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BEHAVIOURAL AND PHYSIOLOGICAL RESPONSES OF SIX-DAY-OLD FEMALE *SARCOPHAGA BULLATA* TO SOLUTIONS OF LIVER EXTRACT AND SUCROSE

Douglas James Simms

A Thesis

in

The Department

of

Biology

Presented in Partial Fulfillment of the Requirements
for the Degree of Master of Science at
Concordia University
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ABSTRACT

Behavioural and physiological responses of six-day-old female *Sarcophaga bullata* to solutions of liver extract and sucrose

Douglas James Simms

In order to examine and compare the response of mated and unmated females of the fleshfly, *Sarcophaga bullata*, to two different types of meal, protein and carbohydrate, dose-response curves were constructed, in which flies were presented with varying concentrations of sucrose and liver extract solutions, and their average consumption over six hours was recorded. The data from these curves were used to determine K_b values for both mated and unmated flies, for both types of food. The K_b values represent the concentrations at which half-maximal consumption was elicited, and were used as a standard at which to compare the responses of the flies. When tested in two-choice behavioural bioassays, 6-day-old females consistently showed a preference for liver extract over sucrose. The equilibrium concentration was also determined from these tests, and represents the concentration of liver extract solution at which the fly consumes equal amounts of the liver extract and of sucrose at its K_b. Electrophysiological analysis suggests that the sensitivity of Cell 1 in the labellar chemosensilla increases in mated flies, supporting the results of an earlier study. In addition, the sensitivity of Cell 2 decreases in mated flies. These changes in sensitivity are likely to function at the level of the peripheral chemoreceptors while being controlled by an unknown central process.

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Finally, I would like to thank the students who worked on graduate degrees in Dr. Albert's lab at the same time as me. Neil Banga is a nice person and a good camper, and Donna Toufexis is a trusted adviser who is very dear to me, despite the fact that she has nagged me incessantly all summer, making me miserable.

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INTRODUCTION

The fleshfly, *Sarcophaga bullata*, has a life cycle that involves two very different types of food source. The larvae feed on the meat of whichever dead animal their mother found on which to deposit them. The adult fly feeds preferentially on the nectar of the yarrow plant, *Achillea lanulosa*. This nectar is made up of carbohydrates and some amino acids (Rathman *et al.*, 1990). The fly also supplements its diet on any meat source that may become available. In the case of the male fly, this meat source is used as a convenient supply of additional nutrition, but it could survive and reproduce without it. The female, on the other hand, must find a blood meal or other suitable protein source, otherwise reproduction is impossible (Dethier, 1976). It needs the protein both as a supply of raw materials for the synthesis of yolk and to trigger the secretion of hormones that are required for egg production. Dethier (1976) refers to this need for protein as a "specific hunger", due to its restriction to a particular stage in the life cycle of the animal, and its relation to a metabolic deficit. Yarrow nectar has an assortment of amino acids that are known to stimulate the receptors of neurons that are located in the chemosensilla of the fleshfly (Rathman *et al.*, 1990). Flies deprived of protein preferred a solution of sucrose containing these amino acids to a solution of sucrose alone. Rathman *et al.* (1990) found that both sexes ate more of the sucrose/amino acid mixture until they were provided with a protein source, when the preference disappeared, and that females reacquired the preference if the protein source was removed. Panzuto (1993) showed that 7- and 15-day-old mated females prefer a sucrose/amino acid mixture over sucrose alone while 2- and 24-day-old mated females show no preference between the two. Unmated females of all four age groups also show no preference between the two stimuli. The same study also showed that of the ten amino acids in the

yarrow mimic, phenylalanine and proline, found in the highest concentrations, are also preferred over the other eight.

The existence of the specific hunger suggests that the female fleshfly must be able to distinguish between a carbohydrate meal and a protein meal. Olfaction plays a role in attracting the fly to its host (Dethier, 1976; Larsen *et al.*, 1966), but it is unclear to what extent odours are involved. Mitchell and Soucie (1993) have shown that olfactory stimulation by itself is insufficient to trigger the initiation of oviposition. The insect must have the opportunity to taste its host as well.

Angioy *et al.* (1983) found that changing ovarian cycles caused shifts in chemosensory response in the blowfly, *Phormia regina*. Increases in sensitivity were discovered in the insect's response to NaCl, a repellant solution. This study did not present the flies with an attractant solution, and thus left unanswered the question of whether a corresponding increase in sensitivity exists in the fly's response to food sources.

