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Fast and slow photoreceptors – a comparative study of the functional diversity of coding and conductances in the Diptera

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Abstract. 1. From a comparison of the photoresponses and membrane properties of photoreceptors from 20 species of Diptera, we conclude that coding in the time domain is matched to the dictates of visual ecology. This matching involves the dynamics of phototransduction and the use of an appropriate mix of potassium conductances to tune the photoreceptor membrane.

2. Rapidly flying, manoeuvrable diurnal Diptera from several families have fast photoreceptors, with corner frequencies (the frequency at which signal power falls by a half) of between 50 and 107 Hz. The ponderous and predominantly nocturnal tipulids have slow photoreceptors with fully light adapted corner frequencies of 16 to 19 Hz.

3. Dark adapted fast photoreceptors have a lower gain (as indicated by lower noise levels), a lower sensitivity, and light adapt more rapidly than dark adapted slow photoreceptors. Fast cells also have much lower input resistances and shorter time constants.

4. Fast photoreceptors rectify more strongly in the steady state because of a weakly inactivating delayed rectifier potassium conductance with fast and slow components of activation. Slow photoreceptors rectify less strongly in the steady state because their membrane properties are dominated by strongly inactivating outward currents with reversal potentials in the range −80 to −90 mV.

5. The differences between potassium conductances match the differing functional requirements of fast and slow photoreceptors. The non-inactivating delayed rectifier promotes the rapid response of fast cells by reducing the membrane time constant. This is an expensive strategy, involving large conductances and currents. Slowly flying nocturnal insects do not require a high speed of response. The potassium conductances in their slow photoreceptors inactivate to avoid costly and unnecessary ion fluxes.

8. Both the dynamics of the photoresponse and photoreceptor membrane properties exhibit sexual dimorphism. Light adapted photoreceptors in the enlarged male dorsal eye of *Bibio markii* have a corner frequency of 42 Hz, compared with 27 Hz for cells in the smaller female eye. This difference in frequency response correlates with the male's higher spatial acuity and is accompanied by consistent differences in potassium conductance activation rate. We conclude that the divison between fast and slow cells is the product of cellular constraints, metabolic costs and the requirements of coding efficiency at different light levels and retinal image velocities.

Key words: Photoreceptor – Response dynamics – Visual ecology – Potassium channels – Diversity

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Introduction

The performance of sensory receptors determines the amount of information that an animal can extract from its environment. Not surprisingly therefore, sense organs and sense cells are usually well adapted for particular requirements. In vision there are many examples of adaptation of form to function, particularly in the predominantly optical stages involving image formation, image sampling, and the tuning of spectral mechanisms (Kirschfeld 1983; Land 1981; Lythgoe 1979; Stavenga 1992). These adaptations operate primarily in the spatial and wavelength domains and many involve the matching of cellular and molecular components, either to each other, or to biologically relevant properties of the image. Our study pursues this well established theme by addressing two questions of retinal design. First, to what extent does the general principle that coding can be matched to visual ecology apply in the time domain? Second, does such matching involve tuning the molecular mechanisms that generate and shape photoreceptors' voltage responses?

The idea that retinal response dynamics matches lifestyle and habitat was established by Autrum's classic

distinction between the fast eyes of rapidly moving diurnal species and the slower eyes of less athletic and nocturnal forms (Autrum 1950, 1984). This distinction, drawn from ERG recordings, was later supported by two comparative studies of the intracellular responses of insect photoreceptors. Predominantly diurnal species have a better temporal resolving power than arrhythmic and nocturnal species, and temporal resolution is associated with the rapidity with which insects move and turn (Howard et al. 1984; de Souza and Ventura 1989). Here we continue this comparative approach by examining the relationship between response dynamics and habitat in the Diptera. We confirm and substantiate the correlation between photoreceptor response dynamics and lifestyle by describing an unexpectedly wide range of temporal coding abilities among light adapted cells from a single order.

How are these differences in photoreceptor response dynamics achieved? This consideration brings us to our second question, the adaptation of cellular and molecular mechanisms to the requirements of efficient coding. The precise tuning of the spectral absorbances of screening and photopigment molecules to the requirements of lifestyle and habitat has been elegantly elaborated (Lythgoe 1979; Chittka and Menzel 1992; Stavenga 1992). The tuning of the subsequent molecular processes generating the photoresponse has received less attention, but there are well established examples. In *Limulus* lateral eye, octopamine, a neuromodulator released by efferent fibres driven by a circadian clock, regulates both the photoreceptor membrane and the processes of phototransduction to match performance to the time of day (Barlow et al. 1989). At the level of the phototransduction cascade, blowfly and dragonfly photoreceptors contain large numbers of phototransduction units to achieve a high signal to noise ratio under bright light conditions (Howard et al. 1987; Laughlin 1989). At the level of membrane conductances, drone bee photoreceptors contain a set of voltage sensitive sodium and potassium conductances that is precisely tuned so that it actively boosts the voltage signals produced by small dark targets, such as queen bees (Coles and Schneider-Pickard 1989; Vallett et al. 1992). In blowflies, signal boosting conductances may also be operating at the level of the photoreceptor's synaptic terminal in the lamina (Weckström et al. 1992).

Potassium conductances are commonly found in arthropod photoreceptors, e.g. *Limulus* (O'Day et al. 1982), *Hermissenda* (Alkon et al. 1985), locust (Weckström 1990), drone bee (Vallett et al. 1992), and in the blowfly *Calliphora* they play an important role in coding (Laughlin and Weckström 1989; Weckström et al. 1991). A powerful delayed rectifier conductance, of a type hitherto unreported in insects, prevents the cell's frequency response from being compromised by the capacitative load imposed by the large area of photosensitive membrane. Because the conductance is voltage sensitive it is able to tune the impedance of the membrane so that it matches the signals produced by the phototransduction cascade over the full range of adaptation states. The fruit fly *Drosophila*, an advanced dipteran with a very different visual ecology from *Calliphora*, possesses a distinctive complement of photoreceptor potassium channels; a prominent A-current coded by unique transcripts in the Shaker gene complex, and two less rapidly inactivating currents, intermediate between the A-current and the *Calliphora* delayed rectifier (Hardie et al. 1991; Hardie 1991). The differences between these two flies add some weight to the proposal that photoreceptor potassium channels are adapted to the requirements of visual ecology (Weckström et al. 1991).

In this study we will test this proposal more rigorously by comparing the membrane properties of fast and slow photoreceptors. We will demonstrate that all of the fast cells exhibit a prominent delayed rectifier with fast and slow components, as typified by *Calliphora*. By comparison, the slow cells of primitive Tipulidae tune their membranes with a different complement of potassium channels. The slow delayed rectifier is effectively absent, and rapidly inactivating conductances, similar to those found in *Drosophila* (Hardie 1991) predominate. This correlation between response dynamics and potassium conductance suggests that the slowly activating delayed rectifier is indeed a specialisation for the fast responses demanded by visual ecology, a proposal that is strengthened by our examination of the sexual dimorphism of photoreceptor responses and membrane properties in the Bibionids.

We conclude that the correlation of response dynamics with visual ecology, along the lines first proposed by Autrum (1950), is dictated by a combination of functional requirements (van Hateren 1992, 1993) and metabolic costs. The tuning of response dynamics to functional requirements requires appropriate sets of potassium channels in the photoreceptor membrane. Thus dipteran photoreceptors may well provide an excellent example of the way in which excitable cells exploit the diversity of potassium channels for functional purposes, by picking and mixing advantageous combinations (Salkoff et al. 1992).

Methods

Animals. The majority of animals were collected in Cambridgeshire or Norfolk. Bibionids were kept for up to 7 days at 4 °C, but all other animals were kept at room temperature and used within 48 h of capture. All experiments were carried out in daytime (10.00–19.00 h), between April and October. Tipulids were prepared in both dark and light adapted states in daytime. Dark adaptation of the day state eye (Williams 1980) had no detectable effect upon the responses of cells when they were subsequently light adapted.

The preparation followed Hardie (1979). Animals were minimally restrained by using tackiwax and insect wax to secure the wings and mouthparts to a small perspex block. A small hole was cut in the cornea of the right eye with a freshly broken razor blade chip and sealed with silicon grease. The indifferent electrode, a chlorided silver wire, was inserted in the left eye. The animal was then placed on the stage of the micromanipulator in a darkened Faraday cage. Animals were normally dark adapted for 30 min before commencing recording.

The recordings followed the intracellular methods described by Weckström et al. (1991). Glass micropipettes, filled with 3.0 *M* KCl and with resistances in the range 100–150 MΩ, were inserted into the retina of the right eye through the sealed hole. The silicon grease prevented fluid from creeping up the outside of the electrode and this reduced the parasitic capacitance. All the fast cells described in this study were classified as short visual fibres (R1–6 type) according to the criteria of Hardie (1979). Signals were amplified using an Axoclamp-2A switched clamp amplifier, and digitised and stored using conventional hardware and software (PClamp system – Axon Instruments).

Current clamp. The switched clamp allowed us to inject current and record membrane voltage with a single electrode. The capacity compensated electrode had a time constant of approximately 50 μs. The switching rate was set to its optimum for each cell, within the range 2.5–4-0 kHz, while continuously monitoring the headstage output. Current pulse delivery, data collection and averaging (generally over 5 or 10 repetitions) were controlled by the PClamp software. The cell time constant at resting potential, or in the light adapted state was determined by fitting exponentials to the response to depolarising and hyperpolarising pulses of 0.1 nA or less. The values obtained from the onset and offset of depolarising and hyperpolarising pulses differed significantly, due to the inescapable action of voltage sensitive conductances, and were averaged to give a single estimate of time constant.

Voltage clamp. The switching amplifier was used to single electrode voltage clamp dark adapted cells. Holding potentials and protocols were controlled by PClamp software, which also collected and averaged the data, generally over 5 or 10 repetitions. Because the single electrode system could not hold the membrane potential perfectly as conductance changed, the true voltage responses were recorded and used for subsequent display and analysis. All currents recorded under voltage clamp were corrected for leakage by subtracting suitably scaled templates. These were obtained by applying negative steps at a holding potential of −95 mV, which is just below the activation range of the voltage sensitive conductances found in fly photoreceptors (Weckström et al. 1991; Hardie 1991). In almost all our experiments this leak subtraction was performed on line, using standard PClamp protocols.

