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DIRECTION-SELECTIVE ADAPTATION IN FLY VISUAL MOTION-SENSITIVE NEURONS IS GENERATED BY AN INTRINSIC CONDUCTANCE-BASED MECHANISM

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Abstract—Motion-sensitive neurons in the blowfly brain present an ideal model system to study the cellular mechanisms and functional significance of adaptation to visual motion. Various adaptation processes have been described, but it is still largely unknown which of these processes are generated in the motion-sensitive neurons themselves and which originate at more peripheral processing stages. By input resistance measurements I demonstrate that direction-selective adaptation is generated by an activity-dependent conductance increase in the motion-sensitive neurons. Based on correlations between dendritic Ca^{2+} accumulation and slow hyperpolarizing after-potentials following excitatory stimulation, a regulation of direction-selective adaptation by Ca^{2+} has previously been suggested. In the present study, however, adaptation phenomena are not evoked when the cytosolic Ca^{2+} concentration is elevated by ultraviolet photolysis of caged Ca^{2+} in single neurons rather than by motion stimulation. This result renders it unlikely, that adaptation in fly motion-sensitive neurons is regulated by bulk cytosolic Ca^{2+}.

Key words: after-hyperpolarization, blowfly, caged Ca^{2+}, Ca^{2+}-activated K^+-channels, input resistance, visual system.

Many sensory cells and neurons react to ongoing stimulation with a change in their response properties (reviews: Fain et al., 2001; Fettiplace and Ricci, 2003; Krekelberg et al., 2006). Such a dependency on stimulus history has been suggested to match neuronal sensitivity and filtering properties to the strength and the statistical properties of the current stimulus distribution (e.g. Dragoi et al., 2002; Benda et al., 2005). In the visual system, adaptation to moving stimuli has been shown to consist of different components, which can be classified according to their selectivity for pattern orientation. A large variety of adaptive properties was found in cat visual cortex V1 and V2, ranging from neurons that adapt to stimuli of any orientation to neurons that adapt only to optimally orientated patterns (Sengpiel and Bonhoeffer, 2002; Crowder et al., 2006). Moreover, it has been reported that adaptation has the potential to shift the orientation tuning of individual neurons, leading to dynamic changes in the map of orientation preference in V1 (Dragoi et al., 2000).

In a group of individually identifiable visual motion-sensitive neurons in the blowfly (*Calliphora vicina*) brain called tangential cells (TCs), several components of motion adaptation have been described (Maddess and Laughlin, 1985; Harris et al., 2000; Brenner et al., 2000; Borst et al., 2005). Many TCs are amenable to electrophysiological and imaging techniques *in vivo* during presentation of sensory stimuli that are well-known to be behaviorally relevant (reviews: Borst and Haag, 2002; Egelhaaf et al., 2002, 2005). This has made fly vision an ideal model system to study both the mechanisms and the functional consequences of motion adaptation.

Most TCs spatially integrate on their retinotopically organized large dendrites the output signals of local motion-sensitive input elements. Thus, TCs respond to visual motion in a fully direction-selective way, being excited by motion in one direction and inhibited by motion in the opposite direction. Adaptation seems to operate on various levels of the motion-detection pathway through mechanisms located presynaptic to TCs, but possibly—although unproven so far—also in TCs themselves. This could be reflected in specific properties of the different components of motion adaptation: Contrast gain has been observed to be reduced by motion in any direction and may therefore originate from stages in the visual pathway prior to the computation of motion direction, i.e. upstream of TCs (Harris et al., 2000). Another component of adaptation only occurs during motion in the preferred direction (PD) of the TC and is associated with membrane after-hyperpolarization (AHP) when the stimulus terminates (Kurtz et al., 2000) and leads to a subtractive shift in the stimulus-response function (Harris et al., 2000). This form of direction-specific adaptation may either originate in TCs themselves or at a processing stage before TCs. The latter possibility would require the underlying mechanism to occur exclusively in elements providing TCs with excitatory inputs but not in those providing inhibitory inputs.

