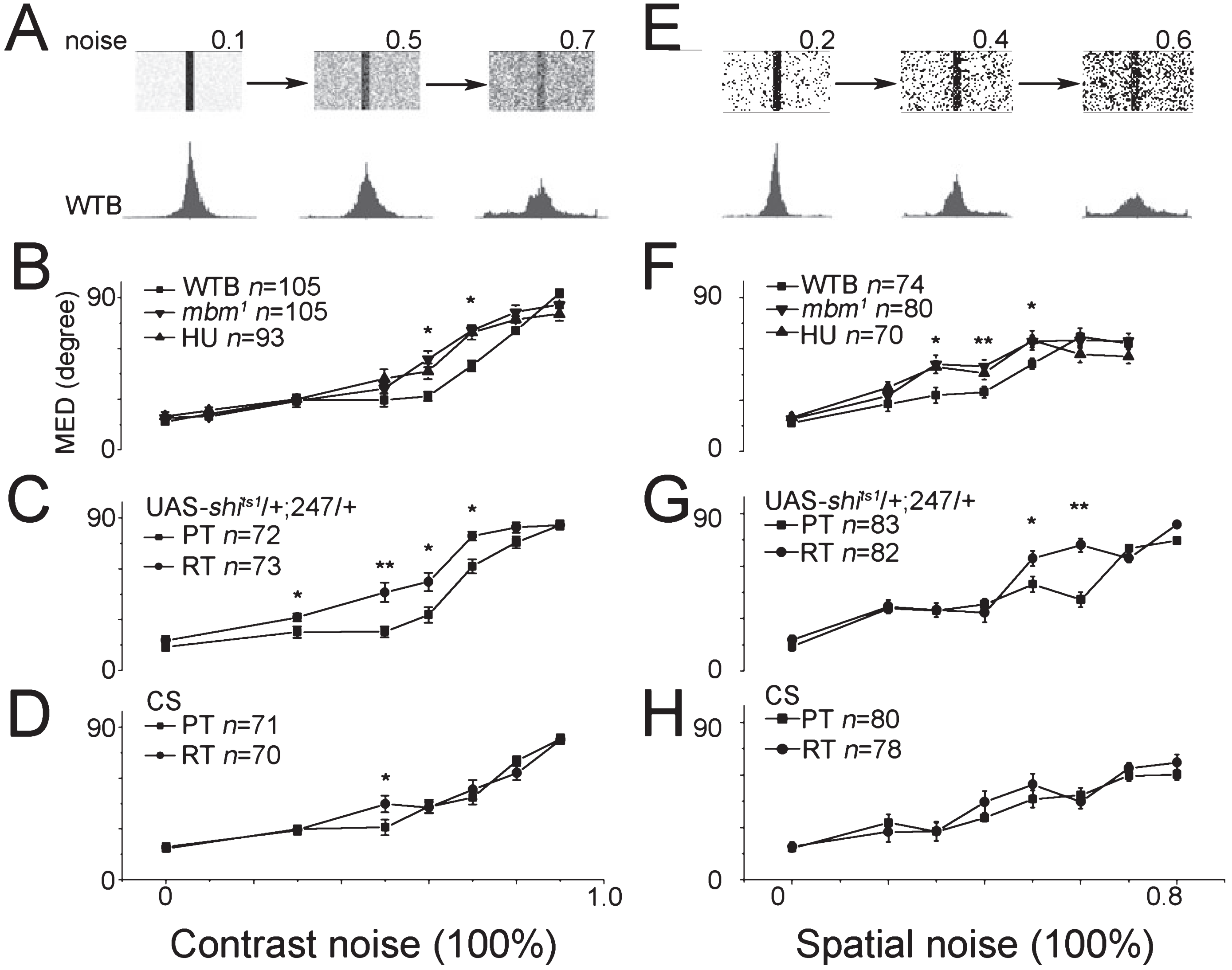

FIG. 3. MBs enhance the salience of objects against a visual background noise. (A) Illustration of visual stimuli with contrast-defined noise and histograms of time spent in various flight directions of an individual wild-type Berlin (WTB) fly. (B) Both mbm^1 and hydroxyurea (HU) flies exhibited poor fixation ability at the 0.6 noise level, but only mbm^1 flies exhibited poor performance at the 0.8 level. (C and D) UAS-shi^{ts1}/247 flies showed significantly different performances at permissive temperature (PT; 25 °C) and restrictive temperature (RT; 30 °C) at several contrast levels, and CantonS (CS) flies also exhibited a significant difference at the 0.5 contrast level. (E) Illustration of visual stimuli with spatial noise and histograms of the flight directions of an individual WTB fly. (F) The fixation performance in MB-deficient flies was worse than that in WTB flies at several noise levels. (G and H) In contrast to CS flies, UAS-shi^{ts1}/247 flies showed a significant difference at PT and RT at several higher noise levels. MED, mean error distance. *$P < 0.05$; **$P < 0.01$.

MB-deficient flies for the olfactory 'distracter' task. To exclude the possible mechanical effect of airflow, we applied odor-free airflow to the flies during the first 3 min of fixation as the control. During the last 3 min, the odor of 1% MCH was randomly delivered at a probability of 0.3. A luminance contrast of 0.5 was selected as the test point because no significant difference was observed amongst all the flies tested at this level (Fig. 2B–D). We found that both mbm^1 and HU flies showed worse fixation performance during the last 3 min with the odor 'distracter' as compared with the first 3 min with airflow alone (Fig. 4A and B). However, the odor 'distracter' did not influence fixation performance in WTB flies (Fig. 4A and B). The Gaussian distributions of the flight histogram demonstrated that the tuning curves in MB-deficient flies were suppressed during the last 3 min due to the odor interference, while the Gaussian distributions in WTB flies remained sharp (Fig. 4A). To exclude the possible effect of flight time on object fixation performance, we compared the performance during the first and the last 3 min in the odor-free airflow paradigm. WTB and MB-deficient flies showed no significant difference in fixation performance (Fig. 4C and D). In addition, the UAS-shi^{ts1}/247 flies showed significantly worse fixation performance in the presence of the odor 'distracter' in the last 3 min than that in the first 3 min at RT. Wild-type CS flies showed similar fixation performance during the first and last 3 min. At PT, both CS and UAS-shi^{ts1}/247 flies demonstrated normal fixation performance. Together with the results of the visual background noise experiments, these data indicate that not only do MBs suppress visual background noise, but they also inhibit the 'distracting' olfactory stimuli in fixation behavior.

