CHARACTERIZATION AND GENETIC ANALYSIS OF *DROSOPHILA MELANOGASTER* PHOTOBEHAVIOR DURING LARVAL DEVELOPMENT

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In *Drosophila melanogaster*, during the mid third instar of development larvae cease foraging and commence a period of increased locomotor activity referred to as wandering behavior. In this study, we quantified the wild type larval response to light during the foraging (first, second, and early third instars) and wandering (late third instar) stages of development. Foraging larvae in the first, second and early third instars exhibited a robust and marked aversion to light (negative phototaxis). From the mid larval third instar larvae showed a decrease in photonegative behavior, until just before pupation when the response of wandering larvae to light became random. The photobehavior of several strains known to affect the adult visual system were also studied. All but four exhibited normal phototaxis in the foraging and wandering stages. *gl* mutant larvae failed to respond to light during the foraging stage likely due to lack of larval photoreceptors. Larvae carrying three different mutations in the rhodopsin RH1 gene continued to express negative phototaxis throughout both the foraging and wandering stages. These results suggest that the transition from negative phototaxis toward photoneutral behavior characteristic of the wandering third instar larva requires vision.

Keywords: larval instars, foraging, wandering, visual system mutants

INTRODUCTION

The *Drosophila* adult and larval visual systems are distinct. The larval visual system is relatively simple, consisting of a pair of bilateral visual organs, the Bolwig's organs, juxtaposed to the cephalopharyngeal skeleton (Bolwig, 1946; Steller et al., 1987; Green et al., 1993; Campos et al., 1995, submitted). Each organ consists of approximately twelve photoreceptor cells, with axons which fasciculate to form the larval optic nerve (or Bolwig's nerve). The larval optic nerve extends posteriorly and ventrally around the ipsilateral brain hemisphere, terminating in the area of the brain destined to become the adult optic ganglia (Bolwig, 1946; Steller et al., 1987; Green et al., 1993; Campos et al., 1995, submitted). The establishment of connectivity in this system follows a stereotypical pattern where guidepost cells located within the optic lobe primordium are likely to be required (Campos et al., 1995, submitted). These guidepost cells or optic lobe pioneers (OLPs), as

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they were first described, are subsequently incorporated into the adult optic ganglia while the larval photoreceptor cells are histolysed during metamorphosis (Tix et al., 1989). Except for the elongation of the larval optic nerve, the larval visual system remains unaltered during larval development (A. R. Campos, unpublished results). The role of the larval photoreceptor cells in the photobehavior in *Drosophila melanogaster* has been largely inferred from analogies with larger flies (Bolwig, 1946).

The visual system of the adult fly consists of two compound eyes, three ocelli and three optic neuropil layers (reviews by Meinertzhagen and Hanson, 1993; Wolff and Ready, 1993). Each compound eye is made up of approximately 800 ommatidia. Each ommatidium is an assembly of eight photoreceptor cells R1 through R8, each of which elaborates a rhodopsin-bearing stack of microvilli, the rhabdomere and 12 accessory cells (Wolff and Ready, 1993).

Adult photobehavior assays have been extensively used in the isolation of genetic variants (Benzer, 1967; Koenig and Merriam, 1977; Pak, 1979; Heisenberg and Wolf, 1984). These assays used in conjunction with mutant strains helped to determine the role of photoreceptor cell types in the performance of different types of photobehavior. Photoreceptor cells R1 through R6 mediate optomotor responses while the photoreceptor R7 is involved in fast phototaxis and some types of slow phototaxis (reviewed by Heisenberg and Wolf, 1984). The various photoreceptor cell types can also be distinguished by their spectral sensitivity. The outer photoreceptors express a blue-absorbing rhodopsin (RH1) while R7, located distally in the retina, expresses either RH3 or RH4 and is ultraviolet-sensitive (Harris et al., 1976; O'Tousa et al., 1985; Zuker et al., 1985; Zuker et al., 1987; Montell et al., 1987). Photoreceptor R8, located proximally in the retina, expresses an unidentified blue-absorbing rhodopsin (Harris et al., 1976; Fortini and Rubin, 1990).

Little is known about how the *Drosophila* larva perceives light. Although the larval photoreceptor cell clusters have an organization somewhat similar to the adult compound eye ommatidium (Green et al., 1993) the functional organization of these cells is not well understood. So, for example, while it has been reported that the larval photoreceptor cell clusters express the same rhodopsin genes as found in the adult compound eye, namely RH1, RH3 and RH4 (Mismer and Rubin, 1987; Pollock and Benzer, 1988), it is not known whether the different rhodopsins are expressed in non-overlapping sets of larval photoreceptor cells. Similarly, it is not known how the two rhodopsin-expressing photoreceptor cell clusters operate to modulate larval locomotion as a function of light.

The *D. melanogaster* larva spends most of its life foraging. It moves through and on the surface of the feeding substrate while shovelling food into its gut with its mouth hooks (Sokolowski, 1980). During this time, it is sensitive to variations in abiotic factors such as light, odors and humidity (Grossfield, 1978). In the late third instar, a general change occurs in larval behavior; larvae cease foraging and commence behavior which is commonly referred to as wandering (Sokolowski et al., 1984). Wandering precedes pupation and presumably occurs in conjunction with a series of strain and condition-dependent behaviors related to the selection of a suitable pupation site (see Rizki and Davis, 1953; Mishima, 1964; Kearsey and Kojima, 1967; Sameoto and Miller, 1968; Grossfield, 1978; Alvarez et al., 1979; Markow, 1979; Manning and Markow, 1981; Fogelman and Markow, 1982; Sokolowski and Hansell, 1983; and Wong et al., 1985; Schnebel and Grossfield, 1986; Sokolowski et al., 1986; Godoy-Herrera et al., 1989).

