ANALYSIS OF THE LIGHT RESPONSE OF THE NEMATODE, *CAENORHABDITIS ELEGANS*, USING A COMPUTER SYSTEM

by

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Analysis of the light response of the nematode,

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ABSTRACT

Despite the small size, the nervous system of *Caenorhabditis elegans* generates and regulates many behaviors; one of them is photosensation. In an previous study, it was reported that monochromatic light stimuli, ranging from 520-600 nm, elicited an avoidance response which included a reversal of locomotion. Because such a response was elicited with a low probability and was superimposed on a background of spontaneous reversals, a large sample size and a great effort were required to achieve significance.

In the experiments reported here, this light response and the spontaneous reversals of *C. elegans* were further analyzed by using a computer-video tracking system that automatically tracks and scores reversals of up to 23 worms simultaneously. A large sample size (~1000) was thereby achieved. From the x and y coordinates recorded along the tracks, the direction and distance moved each second was calculated. These data were analyzed by an algorithm to determine when a reversal bout was initiated. The probability of reversals occurring within the 10 s experimental and control periods was calculated. Paired statistics were used to compare the probability of reversals in 10 s light periods with the preceding 10 s dark periods. Results indicated that the probability of a reversal occurring during the 10 s light period was 21% above the spontaneous reversal rate (0.0473 higher), and the difference between paired experimental and control periods was significantly different from

zero [t=5.57, d.f. = 16, p<0.001]. There was no obvious time dependency of the light-induced reversals during the 10 s light period.

Although filtering of the stimulus beam reduced the infrared to trace levels (< $4x10^{-5}$ of the beam intensity), it was desirable to demonstrate that it was indeed light, not temperature change that elicited the response. The behavior was tested under conditions identical to the preceding experiment except for the addition of a blocking filter which eliminated visible light while transmitting infrared. This effectively eliminated the response. The results confirmed that the observed response was to light and not to a temperature change caused by trace levels of infrared in the stimulus beam.

I did experiments with two mutants aimed at narrowing the possible candidates for the light-sensitive sensory organelle. Possible candidates include the microvillous finger projections of the dendrite of one sensory cell (AFD) and the modified cilia of most of the sensory dendrites of *C. elegans*. I tested *ttx-1* in which the microvilli of AFD are lacking and *daf-19* in which all ciliated endings are absent. The light response of both *ttx-1* and *daf-19* were not significantly different from background spontaneous reversals. The results suggest that both the microvilli of AFD dendrites, and ciliated dendrite endings of one or more sensory neurons may be involved in photosensitivity.

Acknowledgment

I would like to thank my supervisor, Dr. A. H. Jay Burr, for his support and guidance in this thesis. Thanks to Drs. Cathy Rankin and J. M. Webster for their helpful comments and suggestions. Thanks also to Dr. Dave Baillie and his students for their advice and supplies for culturing *C. elegans*. Finally, I thank my parents and Joanne, for their patience and support. This work was partially supported by a grant from the National Science and Engineering Council of Canada, a scholarship from the R. H. Wright Institute for Sensory Sciences and Graduate Fellowships from Simon Fraser University.

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Chapter 1

General

Nematodes are an important group of animals. They are extremely common in most environments and play important ecological roles (Yeates, 1981; Anderson et al., 1981). Hyman (1951) estimates that there are 500,000 nematode species, which is of the same order as is the number of insect species. Nematodes occupy a unique place in the evolution of the nervous system. They are among the simplest of organisms that have a centralized nervous system made up of a specific and countable number of neurons. Nematodes are good subjects for genetic analysis of a variety of biological process because of their simplicity of structure and the ability of some species to self-fertilize.

Brenner (1974) has demonstrated that the bacteriophagous species, *Caenorhabditis elegans*, is well suited for studying the relationship between genome and nervous system. The main advantages of *C. elegans* as an experimental organism, other than its relatively simple anatomy, are its short reproductive cycle, small genome size and the ease with which it can be manipulated genetically. Mutants are readily produced by exposure to ionizing radiation or chemical mutagens. The detailed study of *C. elegans* has motivated much of the recent work on nematode metazoan development and behavior.

Nematode *Caenorhabditis elegans*

The nematode *C. elegans* is a small, free-living soil nematode that is common in many parts of the world. The length of the adult worm is about 1 mm, and it has two sexes, hermaphrodite and male. The hermaphrodites produce both oocytes and sperm and therefore, can reproduce by self-fertilization. The males arise from self-fertilization in the hermaphroditic population, occur at relatively low frequencies (1 in 1,000) (Rose and Baillie, 1979) and have the capacity to fertilize hermaphrodites. The adult hermaphrodite has 959 somatic cells; 302 are neurons which have been classified in 118 types according to their branching and connectivity patterns (White et al., 1986). The nucleus of each neuron can be identified in live animals by differential interference microscopy using a map, and the cell lineage that gives rise to each of these neurons has been described (Sulston and Horvitz, 1977; Sulston et al., 1983). A detailed atlas of the nematode's nervous system has been compiled by reconstructing all neuronal processes and cell bodies from transverse, serial section electron micrographs spanning the length of the animal from the cephalic region to tail (Ward et al., 1975; Ware et al., 1975; White et al., 1986). The haploid genome size is 8×10^7 nucleotide pairs, distributed among five autosomes and one sex chromosome. The genome is estimated to encode about 14,717 genes (*C. elegans* Genome Consortium, personal communication, 1996).

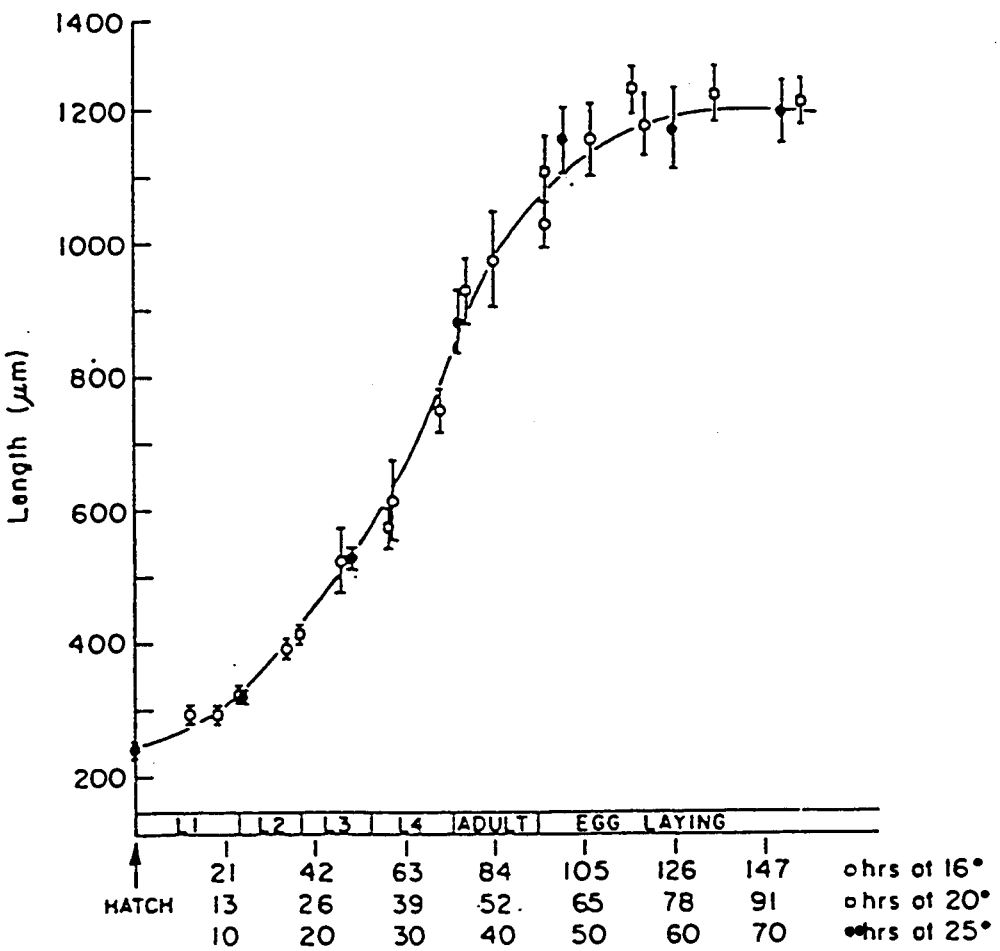
C. elegans develops to adulthood in about 3.5 at 20 °C. A single hermaphrodite that has not mated lays about 300 eggs by self-fertilization during its reproductive life span. Each egg develops into an L1 larva and grows quickly through three additional stages, L2, L3 and L4, and finally molting to become adult animals (Figure 1). Adult hermaphrodites live for about 17 days in culture at 20 °C, although egg laying ceases around 2 days after the final molt.

Nematode behavior and nervous system

Most of the work on nematode behavior has been concerned with locomotory behavior, particularly that related to movement toward or away from stimuli. Basic behavioral activities in *C. elegans* on an agar plate include seven components of body movement (Croll, 1975):

1. Head movements: bending of the head in vertical and horizontal dimensions in an irregular pattern
2. Backward waves: dorsoventral waves that move backward along the animal, propelling it forward
3. Forward waves: dorsoventral waves that move forward along the animal, propelling it backward
4. Omega waves: deep bends in which head and tail are brought close together
5. Tail kinks: sharp bends near the tail

Figure 1. Growth curves of wildtype *Caenorhabditis elegans.* Each point represents measurements from 20 worms, and ±SD is indicated by the vertical bars. The larval stages, which are separated by molting, are indicated as L1 through L4. Adapted from Hirsh et al., 1976.


1400
1200
1000
800
600
400
200
Length (μm)
L1
L2
L3
L4
ADULT
EGG LAYING
HATCH
21 42 63 84 105 126 147 ohrs at 16°
13 26 39 52 65 78 91 ohrs at 20°
10 20 30 40 50 60 70 •hrs at 25°

6. Coiling: most of body bent to the same radius, forming a coil or tight spiral
7. Pause: the animals stop and start moving abruptly

Normal behavior on agar consists of a series of dorsal-ventral, backward waves along the animal that propel it forward by pushing against the surrounding substrate. This behavior is interrupted occasionally by a spontaneous reversal bout of forward waves, which drives the animal backward, followed by a return to forward movement in a new direction. Spontaneous reversal movement is probably triggered by internal stimuli rather than random, external stimuli in the substrate, since the interval between them is the same on agar as in water. This general pattern appears to be a common feature of nematode behavior (Croll, 1977). About 2/3 of the *C. elegans* nervous system is present at hatching, and the motor system in the body changes substantially during postembryonic development. However, the overall sinusoidal pattern of worm movement remains similar throughout postembryonic development.

Despite its small size, the nervous system of *C. elegans* responds to many external stimuli; they include mechanosensation, chemosensation, thermosensation and photosensation .

Mechanosensation includes the touch withdrawal reflex, tap reversal response and anterior tip touch reflex. The touch withdrawal

reflex is mediated by a neural circuit which contains six touch receptors, five pairs of interneurons and 69 motor neurons. A light touch to the anterior body causes the animal to reverse its direction of movement; a touch to the tail region causes the animal to move forward at higher speed (Chalfie and Sulston, 1981). A set of neurons called microtubule cells is required for touch responses in the body. Three anterior microtubule cells (ALML, ALMR, and AVM) are required for the response to touch in the anterior part of the body; the animals no longer respond to light head touch when all of the anterior microtubule cells were killed by laser ablation. Similarly, two posterior microtule cells in the tail (PLMR and PLML) mediate the response to tail touch; when both tail microtubule cells were killed, the tail touch response was lost. (Chalfie and Sulston, 1981).

C. elegans hermaphrodites show a reversal response to a tap on the Petri plate which contains them. Ablations of the sensory neurons and interneurons of the touch circuit confirms that the touch withdrawal reflexes are also primarily involved in the tap withdrawal response (Wicks and Rankin, 1995). Moreover, it was shown the ablation of some neurons (DVA and PVD) outside the touch circuit decreased the magnitude of accelerations produced by worms in response to tap (Wicks and Rankin, 1995). The magnitude of a reversal bout is usually larger with the administration of a short burst of taps. Single tap stimulation repeated 40 times at 10 s interstimulus intervals can result in

habituation, where the response is less than 5% of the initial response (Rankin et al., 1990). Dishabituation of the habituated reversal response was observed following an electrical shock to the agar medium containing the worms. In addition, male *C. elegans* also demonstrate similar habituation and recovery from habituation as found in the hermaphrodite (Mah and Rankin, 1992). These experiments show that *C. elegans* has the same basic form of habituation that is found in higher animals.

Recent studies show that a light touch to the anterior-most tip of the worm (Kaplan and Horvitz, 1993) also initiates backing. There are three classes of neurons (ASH, FLP and OLQ) that sense touch to the anterior-most tip region and are required for this kind of avoidance response. ASH, FLP, and OLQ have sensory endings consisting of modified axonemal cilia embedded in the cuticle. Mutant animals that have defective ciliated sensory endings, as well as laser-operated animals that lack ASH, FLP, and OLQ fail to respond to touch to the most-anterior tip region. The ASH is a polymodal sensory neuron which also acts as a chemosensory neuron that mediates the avoidance of noxious chemicals (Kaplan and Horvitz, 1993).

C. elegans is sensitive to numerous environmental chemical stimuli. The animal responds by chemotaxis to attractive compounds such as its food source and a variety of small, water-soluble molecules including cAMP, biotin and certain amino acids (Ward, 1973; Dusenbery,

1974). Adult animals will orient their movement along the surface of the agar and migrate to the peak of a chemical gradient (Ware, 1973). However, when the animal is presented with a noxious chemical compound, such as copper ions or D-tryptophan (Dusenbery, 1975), it moves away from the chemical stimuli. Chemosensation was earlier thought to be controlled by eleven classes of chemosensory neurons, because electron micrographs showed that these neurons had ciliary endings that were exposed to the environment through specialized sensory channels through the cuticle (Ward et al., 1975; Ware et al., 1975; White et al., 1986). This has been largely confirmed by laser ablation experiments (Bargmann, 1993). Ten of these classes each consist of a pair of bilaterally symmetric neurons, whereas the eleventh class consists of six neurons, for a total of 26 exposed cells.

In *C. elegans*, each member of a pair of sensory organs called amphids contains the ciliated dendrites of 12 sensory neurons plus two support cells called sheath and socket cells (Ward et al., 1975; White et al., 1986). The sheath and socket cell form a cylindrical channel for conducting the dendrite cilia to the surface of the cuticle. Of the 12 amphid neurons, one of them is the AFD sensory neuron that has been suggested to be involved in thermotaxis (Mori and Ohshima, 1995), eight are chemosensory in that their cilia extend into the channel of the socket cell and are exposed to the external medium. The cilia of three neurons (AWA, AWB, AWC) which end in "wing cilia", pass through the channel,

but proximal to where the channel enters the socket cell (Perkins et *al.*, 1986) they separate from the others and invaginate from the channel individually into the sheath cell. Each of the amphid wing cilia has a unique shape (Ward et al., 1975; Ware et al., 1975). The AWC cilium spreads vertically into two sheets, which together with the surrounding sheath cell, fill much of the left and right hemisectors at the anterior tip of the animal. The AWA and AWB cilia are smaller than AWC and comparable in size to the channel cilia. The distal segments of the AWA cilia split into several small projections each containing one or more of the nine doublet microtubules. The AWB dendrite ends in a pair of cilia whose distal segments are flattened and irregular (Perkins et al., 1986). In addition, the dendrite of another amphidial neuron (AFD) invaginates the sheath cell separately from the AWA, AWB and AWC dendrites and without contacting the channel (Ward *et al.*, 1975). The dendritic membrane expands into about 100 microvilli (called fingers) and a short cilium, which invaginate the sheath cell.

Laser ablation experiments have shown that the eight amphidial neurons extending cilia into the channel are required for chemotaxis, chemical avoidance, and normal development (Bargmann, 1993). The ASE, ASG, and ASI cells are important in chemotaxis to several small molecules such as cAMP and biotin, which are known to be produced by bacteria; the ADF and ASK cells participate in chemotaxis to subsets of those small molecules and lysine; ASH and ADL mediate avoidance of

several noxious stimuli such as garlic (Bargmann, Thomas and Horvitz, 1990). The ADF, ASG, ASI, and ASJ are responsible for sensing the crowding pheromone and or food stimulus, stimuli which control entry and exit from the dauer larval stage. The wing cells AWA, AWB and AWC, ending inside the sheath cell, are sensitive to volatile odorants (Bargmann, Hartwieg and Horvitz, 1993).

C. elegans also is thermotatic. When grown at a given temperature in the presence of bacteria, they migrate to that temperature on a radial thermal gradient and then make isothermal circular tracks. Temperature shift during culture results in acclimation to a new temperature within several hours. Conversely, when animals are acclimated at a given temperature in the absence of food, they tend to avoid that temperature on a thermal gradient (Hedgecock and Russell, 1975). This is one manifestation of the dispersion response, which allows *C. elegans* to escape from an unfavorable, local environment to which it might be confined by the usual aggregation thermotaxic behavior (Hedgecock and Russell 1975). Laser ablation experiments show that this complex behavior seems to be mediated by AIB, AIY and AIZ interneurons and two pairs of sensory neurons: the AFDs, and an unidentified sensory neuron class. (Mori and Ohshima, 1995).

Nematode eyes and photobehavior

Most nematodes lack any structures that could be identified as an eye. Of the few that do, most have 2 lateral spots of dense pigment in the anterior, sometimes cup-shaped, each associated with a single, photoreceptor organelle. Phototaxis has been studied in several of these nematode species (Burr and Burr, 1975; Bollerup and Burr, 1979). In the statistical study by Burr (1979) it was shown that nematodes with paired pigment spots, *Oncholaimus vesicarius* and *Enoplus anisospiculus*, preferentially move in the direction away from a source of obliquely-incident, uniform illumination. A similar behavior exists for *Chromadorina bioculata* (Croll et al., 1972). The pigment spot, by shading an adjacent photoreceptor, can provide the mechanism for discriminating the direction of the light source (Burr, 1984). Without the pigment spots, it is unlikely that a nematode can obtain information about the directional distribution of light, since its semi-transparent body would permit the photoreceptor to receive light from any point.

The unique eye of *Mermis nigrescens* has a simple, cylindrical, pigmented region in the anterior tip surrounding a putative photoreceptor (Burr, 1985; Burr *et al.*, 1990). *Mermis* orients towards the source utilizing an unique scanning mechanism (Burr *et al.*, 1990; Burr and Babinszki, 1990). It was found that the anterior 2 mm (head region) of the worm oscillated in a scanning motion involving both vertical and sideways swinging motions. The next 10-20 mm (neck region) are involved in orientation to light (Burr *et al.*, 1990). Such

scanning motion of the head modulates the photoreceptor illumination during phototaxis (Burr and Babinszki, 1990).

Some nematodes appear to be photosensitive although they lack pigment spots, e.g. larvae of the hookworms *Ancylostoma tubaeforme* (Croll and Al-Hadthi, 1972) and *Trichonema* sp. (Croll, 1965, 1966a, 1970, 1971). In *C. elegans*, it was shown that there is an increase in reversal frequency at certain effective light intensities and wavelengths (Burr, 1985). Monochromatic stimuli ranging from 420 to 680 nm at a constant 56 picoeinsteins s^{-1} cm^{-2} were tested. A significant response was elicited in the range of 520-600 nm. At saturating intensities the mean reversal probability was increased by approximately 40% over the background level (Burr, 1985). Burr (1985) investigated all possible sources of radiant heating and concluded that the response to illumination must have been to light and not to temperature changes. This nonoriented type of photoresponse could be beneficial to *C. elegans* as it would enable the animals to avoid the unfavorable conditions present on the soil surface (Coomans and DeGrisse, 1981; Fraenkel and Gunn, 1961).