Visual selection among multiple objects of differing contrast

The most important aspect of selective attention in mammals is the ability to select dominant objects of behavioral relevance from

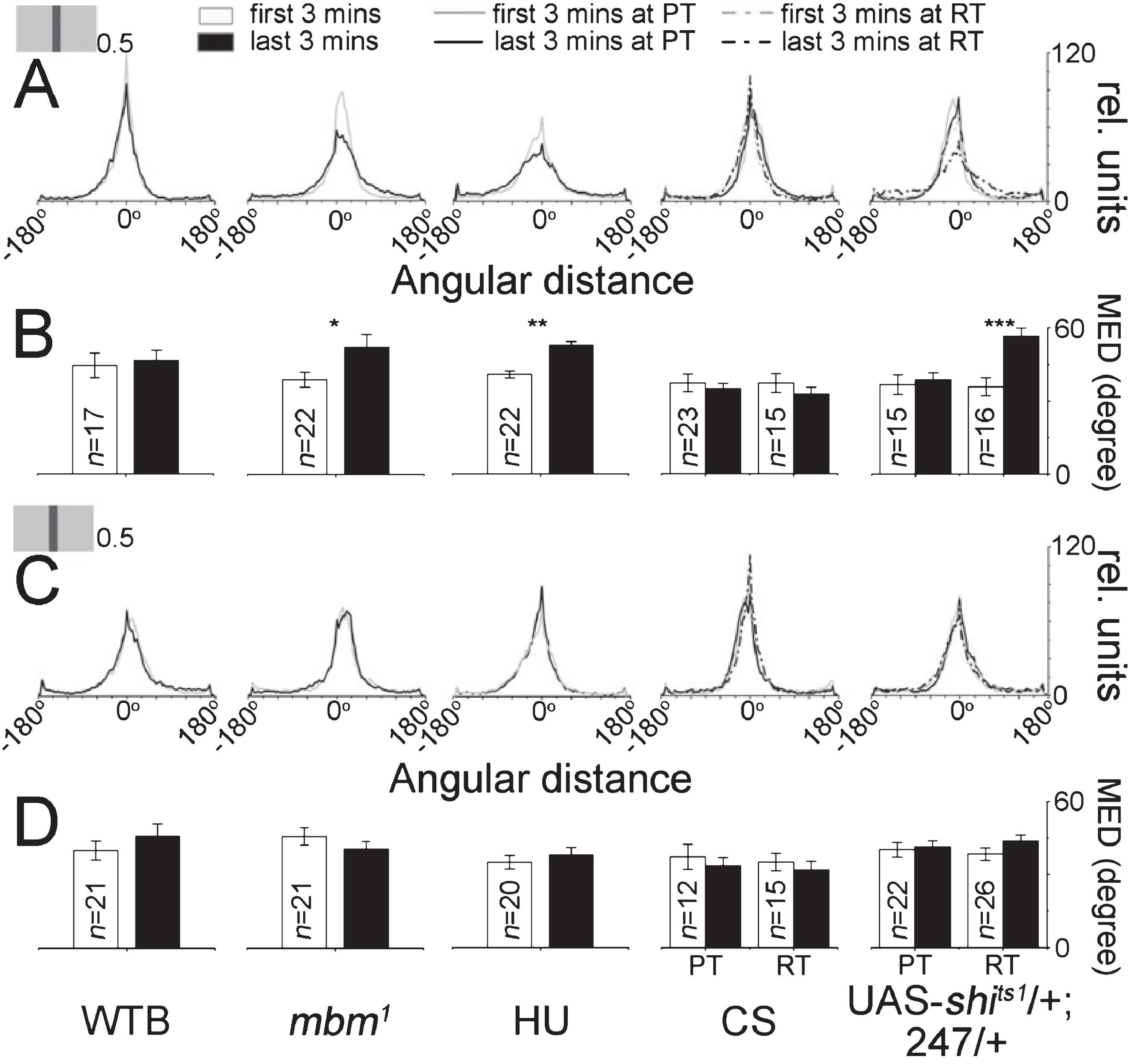

FIG. 4. MBs inhibit olfactory ‘distracters’ in visual fixation behavior. (A and B) MB-deficient flies demonstrated worse fixation performance with an odor distracter (1% MCH) in the last 3 min than that in the first 3 min with airflow alone. The odor distracter had no significant effect on fixation performance in wild-type Berlin (WTB) flies. Further, the UAS-shi^{ts1}/247 flies showed worse fixation performance with the odor ‘distracter’ than with airflow alone at restrictive temperature (RT). The odor ‘distracter’ had no significant effect on fixation performance in CantonS (CS) flies at permissive temperature (PT) and RT, and the UAS-shi^{ts1}/247 flies at PT. (A) Histograms of time spent in flight directions of all the flies. (B) Statistical results of the mean error distance (MED) values. (C and D) All flies exhibited similar fixation performance in the first 3 min and the last 3 min of airflow alone. HU, hydroxyurea. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

multiple competing objects. We wondered whether the fixation performance facing multiple objects would demonstrate some selective properties in flies. To assess this, we used two luminance-contrasted objects positioned at $\psi = \pm 60°$ in a circular panorama as a competing stimuli (Fig. 5A). The SI was used to estimate the selective fixation abilities of the flies (see Materials and methods). For the {0.9, 1.0} contrast set, the histograms of time spent in various flight directions exhibited more partial preference for the weak stimulus in MB-deficient flies than in WTB flies, in addition to the dominant preference for the more salient stimulus (Fig. 5A). However, statistical analysis demonstrated that the SI of WTB flies was not significantly different from those of MB-deficient flies (Fig. 5D). The UAS-shi^{ts1}/247 flies also showed similar results between PT and RT, compared with the CS flies (Fig. 5A and D). These results indicated that both MB-deficient and UAS-shi^{ts1}/247 flies at RT exhibited normal selective fixation to the salient object of contrast 1.0 in the two-object selection paradigm.