One of the behavioral changes associated with the wandering stage involves the larval response to light. Up to 96 hours after hatching at 25°C, *D. melanogaster* displays a negative phototactic response, that is the larva moves away from light (Hotta and Keng, 1984; Lilly and Carlson, 1990; Sawin et al., 1994). Interestingly, Godoy-Herrera et al., (1992, 1994) reported a change from negative to positive photobehavior occurring later in the third larval instar (reviewed by Sawin et al., 1994). Changes in photobehavior during larval development could have evolved in response to different light conditions in the environments inhabited by the larva at different points during development. For example, digging in the food during foraging may be facilitated by negative photobehavior (Godoy-Herrera et al., 1994). In addition, species-specific differences in pupation site choice in *Drosophila* in light as compared to dark environments have been described (reviewed by Schnebel and Grossfield, 1986).

We have begun a genetic dissection of the larval photobehavior response in order to understand how foraging and wandering *D. melanogaster* larvae perceive and respond to light. In the present paper we: 1. characterize the wild type photoresponse throughout larval development including the wandering stage and 2. re-evaluate mutant strains in the larval and adult visual systems in order to begin addressing the functional aspects of the larval visual structures.

MATERIALS AND METHODS

Fly Strains

Fly strains were generously provided by J. C. Hall, J. E. O'Tousa, W. S. Stark, H. Steller, and the Bloomington, Indiana Stock Center courtesy of Kathy Matthews. The visual system mutations disrupted a variety of structural and phototransductive pathways (see Table I). Wild type strains included *Canton-Special* (*Canton-S*), *Oregon R* (*OR*) and an isogenic *for*^{over} strain (*BB*) generated in one of our laboratories (M.B.S.).

Strains were maintained at 25°C ± 1°C on a medium of dead yeast, sucrose, and agar, supplemented with the mold inhibitor propionic acid.

Synchronization of Larval Behavior

Young adult flies aged 1–7 days were allowed to lay eggs for a period of 3 hours on a fresh molasses substrate coated with a yeast paste. This followed a period of 3 hours in which the adult females were encouraged to expel older embryos in the presence of a molasses and yeast substrate. Aged larvae were subsequently collected on the basis of size and mouth hook morphology. All larvae were grown at 25°C ± 1°C and collected and tested as follows: first instar larvae at approx. 36 hours after egg lay (AEL), second instar larvae at approx. 60 hours AEL, early third instar (foraging) larvae at approx. 82 hours AEL, and initially late third instar larvae (wandering) were tested at approx. 110 hours AEL. More detailed tests of wandering larval photobehavior were done in the tube assay (see below) by using larvae collected at the onset of wandering behavior 110 hours AEL and subsequently at 1 to 2 hour intervals until just prior to pupation.

TABLE I

Summary of phenotypes of *Drosophila* mutants with defects in visual physiology

Locus/Allele	Phenotype/Function	Refs.
norpA[*P24*]	*no-receptor-potential* Adults are blind and lack light- elicited receptor potentials in the compound eyes and ocelli. Encodes photoreceptor-specific phopholipase C.	A B C
ninaE[*P118*] *ninaE*[*0117*]	*neither-inactivation-nor-after-potential* Encodes the opsin moiety of the major rhodopsin, RH1. RH1 is expressed in the rhabdomeres of the adult compound eye and in the larval light sensitive organs. An age dependent, light-independent degeneration has been observed.	D E F
ora[*JK84*]	*outer-rhabdomeres-absent* Double mutant in the *ort* and *ninaE* genes in which the electroretinogram lacks on-transients, and off- transients reduced or absent. Mutants show an age dependent degeneration of R1-6 rhabdomeres.	G
rdgA[*BS12*]	*retinal degeneration A* Encodes photoreceptor specific DAG kinase. Adult photoreceptor degeneration.	A H I
rdgB[*KS222*]	*retinal degeneration B* Light-dependent adult photoreceptor degeneration, phospholipase C dependent. Integral membrane protein found in photoreceptors and other neurons.	A J
rdgC[*306*]	*retinal degeneration C* Light-dependent adult photoreceptor degeneration, phospholipase C independent. Encodes a serine/ threonine photoreceptor protein phosphatase directly regulated by calcium.	K L
trp[*CM*]	*transient-receptor-potential* Photoreceptor-specific integral membrane protein represents a class of light-sensitive channel required for inositide-mediated calcium entry necessary for maintained excitation during intense illumination.	M N O
so[*1*]	*sine oculis* Adults lack ocelli, eyes mostly absent, and the optic lobes reduced in size. Larval visual system present in this allele. Homeobox-containing protein required for visual system determination.	P Q R
gl[*60J*]	*glass* Total lack of photoreceptor cells in adult and larval visual systems. Encodes a zinc-finger containing protein known to regulate the expression of R-cell specific genes.	S T

A, Hotta and Benzer, 1970; B, Pak et al., 1970; C, Bloomquist et al., 1988; D, Pak, 1979; E, Zuker et al., 1985; F, O'Tousa et al., 1985; G, O'Tousa et al., 1989; H, Suzuki et al. 1990; I, Inoue et al., 1989; J, Harris et al., 1976; K, Steele and O'Tousa, 1990; L, Steele et al., 1992; M, Cosens and Manning, 1969; N, Montell and Rubin, 1989; O, Hardie and Minke, 1992 P, Fischbach and Technau, 1984; Q, Cheyette et al., 1994; R, Serikaku and O'Tousa, 1994; S, Meyerowitz and Kankel, 1978; T, Moses et al., 1989.

