Grace Tang
Spring 2007

Isolation and Characterization of *Drosophila melanogaster* Central Processing Mutants

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The aim of this study is to isolate and characterize mutant fruit flies (*Drosophila melanogaster*) which are defective in central processing, with the ultimate goals of finding the genes necessary for this process to function, investigating what these genes do, and elucidating the mechanisms of central processing. Mutants that are deficient in central processing, or Type I mutants, are flies that are unable to process any form of sensory information that they receive from their environment, although their sensory receptors are intact. Twenty potential Type I mutants were found, but only one passed the Type I trait to the F1 generation. Further work is being done on this strain, M13, to map the mutant gene and characterize it, i.e. determine its response to other sensory modalities, measure its motility and geotaxis, etc. Assays for measuring responses to various sensory modalities, namely light, benzaldehyde and heat, have been developed, and responses of wild type flies in these assays were recorded. Future work in this study will include finding more Type I mutants, characterizing them, and mapping the genes involved in central processing, as well as development of further assays.

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COVER SHEET

TITLE: Isolation and Characterization of *Drosophila melanogaster* Central Processing Mutants

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Introduction

Organisms are constantly receiving external stimuli from the environment. In animals, the central processing pathway plays a crucial role in interpreting these stimuli in order to elicit an appropriate response. 'Central processing' involves a part of the brain that receives information from the environment and integrates this information to

generate the appropriate response to the stimulus. For example, if an organism comes across an attractant, sensory information coding for the attractant will be relayed to central processing, which will generate a signal instructing the muscles to move the organism towards the attractant.

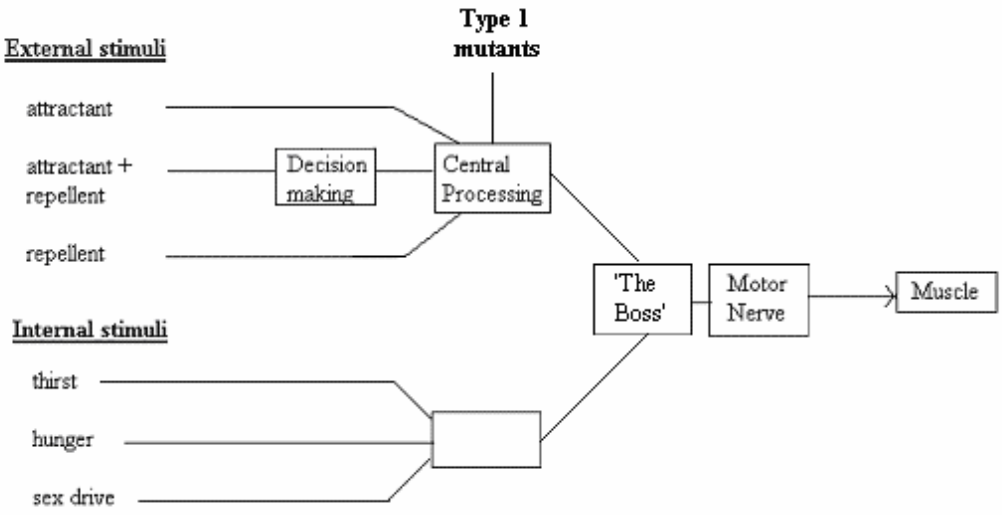

Figure 1: Central processing in Drosophila

Type I mutants are mutants that are defective in central processing, meaning that while their sensory organs are intact and they will be able to receive sensory information from the environment, they will not be able to process and integrate this information in order to perform an appropriate response to this information. The Adler laboratory also aims to isolate mutants in two other areas, namely 'Decision making' (Type II mutants) and 'The Boss' (Boss mutants). These are related to central processing but will not be covered in this report.

Mutants similar to Type I mutants have been isolated in the bacterium *Escherichia coli*. In a study by Armstrong, Adler and Dahl, 40 mutants of *E.coli* were isolated that were nonchemotactic, i.e. the mutants did not move towards attractant chemicals even though they possessed normal flagella and were motile (1967).

The idea of a mutation which inhibits sensory processing in higher organisms than bacteria may seem far fetched to some, but a genetic condition in humans involving multi-sensory impairment does exist (Searle et al. 2005). This condition, also known as CHARGE, results in problems in balance, coordination, taste, touch, smell, vision, hearing and proprioception (Sense 2007). This condition in which many senses are affected is highly similar to our definition of a Type I mutant.

Using known attractants and repellents, we are able to gauge a fly's ability to process a stimulus in each sensory modality by presenting it with an attractant or repellent of each modality. For example, light, an attractant (Hadler 1964), can be used to measure a fly's ability to detect and process visual information. Wild-type flies exhibit strong positive phototaxis; if a fly is presented with light but does not move towards it, this behavior may indicate that the fly is unable to process the visual stimulus. However, an equally plausible explanation of this would be that the fly has faulty visual receptors, while its brain is normal. Because of this, flies are tested in more than one sensory modality. Because it is less likely that detrimental mutations would occur in two or more sensory pathway genes, defects in multiple sensory pathways indicate that the mutation likely does not affect sensory receptors, but rather an area where sensory information converges, in this case, the area of the brain involved in central processing.

Several *Drosophila* attractants and repellants in various sensory modalities are well known. Light is a strong visual attractant (Hadler 1964), with nearly 100% attraction of wild-type flies, and was therefore selected to measure a fly's ability to process visual information. Benzaldehyde is a volatile chemical that acts as a strong olfactory repellent (Ayyub et al. 1990). Benzaldehyde is consistently avoided by flies even at low

concentrations, and was selected to measure olfactory processing. An added advantage of using a repellent is that flies do not have to be starved before the assay. This is in contrast to food attractants, which require prior starvation to be very effective.

In this study, all tests were carried out at 34ºC, because mutants in central processing are likely to be conditional mutants. If a central processing mutant was unable to function under normal conditions, it would be unlikely to mate and produce progeny, since its inability to process stimuli would hinder it from detecting mating calls, pheromones, visual presence of mates, etc. Therefore, assays were carried out at 34ºC to select for flies which displayed the Type I trait at higher temperatures, but behaved normally at room temperature. This way, temperature-sensitive mutants could be isolated which retained the ability to pass down the Type I mutation to future generations.

Currently, the Adler lab has one line of Type I mutants, and this is being mapped and characterized (see Results).

Materials and methods

Mutagenizing flies

Ethyl methane sulfonate (EMS) was used to induce random point mutations (an average of 1 out of every 4000 adenines was affected) in the sperm of male flies by placing them in food medium containing EMS for 24 hours. This mutagenesis was carried out by Robert Kreber in the Barry Ganetzky laboratory. These flies were then mated to virgin females, resulting in progeny (F1 generation) that each possessed mutated genes. Because parent females were not mutagenized, all F1 progeny received only one copy of the mutated genes, therefore mutations detected via the following tests must be dominant. From fall semester 2005 to spring semester 2007, 22 different mutagenizations

were carried out, each about two weeks apart. For each batch, about 150 males were treated with EMS by Robert Kreber, and these were then combined with an equal number of wild-type virgin females. Several thousand F1 flies (about 1500) were tested in each batch, so a total of about 33000 flies have been tested so far.

Type I assays

Canton S flies (*Drosophila melanogaster*) were used in Type I tests. Experiments were carried out with progeny of mutagenized male flies described above, when the progeny flies were about 3-10 days old. Temperature for all experiments for isolation and characterization of Type I mutants (except the heat gradient test and alternative benzaldehyde test) was kept roughly constant at 34ºC, and each setup (including flies) was incubated for 30 minutes at 34ºC before each experiment (excluding heat gradient test and alternative benzaldehyde test). For experiments where light was used as an attractant, the experiment itself and pre-test incubation were carried out in the dark.

