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Small object detection neurons in female hoverflies

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While predators such as dragonflies are dependent on visual detection of moving prey, social interactions make conspecific detection equally important for many non-predatory insects. Specialized 'acute zones' associated with target detection have evolved in several insect groups and are a prominent male-specific feature in many dipteran flies. The physiology of target selective neurons associated with these specialized eye regions has previously been described only from male flies. We show here that female hoverflies (*Eristalis tenax)* have several classes of neurons within the third optic ganglion (lobula) capable of detecting moving objects smaller than 1°. These neurons have frontal receptive fields covering a large part of the ipsilateral world and are tuned to a broad range of target speeds and sizes. This could make them suitable for detecting targets under a range of natural conditions such as required during predator avoidance or conspecific interactions.

Keywords: visual target detection; intracellular electrophysiology; higher-order visual neurons; hypercomplex cells

1. INTRODUCTION

Detection of targets requires high spatial acuity, abundantly available with the simple lens eyes of many taxa, but severely limited in the compound eyes of insects (Land 1997). Despite this limitation, many insects have evolved superb visual target detection and engage in highly aerobatic, visually mediated pursuit of either conspecifics or prey. Being accessible for *in vivo* electrophysiological investigation, insects thus provide an excellent model system to investigate the neurobiology of target detection.

The conflicting demands of eye size, high acuity and wide field of view, have led to evolution of specialized zones within the eyes of many insects that pursue small targets. These acute zones are observed as a male-specific feature in some dipteran flies and in both sexes of many insects that pursue prey (e.g. dragonflies; Land 1997). In some hoverflies, females have a frontal acute zone in which acuity rivals that seen in many male dipterans (Land & Eckert 1985; Warrant 2001).

Higher order target motion detection neurons with fronto-dorsal receptive fields corresponding to acute zones have been described in the third optic ganglion (lobula) of some insects. These include male-specific lobula giant visual neurons (MLGs) in the blowfly and housefly (Hausen & Strausfeld 1980; Strausfeld 1980; Gilbert & Strausfeld 1991) and feature detecting neurons of male hoverflies (Collett & King 1975) and predatory dragonflies (Olberg 1981; O'Carroll 1993). However, to date neurons tuned to the detection of small moving targets have never been described from non-predatory female dipterans.

To test whether females have the neural machinery to detect moving features, we used intracellular recordings from the third optic ganglion of female *Eristalis tenax* and analysed the responses of neurons to small targets. We find neurons tuned to the detection of small moving features very similar to those previously described in male hoverflies (Collett & King 1975).

2. MATERIAL AND METHODS

(a) *Animals*

Female *E. tenax* were collected under permit from the Adelaide Botanic Gardens (City, Wittunga and Mt Lofty). The flies were kept at 4 °C and constant humidity until experimental time.

(b) *Electrophysiology*

The animal was waxed down with the head tilted forward to gain access to the back of the head. A small hole was cut over the left lobula complex, leaving the perineural sheath intact. Neurons were recorded intracellularly from the lobula (determined by positioning electrodes greater than 100 μm below the surface of the brain) with aluminium silicate micropipettes pulled on a Sutter Instruments P-97 puller and filled with 2 M KCl (typical tip resistance 120 MΩ). The flies were mounted in front of a RGB CRT display at a distance of 15 cm. Flies were aligned with the monitor using the planar back surface of the head as a morphological landmark and the animal's equator was assumed to be 90° perpendicular to this. The animal's midline was used to determine the vertical axis. This was used in later analyses to determine receptive fields, and stimuli size and velocity. The display subtended 100° by 75° of the fly's visual field of view, with a resolution of 640 by 480 pixels, permitting targets down to below 0.2° square to be presented, and had a refresh rate of 200 Hz. Visual stimuli were generated by the VisionEgg software (www.visionegg.org). Data were digitized at 5 kHz using a 12 bit A/D converter (National Instruments) and analysed off-line with Matlab.

