Drosophila Mutants that Are Motile but Respond Poorly to All Stimuli Tested

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KEY WORDS: *Drosophila*, motility, behavior, attractants and repellents, sensory reception, processing of sensory stimuli, internal signals, behavioral response

ABSTRACT

Adult *Drosophila melanogaster* fruit flies were placed into one end of a tube near to repellents (benzaldehyde and heat) and away from the other end containing attractants (light and a favored temperature). They escaped from the repellents and went to the attractants. Five motile mutants that failed to do that were isolated. They did not respond to any external attractants tested or external repellents tested. In addition, they did not respond well to internal sensory stimuli like hunger, thirst, and sleep. The mutants failed at both 34°C and at room temperature. Some of the mutants have been mapped. It is proposed that the information from the different sensory receptors comes together at an intermediate, called "inbetween" (Inbet), that brings about a behavioral response.

I. INTRODUCTION

Organisms are constantly exposed to a variety of external attractants and external repellents as well as to a variety of internal sensory stimuli. How organisms respond to these to bring about behavior is a basic question of life.

One approach for discovering how this works is the isolation and study of mutants that fail here. In this report we show that *Drosophila* flies can be mutated in such a way that, although still motile, they no longer respond well to any sensory stimulus tested. This includes various external attractants and various external repellents as well as internal sensory functions like hunger, thirst, and sleep. An account of some of this work has appeared (Vang and Adler, 2016). A preliminary report of some of the results was presented (Adler, 2011; Vang et al., 2012).

It is proposed that information from all the different sensory receptors comes together at a newly found intermediate called "Inbetween" (the products of the *inbet* genes), which sends information to bring about a behavioral response. See Figure 1 and Discussion.

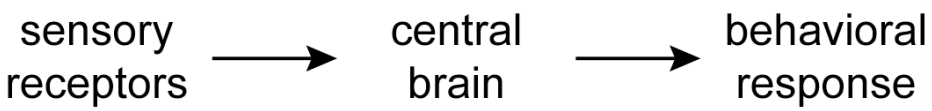

Figure 1. From sensing to response by way of the central brain.

Work by others has shown that in insects the central brain is a part of behaviors such as courtship (Pavlou and Goodwin, 2013), audition (Clemens et al., 2015), and vision (Weir and Dickinson, 2015). The central brain includes the central complex, which is a system of neuropils consisting of the protocerebral bridge, the fan-shaped body, the ellipsoid body, and noduli (Wolff et al., 2015).

II. RESULTS

A. RESPONSES TO EXTERNAL STIMULI

1. RESPONSE TO STIMULI USED TOGETHER

In a 34°C dark room flies were started near two repellents (0.1M benzaldehyde and 37°C) at one end of a tube, away from two attractants (light at 1000 lux and 27°C) at the other end (Figure 2). The parent responded by going away from the repellents and to