Objectives

Although *C. elegans* lacks pigment spots, its photoresponse indicates an ability to detect light. The discovery of photosensitivity raises several questions and opens up new areas for research which

include: 1) Is there a possibility that the response is due to infrared heating rather than light, in spite of the careful filtering of infrared light from the stimulus beams as in the Burr (1985) study. 2) What neuron or neurons are light sensitive? 3) Does the neural circuit responsible for photoreversals have any elements in common with the other reversal reflexes? 4)Are other developmental stages of *C. elegans* photosensitive? Do neuroanatomical changes of *C. elegans* across developmental stages affect photobehavior? Dauer larvae are of particular interest because of their different, dispersal oriented behavior. 5) Is male *C. elegans* sensitive to light? Since adult male *C. elegans* has more neurons than the hermaphrodite and 87 of the 381 neurons are male specific (Hodgkin, 1988), there may be resulting differences in response to light stimuli. In this thesis, I approached question one and two, which lay the groundwork for the others.

In order to approach these questions, a more efficient method for measuring reversal behavior was desirable instead of manual scoring. Therefore a computer video tracking system was developed to facilitate this measurement and to obtain a large sample size in a relatively short period of time. In this aspect, my goal was to 1) develop some of the higher level software for tracking and data analysis, 2) modify existing software to increase the sample speed (up to 5 Hz), 3) modify the apparatus and software to control new hardware (i.e. a loudspeaker source of vibration) so that experiments using other forms of stimuli

could be carried out using the same system, and 4) test the system and analysis software on wildtype *C. elegans* and compare with the results obtained by Burr (1985) who manually followed one worm at a time.

In order to confirm that the observed light response was due to visible light but not infrared heating, the experiment with wildtype *C. elegans* was repeated but with visible light eliminated from the test-beam while allowing any infrared light that had not been filtered out to reach the animals. If such an altered stimulus fails to elicit reversal responses, then the photoresponse must be due to visible light, not infrared-induced temperature change.

Since the light-sensitive organelle and neurons underlying the photobehaviors have not yet been identified for *C. elegans*, I tested two mutants with known defects in their sensory neurons in order to narrow down the possibilities. The mutants I used were *ttx-1* in which the microvilli finger projections in the sensory cell AFD are lacking, and *daf-19* in which all ciliated endings are absent. In almost all animal photoreceptors identified to date, the light-sensitive organelle is either a modified microvillar array or a modified cilium (Burr, 1984). Most sensory neurons of nematodes end in modified cilia, while the only microvillar array of *C. elegans* is found at the terminus of the AFD dendrite.

General Methods

Nematode preparation

There were four experiments in this project; each of the experiments were performed under identical conditions except for the strain of worms that was used and, in one case, a modified test stimulus.

Caenorhabditis elegans hermaphrodites (strains *N2*, *ttx-1* and *daf-19*), obtained from the *C. elegans* Genetics Center, were maintained at 20°C on agar Petri plates seeded with *Escherichia coli* (strain Op50, uracil auxotroph; Brenner, 1974). In order to get synchronous colonies of animals, nematode cultures were initiated by placing 30-60 mature, egg-laying worms on a fresh, *E. coli* seeded Petri plate containing 1.8% agar in MYOB medium (Dave Baillie, personal communication, 1996). They were allowed to lay eggs for 2 to 4 hours then washed off the plate. The plates were then placed into a 20°C (relative humidity 50%) incubator under continuous darkness until the nematodes reached the one to two day old adult stage, 72 to 96 hours after hatching (*N2* and *ttx-1*). Other means of verification of stages included checking the size of the nematodes, using an eyepiece micrometer and the initiation of egg laying. Adult *daf-19* hermaphrodites were obtained 7.5 days after hatching; their size (1.00-1.20 mm) was checked before each experiment.

Adult worms were washed off the food plates into a conical tube using M9 buffer (Wood, 1988). Worms were allowed to settle to the bottom of the tube and then most of the buffer solution was removed

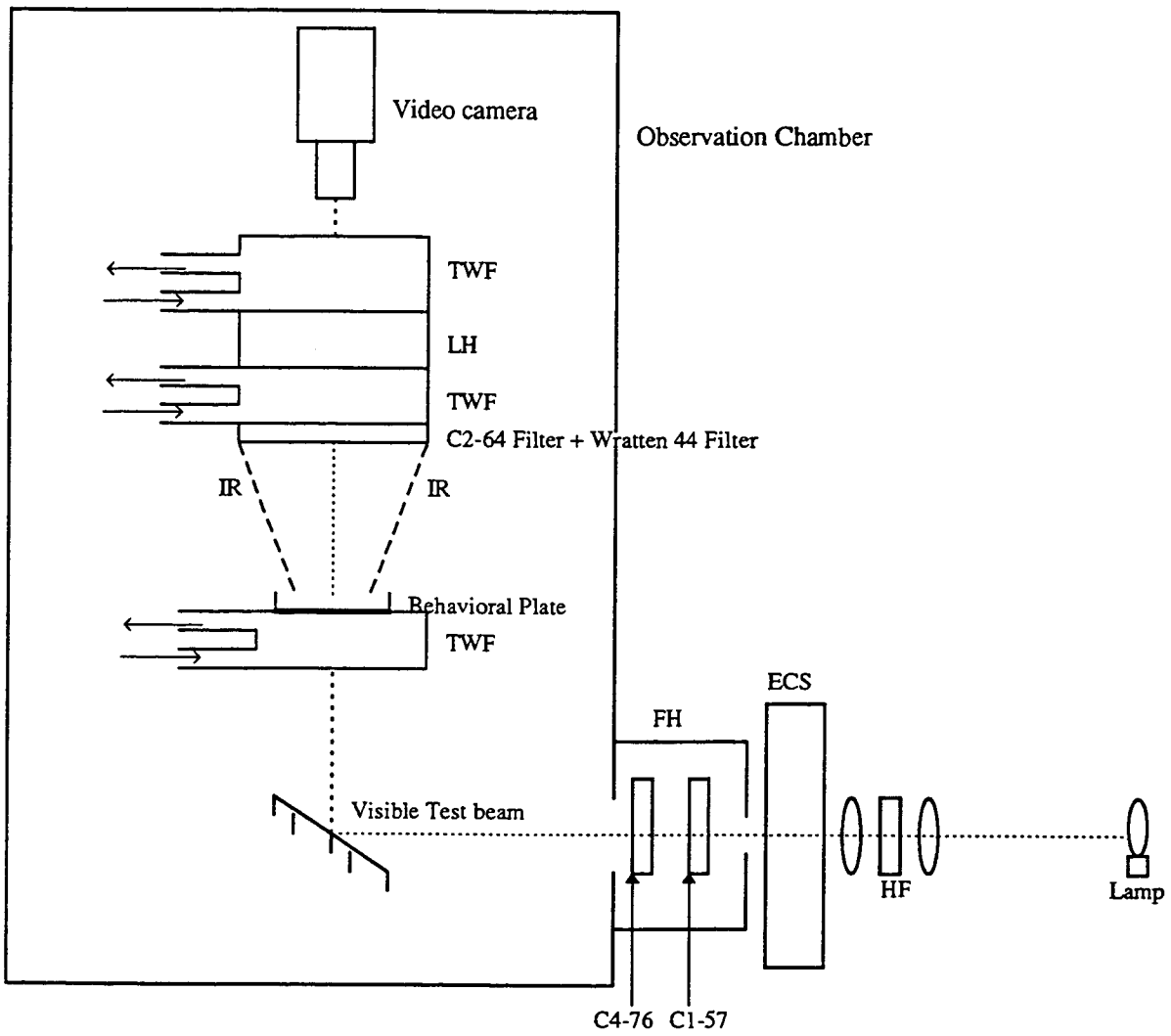
using a Pasteur pipette. Sedimentation was repeated once to wash off most of the bacteria. After the second wash, 60 - 80 worms were transferred with approximate 0.1 ml buffer onto 5 cm Petri plates using a Pasteur pipette. Each of these behavior plates had been filled with 7 ml 2% NGM agar and allowed to evaporate to 90% of initial weight. The plate was put in the observation chamber for 10 min before the beginning of experiment during which time the thin puddle of transferred liquid was absorbed.

Observation chamber

The experiments were carried out in an enclosed observation chamber (see Figure 2). The Petri plate was kept at 20 °C ± 0.05 °C by contact with the glass window of a vessel containing vigorously recirculated, filtered water from a thermostatically controlled water bath. Movement of the nematodes was observed with an infrared-sensitive, Panasonic surveillance camera and monitor. A Cosmicar zoom telephoto lens with a 40 mm extension tube and 2x tele-extender provided a 9 x 12 mm field of view. A Corning C 2-64 filter was placed in front of the camera to block any visible light and to allow infrared light to pass for observational purposes.

There were two kinds of radiant flux passing through the arena (Figure 2). The continuously present infrared observation source provided dark-field, incident illumination with wavelengths of 780-950

Figure 2. Apparatus and optical setup for studies of photobehavior in *C. elegans*. Arrows indicate the flow of filtered and thermostatted water. Dotted lines indicate light paths. ECS, Electronic controlled shutter; FH, Filter house; HF, Heat filter of projector; IR, Infrared observation beam; LH, Infrared lamp house with hole through centre; TWF, Thermostatted water filter; C4-76, Corning C4-76 near-infrared blocking filter; C1-57, Corning C1-57 heat filter.


Video camera
Observation Chamber
TWF
LH
TWF
C2-64 Filter + Wratten 44 Filter
IR
IR
Behavioral Plate
TWF
ECS
FH
Visible Test beam
HF
Lamp
C4-76
C1-57

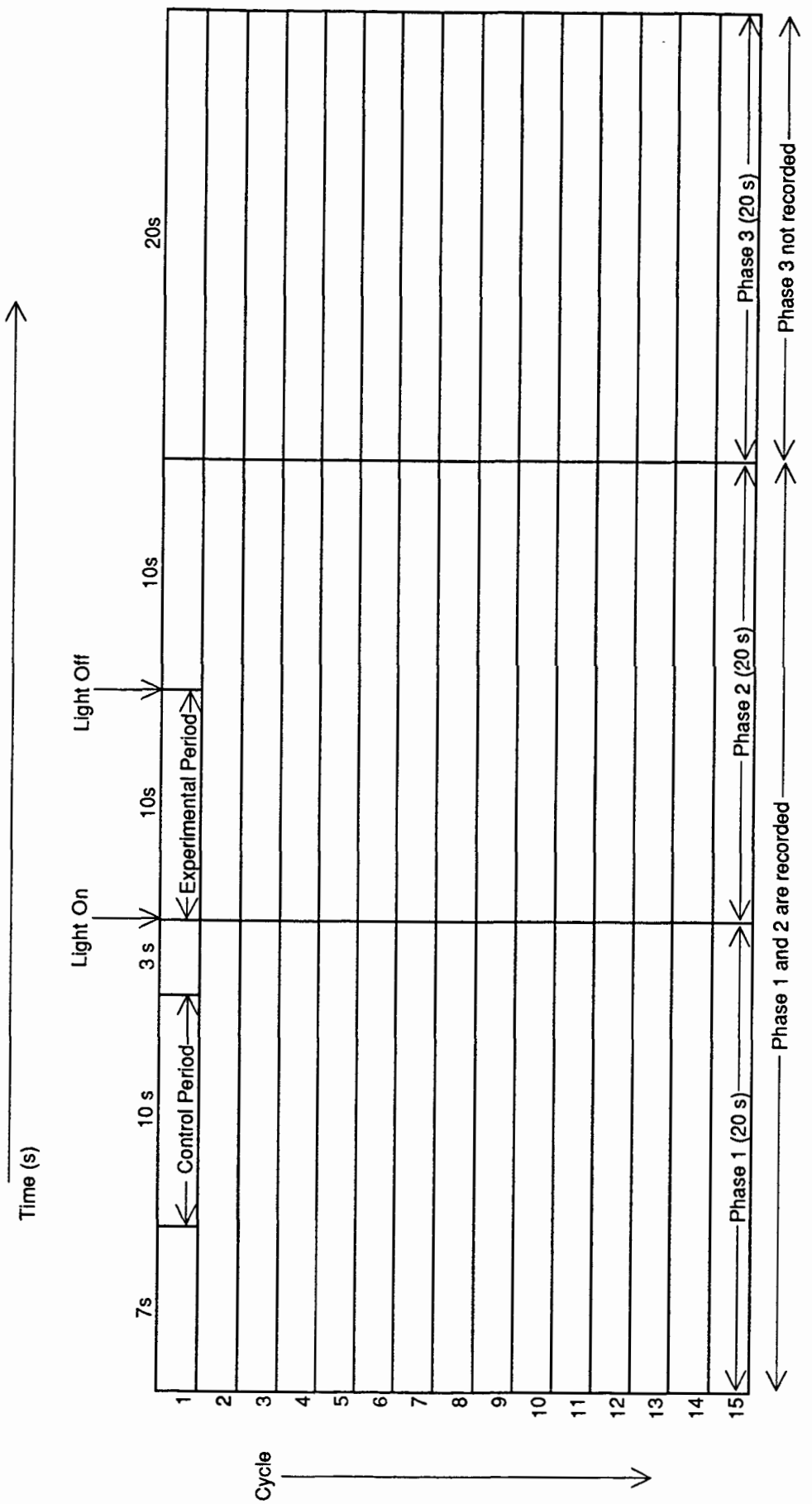
nm, isolated by a Corning C2-64 glass blocking filter, a Wratten 44 gelatin filter and 1 cm water. The test beam was directed perpendicularly from below from a 250 W slide projector and controlled by an electronic shutter. The broad-band (370-610 nm) visible light was isolated by the projector's heat filter, a Corning C 1-57 heat filter, a Corning C 4-76 filter and 5 cm of water in the thermostatically controlled base.

Experimental protocol and data analysis

Track coordinates of up to 23 worms were sampled once per second. Each experiment was divided into 15 cycles. Each 60 s cycle was subdivided into three 20 s phases (Figure 3). Track coordinates were sampled during phase I and II, a total of 40 continuous seconds in each cycle. Paired experimental and control samples were obtained in each cycle. The test stimulus was turned on at the beginning of second 1 of phase II (the experimental period), and was turned off at the end of second 10 of phase II. Ten seconds near the end of phase I was used as the control period. The timing, shutter and sampling were controlled automatically by a GIMIX computer. Paired statistics (see Chapter 2) were applied to remove the variation in spontaneous reversal frequency over the 15 cycles.

All raw data collected by the GIMIX computer was transferred to a PC computer for analysis by APL programs (See Chapter 2). The number

Figure 3. Timing of events during an experiment. Each experiment has 15 cycles. Each cycle is 60 s long and represent by one row. Each cycle consists of 3 20 s phases; phase I, II and III. Coordinates of the animals were recorded in phase I and II but not in phase III. The control period starts at the beginning of second 8 and ends at the end of second 17 of phase I. The first 10 s of phase 2 is the experimental light period. The shutter is opened at the beginning of second 1 of phase II and closed at the end of second ten of phase II.

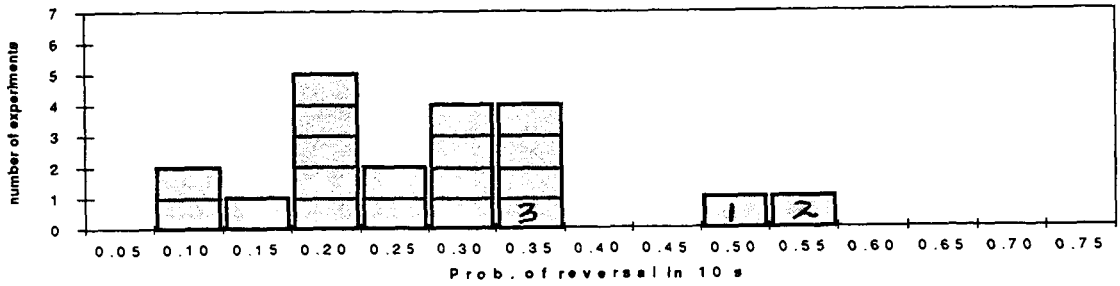

Time (s)
7s
10 s
Control Period
3 s
Light On
10s
Experimental Period
Light Off
10s
20s
Cycle
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Phase 1 (20 s)
Phase 2 (20 s)
Phase 3 (20 s)
Phase 1 and 2 are recorded
Phase 3 not recorded

of reversals in the 10 s experimental and preceding 10 s control periods were analyzed statistically. As well, the differences between the number of reversals in the paired experimental and control periods were analyzed. Data from about 20 experiments were averaged over worms and cycles and analyzed statistically. The mean reversal probability and 95% confidence interval are plotted for each second. The total probability during a 10 s period was obtained by summing over the 10 seconds. The results of a few experiments were clearly outliers (Figures 4 and 5). Because the mean probability of reversals in an experimental or control period was at least 2 standard deviations above the grand mean, these experiments could be excluded from the statistical analysis. Of the 60 experiments described in this thesis, five were thereby excluded.

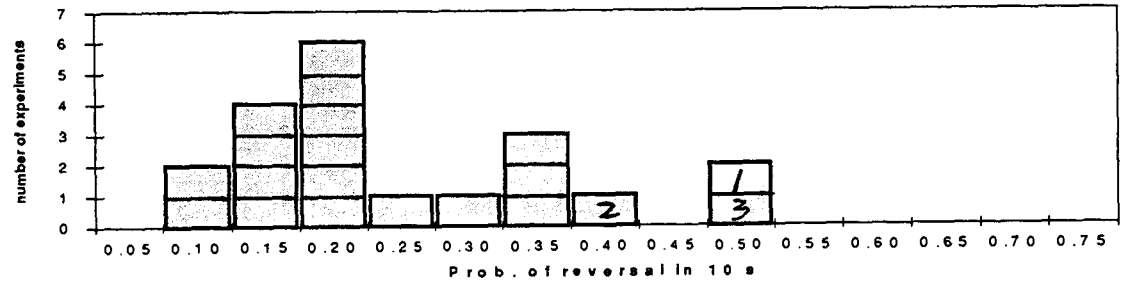
A paired Student's t-test was used to determine if the mean difference in total probability was significantly different than zero, that is, if the effect of a test stimulus differed significantly from the background probability of spontaneous reversals. As averaging over cycles which are probably not independent of each other does not lead to a valid t-test, the data were analyzed using a repeated measures analysis of variance (the proc mixed routine of SAS). A value of t was calculated from the mean difference and standard error provided by the ANOVA.

Figure 4. Summary of mean probability of reversals in wildtype *C. elegans* in experiments described in Chapter 3 and 4. Each rectangle represents one experiment. Any experiment that had probability of reversals at least 2 standard deviations above the grand mean were not included in the calculation. Experiments which were not included in the statistical analysis are marked with a number in the corresponding control and experimental histograms.