Optical stimuli. Cells were stimulated by a point source positioned at the point of maximum sensitivity, the optical axis. The point source was the tip of a fluid filled light guide, which received light from a 450 W Xenon arc lamp that was stabilised and modulated under optical feed-back (Weckström et al. 1991) within a bandwidth of 5 kHz. The cold mirror used to remove infrared also removed most of the energy at wavelengths below 400 nm. The stimulus was calibrated by counting quantum bumps in *Calliphora* photoreceptors, and the unattenuated source was equivalent to an effective capture rate of 8×10^8 photons/receptor/s by a dark adapted cell. This rate compares with an upper photopic level of 10^8 (Howard et al. 1987). Intensities were specified by the attenuation produced by neutral density filters placed in front of the eye. A Uniblitz shutter with high voltage driver was used to generate light pulses down to 3 ms duration. Incremental pulses of known contrast and duration (down to 1 ms) were superimposed on a continuous background by modulating the arc lamp.

The photoreceptor frequency response was derived under a particular condition of adaptation using the method described by Howard et al. (1984). Between 50 and 200 impulse responses to brief (< 2 ms) incremental flashes were collected and averaged. To derive the signal power spectrum, these responses were set to zero baseline and Fourier transformed using software written and executed in MathCad. The impulse responses were less than 5 mV for flashes delivered in darkness, and less than 2.5 mV when flashes were delivered to cells that were light adapted by a continuous background. These values lie within the linear response range, and give a good estimate of signal power spectrum (Howard et al. 1984). Strictly speaking, a cell only remains completely dark adapted when stimuli produce discrete single photon responses (isolated quantum bumps) at a very low rate. Such stringent conditions of dark adaptation were impractical for our wide-ranging comparative study of frequency responses. The dim flashes that we presented in darkness may have slightly light adapted the cells, consequently we refer to the resulting frequency responses as *near dark adapted*, nDA.

Results

Fast cells code high frequencies and slow cells do not

A cell's temporal coding ability is described by its frequency response, determined here by deriving the power spectra of low amplitude (< 5 mV) impulse responses (Fig. 1), averaged over 50–200 repetitions (Methods). We summarise a cell's temporal coding ability in terms of the corner frequency at which the signal power falls to half maximum. The higher the corner frequency, the better the frequency response and the less moving objects are blurred by the dynamics of transduction (rev. Laughlin 1981). Although the corner frequency falls far short of the highest frequency coded by a cell (Fig. 2), this parameter is directly comparable to the standard definition of the cut-off frequency of a low pass filter, as

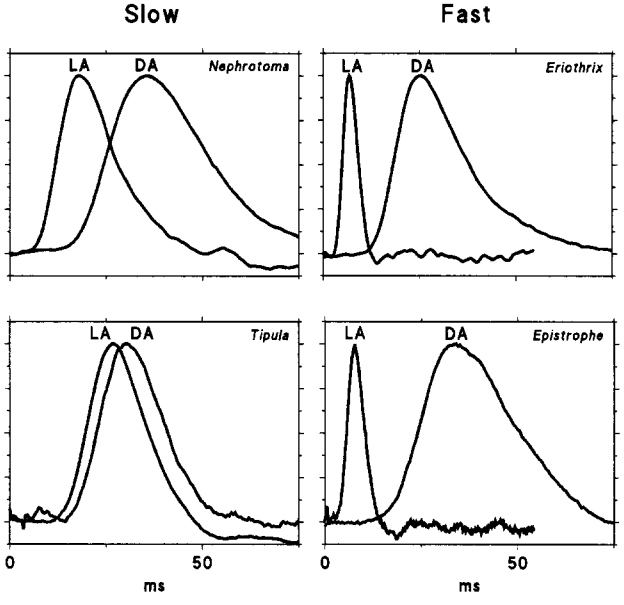

Fig. 1. Impulse responses recorded intracellularly from fast and slow photoreceptors under near dark adapted (*DA*) and light adapted (*LA*) conditions. In each case the stimulus was a brief (1–3 ms) incremental flash, delivered at time = 0 and the response amplitude is normalised to a fraction of the peak. Note that light adaptation greatly reduces the latency and duration of the fast cell responses but has less effect on the slow. The fast cells are from *Eriothrix rufomaculatus* (Tachinidae), response amplitudes – DA = 3.7 mV, LA = 1.3 mV; and *Epistrophe elegans* (Syrphidae), response amplitudes DA = 4.2 mV, LA = 1.1 mV. The slow cells are from *Nephrotoma quadrifaria* (Tipulidae), response amplitudes DA = 3.2 mV, LA = 2.4 mV; and *Tipula paludosa* (Tipulidae), response amplitudes, DA = 4.6 mV, LA = 2.0 mV

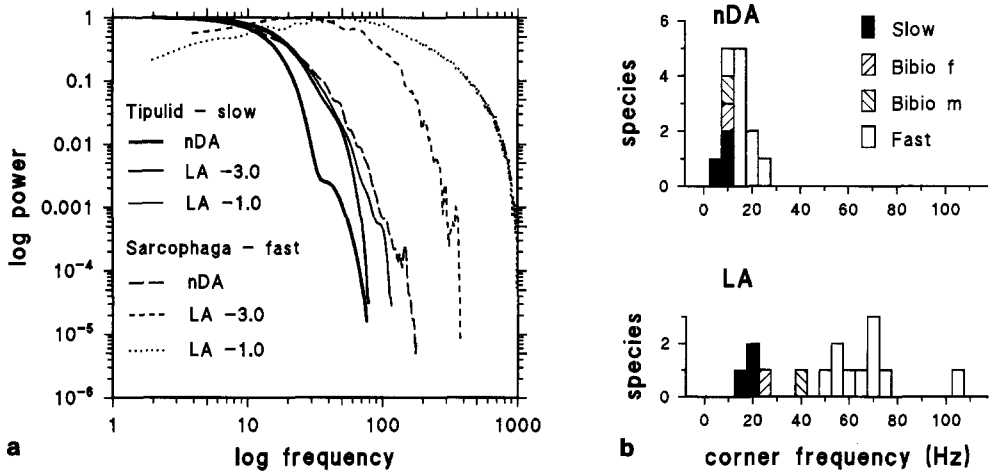

Fig. 2a, b. The large differences in frequency response that typify fast and slow cells. **a** Frequency responses measured under different conditions of adaptation: *nDA*, near dark adapted (see Methods); *LA*, light adapted at a background intensity indicated by the attenuation of the light source, in log units (Methods). The fast photoreceptor from *Sarcophaga* had the best frequency response recorded in this study. The slow cell from *Nephrotoma* was typical of Tipulids (Table 1). **b** The distributions of the corner frequencies measured under near dark adapted (*nDA*) and light adapted (*LA*) conditions, showing fast cells (as classified from light adapted data), slow cells, and male and female Bibionids. Data from Table 1

used to define the frequency response of the photoreceptor membrane.

The dipteran photoreceptors surveyed here fall into one of two distinct classes, depending upon their maximum (fully light adapted) frequency responses (Fig. 2). Fast cells achieve corner frequencies in the range 50–105 Hz while the slow cells from 3 Tipulid species have corner frequencies in the range 16–19 Hz. These two classes cover a surprisingly wide range of frequency response and the distribution of corner frequencies correlates with visual ecology. The fast cells belong to highly manoeuvrable diurnal species whereas the slow cells are from the more cumbersome crepuscular Tipulids. We will consider the one intermediate species, males and females of *Bibio markii*, in a later section.

As expected of arthropod photoreceptors (Fuortes and Hodgkin 1964; Zettler 1969; Pinter 1972; Howard et al. 1984), dark adaptation prolongs the impulse response, so leading to a reduction in frequency response. In the near dark adapted state, fast cell corner frequencies range from 12 to 25 Hz (8 spp.), slow cells from 7–11 Hz, and male and female *Bibio markii* are intermediate at 10–12 Hz (Fig. 2b; Table 1). Much of the variability in near dark adapted corner frequency, particularly among fast cells, results from the fact that it was not practical, in an extensive survey, to routinely dark adapt cells to their fullest extent. Nonetheless, it is clear that dark adaptation has by far the greatest effect on the frequency responses of fast cells, reducing their corner frequencies to a narrow range of values that approaches that of slow cells (Fig. 2b).

Slow cells are more sensitive than fast and light adapt less rapidly

These differences in absolute sensitivity and light adaptation dynamics are plainly exhibited by the step responses to prolonged pulses of light (Fig. 3). The low level responses of slow cells are noisier than the fast, and the noise fluctuations are slower. These differences in noise indicate that the elementary transduction events triggered by photon absorptions are larger in amplitude and longer in duration in slow cells. This suggestion is confirmed by recordings of quantum bumps (Fig. 4). Bumps averaging 2.4 ± 0.8 mV, $n = 59$; and lasting approximately 121 ± 27 ms were recorded from a dark adapted cell of *Tipula paludosa*. These values compare with amplitudes of 1–2 mV and durations of approximately 40 ms recorded from R1–6 in the typical fast eyes of *Musca*, *Lucilia* and *Calliphora* (Hardie 1985; Howard et al. 1987; Laughlin et al. 1987) under more stringent conditions of dark-adaptation. Plots of response amplitude against log intensity (Fig. 5) demonstrate that, as expected from the larger quantum bumps, the dark adapted slow cells are more sensitive to a point source (Table 1). The mean intensities required to elicit a half maximal response for dark adapted fast eyes ranges from $\log I = -4.52$ for a deeply dark adapted *Sarcophaga* cell to $\log I = -1.9$ for *Eristalis* (Table 1). The intensities for half maximal response in the Tipulid species ranged from $\log I = -5.2$ to $\log I = -4.7$ (Table 1).