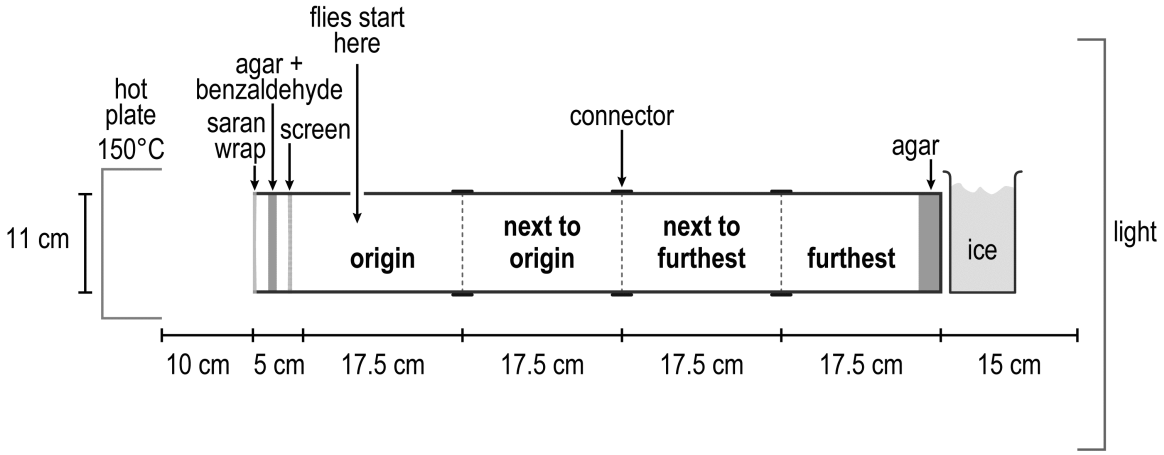

Figure 2. Apparatus for isolating and testing mutants in a 34°C room. At the left end were repulsive 0.1M benzaldehyde and repulsive 37°C (due to a hot plate at 150°C). At the right end were attractive light (1000 lux) and attractive 27°C (due to ice water). The middle was close to 34°C.

the attractants (Figure 3A). Mutants that were not motile were rejected, only the motile

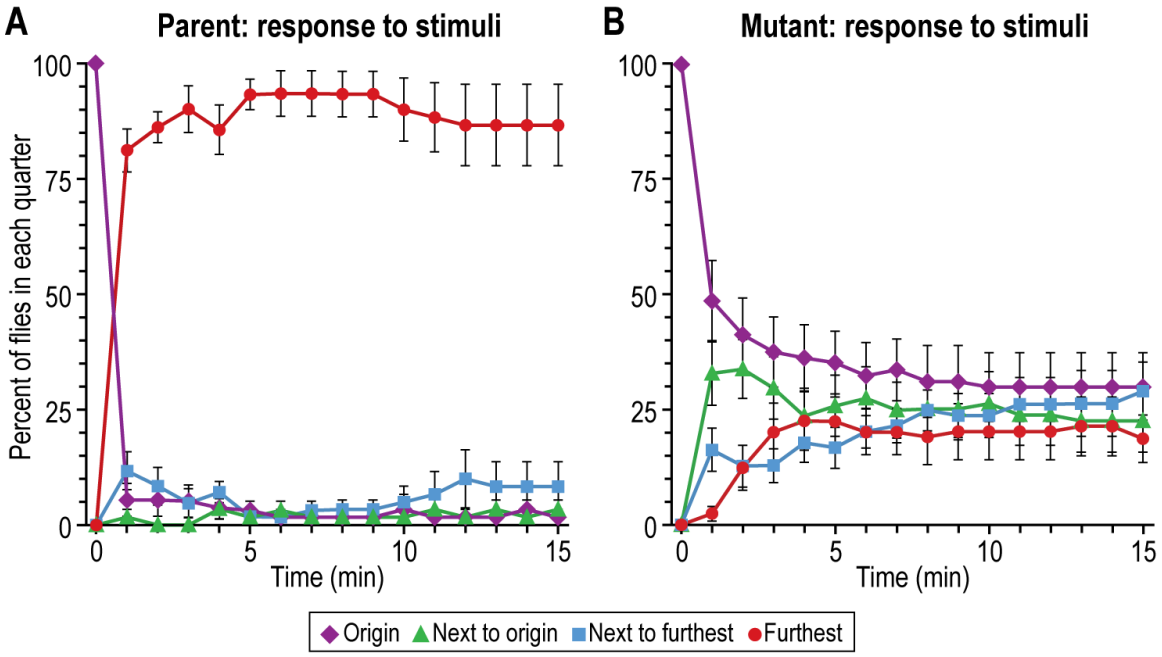

Figure 3. Response to stimuli used together. Repellents (0.1M benzaldehyde and high temperature (37°C) were at the left end, attractants (light, 1000 lux, and a favored temperature (27°C) at the right end. ***A***, Parental response (n=7). ***B***, Mutant 2 (n=8). Flies were tested in a 34°C room with 10 to 20 flies used per trial. Data are mean±SEM.

mutants were studied. This consisted of five mutants, named 1 to 5. Figure 3B shows that such a mutant failed to respond when the four stimuli were together. Each of the five mutants failed to respond to the four stimuli together (Vang and Adler, 2016).