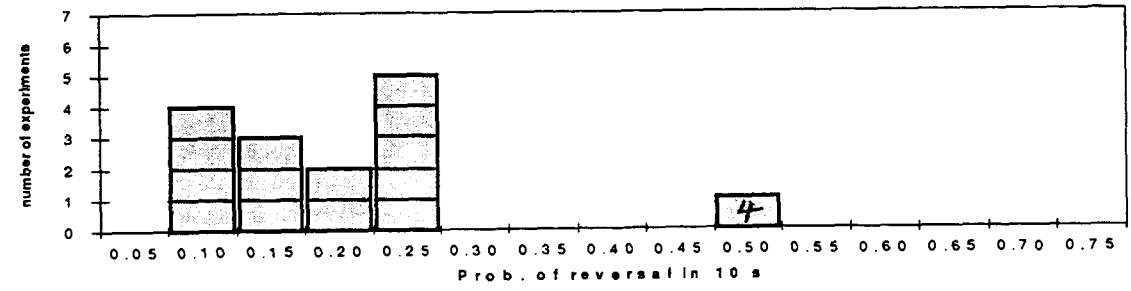
Wildtype Experimental Period



Wildtype Control Period



Filter Experimental Period



Filter Control Period

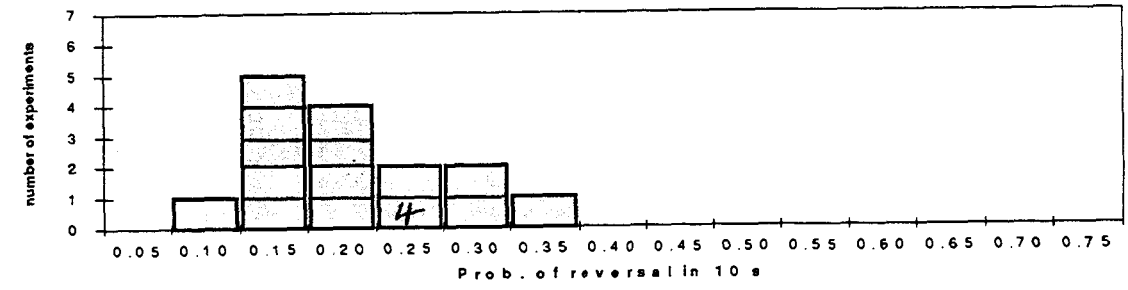
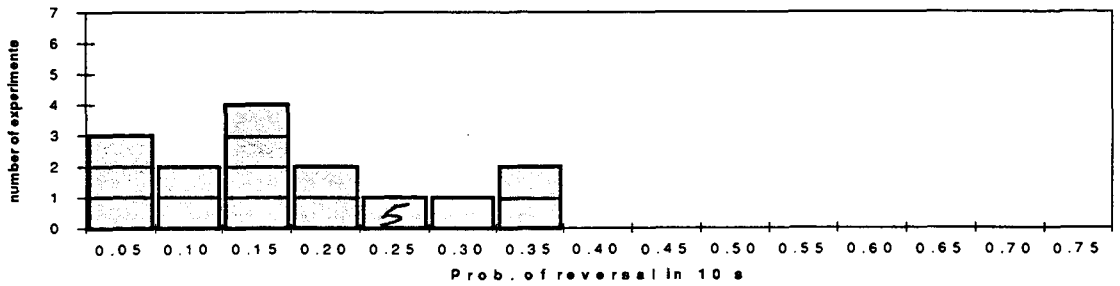
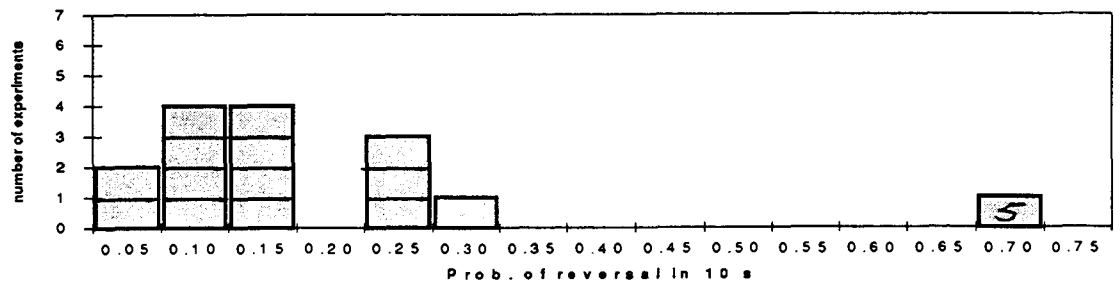


Figure 5. Summary of mean probability of reversals in *C. elegans* mutants in experiments described in Chapter 5. Each rectangle represents one experiment. Any experiment that had probability of reversals at least 2 standard deviations above the grand mean were not included in the calculation. Experiments which were not included in the statistical analysis are marked with a number in the corresponding control and experimental histograms.

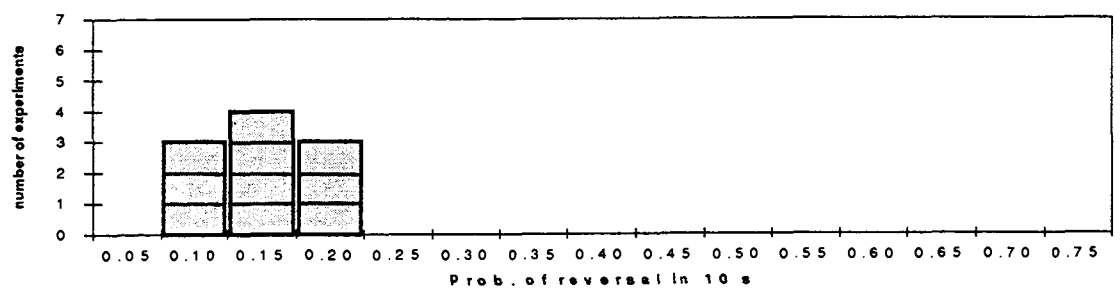
ttx-1 Experimental Period



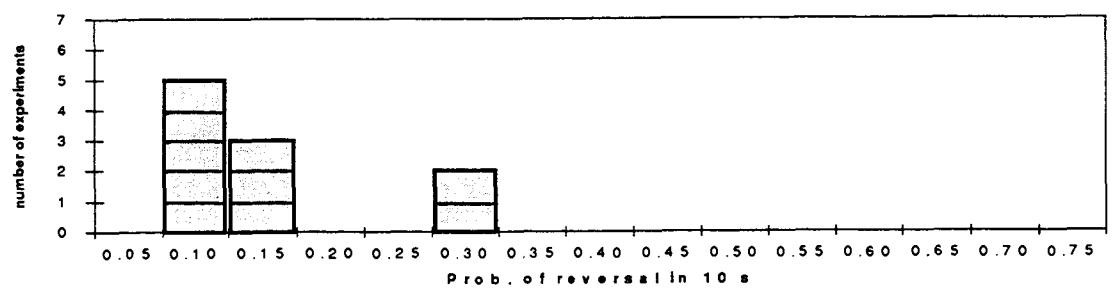
ttx-1 Control Period



daf-19 Experimental Period



daf-19 Control Period



Chapter 2

Computer tracking system

Automatic tracking systems based on image analysis are widely used as tools for automatic analysis of movement of animals or cells (Hader, 1992). One advantage is their high objectivity in contrast to manual assessment performed by a human operator. Moreover, computer-controlled image analysis enhances the speed of the measurements by taking advantage of the speed of today's computer, thus decreasing the time required to obtain a certain amount of data. Computer tracking analysis is particularly useful in the studies of behavior where a large sample size is used to estimate the average behavior of a population.

Computer-controlled image analysis of moving objects is usually based on a sequence of frames that are recorded and processed at discrete time intervals. The movement of the individual objects can be calculated by comparing the position of individual objects in subsequent frames, which allows one to determine the direction of movement. Intervals between subsequently recorded frames are chosen based on the velocity of the subjects being investigated (Hader, 1992). In the cell tracking of gliding microorganisms such as amoebae, the movement can be too slow to be detected between one-second frames, therefore a longer interval between frames is used. In fast movements, short frame

intervals are needed to obtain better resolution of the movement (Hader, 1992). Furthermore, if the interval is too long translocation between frames can be so large that the identification of the new location fails.

In the first step of image analysis, an image is captured by the video camera as an analogue voltage signal. This is converted by a frame-grabber into digital form and stored in computer memory where it can be analyzed. The analog image is digitized at a defined resolution (e.g. 240 x 256 pixels in the system in the Burr laboratory, Simon Fraser University). In the simplest form, each pixel in the array is assigned either bright (one) or dark (zero) during digitization.

In subsequent steps of image analysis, the computer software locates and identifies objects in the memory image. Two different search algorithms are used in the system and nematodes are recognized by contour size and object motion.

The computer tracking system of this investigation consists of different components including suitable illumination, a surveillance camera, frame-grabbing board, a Motorola 6809-based computer by GIMIX with software to control the operation of the frame-grabber and shutter and to search for and identify the nematodes, and an Intel 386 based computer with APL software for analyzing the track coordinates, scoring reversals and doing statistics.

Hardware

In order to achieve a high contrast between the subjects and the background, continuous, dark-field illumination was used. This was projected onto the arena from a ring of halogen lamps near the camera lens (Figure 2). To avoid interference with the photobehavior or heating, all but near-infrared light was filtered from the beams. Light was reflected and scattered by the animals into the camera lens, however the arena was nearly transparent, thus producing an image in which the nematodes were bright against a dim background. Visible light from the stimulus beam was prevented from entering the camera by a Corning C2-64 blocking filter.

The computer used for tracking employed a Motorola 6809 microprocessor. The commercial hardware was from GIMIX. The operating system was OS-9 level I from Microwave Systems Corp. The tracking software was written as 6809 assembly language subroutines to improve speed and minimize size. A BASIC09 program called these subroutines and controlled all aspects of the experiment. When triggered, the frame grabber converted a single video frame into the digitized image 240 (vertical) x 256 (horizontal) array of one bit pixels, the field of view covering a 9 x 12 mm of the test arena. At this magnification 1 mm adult *C. elegans* was represented by approximately 20 pixels. As it was digitized, this array was stored in computer memory by direct memory access (DMA). Another board, when triggered,

reconfigured the memory image into analog form for display on a video monitor. With the aid of this display, two threshold settings were programmed before the experiment was started. If the intensity of a pixel fell between the two thresholds, it was considered to be a bright pixel (a one). On the other hand, any pixels with intensity below the lower threshold were considered not bright (a zero). If background reflections were too high, we could adjust the lower threshold to a higher value in order to eliminate the background from the digitized image. In my experiments, the upper threshold was set at 100 and the lower threshold at 15.

During an experiment, a video signal processor was used to superimpose a bright spot marker on the video image when the scan came to the computed x, y coordinates of each tracked object. Viewed on a video monitor, this provided us a very useful visual indication of how successfully the computer was tracking the worms.

A computer-controlled electronic shutter, placed between the light source and the observation chamber, controlled the dark and light cycle of the experiment (Figure 2). In addition to the electronic shutter, a vibrator system was connected to the microcomputer to provide mechanical vibration to the arena. The vibration was produced by of a 30 cm speaker driven by a multifunction signal generator and audio amplifier. A rod was attached to the speaker armature at one end, and the other end was attached to the behavior plate in the observation

chamber. When the signal generator was triggered, the speaker vibration was transmitted to the Petri plate and agar, and worms were stimulated.

Software

The tracking software runs under OS-9 in the GIMIX computer. The *grab, search* and *follow* assembler modules are called by the BASIC09 main program *track*. Since the tasks done by these modules are the most time consuming and critical part of the tracking procedure, the modules were written in assembly language (by A.H.J Burr). Moreover, assembly language provided communication to I/O ports not readily accessible from higher level languages such as BASIC09.

At the beginning of each second on the computer's clock, *track* runs *grab*, which triggers the frame grabbing board to digitize a new image. Then *follow* finds the new location of worms being tracked. If the pixel at the location identified in the previous second is now dark (the worm has moved), a search is initiated. Adjacent pixels are examined in an ever-widening circle (to a limit of a radius of 9 pixels). Once a point on the edge was found, a new contour of the animal is traced and the new center is recorded. Any objects with a contour too large or too small or which do not move consistently in one direction for more than 3 s are discarded automatically. Once an object passes these tests it is given status as an established worm. When an established worm moves out of the field of view, or when two worms collide, *follow* stops following these

worms. When an established worm is located, *follow* activates the video signal processor board that generates a flash of light on the monitor screen at the address recorded. In the time remaining of each second after *follow* is done, new subjects to track are identified by *search* until either the maximum allowable number of worms is reached or the second has elapsed. A position in the memory image is chosen at random and if that pixel is not bright, adjacent pixels along the row are checked until a bright pixel is found. If that is not near to the stored coordinates of a worm currently being tracked, its coordinates are stored.

The main Basic09 program, *track*, controls the timing and order of the assembler subroutines. *Track* also controls the opening and closure of the electronic shutter or triggering of the vibrator during the 15 min experiments. The frame rate and the maximum number of worms to track are input by the user when the program is executed. The system has sufficient speed (at a 2 Mhz processor speed) that up to 46 worms can be tracked simultaneously at a rate of once a second. With fewer worms being tracked, the system is able to process 5 frames per second. The computer was set to grab one frame per second in all the experiments described here.

The location of each animal is specified by three variables; Y-row, X-column and X-bit. The X-column specifies the horizontal position as one-byte containing 8 adjacent pixels in a horizontal line. The pixel position of the worm within this byte is specified by X-bit. Vertical

position is specified by the Y-column. Each second in each tracking cycle, *follow* stores the pixel coordinates of each subject in RAM and every 10 s *track* moves these data to RAM-disk. In order to maximize the speed of system, all the coordinates are stored as a linear array without further processing.

Data Analysis

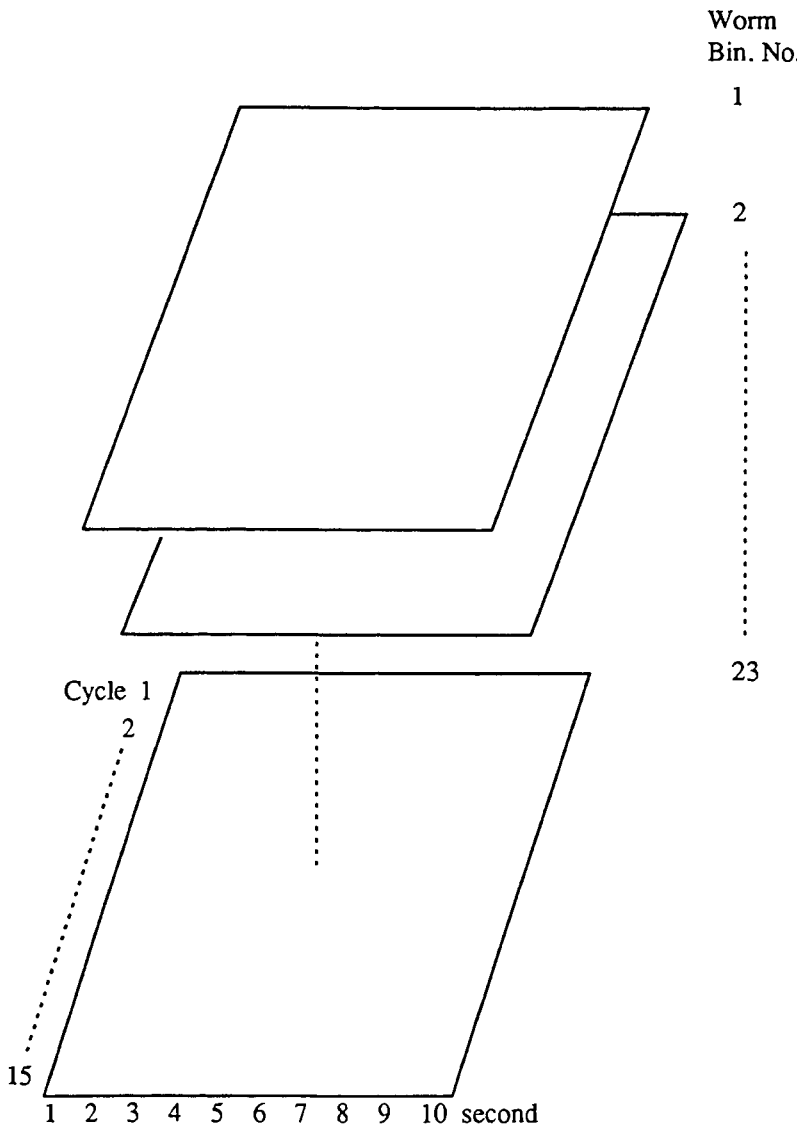
After each 15 min experiment, data were transferred to a 386 computer where programs written in the APL language scored reversals and analyzed the results statistically. The APL interpreter used was APL2 from IBM. The linear arrays of coordinates from the GIMIX system were reconstructed to a 4-dimensional array indexed as to the worm bin number (23), cycles (15), seconds (40) and coordinates(3). The absolute horizontal position X was calculated from Xcolumn and Xbit by the equation X= 8 x (Xcolumn - 1) + (Xbit -1) and stored in a three dimensional array. The y values were stored in another three dimensional array. The change of x and y (Δx and Δy) between each track point, direction and distance moved were calculated. These data were screened to eliminate worms that were not continuously followed throughout the 10 s experimental period, the preceding 5 s and the following 3 s (a total of 18 s), and this subset was stored in a three dimensional array (23 x 15 x 18). A subroutine then analyzed the coordinates to determine the second a reversal bout was initiated, and a

three dimensional array (23 x 15 x 18) was created which contained a one (if reversal) or zero (if not) indexed by worm number, cycle and second. An algorithm eliminated any reversals which happened within 4 s of the previous one because the second change of direction was likely to be part of the same reversal bout. Then the appended initial 5 s and the last 3 s were deleted to give two arrays of the form indicated in Figure 6. One array included the 10 s control period (dark) while the other include the immediately following experimental period (light). Different planes of the three dimensional arrays represented different worm bin numbers. Each plane was subdivided into 15 rows (cycles) and 10 columns (seconds).

Note that the only data used was from worms which were tracked during the entire 18 s that included the control or experimental periods of each cycle. An animal was deemed to have reversed direction if it changed its direction of movement by at least 115 °, moved for at least 1/3 of its body length in the preceding and following second and met other criteria required for accuracy.

A separate program pooled data from many experiments by concatenating the data matrices along the worm bin number dimension (planes). It then calculated the average probability that an animal would reverse in each second within the 10 s experimental and control periods. In calculating the probability of reversals, the 3-dimensional arrays were

Figure 6. Three dimensional data structure of the control period (same structure for the experimental period). Each plane represents one worm bin number (maximum 23). Each plane is divided into 15 rows (cycles) and 10 columns (seconds).


Worm
Bin. No.
1
2
23
Cycle 1
2
15
1 2 3 4 5 6 7 8 9 10 second

summed over worm bin numbers and experiments (planes, Figure 6) resulting in one plane with dimension 15 (cycles) by 10 (seconds). Then this plane was summed down the columns (along the y-axis) to give a linear array of size 10 representing the number of reversals in each second of a cycle. The probability of reversals was calculated by dividing the number of reversals in each second by the total number of worms tracked in each second. Standard deviation and standard errors were calculated similarly.

To obtain paired statistics, a slightly different procedure was used. After summing over planes (worm bin number), the control and experimental arrays of Figure 6 became two 15 x 10 matrices of reversals in each second of each cycle. The data in the control matrix were subtracted from that in the experimental matrix to give a matrix of differences. The weighted average over cycles was calculated by multiplying the values in each cycle by a weighting factor derived from the number of worms in the corresponding cycles of the experimental and control arrays $(1/ne + 1/nc)^{-1}$. The weighted standard deviation was calculated similarly.

Results of the statistical analysis were used to prepare graphs using Microsoft Excel. The mean number of reversals are plotted with error bars indicating 95% confidence intervals.

