malized") and probit analysis is applied (*21*), the EDD titer giving 50 percent effect was 0.059 mg of protein per milliliter, with 95 percent confidence limits of 0.058 and 0.060 mg of protein per milliliter and a slope of 1.93.

DENNIS W. MURPHY
S. S. SOHI
P. G. FAST

Insect Pathology Research Institute, Environment Canada, Sault Ste. Marie, Ontario, P6A 5M7, Canada

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Photoreceptor Mutant of *Drosophila*: Is Protein Involved in Intermediate Steps of Phototransduction?

Abstract. *In* norpA *mutants of* Drosophila melanogaster *the phototransduction process is either partially or completely blocked. By using a temperature-sensitive allele, we have found that the* norpA *mutation has little or no effect on either the rhodopsin-metarhodopsin transition or the machinery of quantum bump production. Thus, the* norpA *lesion appears to be localized in the intermediate process of phototransduction. Because a temperature-sensitive allele of* norpA *has been isolated, the* norpA *gene probably encodes a protein involved in the process.*

One of the main functions of the photoreceptor is to convert light signals to physiological responses of the photoreceptor membrane—the phototransduction process. Although much is known about the physiology of the photoreceptor, the mechanism of transduction itself has proved to be elusive. We thought that the use of mutants might be of considerable value in the study of the photoreceptor, because the technique would allow manipulation of any given physiological process at the molecular level. Thus, we set out to mutagenize fruit flies of the species *Drosophila melanogaster* and to isolate mutants with abnormal receptor function (*1*, *2*).

We reported previously that the *norpA* cistron, mapping at about 6.5 units from the tip of the X chromosome, appears to be involved in phototransduction (*2–4*). There are now over 40 independently derived *norpA* mutants. The homozygous phenotypic expression of these alleles varies from a complete absence of the electroretinogram to only a slight change in time course or amplitude of the potential. Alawi *et al.* (*3*, *5*) applied intracellular recording techniques to the photoreceptors of *norpA* mutants to show directly that the mutation indeed affects the receptor potential. They further showed that the photoreceptors of even the most extreme alleles of *norpA* have apparently normal membrane potential and resistance in the dark, but, unlike the wild-type photoreceptors, the potential and resistance do not change when a light stimulus is applied (*3*, *5*). Furthermore, the results of histological and spectrophotometric studies have suggested that the absence of the receptor potential in these mutants cannot be attributed to either morphological abnormality or the lack of the visual pigment (*3*, *6*).

One of the *norpA* alleles, $norpA^{11\,52}$, has been found to be reversibly temperature sensitive (*7*). At 17°C, the electroretinogram and the intracellularly recorded receptor potential of this mutant are very nearly normal. Above 29°C, however, these responses deteriorate, and no response is obtained above 34°C. These temperature-induced changes occur very rapidly and are reversible except when the mutants are exposed to more extreme treatments in temperature, duration, or both. These results have been interpreted to suggest that the *norpA* gene product (presumably a protein) is somehow involved in the generation of the photoreceptor response and that in $norpA^{11\,52}$ the conformation of the *norpA* gene product has become highly sensitive to temperature (*7*). In the present work (*8*), the temperature-sensitive allele, $norpA^{11\,52}$, has been used to localize more precisely the stage of phototransduction affected by the *norpA* gene.

The transduction events in the *Drosophila* photoreceptor may be summarized as follows. The dominant class of visual pigment rhodopsin (*9*) in the fly compound eye absorbs maximally (λ_{max}) at about 485 nm and is photoconverted to a stable photoproduct with a λ_{max} of about 580 nm (*6*, *10*). By still unknown mechanisms, photoexcitation of rhodopsin molecules leads to a change in permeability of the photoreceptor membrane, giving rise to a depolarizing receptor potential. Studies on other invertebrate photoreceptors have shown that most of the receptor current is carried by Na^+ ions (*11*). Moreover, several lines of evidence suggest that the receptor potentials of many invertebrates, including *Drosophila*, arise from the summation of a large number of small membrane voltage fluctuations, commonly referred to as quantum bumps (*12*, *13*). The nature of the intermediate process linking the excitation of rhodopsin and the production of bumps is not known.