The step responses of fast and slow cells have different waveforms. In fast cells the response to dimmer steps is monophasic, with the membrane potential level remaining relatively constant throughout the light pulse (Fig. 3; c–f). In the slow cells (Fig. 3a, b), the level of depolarisation increases steadily during responses of similar amplitude. We show later that this gradual rise can be attributed, at least in part, to the inactivation of a voltage sensitive potassium conductance. At higher light levels both fast and slow cells adapt, the response declining from an initial peak to a stable plateau. In fast cells this adaptation is rapid and powerful, typically reducing a saturated response by 40–60% of its initial amplitude in the first 50–200 ms of the light step. Ex-

Table 1

Family	Species	Cell type	R_{in} MΩ		T_m ms		cf		$\bar{V}_{max}$	$logI_{50}$	FDR	SDR	IOC	*n*
			DA	LA	DA	LA	DA	LA						
Tipulidae	*Tipula lunata*	Slow	52		14					−4.7	−	−	+	5
	Tipula paludosa	Slow	121	40	25	11	11	19	30	−5.5	−	−	+	28
	Tipula marmorata	Slow	68	46	12	10	7	16	15		+	−	+	7
	Nephrotoma quadrifaria	Slow	48	27	7	3	10	19	22	−5.2	−	−	+	4
	Ctenophora atrata	Slow	>100		20						−	−	+	3
	Limonia nubeculosa	Slow	60		11						−	−	+	2
Bibionidae	*Bibio markii* male	Fast?	43	9	16	3	12	42	19		+	+	−	19
	female	Slow?	60	21	4	2	10	27	18	−4.0	+	−	−	10
Therevidae	*Thereva nobilitata*	Fast	19		2						+	+	−	3
Asilidae	*Philonicus albiceps*	Fast	15	8	2	0.7	20	71	11	−3.1	+	+	−	12
	Asilid spp.	Fast	21		3		21	56	11	−3.0	+	+	−	4
Syrphidae	*Episyrphus balteatus*	Fast	13		2		15	77	19	−1.9	+	+	−	8
	Volucella pelluscans	Fast	18	4	4	1.7	15	50	21	−4.4	+	+	−	2
	Eristalis tenax	Fast	10		3		15	55	19	−1.9	+	+	−	4
	Epistrophe elegans	Fast	21	7	2	0.9	12	65	13	−3.7	+	+	−	3
Scatophagidae	*Scatophaga sterocorarium*	Fast	40		3.3			>38			+	+	−	6
Tachinidae	*Eriothrix rufomaculatus*	Fast	23	11	2.4	1	14	69	11	−2.7	+	+	−	8
Muscidae	*Fannia canicularis*	Fast	18		2.8			60	16	−2.7	+	+	−	17
Calliphoridae	*Sarcophaga carnaria*	Fast	26	8	2	1	15	104	25	−4.5	+	+	−	14
	Calliphora vicina[a]	Fast	32	9	4	0.8	25	72	21		+	+	−	70
Hippoboscidae	*Crataerina pallida*	Fast?	36		4					−4.3	+	+	−	9

Coding properties and membrane parameters for the 20 species surveyed in this study. [a] = data from Weckström et al. (1991). The photoreceptor cell type, fast or slow, is assigned either according to the frequency response or, in the few cases where these measurements are absent, according to the response waveform and current clamp response. R_{in} = photoreceptor input resistance, measured with a hyperpolarising pulse, average of all measurements. DA = dark adapted state, LA = light adapted. T_m = cell time constant, average for all measurements, DA = dark adapted, LA = light adapted. cf = corner frequency of the signal power spectrum, average for all measurements. $\bar{V}_{max}$ is the amplitude of the steady state response to daylight backgrounds, average of all measurements. $logI_{50}$ = the log of the intensity required to elicit a half-maximal peak response from a dark adapted cell. FDR, SDR and IOC signify the presence (+) or apparent absence (−) of a fast activating delayed rectifier, a slow activating delayed rectifier and an inactivating outward current respectively. *n* = the number of cells recorded from the species. The photoreceptors of *Scatophaga* were not fully light adapted and the corner frequency is a lower bound

treme examples of rapid adaptation are provided by the Asilids (e.g. *Philonicus albiceps* in Fig. 3d) and the Tachinid *Eriothrix rufomaculatus*. By comparison, the adaptation of slow cells is weaker and more sluggish. Even with saturated responses, it is rare to see a reduction of more than 30% in the first 500 ms and adaptation often takes several seconds to complete (Fig. 4). In support of this finding, the Tipulid V/log I curves have steeper slopes (Fig. 5), indicative of less light adaptation during the rising phase of the response (Matic and Laughlin 1981). The weaker adaptation of slow cells results in larger steady state responses to sustained backgrounds with values from 22–30 mV, as compared with a range from 11–25 mV in fast cells. Indeed, the fast

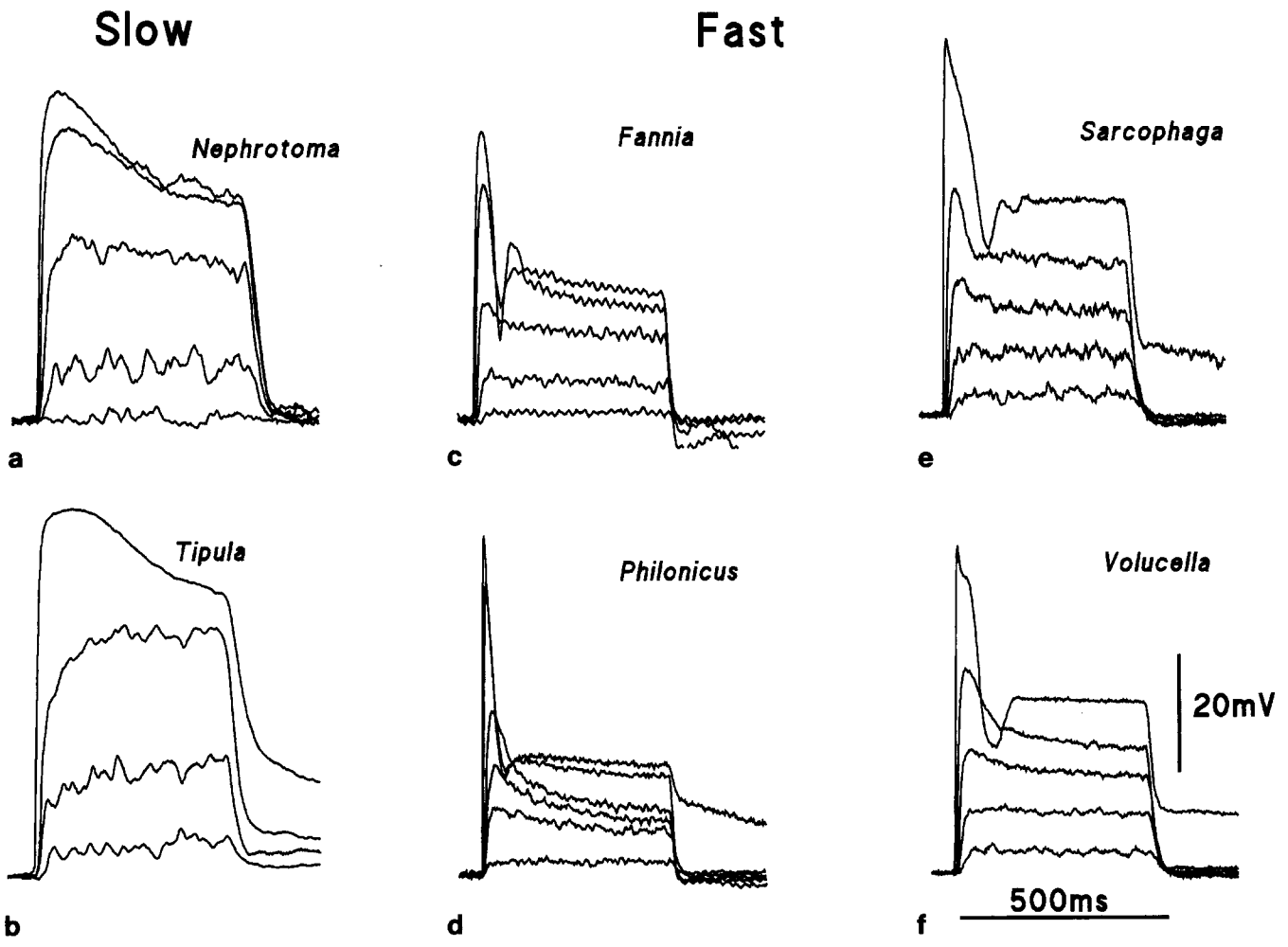

Fig. 3a–f. Fast and slow cells differ markedly in their responses to 500 ms light pulses, delivered in darkness. In each case a series of responses is illustrated, spanning the dark adapted response range. Note the noisier low level responses of slow cells and the rapid and pronounced adaptation of fast cells. **a** *Nephrotoma quadrifaria* (Tipulidae); **b** *Tipula paludosa*; **c** *Fannia canicularis* (Muscidae); **d** *Philonicus albiceps* (Asilidae); **e** *Sarcophaga carnaria*; **f** *Volucella pelluscans* (Syrphidae)

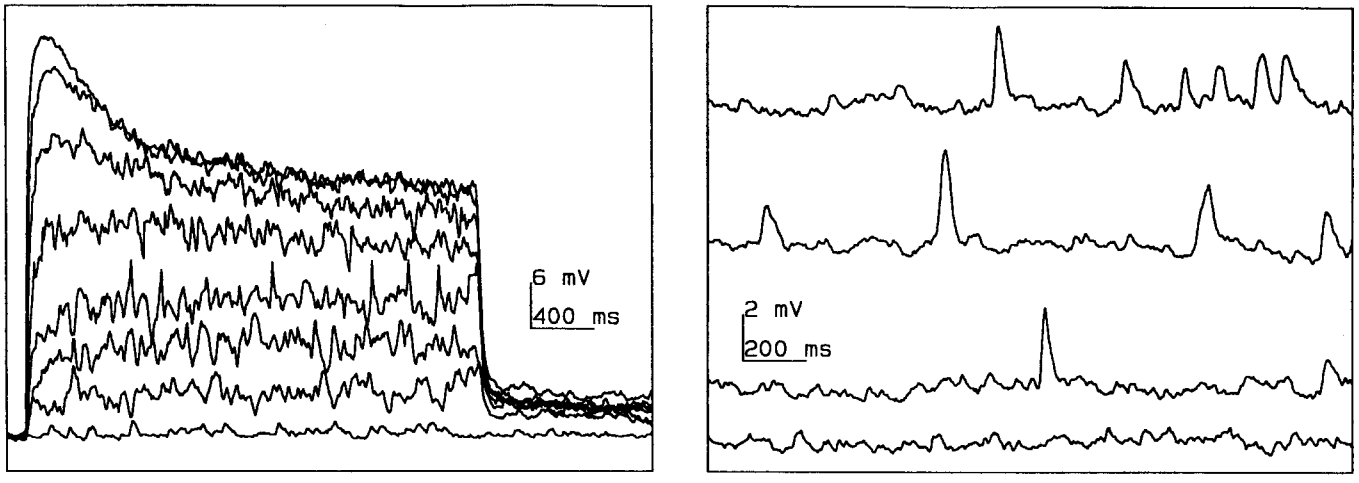

Fig. 4. Slow cells adapt relatively weakly, as illustrated from a series of prolonged responses recorded from a dark adapted cell in the retina of *Tipula lunata*. Slow cells have a high gain when dark adapted, as illustrated by these large quantum bumps recorded from *Tipula paludosa*