Light box test: Separating flies that are responsive and unresponsive to light at 34ºC

To separate flies that were responsive to light from those that were unresponsive, the following setup was used:

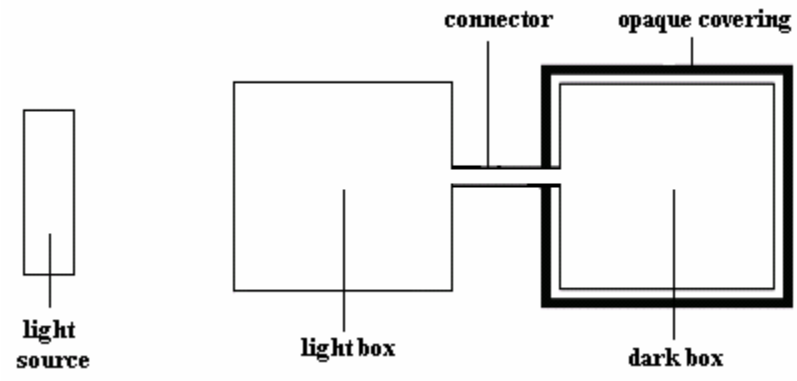

Figure 2: Light box test

Approximately 500 flies were placed in the dark box (8"x 8"x 8") which consisted of a transparent Lucite box surrounded by a cardboard box. After 30 minutes at 34ºC, at time = 0, the dark box was connected by a 34ºC transparent tube (12mm x 70mm) to another 34ºC transparent Lucite box (8"x 8"x 8") called the light box, allowing the flies to move freely between boxes. A fluorescent light source (General Electric Premium Cool White, 18 inch, 15 watt) was placed 6cm from the light box and was switched on after connecting the boxes. After 45 minutes, the two boxes were separated. The flies that crossed over to the light box were called $L^{+}_{box,}$ and it was assumed that these flies were attracted to light, while those that remained in the dark box, called L^{-}_{box}, were assumed to be unresponsive to light. If many flies remained in the dark box, the process was repeated a second time using only the L^{-}_{box} flies to see if any more flies cross over to the light box.

Light tube test: Confirming unresponsiveness to light at 34ºC

The following setup was used to confirm unresponsiveness to light:

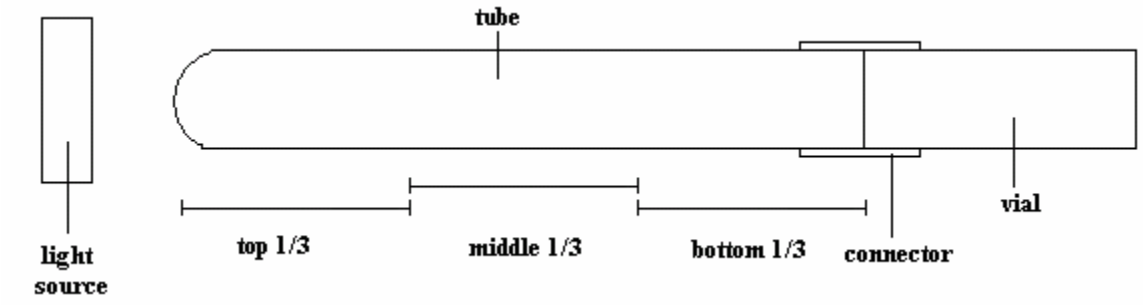

Figure 3: Light tube test

About 20 L^{-}_{box} flies were placed into the vial (95 x 25mm). After 30 minutes at 34ºC, at time=0, the vial was connected to the tube (200 x 25 mm) by means of a transparent plastic connector (tight fitting around the tube and vial, made from cutting Beckman 1 x 3.5 UC tubes into 2 cm lengths). A light source located 15cm away from

the tube was switched on and the number of flies in the top 1/3, middle 1/3, bottom 1/3 and vial were noted every 15 seconds for the first minute, and then every minute for the next four minutes or until it was clear which flies were attracted to light and which were not. Those that were not attracted to light were called L^-_{tube} flies, and were separated from flies in the same tube which were responsive to light, called L^+_{tube} flies. The L^-_{tube} flies were then tested for unresponsiveness to benzaldehyde.

To make it easier to isolate L^-_{tube} mutants from a large number of flies, a transparent funnel (made with overhead projector transparency, wider diameter 25mm, mouth diameter 5mm) was inserted at the mouth of the vial, and the setup was arranged as shown:

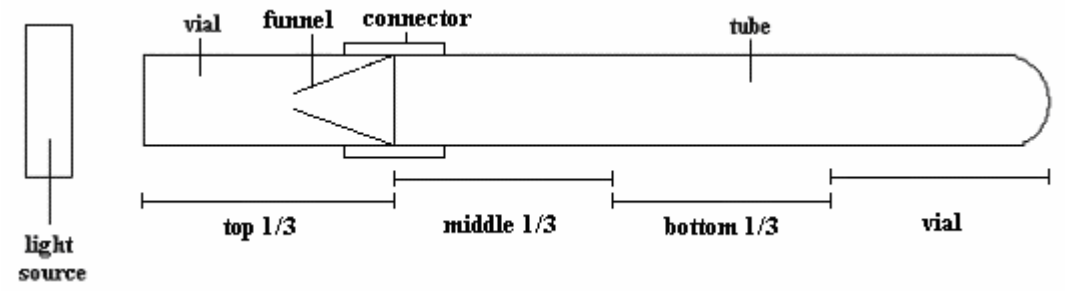

Figure 4: Light tube test with funnel

To make it easier to insert and remove the funnel, the setup was turned around (the vial was placed nearer the light source while the tube was further, see Figure 4) when the funnel was used in the light tube test. The names of the parts of the setup were kept in their original order for consistency (the vial was called the 'top 1/3', the end of the tube furthest from the light was called the 'vial', and so on). Flies that did not cross through the funnel into the vial within five minutes after the light was switched on were considered to have a decreased response to light compared to wild-type flies.

Benzaldehyde tube test: Isolating flies unresponsive to light and benzaldehyde at 34ºC

The following setup was used with the flies unresponsive to light to test for unresponsiveness to benzaldehyde:

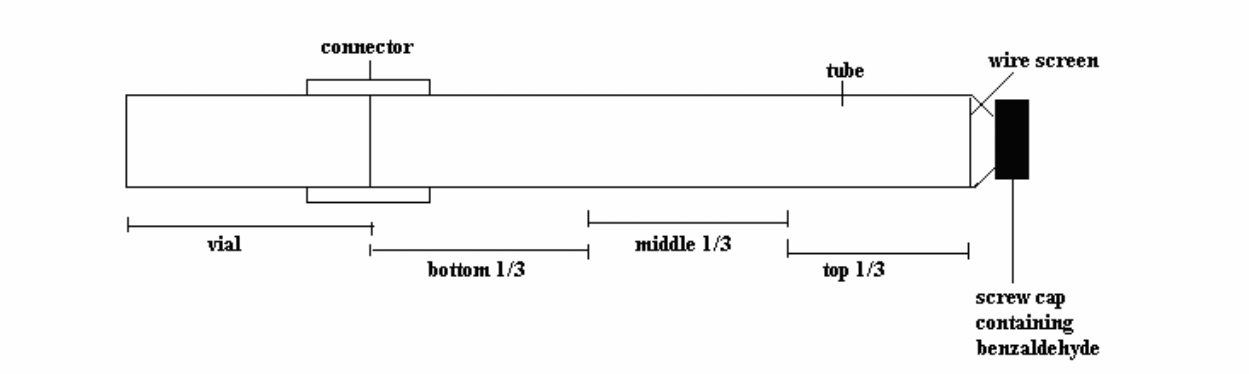

Figure 5: Benzaldehyde tube test

About 20 L^-_{tube} flies were placed in a vial (95 x 25mm). The vial, a tube (220 x 25mm) with a wire screen, and a screw cap containing 1ml of agarose with 10^{-1}M benzaldehyde (wrapped in Saran wrap to prevent evaporation) were incubated separately at 34ºC for 30 minutes. At time=0, the vial was connected to the tube by means of a transparent plastic connector, and the screw cap with 1ml of agarose with 10^{-1}M benzaldehyde was attached to the end of the tube with the wire screen. The wire screen was placed in front of the agarose to prevent flies from coming into contact with the benzaldehyde, which is toxic to flies. Flies were tapped down to the wire screen. The number of flies in the top 1/3, middle 1/3, bottom 1/3 and vial were noted every minute for 10 minutes. Flies that spent the majority of the 10 minutes in the vial were considered to be repelled by benzaldehyde. Flies that were not repelled by benzaldehyde (i.e. flies that spent more time in the top 1/3 and middle 1/3 of the tube compared to wild type) at this stage were considered possible Type I mutants unresponsive to both light and benzaldehyde, called L^-_{tube} B^-_{tube}, since the flies used here were the ones that were not attracted to light in the test immediately above.

Alternative benzaldehyde tube test at room temperature

An alternative benzaldehyde tube test was developed that did not require modification of commercially available equipment:

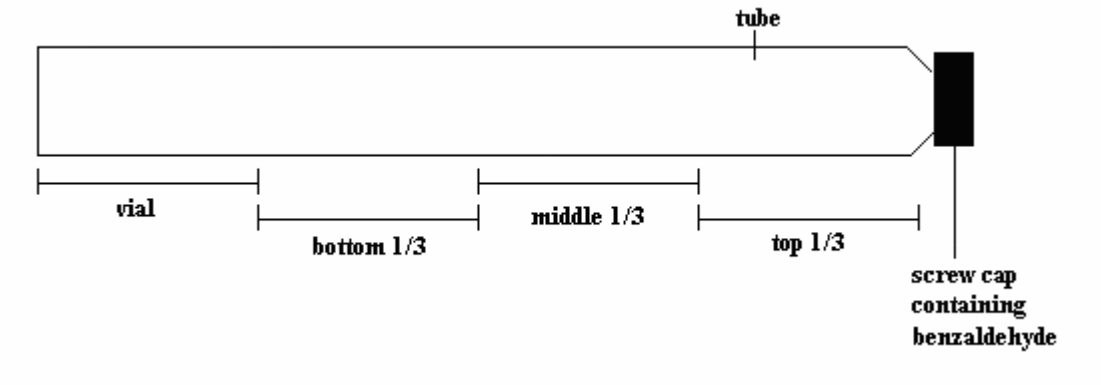

Figure 6: Alternative benzaldehyde tube test

This setup was not used in the isolation of mutants, but was developed to enable others without specialized equipment to more easily carry out a benzaldehyde assay. The same labels 'vial', 'top 1/3', 'middle 1/3' and 'bottom 1/3' as used in the previous benzaldehyde test were used to describe sections of this setup to make it easier to compare results of both versions of the benzaldehyde tube test.

About 20 L^{-}_{tube} flies were placed into the tube (200 x 25mm) and an empty screw cap was attached to the open end. This was incubated at room temperature for 30 minutes and flies were allowed to randomize. At time=0, the empty screw cap was replaced by a screw cap containing 1ml of agarose with 10^{-1}M benzaldehyde. The number of flies in the top 1/3, middle 1/3, bottom 1/3 and vial were noted every minute for 10 minutes. Flies that spent the majority of the 10 minutes in the vial end were considered to be repelled by benzaldehyde, B^{+}_{tube}. Flies that were not repelled by benzaldehyde (i.e. flies that spent more time in the top 1/3 and middle 1/3 of the tube compared to wild type) were considered unresponsive to benzaldehyde, B^{-}_{tube}.

Heat response test: Isolating flies unresponsive to a heat gradient

The following setup was used to measure responses to a temperature gradient:

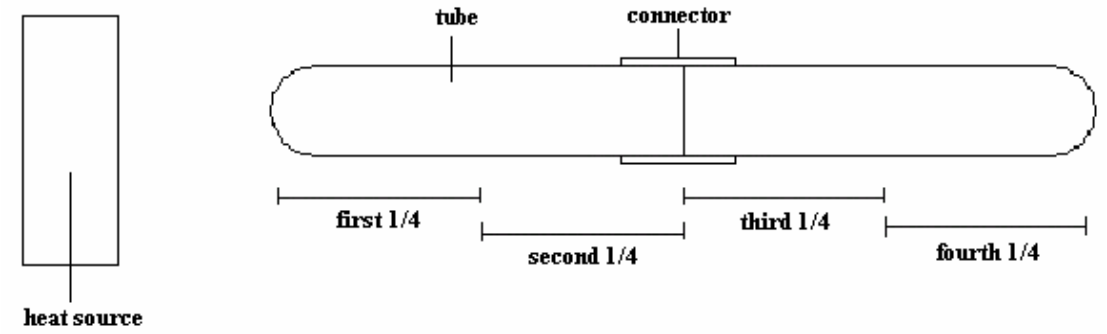

Figure 7: Heat response test

About 20 flies were placed in a tube (130 x 25 mm), and this was connected to an identical tube by a plastic connector. The tubes were placed 8cm from a heat source (Dakota Designs oil-filled radiator model 3LY45), oriented perpendicularly to the heat source. A light source was placed parallel to the tubes at a distance of 30cm, and the setup was incubated for 30 minutes such that at time=0, the temperature in the first ¼ and fourth ¼ were relatively stable (±0.1ºC). The temperature was about 36ºC in the first ¼ and about 33ºC in the fourth ¼. At time=0, the flies were tapped down to the first ¼ and the position of flies within the tube was then recorded every minute for ten minutes. The temperature was also monitored to ensure that it did not vary significantly from the initial measurements.

Starting the F2

Once a Type I mutant is found, it is mated by putting it together with 10 to 20 wild-type virgin flies of the opposite sex. The progeny of this cross is the F2 generation, which is tested with the above assays to determine if the mutant trait is passed down from the parent generation, i.e. is heritable. If the trait was passed down from the F1 to the F2,

it is expected that 50% of the flies in the F2 generation will possess the mutant trait, since it would be a dominant mutation, while the other half will be wild type.

Mapping

Mutants from the F2 are mated with flies possessing balancer chromosomes in order to determine which chromosome the gene is on. Robert Kreber is carrying this out.

Room temperature assays

The light tube test and benzaldehyde tube test carried out at 34ºC described above were also carried out at room temperature to determine the responses of wild-type flies at this lower temperature. The heat response test was also carried out at a lower temperature range of 22.4 ºC to 26.8 ºC.