Phototaxis Assays

Measurements of foraging larval photobehavior were made on a plate assay modified from Lilly and Carlson (1990), and on a new tube assay described here (Figure 1). The plate assay consisted of a plastic Petri plate (100 mm × 15 mm; Fischer Scientific) .45 ml of 1% agarose was poured into each plate. Agarose was poured into the plates slowly to create a smooth surface for larval locomotion. The plates were then allowed to equilibrate to room temperature for a period exceeding 1.5 hours. Each plate was then placed upon a template fit to its circumference. This template sectioned the plate into four equal quadrants two diametrically opposed dark quadrants/sections blocking out all light, and two diametrically opposed light quadrants/sections permitting the transmission of light. The template and dish were then placed on a fluorescent light box. At the appropriate time larvae were removed, using a moist paintbrush, from several culture dishes and carefully rinsed with distilled water and blotted to remove excess water and yeast. Each culture dish contained 35 ml of medium which had been seeded with approx. 100 newly hatched larvae.

Approximately 100 larvae were placed in the center of the plate assay. Ambient light in the room was extinguished and the plate was lit via the fluorescent light box from below (a smaller (35 mm × 10 mm; Fischer Scientific) plate assay was used for the first and second instar larval tests, with 10 ml of 1% agarose). Larvae were left on the plate assay for 5 minutes. At the end of this test period the number of larvae in the light sections were recorded. First, second and early third instar larvae do not leave the agar surface. They remain on moist substrates. If they are placed on plates without agar, they try to leave the plates. There was no significant difference between the surface temperatures of the clear and dark quadrants after five minutes on the light source (data not shown).
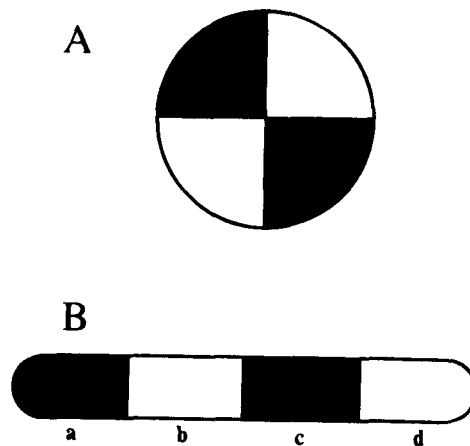

FIGURE 1   Assays for the measurement of larval photobehavior in *D. melanogaster*. A: Measurements of larval foraging photobehavior were made on this assay modified from Lilly and Carlson (1990), 100 larvae were placed in the middle of the petri dish at the onset of testing. B: Assay used for the measurement of both foraging and wandering photobehavior, with black adhesive tape covering sections a and c, tubes were joined between b and c with tape. Ten larvae were placed in section b at the beginning of the test period.

The plate assay described above did not allow us to test wandering photobehavior since wandering larvae leave the agar surface in search of dry substrates. Wandering larvae were found on the lid of the plate assay whose bottom contained an agar surface. The differences in behavior between foraging and wandering larvae necessitated that we measure their photobehavior on different surfaces. We therefore used a test tube assay for the quantification of wandering larval photobehavior.

The tube assay consisted of two apposed glass test tubes (16 mm × 100 mm each) joined by black adhesive tape between sections **b** and **d** (see Figure 1). This tape was applied to the test tubes to create alternate dark sections, and clear sections. The testing of larval photobehavior consisted of placing 10 larvae in section **b** of one tube which was then sealed to an apposed tube containing sections **c** and **d**. The tube was placed on an illuminated fluorescent light box in the absence of ambient light in the room. After 5 minutes the number of larvae in sections **b** and **d** were counted. Behavioral measures were taken in both assays once larval movement had reached an equilibrium. Measurements of photobehavior in the plate and tube assays were similar. For example, the photobehavior of *Canton-S* foraging larvae did not significantly differ in the dish and tube assays ($F_{(1,37)} = 0.23$, NS).

Statistical Analysis

All data is presented as means plus or minus one standard error (X ± SEM). Analysis of variances (ANOVAs) were performed on the raw data (mean number of larvae in light) but in order to compare data from the plate assay where N = 100 per plate and the tube assay where N = 10 per tube, mean percentages ± SEM were used. Chi-squared tests for homogeneity were performed according to Stalker (1942). Transformation of the data was unnecessary because variances did not differ significantly (F_{max} test, Zar, 1984). Statistical analyses were done using SAS procedures (SAS Institute Inc., 1985).

RESULTS

Phototaxis Throughout Larval Development in Drosophila melanogaster

Synchronized first, second and third instar larvae were tested according to Methods. Third instar larvae were collected as wandering irrespective of the time of onset of the wandering behavior. First, second and foraging third instar larvae were tested in the plate assay while wandering third instar larvae were tested in the tube assay. In the plate assay, larvae showing negative phototaxis migrated away from their initial position in the center of the plate, towards the dark quadrants. Likewise, in the tube assay, negatively phototactic larvae migrated away from their initial placement in lighted section b towards dark sections a and c (Figure 1). The number of larvae in the light sections for the plate assay could range from 0 (absolute negative phototaxis) to 50 (neutral phototaxis) to 100 (absolute positive phototaxis). For the tube assay the values similarly ranged from 0 to 5 to 10. Data are shown as percentages for ease of comparison between the assays.