(c) *Receptive field analysis*

To determine receptive fields, we scanned the entire monitor with a black 0.8° square target. Scans were performed with a 2 s horizontal rightward scan (50° s^{-1}), followed by a 1 s rest,

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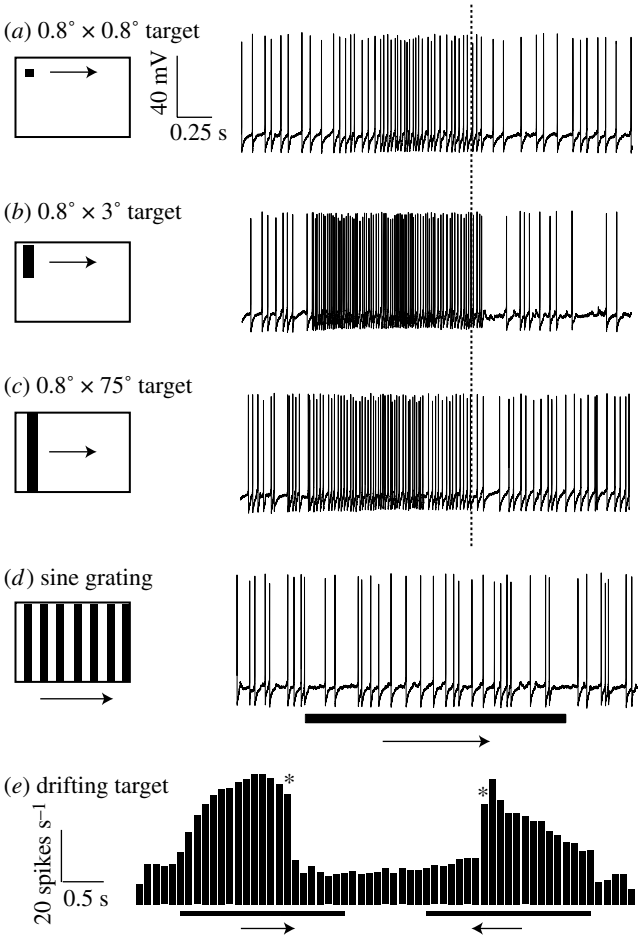

Figure 1. Raw responses of a hoverfly (type II) neuron tuned to small targets. (*a–c*) Response to a 0.8° wide black target presented at 50° s^{-1} (stimulus period indicated by black bar) and three different vertical heights. The dotted line indicates the time the target crossed the midline and entered the contralateral visual field. (*d*) A control stimulus, consisting of a drifting sinusoidal grating (1 Hz, 0.1 cycles per degree). (*e*) A histogram of responses to 42 presentations of a target that drifts from left to right and then back again. Asterisks (*) indicate when the target crossed the midline.

and a 2 s leftward scan back over the same path at 50° s^{-1}. Following a 3 s rest, a new semi-randomly chosen elevation was scanned in the same way, until the entire monitor had been covered at 21 elevations. The monitor was also scanned in 21 azimuth positions in a vertical orientation to determine direction selectivity. Spontaneous rates were subtracted from spiking rates after binning data into 100 ms bins. This permitted a two-dimensional matrix of spike rates to be produced, representing the area of the stimulus display, with spike rates mapped to different colours. Where neurons gave graded responses, we used a similar analysis of the membrane potential averaged across 100 ms bin, expressed relative to the resting potential. For both map types, the data for each bin was transformed into azimuth and elevation coordinates, using the calibrated position of the fly and the angle of its head. Azimuths are negative left (ipsilateral) of the midline, and elevations are positive dorsal to the equator.

(d) *Size and velocity tuning*

To estimate selectivity for small targets (size tuning) 0.8° wide targets were drifted across the receptive field at 50° s^{-1} for 2 s, followed by a minimum 2 s rest before the next

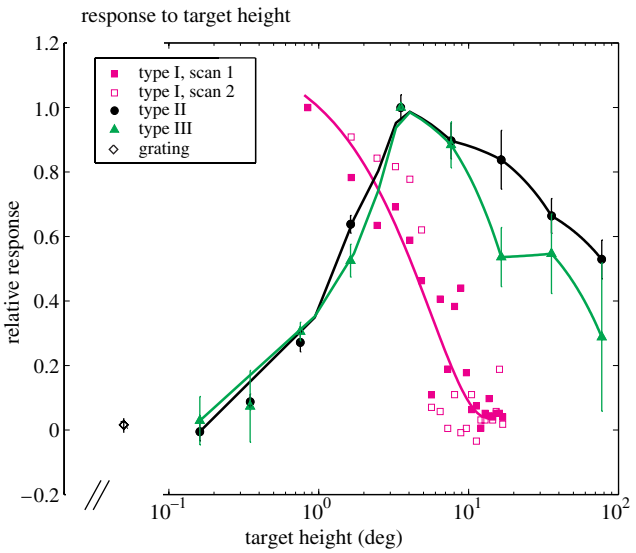

Figure 2. Height tuning for three classes of STMD neurons. Normalized responses (see §2) are shown averaged from the time the target traversed the receptive field. For STMD type I we show responses for length tuning in two parts of the receptive field—open squares denote scans at an elevation of 60°, while filled squares show the same tuning at 40° elevation. Inset shows the response to drifting sinusoidal gratings (1 Hz, 0.1 cycles per degree, $n=7$). Errorbars denote standard error of the mean averaged from several neurons ($n=1$ for type I, $n=5$ for type II, $n=3$ for type III).