Results

A. Assay results for wild type at 34ºC

The following are the results for the assays performed with wild-type Canton S flies at 34ºC (or at a higher temperature gradient of 33.1ºC to 36.3ºC for heat test):

Light tube test

The response of wild-type flies to a light gradient (see method in Figure 3) at 34ºC is as follows:

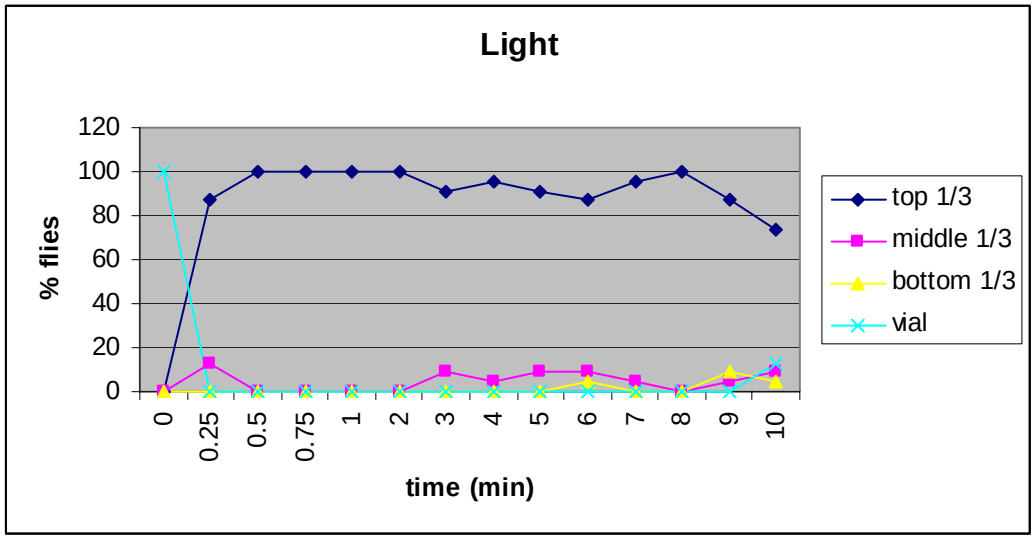

Figure 8: Results of light tube test at 34ºC

Wild-type flies are strongly attracted to light (Figure 8). Flies started in the vial end of the setup (furthest from light), and by 30 seconds (0.5 minutes in graph), all had moved to the top 1/3. However, after about 2 minutes, a small percentage of flies began moving back out of the top 1/3, presumably because of adaptation to the light. In many other experiments, this moving to the back was much more pronounced. Wild-type flies are therefore most reliably attracted to light within the first 2 minutes of the light tube test.

A comparison of the light tube test with a funnel (see method in Figure 4) and without a funnel at 34ºC follows:

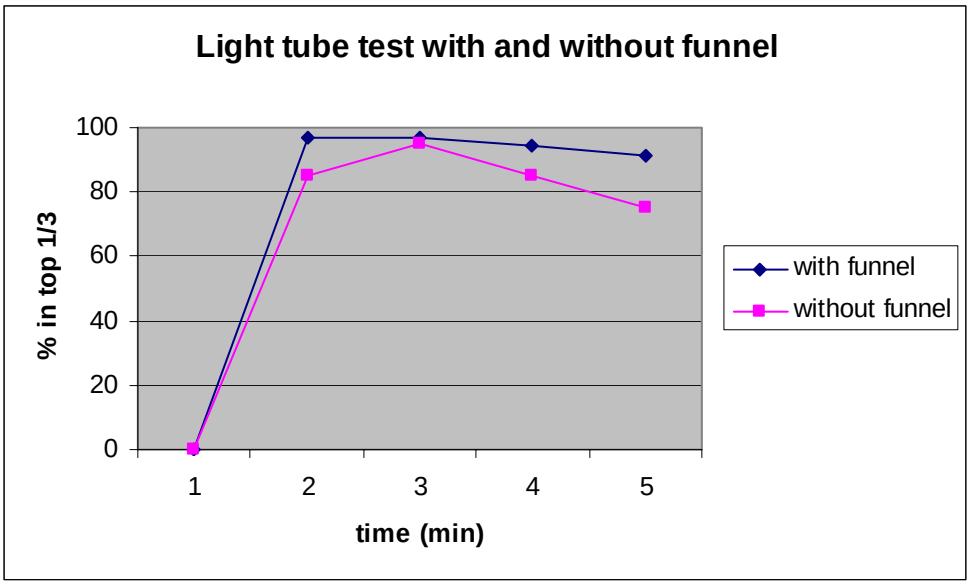

Figure 9: Comparison of light tube test results with and without funnel addition at 34ºC

Adding a funnel did not significantly improve or worsen the light tube test (Figure 9).

Benzaldehyde tube test at 34ºC

The response of wild-type flies to 0.1M benzaldehyde (see method in Figure 5) at 34ºC is as follows:

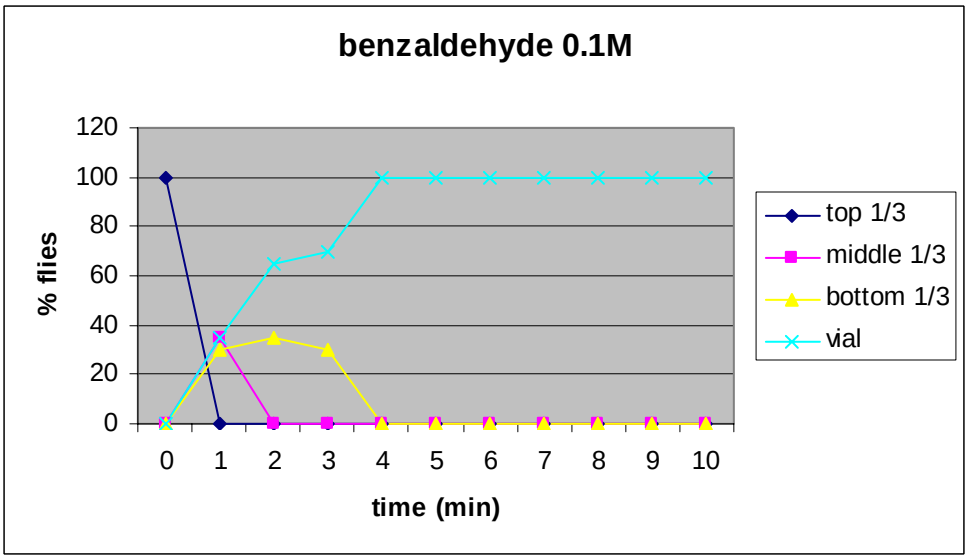

Figure 10: Results of benzaldehyde tube test for concentration of 0.1M at 34ºC

Wild-type flies are strongly repelled by 0.1M benzaldehyde (Figure 10). Flies are started in the top 1/3 (section nearest the benzaldehyde) of the setup, and by 4 minutes all had moved to the vial end of the setup.