2. RESPONSE TO INDIVIDUAL STIMULI

A single stimulus was presented to flies that were derived from ones that had already experienced the four stimuli used together. For example, the parent went to light only (Figure 4A) while a mutant did not (Figure 4B). Each of the five mutants failed to

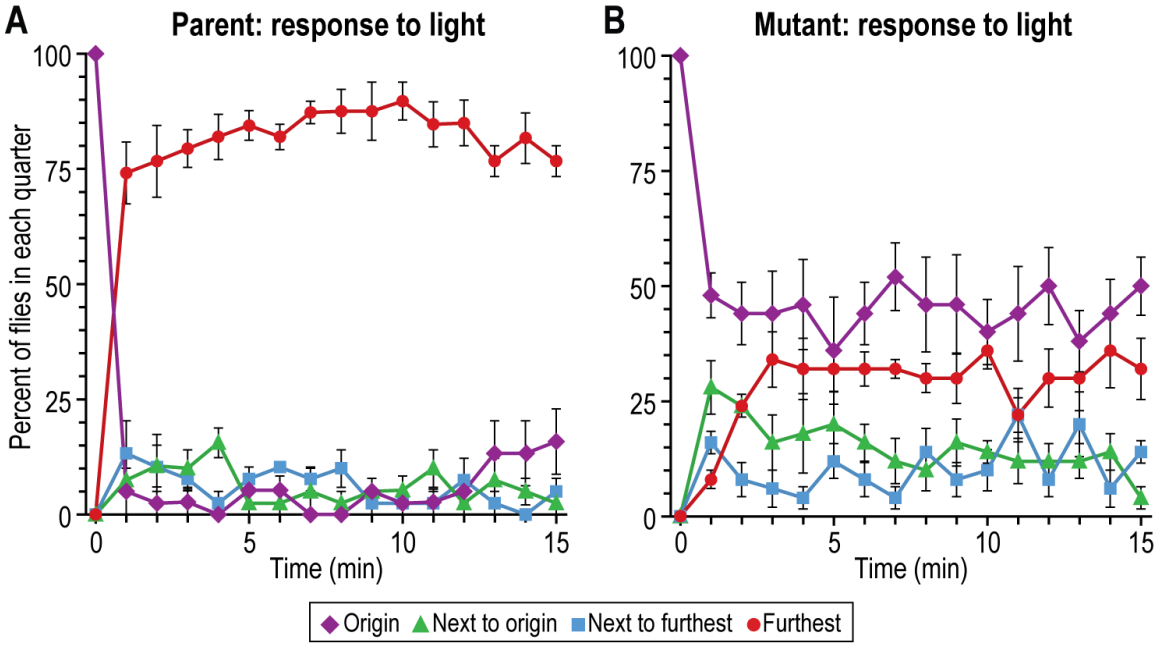

Figure 4. Response to light alone. Light (1000 lux) was placed at the right end as in Fig. 2. ***A***, Parental response (n=4). ***B***, Mutant 1 response (n=5). Flies were tested at 34°C with 10 to 20 flies used per trial. Data are mean±SEM.

respond to light only (Vang and Adler, 2016).

For heat alone, the parent was repelled (Figure 5A) but the mutant was not repelled (Figure 5B). That was the case for Mutants 1 and 2 (Vang and Adler, 2016). (The other mutants were not tested for this.)