presentation. The vertical height of the target was semi-randomly varied from less than 1° to a height covering the entire monitor. To estimate velocity tuning we drifted a 0.8° black square target across the centre of the receptive field at velocities between 8 and 800° s^{-1}. The targets were presented moving horizontally, and included a minimum 3 s rest between presentations. For both experiments, we measured the spike rate during the time the target traversed the receptive field, as a function of the varied parameter. For 'optimal' stimuli, maximum firing rates of different neurons varied from 50 to over 150 spikes per second. To generate meaningful error bars in data averaged from several different neurons, we first normalized the data to account for these differences in overall firing rates by subtracting the average spontaneous spike frequency from the response, and then dividing by the sum of responses to all conditions. The averaged data from all cells were then re-scaled to a maximum of 1. Velocity data were plotted both as the peri-stimulus spike frequency as the target traversed the receptive field and by transforming responses into angular coordinates, i.e. spikes fired per degree of the visual field traversed.

3. RESULTS

(a) *Spatial tuning*

Figure 1 shows responses of a typical small target motion detector (STMD) neuron recorded from the left lobula of a female *E. tenax*. We distinguished such neurons by a strong spiking response to small stimuli (less than 5° of the visual field of view, figure 1*a*), but no response above spontaneous levels to wide-field stimuli such as sine-wave gratings (figure 1*d*) that generate powerful responses in other neuron classes described from female flies (e.g. lobula plate tangential cells, LPTCs). To confirm a possible role in target detection, we analysed size-tuning

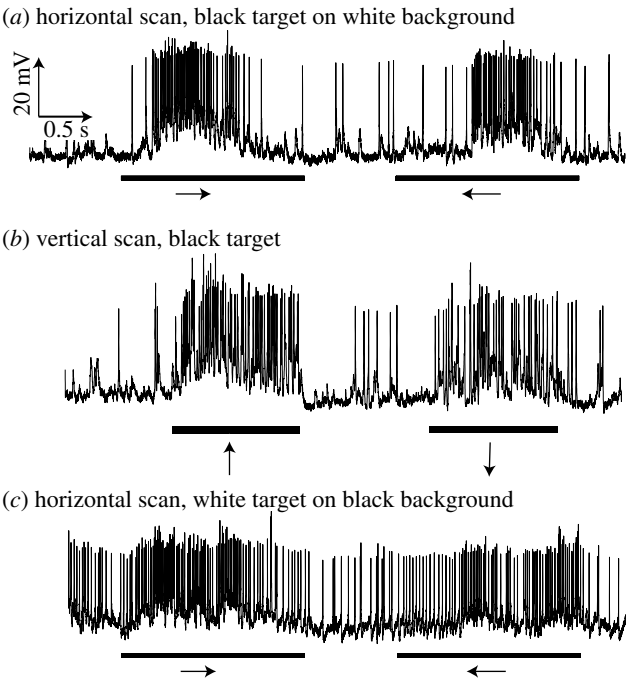

Figure 3. Raw responses from a graded type III STMD neuron to (*a*) horizontal and (*b*) vertical motion of black targets on white backgrounds. (*c*) The response to horizontal motion when the target is white, traversing a black background.

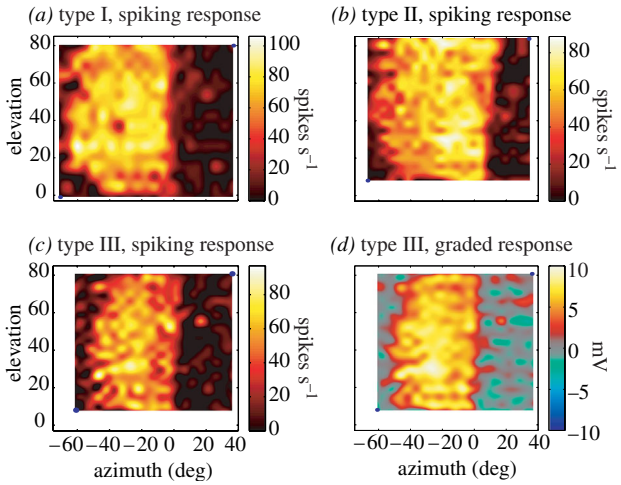

Figure 4. Receptive fields for three classes of STMD neurons. (*a*, *b*) STMD type I and II gave purely spiking responses, while (*c*) STMD type III responded with spikelets, (*d*) riding on depolarization. The maps show data from individual recordings to target motion from left to right. The response is colour coded to show (*a–c*) the response spike frequency or (*d*) membrane potential change from baseline.