To further investigate the MB function in more complex visual selection, we used three symmetrically distributed luminance-contrasted objects positioned at $\psi = 0°$ and $\pm 120°$ in the circular panorama (Fig. 5B and C). We observed that both wild-type flies and

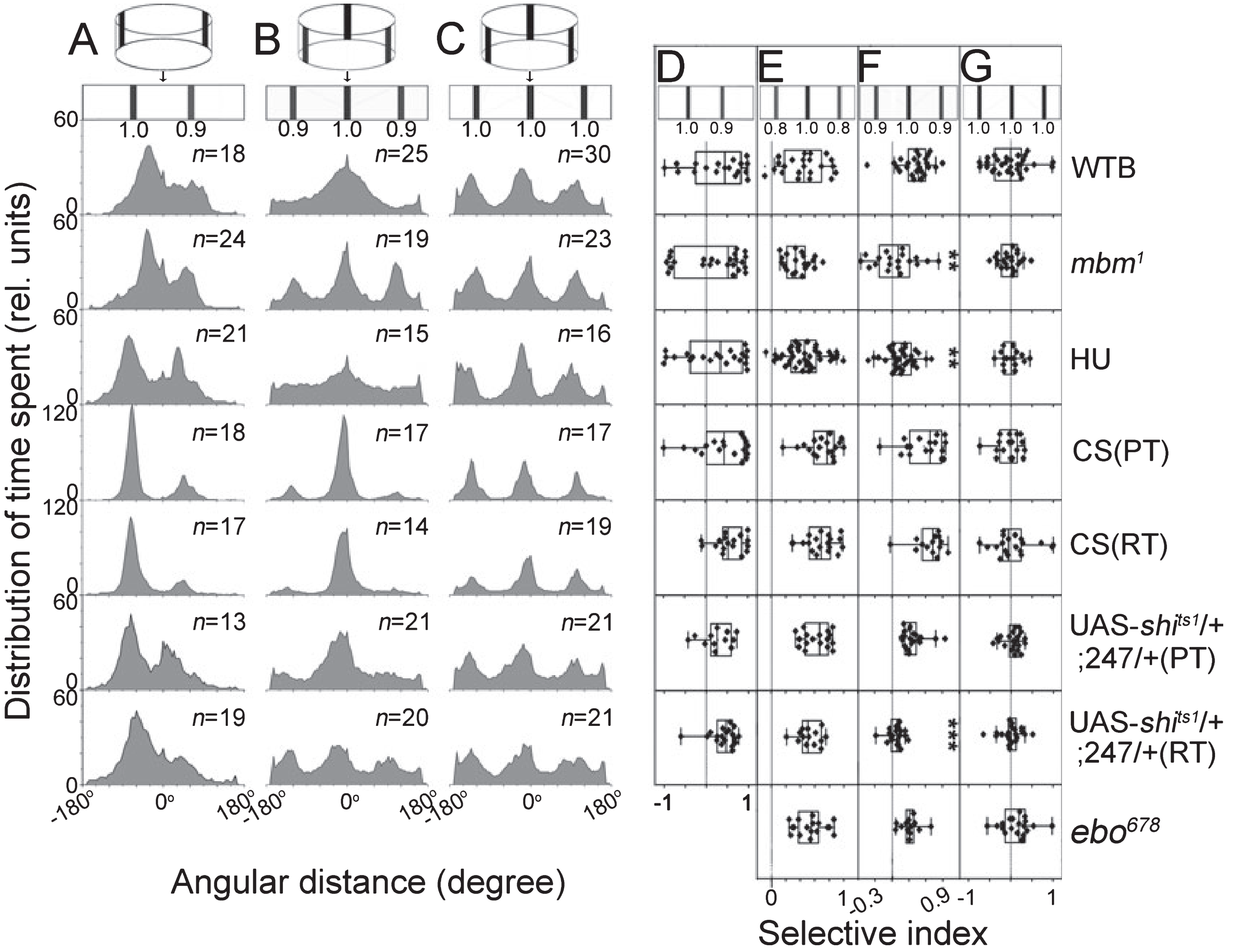

FIG. 5. MBs facilitate visual selection among three competing objects. (A) Histogram of the flight directions of all the flies during visual selection between two objects of contrast 0.9 and 1.0. Both wild-type Berlin (WTB) flies and MB-deficient flies showed dominant preference for the stronger stimulus, but the MB-deficient flies exhibited a slightly greater preference for the weaker stimulus as compared with the WTB flies. (B) Histogram of the time spent in flight directions during visual selection among three objects of the contrast set {0.9, 1.0, 0.9}. Wild-type flies showed one dominant peak of time spent fixating on the strongest stimulus, whereas MB-deficient and UAS-shi^{ts1}/247 flies at restrictive temperature (RT) showed three similar peaks corresponding to the three objects. (C) Histogram of the time spent in flight directions during selection among three objects of 1.0 contrast. Both WTB and MB-deficient flies showed no preference among the three identical objects. Canton S (CS) and UAS-shi^{ts1}/247 flies showed no preference between the objects at permissive temperature (PT) and RT. (D) There were no significant differences in SI among wild-type flies and MB-deficient flies for the {0.9, 1.0} contrast sets. (E) For the contrast sets {0.8, 1.0, 0.8}, all flies showed significant preference for the strongest stimulus, with no statistical difference in SIs among flies. (F) For the {0.9, 1.0, 0.9} contrast set, WTB flies showed significantly higher SI than MB-deficient flies. The UAS-shi^{ts1}/247 showed significantly higher SI at PT than that at RT. (G) When selecting among three identical objects, none of the flies showed significant selection and the SIs were close to zero. The ebo^{678} flies showed normal selective fixation performance, as compared with the WTB flies. Dashed lines indicate the zero position. HU, hydroxyurea. **$P < 0.01$; ***$P < 0.001$; Kolmogorov–Smirnov Z-test.