Chapter 3

Light response and spontaneous reversal activity

Caenorhabditis elegans produces sinusoidal waves on agar. Most of the movement is forwards, resulting from backwardly moved sinusoidal waves. However, the forward movement is periodically interrupted by forward waves, which results in backward movement or reversal. Croll (1975) found that on average one spontaneous reversal bout occurred after every 36 backward waves. There was a high degree of individual variation among animals where the mean backward waves between forward waves (reversals) was between 6.4 and 106 among 20 animals. Reversal periods usually consisted of only one or a few waves. The first forward wave of a reversal bout was always very rapid, approaching twice the rate of a backward wave. When a series of consecutive forward waves occurred the rate of subsequent waves decreased to that of backward waves. Moreover, reversal bouts could start at any phase in movement (Croll, 1975).

In addition to reversals which arise spontaneously, similar bouts can be elicited in *C. elegans* by at least five types of sensory stimuli, including touch to the anterior (Chalfie et al., 1981), vibration (Chiba and Rankin, 1989), collision with an object (Croll, 1976), a sudden decrease in concentration of salt or oxygen (Dusenbery, 1980a,b) or the presentation of light stimulus (Burr, 1985). In the latter case, Burr (1985) demonstrated that wildtype adult *C. elegans* hermaphrodites showed an increase in reversal frequency in the presence of a light stimulus. Moreover, such a response was not due to chance observation of spontaneous reversals or temperature change.

My first experiment was designed to examine the frequency of spontaneous reversals and light response in wildtype adult hermaphrodites using the computer tracking system. In my experiments, I used the same optical filters as used by Burr (1985). However, using a computer tracking system instead of manual scoring had several advantages: 1) A time dependency of the light behavior could be measured. 2) The large number of samples required to achieve significance could be obtained in a relatively short period of time. 3) There can be no observer bias and 4) the light stimulus could be precisely controlled by the computer system.

Methods

In each experiment, 60-80 1-day old hermaphrodites were tested using the procedures described in Chapter 1. Immediately following transfer to a test plate, animals were allowed to acclimate to the environment inside the experimental chamber (20°C in darkness) for 10 min. The behavioral plate was then placed onto the glass surface below the camera lens. At the start, the threshold of frame grabbing was adjusted until the digitized image displayed on a monitor showed a good representation of the actual animals. Tracking was then started and a fifteen minute period of alternating dark and light periods was then presented as described in Chapter 2.

Results

The results are summarized in Table 1 and Figures 7-9. Spontaneous reversals occurred at similar rates across the 10 s period (Figure 7). The average rate was 0.0207 per second (1.2 reversals per

Table 1: Photoreversal response and spontaneous reversal activity of wildtype *Caenorhabditis elegans*

	Second											
	1	2	3	4	5	6	7	8	9	10	Summary[a]	
Experimental period												
Reversal probability	0.0256	0.0204	0.0266	0.0174	0.0225	0.0245	0.0286	0.0153	0.0256	0.0215	Sum over 10 s	0.2509[b]
Standard deviation	0.1579	0.1416	0.1609	0.1308	0.1484	0.1548	0.1668	0.1230	0.1579	0.1450		
Standard error	0.0050	0.0045	0.0051	0.0042	0.0047	0.0049	0.0053	0.0039	0.0050	0.0046	Standard Error	0.0083
1.96 x standard error	0.0098	0.0088	0.0100	0.0082	0.0092	0.0096	0.0104	0.0076	0.0098	0.0090		
Number of samples	978	978	978	978	978	978	978	978	978	978		
Control period												
Reversal probability	0.0266	0.0187	0.0197	0.0158	0.0138	0.0158	0.0256	0.0187	0.0168	0.0197	Sum over 10 s	0.2073[b]
Standard deviation	0.1611	0.1357	0.1391	0.1247	0.1167	0.1247	0.1581	0.1357	0.1285	0.1391		
Standard error	0.0051	0.0043	0.0044	0.0039	0.0037	0.0039	0.0050	0.0043	0.0040	0.0044	Standard Error	0.0094
1.96 x standard error	0.0100	0.0084	0.0086	0.0076	0.0073	0.0076	0.0098	0.0084	0.0078	0.0086		
Number of samples	1014	1014	1014	1014	1014	1014	1014	1014	1014	1014		
Difference	0.0014	0.0011	0.0074	0.0017	0.0088	0.0090	0.0031	-0.0036	0.0083	0.0024	Sum over 10 s	0.0473[c]
Standard deviation	0.0074	0.0076	0.0078	0.0054	0.0047	0.0054	0.0076	0.0047	0.0076	0.0053		
Standard error	0.0019	0.0020	0.0020	0.0014	0.0012	0.0014	0.0020	0.0012	0.0020	0.0014	Standard Error	0.0085
2.14 x standard error	0.0041	0.0043	0.0043	0.0030	0.0026	0.0030	0.0043	0.0026	0.0043	0.0030		

[a]Statistics based on a repeated measures analysis of variance (the proc mixed routine of SAS)

[b]Separately determined experimental and control probabilities significantly different ($t=4.93$, $p<0.001$, d.f. 996)

[c]Difference between paired experimental and control probabilities significantly different from zero ($t=5.57$, $p<0.001$, d.f. 16)

Figure 7. The probability of spontaneous reversals of wildtype *Caenorhabditis elegans* during the 10 s dark (control) period. Number of samples (worms x cycles) were 1014. Points indicate the mean probability at that particular second. Error bars indicate 95% confidence intervals (1.96 x standard error).

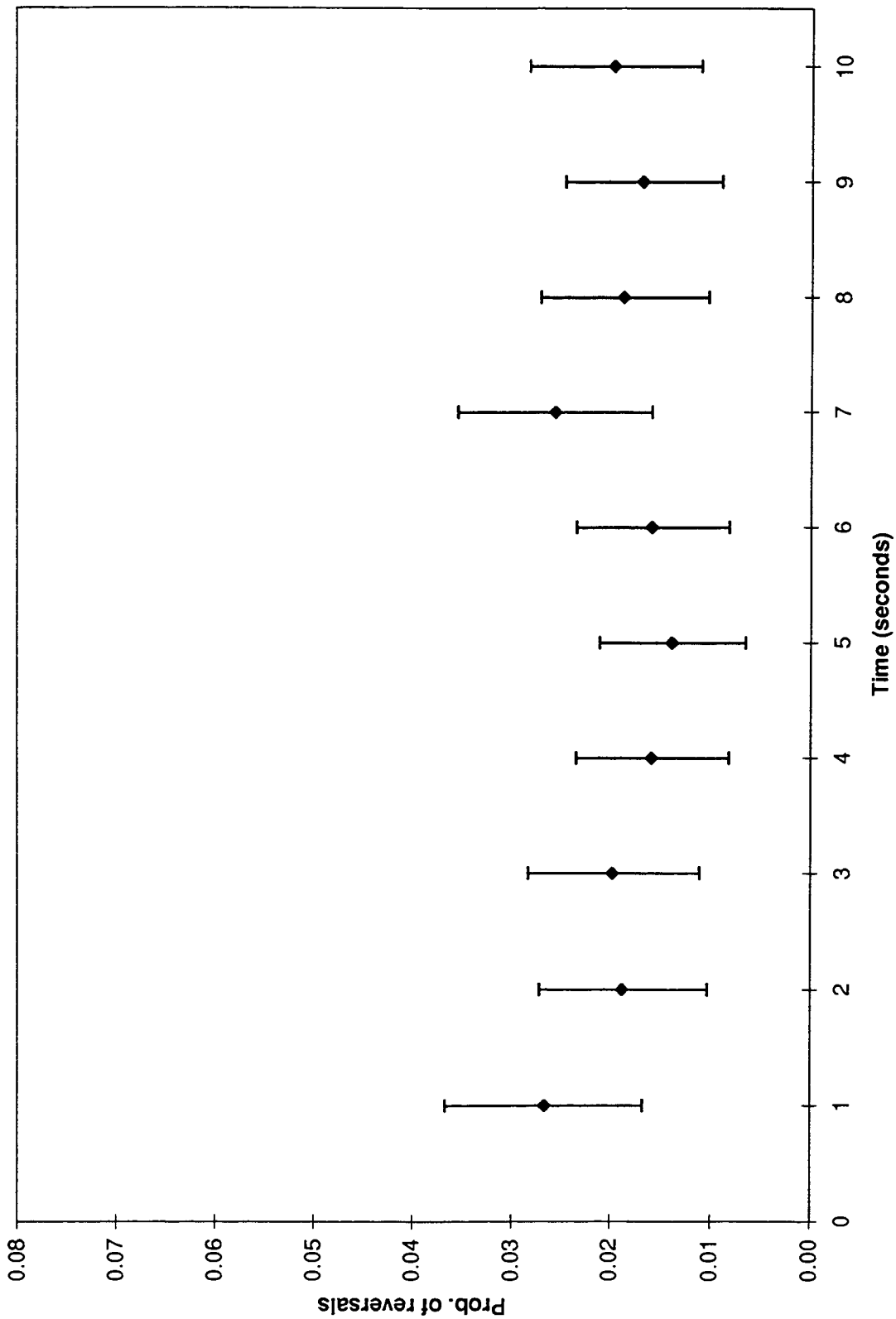

Prob. of reversals
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0
1
2
3
4
5
6
7
8
9
10
Time (seconds)

Figure 8. The probability of reversals of wildtype *Caenorhabditis. elegans* during the 10 s light (experimental) period. Number of samples (worms x cycles) were 978. Data plotted as in Figure 7.

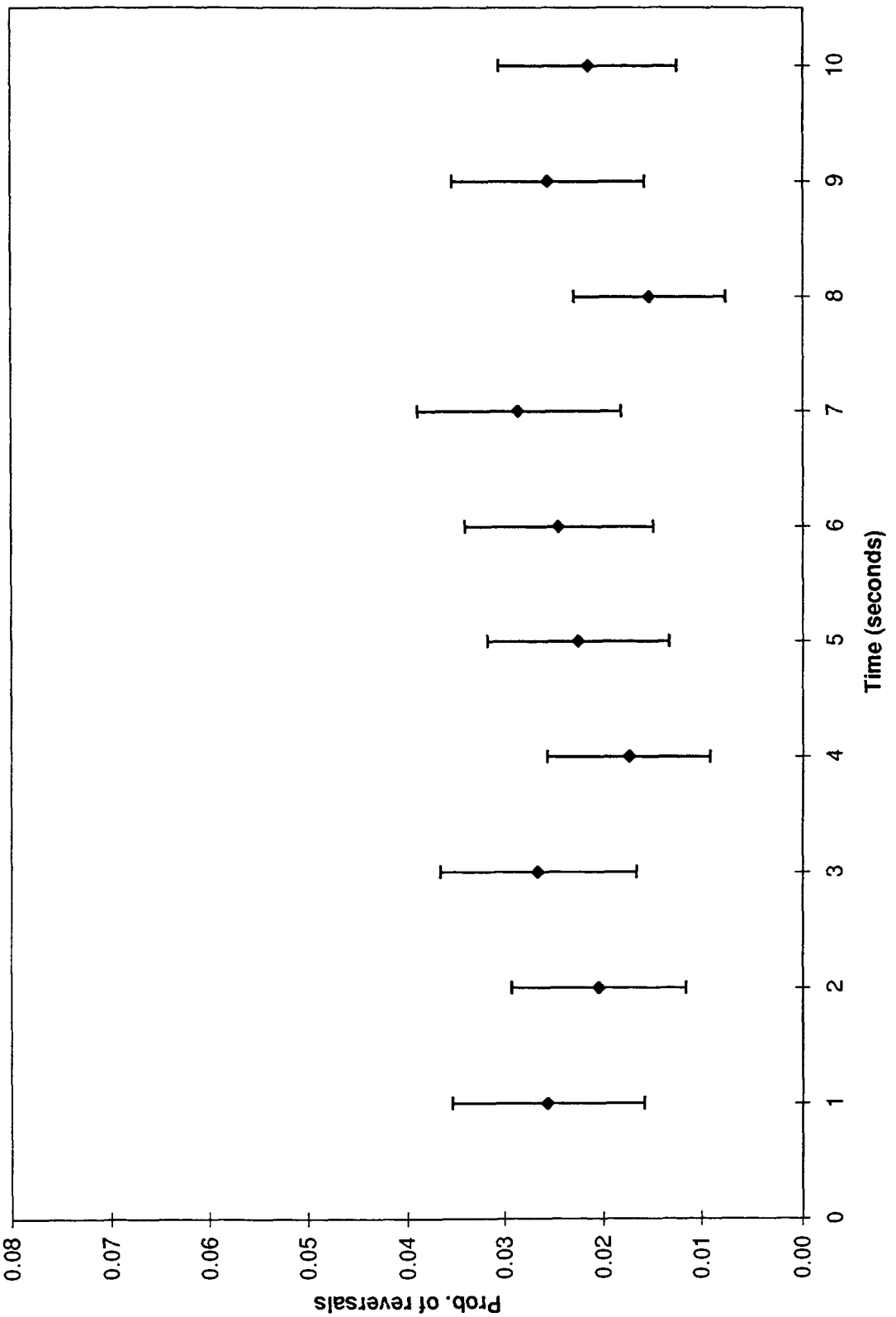
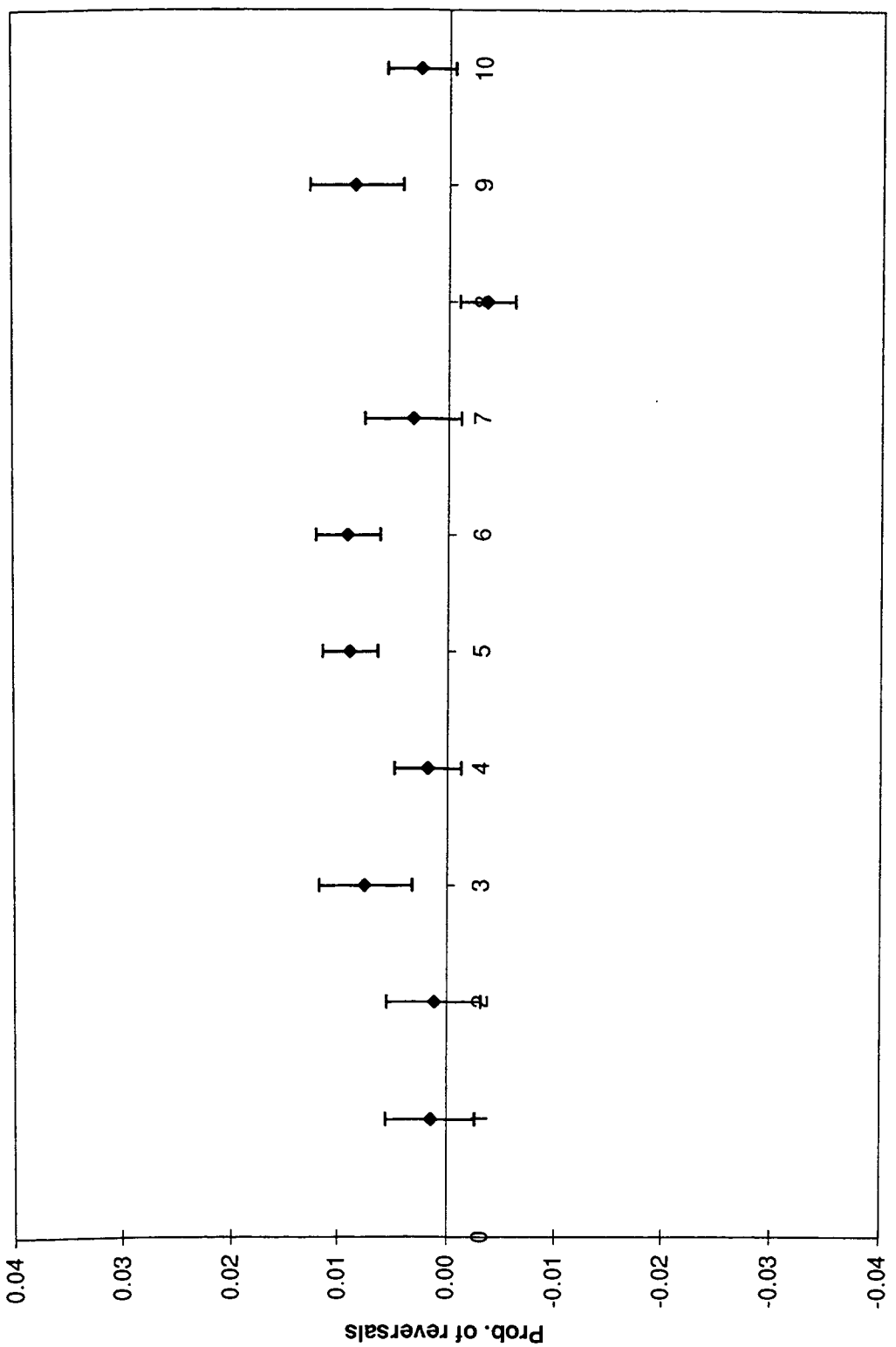

Prob. of reversals
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0
1
2
3
4
5
6
7
8
9
10
Time (seconds)

Figure 9. Differences in reversal probabilities between paired dark and light periods of Figure 7 and 8. Points indicate the mean difference in probability at that particular second. Error bars indicate 95% confidence intervals (2.14 x standard error; degrees of freedom = 16). Where the confidence intervals include zero, the difference is not significant.


0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
-0.03
-0.04
Prob. of reversals
0
1
2
3
4
5
6
7
8
9
10
Time (seconds)

min) (Table 1). This value is included by the 95% confidence intervals of each of the 10 seconds (Figure 7), therefore there was no significant variation over time.

The probability of a reversal during the 10 s of light was 0.0473 higher, on the average, than that during the dark periods (Table 1). The separately computed probability of reversals in the experimental and control periods differed significantly [$t=4.93$, $p<0.001$; $df=996$). A variation over time (Figure 8) was not detectable when the experimental data were analyzed separately.

The time dependence of the mean difference between paired light and dark periods is shown in Figure 9. Where the 95% confidence interval does not include zero, the mean difference is significantly different from zero. It is observed that the probability of a light induced reversal was significantly above background 3, 5, 6 and 9 s after the light stimulus was turned on while second 8 was significantly below background. The summed probability of a response to the light stimulus was 0.0473 ± 0.0085 (Table 1). This is significantly different from zero ($t=5.57$, $p<0.001$; $df=16$)

Discussion

The above results show that *C. elegans* responds to light by an increase in reversal probability from a background level of spontaneous reversals.

The probability of spontaneous reversals of the animals during the dark periods was essentially uniform across the 10 s period (Figure 7) as would be expected in the absence of any treatment. In the previous study of the light response of *C. elegans* (Burr, 1985), it was found that

the probability of spontaneous reversals occurring in a 10 s dark interval was 0.12. In my experiments, the probability in an equivalent dark period was 0.21 (Table 1). The higher background probability in my experiments could be due to the slightly different experimental protocol, or due to the presence of more worms on the behavioral plate. It is reasonable that the density of worms on the behavioral plate could affect the spontaneous reversal frequency, since *C. elegans* secretes a crowding pheromone. This is known to affect larval development (Golden and Riddle, 1984), but other effects have not been reported.

The probability of a reversal occurring during 10 s light periods was significantly higher than in paired dark periods. The probability in the light was only 21% above the spontaneous reversal rate (increased from 0.2073 to 0.2509), therefore, the very large sample size (about 1000) was necessary to achieve significance. In my experiments, the increase in probability, 0.047 was about half that observed by Burr (1985), even though the wavelengths and intensity of the stimulus was identical.