At what stage of transduction does the *norpA* gene exert its effect? One possibility is that the visual pigment rhodopsin fails to make its normal transition to metarhodopsin when photoactivated. This possibility was tested by performing spectrophotometry on the temperature-sensitive mutant $norpA^{11\,52}$ at a temperature of 35°C. At a temperature as high as this, no trace of receptor potential can be detected in this mutant (*7*).

The spectrophotometry was performed on the entire eye in a decapitated preparation (*6*). For this purpose, the screening, eye color pigments in the compound eye were removed genetically from both the wild-type and *norpA*^{H52} flies to prevent these pigments from contributing to spectral measurements of visual pigment.

Figure 1, A and B, shows the results obtained with *NorpA*^{H52} and wild-type *D. melanogaster*, respectively. Spectrum 1 shows that blue irradiation resulted in a decrease in absorbance in the blue and an increase in absorbance in the orange, indicating that rhodopsin had been photoconverted to metarhodopsin. Spectrum 2 (Fig. 1, A and B) shows that orange irradiation photoconverted the metarhodopsin back to rhodopsin, as indicated by an absorbance decrease in the orange and an increase in the blue. Difference spectra 3 and 4 were obtained at 35°C under conditions similar to those used to obtain spectra 1 and 2. Even at this elevated temperature, rhodopsin was photoconverted to metarhodopsin by blue light and photoregenerated back by orange light both in wild-type *D. melanogaster* and *norpA*^{H52} (spectra 3 and 4) (*14*). Thus, elevation of the temperature to 35°C did not block phototransition between rhodopsin and metarhodopsin in either preparation, even though no receptor potential was present in *norpA*^{H52} at 35°C. These results lead to the conclusion that the *norpA* mutation does not affect the phototransition between rhodopsin and metarhodopsin. Rather, it appears to affect some later step or steps to block receptor potential.

We next considered the possibility that the *norpA* mutation affects the machinery of quantum bump production. In this hypothesis, the entire sequence of phototransduction steps are thought to be normal in *norpA* except for the very last step involving the opening of sodium channels to produce quantum bumps. The receptor potential is present in many of the less extreme alleles of *norpA*, but it has a very prolonged time course compared to that of the wild-type receptor potential, particularly at the stimulus offset. In these mutants the receptor potential often proceeds for tens of seconds to minutes after the stimulus has been turned off (Fig. 2B). Such a receptor potential is obtained, for example, from the temperature-sensitive mutant *norpA*^{H52} near room temperature, particularly if the preparation has previously been exposed to elevated temperatures (Fig. 2B). We reasoned that if the primary effect of *norpA* mutation is on the machinery of quantum bump production and if, indeed, individual bumps sum to produce the receptor potential, the same *norpA* phenotype expressed at the receptor potential level should be recognizable at the quantum bump level. That is, since the receptor potential has a prolonged time course, each bump should have a prolonged time course also. Accordingly, quantum bumps were examined in *norpA*^{H52} at 25°C and compared to those obtained from wild-type *D. melanogaster* at the same temperature. Bumps were recorded intracellularly (*13*) from the retinula cells by means of glass pipette microelectrodes filled with 2*M* KCl.

Figure 2B shows the prolonged time course of the *norpA*^{H52} receptor potential. When the wild type and the mutant were stimulated with a dim light of 10-msec duration at room temperature (Fig.

