CALCIUM ENABLES PHOTORECEPTOR PIGMENT MIGRATION IN A MUTANT FLY

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Upon bright illumination, pigment granules in the photoreceptors of the fly compound eye migrate transversely toward the rhabdomeres (Kirschfeld & Franceschini, 1969). The granules absorb light passing down the rhabdomeric light guide, thereby reducing the light available to the visual pigment. This light-regulating mechanism is found in a number of invertebrate eyes (Stavenga, 1979) and has been termed the 'longitudinal pupil' (Franceschini, 1972).

Pigment migration is triggered by the absorption of light by the visual pigment (Franceschini, 1975) but is not directly caused by the accompanying depolarization of the photoreceptors (Kirschfeld & Vogt, 1980). Kirschfeld & Vogt showed that the extracellular application of the calcium-sequestering agent EGTA to the fly retina disables pigment migration. They suggested that an increase in intracellular free Ca$^{2+}$ concentration, which is known to accompany strong illumination of invertebrate photoreceptors (Brown & Blinks, 1974; Muijser, 1979), triggers pigment movement. Here I present evidence in support of this hypothesis by using Ca$^{2+}$ to promote pigment migration in a transduction mutant of the fly Lucilia which does not normally exhibit pigment migration: this is the converse to Kirschfeld & Vogt's experiment.

Pigment migration was monitored in restrained, living flies by observing light-induced changes in reflection from the deep pseudopupil (Franceschini & Kirschfeld, 1971). In wild-type Lucilia, the pupil reflects yellow light after adaptation with bright white light. The time constant of the reflection change from the dark-adapted to the light-adapted state is about 7 s at room temperature (Fig. 1A). The receptor potential in the wild-type fly has a characteristic initial transient followed by a steady-state plateau (Fig. 1B). As reported by Kirschfeld & Vogt (1980), infiltration of the retina with EGTA (0.1 M dissolved in 0.15 M-NaCl and neutralized to pH 7 with NaOH) blocks pigment migration without decreasing the amplitude of the receptor potential. The pigment remains in the 'dark-adapted' position independent of the light regime. A drop of solution, the dimensions of which were measured under the dissection microscope and the volume of which was calculated to be about 1 nl, was absorbed into the eye, which has a volume of about 250 nl, through a small hole cut in the cornea. EGTA takes effect about 1 min after infiltration and can be reversed by subsequent

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Fig. 1. Time course of reflection (top traces) and membrane potential in wild-type (A,B), nss (C,D) and nss in which calcium had been infused into the retina (E,F). Reflection was measured using a Leitz MPV3 microphotometer. Intracellular recordings were made using standard techniques (see e.g. Muijser 1979). C,E and D,F are from the same fly, but different flies were used for the reflection and voltage measurements. There was little variability among the week-old flies used. Upward deflection of the lower traces corresponds to time during which the light is on.

Fig. 2. (A) Receptor potential to increasing steps of light in the mutant nss w (white-eyed). At low light intensity, the steady-state component is noisy due to the superposition of single-photon responses. The lowest intensity corresponds to approximately 10 photon-responses per second. The nss and wild-type eyes have the same quantum capture efficiency. (B) Change in conductance in the nss photoreceptor measured using the bridge-balance technique. Note the change in time calibration. (C) ERG from nss w.

infiltration with 1 nl of 0·5 M-CaCl₂. This experiment confirms, for Lucilia, that the presence of extracellular calcium is a prerequisite for pigment migration.

Pigment migration does not normally occur in the nss (no steady-state receptor response) mutant of Lucilia (Fig. 1C). The photoreceptors of flies carrying this fully
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Recessive gene on the fourth chromosome (Howard, 1982) respond normally to dim light, but following the onset of moderate and high intensity illumination the steady-state component of the receptor potential decays over a few seconds to the dark level (Figs 1D, 2A). The photoreceptors are then insensitive to further changes in light intensity and in particular show little or no off-response (Fig. 2A). In white-eyed *nss* flies, in which all receptors are stimulated, there is no sign of an off-response as seen in the ERG (Fig. 2C). Membrane conductance also decays to its pre-stimulus value (Fig. 2B), indicating that the decay is caused by a decrease in the light-dependent Na⁺ conductance, rather than an increase in conductance to other ions, such as K⁺, which have an equilibrium potential close to the resting potential. Sensitivity returns after about 1 min in the dark (Howard, 1982).