characteristics of all neurons encountered, to determine the response dependence on the height of the target. All neurons that we classified as STMDs gave stronger responses to small targets (figure 1*b*) compared with bars extended to cover the whole screen (0.8×75°, figure 1*c*). In some cases, such larger stimuli elicit no measurable response.

We recorded from 44 neurons in 42 female hoverflies that we can classify as STMDs based on strong responses to small targets but weak or absent responses to large targets and wide-field motion stimuli (as in figure 1).

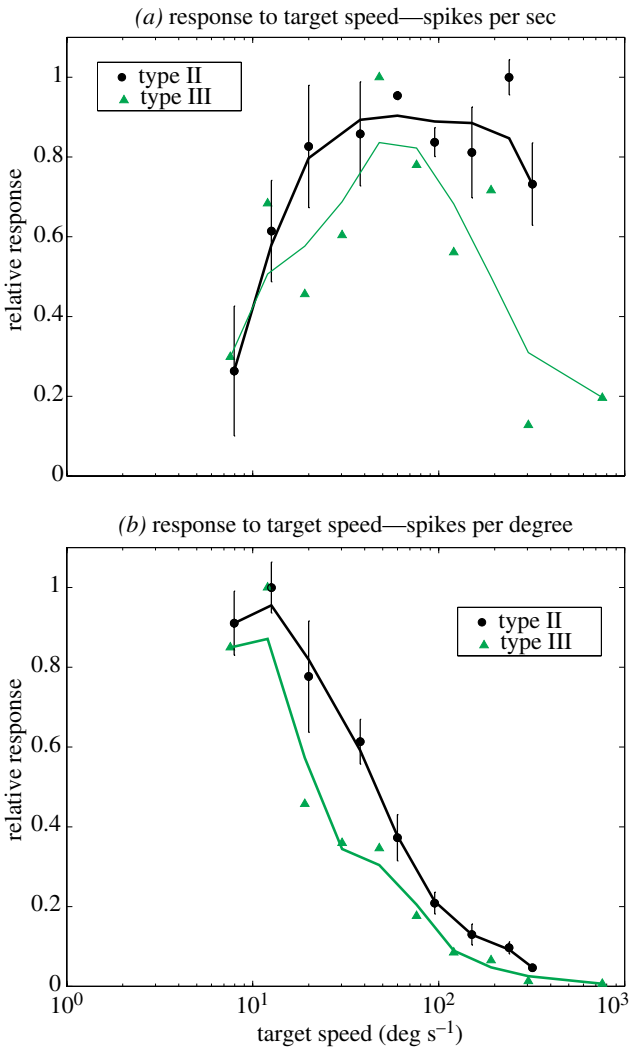

Figure 5. Speed tuning of type II and III STMD neurons, averaged from the time the target (a 0.8° dark square) was traversing the receptive field. (*a*) Data plotted as normalized spike rate. (*b*) The same data after transforming into spatial coordinates (spikes/degree). Error bars denote standard error of the mean ($n=4$ for type II, $n=1$ for type III) and the lines join the low-pass filtered mean.

Although encountered in most subjects, these STMDs represent a small fraction of the neurons encountered during these experiments, and are clearly a rare class. We obtained extensive data in 14 cases that allows us to further classify the neurons. Not all of this group of STMDs have the same selectivity for small targets. Two distinct forms of size-tuning were observed in female STMDs (figure 2). The first neuron class (STMD type I) showed a peak response to the smallest target presented (≈0.8° square), and strong suppression of response if targets were elongated to even a few degrees of visual angle. Two additional neuron classes (STMD type II, III) gave maximal responses to targets with a length of *ca* 3° of the visual field and less excitation to elongated targets. Type III neurons were distinguished by irregularly shaped and relatively small 'spikelets', which ride upon a pronounced, graded response to target motion (figure 3).