Figure 2 shows the mean percentage ± SEM of the first, second, early third and wandering wild type *Canton-S* larvae found in the light sections of the phototaxis assays. First through early third instar (foraging) larvae exhibited negative phototaxis (ANOVA, $F_{(2,20)}$
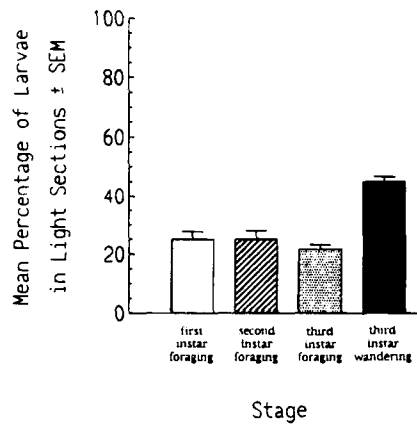
FIGURE 2   Wild type *Canton-S* photobehavior of first instar (unfilled bar) (N = 3), second instar (hatched bar) (N = 3), third instar foraging (dotted bar) (N = 31) and third instar *D. melanogaster* wandering larvae (filled bar) (N = 130) ± SEM. The populations of foraging larvae and wandering larvae varied in age by ± 3.5 hours and ± 6 hours respectively.

= 2.44, NS). The behavior of wandering larvae differed significantly from that of the foraging larvae (ANOVA, $F_{(3,184)}$ = 34.31, p < 0.0001) (Figure 2) and appeared to be random in response to the light stimulus. Wandering larvae of the strains *norpA*[p24], *rdgA*[BS12], *rdgB*[KS222], *rdgC*[306], *trp*[CM], *so*[1] and *gl*[60j] showed no response to light, that is, they distributed themselves randomly in the assay (Chi-square, $X^2{}_{357}$ = 43.8, p < 0.0001). There were no significant strain differences (Student-Newman-Keuls (SNK), alpha = 0.05, df = 357).

Photobehavior of Wild Type Strains During the Wandering Stage of the Third Larval Instar

Given previous reports of positive phototaxis in the late third larval instar (Godoy-Herrera *et al.*, 1992, 1994) we decided to examine the response to light of larvae collected and aged from the onset of wandering. Figure 3A, B and C shows the larval phototactic response from early third instar up to 0, 3, 6 and 10 hours post the onset of wandering in three wild type strains. The wandering larval photobehavior in these strains displayed a highly significant time effect (ANOVA, $F_{(3,201)}$ = 10.16, p < 0.0001). As wild type larvae age their wandering behavior became significantly positively correlated with the number of larvae in light sections (Correlation Coefficient for *Canton-S*, r = 0.4, p < 0.0001, N = 130; *Oregon R*, r = 0.3, p < 0.0001, N = 57; *BB*, r = 0.5, p < 0.003, N = 28). The final outcome was the random distribution of larvae in the light and dark sections of the tube.

Photobehavior of Visual System Mutants During the Foraging Stage of the Third Larval Instar