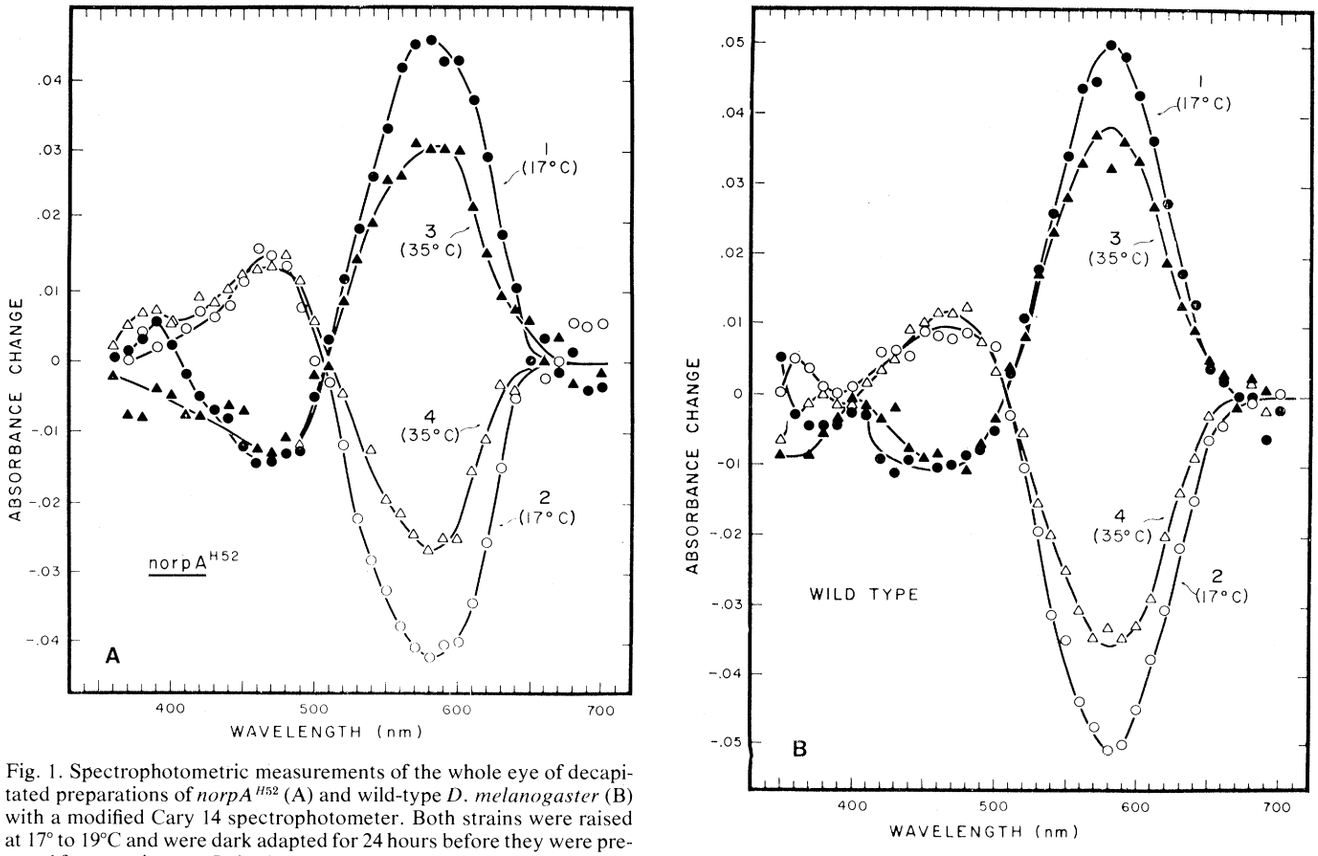

Fig. 1. Spectrophotometric measurements of the whole eye of decapitated preparations of *norpA*^{H52} (A) and wild-type *D. melanogaster* (B) with a modified Cary 14 spectrophotometer. Both strains were raised at 17° to 19°C and were dark adapted for 24 hours before they were prepared for experiments. In both (A) and (B), spectra 1 and 2 were obtained at 17°C and 3 and 4 at 35°C. Spectrum 1 is a difference spectrum between the initial spectral measurement on the dark-adapted eye and that following 2 minutes of intense blue irradiation with a Balzer broadband K2 filter. The eye was then irradiated for 2 minutes with intense orange light (Balzer broadband K5 filter). Spectrum 2 was obtained by taking the difference between the spectral measurements obtained before and after the orange irradiation. The temperature of the fly was then raised to 35°C. The difference spectra 3 and 4 were obtained at the new temperature in a manner similar to that used to obtain 1 and 2.