Prompted by correlations between dendritic Ca^{2+} accumulation and membrane AHP following excitatory stimulation, a Ca^{2+}-dependent inhibitory conductance, such as Ca^{2+}-activated K^+-channels, has been proposed as a physiological basis of direction-selective adaptation (Kurtz et al., 2000). Alternative to Ca^{2+}, Na^+ could also act as mediator of activity-dependent adaptation (review: Bhatta-

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Abbreviations: AHP, after-hyperpolarization; BAPTA, 1,2-bis(o-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; CCD, charged-coupled device; CH, centrifugal horizontal; DCC, discontinuous current clamp; HS, horizontal system; HSE, horizontal system equatorial; ND, null direction; NP-EGTA, o-nitrophenyl ethylene glycol bis(2-aminoethyl ether)-*N*,*N*,*N*′*N*′-tetraacetic acid; PD, preferred direction; R_{in}, input resistance; TC, tangential cell; UV, ultraviolet; VS, vertical system.

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charjee and Kaczmarek, 2005). Although evidence for the involvement of Na^+ in adaptation is still scarce, Na^+-regulated adaptation in TCs remains distinctly possible, since voltage-clamp experiments and pharmacology suggest that Na^+-activated K^+-channels exist in TCs (Haag et al., 1997).

In the present study, I demonstrate that direction-specific adaptation is intrinsically generated in TCs by an activity-dependent mechanism. Furthermore, by directly manipulating cytosolic Ca^{2+} concentrations by ultraviolet (UV) photolysis of caged Ca^{2+} I show that direction-specific adaptation is probably not controlled by bulk cytosolic Ca^{2+}. Alternative explanations are the control of adaptation by a Na^+-regulated conductance, or by Ca^{2+}-regulated channels which co-localize with Ca^{2+} channels and which therefore temporarily experience much higher Ca^{2+} concentrations than during flash photolysis.

EXPERIMENTAL PROCEDURES

Preparation and electrophysiology

All experiments were carried out at room temperature (18–25 °C) on ≤3-day-old female blowflies, bred in the department's stock. After dissection as described in (Dürr and Egelhaaf, 1999) the fly was mounted under an upright fixed-stage microscope (Axioskop FS, Zeiss, Oberkochen, Germany) to view the fly brain from behind. Membrane potential recordings, Ca^{2+} imaging, and UV photolysis of caged Ca^{2+} were performed *in vivo* on TCs in the third visual neuropile of the fly, the lobula plate. Identification of individual TCs was based on their receptive field properties, specific characteristics of their electrical responses, and their anatomy, if visualized with fluorescent Ca^{2+} dyes.

Intracellular recordings from TCs were made using sharp borosilicate glass electrodes (GC100TF-10, Clark Electromedical, Edenbridge, UK) pulled on a Brown-Flaming Puller (P-97, Sutter Instruments, San Rafael, CA, USA). Electrode resistance was 20–40 MΩ when filled with 1 M KCl and 30–80 MΩ when the electrode tip contained Ca^{2+} dye and caged Ca^{2+} (see below). Electrode signals were amplified with an Axoclamp 2A (Axon Instruments, Foster City, CA, USA) operated in bridge mode and sampled at rates of 3 or 4 kHz with an amplitude resolution of 0.0244 mV by an analog-to-digital converter (DT2801A, Data Translation, Marlboro, MA, USA). Recording duration was approximately 10–40 min.