(b) *Receptive field*

In male flies, previously described STMD neurons respond primarily to target motion within the fronto-dorsal part of the visual field (Collett & King 1975;

Gilbert & Strausfeld 1991; Grönenberg & Strausfeld 1991) corresponding to the male-specific acute zone. In contrast to the compact fronto-dorsal receptive fields described for male STMDs, we found that all three classes of female STMDs have relatively large frontal receptive fields (figure 4). The response of these neurons extended further ventrally and dorsally than we could stimulate with our set-up. In some neurons, responses fall off at around 40° ipsilaterally (e.g. figure 4*c*), while in others they extend almost to the limits of our display (*ca* 70° ipsilateral, figure 4*b*). These neurons also had a sharp receptive field boundary at the midline between the eyes (i.e. no binocular input). In some cases, we observed that responses were inhibited after the target crosses this midline (dashed line in figure 1*a–c*) into the contralateral field. A histogram of responses averaged from 42 repetitions of the target drifting from right to left and then back again across this midline reveals no evidence of inhibition when the target crosses this same region from the near-contralateral part of the screen (figure 1*e*) so this may represent a post-excitatory rebound phenomenon caused by the sharp boundary of the receptive field, rather than an inhibitory receptive field sub-region. The graded receptive field of STMD type III neurons (figure 4*d*) is similar to a receptive field constructed from its own spiking response (figure 4*c*) and also shows no evidence for sub-threshold inhibition in the contralateral area. The STMDs described here were excited by target motion in all four directions tested (figure 3*a*,*b*). However, the response was slightly stronger to target motion up and to the right (towards the midline) over the receptive field in all neurons when recorded from the left lobula.

The longitudinal extents of the receptive fields (figure 4) were much larger than the optimally sized targets (figure 2) in all neuron classes. This indicates that the size tuning of these neurons does not derive from simple receptive field properties. Responses are selective for small targets independent of where they are placed within the receptive field. This is illustrated in figure 2 by data for a type I neuron obtained from two receptive field locations.

(c) *Velocity tuning*

Most hoverflies are excellent fliers and can easily reach speeds of 10 m s^{-1} at least for short distances (Collett & Land 1978), which means that the eyes need to be suitably adapted to enable feature detection during flight. For STMD types II and III we saw velocity tuning with a broad peak response plateau between 20 and 120° s^{-1} (figure 5*a*), and reduced responses as the velocities were increased further.

As the time a given target spends traversing the receptive field of a neuron decreases with increasing speeds (even though the spike frequency during this time might increase), the actual number of spikes per unit of space might decrease. We therefore analysed the velocity data in a second way by calculating the spikes per degree of the visual field. Interestingly this analysis gave a nearly monotonic logarithmic decline in response with increasing target speed (figure 5*b*) with a peak at about 15° s^{-1}. This indicates that per unit of space, the most reliable signal is achieved when viewing low-velocity features.

(d) *Additional neuron classes*

We have described data here for three classes of STMD neurons in female *Eristalis*, but these are not the only classes we encountered. We also recorded responses of neurons with much smaller frontal receptive fields, but recordings from this class are of short duration, and we have yet to obtain sufficient data to definitively characterize these cells. Nevertheless, it is clear that the diversity of STMD neuron classes in females is smaller than in males. In addition to the male-specific neurons described in earlier work, we have used similar methods to classify at least 20 different classes of STMDs in male *Eristalis* (Nordström *et al*. in press). Of these, one is similar to the type III neuron described here, while the others have significantly smaller fronto-dorsal receptive fields.

4. DISCUSSION

(a) *Spatial tuning of object motion detectors in flies*

How does spatial tuning of the female neurons described here compare with other classes of object detectors described in previous work on insects? In blowflies (*Sarcophaga bullata*) the male-specific lobula giant neurons (MLGs) with receptive fields located within the acute zone have been implied as playing a role in conspecific detection (Gilbert & Strausfeld 1991). The size tuning of MLGs is not extensively described and the receptive fields are only deduced from the dendritic arborizations and hand stimulus methods, which makes comparisons to the STMDs described here difficult. Collett & King (1975) also described neurons in male hoverflies with fronto-dorsal receptive fields and peak responses to targets around 2.5° square—similar to the spatial optimum seen in these female neurons. Interestingly, morphologically similar neurons to MLGs have also been described in female blowflies (Strausfeld 1980), but as no physiology accompanied the anatomy, it is premature to classify these female lobula giants (FLGs) as STMDs. However, the anatomically predicted frontal receptive fields of FLGs overlap with those described here (figure 4).