The organs of gustation in the fleshfly are located mainly on the tarsi and on the labellar lobes of the proboscis (Dethier, 1976). These organs are hairlike projections with hollow tips, along the inner shafts of which are found the dendrites of four chemosensitive neurons (Zacharuk, 1985). The membranes of these dendrites possess receptors specific for several different types of molecule. By way of action potentials, the neurons transmit information on the nature of the stimulating substance to the central nervous system. A number of studies have used electrophysiological techniques to understand the code used in transmitting this information (reviewed in Dethier, 1976; Mitchell *et al.*, 1990; Albert *et al.*, 1991).

Mitchell *et al.*, (1990) used behavioural bioassays as well as electrophysiological studies to examine the response of 2-day-old female

Sarcophaga bullata to solutions of sucrose, fish meal and liver powder. They showed that flies consumed greater quantities of liver and fish solution than they did of sucrose, but analysis of the mean firing rates of the chemosensitive cells failed to reveal information that was correlated with the behavioural results. The only difference in the response, that correlated with the behavioural results, was the variance of the response of the first cell which was greater for sucrose than for the other two substances. Mitchell *et al.*. (1990) hypothesized that this variance itself gave information to the central nervous system that allowed it to distinguish a simple sucrose solution from a complex liver or fish solution. One of the difficulties associated with this study was the lack of a standard concentration for comparison of the three solutions. 100 mM/l sucrose was used, along with 10% w/v liver powder and 40% w/v fish meal. An analysis of the constituent parts of the liver and fish meal extracts has not been done, and this eliminates molarity as the most likely standard by which to compare the different substances.

The focus of the present project was to compare mated and unmated females in their response to two types of food stimulus. Liver powder in solution was used to represent the protein meal that the flies require in order to allow development of their eggs. Sucrose in solution was used to represent the carbohydrate meal. Six-day-old females were used, as this is an age at which they will soon be ready to produce larvae, thus they are attracted to a protein source. Both mated and unmated females were studied, to measure the effect of mating on the ingestion of each solution, as well as on the electrophysiological response to the two solutions.

One method of deriving a standard concentration at which to compare the response of flies to different types of food stimulus is to construct behavioural assay curves of quantity ingested versus concentration of solution. The

concentrations that elicit a half-maximal consumption of a particular substance can be referred to as K_b values. The K_b values obtained from these curves can then be considered standard concentrations. K_b values are likely to differ between mated and unmated flies, therefore curves were constructed for each. Thus, I performed four sets of behavioural bioassays, resulting in K_b values for mated and unmated flies when responding to sucrose, as well as those for mated and unmated flies when responding to liver xtract.

I then conducted studies using two-choice behavioural bioassays in which flies were given a choice of sucrose and liver solutions, each at their respective K_b concentration. Once the preference of the insects was determined, the concentration of the preferred solution was manipulated in further tests, in order to arrive at a concentration where the two solutions were consumed in equal quantities. This concentration was dubbed the "equilibrium" concentration.

The next objective of the project was to compile recordings of the electrophysiological responses of both mated and unmated flies to each solution at its respective K_b, as well as the equilibrium concentration and 100 mM/l KCl, a solution that is known to be a deterrent to feeding. An analysis of the data thus obtained would reveal any differences in the mean firing rate of neurons that have been stimulated with the different types of solution.

In examining the collected results, I was then able to explore the differences between unmated and mated female flies in their behavioural response to the different solutions, and to look for an electrophysiological basis that could explain these differences. I was also able to look for changes in sensitivity to both deterrent and attractant solutions, and to explore and expand upon the concept of shifts in chemosensitivity caused by changes in the reproductive state.

MATERIALS AND METHODS

Insects. Fleshflies (*Sarcophaga bullata*) were reared in a colony maintained at 23°C on a 16L:8D photoperiod. Water, powdered milk and sucrose were provided *ad libitum*. Female flies deposited their larvae on raw liver, that was replenished on a daily basis. Sated larvae crawled away from the liver and burrowed into a bed of vermiculite, where they pupated. I placed the pupae individually into 20 ml glass vials to ensure that the flies would not mate upon emerging from the pupa. I then moved the adult flies into 1 L plastic jars, with no more than 50 flies in a jar. In some cases all of these flies were female, in others half were female and half were male. This was done to provide both mated and unmated females for testing. Sucrose, powdered milk and water were again provided. After tests were completed on mated females in the one-choice behavioural bioassays, each of the tested flies was confined to an individual 1 L plastic jar and provided with sucrose, powdered milk and water. To ensure that the females in these tests were indeed mated, small pieces of raw beef or pork liver were placed in the jar on a daily basis until the female larviposited. This procedure was discontinued after more than 95% of the females used in the first few tests were shown to have mated. Of the remaining 5%, most had died or had escaped before larviposition.