The response of wild-type flies to 0.01M benzaldehyde is as follows:

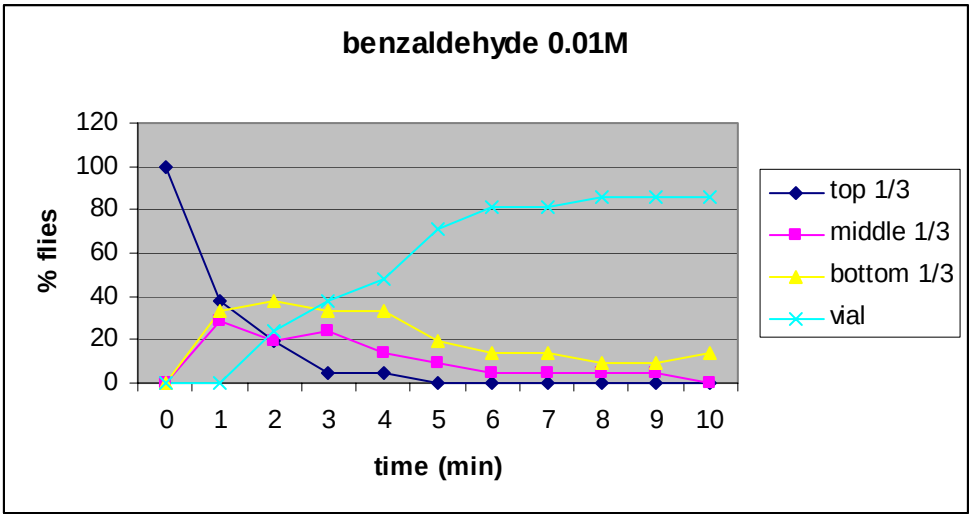

Figure 11: Results of benzaldehyde tube test for concentration of 0.01M at 34ºC

As in the 0.1M benzaldehyde test, flies are started in the top 1/3 (section nearest the benzaldehyde) of the setup (Figure 11). The majority of flies move to the vial end of the setup by 5 minutes. This shows that wild-type flies are repelled by 0.01M benzaldehyde, although not as strongly as by 0.1M benzaldehyde.

Heat response test

The response of wild-type flies to a heat gradient (see method in Figure 7) from 33.1ºC to 36.3ºC is as follows:

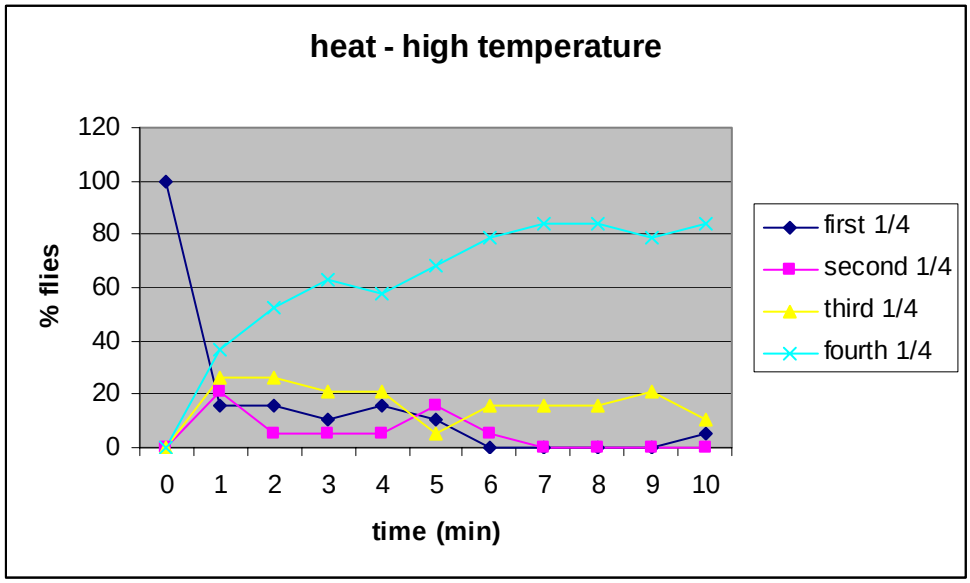

Figure 12: Results of heat gradient test for a gradient from 33.1ºC to 36.3ºC

Flies are started in the first ¼ (section nearest the heat) of the setup (Figure 12). The majority of flies move to the fourth ¼ by 6 minutes. Several of the flies that initially move to the cooler end periodically return to the first ¼, but once there, immediately turn back to the cooler end. Therefore, it will be easy to differentiate flies which are repelled by heat versus those that are not repelled by heat, by looking for flies that stay in the hot end for longer periods of time. Overall, this shows that wild-type flies are repelled by heat, although some periodically move to the hot end for short periods of time.

B. Assay results for wild type at room temperature

The following are the results for the assays performed with wild-type Canton S flies at room temperature (or at a lower temperature gradient of 22.4 ºC to 26.8 ºC for heat test):

Light tube test at room temperature

The response of wild-type flies to a light gradient (see method in Figure 3) at room temperature is as follows:

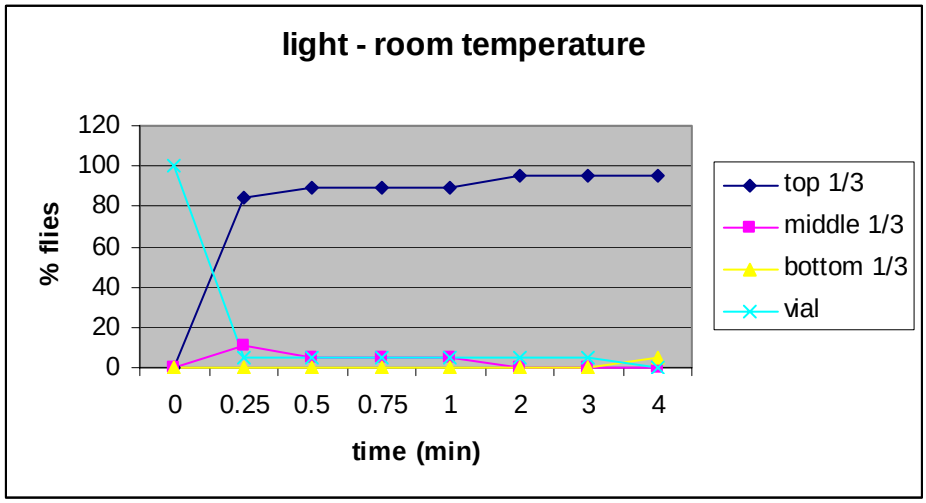

Figure 13: Results of light tube test at room temperature

As in the previous light tube test at 34°C (Figure 8), flies started in the vial end of the setup (furthest from light), and by 30 seconds (0.5 minutes in graph), about 90% of the flies had moved to the top 1/3. Unlike the light tube test at 34°C, there was not 100% attraction, but there was also less adaptation than at 34°C.

Benzaldehyde tube test at room temperature

The response of wild-type flies to 0.1M benzaldehyde (see method in Figure 5) at room temperature is as follows:

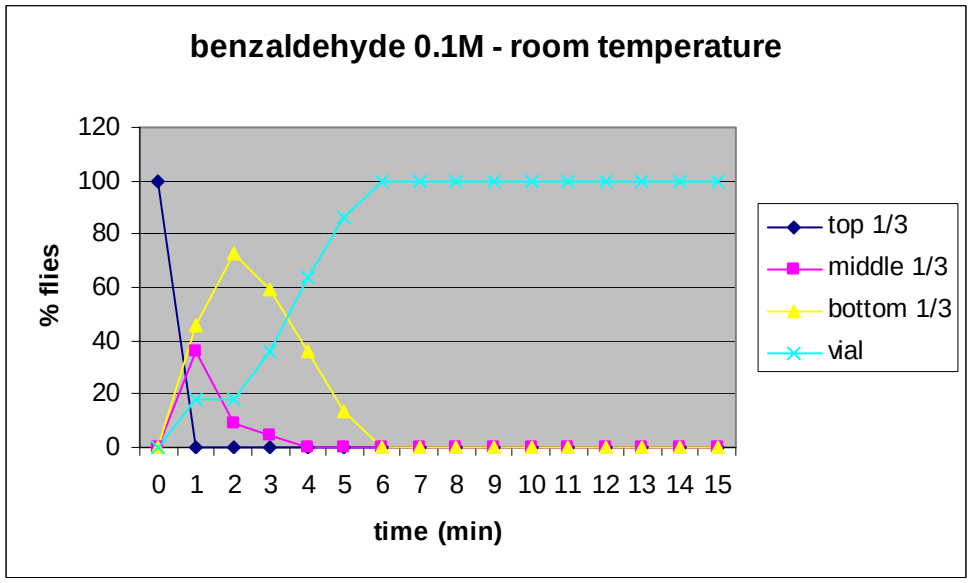

Figure 14: Results of benzaldehyde tube test for concentration of 0.1M at room temperature

As in the benzaldehyde tube test at 34ºC, wild-type flies are strongly repelled by 0.1M benzaldehyde at room temperature(Figure 13). Flies are started in the top 1/3 (section nearest the benzaldehyde) of the setup, and by 6 minutes all had moved to the vial end of the setup.