Given the results above we decided to re-evaluate the phototactic response of several mutations known to disrupt adult visual system function. Figure 4 shows the mean percentage of early third instar foraging larvae in light sections ± SEM for the mutant
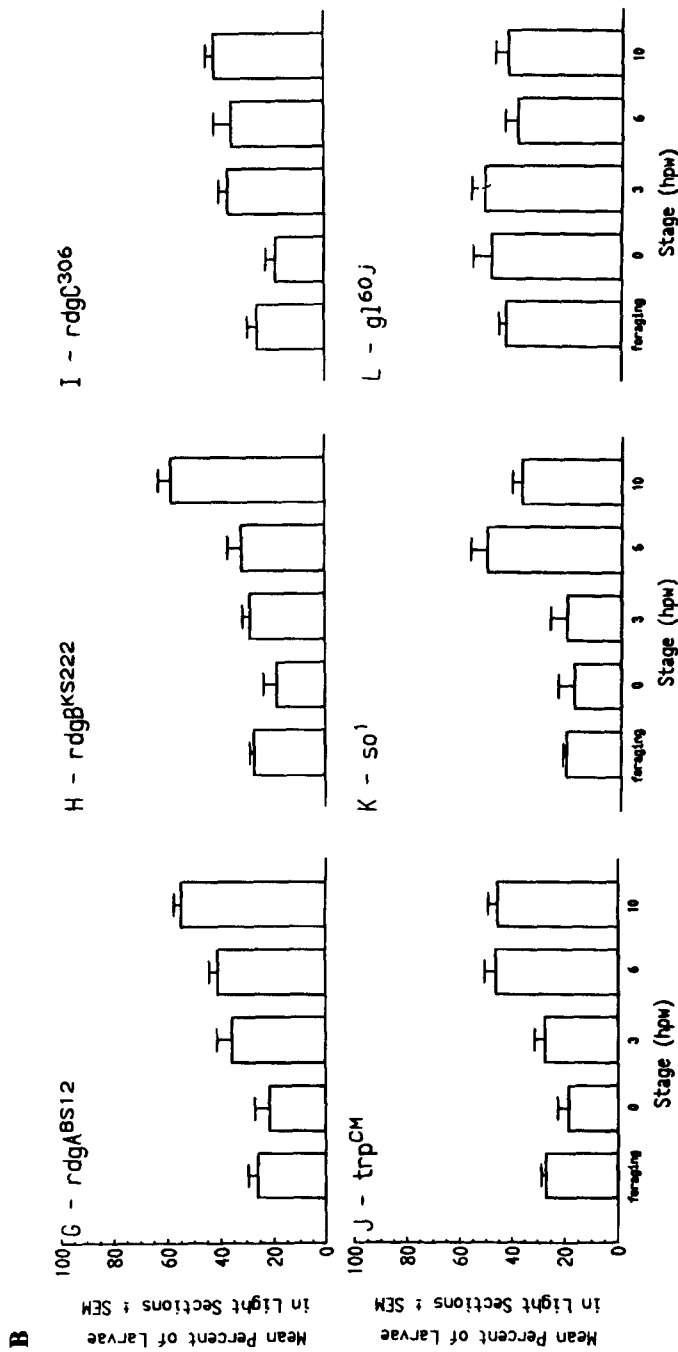
B
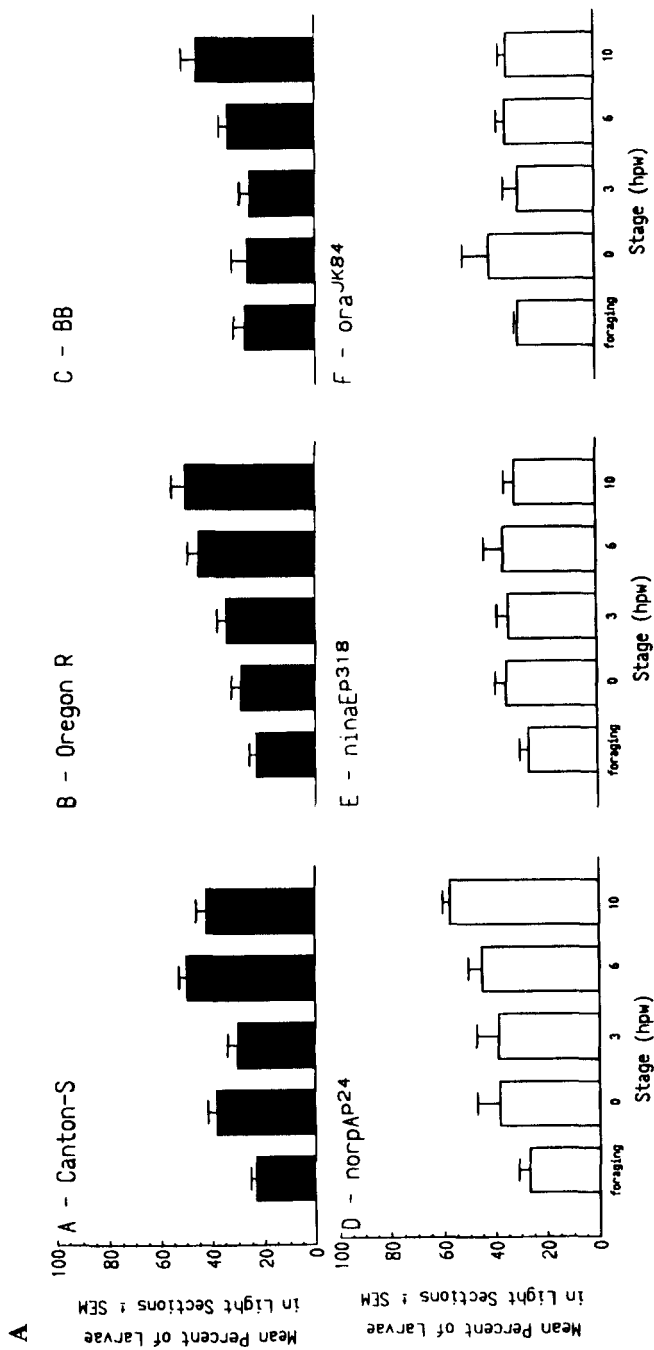
FIGURE 3   Summary of *Drosophila* larval photobehavior throughout the third instar of development in the foraging and wandering stages (hours post wandering = hpw) showing mean percent of larvae in light sections ± SEM. A: *Canton-S*, B: *Oregon R*, C: *BB*, D: *norpA^{P24}*, E: *ninaE^{118}*, F: *ora^{JK84}*, G: *rdgA^{BS12}*, H: *rdgB^{KS222}*, I: *rdgC^{306}*, J: *trp^{CM}*, K: *so^1*, L: *gl^{60j}*.
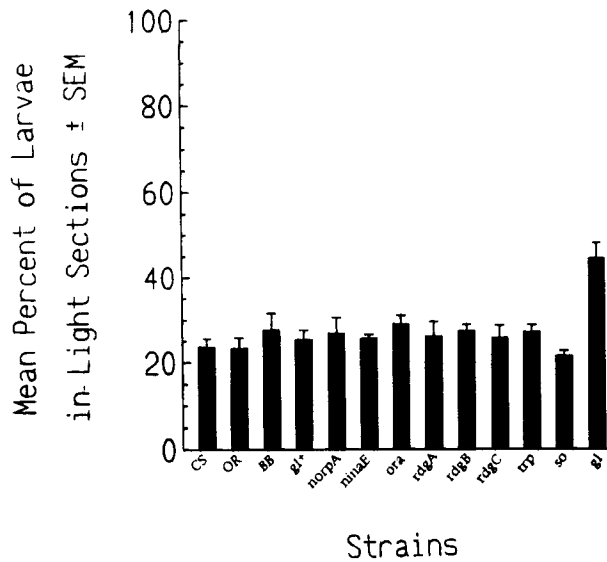
FIGURE 4   *Drosophila* larval photobehavior during the early third instar foraging stage in visual system mutants (mean percent of larvae in light sections ± SEM). The number (N) of dish assays each containing 100 larvae was N = 31 for the wild type *Canton-S* strain, N = 3 for *Oregon R, BB, P[10Kb Sal, ry]; gl^MI* (referred to as *gl⁺*), N = 6 for *norpA^H24*, N = 3 for *ninaE^P11X*, *ora^JK84*, *rdgA^RS12*, *rdgB^KS222*, *rdgC^H06*, *trp^CM* and *so'*, and N = 6 for *gl^MI*.
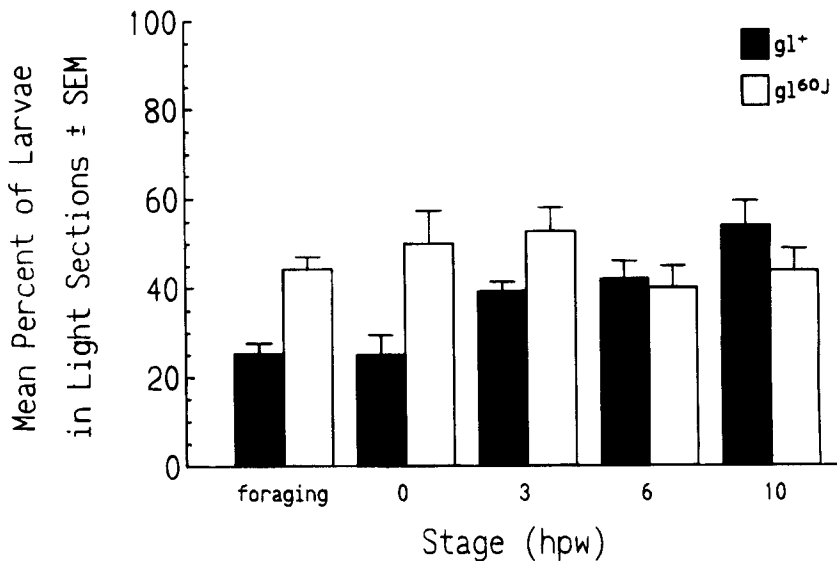