Figure detection (FD) neurons of the lobula plate are another group of blowfly object detectors that have been described previously from female flies (Egelhaaf 1985*a*), and are proposed to mediate yaw torque responses to motion of frontal objects. No length-tuning data for such neurons directly comparable to that presented here have been published for FD neurons, but experiments with grating patches suggest that they are tuned to much larger stimuli, with an optimum response to 6° square stimuli (greater than 10 times the area of the 0.8° wide target stimuli used here) and a gentler roll-off in response for larger stimuli (Egelhaaf 1985*b*). FDs are also direction-opponent, i.e. they are excited by one direction of motion and inhibited by the opposite (anti-preferred; Egelhaaf 1985*a*), while the three classes of STMD described here are (at best) weakly direction-selective (figure 3*a*,*b*). Finally, the receptive fields of the FDs are located in the ventral visual field (Egelhaaf 1985*a*), while those described here extend well into the dorsal hemisphere (figure 4).

Other classes of neuron responding to object motion have also been described from insects. In dragonfly species, several previous studies described neurons tuned

to small targets and presumably involved in target pursuit (Olberg 1981; O'Carroll 1993; Olberg *et al.* 2000). The data we present for type I neurons here (figure 2) show similar size tuning to these dragonfly neurons. The limited optical acuity of dipteran eyes suggests targets smaller than 1° would be significantly blurred (Land 1997; Stavenga 2003). A peak response to targets as small as 0.8° square seen in type I STMDs is thus an amazing feat.

While the other two classes are more broadly tuned, they are still selective for small targets, particularly by comparison with some other classes of object motion detectors including orientation-selective, bar-tuned cells described from dragonflies (O'Carroll 1993) and bees (*Apis mellifera*) (Maddess & Yang 1997) and the looming object detectors of the locust (*Schistocerca gregaria*) lobula (for review see Rind & Simmons 1999). It is clear that there are numerous classes of object-specific motion detector in insects, hence our adoption of the term STMD to distinguish neurons that give the largest responses to small targets (in some cases on the order of just one or two photoreceptors in the retinal image).

(b) *Could female target neurons be used for conspecific detection?*

Our study is the first description of STMD neurons in female flies, which begs the question: why do female flies require neural pathways for small target detection? The sex-specific nature and receptive fields of male STMDs is consistent with a behavioural role in conspecific detection and pursuit (Collett & Land 1975; Gilbert & Strausfeld 1991). The female neurons described here are similar, although their receptive fields are larger and more equatorial. An interesting hypothesis is that female hoverflies might use STMD neurons to detect hovering males—thus playing a more active role in courtship and mating than previously described. This role is supported by the impressive visual acuity described for large syrphids: female *Volucella pellucens* (Warrant 2001) and *E. tenax* (E. J. Warrant 2005, personal communication) both have pronounced acute zones located frontally—corresponding directly to the location of the receptive fields of STMDs that we describe here. Such acute zones are lacking in females of most other non-predatory dipteran species, yet rival the acuity seen in the sex-specific acute zones of male dipterans (Land & Eckert 1985). Eristaline males have been described hovering above feeding females in a stereotyped fashion, at times displaying a hovering jerky dance (Stubbs & Falk 1996). In *E. tenax* (the species studied here) males normally occupy distinct hovering territories within a shaft of sunlight that makes them conspicuous against dark foliage (Fitzpatrick & Wellington 1983). Interestingly, the female STMD neurons respond to either dark objects against a bright background or vice versa (albeit less strongly, figure 3*a*,*c*) and so would be suited to detection of conspecifics against either sky or background foliage. The 0.8° stimulus within the velocity-tuned plateau (figure 5*a*) that produces a powerful stimulus for the female STMDs corresponds approximately to a conspecific at a distance of 1.1 m, flying at a velocity of 0.4–4 m s^{-1}—typical of the forward flight speed of active *Eristalis* (Collett & Land 1978). Interestingly, a similar stimulus would be generated by a female fly moving at the same speed past a hovering male.

While conspecific detection is one possible role for female STMDs, neurons with a wide field of view, and tuned to small objects, have been suggested to be useful in a range of other behavioural scenarios, such as rival surveillance and predator avoidance (Hemmi & Zeil 2003). As a bee mimic (Golding *et al.* 2001), predation has probably been a strong selective pressure in the evolution of *Eristalis*. Together with the broad size and velocity tuning of the type II and III STMDs described here, these neurons would be appropriate for visual predator detection and subsequent avoidance.

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