2, C and D) individual light-induced bumps were often clearly recognizable, and the time course of bumps recorded from *norpA*^{H52} was not substantially different from that of wild-type bumps (*15*). Thus, the prolonged time course of the *norpA*^{H52} receptor potential cannot be due to an abnormal time course of individual bumps. Instead, it appears to originate from abnormal distribution of bumps. In the wild-type animal, the light-induced bumps occur more or less in synchrony with the light stimulus (Fig. 2C). The *norpA*^{H52} bumps, on the other hand, display considerable latency dispersion with respect to light stimulus (Fig. 2D). These results are consistent with the interpretation that (i) the machinery of bump production apparently is intact in the *norpA* mutant and (ii) the mutation affects a step which precedes the bump production. This step may, for example, be involved in the release of a substance which in turn produces the quantum bumps (*16*). Thus, we have bracketed the lesion in the *norpA* mutants to one of the intermediate steps of phototransduction, subsequent to the rhodopsin to metarhodopsin transition but previous to the bump production.

We conducted the present study with a temperature-sensitive allele of the *norpA* gene. In the case of microorganisms, evidence suggests that temperature-sensitive mutations arise from the formation of an altered protein which is still functional but more heat labile than the wild-type protein (*17*). Likewise, Suzuki and co-workers (*18*) have argued that the most frequent basis for temperature sensitivity in *Drosophila* is a missense mutation affecting the thermostability of the gene product, presumably a protein. While other interpretations are possible, our results indicate that the *norpA* gene encodes a macromolecule (presumably a protein) involved in the intermediate process of phototransduction. Although the intermediate steps of phototransduction have been argued for on many grounds (*19*), little direct evidence has been obtained for them in any system. The existence of a mutant whose gene product is apparently involved in one of the intermediate steps thus raises the possibility that the process may be studied more directly than was possible before.

W. L. PAK
S. E. OSTROY
M. C. DELAND*
C.-F. WU†

Department of Biological Sciences,
Purdue University,
West Lafayette, Indiana 47907