The response of wild-type flies to 0.1M benzaldehyde using the alternative method without a screen (see method in Figure 6) at room temperature is as follows:

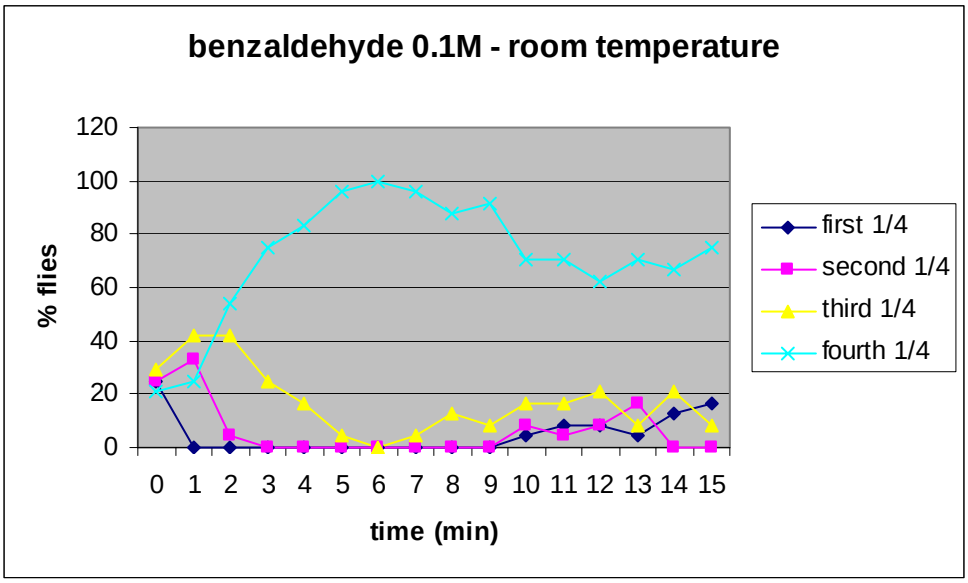

Figure 15: Results of benzaldehyde tube test for concentration of 0.1M at room temperature using alternative setup

At the beginning of the experiment, flies were distributed randomly in the setup (Figure 15). The majority of flies move to the vial end of the setup by 6 minutes. However, flies started to adapt quickly after this and moved back closer to the benzaldehyde (see Discussion). Overall, repulsion from benzaldehyde was still strong.

Heat response test near room temperature

The response of wild-type flies to a heat gradient from 22.4 ºC to 26.8 ºC is as follows:

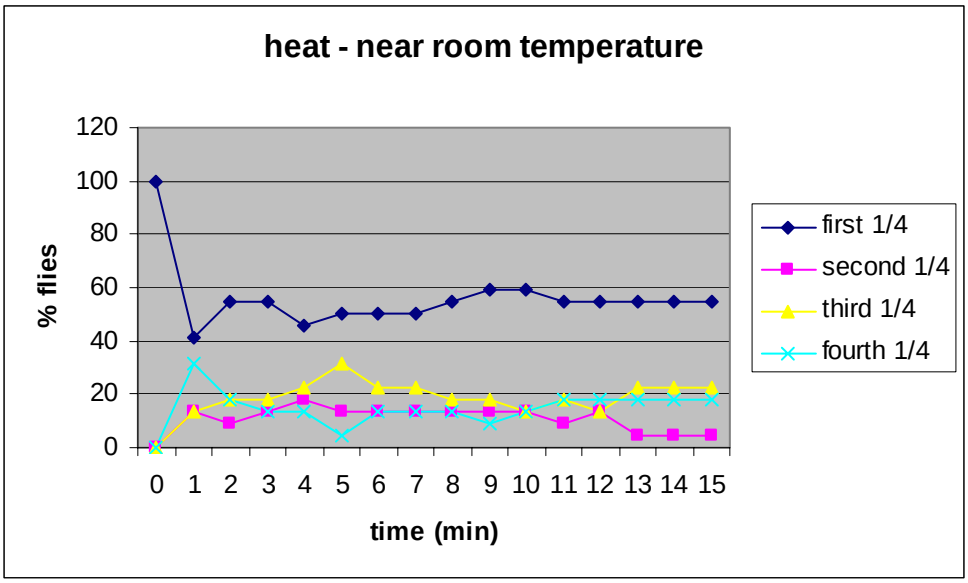

Figure 16: Results of heat gradient test for a gradient from 22.4 ºC to 26.8 ºC

As in the heat test carried out at a higher temperature (Figure 12), flies are started in the first ¼ (Figure 16). However, at a lower temperature, more than half the flies stayed in the hotter end of the setup. This shows that flies are not as repelled by heat at lower temperatures (see Discussion).

C. Type I mutants

Since fall semester 2005, about 33000 different mutants have been assayed for response to light and response to benzaldehyde, and 26 Type I mutants have been found. However, 25 of these 26 Type I mutants were found only to be lost due to unsuccessful mating, non-inheritable traits, loss of Type I trait in later generations, double mutations (mutations in more than one gene, such as a double mutation in vision and smell receptors) or others reasons. Currently there is one mutant strain, M13, for which the F1 and future generations are showing Type I traits (unresponsiveness to light and benzaldehyde, as well as humidity; assay for humidity not described here, see report by Sonya Bute). The following table summarizes these results:

Table 1. Type I mutants

Mutant Number	Mating of F1 with wild type	Date F2 adults start hatching	Results
M1	successful	8/14/06	50% were unresponsive to light: however, mutation was lost due to wild type contamination in F3
M2	unsuccessful	-	-
M3	successful	8/17/06	Type I trait did not appear in later generations
M4	successful	8/17/06	Parent had poor motility; Type I trait did not appear in F2
M5	successful	8/17/06	Parent had poor motility; Type I trait did not appear in F2
M6	successful	8/17/06	Parent had poor motility; Type I trait did not appear in F2
M7	unsuccessful	-	-
M8	unsuccessful	-	-
M9	unsuccessful	-	-
M10	successful	9/8/06	Type I trait did not appear in later generations
M11	successful	9/14/06	Type I trait did not appear in later generations
M12	successful	9/9/06	Type I trait did not appear in later generations
M13	Mating with wild type successful	9/14/06	F2 were Type I
M14	successful	9/21/06	F2 were L^- but B^+ (Not Type I)
M15	unsuccessful	-	-
M16	successful	11/29/06	Type I trait did not appear in later generations
M17	successful	11/29/06	Type I trait did not appear in later generations
M18	successful	11/30/06	Type I trait did not appear in later generations
M19	successful	3/28/07	Type I trait did not appear in later generations
M20	successful	3/28/07	Turned out to be Shaker mutation

Six more apparent Type I mutants were found from fall semester 2005 to spring semester 2006 that ultimately did not pass down the Type I trait to subsequent generations as well (data not shown).