Although the probability of reversals in light was higher than in dark periods, I could not rule out the possibility that light could have affected the rate of spontaneous reversals. If the presence of light lowers the probability of spontaneous reversals, the animals may have a stronger light response than that calculated (i.e. total light response = increased reversal response to light + the reduction of spontaneous reversal due to light). On the other hand, if light does not affect the rate of spontaneous reversals, the increased reversals to light simply add to a constant background level of spontaneous reversals.

There was no regular time dependency of the light response (Figure 9). No significant response to light occurred in the first two seconds whereas over 90% of responses to tap stimuli occur within the first second (Rankin, Beck and China, 1990).

The strength of response to light was very different from mechanical touch to the head region. The reversal probability was only 4.7% higher during the 10 s light period, while over 99 percent of touch stimuli to the head region of the nematode produce a reversal response (Chalfie and Sulston, 1981).

Chapter 4

Confirmation that the observed response is to visible light not infrared heating.

Since *C. elegans* is very sensitive to small changes (0.05 °C) in temperature (Hedgecock and Russell, 1975), it was necessary to prove that the increase in probability of reversals discussed in Chapter 3 was not due to temperature change by the stimulus. In several different ways, Burr (1985) showed that when using the same blocking filters used in this thesis project, the response could not be due either to absorbed visible light converted to heat in the arena or nematode, or to absorption of infrared light that might be leaked by the filters. In particular, the following points were made. 1) The fraction of visible light absorbed by the plastic and agar inside the arena in the 10 s period would increase the temperature < 3×10^{-6} °C (assuming no heat loss), which was on the order of or below the level of natural temperature fluctuations. 2) The estimated temperature increase inside the anterior segment of the worm during 10 s of absorption of the visible light beam would be less than 0.18×10^{-6} °C, which is smaller than natural temperature fluctuations (13×10^{-6} °C). 3) The wavelength dependence of the light response of *C. elegans* in the region between 520 - 600 nm was different from that of light absorption by the arena or nematode. 4) The broad-band stimulus contained <0.004% of near-infrared radiation and because of filtration through the 5 cm water jacket, negligible far-infrared radiation would reach the plate. Any leaked infrared absorbed by the arena would increase the arena temperature less than 10^{-9} °C s^{-1} .

In the previous experiment (Chapter 3) it was shown that reversal probability is increased by the light stimulus. The following experiment was done to show that the response was to visible light in the stimulus, and not to possible infrared contamination of the beam or vibration due to the shutter, or any other unknown phenomenon correlated with the experimental period.

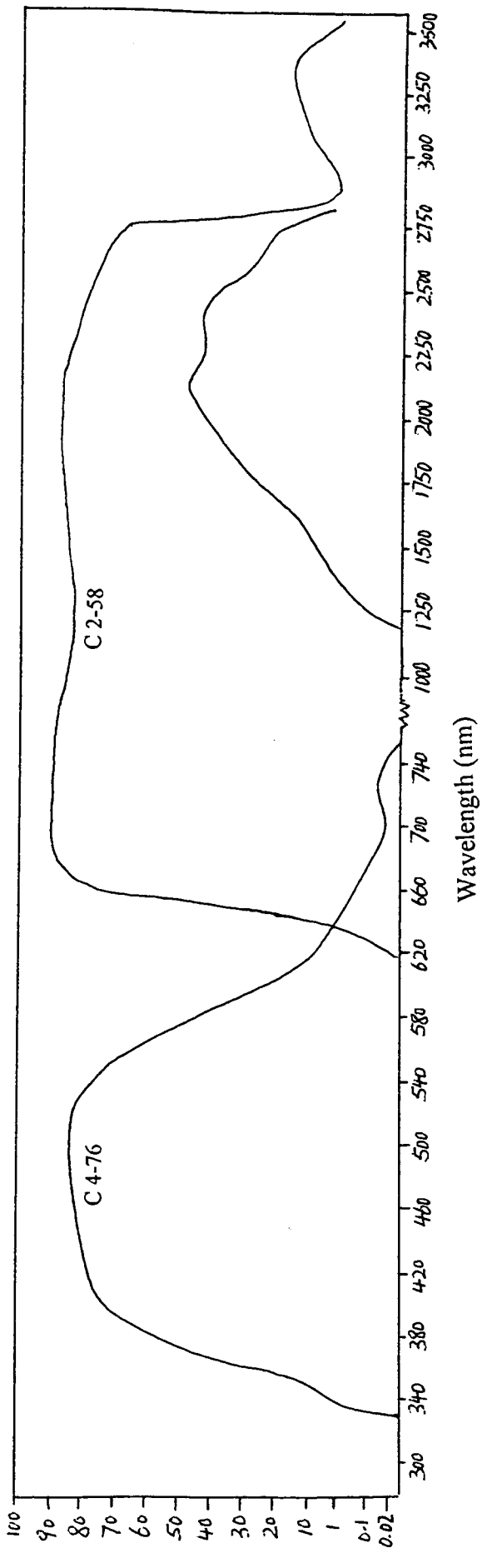
Methods

Animals were tested under the identical conditions as in Chapter 3 except a Corning C 2-58 filter was placed in the test beam in addition to the Corning C 1-57 heat filter and C 4-76 near-infrared blocking filter (Figure 2). This filter absorbed the broad-band visible spectrum passed by the other filters without affecting any infrared that might have been present in the beam. The C 2-58 filter blocked > 90% of light at wavelengths below 635 nm while transmitting most of the infrared light that is emitted by a halogen lamp (Figure 10). Thus if the response was due to infrared leaked by the 5 cm water and glass filters, the response would be little changed. However if the response was due to visible light in the range blocked by the C 2-58 filter, then the increment in reversal probability during the experimental (shutter open) period should be eliminated by the filter. The projector which provided the light stimulus was turned on during the experiment and the electronic shutter was opened and closed as previously.

Results

The results are summarized in Table 2. The average spontaneous reversals rate was 0.0181 s^{-1} (1.09 reversals per min) (Table 2).

Figure 10. Transmittance spectra of Corning C 2-58 and C 4-76 filters. Adapted from Corning Color Filter Glasses handbook, Kopp Glass Inc.


C 2-58
C 4-76
Wavelength (nm)
100
90
80
70
60
50
40
30
20
10
1
0.1
0.02
300
340
380
420
460
500
540
580
620
660
700
740
1000
1250
1500
1750
2000
2250
2500
2750
3000
3250
3500

This value is included by the 95% confidence intervals of each of the 10 seconds (Figure 11), therefore there is no significant variation over time.

The probability of reversals during the light period was 0.0184 per second (1.10 reversals per min) (Table 2). The probability of a reversals during the 10 s light was 0.0035 higher, on the average, than that during the dark periods (Table 2). A variation over time (Figure 12) was not detectable when the experimental data were analyzed separately.

The time dependence of the mean difference between paired light and dark periods is shown in Figure 13. It is observed the probability of a light induced reversals was significantly above background 2, 4 and 10 s after the light stimulus was turned on while second 6, 8 and 9 were significantly below background. The summed probability of a response to the light stimulus was 0.0035 ± 0.009 (Table 2). This is not significantly different from zero ($t=0.38$, $p>0.5$, $df=13$).

Discussion

The mean difference in reversal probability between the paired light and dark period was not significant compared with a significant 21% higher in the experiment of Chapter 3. Thus the addition of the visible light blocking filter effectively eliminated the response to the light stimulus. This demonstrates that the increase in reversal behavior in the previous experiment was caused by radiation in the visible spectrum. Moreover, we eliminated the possibility that any increase in reversal rate was due to mechanical vibration or other unknown factors correlated with the shutter opening, since the experimental conditions were exactly the same except for the filter.

The reversal activity during the experimental period was probably due purely to spontaneous reversals, since the treatment during the experimental period was rendered ineffective by the filter. The mean levels of spontaneous activity during experimental and control periods were similar, 0.0184 and 0.0181 s^{-1} (Table 2), similar to that of previous experiment (0.0207) of Chapter 3 and they were not significantly different (t= 0.696, p=0.5].

The large and significant deviation from zero of the differences (Figure 13) are difficult to explain. It appears to be primarily due to fluctuations during the control period (Figure 11).

Table 2: Lack of photoreversal response of wildtype *Caenorhabditis elegans* in the absence of visible light

	Second											
	1	2	3	4	5	6	7	8	9	10	Summary[a]	
Experimental period												
Reversal probability	0.0153	0.0180	0.0139	0.0208	0.0208	0.0139	0.0208	0.0083	0.0111	0.0277	Sum over 10 s	0.1844[b]
Standard deviation	0.1227	0.1332	0.1170	0.1428	0.1428	0.1170	0.1428	0.0909	0.1048	0.1643		
Standard error	0.0046	0.0050	0.0044	0.0053	0.0053	0.0044	0.0053	0.0034	0.0039	0.0061	Standard error	0.0073
1.96 x standard error	0.0090	0.0098	0.0086	0.0104	0.0104	0.0086	0.0104	0.0067	0.0076	0.0120		
Number of samples	721	721	721	721	721	721	721	721	721	721		
Control period												
Reversal probability	0.0168	0.0112	0.0154	0.0126	0.0209	0.0265	0.0223	0.0182	0.0168	0.0140	Sum over 10 s	0.1810[b]
Standard deviation	0.1285	0.1052	0.1231	0.1115	0.1433	0.1608	0.1479	0.1336	0.1285	0.1174		
Standard error	0.0048	0.0039	0.0046	0.0042	0.0054	0.0060	0.0055	0.0050	0.0048	0.0044	Standard error	0.0066
1.96 x standard error	0.0094	0.0076	0.0090	0.0082	0.0106	0.0118	0.0108	0.0098	0.0094	0.0086		
Number of samples	716	716	716	716	716	716	716	716	716	716		
Difference	-0.0012	0.0075	-0.0011	0.0079	0.0007	-0.0127	-0.0014	-0.0100	-0.0058	0.0131	Sum over 10 s	0.0035[c]
Standard deviation	0.0066	0.0067	0.0074	0.0052	0.0070	0.0072	0.0063	0.0045	0.0053	0.0041		
Standard error	0.0017	0.0017	0.0019	0.0013	0.0018	0.0019	0.0016	0.0012	0.0014	0.0011	Standard error	0.0090
2.14 x standard error	0.0036	0.0036	0.0041	0.0028	0.0039	0.0041	0.0034	0.0026	0.0030	0.0024		

[a]Statistics based on a repeated measures analysis of variance (the proc mixed routine of SAS)

[b]Separately determined experimental and control probabilities not significantly different (t=0.49, p>0.5, d.f. 719)

[c]Difference between paired experimental and control probabilities not significantly different from zero (t=0.38, p>0.5, d.f. 13)

Figure 11. The probability of spontaneous reversals of wildtype *Caenorhabditis elegans* during the 10 s dark (control) period. Number of samples (worms x cycles) were 716. Points indicate the mean probability at that particular second. Error bars indicate 95% confidence intervals (1.96 x standard error).

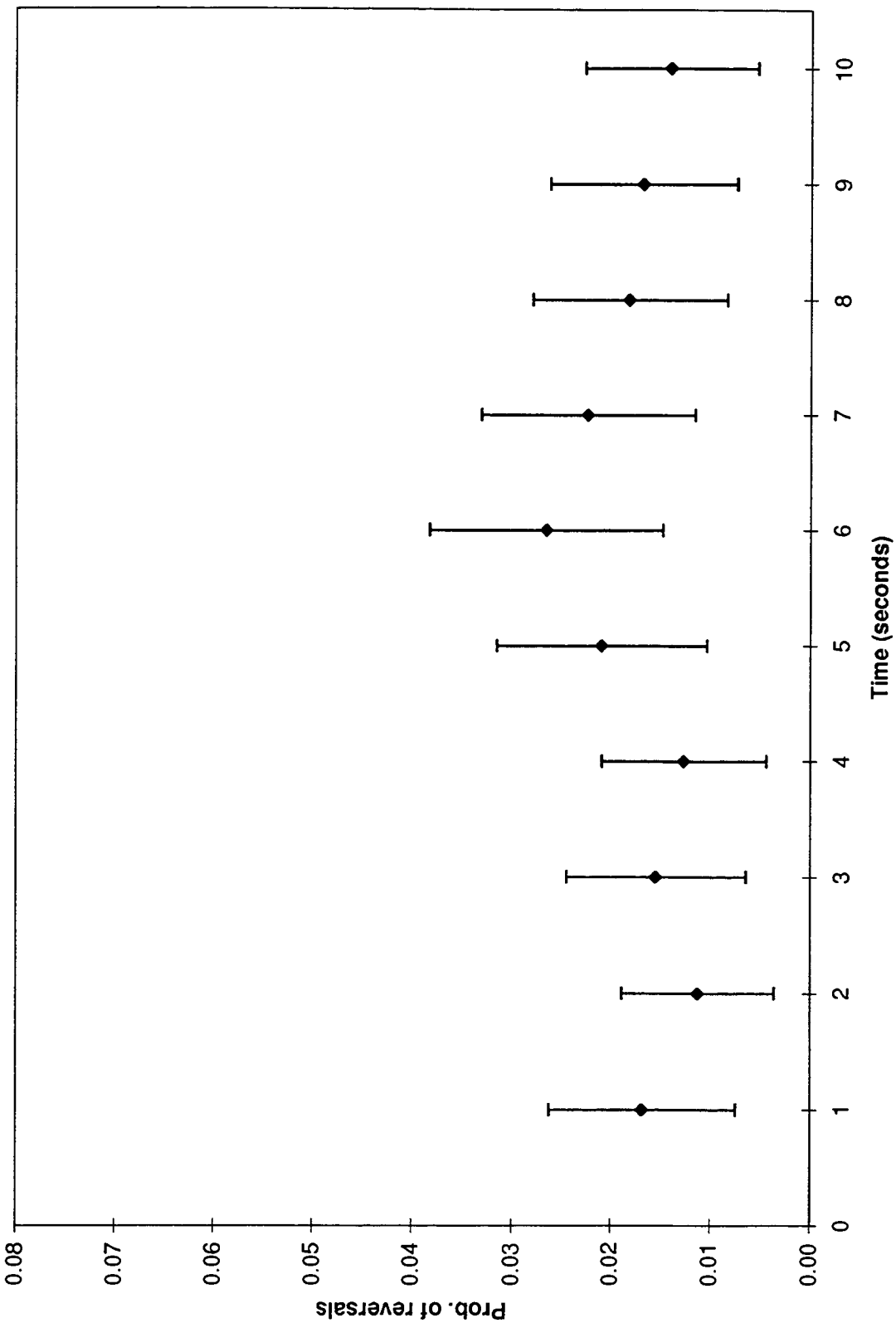

0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Prob. of reversals
0
1
2
3
4
5
6
7
8
9
10
Time (seconds)

Figure 12. The probability of spontaneous reversals of wildtype *Caenorhabditis elegans* during 10 s shutter-open (experimental) period. Visible light was eliminated by a Corning C2-58 filter. Number of samples were 721. Data plotted as in Figure 11.

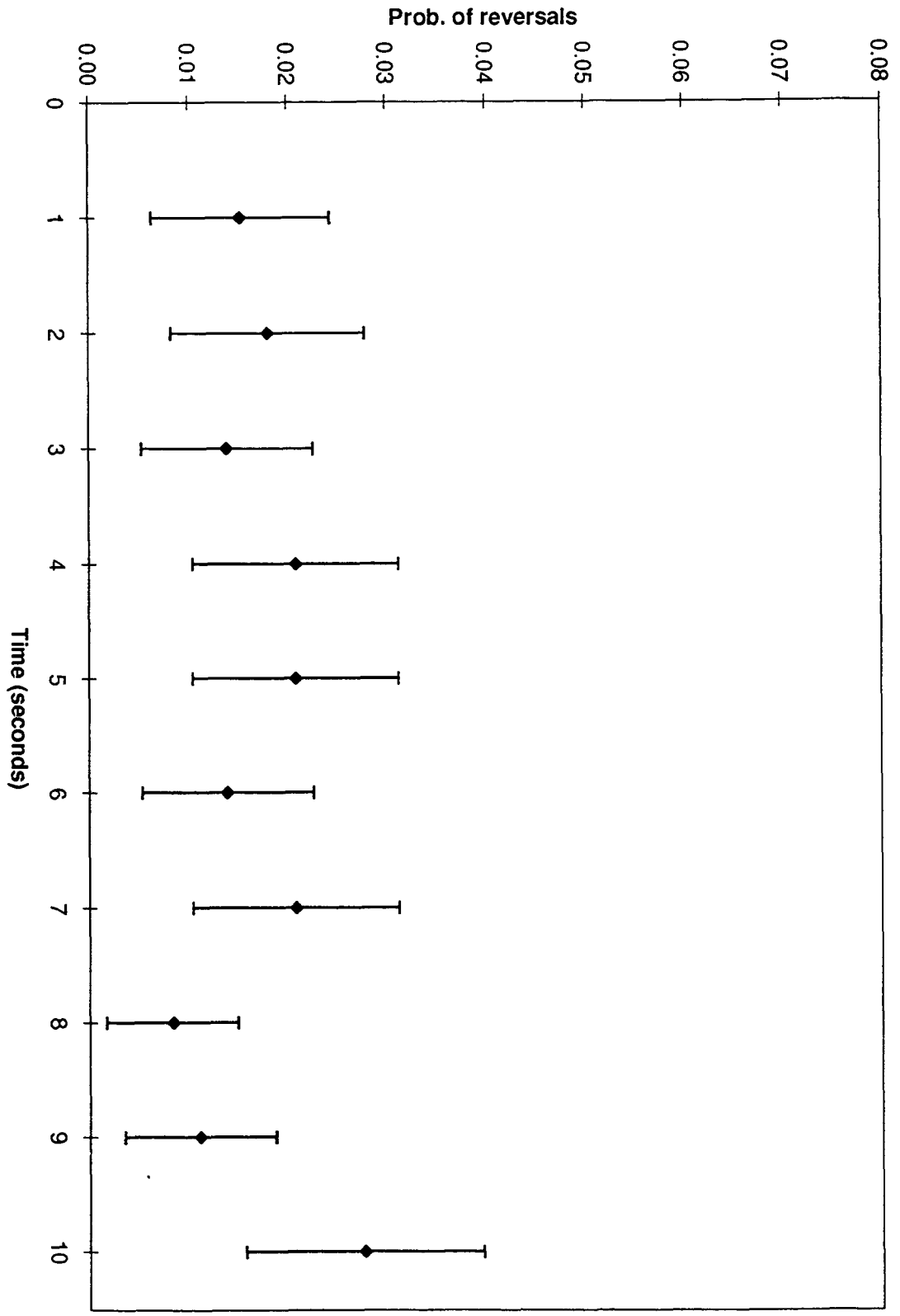
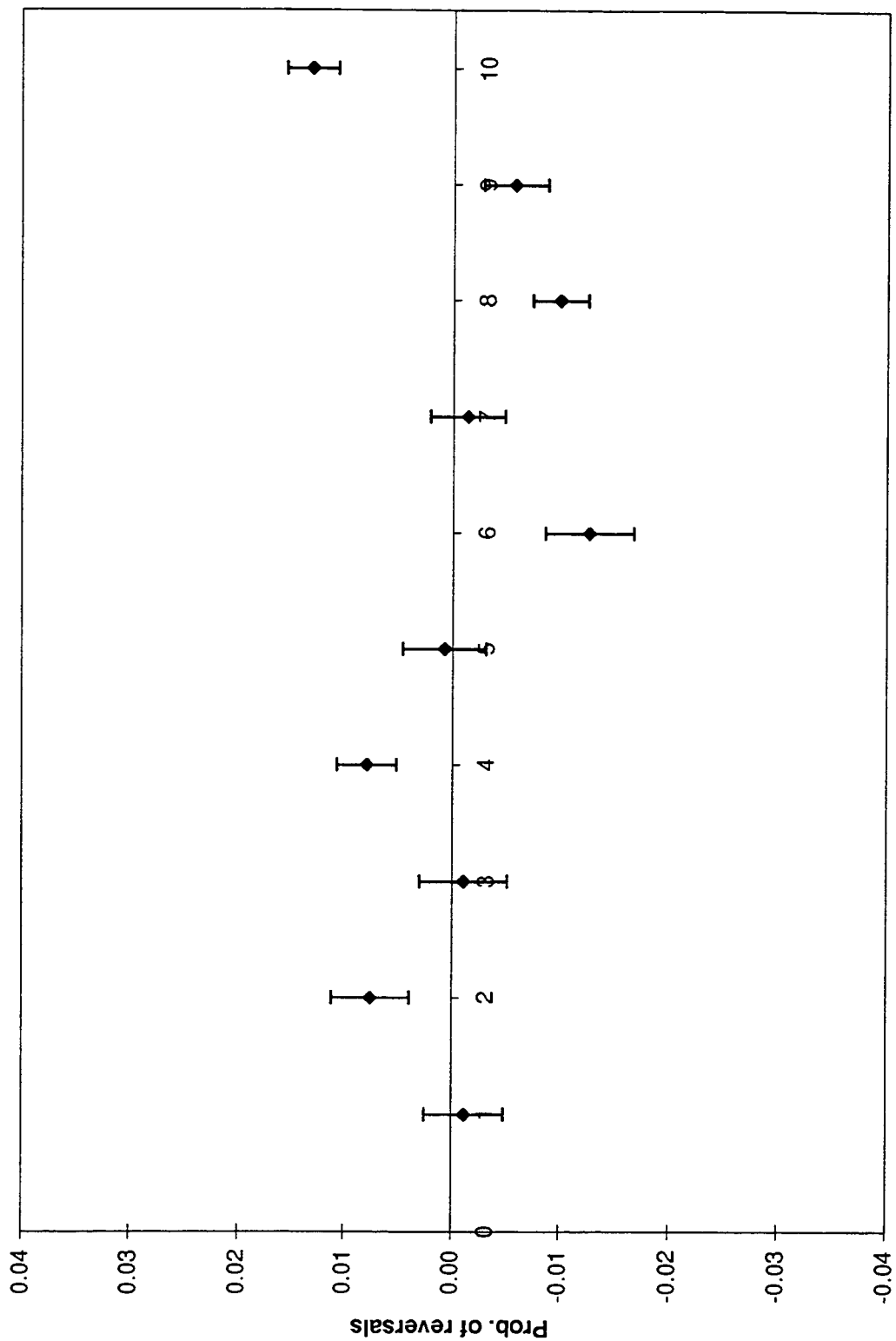