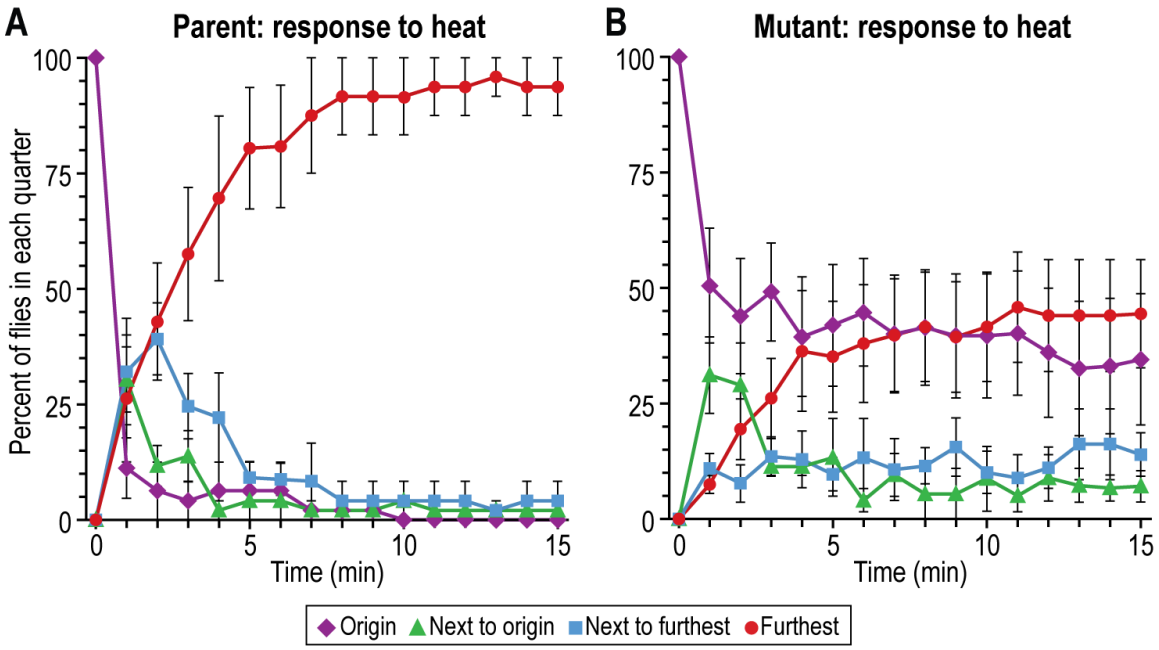

Figure 5. Response to heat gradient alone. The heat source was placed at the left end as in Figure 2. ***A***, Parental response (n=4). ***B***, Mutant 1 response (n=5). Flies were tested at 34°C with 10 to 20 flies per trial. The warm side measured 37°C and the cool side 27°C. Data are mean±SEM.

A similar result was found for benzaldehyde alone: the parent was repelled by benzaldehyde while Mutants 1 and 2 were not repelled. See (Vang and Adler, 2016) for the figures. (The other mutants were not tested for this.)

Thus the mutants were defective not only for the four stimuli used together but also for each stimulus used alone.

3. RESPONSE TO OTHER EXTERNAL STIMULI

These mutants were in addition tested with stimuli that were not among those four used to obtain the mutants:

The mutants were tested for response to the attractant sucrose after starvation (Edgecomb et al., 1994) for 17 to 20 hours. Compared to the wild-type, both Mutants 1 and 2 consumed less sucrose, about 20% as much as the wild-type after subtraction of movement without any added stimuli. See (Vang and Adler, 2016) for the figures. (The other mutants were not tested for this.)

In the case of the repellent quinine, flies were started in a 0.1M quinine half and then they had the opportunity to go into a non-quinine half (see Vang et al., 2012, for details of the method). The parent went into the non-quinine half but Mutant 1 and Mutant 2 did not. See (Vang and Adler, 2016) for the figures. (The other mutants were not tested for this.)

To test response to gravity, these flies were placed into a vertical tube and pounded down, then at every minute the flies in each third of the tube were counted (see Vang et al., 2012, for details of the method). The parent responded by climbing up while Mutants 1 and 2 climbed up 10% as well after subtraction of movement without any added stimuli. See (Vang and Adler, 2016) for the figures. (The other mutants were not tested for this.)

Thus these mutants, isolated by use of the four stimuli, were defective even for stimuli that were not present during their isolation.