MB-deficient flies showed significant preference for the most salient object (contrast 1.0) in the contrast set {0.8, 1.0, 0.8} (Fig. 5E). The UAS-shi^{ts1}/247 and CS flies also showed significant preference for the salient object (contrast 1.0) in this contrast set both at PT and RT (Fig. 5E). However, WTB flies spent more time fixating to the most salient object as compared with MB-deficient flies for the {0.9, 1.0, 0.9} contrast set (Fig. 5B). The SI of WTB flies was significantly higher than that of MB-deficient flies (Fig. 5F). The histogram of the time spent in flight directions of HU flies resembles that of the WTB flies, probably because the MB of some HU flies were not completely ablated (Armstrong *et al.*, 1998). The UAS-shi^{ts1}/247 flies also spent more time fixating to the salient object at PT than that at RT. Similarly, the SI of the UAS-shi^{ts1}/247 flies at PT was significantly higher than that at RT (Fig. 5B and F). To exclude the possible side-effects of data average in the population flies, we also examined the selection behavior in the individual flies. We found in most cases, a single fly spent certain times on switching the tracked objects in the contrast set {0.9, 1.0, 0.9} during the 6-min flight (supplementary Fig. S5). The averaged fixation data over the total 6 min were consistent with the averaged data over population flies. For the {1.0, 1.0, 1.0} contrast set, all the flies showed almost similar visual selections (Fig. 5C and G). These data indicated that MBs only play a role in more complex paradigms with the slight contrast difference among three objects. Our results imply that MBs play a sensory gating function that allows only salient inputs to pass through, whereas weaker inputs are suppressed. To determine whether this salience-based visual selection is specifically modulated by MBs rather than by global brain regions, we tested ebo^{678} mutant flies (Strauss & Heisenberg, 1993), in which the ellipsoid body of the central complex is damaged. We found that *ebo*^{678} mutant flies showed visual selection similar to that of WTB flies in selective fixation behavior (Fig. 5). This result indicated that salience-based visual selection is a brain area-specific behavior and MBs could be the major contributor to this behavior.

Our previous visual selection data involving two and three competing stimuli demonstrated that MBs only functioned in the three-object paradigm. The results in the two-object selection paradigm can be treated as a control to show that MB-deficient flies can discriminate the contrast difference between 0.9 and 1.0 in white background and track the salient object. However, the competition among three objects may be more complex and distractive than that between two objects, which may require higher-level cognitive functions. Therefore, our data suggest that during selective fixation behavior, MBs may be only involved in the processing of higher-order complexities.

Olfactory and visual cross-modal synergistic integration in fixation behavior

Attention can improve the perception of information from multiple sensory modalities in mammals when stimuli are semantically congruent and occur synchronously (McDonald *et al.*, 2000; Gottfried & Dolan, 2003). A recent study demonstrated that the acquisition and subsequent retrieval of memory in fruit flies are strongly influenced by inputs from multiple sensory modalities (Guo & Guo, 2005). To investigate whether the fly exhibits cross-modal synergistic integration during fixation behavior, we designed two cross-modal hint paradigms in the flight simulator. The first was an odor hint paradigm for visual object fixation at a contrast-defined noise level of 0.7 (Fig. 6A, left panel). At this noise level, the object was not fuzzy and could be tracked by the flies. A neutral odor of 0.15% OCT was administered to the front of the fly at a probability of 0.3 whenever the visual object appeared in the frontal 30° sector of its