Solutions. For the one-choice and two-choice behavioural bioassays, sucrose solutions were made by dissolving molar fractions of sucrose in distilled water. Liver solutions were made by dissolving liver concentrate powder (Sigma, Lot # 50HO376) by percent weight per unit volume in distilled water. For the electrophysiological studies, KCl solutions were made by dissolving molar fractions of KCl in distilled water. Sucrose solutions were made by dissolving molar fractions of sucrose in 100 mM/l KCl. Liver solutions were made by

dissolving liver concentrate powder by percent weight per unit volume in 100 mM/l KCl.

Behavioural bioassays. In the one-choice tests, dose-response curves for both liver and sucrose were constructed by isolating individual female flies in 25 ml glass jars, stoppered with plastic lids. These lids were punctured with several small holes for aeration, as well as one larger hole, through which protruded the tip of a 0.1 ml pipette filled with the test solution. I recorded the amount consumed after 15 minutes, 1 hour, 2, 4, and 6 hours. Evaporation was limited by injecting a small amount of water at the end of the pipette that extended away from the jar. Control jars with no fly were also included in the test, with roughly one control jar being included for every three test jars. I subtracted the average amount of evaporation from the control jars from the average amount of solution missing from the test jars to arrive at the average amount consumed by the flies. For each concentration of test solution, I attempted to reach a sample size of 25-30 flies, and from 10-12 control jars. In the two-choice tests, taste preferences of the flies were measured in the same way as with the one-choice tests, with the exception that two pipettes protruded into the test jar, each filled with a different solution. This apparatus was modified from Belzer (1970).

Calculations. K_b values for each of the solutions, and for both mated and unmated flies, were calculated from the behavioural data as follows. I constructed double-reciprocal plots with the data from each of the dose-response curves. The result was a straight line that passed through the positive phase of the vertical axis and intercepted the horizontal axis. The K_b value is equal to the negative inverse of the x-intercept. The assumption is that mechanisms of insect chemoreception are analogous to those of enzyme-substrate reactions. This technique has been successfully applied in several studies of insect chemoreception (Mitchell & Gregory, 1979; Hansen & Kühner, 1972; Kijima *et*

al., 1973).

Electrophysiology. Electrophysiological studies were done using the tip recording technique first described by Hodgson *et al.* (1955). Female flies were anaesthetised by being placed at 0° C in a freezer for between 3 and 4 minutes. Their probosces were then removed and mounted on the tip of a pulled capillary tube filled with insect Ringer solution (Schnuch and Hansen, 1990). This tube was then placed over a Ag/AgCl reference electrode. Individual chemosensory hairs were stimulated by having the tip of another pulled capillary micropipette placed briefly over the tip of the hair. This second micropipette was filled with the test solution, and placed over a Ag/AgCl recording electrode. The chemosensilla were stimulated with the solutions of sucrose and liver in random order. One hundred mM/l KCl was presented first (KCl_a) and last (KCl_b) as a control, to ensure that there was no deterioration of the proboscis. Sample sizes ranged from 11 to 18 sensilla tested. There were no differences between the first and last KCl stimuli (t-test; p=0.80) and consequently these data were pooled for subsequent analyses. During the course of the experiment a 3-minute interval was used between stimulations of the same hair, to guard against the effects of adaptation. I made recordings from as many hairs as possible on the proboscis of each fly, and the responses of between one and thirteen hairs were thus recorded from each of the flies used in the study. The action potentials that were elicited by this stimulation were recorded on digital audio tape. The Sapid Tools computer program (Smith *et al.*., 1990) was used to digitize and print the recordings, and the first 1 s of the recording was analysed through a visual counting and characterization of the action potentials.

The use of the unfiltered signal presented an advantage in the form of a better ability to distinguish spike shapes. DC recording allows us to avoid the distortion of the impulses that is found in filtered recordings (Schnuch and

Hansen, 1990). This distortion can alter the shapes of spikes, making identification of cells less reliable. Figure 1 shows the shapes of spikes from a recording of the stimulation of a small hair on a mated fly with sucrose at its K_b. The shapes of spikes from Cell 1 and Cell 2 are very similar, but those from Cell 1 can be seen to have a deeper refractory period. Another advantage of the use of unfiltered signals resulted from the inclusion of the initial 0.3s segment of the recording, and can be explained as follows.

One of the cells that is found within the chemoreceptor has been traditionally described as the "salt cell", while others have been described as the "water" and "sugar" cells (reviewed in Dethier, 1976). These terms will not be used in the present work in favour of the idea that each cell is capable of responding to a variety of substances. Nonetheless, the cells remain distinct in that the action potentials of each have a characteristic shape and firing pattern. The salt, sugar and water cells will be referred to in the present study as Cells 1, 2 and 3. The shapes of the action potentials of Cells 1 and 2 are very similar. The two cells, however, can be discerned by noting that Cell 2 produces a characteristic initial burst of numerous spikes, followed by a steady stream of spikes at gradually increasing intervals (Figure 2). Cell 1 lacks the initial burst and fires instead at a uniform, slowly decreasing rate. Thus by including the first 0.3s of a response, the cells can be distinguished by examining the pattern of firing.