4. MOVEMENT WITHOUT ANY ADDED STIMULI

In the absence of any stimulus added by the experimenters, the parent (Figure 6A) and the mutant (Figure 6B) moved similarly, indicating that motility alone is about the

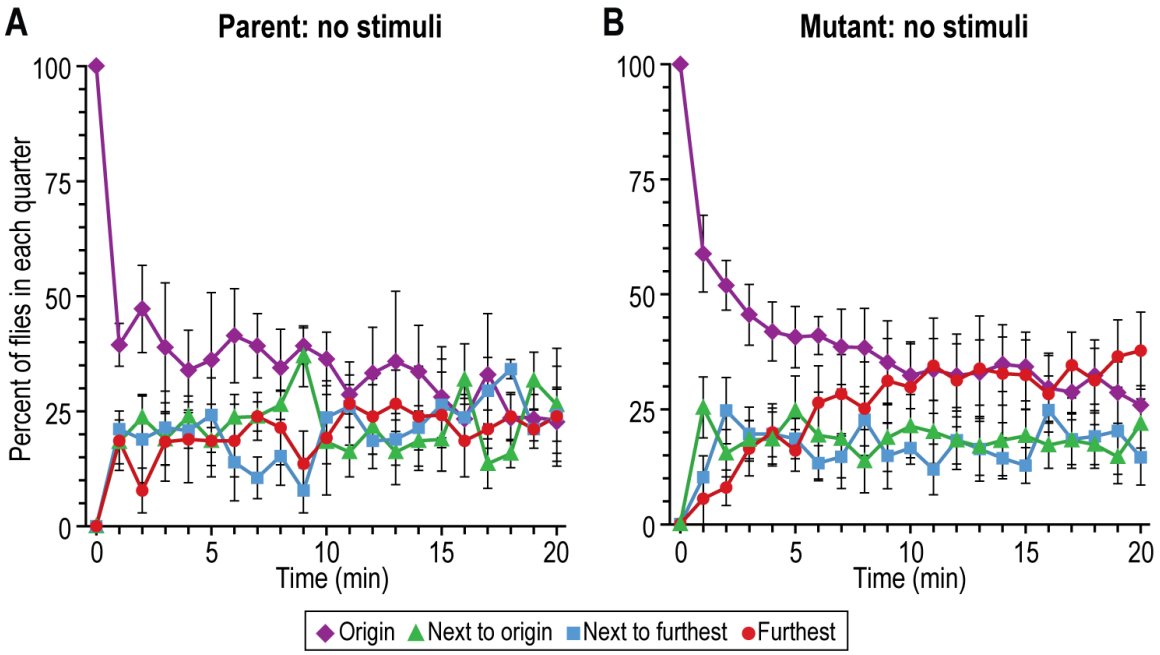

Figure 6. Response without added stimuli**.** ***A***, Parental response (n=4). ***B***, Mutant 1 response (n=6). Flies were tested at 34°C with 10 to 20 flies used per trial. Data are mean±SEM.

same in parent and mutant. This was found also for Mutants 2, 3, 4 and 5 (Vang and Adler, 2016). Aside from our seeing the flies, these results tell that the mutants are motile.

5. EFFECT OF INCUBATION TEMPERATURE

All the work reported above was carried out in a 34°C room in order to allow, if necessary, isolation and study of conditional mutants, i.e. mutants defective at 34°C but not defective at room temperature. We measured response to light (1000 lux) at room temperature (21 to 23°C). The parent responded to light but all five of the mutants failed to respond to light or responded only 10% as well as the parent, just as they did at 34°C (Vang and Adler, 2016). Thus the mutations are not conditional.

Presumably these mutants are defective to all stimuli at room temperature, not just to light. Figures below show defects at room temperature for hunger, thirst, and sleep. Then how could the mutants survive and grow at room temperature? It must be that the mechanism studied here is not an essential one: flies live and reproduce without it.

B. RESPONSES TO INTERNAL STIMULI

1. HUNGER

Here we focus on hunger (Edgecomb et al., 1994; Melche et al., 2007; Fujikawa et al., 2009; Farhadian et al., 2012; Hong et al., 2012; Itskov and Ribeiro, 2012). To

measure hunger we used an apparatus (Figure 7), inspired by and modified from an earlier design (Browne et al., 1960), that we described (Vang and Adler, 2016).