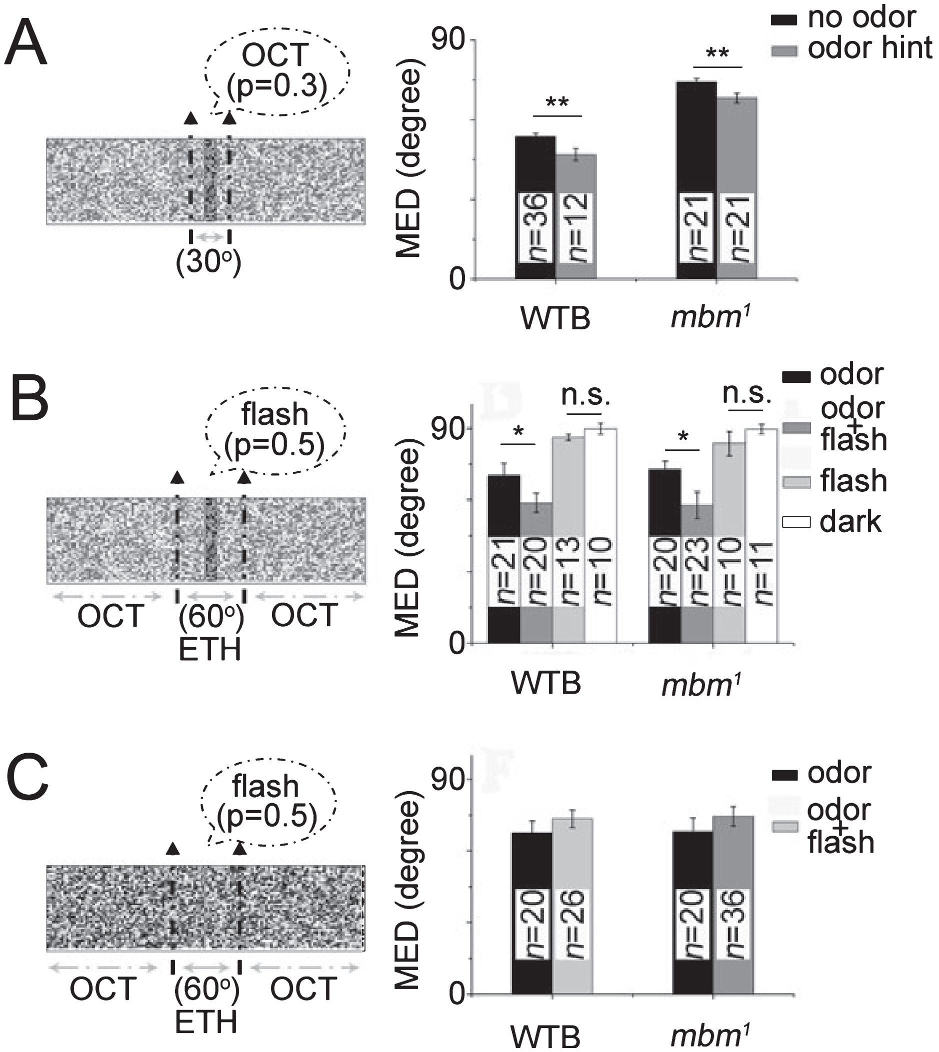

Fig. 6. MBs are not required for cross-modal synergistic integration. (A) (Left) In the visual fixation paradigm, a stripe was used and an odor hint [0.15% 3-octanol (OCT)] was delivered when the fly orientated to the frontal 30° sector of its visual field. (Right) Fixation performance in both wild-type Berlin (WTB) and *mbm*^{1} flies was significantly improved by the odor hint. (B) (Left) In the odor-fixation paradigm, an attractive odor [0.1% ethanol (ETH)] was delivered in the dark when the fly orientated to the frontal 60° sector. In the remaining sectors, an aversive odor (0.9% OCT) was delivered. A stripe was flashed as a hint when the fly orientated to the ETH sector. (Right) The fixation performance in both WTB and *mbm*^{1} flies was significantly improved by the visual hint. A visual flash without odor cues induced fixation performance similar to random flight in the dark. (C) (Left) A visual hint without a salient stripe during odor fixation. (Right) Without the salient stripe, the flash of random dots has no effect on odor-fixation performance in WTB and *mbm*^{1} flies. $*P < 0.05$; $**P < 0.01$.

visual field. This probability level was chosen because it would not trigger odor adaptation and memory formation (Guo & Guo, 2005). With the odor hint, both WTB and MB-deficient flies demonstrated a significant improvement in visual fixation performance (Fig. 6A, right panel). The results indicate that the olfactory signal synchronized with the visual object can synergistically improve visual object fixation.

The second paradigm used a visual hint (a stripe at a contrast-defined noise level of 0.7) for odor fixation (Fig. 6B, left panel). In the dark, an attractive odor of 0.1% ETH was delivered to the fly whenever the flight direction was orientated to the frontal 60° sector of its visual field, and an aversive odor of 0.9% OCT was delivered to the other sectors. Compared with performance in a pure odor-fixation paradigm, both WTB and MB-deficient flies demonstrated significantly improved fixation performance to ETH odor when the visual hint was synchronously flashed at 50% probability (Fig. 6B, right panel). However, the visual hint alone without odor cues was not sufficient to induce stable fixation performance, as indicated by a MED value similar to that in the dark without any visual and olfactory cues (Fig. 6B, right panel). Furthermore, a visual flash of random dots alone without the stripe also had no effect on odor fixation in both WTB and *mbm*^{1} flies (Fig. 6C). These data indicate that a salient visual signal synchronized with an olfactory signal can enhance odor-fixation ability in *Drosophila*.

Taken together, the results of both the visual and olfactory hint paradigms indicate that the flies are capable of cross-modal synergistic integration in fixation behavior. Our results suggest that the MBs suppress olfactory distracters in visual object fixation behavior, but do not contribute to cross-modal integration between visual and olfactory perception.

Discussion

The basic function of the MBs has been proposed to be inhibition, which is supported by MB lesion studies examining the locomotor activity and singing behavior in crickets (Huber, 1963, 1965; Otto, 1971) and grasshoppers (Wahdepuhl, 1983), and by studies of locomotor activity in MB mutant flies (Martin *et al.*, 1998). In addition, recent data indicate that MBs promote sleep in *Drosophila* (Joiner *et al.*, 2006; Pitman *et al.*, 2006), a state that is correlated with global changes in the brain activity in adult flies (Nitz *et al.*, 2002; van Swinderen *et al.*, 2004). This result suggests that MBs may be central to the suppression of locomotor activity at night. Our results demonstrate that *Drosophila* MBs not only suppress background noise and distracters, but also improve visual selection among multiple objects by inhibiting unrelated signals. The modulatory effects of MBs in fixation towards single and multiple objects provide strong evidences that MBs play a gating role in *Drosophila* fixation behavior.

In other cognitive behaviors, an increasing number of divergent phenomena have been accumulated to support the idea that MB provides an inhibition gating mechanism in flies at functional level. In visual context generalization, MB may inhibit the changing of the context (Liu *et al.*, 1999); in experience-dependent feature extraction, MB may inhibit the unconditioned features (Peng *et al.*, 2007); and in decision-making, dopamine-MBs circuit may inhibit the less salient cues and dramatically enhance the more salient cues to get sharp and complete transit (Tang & Guo, 2001; Zhang *et al.*, 2007). Thus, the universal functions of MBs could be interpreted to serve as a filter to inhibit background or unrelated information. Why are MBs not required to gate an olfactory cue during visual fixation as shown in Fig. 6? Briefly, the brain centers for inhibitive and synergistic interaction between two cues may be different. Together, these findings imply that MBs generally play a higher inhibitory function, which can suppress locomotor activity, weight the input signals, and bias the output to filter out the background noise and unrelated signals in the late information process in fly's brain. Quite recently, a result suggested that an inhibitory system on MBs may be a noise filter for olfactory learning (Liu *et al.*, 2007).