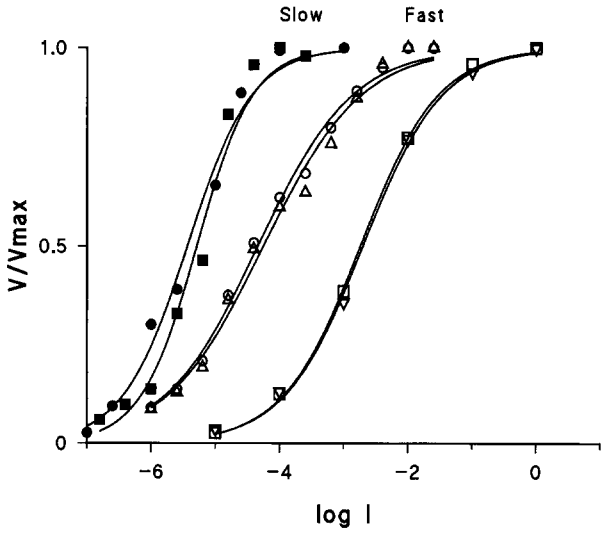

Fig. 5. Dark adapted slow cells (*closed symbols*) are more sensitive to an axial point source than fast cells (*open symbols*), and their V/log I curves are steeper. Each set of data points has been normalised to a proportion of maximum response, V_{max}, and fitted with a hyperbolic function of the form $V/V_{max}=I^n/(I^n+I_{50}{}^n)$ (Lipetz 1971) where n is an exponent governing the slope and I_{50} is the intensity required for half-maximal response. The slow cells are from *Tipula paludosa* (●), $I_{50}=3\cdot10^{-6}$; n=0.82; and *Tipula lunata* (■), $I_{50}=3.6\cdot10^{-6}$, n=1.0. The fast cells are from *Sarcophaga carnaria* (△), $I_{50}=4.9\cdot10^{-5}$, n=0.57; *Volucella pelluscans* (○) $I_{50}=4.1\times10^{-5}$, n=0.60; *Eriothrix rufomaculatus* (▽) $I_{50}=2\times10^{-3}$, n=0.71; and *Fannia canicularis* (□) $I_{50}=1.7\times10^{-3}$, n=0.72

cells of the Asilids and Tachinids produce surprisingly low levels (11 mV) of sustained response to the brightest background lights (Table 1). Extracellular recordings made immediately after withdrawing the electrode from these cells show that this low level of sustained response cannot be attributed to contamination by the hyperpolarising field potential that is commonly recorded in the retina (Shaw 1975; Howard et al. 1987). However, these strongly adapting cells often show a large negative after-hyperpolarisation following the cessation of a light response, which suggests that electrogenic pumps (Jansonius 1990) could be making an important contribution to membrane repolarisation during light adaptation.

The differences in response waveform between fast cells and slow cells are pronounced and characterise these two classes. The smaller differences observed among the members of a single class (e.g. Fig. 3) are more difficult to interpret. In our experience, the amplitude and duration of the on transient is sensitive to the depth of dark adaptation, and the amplitude of the plateau potential varies considerably from cell to cell within the same species (e.g. Howard et al. 1987). Given these uncontrolled differences only extreme specialisations, such as the sharp on transients of Asilids (Fig. 3d) can be said to typify a particular group of insects or a species. The significance of smaller differences, such as *Nephrotoma* cf *Tipula* (Fig. 3a, b) and *Sarcophaga* cf *Volucella* (Fig. 3e, f) cannot be assessed from present evidence.

Slow cells have higher input resistances, longer time constants and rectify less

Current pulses injected via the single electrode switching clamp (Methods) allowed us to measure photoreceptor membrane resistances and time constants, and to observe the effects of voltage sensitive conductances. Both slow and fast photoreceptors rectify, particularly close to resting potential (Figs. 6, 7). As a result, the measurements of membrane resistance made by hyperpolarising cells give higher values of input resistance than those made by depolarising them. To take account of rectification, input resistances are specified with respect to negative (−) and positive (+) current injections. At rest the slow photoreceptors have high input resistances, ranging in total between (−) 200 MΩ and (−) 27 MΩ for hyperpolarising current and (+) 80 MΩ and (+) 22 MΩ for positive current. The mean input resistances for the slow cells of the most extensively investigated Tipulid, *Tipula paludosa* are (−) 121.5±37.3; (+) 72.7±8.4 MΩ. By comparison the input resistances of resting fast cells are usually much lower, ranging from (−) 52 to (+) 12 MΩ, with means of (−) 23.5±9.3 MΩ and (+) 16.6±9.0 MΩ taken across all fast cells. The higher resistances of dark adapted slow cells are accompanied by longer time constants (Table 1).

It can be argued that the lower resistances of fast cells result from a greater susceptibility to electrode damage. In several species this hypothesis was tested directly by measuring input resistances when cells were hyperpolarised with a tonic current to −80 or −90 mV. In all cases we tested, hyperpolarisation increased the input resistance by a factor of at least two. For example, in *Fannia canicularis* a cell with an input resistance of (−) 13 MΩ at resting potential (−65 mV), had a resistance of (−) 32 MΩ when hyperpolarised to −80 mV. In the Asilid spp. a cell with an input resistance of (−) 14 MΩ at r.p.= −61 mV attained (−) 50 MΩ when hyperpolarised to −90 mV. These findings demonstrate that the activation of voltage sensitive conductances is primarily responsible for the lower input resistance of fast cells. I/V curves (Fig. 7) obtained by plotting steady state polarisation versus applied current confirm that voltage sensitive conductances are more powerful in fast cells, giving a more pronounced rectification. In addition, there are consistent differences among the fast eyes, with the Muscid *Fannia* exhibiting the strongest rectification (Fig. 7).

Current clamp indicates that fast and slow cells have different types of voltage sensitive conductance

Rectification suggests that the membranes of both fast and slow cells contain conductances that are activated by depolarisation. Moreover, the marked differences between the waveforms of the voltage responses to injected current (Fig. 6) suggest that different sets of conductances inhabit the membranes of fast and slow cells. Fast cells depolarise tonically to small currents and, in *Calliphora*, this has been shown to be symptomatic of a rapid-

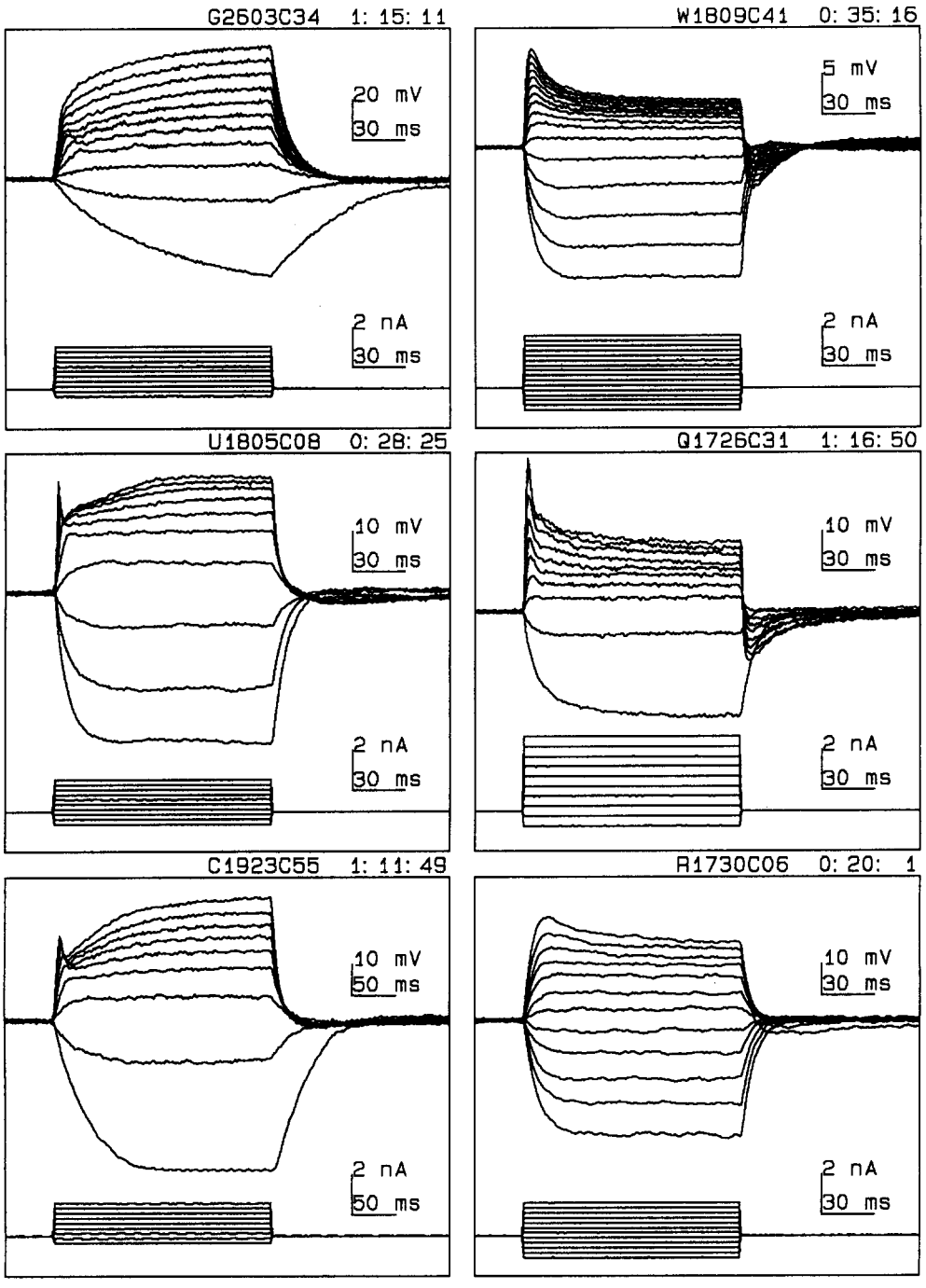

Fig. 6. Slow cells (*left hand column*) and fast cells (*right hand column*) produce distinctly different responses to injected current. The upper traces in each panel show the voltage responses and the lower traces the applied current. The cells tested are, from top to bottom: slow, *Tipula lunata*, *Nephrotoma quadrifaria*, *Tipula paludosa*; fast, *Fannia canicularis*, Asilid spp., Dolichopodid spp.. Note that the Dolichopodid does not appear in Table 1. Its retina was extremely fragile and difficult to record from but cells could be held long enough to provide the unequivocal current clamp data shown here

ly activating component of the delayed rectifier. Larger currents injected into fast cells produce a depolarisation that rises to a peak and then decays to a stable plateau. In *Calliphora* this transience is associated with a more slowly activating component of the delayed rectifier (Weckström et al. 1991).