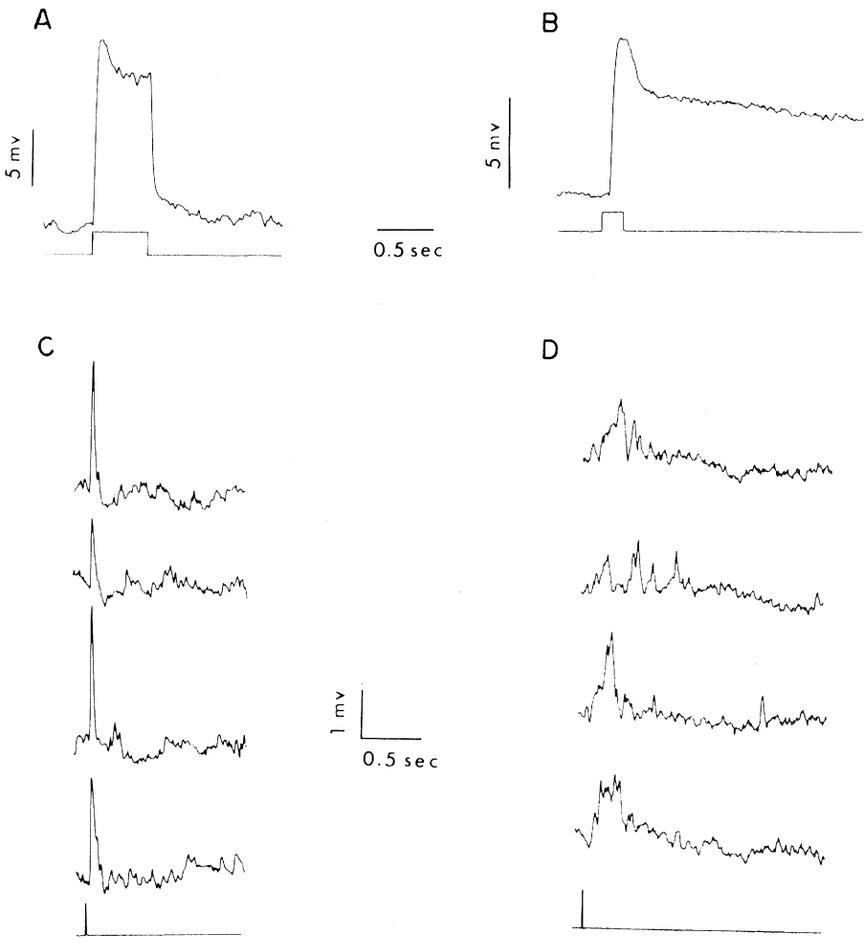

Fig. 2. Intracellular recordings obtained at room temperature from the photoreceptors of wild-type *D. melanogaster* (A and C) and *norpA*^{H52} (B and D). (A) Response of wild type to a 0.5-second, 475-nm stimulus (log $I = -1.5$). (B) Response of *norpA*^{H52} to a 0.2-second, 500-nm stimulus (log $I = 0$). (C) A series of four responses of the wild type to 10-msec, 475-nm flashes (log $I = -4.0$). (D) A series of four *norpA*^{H52} responses to 10-msec, 500-nm flashes (log $I = -1.5$). Somewhat higher stimulus intensities were used for the mutant because its threshold is somewhat higher than the wild type.

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9. There are three classes of photoreceptors in the *Drosophila* compound eye, R_{1-6}, R_7, and R_8. In measurements performed on the whole eye, the visual pigment contained in R_{1-6} makes predominant contribution to absorption or action spectra, because R_{1-6} receptors outnumber the other classes of receptors and have larger rhabdomeric membrane areas. Rhodopsin contained in R_{1-6} is referred to in this report as the "dominant class of visual pigment."
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wild-type *Drosophila* and the mutant. Therefore, it cannot be attributed to the effect of the mutation.
15. For quantitative verification of this statement, autocovariance functions of the bump noise were calculated for both the wild type and the mutant, $norpA^{H52}$. The autocovariance function $C(\tau)$ gives a picture of how the signal correlates to its future (or past) at an interval τ. If the occurrence of bumps is totally uncorrelated, the autocovariance function would describe the time course of individual bumps. In the above calculation, the autocovariance functions of bump noise for the wild type and the mutant were found to have very similar dependence on τ, suggesting that the time course of individual bumps is similar.
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* Present address: Department of Genetics, Monash University, Clayton, Victoria, Australia 3168.
† Present address: Division of Biology, California Institute of Technology, Pasadena 91109.

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Prenatal Experience and Avian Development: Brief Auditory Stimulation Accelerates the Hatching of Japanese Quail

Abstract. *A single 2-hour exposure to auditory stimulation at any point during the final 3 days of incubation accelerates the hatching of Japanese quail. The 3-day sensitive period includes both prenatal and perinatal stages of incubation. So far as is known these results provide the first unequivocal evidence that short-term prenatal sensory stimulation can affect the development of an avian embryo.*

Brief (*1*) periods of sensory experience can profoundly affect a developing organism. The evidence for this in vertebrates has been limited to studies of the visual system after birth (*2*). We now report that brief stimulation of the auditory system can also affect vertebrate development. Moreover, this sensitivity exists during the prenatal period of development. Our results indicate that brief prenatal exposure of an avian embryo to auditory stimulation can accelerate the time of hatching, reducing the normal incubation time by as much as 10 percent.