Measurements of the neuronal input resistance (R_{in}) were performed by probing the neurons' responses to rectangular current pulses of 80 ms duration. Hyperpolarizing currents of small amplitude (−1 nA) were used to minimize the involvement of active currents (Haag et al., 1997). A linear relationship between injected current and resulting voltage was found to hold for TCs in the hyperpolarized voltage range (Borst and Haag, 1996). Bridge recording mode was used instead of discontinuous current clamp (DCC) to measure R_{in}, since the same current level was used for all injections. In this case, DCC would provide no advantages over bridge mode but would add more noise to the recording. The bridge balance was precisely calibrated to the −1 nA current injections. The average membrane potential was determined in 60 ms time windows centered on phases with and without current injection. For each time window with current injection the membrane potential values were subtracted from the average value of the two neighboring time windows without current injection and R_{in} values were calculated according to Ohm's law. Under- and over-compensation of the electrode's resistance by the amplifier's bridge circuit might have led to over- and underestimation of R_{in} values. Such electrode compensation errors would leave the estimation of sign and amplitude of changes in neuronal R_{in} unaffected. Note however, that when plotting R_{in} changes relative to resting values, electrode over- and under-compensation would lead to over- or underestimation of relative resistance changes, respectively. Such errors are small when R_{in} changes are small in relation to resting values as is the case for R_{in} changes after the cessation of motion stimulation (see Results). Moreover, the magnitude of measured R_{in} changes might depend on impalement site. Since the electrode was placed in the axon, R_{in} changes located in the dendrite might have been underestimated. However, the classes of TCs recorded in this study possess large axon diameters of 10–25 μm (e.g. Hausen, 1982). In a compartmental model study on these TCs 20–80% of current injected into the axon was estimated to reach the dendritic tips (depending on TC class, see Borst and Haag, 1996).

UV photolysis of caged Ca^{2+} and Ca^{2+} imaging

Photolysis of caged Ca^{2+} and Ca^{2+} imaging followed Kurtz (2004), apart from some minor modifications as detailed below. Caged Ca^{2+} and fluorescent Ca^{2+} dye was injected from the electrode tip into single TCs during intracellular recording by applying 1–3 nA hyperpolarizing current for 5–10 min and left to diffuse throughout the cytoplasm for ≥5 min. The electrode tips contained 5 mM KOH and 101–127 mM o-nitrophenyl EGTA (NP-EGTA) tetrapotassium salt and 5–6 mM Oregon Green 488 1,2-bis(o-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA)-1 hexapotassium salt (both from Molecular Probes, Eugene, OR, USA; concentration ranges indicate variations between experiments). With this procedure, step-like increases of the cytosolic Ca^{2+} concentration could be induced repetitively by delivering filtered light ($\lambda<360$ nm or $\lambda<380$ nm) from a xenon flash lamp (JML-C2, Rapp Optoelectronics, Hamburg, Germany) via a quartz light guide (diameter 100 or 200 μm). In addition to UV flashes, continuous illumination with filtered light ($\lambda<360$ nm) from a UV lamp (UVICO, Rapp Optoelectronics) was used to release caged Ca^{2+}. Due to the fly's photoreceptors' sensitivity to UV light (Kirschfeld and Franceschini, 1977), visual responses are observed in TCs during UV photolysis of caged Ca^{2+}. I applied two kinds of controls to distinguish cellular responses, which are elicited by increases in the cytosolic Ca^{2+} concentration from those elicited by unwanted visual stimulation. First, I recorded control traces with UV illumination before injection of caged Ca^{2+}. Alternatively, the flash or continuous-light lamp system was used with long-wavelength filtering ($\lambda>475$ nm). This filtering produced equally strong visual excitation but no photorelease of caged Ca^{2+}.

Relative cytosolic Ca^{2+} concentration changes in single TCs were monitored by epifluorescence imaging of Oregon Green 488 BAPTA-1 emission. I used long working-distance objectives (Achroplan 20× NA 0.50W and 40× NA 0.75W, Zeiss) at an upright fixed-stage microscope (Axioskop FS, Zeiss; filter settings: excitation 475±20 nm, dicroic mirror 500 nm, emission 530±20 nm) equipped with a cooled frame-transfer charged-coupled device (CCD) camera (Quantix 57, Photometrics, Tucson, AZ, USA), operated with 4×4 pixel binning at a frame rate of 14 Hz.

Visual stimulation and data analysis

A light emitting diode board was used to present a moving high-contrast square wave grating in the receptive field of TCs (for details see Kurtz et al., 2001). The angular extent of the pattern was approximately 50×60°, with the larger extent perpendicular to the axis of motion. The temporal frequency of pattern motion was 4 Hz.

Ca^{2+} concentration signals were evaluated as fluorescence changes of the Ca^{2+}-sensitive dye relative to resting values obtained from the first image (ΔF/F). Background values were calculated by averaging the signal from unstained regions in the