FIGURE 5   *Drosophila* larval photobehavior in *gl^MI* mutants (unfilled bar), and a *gl⁺* strain containing a *gl*-rescuing transposon *P[10Kb Sal, ry]; gl^MI* (filled bar). Hours post wandering is indicated by hpw.

strains (listed in Table I), and the wild type strains (*Canton-S, Oregon R* and *BB*). ANOVA revealed significant variation among the photobehavior of the strains tested ($F_{(11,61)}$ = 8.70, p < 0.0001). All strains, with the exception of gl^{60j}, exhibited negative photobehavior indistinguishable from the wild type response to light at this point in development. Significantly higher numbers of foraging gl^{60j} mutant larvae were found in the light sections of the plate than in all other wild type and mutant strains (SNK alpha = 0.05, df = 11). gl^{60j} larvae distributed themselves randomly in light and dark quadrants (X^2_5 = 0.82, p < 0.025), that is they failed to respond to light. Figure 5 shows the gl^{60j} mutant strain compared with the wild type pattern of responding exhibited by a gl^{60j} mutant strain carrying a P-element containing a wild type copy of the *gl* gene *P[10Kb Sal, ry]; gl^{60j}* shown to completely rescue the adult phenotype of gl^{60j} (Moses and Rubin, 1991).

Photobehavior of Visual System Mutants During the Wandering Stage of the Third Larval Instar

The mutant strains *norpA*[P24], *rdgA*[BS12], *rdgB*[KS222], *rdgC*[306], *trp*[CM] and *SO*[1] showed a decrease in negative phototaxis from the onset of wandering culminating in random photobehavior indistinguishable from the response of wild type strains tested (Figure 3). Significant correlations in this pattern were found for *norpA*[P24] (r = 0.4 p < 0.007, N = 43), *rdgA*[BS12] (r = 0.6 p < 0.0001, N = 41), *rdgB*[KS222] (r = 0.7 p < 0.0001, N = 35), *rdgC*[306] (r = 0.4 p < 0.005, N = 43), *trp*[CM] (r = 0.6 p < 0.0001, N = 36), and *so*[1] (r = 0.4 p < 0.01, N = 44). In contrast,
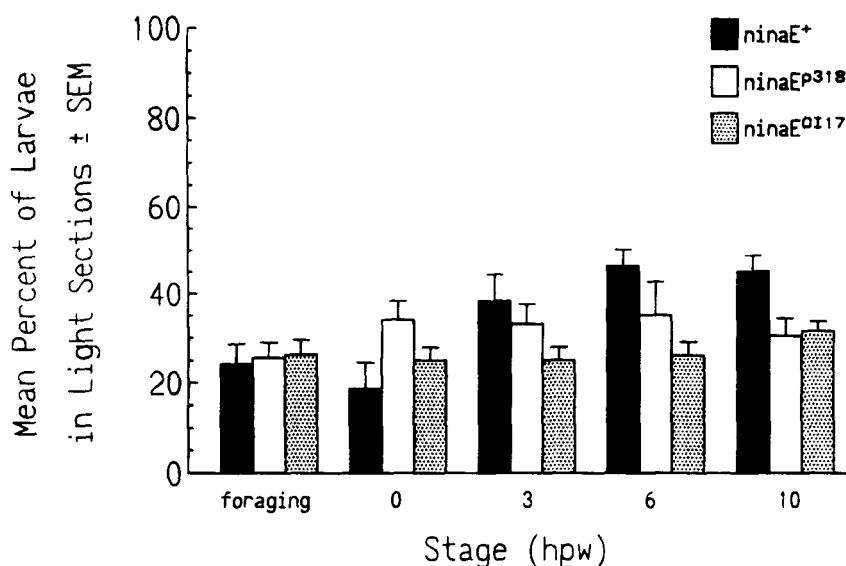

FIGURE 6 Photobehavior in strains of *Drosophila* larvae with two mutant alleles; *ninaE*[P318] (unfilled bar) and *ninaE*[OI17] (dotted bar) and a *ninaE*+ strain containing a wild type transgene in a *ninaE*[OI17] background (solid bar). Wild type strains are indicated by filled bars, mutant strains exhibiting the wild type pattern of responding are indicated by hatched bars, and mutant strains showing a mutant response are unfilled bars.