Many species of precocial ground-nesting birds brood egg clutches whose eggs hatch within a short time of one another (*3*). This synchronization of hatching is of putative adaptive advantage in that it reduces the period during which the parents must choose between care for unhatched eggs and newly hatched young. That is, during incubation the eggs must be maintained within a narrow range of environmental conditions (*4*). The behavior required of the parents to provide these incubation conditions is in conflict with the behavior required later for the care of the highly mobile, precocial brood. After the eggs hatch the parents must utilize new tactics to protect the young from predators and must introduce the young to available food resources before their embryonic reserves are exhausted. The strategy of synchronization of hatching allows the most efficient division of the demands on the parents for care of the young before and after hatching.

Synchronization of hatching is mediated by acoustic communication between the individual members within a clutch. Audible clicks, which are correlated with the embryo's respiratory cycle (*5*), provide the basis for the inter-egg communication (*6*). Acoustic presentation of synthetic clicks (such as brief, broad-band bursts of noise) has shown that the hatching of embryos following acoustic stimulation is accelerated or retarded depending on the click repetition rate (*7*), the duration of the stimulating period, and gestational age of the embryo.

Under normal incubation conditions, Japanese quail (*Coturnix coturnix japonica*) hatch on day 17 of incubation. Continuous exposure during the final 2 days of incubation to synthetic clicks at stimulation rates of approximately three per second optimally advances the hatching of Japanese quail, while rates above or below the optimum produce less significant hatching advancement or even hatching retardation, as compared to unstimulated controls (*7*).

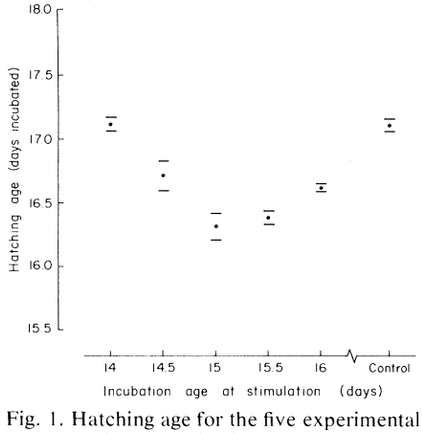

Fig. 1. Hatching age for the five experimental groups and the unstimulated control group. Experimental groups were exposed to auditory stimulation for 2 hours at the incubation age indicated (*9*). Means and standard errors are shown. Sample sizes for experimental groups days 14 through 16 and controls are 35, 46, 41, 39, 39, and 96, respectively.

We first studied the developmental stages at which exposure of the embryo to synthetic clicks at the optimal repetition rate would accelerate the hatching of Japanese quail, and found that the hatching time could be advanced only if the embryos were stimulated during the final 3 days of incubation. We then systematically examined the effects of stimulation during this interval and report here that very brief exposures to synthetic clicks at any point within this 3-day sensitive period significantly advance the hatching time.

Japanese quail eggs (Cornell University, Department of Poultry Science) were incubated in Jamesway forced-draft incubators (37.5°C, 60 percent relative humidity), in which the eggs were turned automatically every 3 hours (*8*). Before day 14 the eggs were checked for viability (candled), and the viable embryos were randomly grouped into clutches of six eggs. The eggs within a clutch were formed into a 2 by 3 array, with each egg in contact with its nearest neighbors.

Experimental clutches were stimulated for 2 hours at 14, 14.5, 15, 15.5, or 16 days of incubation (*9*). In the procedure for sound presentation, the tray containing the clutch was rapidly transferred from the control incubator to a second incubator that was equipped with a KLH model 11 loudspeaker. The temperature and relative humidity of both incubators were identical. The speaker was centered 12.5 cm above the clutch and the stimuli were presented at 80-db sound pressure level peak (General Radio model 33 sound level meter) at the optimal rate of three per second (*7*). The stimuli were 37-msec, broad-band (0.1 to 8 khz) bursts of noise generated by a unijunction relaxation oscillator, whose resistance-capacitance values were chosen to maximize the spectral flatness of the speaker output. Control clutches were treated identically but not exposed to the 2-hour stimulation period.

After 16 days of incubation the eggs were placed on an automated device that recorded the individual hatching times. Each egg was placed on a separate balanced spoon that held down a microswitch. When the animal hatched, the mi-