The current clamp responses of slow cells are inconsistent with the action of fast and slow delayed rectifier conductances. Instead of rising to an initial peak and then decaying, the slow cells's membrane potential exhibits a sharp spike and then increases steadily during the first few hundred milliseconds of depolarisation (Fig. 6). As will be demonstrated below, this slow increase indicates the inactivation of a fast potassium current, quite unlike the delayed rectifier of fast cells, but resembling currents described in *Drosophila* (Hardie 1991). The small spike, seen in some slow cells at the initiation of depolarisation (Fig. 6), is discussed in connection with voltage clamp observations in the next section.

Fast and slow cells produce voltage gated outward currents with different dynamics

In *Calliphora*, the currents measured by applying single electrode voltage clamp techniques to resting cells in the

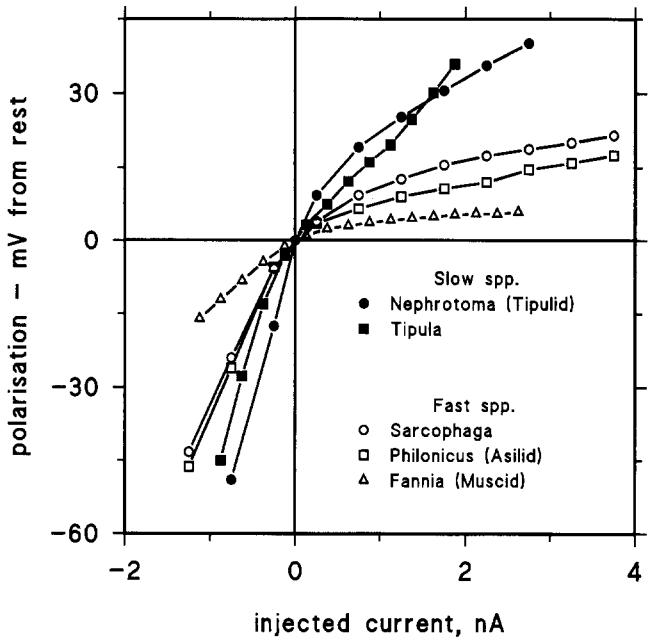

Fig. 7. Steady state I/V curves demonstrate the slow cells have higher input resistances and rectify less than fast cells. Membrane depolarisation, from resting potential, was measured 200 ms after the application of a current pulse. The slow cells were from *Nephrotoma quadrifaria* and *Tipula paludosa*. The fast cells were from *Sarcophaga carnaria*, *Philonicus albiceps* and *Fannia canicularis*

intact retina are very similar to those measured by the rigorous application of patch and whole cell clamp techniques to isolated photoreceptors (Weckström et al. 1991). This suggests that the single electrode voltage clamp of intact cells can provide a reasonable qualitative account of voltage sensitive potassium conductances in insect photoreceptors. We applied this technique to the photoreceptors of all the species reported here and found that, in line with rectification, the depolarisation of both fast and slow photoreceptors always elicited substantial outward currents.

In the fast cells the outward current exhibits the two kinetic components, first described in *Calliphora* (Weckström et al. 1991) (Fig. 8). The fast component activates rapidly and dominates the response close to resting potential. The slow component activates more slowly and increases in relative size when the cell is more depolarised (Fig. 8). As in *Calliphora*, the progressive activation of these two components accounts for the change in waveform of the current clamp response, from tonic close to resting potential, to phasic at higher levels of depolarisation (Fig. 6). There is a tendency for the outward current to inactivate slightly at the highest holding potentials and the time course and precise extent varied from cell to cell and species to species (Figs. 8, 9). Where prominent tail currents are seen, these are abolished or reversed at holding potentials in the range of −85 to −95 mV. Similar currents (Fig. 8) are elicited from *Calliphora* photoreceptors and, on the basis of their reversal potential, sensitivity to TEA and single channel properties, have been shown to result from the activation of voltage sensitive potassium channels (Weckström et al. 1991). Given this similarity in the activation range, kinetics (Fig. 8) and reversal potentials, the outward currents recorded here from fast photoreceptors are attributed to the non-inactivating fast and slow delayed rectifier potassium channels, similar to those discovered in *Calliphora* (Weckström et al. 1991).

The outward currents elicited by depolarising slow cells are very different, because they always activate rapidly and then inactivate strongly, even at moderate holding potentials (Fig. 9). This transient activation explains the slow increase in the current clamp response (Fig. 6), and contributes to the steady rise in receptor potential during low intensity light pulses (Fig. 3). The time constant of inactivation of these currents, as judged from their decay, is in the order of 50–100 ms. A smaller sustained outward current is seen in the slow cells. In *Tipula marmorata* this non-inactivating current could be observed in isolation by holding the cell at a level of depolarisation that inactivated the much larger transient currents. The sustained current activates rapidly in response to step depolarisations and on this basis it is categorised as a fast delayed rectifier (FDR – Table 1). In *Tipula paludosa* and *Nephrotoma quadrifaria* the tail currents reversed in the region of −80 to −95 mV, suggesting that, as proved for similar currents in *Drosophila* (Hardie 1991), these outward currents are carried by potassium ions.

In several slow cells, and very occasionally in some fast cells, depolarisation triggered an inward current that was too fast to be properly resolved by the single electrode clamp. In some cases this inward current was accompanied by a rapid transient outward current. For example, in a number of Tipulid cells, steps from a holding potential of −90 mV revealed a very rapid transient outward current. Because of the limitations imposed by the time constants of these cells the precise rate of decay could not be determined but it was less than 5 ms. These properties suggest an A-current, similar to the one found in *Drosophila* (Hardie et al. 1991; Hardie 1991). The cells that exhibited these rapid inward and outward currents showed rapid transients on the rising phase of current clamp response (Fig. 6), particularly when the cell was hyperpolarised prior to application of the depolarising pulse. However, the technical limitations of single electrode voltage clamping, and our inability to rapidly and reliably change extracellular ion concentrations in an intact preparation, precluded further analysis of these currents. Similar observations have been made when recording from photoreceptor synaptic terminals in the blowfly lamina (Weckström et al. 1992).

Light adapted slow cells have a much lower conductance than the fast

Measurements of the input resistances and time constant of light adapted cells (Fig. 10) confirm that fast and slow photoreceptors have opted for distinctive strategies of signalling and membrane tuning. Light adapted fast cells have a relatively low input resistance (4–12 MΩ; Table 1)

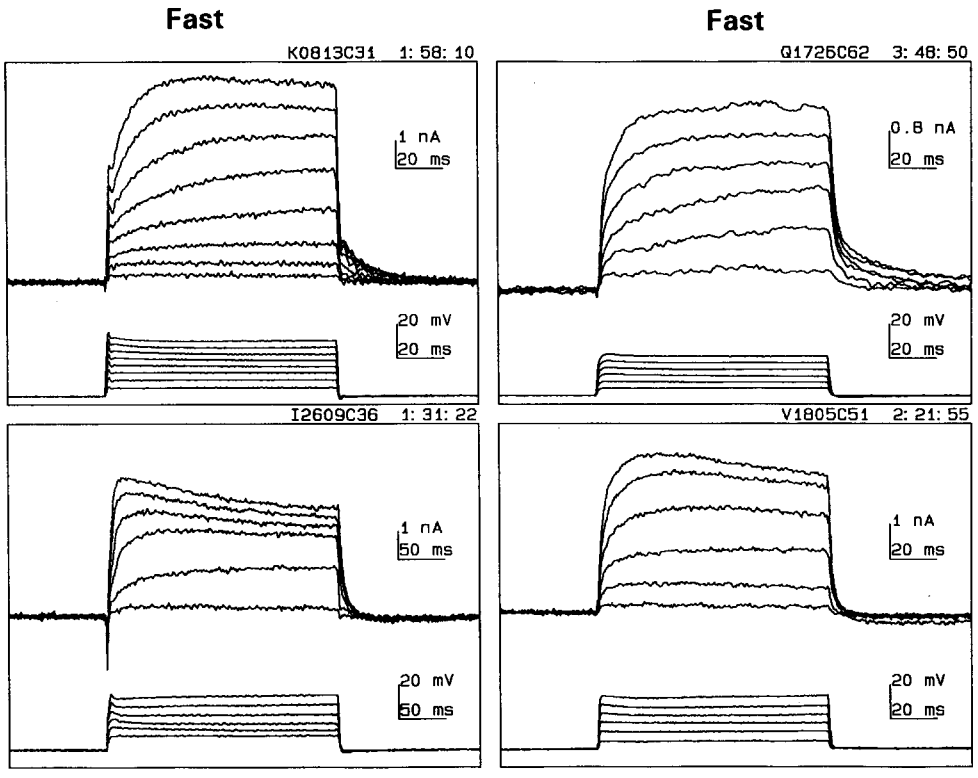

Fig. 8. Under voltage clamp, fast cells produce outward currents (*top traces* in each panel) in response to depolarisation (*bottom traces* in each). These currents exhibit the fast and slow activating components, and inactivate weakly (if at all), as typified by the delayed rectifier of *Calliphora vicina* (Weckström et al. 1991) – shown top left. The remaining examples are, top right – Asilid spp.; bottom left *Volucella pelluscans*, bottom right *Eriothrix rufomaculatus*. In all cases, voltage steps were made from a holding potential of −65 mV (close to resting potential) and currents were corrected for leakage (Methods)