How is the gain gating function implemented in MBs at the circuit level? Although it is extremely difficult to conduct electrophysiological recordings in the fly brain, some electrophysiological studies in insects have helped us understand how MB circuits may work in salience-based fixation behavior. In locusts, a LFP of 20–30 Hz oscillation was induced in the MB calyx by an odor puff to the antenna (Laurent & Davidowitz, 1994; Laurent & Naraghi, 1994; Laurent, 1996). Recent reports reveal that the behavioral response to salient stimuli correlates with a physiological signature of 20–30 Hz activity in the medial protocerebrum of fruit flies (van Swinderen & Greenspan, 2003). In addition, the outputs of a subset of MB neurons are required for both fixation behavior and for the 20–30 Hz oscillations (van Swinderen & Greenspan, 2003). This oscillation, which correlates with the salient stimulus in insects, resembles the transient gamma (30–80 Hz) activity in human electroencephalograms (EEGs), which is also modulated by salience (Zeman, 2001). Furthermore, LFP recordings in the brains of flies corresponding to visual novelty and optomotor response in the fixation behavior revealed that attention-like processes were also modulated by genes involved in short-term memory formation, namely, *dunce* and *rutabaga* (van Swinderen, 2007). A previous report demonstrates that both these short-term memory genes are preferentially expressed in MBs (McGuire *et al.*, 2005). Therefore, our findings are in agreement with these previous studies that indicate that MB circuits are tuned to oscillation activity that might be triggered by olfactory or visual salience.

A direct anatomical link between the MBs and the visual system in *Drosophila* has not yet been reported. In addition, previous works demonstrate that *Drosophila* MBs are not required for visual, tactile and motor learning (Wolf *et al.*, 1998). However, our findings that MBs modulate salience in selective fixation behavior offer new perspectives into the roles of *Drosophila* MBs in visual perception. In other insects, such as crickets (Schildberger, 1984), cockroaches (Li & Strausfeld, 1997) and honeybees (Ehmer & Gronenberg, 2002), the MBs integrate many sensory modalities, including olfactory, visual, tactile and acoustic input. Further evidence states that various sensory modalities reach the lobes and calyces of MBs indirectly through protocerebral neuropils in cockroaches (Li & Strausfeld, 1997; Strausfeld *et al.*, 1998). Therefore, we believe that *Drosophila* MBs also receive visual inputs from the visual system, probably through an indirect neural projection.

Selective fixation behavior in flies shares some similarities with human attention, such as orienting, expectancy, stimulus differentiation and sustainability (van Swinderen & Greenspan, 2003; Greenspan & van Swinderen, 2004; Swinderen, 2005). Several models have been proposed to explain the mechanism of attention modulation in primates. A contrast gain model postulates that attention causes a multiplicative increase in the effective contrast of a stimulus by increasing neuronal contrast sensitivity for the attended stimulus, which results in a leftward shift in the contrast–response function (Reynolds *et al.*, 1999; Martinez-Trujillo & Treue, 2002; Reynolds & Chelazzi, 2004). Our current data reveal that MBs caused a similar rightward shift of the contrast–response function in visual object fixation by enhancing the salience of the relevant object (Figs 2 and 3, and supplementary Fig. S1). Consistent with the previous cognitive work on MBs, it appears that MBs facilitate the context generalization for memory retrieval after visual learning (Liu *et al.*, 1999). This facilitation of object discrimination from the background is in accordance with the promotion of fixation in complex condition in our results, which is MB dependent. Subsequently, this modulation may decrease the contrast threshold and increase sensitivity in object discrimination, which is consistent with the contrast gain model established in the contrast response function of attention in the V4 and MT neurons in monkeys (Reynolds *et al.*, 1999, 2000; Martinez-Trujillo & Treue, 2002; Reynolds & Chelazzi, 2004). Together, this suggests that a similar neural mechanism underlies the modulation of salience-based fixation behavior in *Drosophila*. Our findings not only provide important insights into the inhibitory function of MBs, but also provide a basis for further studies on the mechanisms underlying selective fixation behavior in *Drosophila*.

Supplementary material

The following supplementary material may be found on http://www.blackwell-synergy.com

Fig. S1. MBs modulate the fixation response gain.

Fig. S2. Fixation performance of the transgenic heterozygote strains.

Fig. S3. The optomotor response to the moving stimulus of contrast noise 0.7.

Fig. S4. Fixation performance to the 2.5° stripe in a white background and a contrast noise 0.5.

Fig. S5. Fixation selection among three objects of the contrast set {0.9, 1.0, 0.9} in individual fly.

Appendix S1. Optometer response test.

Acknowledgements

This research was supported by grants from the National Science Foundation of China (Grant 30270341, 30630028, 30621004 and 30770511), the Multidisciplinary Research Program (Brain and Mind) of Chinese Academy of Sciences, the National Basic Research Program of China '973' Projects (G2000077800, 2006CB806600 and 2006CB911003), and the Knowledge Innovation Engineering Project of Chinese Academy of Sciences (Grant No. KJCX1-09-03 and KSCX2-YW-R-28). We thank Dr Woo N. (NIH, USA) for critical reading of the manuscript.

Abbreviations

CS, CantonS; ETH, ethanol; HU, hydroxyurea; LFP, local field potential; MBs, mushroom bodies; MCH, 4-methylcyclohexanol; MED, mean error distance; OCT, 3-octanol; PT, permissive temperature; RT, restrictive temperature; SI, selective index; WTB, wild-type Berlin.