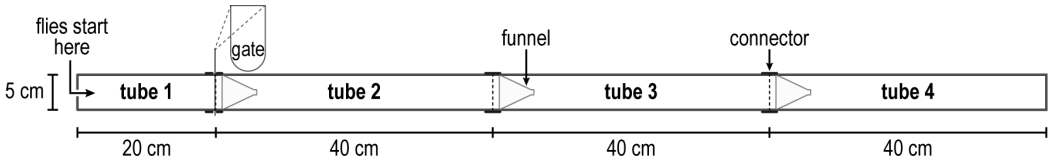

Figure 7. Apparatus for measuring hunger and for measuring thirst. For details see (Vang and Adler, 2016). Tube 1 is called "origin". Flies were tested at room temperature (21-23°C) for up to 40 hours.

Briefly, in a dark room at 21-23°C male flies – parent or mutants - were transferred into one end (tube 1) of a 5 x 140 cm apparatus containing throughout its length a 5 cm wide strip of wet paper to satisfy thirst but containing no food. Starvation for food began once the flies were put in. Every 10 hours the location of the flies was measured with light on for a few seconds.

At 20 hours the parent had largely left the origin (tube 1) and had begun to accumulate at the end (tube 4) (Figure 8A, solid bars), while the mutant had moved towards

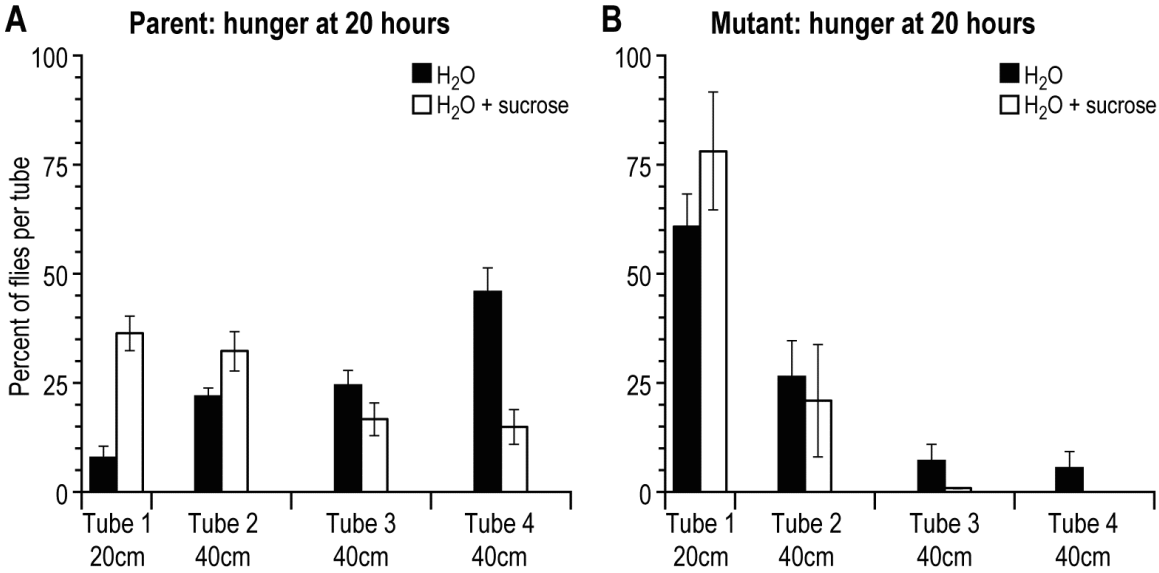

Figure 8. Movement of flies at 20 hours in search for food. Solid: water but no food (no sucrose). Open: water and food (0.1M sucrose). ***A***, Parental response with water only (n=5) and with water + sucrose (n=9). ***B***, Mutant 2 response with water only (n=5) and with water + sucrose (n=4). Data are mean±SEM. See (Vang and Adler, 2016) for Mutant 1; the other mutants were not tested for this. Flies were tested at room temperature (21-23°C) with 40 to 60 flies used per trial.

the end very little (Figure 8B, solid bars). This is interpreted to mean that the parent is searching for food while the mutant is defective in searching for food.