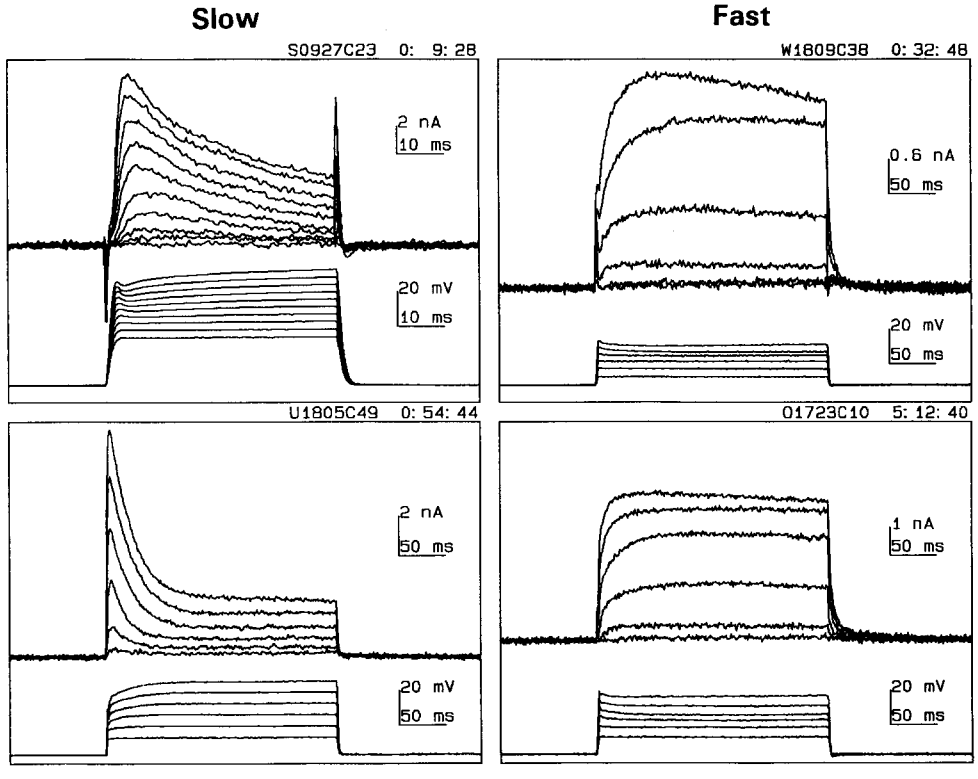

Fig. 9. Under voltage clamp, slow cells elicit transient outward currents, as opposed to the sustained currents produced by fast cells. The slow cells are (*top left*) *Tipula paludosa* holding potential −95 mV, note the shorter time scale, (*bottom left*) *Nephrotoma quadrifaria* holding potential −65 mV. The fast cells are (*top right*) *Fannia canicularis* and (*bottom right*) *Thereva nobilitata*, both held at −65 mV. All current traces have been corrected for leakage (Methods)

and, as in *Calliphora* (Weckström et al. 1991; Jansonius 1990) this is associated with a relatively low level of steady depolarisation, generally in the range 10–25 mV. These low input resistances suggest that a substantial light gated conductance is vigorously opposed by the non-inactivating voltage gated potassium conductances that typify this class of cell. As a result, the membrane time constant is reduced to a point where it does not significantly band-limit visual signals (0.7–1.7 ms; Table 1).

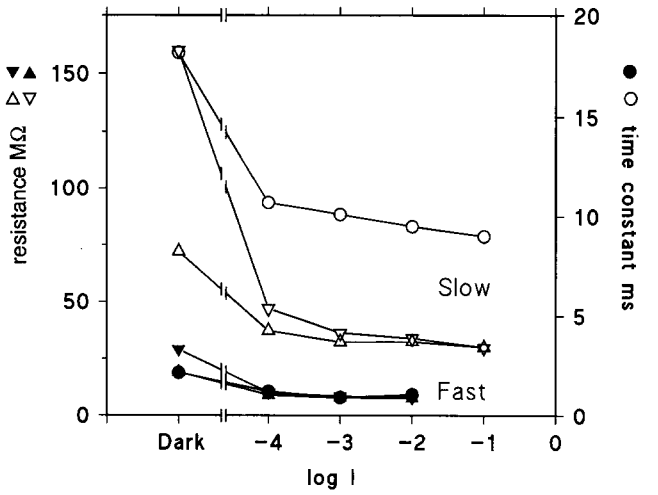

Fig. 10. A comparison between the membrane resistances (*triangles, left hand abscissa*) and time constants (*circles, right hand abscissa*) of a fast and a slow cell, when dark adapted, and stably light adapted at the log background intensities indicated. The slow cell, from *Tipula paludosa* (*open symbols*) has a consistently higher resistance and longer time constant than the fast cell, from *Sarcophaga carnaria* (*closed symbols*). (▼, ▽) = resistances measured by hyperpolarising the cell and (▲, △) = resistances measured by depolarising

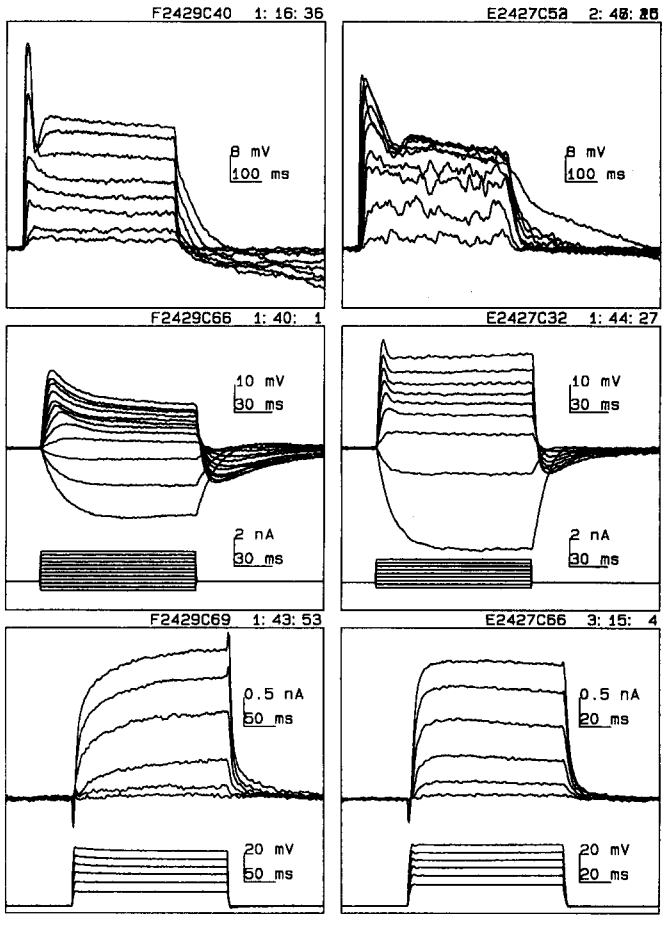

Fig. 11. Sexual dimorphism in photoreceptor response in *Bibio markii*, male responses on the *left* and female on the *right*. Differences can be observed in the response of dark adapted cells to light pulses (*top panels*), to applied current (*middle panels*) and in the leak corrected voltage clamp responses to depolarisation from a holding potential of −65 mV (*bottom panels*). Note that the male cell exhibits characteristics of fast cells, namely a less noisy light response, more pronounced adaptation (*top panels*), greater delayed rectification (*middle panels*), and both fast and slowly activating outward currents (*bottom panels*). The female cell's light response is noisier and adapts a little more slowly (*top panels*). Her response to injected current shows a fast initial notch but no delayed rectification, and in voltage clamp there is little evidence of a slowly activating component of the sustained outward current

Slow cells have a high input resistance and long time constant when both dark and light adapted. Thus, in bright light both the steady state light gated conductance, and the conductances that oppose it are much smaller. The higher plateau potential of slow cells suggests that, in the steady state, their voltage activated conductance is a smaller proportion of the light activated. These measurements of light adapted membrane resistances agree with the findings that steady state rectification is less pronounced in slow cells, and that a substantial proportion of the voltage induced outward current inactivates. Slow cells with a high light adapted input resistance retain a long time constant that significantly restricts their signal bandwidth. The average membrane time constant of 11.1 ms in *Tipula paludosa* yields a membrane cut-off frequency of 14 Hz, as compared to an average corner frequency for light signals of 19 Hz (Table 1).

Bibionids demonstrate sexual dimorphism in photoreceptor physiology

If, as our observations suggest, photoreceptor performance and membrane physiology follow the dictates of lifestyle and habitat, then one should find differences between closely related individuals with distinctive visual ecologies. The primitive family, the Bibionidae, provide the opportunity to test this suggestion. Male Bibionids have a greatly enlarged dorsal eye, specialised for the detection, tracking and interception of females, prior to mating, and this sexual dimorphism is a response to sexual selection (Zeil 1983a, b). Our recordings show that male and female photoreceptors differ, both in their responses to light, and in the electrical properties of their membranes. Responses to light steps (Fig. 11) show that the female cells are more sensitive, as judged by the higher noise levels. In addition the female photoreceptors light adapt less quickly, and have a relatively poor frequency response when light adapted, with a corner frequency intermediate between the fast and slow cells, of 26.7 ± 3.4 Hz, $n = 4$. By comparison (Fig. 11), the male dorsal cells are insensitive, with relatively low noise levels. Male dorsal photoreceptors light adapt very quickly (Fig. 11) and have a better light adapted frequency response than the female, with a corner frequency of 42.4 ± 5.3 Hz, $n = 9$, at the low end of the fast cell range. In our experiments the low sensitivity of male dorsal cells, as indicated by noise levels, is compounded by a lack of stimulus energy (Methods) at the short wave-

lengths that best drive these cells (Burkhardt and de la Motte 1972). By moving the light guide as close as possible to the eye we could just saturate the response of dark adapted male dorsal cells. This weaker stimulation raises the possibility that the male cells were not fully light adapted and, as a result, their frequency response has been underestimated.

These differences between male and female light responses are accompanied by differences in the electrical properties of the photoreceptor membrane. Although female cells have higher resistances, they have shorter time constants – presumably because they are shorter and have much smaller rhabdoms than male cells. Female cells are more weakly rectifying than male (Fig. 11) and their response to the injection of positive current is almost entirely tonic (Fig. 11). In the male the response is decidedly phasic under comparable conditions. Voltage clamp confirmed that the differences in current clamp response resulted from differences in the activation kinetics of voltage gated potassium conductances. The outward currents elicited by depolarising female photoreceptors (Fig. 11) are rapidly activating at all but the very highest holding potentials and inactivate slowly, if at all. By comparison (Fig. 11), the male photoreceptor outward currents are dominated by a slowly activating component. Thus both the responses and membranes of photoreceptors are sexually dimorphic and, as discussed below, this physiological dimorphism can be directly related to functional demands and constraints.