Additionally, five more previously studied (1999) strains were tested for the Type I trait and were found to not be Type I mutants or at least not useful:

Table 2: Previously isolated mutants

Mutant strain	Results
11-58	Responsive to benzaldehyde; not Type I mutant
9-124	Responsive to benzaldehyde; not Type I mutant
251	Responsive to light; not Type I mutant
9-104	Responsive to light; not Type I mutant
12-65	Responsive to 0.1M benzaldehyde, partially responsive to 0.01M benzaldehyde and light; discarded due to weakness of Type I trait

M13

Further work on M13 was carried out by Lar Vang (2007). The following section contains his results:

F2 progeny of M13 were immediately sorted and separated into virgin female and male vials after eclosure; the flies were then assayed using the light tube and benzaldehyde tube tests to isolate the flies which carried the mutant gene. It was expected, at best, that 50% of the F2 offspring should carry the gene, but less was acceptable. It was noted that mutant F2 flies were appearing later in the batch of flies and in low numbers. Often, the mutant fly yield was very low (5% or less) with respect to the expected 50%; these results are not shown.

M13: F3 Progeny

F2 M13 flies which displayed the Type I trait were given to Robert Kreber to mate with balancer flies containing the genetic markers curly (Cy), scutoid (sco), and

tubby (Tb) to produce an F3 progeny. The dominant mutations Cy and sco are located on chromosomes 2 and the Tb mutation is located on chromosome 3. The goal of mating the flies of interest with flies carrying balancer chromosomes is to 1) create a homogenous stock of flies, each carrying the mutation of interest, 2) create a homogenous stock so mass transfer can be performed, negating the necessity of constantly outcrossing flies to produce the desired fly of interest, and 3) to prevent recombination in the flies to maintain the mutation of interest.

The F3 progeny was then assayed using the light tube and benzaldehyde tube tests to determine what proportion of the F3 flies containing genetic markers carried the mutation, in an attempt to create a balanced stock and to locate where the mutation was located. The results of the F3 tests are shown in Table 3. Flies showing the mutation of interest were given to Robert Kreber for further analyses.

Table 3: Results of the F3 progeny screening

No.	Vial	Light Tube Test	Benzaldehyde Tube Test	Conclusion
1	M13-2	0 of 2 L^-	--	Discarded
2	M13-3a	6 of 11 L^-	1 of 6 B^-	1 L-B-; gave to Robert Kreber
3	M13-3a	0 of 1 L^-	--	Discarded
4	M13-4	13 of 21 L^-	2 of 10 B^-	2 L-B-; gave to Robert Kreber
5	M13-4 V♀	1 of 2 L^-	0 of B^-	Discarded
6	M13-3 V♀	0 of 1 L^-	--	Discarded
7	M13-3	0 of 2 L^-	--	Discarded
8	M13-4 V♀	1 of 4 L^-	0 of 1 B^-	Discarded
9	M13-4	3 of 6 L^-	1 of 3 B^-	1 L^- B^-; gave to Robert Kreber
10	M13-3b V♀	1 of 1 L^-	1 of 1 B^-	1 L^- B^-; gave to Robert Kreber
11	M13-2 V♀	1 of 2 L^-	1 of 1 B^-	1 L^-B^-; gave to Robert Kreber

Only those that were L^- were assayed for benzaldehyde response in this and subsequent tables.

Of the 11 received vials, 5 of them seemed to carry the mutation of interest (No. 2, 4, 9, 10 and 11). These were subsequently given to Robert Kreber to further mate.

M13: F4 Progeny

Isolated F3 flies were given to Robert Kreber for the introduction of more balancer chromosomes via mating, producing an F4 progeny. These flies were then assayed using the light tube test, benzaldehyde tube test, and a humidity test, which was performed by Sonya Bute in the Fall semester of 2006; a separate report was written detailing the humidity test. It was found that three mutants, No. 2, 5 and 6 (all of the M13 line) failed to be attracted to light, repelled by benzaldehyde, or attracted to a humidity of approximately 70%, where as wild-type flies are attracted (see Table 4). These flies were subsequently given to Robert Kreber for further analyses.

Table 4: Results of the F4 progeny screening

No.	Vial	Light Tube Test	Benzaldehyde Tube Test	Humidity Test	Conclusion
1	"sco" M13-1 sco ♂	5 of 7 L^-	0 of 2 B^-	--	Discarded
2	sco+ M13-1 sco ♂	4 of 11 L^-	3 of 4 B^-	2 of 2 H^-	2 L^-B^- H^-; gave to Robert Kreber
3	Tb ♂	6 of 14 L^-	0 of 6 B^-	--	Discarded
4	Tb V♀	0 of 1 L^-	--	--	Discarded
5	Cy V♀	1 of 1 L^-	1 of 1 B^-	1 of 1 H^-	1 L^-B^- H^-; gave to Robert Kreber
6	Cy V♀	1 of 2 L^-	1 of 1 B^-	1 of 1 H^-	1 $L^-B^-H^-$; gave to Robert Kreber
7	Cy ♀ (M13-1 CyTb ♂)	0 of 3 L^-	--	--	Discarded

M13: F5 Progeny

Flies isolated from the F4 progeny were assayed using the light tube test and benzaldehyde tube test. Flies of vials No. 5 and 6 died, having been stuck to the food medium and flies of vial No. 1 were not assayed due to the vial having been broken.

Vials No. 2, 3, 4 and 7 did not display both the light and benzaldehyde mutation and were subsequently discarded (see Table 5 for results).

Table 5: Results of the F5 progeny screening

No.	Vial	Light Tube Test	Benzaldehyde Tube Test	Conclusion
1	M-13-4 V♀	Vial broke	--	Vial broke, discarded
2	M13-4 sco ♂	0 of 2 L^-	--	Discarded
3	M13-4 z/cy Tb^+♀	0 of 2 L^-	--	Discarded
4	M13-4 pairs 6♀	1 of 10 L^-	0 of 1 B^-	Discarded
5	M13 sco ♂	Died	--	Died, discarded
6	M13-4 Cy^+Tb^+ ♂	Died	--	Died, discarded
7	M13-4 pair Cy^+Tb^+ ♀ 3♀	2 of 4 L^-	0 of 2 B^-	Discarded

M13: New F1 Progeny

Since no flies of the F5 progeny were isolated, it was necessary to return to the original M13 stock of flies to restart the process. Using the light tube test and benzaldehyde tube test, several flies of vial No. 1 and 2 were isolated; 7 flies from No. 1 and 3 flies from No. 2 carried the mutation of interest (see Table 6 for results). These flies were subsequently given to Robert Kreber for further analyses.

Table 6: Results of a new F1 progeny screen

No.	Vial	Light Tube Test	Benzaldehyde Tube Test	Conclusion
1	♀	8 of 28 L^-	7 of 8 B^-	7 L^-B^-; gave to Robert Kreber
2	all siblings	13 of 39 L^-	3 of 13 B^-	3 L^-B^-; gave to Robert Kreber

M13: F2 Progeny

Viable progeny from the new F1 progeny were assayed accordingly (see Table 7 for results). It was found that 4 flies seemed to carry the mutation of interest and were

thus isolated for further analyses. These flies were mated with additional balancer flies to by Robert Kreber to produce an F3 progeny.

Table 7: Results of the F2 progeny screening

No.	Vial	Light Tube Test	Benzaldehyde Tube Test	Conclusion
1	Cy ♂	10 of 42 L^-	4 of 10 B^-	4 L^-B^-; gave to Robert Kreber

M13: F3 Progeny

Flies of the F2 progeny were able to mate and produce a viable F3 progeny and were assayed accordingly (see Table 8 for results). Of the received flies, vial No. 4 and 5 seemed to carry the mutation of interest, whereas the others did not. Flies of interest were thus isolated and further mated to produce an F4 progeny.