When food (0.1M sucrose) was added throughout the tube along with the wet strip of paper, the parent moved less far (rather than accumulating at the end) (Figure 8A open bars), while the mutant remained mostly where placed (Figure 8B, open bars). Since sucrose inhibited the movement of the parent, it is supposed that movement without sucrose is due largely to hunger. From these results we conclude that the mutants are defective in hunger.

2. THIRST

To study thirst, flies were deprived of water. The procedure is the same as for hunger except that water was omitted and solid sucrose was layered throughout (Vang and Adler, 2016). Mutants 1 and 2 were tested, the other mutants not (Vang and Adler, 2016).

By 30 hours the parent had moved out, presumably to search for water since addition of water inhibited this (Vang and Adler, 2016). The mutant moved out less well than the parent (Vang and Adler, 2016), so we conclude that the mutants are defective in thirst.

3. SLEEP-WAKE

The parent and mutants isolated here were studied for sleep and wake according to the procedure of Pfeiffenberger et al. (Pfeiffenberger et al., 2010). The parent was different from the mutants (Figure 9). The parent showed greatest activity at the start and

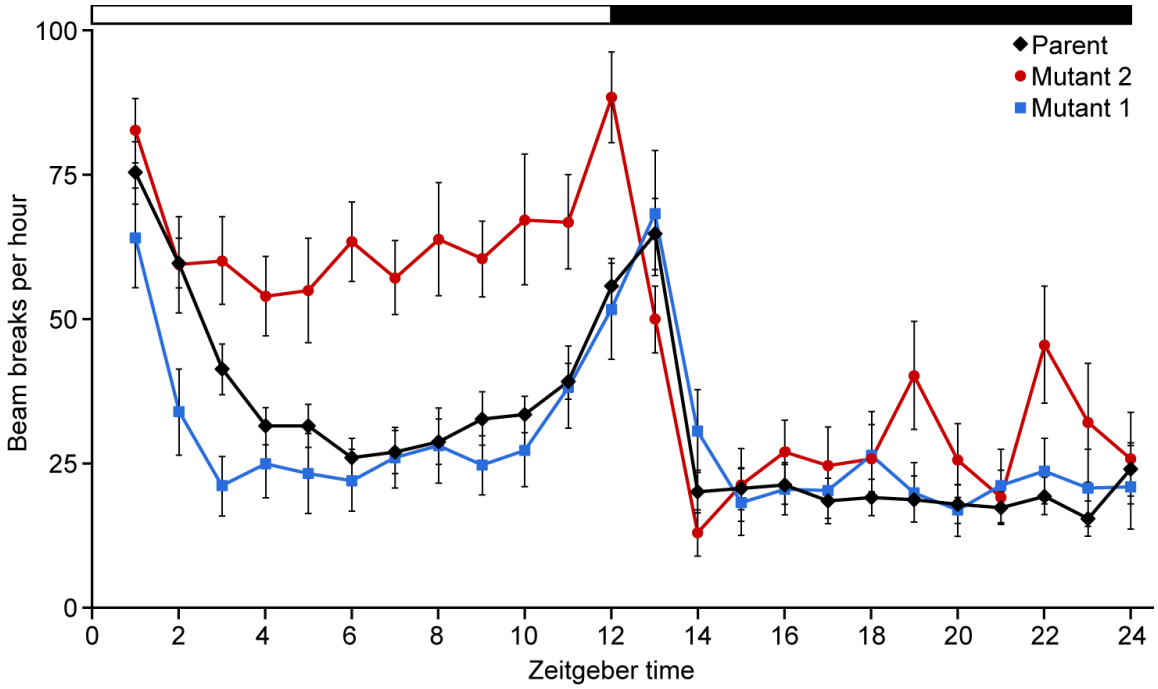

Figure 9. Circadian response. Individual flies are placed into a tube (5 x 20 mm) with an infrared light beam intersecting at the middle of the tube. Mutant 1 (n=24), Mutant 2

(n=24), and parental response (n=24) are recorded over a 24 hour period at 22° C. Data are mean±SEM. There are smaller differences between the parent and the three other mutants (Vang and Adler, 2016).

end of the day but not in the middle of the day. Mutant 2 showed high activity throughout the day. Mutant 1 was less active than the parent at the start of the day.