Discussion

Fast and slow photoreceptors in the Diptera

Our survey of 20 species of Diptera, covering several major groups, reveals differences in photoreceptor frequency response that are much larger than those found in the one comparable study of light adapted cells (Howard et al. 1984). The majority of cells are fast, with a frequency response that increases with light adaptation from near dark adapted corner frequencies of 12–25 Hz to fully light adapted values of 50–104 Hz, depending upon the species (Fig. 2; Table 1). In addition to this marked improvement in frequency response, these fast cells share with the more intensively studied R1-6 cells of *Calliphora* (Jansonius 1990; Weckström et al. 1991) the following properties. Light adaptation is fast and effective, leading to low values of plateau potential in the fully light adapted state, a low input resistance, and a short membrane time constant. This low resistance can be partly attributed to the presence of a powerful non-inactivating delayed rectifier conductance that produces a strong rectification in the response of the membrane to current. Cells recorded from the retinas of tipulids have very different coding characteristics and membrane properties. Tipulid cells are slow, with near dark adapted corner frequencies of 7–11 Hz, and light adapted values of less than 20 Hz (Fig. 2; Table 1). This very inferior temporal resolving power is accompanied by a number of other distinguishing characteristics. In slow cells, light adaptation is slower and less pronounced than in fast cells, giving higher steady state plateau potentials in bright light. Slow cells have much higher input resistances and time constants under all conditions of adaptation. In the dark adapted state this is associated with larger quantum bumps and a higher sensitivity. The higher light adapted input resistance results, in part, from the fact that the voltage sensitive potassium conductances in the slow cell membranes are smaller in magnitude and activate transiently – a difference that is reflected in the smaller degree of steady state rectification shown by slow cells.

The distinction between fast and slow cells in a single insect order was first drawn by de Souza and Ventura (1989), who compared green sensitive cells among Hymenoptera. They measured temporal coding ability in the near dark adapted state by determining the linear integration time of the photoresponse. Two species of slow moving nocturnal ant have slow photoreceptors with long integration times while one rapidly moving diurnal ant and three diurnal bees have fast photoreceptors with shorter integration times. A slower diurnal ant is intermediate. The Hymenopteran photoreceptors show the same distinctive differences in sensitivity and waveform as our Dipteran cells. Fast cells have low noise levels, shallow V/log I curves and light adapt rapidly. Slow cells fail to light adapt rapidly and the response waveform one Hymenopteran, *Camponotus*, is very similar to the Tipulid, with large bumps and a high noise level. The second slow species *Atta sexta* has a steeper V/log I curve. Thus our study of the Diptera supports de Souza's and Ventura's (1989) distinction and extends the comparison of fast and slow types to the light adapted state, and to the level of the photoreceptor membrane.

The differences between fast and slow cells raise a number of questions and we will discuss the following. What roles do voltage gated potassium channels play in phototransduction? What is the functional significance of the different types of conductance found in fast and slow cells? What factors constrain the performance of photoreceptors, and how do these constraints relate to the distinction between fast and slow photoreceptors?

The role of potassium channels in phototransduction

The comparison of fast and slow cells supports our proposal that potassium channels play an important role in adaptation and coding. Our study of the archetypical fast cells of the blowfly *Calliphora* (Weckström et al. 1991) suggested that potassium conductances tune the photoreceptor membrane to meet the demands of habitat and lifestyle. Blowfly photoreceptors respond effectively over a wide dynamic range. Near darkness they produce quantum bumps of 1 to 2 mV amplitude but in full daylight 10^5 bumps sum to produce a response of 20–30 mV (Howard et al. 1987). As commonly found in photoreceptors, this reduction in gain is accompained by a profound improvement in frequency response

(Zettler 1969) that has been associated with a reduction in the time course and latency distribution of events in the phototransducing cascade (Wong 1978; Howard et al. 1987). The *Calliphora* delayed rectifier potassium channels match the impedance of the photoreceptor membrane to the currents generated by light gated channels as they change with adaptation state. This delayed rectifier has three properties that enable it to operate effectively. First, its wide activation range encompasses the normal range of light responses. Thus the membrane has a high input resistance and long time constant when the cell is at resting potential, so matching the high gain and slower response of dark adapted quantum bumps. The conductance then activates progressively as the cell is depolarised by steady background lights. The resulting drop in membrane resistance lowers its gain and time constant to values appropriate for operations at high light levels. Pharmacological intervention shows that the activation of the delayed rectifier is essential for coding the high frequency signals generated by the light adapted cascade (Weckström et al. 1991). A second, and most important advantage, is the failure of the delayed rectifier to inactivate significantly. Thus the membrane retains its low gain and excellent frequency response for as long as it is depolarised by light. Third, the activation kinetics of the delayed rectifier are advantageous. Close to resting potential (i.e. in dim conditions), the rapidly activating, fast component dominates. At the higher levels of depolarisation (i.e. in brighter light) the fast component is superseded by the slow, and the longer activation time constant converts the membrane into a high pass filter that spares high frequency signals. Given that these specialisations are advantageous because they promote the efficiency with which voltage signals are coded (Weckström et al. 1991), one might expect this type of delayed rectifier to be incorporated into the membranes of many, if not all, fast photoreceptors.

Our comparative study strongly supports this expectation. Rapidly flying diurnal Diptera have cells that light adapt quickly to attain a good frequency response. These fast cells all contain a prominent delayed rectifier that resembles *Calliphora*'s. By comparison, the photoreceptors of slowly flying nocturnal forms are slow, both in their frequency response and in their speed of light adaptation. The membrane properties of these slow cells are dominated by rapidly inactivating currents and, when the non-inactivating delayed rectifier can be unambiguously detected (e.g. *Tipula marmorata*, Table 1), only the rapidly activating, fast component is seen. The functions of the rapidly inactivating potassium currents that dominate slow cells have not been established, but they could help to suppress the large transients generated in cells with a high sensitivity. Furthermore, the high input resistances of slow cell somata may be allowing us to observe the conductances employed at the photoreceptor synaptic terminal in the lamina (Weckström et al. 1992) – a suggestion that could also explain the observation of a rapid inward current in slow cells. Despite these uncertainties, the correlation between membrane conductance and response dynamics found here, demonstrates that fast and slow cells use different sets of potassium channels to tune their membranes in a functionally appropriate manner. Furthermore, our data provides strong comparative evidence that the slowly activating component of the delayed rectifier is specifically incorporated within the fast cell membrane to permit the rapid responses needed to guide agile flight.

Our proposal that, within the Diptera, both the dynamics of the phototransduction cascade and the photoreceptor membrane are tuned to functional requirements must be considered more critically. We only found the slow photoreceptor type in the most primitive family of Diptera, the Tipulidae. One could argue, therefore, that the distinction between fast and slow cells is phylogenetic rather than functional. The phylogenetic argument can be countered by three observations, First, another primitive Nematoceran group, the Bibionidae, have faster cells with the characteristic non-inactivating delayed rectifier. Thus any phylogenetic division between transduction cascade properties or potassium channel genes would have to have occurred at the base of the Diptera. Second, an advanced slowly flying dipteran that prefers dim conditions, *Drosophila* (Shorrocks 1972), lacks *Calliphora*'s delayed rectifier and exhibits rapidly inactivating currents (Hardie 1991), similar to the Tipulids'. Third, a similar distinction between fast and slow cells has been made in the Hymenoptera (de Souza and Ventura 1989). Nonetheless, in the absence of data from nocturnal Diptera from more advanced families, a strictly phylogenetic argument cannot be totally dismissed. A second apparent anomaly is that the photoreceptors of the Hippoboscid, *Crataerina*, light adapt rapidly and display the delayed rectifier that typifies fast cells. Unfortunately, fully light adapted frequency responses could not be determined in recordings from this very small eye. This advanced Dipteran is a flightless parasite of swifts, and the ecological significance of its fast cell properties is not apparent, unless one chooses to believe that a parasite likes to see where it's host is flying. Having noted these qualifications, we will now review the evidence that the distribution of potassium channels among Dipteran photoreceptors is an evolutionary response to functional requirements.

A functional basis for the diversity of potassium channel expression?

Molecular studies suggest that a set of 4 basic potassium channels, *Shaker*, *Shal*, *Shab* and *Shaw* existed at the time of the Cambrian radiation (Salkoff et al. 1992). The repertoire of this basic set of genes, first found in the Dipteran *Drosophila*, is greatly extended by gene duplications (particularly in mammals) and by the splicing of alternative transcripts. For example, the *Shaker* A-current is exhibited by *Drosophila* photoreceptors R1–6 and has a distinctive activation range that results from a unique pattern of transcript expression (Hardie et al. 1991). Thus all Diptera probably have the potential to specify a wide range of potassium channels and these channels can, in principle, be mixed to tailor the membrane to meet the specific needs of particular cells.

Our present knowledge of potassium currents in dipteran photoreceptors indicates that, as a class, these cells could be expressing 3, if not 4, of these basic classes of potassium channel. Amplification by PCR shows that *Shaker* is expressed in *Drosophila* photoreceptors (Hardie et al. 1991). This channel is accompanied by two more slowly inactivating potassium conductances, with inactivation time constants of 120 ms and 500 ms respectively and their properties resemble *Shal* products expressed in oocytes (Hardie 1991). The inactivating outward currents seen in Tipulids also appear to have these putative *Shal* properties, although the present limitations of single electrode clamp preclude a quantitative comparison of activation ranges and time constants. The delayed rectifier conductance described in *Calliphora* (Weckström et al. 1991) resembles *Shaw* in as much as it has a wide activation range and inactivates weakly. The slow partial inactivation of the delayed rectifier seen in certain fast cells (Fig. 8) could be obtained by adding the 4th type of channel, *Shab*.

We have already discussed the striking correlation between potassium conductance and photoreceptor function. The rapidly inactivating channels dominate in the slow photoreceptors of nocturnal Tipulids, and in the photoreceptors of the fruit fly, *Drosophila*, an animal that is rarely trapped outdoors in the middle of the day (Shorrocks 1972). The slowly or non-inactivating channels dominate the membranes of diurnal species and play a critical role in tuning the membrane for a fast response. Taken together this evidence suggests that dipteran photoreceptors express on their membranes the mix of potassium channel gene products that is most advantageous for coding optical signals. This suggestion that cells pick and mix components from a rich repertoire of gene products (Salkoff et al. 1992; Hardie et al. 1991) is strengthened by the observation that at least one Tipulid, *T. marmorata*, expresses the fast component of the delayed rectifier. Thus a means to achieve a faster response, reducing the long membrane time constant (Table 1), is available to Tipulid photoreceptors, but they choose not to increase the magnitude of this particular non-inactivating conductance.