Within 2 min of infiltration of the *nss* retina with about 1 nl of 0.5 M-CaCl₂, illumination causes a transient pigment migration (Fig. 1E). The pigment moves toward the normal light-adapted position with a similar time course to that of the wild-type fly; but before reaching its fully light-adapted position, the pigment returns to the ‘dark-adapted’ position within about 30 s. The transient pigment migration that is enabled by calcium in the *nss* fly has a similar time course to the pupil response that is observed in the wild-type fly when a brief flash of light is used. Clearly both the pigment and the machinery for moving it are present in *nss*. Calcium infiltration has little effect on the receptor potential of *nss*, except to decrease the post-transient plateau depolarization from which the response decays (see also Muijser, 1979). Note that the receptor potential has declined by the time that the pigment migration begins: this is further evidence that the pupil is not driven directly by depolarization. As controls for any possible effects of Cl⁻, infiltration of the *nss* retina with either 0.5 M-KCl or NaCl has no effect on the already inactive pupil mechanism.

If calcium plays a role in pigment migration, we might expect other divalent cations to affect the pupil mechanism. In *nss*, infiltration with 1 nl of 0.5 M-SrCl₂ mimics the effect of CaCl₂. But neither BaCl₂, MgCl₂, CoCl₂ nor NiCl₂ activate the pupil mechanism of *nss*. In the wild-type fly, neither CaCl₂, SrCl₂ nor the controls KCl and NaCl affect pigment migration. But infiltration of the wild-type retina with 1 nl of 0.1 M-BaCl₂, MgCl₂, CoCl₂ or NiCl₂ has the same effect as EGTA: the pupil is pushed into its dark-adapted position irrespective of the light regime.

These experiments are consistent with the following model: illumination causes an influx of Ca²⁺ ions through channels in the plasma membrane which, in turn, triggers the migration of the pigment granules.

That calcium enables, at least transiently, the pupil mechanism in *nss* is interpreted as follows. The rapid peak-to-plateau transition of the receptor potential which is known to be caused by increased intracellular free Ca²⁺ (Muijser, 1979) is normal in *nss* – the decay follows the transition. Therefore the initial calcium flow is present in this mutant. But in *nss* the calcium channels are open for insufficient time for intracellular free Ca²⁺ to reach a high enough concentration to trigger pigment migration. Increasing extracellular Ca²⁺ allows sufficient Ca²⁺ to enter the cytosol during the initial depolarization. Following the response decay, Ca²⁺ ions no longer enter the cytosol and Ca²⁺ transport (Fain & Lisman, 1981; Armon & Minke, 1983) or sequestering (Walz, 1982) mechanisms reduce intracellular free Ca²⁺ and hence the pupil returns to its ‘dark-adapted’ state. In the *Drosophila* mutant *trp* (Minke, 1982), which
is phenotypically similar though not identical to \textit{nss} (Howard, 1982), pigment migration is transitory (Lo & Pak, 1981) and as pointed out by Kirschfeld & Vogt (1980), this is consistent with a transient increase in intracellular free Ca$^{2+}$.

That Sr$^{2+}$ mimics Ca$^{2+}$ is not surprising: the voltage-dependent Ca$^{2+}$ channel has almost the same selectivity to Sr$^{2+}$ (Fain & Lisman, 1981; Hagiwara & Byerly, 1981) and Sr$^{2+}$ can replace Ca$^{2+}$ in triggering the release of transmitter at the neuromuscular junction, as well as secretion from other neuroendocrine cells (Cuthbert, 1970; Rubin, 1974). Ba$^{2+}$ acts as a less effective agonist in some systems, while in other systems it acts as an antagonist (Rubin, 1974). The other divalent cations used in these experiments act as competitive blockers of the Ca$^{2+}$ channel and inhibit neuroendocrine secretion.

Three considerations favour the hypothesis that Ca$^{2+}$ influx is from the extracellular space. Firstly EGTA does not penetrate cells; secondly the antagonizing divalent cations presumably block Ca$^{2+}$ entry at the plasma membrane; and thirdly all agents act within a few minutes, which is approximately the time needed for diffusion from the site of application to that of pupil monitoring. But $^{45}$Ca$^{2+}$ incorporation into mitochondria from extracellular space can be rapid, and in rat fat-cells is completed within 2 to 5 min (Severson, Denton, Bridges & Randle, 1976).

The detailed mechanism of how calcium could trigger pigment migration or neuroendocrine secretion is unknown. Diffusion by Brownian motion of 150 nm diameter pigment granules (Boschek, 1971) in cytoplasm of viscosity 0.006 Pa s will result in a mean displacement of about $\sqrt{t}$ \textmu m, where t is time in seconds (Cuthbert, 1970). In the fly, the pigment granules move about 2 \textmu m to abut the rhabdom, so that diffusion is fast enough to account for the migratory process. But pigment migration in \textit{Limulus} can be disabled by the disruption of the microtubule network using colchicin (Miller, 1979).

An increase in intracellular free calcium causes changes in the transduction mechanism of invertebrate photoreceptors associated with light adaptation: a decrease in sensitivity and shortening of the time course (Fein & Szuts, 1982). In this context it is natural that calcium also activates the longitudinal pupil.

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**REFERENCES**


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