References

Armstrong, J.D., de Belle, J.S., Wang, Z. & Kaiser, K. (1998) Metamorphosis of the mushroom bodies; large-scale rearrangements of the neural substrates for associative learning and memory in Drosophila. *Learn. Mem*, **5**, 102–114.

de Belle, J.S. & Heisenberg, M. (1994) Associative odor learning in Drosophila abolished by chemical ablation of mushroom bodies. *Science*, **263**, 692–695.

de Belle, J.S. & Heisenberg, M. (1996) Expression of Drosophila mushroom body mutations in alternative genetic backgrounds: a case study of the mushroom body miniature gene (mbm). *Proc. Natl Acad. Sci. USA*, **93**, 9875–9880.

Chen, M.S., Obar, R.A., Schroeder, C.C., Austin, T.W., Poodry, C.A., Wadsworth, S.C. & Vallee, R.B. (1991) Multiple forms of dynamin are encoded by shibire, a Drosophila gene involved in endocytosis. *Nature*, **351**, 583–586.

Davis, R.L. (1993) Mushroom bodies and Drosophila learning. *Neuron*, **11**, 1–14.

Desimone, R. & Duncan, J. (1995) Neural mechanisms of selective visual attention. *Annu. Rev. Neurosci.*, **18**, 193–222.

Ehmer, B. & Gronenberg, W. (2002) Segregation of visual input to the mushroom bodies in the honeybee (Apis mellifera). *J. Comp. Neurol.*, **451**, 362–373.

Everling, S., Tinsley, C.J., Gaffan, D. & Duncan, J. (2002) Filtering of neural signals by focused attention in the monkey prefrontal cortex. *Nat. Neurosci.*, **5**, 671–676.

Goetz, K.G. (1964) Optomotorische Untersuchung des visuellen Systems einiger Augenmutanten der Fruchtfliege Drosophila. *Kybernetik*, **2**, 77–92.

Goetz, K.G. (1994) Exploratory strategies in *Drosophila*. In Schildberger, K. & Elsner, N. (Eds), *Neural Basis of Behavioral Adaptations. International Symposium.* Tuzing, Germany, pp. 47–59.

Gottfried, J.A. & Dolan, R.J. (2003) The nose smells what the eye sees: crossmodal visual facilitation of human olfactory perception. *Neuron*, **39**, 375–386.

Greenspan, R.J. & van Swinderen, B. (2004) Cognitive consonance: complex brain functions in the fruit fly and its relatives. *Trends Neurosci.*, **27**, 707–711.

Guo, J. & Guo, A. (2005) Crossmodal interactions between olfactory and visual learning in Drosophila. *Science*, **309**, 307–310.

Guo, A., Li, L., Xia, S.Z., Feng, C.H., Wolf, R. & Heisenberg, M. (1996) Conditioned visual flight orientation in Drosophila: dependence on age, practice, and diet. *Learn. Mem.*, **3**, 49–59.

Heisenberg, M. (1998) What do the mushroom bodies for the insect brain? *Learn. Mem.*, **5**, 1–10.

Heisenberg, M. (2003) Mushroom body memoir: from maps to models. *Nat. Rev. Neurosci.*, **4**, 266–275.

Heisenberg, M. & Wolf, R. (1984) *Vision in Drosophila*. Springer, Berlin.

Heisenberg, M., Wolf, R. & Brembs, B. (2001) Flexibility in a single behavioral variable of Drosophila. *Learn. Mem.*, **8**, 1–10.

Horn, E. (1978) The mechanism of object fixation and its relation to spontaneous pattern preferences in Drosophila melanogaster. *Biol. Cybern.*, **31**, 145–158.

Huber, F. (1963) The role of the central nervous system in Orthoptera during the co-ordination and control of stridulation. In Busnel, R.G. (Ed.), *Acoustic Behavior of Animals*. Elsevier, Amsterdam, pp. 440–488.

Huber, F. (1965) Brain controlled behavior in orthopterans. In Treherne, J.E. & Beament, J.W.L. (Eds), *The Physiology of the Insect Central Nervous System*. Academic Press, London, pp. 233–246.

Joiner, W.J., Crocker, A., White, B.H. & Sehgal, A. (2006) Sleep in Drosophila is regulated by adult mushroom bodies. *Nature*, **441**, 757–760.

Kanwisher, N. & Wojciulik, E. (2000) Visual attention: insights from brain imaging. *Nat. Rev. Neurosci.*, **1**, 91–100.

Kitamoto, T. (2001) Conditional modification of behavior in Drosophila by targeted expression of a temperature-sensitive shibire allele in defined neurons. *J. Neurobiol.*, **47**, 81–92.

Koenig, J.H., Saito, K. & Ikeda, K. (1983) Reversible control of synaptic transmission in a single gene mutant of Drosophila melanogaster. *J. Cell Biol.*, **96**, 1517–1522.

Land, M.F. (1997) Visual acuity in insects. *Annu. Rev. Entomol.*, **42**, 147–177.

Laurent, G. (1996) Dynamical representation of odors by oscillating and evolving neural assemblies. *Trends Neurosci.*, **19**, 489–496.

Laurent, G. & Davidowitz, H. (1994) Encoding of olfactory information with oscillating neural assemblies. *Science*, **265**, 1872–1875.

Laurent, G. & Naraghi, M. (1994) Odorant-induced oscillations in the mushroom bodies of the locust. *J. Neurosci.*, **14**, 2993–3004.

Li, Y. & Strausfeld, N.J. (1997) Morphology and sensory modality of mushroom body extrinsic neurons in the brain of the cockroach, Periplaneta americana. *J. Comp. Neurol.*, **387**, 631–650.

Liu, X., Krause, W.C. & Davis, R.L. (2007) GABA(A) receptor RDL inhibits Drosophila olfactory associative learning. *Neuron*, **56**, 1090–1102.

Liu, L., Wolf, R., Ernst, R. & Heisenberg, M. (1999) Context generalization in Drosophila visual learning requires the mushroom bodies. *Nature*, **400**, 753–756.