C. MAPPING OF THE MUTANTS

We found that Mutant 1 maps in a small gap between 12E3 and 12E5 on the X-chromosome (Vang and Adler, 2016) which we call "*inbetween A"* (*inbetA)*. We found that Mutant 2 maps in the *CG1791* gene, a part of the fibrinogen gene of the X-chromosome, or next to it (Vang and Adler, 2016), which we call "*inbetween B*" (*inbetB)*.

III. DISCUSSION

Here we have described the isolation and some properties of *Drosophila* mutants that are motile but yet they each fail in response to all external attractants and repellents tested (Figures 3-5) and also they are deficient in response to internal stimuli tested (Figures 8 and 9). Thus, although the mutants are motile, they have:

decreased responsiveness to light
decreased responsiveness to heat and to favorable temperature
decreased responsiveness to repulsive chemicals (like benzaldehyde)
decreased responsiveness to sweet tastants (like sucrose)
decreased responsiveness to bitter tastants (like quinine)
decreased responsiveness to gravity
decreased responsiveness to hunger
decreased responsiveness to thirst
abnormality in some sleep

Because all of these different behaviors are defective, it seems reasonable to say that there is a single place that is responsible, rather than a defect in each of the many different sensory receptors. One possibility for this place is the interaction between sensory receptors and processing of sensory stimuli (Vang and Adler 2016). Another possibility is that this place is in the central brain (Figure 1) between sensing and response , where all the sensory information comes together at a newly discovered place we call "Inbetween", the proteins of the *inbetA* and *inbetB* genes.

The mechanism and function of these Inbetween proteins need to be determined further.

An analogy can be made between *Drosophila* missing the part of the central brain studied here and *Escherichia coli* mutants missing their chemotaxis mechanism (Armstrong, Adler, and Dahl 1967, Parkinson 1976). As stated above (see 5. EFECT OF

INCUBATION TEMPERATURE), the process studied in this report is not an essential one: the flies live and reproduce without it. Similarly, *E. coli* mutants missing their chemotaxis mechanism are motile and live and reproduce without it. So these are parts of an organism that are useful but not essential.

A further analogy can be made with humans. Brenda Milner studied a patient who had amnesia as a result of a bilateral ablation of parts of the hippocampus; after this operation the patient failed to remember all stimuli but was otherwise "normal" (Scoville and Milner 1957). The patient lived from 1926 to 2008 and his operation was in 1953. The patient's brain after death was examined by histological sectioning to identify the affected parts in the medial temporal lobes and to a small degree in the orbitofrontal cortex (Annese et al. 2014). See Adler, 2016.

It would appear that all three organisms – bacteria, flies, and people – may have a similar mechanism for relating sensory stimuli to behavioral responses.

In each case, when the behavior was removed the organism was still alive: behavior is not essential to life, though of course the defective organism is severely handicapped.

IV. METHODS

Details of methods used here are found in in the previous paper (Vang and Adler, 2016): A. Isolation of mutants. B. How to study response to external stimuli. C. How to study response to internal stimuli.

ACKNOWLEDGEMENTS

We are grateful to the Camille and Henry Dreyfus Foundation for six years of grants in support of 31 undergraduate research students named in Vang and Adler, 2016. Lar Vang is currently associate research specialist in the Adler laboratory. Robert A. Kreber, a research specialist in Barry Ganetzky's laboratory, has helped us in studies of the genetics of our mutants. We are grateful to Erin Gonzales and Jerry Yin for showing us how to use the *Drosophila* activity monitory system. Julius Adler thanks Barry Ganetzky for teaching him about fruit flies. Thanks to Millard Susman for criticism. We are thankful to Laura Vanderploeg for the art work.

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