Prob. of reversals
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
Time (seconds)
0
1
2
3
4
5
6
7
8
9
10

Figure 13. Mean of differences in reversal probabilities between paired dark and light periods of Figures 11 and 12. Points indicate the mean difference in probability at that particular second. Error bars indicate 95% confidence intervals (2.14 x standard error; degrees of freedom = 13). Where the confidence intervals include zero, the difference is not significant.


0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
-0.03
-0.04
Prob. of reversals
0
1
2
3
4
5
6
7
8
9
10
Time (seconds)

Chapter 5
Behavior analysis using mutants

The nervous system is the computing machinery underlying animal behavior. An organism's response to a stimulus in the ambient environment depends on the anatomical organization and biochemical features of its nervous system as well as on the nature of the stimulus. Isolation of behavioral mutants is one approach to studying the function of individual components of the nervous system.

In *C. elegans*, most of the neurons of the nervous system are not essential for development under laboratory conditions. Therefore, many mutations leading to defects in the nervous system do not have lethal phenotypes. The different types of *C. elegans* behavior that have been studied by mutational analysis include coordinated movement, chemotaxis, thermotaxis, osmotic avoidance, male mating, egg laying, and mechanosensation. Some of the existing sensory mutants have well-described specific anatomical defects, and I have taken advantage of two of these, *ttx-1* and *daf-19*, in order to help identify the photoreceptive organelle and neuron involved in the photoreversal response.

Light response of *ttx-1* mutants

A significant question is whether the AFD fingers serve a photoreceptive function, since such a microvillar structure is associated with a photoreceptive function in many other invertebrates (Burr, 1984). The AFD dendrites of the cryophilic *ttx-1* mutants lack the numerous microvillar projections in the sensory ending of the AFD neurons. The structure resembles that of a fingerless sack of membranes which

protrudes from the dendrite just below the cilium (Perkins et al., 1986). The mutant cilia are approximately three times longer than their wild-type counterparts, and instead of projecting directly anterior, the *ttx-1* cilia are tilted ventrally at their bases. The modified cilia of the other neurons associated with the amphid, the wing and channel cilia and the various mechanosensilla of the head are morphologically normal in *ttx-1* mutants (Perkins et al., 1986). When both AFD neurons were killed by a focused laser beam, the animals showed cryophilic, athermotactic or an intermediate phenotype, and isothermal movement in a thermal gradient was abolished similar to the *ttx-1* behavior. When a single AFD neuron was killed, the defects became less severe and some animals could move isothermally along their cultivation temperature. These results suggest that both AFD neurons are necessary for normal thermotaxis (Mori and Ohshima, 1995). As another *C. elegans* neuron (ASH) has been shown to have a dual sensory modality (Kaplan and Horvitz, 1993), it is possible that the AFD may have a second, photosensory function.

Methods

Ttx-1 cultures were maintained at 20 °C under continuous darkness until the animals reached the one-day-old adult stage. The animals were washed off the bacterial plates and put onto 5-cm behavioral plates for light experiments as described in Chapter 1. In each experiment, 60 - 80 hermaphrodites were put on one plate.

Results

The results are summarized in Table 3. The average probability of reversals was 0.01534 s^{-1} (0.92 reversal per min) during the 10 s dark period and 0.01405 s^{-1} (0.84 reversal per min) during the 10 s light period. A large variation of the probability of reversals over time was observed in the both the control and experimental periods (Figures 14 and 15).

The time dependence of the mean difference between paired light and dark periods is shown in Figure 16. The mean difference in the probability of reversals was -0.0167, which is a 10% decrease over the background of spontaneous reversals. The decrease was not significant [t=-1.62, p=0.2, d.f = 13].

Table 3: Photoreversal response and spontaneous reversal activity of *Caenorhabditis elegans* mutant *ttx-1*

	Second											
	1	2	3	4	5	6	7	8	9	10	Summary[a]	
Experimental period												
Reversal probability	0.0157	0.0059	0.0138	0.0079	0.0196	0.0216	0.0177	0.0039	0.0157	0.0216	Sum over 10 s	0.1405[b]
Standard deviation	0.1245	0.0766	0.1166	0.0884	0.1389	0.1456	0.1319	0.0626	0.1245	0.1456		
Standard error	0.0055	0.0034	0.0052	0.0039	0.0062	0.0065	0.0058	0.0028	0.0055	0.0065	Standard Error	0.0075
1.96 x standard error	0.0108	0.0067	0.0102	0.0076	0.0122	0.0127	0.0114	0.0055	0.0108	0.0127		
Number of samples	509	509	509	509	509	509	509	509	509	509		
Control period												
Reversal probability	0.0191	0.0153	0.0115	0.0191	0.0172	0.0076	0.0210	0.0057	0.0115	0.0134	Sum over 10 s	0.1534[b]
Standard deviation	0.1371	0.1228	0.1066	0.1371	0.1302	0.0872	0.1436	0.0756	0.1066	0.1150		
Standard error	0.0060	0.0054	0.0047	0.0060	0.0057	0.0038	0.0063	0.0033	0.0047	0.0050	Standard Error	0.0088
1.96 x standard error	0.0118	0.0106	0.0092	0.0118	0.0112	0.0074	0.0123	0.0065	0.0092	0.0098		
Number of samples	523	523	523	523	523	523	523	523	523	523		
Difference	-0.0031	-0.0092	0.0029	-0.0107	0.0026	0.0141	-0.0028	-0.0013	0.0044	0.0083	Sum over 10 s	-0.0167[c]
Standard deviation	0.0108	0.0049	0.0105	0.0069	0.0073	0.0058	0.0064	0.0049	0.0070	0.0076		
Standard error	0.0028	0.0013	0.0027	0.0018	0.0019	0.0015	0.0016	0.0013	0.0018	0.0020	Standard Error	0.0103
2.14 x standard error	0.0060	0.0028	0.0058	0.0039	0.0041	0.0032	0.0034	0.0028	0.0039	0.0043		

[a]Statistics based on a repeated measures analysis of variance (the proc mixed routine of SAS)

[b]Separately determined experimental and control probabilities not significantly different (t=-1.58,p=0.20, d.f. 516)

[b]Difference between paired experimental and control probabilities not significantly different from zero (t=-1.62, p=0.20, d.f. 13)

Figure 14. The probability of spontaneous reversals of *Caenorhabditis elegans* mutant *ttx-1* during the 10 s dark (control) period. Number of samples (worms x cycles) were 523. Points indicate the mean probability at that particular second. Error bars indicate 95% confidence intervals (1.96 x standard error).

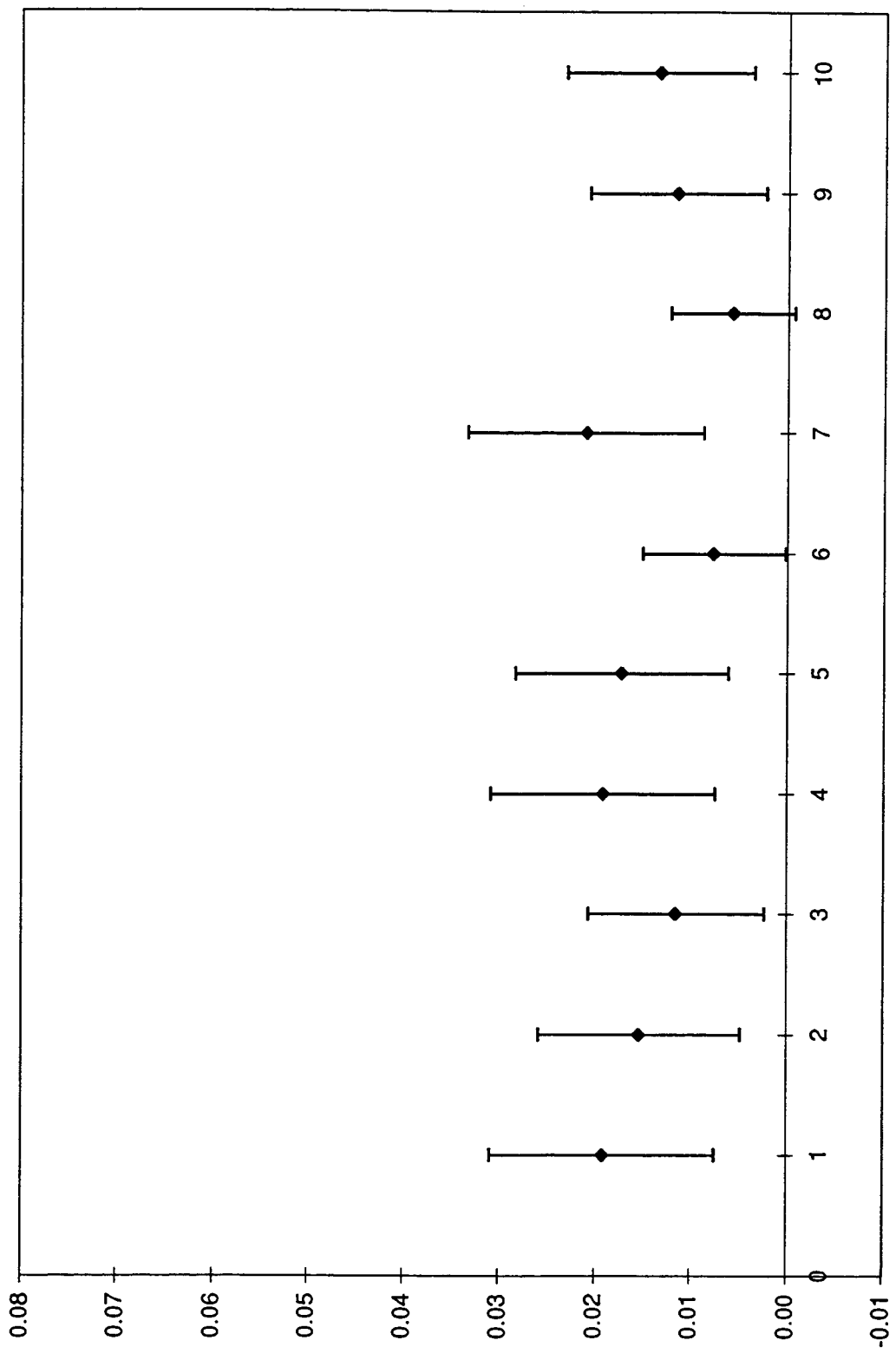

Prob. of reversals
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
0
1
2
3
4
5
6
7
8
9
10
Time (seconds)

Figure 15. The probability of reversals of *Caenorhabditis elegans* mutant *ttx-1* during the 10 s light (experimental) period. Number of samples (worms x cycles) were 509. Data plotted as in Figure 14.

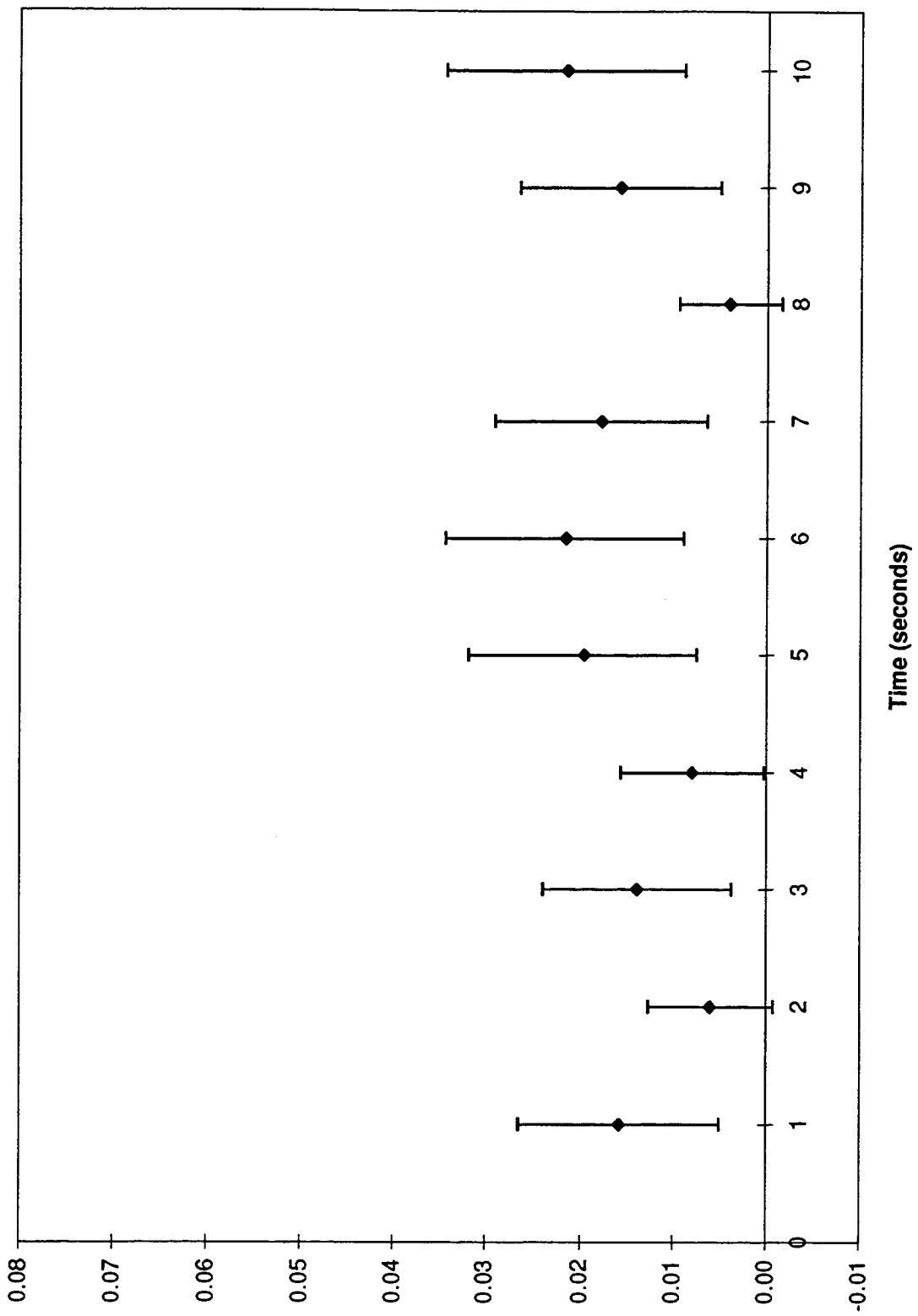
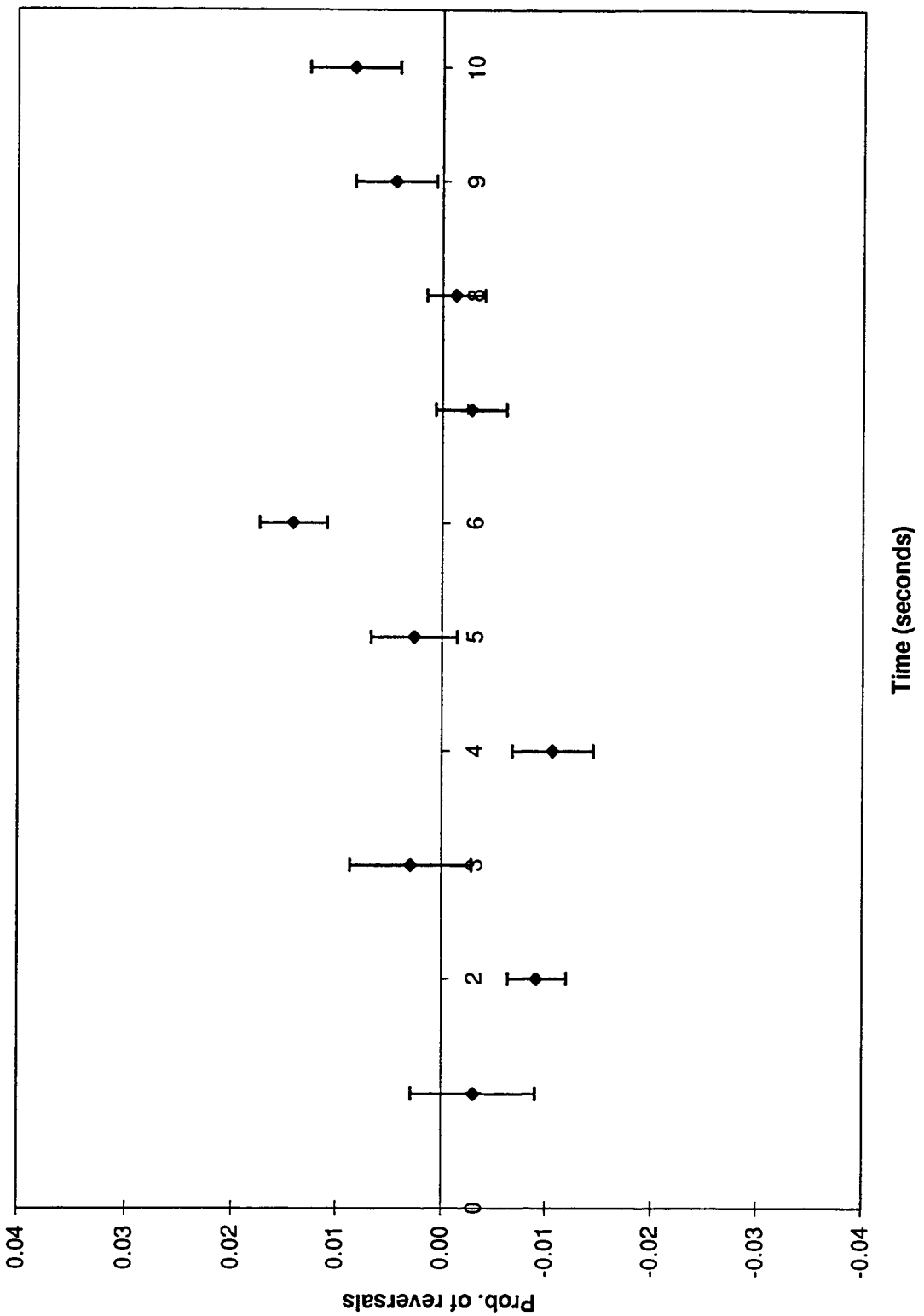

0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
Prob. of reversals
0
1
2
3
4
5
6
7
8
9
10
Time (seconds)

Figure 16. Differences in reversal probabilities between paired dark and light periods of Figure 14 and 15. Points indicate the mean difference in probability at that particular second. Error bars indicate 95% confidence intervals (2.14 x standard error; degrees of freedom = 13). Where the confidence intervals include zero, the difference is not significant.