ninaE[P318], *ora*[JK84] and *gl*[60j] mutants did not show the wild type pattern of phototaxis (*ninaE*[P318] r = –0.1, NS, N = 48; *ora*[JK84] r = 0.02, NS, N = 32; *gl*[60j] r = –0.2, NS, N = 28). These mutant larvae failed to exhibit a significant relationship between wandering and photobehavior. *ninaE*[P318] and *ora*[JK84] mutant larvae continued to exhibit the negative phototaxis characteristic of the foraging stage whereas *gl*[60j] mutant larvae remained unresponsive to light (Figure 3 panel L). The duration of time spent wandering prior to pupation was evaluated for these three mutant strains to determine if these larvae were developmentally delayed. *ninaE*[P318], *ora*[JK84] and *gl*[60j] mutant larvae spent the same amount of time wandering as did the wild type strain *Canton-S* (data not shown).

An additional *ninaE* mutant strain (*ninaE*[OII7]) was tested in order to confirm the requirement for a functional *ninaE* gene in phototaxis during the wandering stage. As a control we tested the *ninaE*[OII7] mutant strain carrying a P-element containing a wild type copy of the *ninaE* gene. This transposon has been previously shown to completely rescue the adult phenotype of *ninaE*[OII7] mutant alleles (Zuker *et al.*, 1988). These results are shown in Figure 6. For ease of comparison the results of the *ninaE*[P318] mutant strain previously presented in Figure 3E are also shown. Both *ninaE*[P318] and *ninaE*[OII7] alleles failed to exhibit a significant relationship between the duration of wandering and an increase in photoneutral behavior (r = –0.1, NS, N = 48; r = 0.2, NS, N = 30 respectively). The *ninaE*[OII7] mutant strain carrying the transposon showed the wild type response with increasingly positive phototaxis directly correlated with the duration of wandering behavior (Correlation Coefficient r = 0.3, p < 0.001, N = 32; Figure 6). We concluded that the continued negative phototaxis displayed by *ninaE* mutant strains is due to lack of *ninaE* gene function.

DISCUSSION

The major findings in this study were 1) *D. melanogaster* larvae during the foraging stage of development (first, second and early third larval instar) showed a marked aversion to light. 2) Wild type *D. melanogaster* larvae during the wandering stage of development (from mid third larval instar until just prior to pupation) showed a shift in photobehavior from negative phototaxis to a random response to light. 3) The analysis of photobehavior of visual system mutants revealed a failure to respond to light in *gl*[60j] mutant larvae throughout the third larval instar. 4) Mutant strains *ninaE*[P318], *ninaE*[OII7] and *ora*[JK84] displayed negative photobehavior which is characteristic of the foraging stage throughout the wandering stage. 5) Visual system mutants *norpA*[P24], *rdgA*[BS12], *rdgB*[KS222], *rdgC*[306], *trp*[CM] and *so*[1] exhibited the wild type pattern of photobehavior throughout the foraging and wandering stages.

A characterization of larval photobehavior has been previously reported by Godoy-Herrera and colleagues (1992, 1994). Their results differed from those found in the present study. These authors reported a complex and variable pattern of photobehavior throughout larval development, and a reversal in larval photobehavior from negative to positive phototaxis at 96 hours AEL (mid third larval instar, just prior to the onset of wandering behavior). This may have resulted in part from differences in experimental design. In the aforementioned author's assay, larvae were tested while foraging on a food substrate, whereas ours were tested on non-nutritive substrates; agarose in the foraging stage

and glass in the wandering stage. In Godoy-Herrera's studies larvae were tested in perspex boxes. The use of such boxes may have affected larval behavior since boxes have corners which cause larvae to change their behavior (for example, dig) (Sokolowski et al., 1984). The plate assay and tube assay utilized in our experiments contained no edges or corners. Finally, the larvae utilized in their analyses carried morphological markers (e.g. yellow) which may alter locomotory behavior. Hotta and Keng (1984) likewise examined larval photobehavior in the third larval instar, however, the stage of development within the third larval instar was not determined.

Photobehavior during Foraging

D. melanogaster displayed a robust negative phototactic response throughout the foraging stage of development (first, second, early and mid third larval instars). The negative phototaxis of larvae during foraging may serve to keep young larvae in close proximity to a food source. Additionally, a propensity to avoid light could be necessary not only in the location of food, but also in the avoidance of desiccation and predation (Sokolowski, 1985).

The gene products of the genes *norpA*, *ninaE*, *rdgA*, *rdgB*, *rdgC* and *trp* have been shown to participate in phototransduction in adult flies (reviewed by Smith et al., 1991 and Zuker, 1992; see Table I for a list of mutations, phenotypes, and gene products). Mutations in the genes *norpA*, *ora* and *trp* were first isolated as ERG-defective (Pak et al., 1970; Koenig and Merriam, 1975; Cosens and Manning, 1969), while *rdgA*, *rdgB* and *so* mutants were identified as nonphototactic adult flies (Hotta and Benzer, 1970; Benzer, 1967). All of these visual system mutants fail as adults to exhibit some aspect of light-induced behaviors (Benzer, 1967; Cosens and Manning, 1969; Hotta and Benzer, 1970; Pak et al., 1970; Koenig and Merriam, 1975). *ora^JKM* is a double mutant in the *ninaE* and *ort* (*ora transientless*) genes, certain effects of *ora^JKM* on visually mediated behaviors, such as the absence of blue-light influenced phototaxis (Willmund and Fischbach, 1977), or the absence of R1-6-dependent optomotor responses (Heisenberg and Buchner, 1977) could be due to mutations in either of the *ninaE* and *ort* genes (O'Tousa et al., 1989). Young adult *rdgC* mutant flies show normal visual function, but develop photoreceptor degeneration and a degraded response to light stimuli if the flies are maintained in a lighted environment (Steele et al., 1992).