Are there more subtle differences between photoreceptor potassium conductances that can be related to function? Consider the photoreceptors of male and female Bibionids. The male dorsal eye is greatly enlarged for the express purpose of detecting and tracking females (Zeil, 1983a, b). To this end the male photoreceptors are longer, have narrower acceptance angles, a higher sampling density and are sensitive to short wavelengths. We can now add to this list of specialisations our finding that the male dorsal cells have a better frequency response. The male corner frequency is 42 Hz, compared with 27 Hz in the female (Table 1). In addition the male cells show a more transient and rapidly adapting response. These differences in photoreceptor physiology reflect the intense sexual selection among swarming males for the reliable detection and tracking of females, and it is interesting to note that the flicker fusion frequency of honeybee drones is approximately twice that of workers (Edrich 1991). In principle, the better frequency response increases the signal power received from a moving point source. The higher frequency response also improves the spatial localisation of moving targets by reducing motion blur (rev. Laughlin 1981). The male eye has larger facets and narrower interommatidial angles (Zeil 1983a) to improve spatial acuity. If one is tracking moving targets this improvement should be accompanied by an increased frequency response because there is little point in improving spatial acuity when motion blur is limiting. It is interesting in this respect that our measured ratio of male:female temporal cut-off frequencies of 1.5:1 is similar to the ratio of spatial acuities of 1.6:1, taken as the reciprocal of the ratio between the inter-ommatidial angles given by Zeil (1983a). Thus the blurring of moving images induced by the photoreceptor response dynamics is scaled to match the spatial sampling density.

This sexual dimorphism in photoreceptor frequency response is accompanied by a consistent difference in the kinetics of voltage activated outward currents. The male exhibits both the rapidly and the slowly activating components, typical of fast cells, with the slow tending to dominate (Fig. 11). In the female, this slow component is barely detectable and the fast dominates (Fig. 11). This difference between male and female photoreceptor membranes suggests that the slow component is associated with the coding of higher frequencies and the fast component with coding lower frequencies. This division is strikingly similar to observations made on fast cells, both in *Calliphora* (Weckström et al. 1991) and in this study. The fast component of the delayed rectifier dominates when the cell operates in dim light, close to resting potential, a condition in which responses are slowed by partial dark adaptation. The slow component of the outward current dominates at higher potentials, corresponding to adaptation by bright light and hence a fast response. What advantages could there be in using different kinetic components of potassium conductance under different conditions of response? As pointed out above, the slowly activating component converts the membrane into a high pass filter that selectively transmits the high frequency signals generated by the light adapted phototransduction cascade. At low light levels such a high pass filter is undesirable because it will selectively transmit shot noise and attenuate the slower signals. Under these conditions it may be preferable to use a fast conductance and rely on the membrane time constant and more central mechanisms to suppress high frequency noise (Weckström et al. 1991). The extent to which the difference in balance between fast and slow components in Bibionids, and in other flies, results from differential gene expression or from another factor responsible for expanding the channel repertoire, modulation, is an open question.

Our data from bibionids also suggest that the distinction between fast and slow cells is not rigidly defined by sets of mutually exclusive factors. The male and female corner frequencies (42 Hz and 27 Hz, respectively) bridge the gap between the slowest fast cells (50 Hz) and the fastest slow cells (19 Hz). Moreover the female photoreceptor employs a slightly different mix of potas-

sium conductances. An intermediate cell was also reported in the Hymenoptera (de Souza and Ventura 1989). These intermediate forms suggest that the slow Tipulid cells are at one end of a continuum of photoreceptor properties, with the fastest Asilid and Calliphorid cells at the other. Note that there are considerable differences among the frequency responses and conductance properties of fast cells and that our voltage and current clamp records indicate widespread differences in the balance between fast and slow components and in the activation time constants of the slow component. One of our lasting impressions of this survey is that the photoreceptor of every species has a unique physiology, in terms of the response to light, the response to current, and its behaviour under voltage clamp. Such subtle differences between cells would not surprise a comparative anatomist, used to relating photoreceptor structure to function.

These physiological differences between photoreceptors could, in principle, be exploited to throw further light on potassium channel diversity. Among the fast cells, potassium channel properties might be fine tuned to suit the requirements of particular cell types. This possibility could be tested for by comparing differences in channel kinetics and operating range with the differences in frequency response and light adapted membrane potentials. Regrettably, given the uncontrolled differences in photoreceptor dimensions and the imperfect space clamp achieved by our single electrode technique, an attempt to use our existing data set for a detailed comparison is premature. Nonetheless, one particular result is suggestive. Of all the photoreceptors that we examined, those of *Fannia canicularis* rectify most strongly. *Fannia* is remarkable for hovering in dappled shade and darting rapidly back and forth between shadow and sunfleck (Colyer and Hammond 1968). This habit distracts predators and might require a rapid and powerful means of light adaptation – hence the strong rectification.

Fast and slow cells and the constraints governing phototransduction

Why are some cells fast and others slow? There is a clear correlation, both in the Diptera and in the Hymenoptera (de Souza and Ventura 1989), between visual ecology and speed of photoreceptor response, with nocturnal and slow moving species using slow cells. Nonetheless, why is it advantageous for the female *Bibio* to have photoreceptors that are slower than the male's? One can appreciate that the extra temporal resolution is not so essential for the female, but it might still help her to see better. This observation suggests that significant penalties are paid for improving the temporal bandwidth of vision. In addressing the question of costs and benefits, we take as our starting point the observation that all dipteran photoreceptors are slow when dark adapted (Fig. 2; Table 1). It follows that slow cells simply fail to improve their frequency response to the same extent as the fast during light adaptation. We suggest that this failure occurs because the cost of improving photoreceptor frequency response outweighs the advantages.

It is energetically expensive to operate an insect photoreceptor with the high signal to noise ratio and good frequency response required for acute vision in a moving world. A high signal to noise ratio requires that one employs a large number of transduction units to register photons at a rapid rate (Howard et al. 1987). To this end, rapidly flying diurnal insects have longer photoreceptors than less active and nocturnal forms because the longer rhabdom, with more microvilli, provides more transduction units and hence a better signal to noise ratio in daylight (Laughlin 1989). The numbers of photons required to attain a good signal to noise ratio are large. In daylight, a fast blowfly photoreceptor is transducing photons at rates as high as 10^6–$10^7 \cdot s^{-1}$ to achieve an rms noise level that is equivalent to a contrast of 1–2% (Howard et al. 1987). With a minimum requirement of one light gated channel per photon, this necessitates a large light gated conductance which, in turn, must be opposed by a large antagonistic conductance, the delayed rectifier. As we have seen, activation of this large potassium conductance is also required to reduce the membrane time constant so that the cell can transmit the high frequency signals generated by the light adapted phototransduction cascade (Weckström et al. 1991). Thus acute vision demands the large conductances found in fast cells (Table 1), resulting in ion fluxes that place extreme demands upon the photoreceptor, both in terms of the density of Na/K ATPase molecules required to maintain ionic homeostasis (Jansonius 1990), and in terms of oxygen consumption (Howard et al. 1987; Hamdorf et al. 1988).

For a slowly flying and primarily nocturnal insect the benefits of this expensive fast cell strategy are decidedly limited. Night vision is primarily restricted by the number of available photons. The magnitude of the light gated conductance has little influence on the photoreceptor signal to noise ratio, although the size of quantum bumps may be important for higher order detection processes (Laughlin 1981). Furthermore, the cumbersome flight of Tipulids restricts the range of retinal image velocities. With a 1/f amplitude spectrum for natural scenes, much less information is available to sluggards at high temporal frequencies (van Hateren 1992, 1993), so that relatively little signal is gained by increasing the frequency response. Moreover, a fast response is undesirable when high frequencies carry more noise than signal (Laughlin 1981) and, under conditions of low average retinal velocity and a relatively poor signal to noise ratio, the slower response codes optimally (van Hateren 1992). In other words, a fast response can be both expensive and disadvantageous for an insect that turns slowly in dim light. It follows that the photoreceptor membrane time constant does not have to be reduced to permit high frequency signal transmission. Nor need the phototransduction cascade be so highly specialised to achieve a good frequency response. In addition, if the Tipulid did choose to greatly increase the photoreceptor signal bandwidth, the costs would be greater than those incurred by a diurnal fast cell. The Tipulid has a large

diameter rhabdom to catch more photons (Williams 1980), so increasing the capacitance per unit photoreceptor length, and necessitating a correspondingly greater increase in membrane conductance to reduce the photoreceptor time constant. Similarly, the Hymenopteran slow cells have wider rhabdomeres, raising the possibility that the diffusion of a component of the phototransduction cascade could be limiting temporal resolving power (de Souza and Ventura 1989).

We conclude that, irrespective of the dynamic constraints imposed by phototransduction, slow cells have opted for a strategy of low conductance, and hence low metabolic cost. This strategy is appropriate for both their visual ecology and their photoreceptor morphology, and involves the use of an appropriate set of potassium channels. Tipulid slow cells use strongly inactivating conductances and avoid the metabolically expensive currents associated with the non-inactivating potassium conductances. In other Diptera, fast flight demands fast cells with a rapid and reliable response in bright light. Their excellent frequency response must involve some considerable specialisation of both the molecular components of the phototransduction cascade and their structural organisation close to the rhabdomere. Specialisation of the cascade must be accompanied by a reduction in the effects of the capacitance imposed by photoreceptive membrane, otherwise the high frequencies are attenuated by the photoreceptor's time constant. This capacitative load is considerable because large numbers of microvilli are required to provide an adequate quantum capture efficiency and to transduce photons at a high rate. The reduction of time constant necessitates a high conductance, as does a good signal-to-noise ratio (Howard et al. 1987). The implementation of this high conductance strategy requires the expression of a specific set of non-inactivating potassium channels in the photoreceptor membrane and these have properties that are uniquely well suited to their function (Weckström et al. 1991). We suggest that the distinction between fast and slow cells in insects (de Souza and Ventura 1989) occurs, not because a slow response is inevitably associated with a high gain in the cascade, but because a fast response is expensive, is unnecessary for a slow moving insect and, under some conditions, reduces coding efficiency (van Hateren 1992, 1993). Thus we are led to the proposal that cellular constraints, metabolic costs, coding efficiency and distinctive visual ecologies all help to explain Autrum's classical distinction between the fast and slow eyes of insects (Autrum 1950, 1984).

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