0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
-0.03
-0.04
Prob. of reversals
0
2
3
4
5
6
8
9
10
Time (seconds)

Light response of daf-19 mutants

In nematodes, cilia are found only in the nervous system, where they are modified for sensory reception of diverse modalities (Ward et al., 1975; Ware et al., 1975; Perkins et al 1986). Modified cilia are found at the terminus of sensory dendrites of many but not all sensory neurons of *C. elegans*. In other vertebrates ciliary receptor organelles are found in the rod and cone cells of the eye, the hair cells of the ear, and olfactory receptors of nasal mucosa. Of the 118 classes of neurons in *C. elegans* hermaphrodites, about one third are sensory and of these, 24 classes have cilia (White et al., 1986; Perkins et al., 1986). Sensory cilia consist of a membrane-bound cylinder of nine doublet microtubules that usually extend from a basal body. In *C. elegans*, *daf-19* is one of the genes required for normal morphology of all sensory cilia in the animal. The sensory dendrites in *daf-19* mutants entirely lack cilia, including the transition zones, whereas nonciliated sensory endings are not affected (Albert et al 1981; Perkins et al, 1986). Vestigial basal bodies without membrane attachments are found in a few of the amphidial dendrites. The amphidial dendrites and most of the mechanosensory dendrites terminate in club-shaped endings after invaginating, and form normal belt-shaped junctions with their respective sheath cells. The fingers of the AFD neuron appear normal although its cilium is affected. *Daf-19* mutants are found to be chemotaxis-defective, defective in avoiding chemical repellents, and dauer formation-defective (Bargmann, 1993). The structural and behavioral defects in *daf-19* indicate that ciliated amphidial neurons are important in chemosensory responses. This has been confirmed by laser ablation experiments (Bargmann, 1993). Thermotaxis is normal so the thermosensory neurons do not require

modified cilia for function (Perkins et al 1986). Since *daf-19* mutants lack all classes of cilia, it is a good candidate to test whether a ciliated sensory neuron is involved in the photosensation of *C. elegans.*

The growth rate of *daf-19* mutants is much slower than the *N2* strain. *Daf-19* mutants develop to adulthood in about seven and a half days at 20 °C, and they develop to mostly dauers at higher temperature.

Methods

Daf-19 mutants were maintained at 20 °C under continuous darkness in 9 cm NGM agar plate seeded with *E. coli* on the entire surface. Since the life cycle of daf-19 mutants is 7 ½ days instead of 3 ½ days in N2 strain, more food was needed during extended incubation time. Newly hatched L1s were transferred onto the bacterial plate. Adults were obtained after 7½ days of incubation and were transferred onto behavior plate for photobehavior analysis as described in Chapter 1.

Results

The results are summarized in Table 4. The average reversal rate during the 10 s dark period was 0.01343 per second (0.81 reversal per min) and the average probability of reversals during the 10 s light period was 0.01454 per second (0.87 reversal per min). Variation of the probability of reversals over time in both control and experimental periods was not significant except second 1 of the control periods (Figure 17 and 18). The time dependence of the mean difference between paired light and dark periods is shown in Figure 19. The mean difference in the probability of reversals was 0.0033, which is a 2.5% increase over the

Table 4: Photoreversal response and spontaneous reversal activity of *Caenorhabditis elegans* mutant *daf-19*

	Second											
	1	2	3	4	5	6	7	8	9	10	Summary[a]	
Experimental period												
Reversal probability	0.0085	0.0068	0.0136	0.0119	0.0136	0.0239	0.0153	0.0136	0.0204	0.0102	Sum over 10 s	0.1454[b]
Standard deviation	0.0920	0.0823	0.1160	0.1086	0.1160	0.1527	0.1230	0.1160	0.1416	0.1007		
Standard error	0.0038	0.0034	0.0048	0.0045	0.0048	0.0063	0.0051	0.0048	0.0058	0.0042	Standard Error	0.0058
1.96 x standard error	0.0074	0.0067	0.0094	0.0088	0.0094	0.0123	0.0100	0.0094	0.0114	0.0082		
Number of samples	587	587	587	587	587	587	587	587	587	587		
Control period												
Reversal probability	0.0016	0.0082	0.0179	0.0147	0.0114	0.0131	0.0163	0.0147	0.0147	0.0212	Sum over 10 s	0.1343[b]
Standard deviation	0.0404	0.0900	0.1329	0.1204	0.1063	0.1136	0.1268	0.1204	0.1204	0.1442		
Standard error	0.0016	0.0036	0.0054	0.0049	0.0043	0.0046	0.0051	0.0049	0.0049	0.0058	Standard Error	0.0087
1.96 x standard error	0.0031	0.0071	0.0106	0.0096	0.0084	0.0090	0.0100	0.0096	0.0096	0.0114		
Number of samples	613	613	613	613	613	613	613	613	613	613		
Difference	0.0067	-0.0007	-0.0047	-0.0025	0.0030	0.0091	-0.0003	-0.0008	0.0063	-0.0121	Sum over 10 s	0.0033[c]
Standard deviation	0.0029	0.0039	0.0062	0.0087	0.0082	0.0069	0.0071	0.0054	0.0061	0.0083		
Standard error	0.0008	0.0010	0.0016	0.0023	0.0021	0.0018	0.0018	0.0014	0.0016	0.0021	Standard Error	0.0113
2.14 x standard error	0.0017	0.0021	0.0034	0.0049	0.0045	0.0039	0.0039	0.0030	0.0034	0.0045		

[a]Statistics based on a repeated measures analysis of variance (the proc mixed routine of SAS)

[b]Separately determined experimental and control probabilities not significantly different (t=1.53, p=0.20, d.f. 600)

[c]Difference between paired experimental and control probabilities not significantly different from zero (t=0.29, p>0.5, d.f. 9)

Figure 17. The probability of spontaneous reversals of *Caenorhabditis elegans* mutant *daf-19* during the 10 s dark (control) period. Number of samples (worms x cycles) were 613. Points indicate the mean probability at that particular second. Error bars indicate 95% confidence intervals (1.96 x standard error).

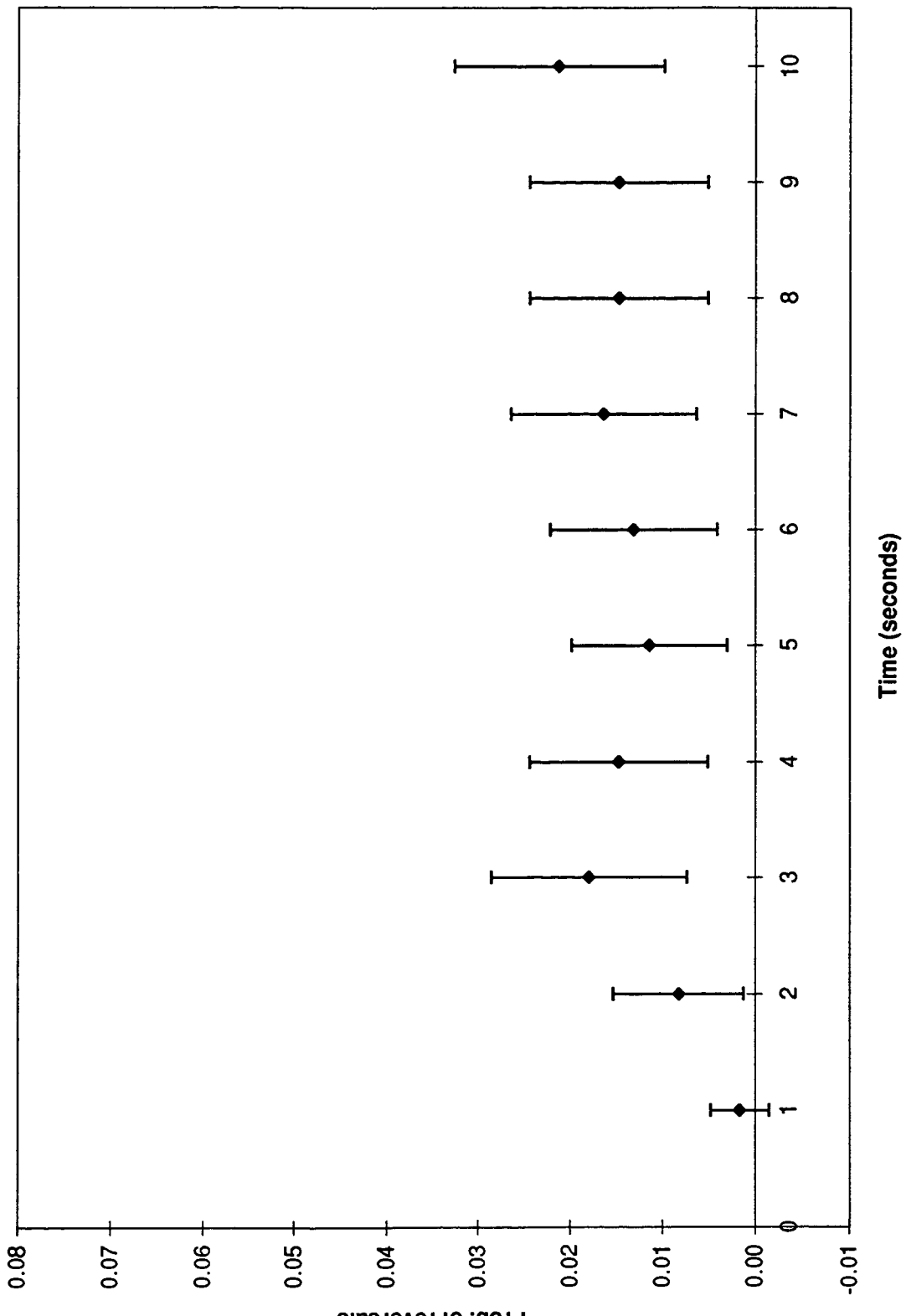

Prob. of reversals
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
0
1
2
3
4
5
6
7
8
9
10
Time (seconds)

Figure 18. The probability of reversals of *Caenorhabditis elegans* mutant *daf-19* during the 10 s light (experimental) period. Number of samples (worms x cycles) were 587. Data plotted as in Figure 17.

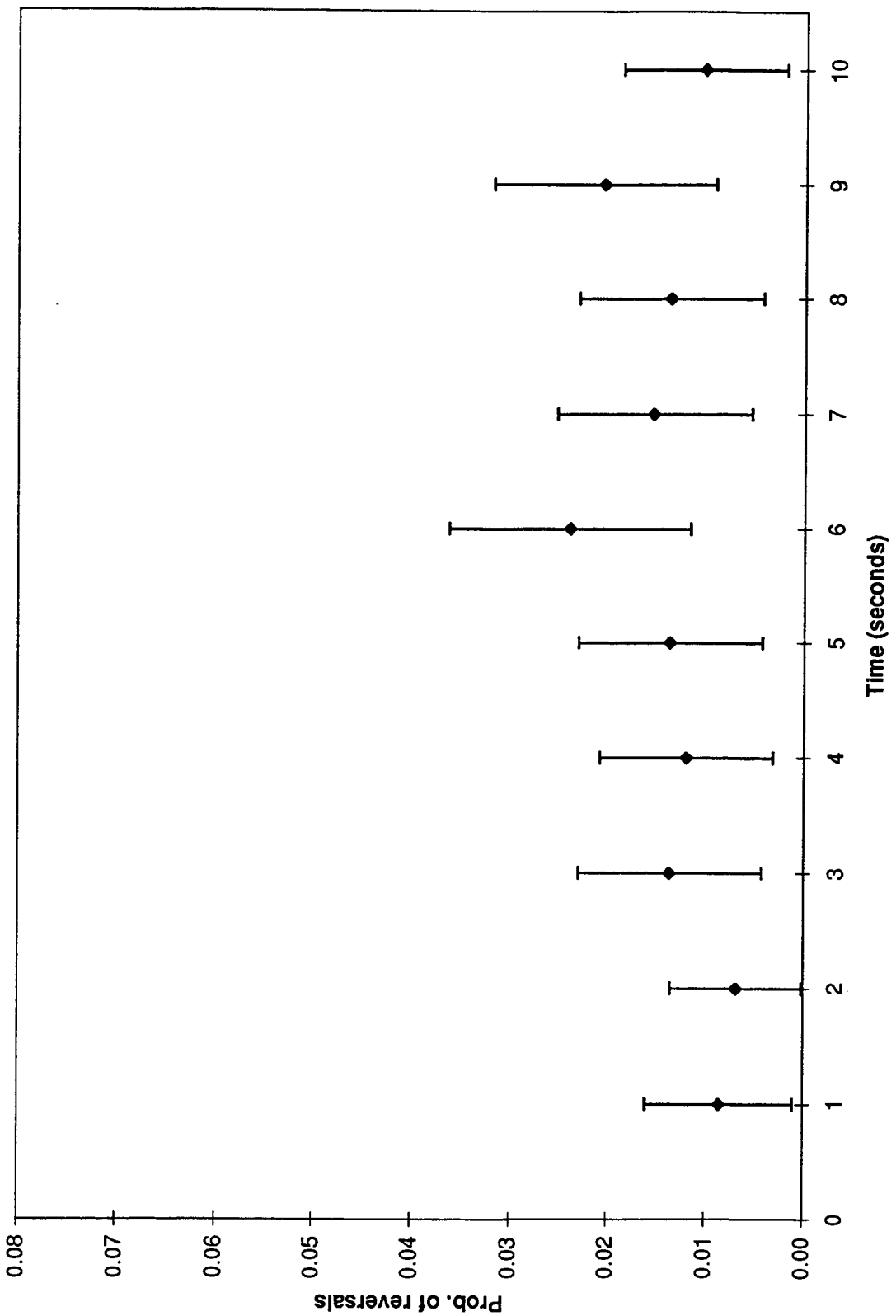
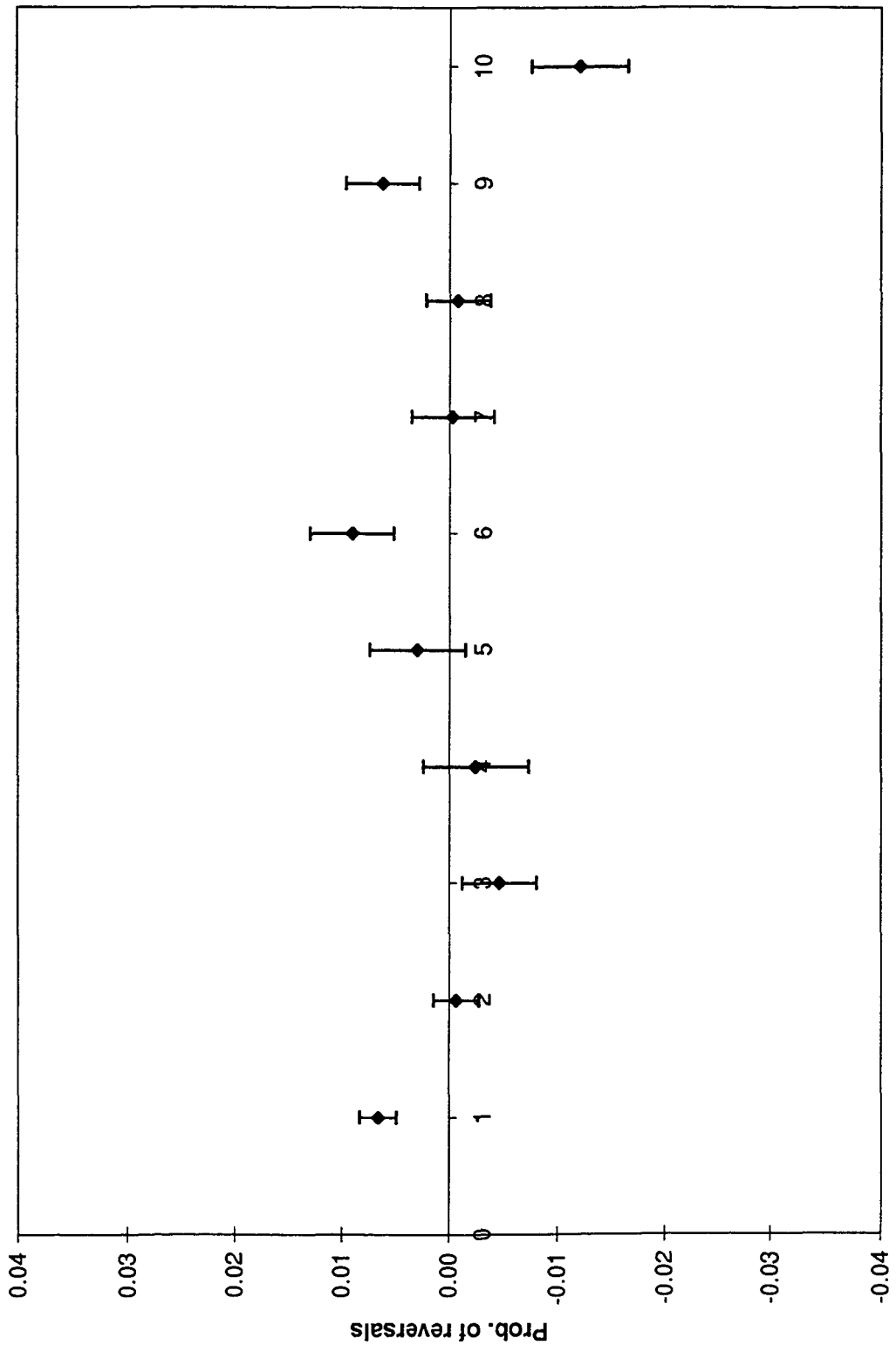

Prob. of reversals
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0
1
2
3
4
5
6
7
8
9
10
Time (seconds)

Figure 19. Differences in reversal probabilities between paired dark and light periods of Figure 17 and 18. Points indicate the mean difference in probability at that particular second. Error bars indicate 95% confidence intervals (2.14 x standard error; degrees of freedom = 9). Where the confidence intervals include zero, the difference is not significant.