Mutations in these genes did not disrupt negative phototaxis in the early third instar foraging larvae suggesting the existence of a separate and as yet undetermined phototransduction pathway functioning in larval phototaxis as measured by our assays. The apparent lack of mutant phenotype in *trp* mutants however, should be taken with caution. In adult flies carrying mutations in the *trp* gene, phototaxis is normal in low ambient light, however, these flies behave as though they were blind in bright light (Cosens and Manning, 1969). Hence it is possible that modifications in the intensity of the light source may uncover a mutant phenotype in the larval phototactic response of *trp* mutants.

Our results suggest that RH1, a blue-absorbing rhodopsin whose protein moiety opsin is encoded by the gene *ninaE* (Scavarda et al., 1983), serves no observable function with respect to larval foraging photobehavior as defined by our assay. Two other rhodopsins are also found in the larval photoreceptor cells, ultraviolet-absorbing rhodopsins RH3 and RH4 (Mismer and Rubin, 1987; Pollock and Benzer, 1988; Fortini and Rubin, 1990).

There are no known mutations in the structural genes for either RH3 or RH4. Given that the spectral sensitivity of the larval phototactic response is not known it is possible that either one of these rhodopsins are involved in phototaxis in the foraging larva.

Evidence for extra-ocular photoreception comes from experiments with circadian rhythms in which several mutations that disrupt adult visual system function have been reported to leave the adult fly with functioning circadian photoreceptors (Helfrich, 1986; Dushay et al., 1989; Wheeler et al., 1993). Both *norpA* and *so* mutants have been found to be entrainable to light:dark cycles (Wheeler et al., 1993).

Previous work using phototransduction mutants to examine the larval visual system (Hotta and Keng, 1984) attributed aberrant photobehavior to strains carrying mutations in the genes *norpA*, *rdgA* and *rdgB*. However, the larvae collected by these authors were assayed as third instar, without distinguishing between foraging and wandering larvae. Pleiotropic effects of the mutations on the developmental timetable could affect the proportion of the third instar larvae in the foraging and wandering stages. Our findings that wandering photobehavior is significantly less photonegative than foraging photobehavior (Figure 2) may explain the discrepancy between our results and those of Hotta and Keng (1984).

Our results demonstrated that gl^{60j} mutant larvae failed to respond to light during the foraging stage. Rescue of this mutant phenotype by a transposon containing a wild type copy of the *glass* gene demonstrates that the behavioural phenotype is indeed due to lack of *glass* gene function. Disrupted larval photobehavior of gl^{60j} mutants is likely due to the absence of larval photoreceptors, however, the possibility that *glass*-expressing cells in the central brain (Moses and Rubin, 1991; Ellis et al., 1993) also have a role in larval photobehavior can not be excluded. Mosaic analysis in which larvae carrying mutant patches in the central brain area where the *glass*-expressing cells are located should aid in defining the role of these cells in larval phototaxis.

Photobehavior during Wandering

The duration of larval wandering was found to be positively correlated with the number of larvae in light sections for the three wild type strains examined. From the onset of wandering behavior, larvae became less repelled by light until achieving photoneutral behavior as seen by the equal distribution of larvae in light and dark sections. Changes in the development of larval photobehavior may be associated with the different light environments inhabited by the larva at different points in its development. It is possible that the migration away from the food source that characterizes the onset of wandering behavior is somewhat influenced by the larval response to light.

The transition from foraging to wandering photobehavior was found to be abnormal in $ninaE^{p318}$, $ninaE^{0117}$ and ora^{JK84} mutant larvae, all of which display a marked reduction in the rhodopsin RH1. These mutant strains continued to express negative phototaxis throughout the wandering stage, failing to achieve a significant correlation between duration of wandering and unresponsiveness to light. Larval phototaxis in the $ninaE^{p318}$, $ninaE^{0117}$ and ora^{JK84} mutant strains at the wandering stage was not significantly different from that at the foraging stage (Figures 3E and 6). A transposable element containing a wild type copy of the *ninaE* gene was sufficient to restore the wild type phenotype. This result demonstrates that the defect in behavior seen in the *ninaE* mutant strains resulted specifically from abnormal

ninaE gene function. In the case of the *ora* mutant, the mutation in the *ort* gene may additionally contribute to the mutant behavioral phenotype. Thus, it appears that RH1 plays a significant role in the larval visual system for the reduction in negative phototactic behavior observed from the onset of the wandering stage. These results strongly suggest that active reception of light and vision are necessary for the observed shift in phototaxis.

The adult photoreceptor axons first enter the developing optic lobes at the same time when the shift in phototaxis was observed (Wolff and Ready, 1993). Our results however do not support the hypothesis that changes occurring in the developing optic lobe due to adult R-cell innervation trigger the observed behavioral shift. In *so¹* mutant larvae which lack the adult compound eyes or ocelli but not the larval visual system (Cheyette et al., 1994; Serikaku and O'Tousa, 1994) the phototaxis response from the onset of wandering behavior was indistinguishable from wild type.

The *Drosophila* larval visual system is well suited for the genetic dissection of development and behavior. Here we report the wild type response to light at different stages during larval development and an initial analysis of mutations previously determined to disrupt adult visual function. Fundamental questions regarding the role of various rhodopsins and the spectral sensitivity of larval phototaxis during the foraging and wandering stages remain to be addressed. The generation of additional mutants that disrupt phototaxis during both larval stages will be instrumental in dissecting the components required for the performance and modulation of phototaxis.

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