Martin, J.R., Ernst, R. & Heisenberg, M. (1998) Mushroom bodies suppress locomotor activity in Drosophila melanogaster. *Learn. Mem.*, **5**, 179–191.

Martinez-Trujillo, J. & Treue, S. (2002) Attentional modulation strength in cortical area MT depends on stimulus contrast. *Neuron*, **35**, 365–370.

McDonald, J.J., Teder-Salejarvi, W.A. & Hillyard, S.A. (2000) Involuntary orienting to sound improves visual perception. *Nature*, **407**, 906–908.

McGuire, S.E., Deshazer, M. & Davis, R.L. (2005) Thirty years of olfactory learning and memory research in Drosophila melanogaster. *Prog. Neurobiol.*, **76**, 328–347.

Nitz, D.A., van Swinderen, B., Tononi, G. & Greenspan, R.J. (2002) Electrophysiological correlates of rest and activity in Drosophila melanogaster. *Curr. Biol.*, **12**, 1934–1940.

Otto, D. (1971) Untersuchugen zur zentralnervosen kontrolle der lauterzeugung von grillen. *Z. Vergl. Physiol.*, **74**, 227–271.

Peng, Y., Xi, W., Zhang, W., Zhang, K. & Guo, A. (2007) Experience improves feature extraction in Drosophila. *J. Neurosci.*, **27**, 5139–5145.

Pitman, J.L., McGill, J.J., Keegan, K.P. & Allada, R. (2006) A dynamic role for the mushroom bodies in promoting sleep in Drosophila. *Nature*, **441**, 753–756.

Poggio, T. & Reichardt, W. (1976) Visual control of orientation behaviour in the fly. Part II. Towards the underlying neural interactions. *Q. Rev. Biophys.*, **9**, 377–438.

Rensink, R.A., O'Regan, J.K. & Clark, J.J. (1997) To see or not to see: the need for attention to perceive in scenes. *Psychol. Sci.*, **8**, 368–373.

Reynolds, J.H. & Chelazzi, L. (2004) Attentional modulation of visual processing. *Annu. Rev. Neurosci.*, **27**, 611–647.

Reynolds, J.H., Chelazzi, L. & Desimone, R. (1999) Competitive mechanisms subserve attention in macaque areas V2 and V4. *J. Neurosci.*, **19**, 1736–1753.

Reynolds, J.H., Pasternak, T. & Desimone, R. (2000) Attention increases sensitivity of V4 neurons. *Neuron*, **26**, 703–714.

Schildberger, K. (1984) Multimodal interneurons in the cricket brain: properties of identified extrinsic mushroom body cells. *J. Comp. Physiol. A*, **154**, 71–79.

Strausfeld, N.J., Hansen, L., Li, Y., Gomez, R.S. & Ito, K. (1998) Evolution, discovery, and interpretations of arthropod mushroom bodies. *Learn. Mem.*, **5**, 11–37.

Strausfeld, N.J., Sinakevitch, I. & Vilinsky, I. (2003) The mushroom bodies of Drosophila melanogaster: an immunocytological and golgi study of Kenyon cell organization in the calyces and lobes. *Microsc. Res. Tech.*, **62**, 151–169.

Strauss, R. & Heisenberg, M. (1993) A higher control center of locomotor behavior in the Drosophila brain. *J. Neurosci.*, **13**, 1852–1861.

Strayer, D.L., Drews, F.A. & Johnston, W.A. (2003) Cell phone-induced failures of visual attention during simulated driving. *J. Exp. Psychol. Appl.*, **9**, 23–32.

Swinderen, B. (2005) The remote roots of consciousness in fruit-fly selective attention? *Bioessays*, **27**, 321–330.

van Swinderen, B. (2007) Attention-like processes in Drosophila require short-term memory genes. *Science*, **315**, 1590–1593.

van Swinderen, B. & Flores, K.A. (2007) Attention-like processes underlying optomotor performance in a Drosophila choice maze. *Dev. Neurobiol.*, **67**, 129–145.

van Swinderen, B. & Greenspan, R.J. (2003) Salience modulates 20–30 Hz brain activity in Drosophila. *Nat. Neurosci.*, **6**, 579–586.

van Swinderen, B., Nitz, D.A. & Greenspan, R.J. (2004) Uncoupling of brain activity from movement defines arousal states in Drosophila. *Curr. Biol.*, **14**, 81–87.

Tang, S. & Guo, A. (2001) Choice behavior of Drosophila facing contradictory visual cues. *Science*, **294**, 1543–1547.

Treue, S. (2001) Neural correlates of attention in primate visual cortex. *Trends Neurosci.*, **24**, 295–300.

Wahdepuhl, M. (1983) Control of grasshopper singing behavior by the brain responses to electrical stimulation. *Z. Tierpsychol.*, **63**, 173–200.

Wolf, R., Wittig, T., Liu, L., Wustmann, G., Eyding, D. & Heisenberg, M. (1998) Drosophila mushroom bodies are dispensable for visual, tactile, and motor learning. *Learn. Mem.*, **5**, 166–178.

Wu, Z., Gong, Z., Feng, C. & Guo, A. (2000) An emergent mechanism of selective visual attention in Drosophila. *Biol. Cybern.*, **82**, 61–68.

Ye, Y., Xi, W., Peng, Y., Wang, Y. & Guo, A. (2004) Long-term but not short-term blockade of dopamine release in Drosophila impairs orientation during flight in a visual attention paradigm. *Eur. J. Neurosci.*, **20**, 1001–1007.

Zars, T. (2000) Behavioral functions of the insect mushroom bodies. *Curr. Opin. Neurobiol.*, **10**, 790–795.

Zeman, A. (2001) Consciousness. *Brain*, **124**, 1263–1289.

Zhang, K., Guo, J.Z., Peng, Y., Xi, W. & Guo, A. (2007) Dopamine-mushroom body circuit regulates saliency-based decision-making in Drosophila. *Science*, **316**, 1901–1904.