0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
-0.03
-0.04
Prob. of reversals
0
1
2
3
4
5
6
7
8
9
10
Time (seconds)

background of spontaneous reversals. The increase was not significant [t=0.29, p>0.5, d.f = 9].

Discussion

The spontaneous reversal frequencies of *ttx-1* and *daf-19* were similar (0.1534 and 0.1343 in a 10 s period) but about 30% lower than that observed in the wildtype (0.2073). It is not known what structures in *C. elegans* control spontaneous reversal. These results suggest that spontaneous reversals are partly controlled by cilia or microvilli since the absence of either of these structures resulted in a lower spontaneous reversal frequency. It would be interesting to investigate the spontaneous reversal frequency of the double mutant.

The probability of reversal during the light period was not significantly different from the dark period in both mutants. However, it is possible that light sensitivity in the mutants would be detectable with a larger sample size. The light responses were 35% and 7%, respectively below that of the wildtype (*ttx-1* -0.0167; *daf-19* 0.0033; wildtype 0.0473). Evidently the mutants do not sense light as effectively as wildtype animals and it is tempting to conclude that this is due to the absence of cilia in sensory dendrites of *daf-19* mutants and the absence of microvillous finger projections in *ttx-1*. Alternatively there may be some unknown defect in their locomotory or neuronal system which affect their reversal behavior, both spontaneously and sensory-induced.

Chapter 6

General Discussion

Computer tracking

One of the objectives of this thesis was the development of a computer tracking system that can automatically track and analyze the movement of *C. elegans* on a behavioral plate. My contribution was to develop higher level software, written in BASIC09 and APL, to control peripheral hardware and facilitate data analysis. A large part of this work was testing the programs. The huge number of track coordinates collected in each experiment (41,400) were analyzed by an APL program which calculated the direction and distance moved between points along the track and scored reversal bouts. Another APL program pooled the data from the 20 or so experiments and calculated paired statistics.

Although the system could track adult *C. elegans* reliably at the current magnification (an adult worm was represented by 12 - 15 pixels in computer memory), early stages of the animals (e.g. L1 and L2 larvae) were too small to be tracked. One way to get around this problem is to use a longer extension tube with the lens. The drawback of using higher magnification is that the field of view is smaller, fewer worms can be tracked simultaneously and the worms crawl out of the field of view more frequently. Moreover, it was found that putting a smaller number of worms onto the behavioral plate increased the time length that a particular worm could be continuously tracked. Fewer worms present per unit area lowered the probability of collisions between worms which necessitate the termination of the tracking of those worms.

In some of the experiments (5 out of 60) the mean reversal probability of either the light or dark (or both) periods was exceptionally high (e.g. > 0.5 in a 10 s interval) and were clearly outliers (Figures 4 and 5). In such cases, the reversal probability was usually also higher than average. Such a high reversal probability could have been due to the presence of too high a density of worms on the behavioral plate such that when two worms came close enough (but not touching each other), the computer might have picked up the wrong worm during tracking and falsely scored a reversal.

Although the computer tracking system is capable of tracking worms for an extended period of time, the time for one experiment should not be much over 15 minutes: 1) The absence of food on the behavioral plate may affect the behavior of the worm after a time. 2) Since adult *C. elegans* hermaphrodites start laying eggs a short time after being transferred onto behavioral plate, the increasing number of eggs with time can decrease the efficiency of tracking worms because it takes time for the program to eliminate such stationary objects. 3) It is possible that repeated presentation of the stimulus for longer times will cause habituation.

The method of pairing the control and experimental periods when the mean difference of reversal probability is calculated allows a small effect of the stimulus to be detected even though the background level of spontaneous reversals may vary from cycle to cycle. This greater power of paired statistics is evident when comparing the results of a paired t-test with the t-test based on separately determined experimental and control means (footnotes b and c in the tables).

Photosensitivity of wildtype *C. elegans*

One main objective was to compare the photosensitivity of *C. elegans* using the computer tracking system with that obtained manually by Burr (1985). A second objective was to investigate the possibility that the response was due to infrared radiation.

The results from Chapter 3 confirmed that in *C. elegans* the probability of reversal was increased when the animals were presented with visible light stimuli. The 10 s visible light stimulus increased the probability of reversals by 21% to 0.047 above the background spontaneous reversal rate. By using the computer tracking system, a sample size of ~1000 was obtainable, and repeated measures analysis of variance show that the probability of reversal during the light period was significantly higher than the paired dark period [$t=5.57$, $p<0.001$]. When comparing my results with findings by Burr (1985), it was found that the probability of spontaneous reversal in a 10 s dark period was higher in my results (0.21 vs 0.12). Since the apparatus were the same except slightly different experimental protocol, it is possible that the presence of more than one worm on the behavioral plate might have increased the probability of spontaneous reversal.

In order to eliminate the possibility that the reversal response was due to the leakage of infrared from the light source, a blocking filter was added which removed all visible light from the light stimulus but not infrared. This eliminated the increase in reversal probability during the experimental period and thus demonstrated that the increased response was due to visible light and not infrared heating.

The probability of spontaneous reversal during the 10 s dark period of wildtype with light (0.2073) differs from that of wildtype without

light (0.1810). The difference of 0.0263 is significant [t=2.20, p=0.05] but is smaller than the increase due to light (0.047) found in wildtype animals. Since the addition of a visible light blocking filter should not change the spontaneous reversal in the dark period, the difference could be due to the variation among populations of worms being tested.

Although it was shown that the light stimulus increased the probability of reversals, it is unknown whether the animals were sensitive to the change from "dark to light" or simply to the presence of light for an extended period of time. To determine if the animals are sensitive primarily to changes, a small modification of the protocol and APL programs can be made so that the probability of reversals just after the light period is analyzed. If there is an increase of probability of reversals after the light stimulus is turned off, it is likely that the animals are sensitive to a change in intensity of light no matter whether the change is positive or negative.

Since *C. elegans* exhibits habituation when exposed to a repeated stimulus such as mechanical taps to the Petri plate containing the animals (Rankin, Beck and Chiba, 1990; Rankin and Chiba, 1988), it will be interesting to find if *C. elegans* exhibits a decrement in responsiveness during repeated light stimuli. It would be interesting also to investigate other forms of nonassociative learning such as dishabituation and sensitization.

***ttx-1* and *daf-19* mutants**

One objective in the design of the computer tracking system was to enable determination of the time dependence of reversal behavior following the introduction of a stimulus. This appears to have been successful in the test of photobehavior of wildtype animals (Figure 9, Table 1), although it is possible that a higher sample size may have improved the result. The time dependence recorded in the other experiments is unsatisfactory, when considering the wide variation of the points with time in the difference plots (Figure 13, 16, 19). This appears to be due to the small number of reversals recorded each second. In Figures 16 and 19, the time dependence of the differences is inversely related to that of the corresponding control measurements (Figure 14 and 17). When the corresponding results in Table 3 and 4 were examined carefully, it was found that the number of reversals in each second in the dark (control) period was between 3 - 11 in *ttx-1* and 1 -13 in *daf-19*. Since the number of reversals in a particular second could be so low, it is likely that the fluctuations in the difference plots of Figure 16 and 19 were due to the small number of reversals recorded. On the other hand, there were 14-27 reversals in each second in wildtype animals and no unexpected fluctuation in the corresponding differences plot (Figure 9). Since the mutants had a lower probability of spontaneous reversals, even more replicates of experiments are required to get the same number of reversals as for the wildtype.

The spontaneous reversal frequencies of *ttx-1* and *daf-19* were about 30% lower compared with wildtype animals. The significantly lower probability [t=4.04, p=0.001 for *ttx-1*; t=5.05, p=0.001 for *daf-19*] of spontaneous reversals might be related to some innate defects in the

mutants. Since the fingers of the AFD neurons in *ttx-1* mutants are entirely missing and *daf-19* mutants lack all cilia in the sensory dendrites, it is possible that spontaneous reversals of the mutants are influenced by input from the AFD fingers or cilia of sensory dendrites.

The probability of reversals in a 10 s light period was 10% below (*ttx-1*) and 2.5% above (*daf-19*) the background level of spontaneous reversals, and the differences were not significant in repeated measures analysis of variance [t=-1.62, p=0.2 in *ttx-1*; t=0.29, p>0.5 in *daf-19*]. Therefore, it would appear that cilia in sensory dendrites and the microvillar finger projections in AFD dendrites both play a role in light sensitivity. Since the AFD dendrite projects both microvilli and a cilium, the amphid sensory neuron AFD is a good candidate as a photoreceptor in *C. elegans*. Both microvillar and ciliary photoreceptors are found in other invertebrate phyla (Burr, 1984).

The two mutants with low spontaneous reversal probability also exhibited no light response. It is possible that there is a functional connection between these two effects. The mutants may have slight defects in the interneurons or motor neurons that affect reversal behavior.

Since several nematode sensory organs have previously been reported to have a dual or bimodal function (Burr and Burr, 1975; Kaplan and Horvitz, 1993), it is not surprising that the AFD may function as a bimodal sensory cell. Experiments by Mori and Ohshima (1995) showed that the AFD is a major thermosensory neuron and plays a critical role in thermotaxis. In order to investigate if AFD neurons are involved in photosensitivity, laser ablation experiments can be carried out by killing AFD neurons in live animals and subsequently examining

their photoresponse. Experiments that can be done in the future include: 1) Killing of both left and right AFD neurons and test if light response is eliminated. 2) If the light response is eliminated in 1), all amphid sensory neurons except AFD can be killed and the photoresponse tested. This experiment would show whether the AFD is the site of photosensation or just a regulator of other photosensory neurons elsewhere in the amphid sensillum. 3) Killing of either left or right AFD neurons and test for any decrease response to light.

Conclusions

By using the computer tracking system, it was possible to collect and analyze data from a large sample size in experiments on the photoresponse of wildtype *C. elegans*, and it was confirmed that wildtype *C. elegans* respond to light stimulus by the increasing probability of reversal. The photoresponse is to a visible light stimulus and not to infrared heat radiation. Results from the photoresponse of *ttx-1* and *daf-19* mutants suggest that the amphidial sensory neurons AFD are a good candidate as a photoreceptor and further investigations are required to confirm this possibility.

LITERATURE CITED

Albert, P. S., Brown, S., and Riddle, D. L. (1981). Sensory control of dauer larva formation in *Caenorhabditis elegans*. *J. Comp. Neurol.* 198: 435-451.

Anderson, R. V., Coleman, D. C., Cole, C. V., and Elliott, E. T. (1981). Effect of the nematodes *Acrobeloides* sp. and *Mesodiaplogaster iheritieri* on substrate utilization and nitrogen and phosphorous mineralization in soil. *Ecology* 62: 549-555.

Bargmann, C. I., Thomas, J. H., and Horvitz, H. R. (1990). Chemosensory cell function in the behavior and development of *Caenorhabditis elegans*. *Cold Spring Harbor Symp. Quant. Biol.* 55: 529-538.

Bargmann, C. I. (1993). Genetic and cellular analysis of behavior in *C. elegans*. *Annu. Rev. Neurosci.* 16: 47-71.

Bargmann, C. I., Hartwieg, E., and Horvitz, H. R. (1993). Odorant-selective genes and neurons mediate olfaction in *C. elegans*. *Cell* 74: 515-527

Bollerup, G., and Burr, A. H. (1979). Eyespot and other pigments in nematode esophageal muscle cells. *Can. J. Zool.* 57: 1057-1069.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* 77: 71-94.

Burr, A. H., and Burr, C. (1975). The amphid of the nematode *Oncholaimus vesicarius*: Ultrastructural evidence for a dual function as a chemoreceptor and photoreceptor. *J. Ultrastruct. Res.* 51: 1-15.

Burr, A. H. (1979). Analysis of phototaxis in nematodes using directional statistics. *J. Comp. Physiol.* 134: 85-93.

Burr, A. H. (1984). Photomovement behavior in simple invertebrates. In: Ali, M. A. (ed.). Photoreception and Vision in Invertebrates. Plenum Press, New York, p. 179-215.

Burr, A. H. (1985). The photomovement of *Caenorhabditis elegans*, a nematode which lacks ocelli. Proof that the response is to light not radiant heating. *Photochem. Photobiol.* 41: 577-582.

Burr, A. H. J., and Babinszki, C. P. F. (1990). Scanning motion, ocellar morphology and orientation mechanisms in the phototaxis of the nematode *Mermis nigrescens*. *J. Comp. Physiol. A.* 167: 257-268.

Burr, A. H. J., Babinszki, C. P. F., and Ward, A. J. (1990). Components of phototaxis of the nematode *Mermis nigrescens*. *J. Comp. Physiol. A.* 167: 245-255.

Chalfie, M., and Sulston, J. (1981). Developmental genetics of the mechanosensory neurons of *Caenorhabditis elegans*. *Dev. Biol.* 82: 358-370.

Coomans, A. and DeGrisse, A. (1981). Sensory structures. In: Plant Parasitic Nematodes, Vol III. B. M. Zuckerman and R.A. Rhode (eds.). Academic Press, New York, 127-174.

Croll, N. A. (1965). The klinokinetic behavior of infective *Trichonema* larvae in light. *Parasitol.* 56: 579-582.

Croll, N. A. (1966a). Activity and the orthokinetic response of larval *Trichonema* to light. *Parasitol.* 56: 307-312.

Croll, N. A. (1970). Sensory basis of activation in nematodes. *Exp. Parasitol.* 27: 350-356.

Croll, N. A. (1971). Movement patterns and photosensitivity of *Trichonema* spp. infective larvae in non-directional light. *Parasitol.* 62: 467-478.

Croll, N. A., Riding, I. L. and Smith, J. M. (1972). A nematode photoreceptor. *Comp. Biochem. Physiol.* 42A: 999-1009.

Croll, N. A. and Al-Hadthi, I. (1972). Sensory basis of activity in *Ancylostoma tubaeforme* infective larvae. *Parasitol.* 64: 279-291.

Croll, N. A. (1975). Components and patterns in the behavior of the nematode *Caenorhabditis elegans*. *J. Zool. (Lond.)* 176: 159-176.

Croll, N. A. (1976). When *Caenorhabditis elegans* (Nematoda: Rhabditidae) bumps into a bead. *Can. J. Zool.* 54: 566-570.

Dusenbery, D. B. (1974). Analysis of chemotaxis in the nematode *Caenorhabditis elegans* by countercurrent separation. *J. Exp. Zool.* 188: 41-47.

Dusenbery, D. B. (1975). The avoidance of D-tryptophan by the nematode *Caenorhabditis elegans*. *J. Exp. Zool.* 193: 413-418.

Dusenbery, D. B. (1980a). Behavior of free-living nematodes. In: Nematodes as Biological Models, Vol. 1. B. M. Zuckerman (ed.). Academic Press, New York, p. 127-158.

Dusenbery, D. B. (1980b). Responses of the nematode *Caenorhabditis elegans* to controlled chemical stimulation. *J. Comp. Physiol.* 136: 327-331.

Fraenkel, G. S. and Gunn, D. L. (1961). The orientation of animals. Clarendon Press, Oxford; Dover, New York.

Golden, J. W., and Riddle, D. L. (1984). The *Caenorhabditis elegans* dauer larvae: developmental effects of pheromone, food, and temperature. *Dev. Biol.* 102: 368-378.

Hader D. P. (1993). Real-time tracking of microorganisms. In: Hader D. P. (ed.). Image Analysis in Biology. CRC Press, USA, p. 289-313.

Hedgecock, E. M., and Russell, R. L. (1975). Normal and mutant thermotaxis in the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* 72: 4061-4065.

Hirsh, D., Oppenheim, D., and Klass, M. (1976). Development of the reproductive system of *Caenorhabditis elegans*. *Dev. Biol.* 49: 200-219.

Hodgkin, J. (1988). Sexual dimorphism and sex determination. In W. B. Wood (ed.), *The Nematode Caenorhabditis elegans*. Cold Spring Harbour Laboratory, Cold Spring Harbour, NY, p. 243-279.

Hyman, L. H. (1951). The Invertebrates: Acanthocephala, Aschelminthes, and Entoprocta. The Pseudocoelomate Bilatera, Vol. III. McGraw-Hill, New York.

Kaplan, J. M., and Horvitz, H. R. (1993). A dual mechanosensory and chemosensory neuron in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA*. 90: 2227-2231.

Mah, K. M., and Rankin, C. H. (1992). An analysis of behavioral plasticity in Male *Caenorhabditis elegans*. *Behav. Neuro. Biol.* 58: 211-221.

Mori, I., Ohshima, Y. (1995). Neural regulation of thermotaxis in *Caenorhabditis elegans*. *Nature* 376: 344.

Perkins, L. A., Hedgecock, E. M., Thomson, J. N., and Culotti, J. G. (1986). Mutant sensory cilia in the nematode *Caenorhabditis elegans*. *Dev. Biol.* 117: 456-487.

Rankin, C. H., and Chiba, C. M. (1988). Short and long term learning in the nematode *C. elegans*. *Soc. Neurosci. Abstr.* 14: 607.

Rankin, C. H., and Broster, B. S. (1990). Factors affecting habituation and recovery from habituation in *Caenorhabditis elegans*. *Soc. Neurosci. Abstr.* 26:626

Rankin, C. H., Beck, C. D. O., and Chiba, C. M. (1990). *Caenorhabditis elegans*: A new model system for learning and memory. *Behav. Brain. Res.* 37: 89-92.

Rose, A. M., and Baillie, D. L. (1979). The effect of temperature and parental age on recombination and nondisjunction in *Caenorhabditis elegans*. *Genetics*. 92:409-418.

Sulston, J. E., and Horvitz, H. R. (1977). Post-embryonic lineages of the nematode, *Caenorhabditis elegans*. *Dev. Biol.* 56: 111-156

Sulston, J. E., Schierenberg, E., White, J. G., and Thomson, J. N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* 100: 64-119.

Ward, S. (1973). Chemotaxis by the nematode *Caenorhabditis elegans*: Identification of attractants and analysis of the response by use of mutants. *Proc. Natl. Acad. Sci. USA* 70: 817-821.

Ward, S., Thomson, N., White, J. G. and Brenner, S. (1975). Electron microscopic reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. *J. Comp. Neurol.* 160: 313-338.

Ware, R. W., Clark, D., Crossland, K., and Russell, R. L. (1975). The nerve ring of the nematode *Caenorhabditis elegans*: Sensory input and motor output. *J. Comp. Neurol.* 162: 71-110.

White, J. G., Southgate, E., Thomson, E., and Brenner, S. (1986). The structure of the nervous system of *Caenorhabditis elegans*. *Phil. Trans. R. Soc. Lond. B.* 314: 1-340.

Wicks, S. R. and Rankin, C. H. (1995). Integration of mechanosensory stimuli in *Caenorhabditis elegans*. *J. Neurosci.* 15: 2434-2444.

Wood, W. B. (1988). The nematode *Caenorhabditis elegans*. Cold Spring Harbour Laboratory, Cold Spring Harbour, NY.

Yeates, G. W. (1981). Nematode populations in relation to soil environmental factors: A review. *Pedobiologia* 22: 312-338.