Table 8: Results of the F3 progeny screen

No.	Vial	Light Tube Test	Benzaldehyde Tube Test	Conclusion
1	#3 sm6 V♀	1 of 2 L^-	0 of 1 B^-	Discarded
2	#3 sm6 Cy ♂	2 of 2 L^-	0 of 2 B^-	Discarded
3	#1 sm6 ♂	0 of 3 L^-	--	Discarded
4	#1 sco ♂	1 of 4 L^-	1 of 1 B^-	1 L^-B^-; gave to Robert Kreber
5	#1 sm6 Cy V♀	1 of 3 L^-	1 of 1 B^-	1 L^-B^-; gave to Robert Kreber
6	#1 Cy/sm6 V♀	2 of 2 L^-	0 of 2 B^-	Discarded
7	sm6 Cy V♀	0 of 1 L^-	--	Discarded

Current M13 Progeny: F1

Of the F3 flies, none were able to produce viable progeny, either due to age or the nature of the mutation (data not shown). Since flies of the F3 progeny were not able to mate, it was therefore necessary to restart the process. Returning to the original M13 stock of flies, flies were assayed accordingly and it was found that the mutation of interest was still present (see Table 9). Flies from vial No. 1 and 2 were found to

apparently be non-phototatic, non-chemotactic and non-thermotactic. Past data from previous assays (see Table 4) has also demonstrated that M13 flies may be non-hygrotactic also. At present, it would seem that M13 flies may be unresponsive to light, benzaldehyde, 70% relative humidity and high temperature (~38° C).

Table 9: Results of the F1 progeny screen

No.	Vial	Light Tube Test	Benzaldehyde Tube Test	High Temperature Avoidance Test	Conclusion
1	bw	8 of 19 L^-	3 of 8 B^-	2 of 2 T^-	2 $L^-B^-T^-$; gave to Robert Kreber
2	+	3 of 18 L^-	2 of 3 B^-	1 of 1 T^-	1 $L^-B^-T^-$; gave to Robert Kreber

Discussion

Problems with the assays

One of the problems faced during the course of this study was the randomization of mutants. For example, in trying to isolate mutants that are unresponsive to benzaldehyde, mutants would not necessarily stay near the benzaldehyde, but might move away from it by chance. This makes them hard to separate from non-mutants as mutants and non-mutants would mix with each other. This also means it is possible that there were more mutants present that we failed to detect.

Another problem was the adaptation of non-mutants to the stimulus. After exposure to the stimuli (especially light) for a certain period of time, even flies that were initially responsive to the stimulus would adapt to it and stop displaying their responsiveness. This resulted in the non-mutant flies mixing with the mutants. Many retests using a smaller number of flies (which makes it easier to keep track of individual flies) and of a shorter duration are needed before mutants could be confidently isolated. Another factor that could have led to the non-mutants being mistaken for mutants were

the age of flies and the number of flies per trial: very young and very old flies do not exhibit phototaxis or repulsion from benzaldehyde as well as medium-aged flies, so young non-mutants could be mistaken for mutants. Yet another factor pertaining to the number of flies per trial is that if too many flies were used in a single setup, non-mutant flies may avoid very crowded areas of the setup. For example, the flies might avoid the light end of the tube if too many flies are gathered there due to the light. Also, it was often observed that flies were attracted to gaps where the tube or vial are joined by the connector, perhaps because the flies were attracted to fresh air. All of these factors could have resulted in a non-mutant fly appearing to be unresponsive to light, heat or benzaldehyde, when in fact it was staying away from light or staying near heat or benzaldehyde for reasons other than a mutation.

Multiple mutations

As stated above, a number of potential Type I mutants actually turned out to be flies with multiple mutations in various sensory pathways. For example, a double mutation in both visual receptors and smell receptors would result in a fly that would not respond to either light or benzaldehyde, giving the appearance of a Type I mutant, although it is not one. Up till recently, it was assumed that EMS created only a single point mutation in the entire genome. However, it is now known that EMS affects between 1/1000 to 1/10000 adenines in the genome (Kreber 2007). This explains the prevalence of flies with multiple mutations in our study.

Delayed hatching of mutants

As mentioned in the results for strain M13, it was often observed that mutant flies appeared later than their wild-type sibs (from a batch of eggs laid in one vial from one

mating pair). This could have been due to slower development of the mutant flies, leading to later pupation and eclosure. The low percentage of mutant flies may also have been due to the mutant flies being outcompeted by their wild-type sibs, perhaps in larval stage.

Modified light tube test

As mentioned above, in the classic light tube test, flies adapted to the light relatively quickly (by 1-2 minutes) after it was switched on. This made it hard to separate L^-_{tube} flies from L^+_{tube} flies as flies that were responsive to light would begin to randomize and mix with the flies that were not responsive to light. A funnel was therefore added to the light tube test in order to prevent flies that had been attracted to the light from returning to the darker part of the setup. It also lessened that chances that a mutant would move right to the light as it had to pass through a smaller hole. Although this method did not completely solve the problem of mutants randomizing through-out the setup and crossing through the funnel (see Figure 6), it did at least make it easier to separate flies that were attracted to the light from those that were not attracted at all. The funnel was placed as near to the light source as possible to minimize the number of light-unresponsive flies that crossed through it due to random movement.

Heat response test

In the heat response test developed this semester, wild-type flies were repelled from the hot end when this test was carried out at higher temperatures (see Figure 11). However, it was not as effective at lower temperatures (see Figure 12). The maximum temperature in this case was 26.8ºC, which is near room temperature, making it possible that flies are able to tolerate the entire range of temperatures in the setup when the test is carried out at a lower temperature.

Alternative benzaldehyde test

In the alternative benzaldehyde test, rapid adaptation was observed after a peak repulsion at 6 minutes (see Figure 15). This could have been due to the shorter length of the setup as compared to the classic benzaldehyde test setup, in which no adaptation was observed (see Figure 14). In this shorter setup, the benzaldehyde may have diffused and filled the entire tube, thus eliminating the benzaldehyde gradient, in a shorter amount of time. The establishment of a more uniform benzaldehyde concentration though out the setup can explain why flies adapted quickly in the alternative setup.

Isolation of mutants

To date, 26 potential Type I mutants have been tested for Type I trait. Of these, 25 were interesting but did not ultimately qualify and one is a Type I mutant (see Results). This one mutant, M13, has not reproduced well, and only a small percentage of progeny in future generations display the Type I trait. M13 could be a double mutant in light response (L^-) and benzaldehyde response (B^-), rather than a single mutation in central processing. The fact that not all L^- were B^- tends to support the idea that L^- and $B^-$are separate mutations, but the fact that the mutant is also insensitive to heat and humidity tends to support a single mutation in central processing. Another explanation for the low yield of progeny with the Type I trait could be that Type I flies are slower to develop relative to wild type. More work needs to be done to resolve this issue.

The flies that qualify as Type I based on failure to respond to light, benzaldehyde, heat, and humidity will then be tested with various other attractants and repellents such as quinine. These further assays test attraction and repulsion in other sensory modalities such as taste to confirm that the inherited defect is in central processing.

Future work

Future work on this will include further efforts to improve assays, isolating more Type I mutants, mapping the genes responsible for the abnormal phenotype, and investigating the role of these genes in central processing. If such genes are found, research on these genes can be extended beyond *Drosophila* to other organisms such as mice and humans. A clearer understanding of how central processing works, especially in humans, will no doubt have an impact on the fields of medicine and psychology.

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