Statistics. Data for both the bioassays and electrophysiology were analyzed with the Number Cruncher Statistical System (J.L. Hintze, 865 East North, Kaysville, UT 84037, USA). The Two Sample Sign test was used to analyze results from the two-choice tests. A 3-way Anova and t-tests were used to compare mean firing frequencies of the electrophysiological results.

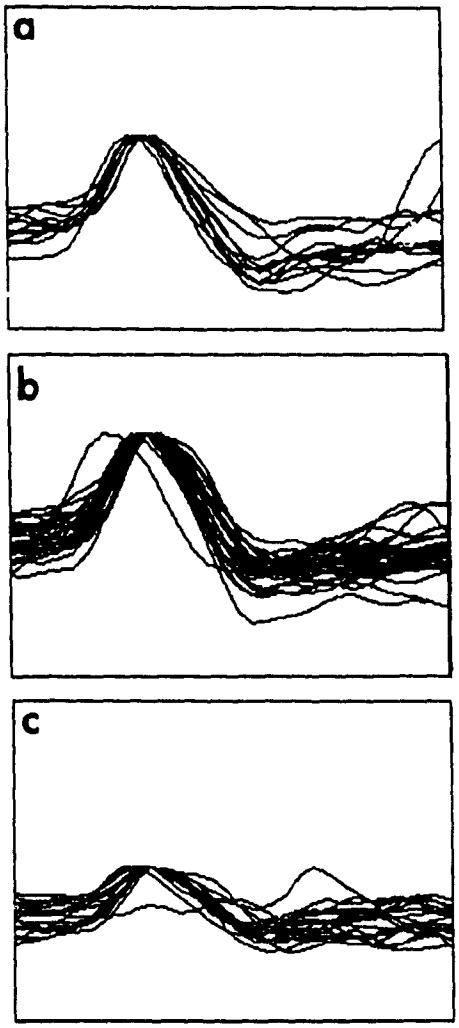

Figure 1. Profiles of action potentials from a representative electrophysiological recording. Stimulation of a small hair on an unmated fly with 70 mM/l sucrose. a, Cell 1; b, Cell 2; c, Cell 3.

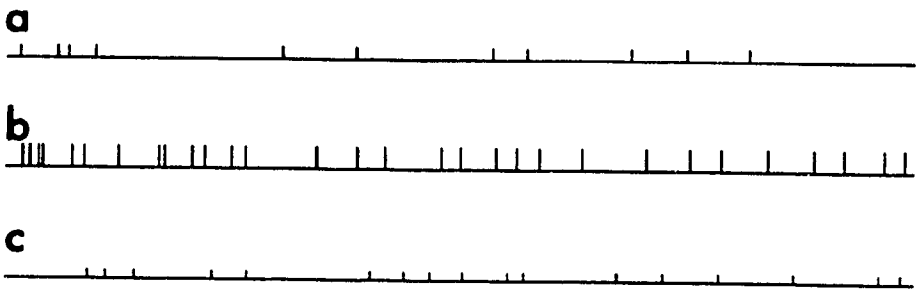

Figure 2. Patterns of firing of three cells from a representative 1 s electrophysiological recording. Stimulation of a small hair on an unmated fly with 70 mM/l sucrose. a, Cell 1; b, Cell 2; c, Cell 3.

RESULTS

One-Choice Bioassays

Figure 3 shows the dose-response curves obtained for both mated and unmated 6-day-old female fleshflies, when presented with a range of concentrations of liver extract in solution.

Figure 4 is a double-reciprocal plot of the data from the liver extract dose-response curves. I constructed this plot to derive K_b values. By finding the negative inverse of the X-intercepts, K_b values of 1.43% w/v and 2.63% w/v were calculated for the unmated and mated flies, respectively. Unmated females respond more strongly at lower concentrations.

Figure 5 shows the dose-response curves obtained for both mated and unmated 6-day-old female fleshflies, when presented with a range of concentrations of sucrose in solution. The unmated flies are less responsive than the mated flies.

Figure 6 is a double-reciprocal plot of the data from the sucrose dose-response curves. The negative inverses of the X-intercepts give K_b values of 70 mM/l and 135 mM/l for the unmated and mated flies, respectively.

Two-Choice Bioassays: Equilibrium Concentration

In the next experiment I gave the flies a choice between sucrose and liver extract. Figure 7 shows relative consumption by unmated females of sucrose to liver extract at varying concentrations of liver extract solution. In each test, the concentration of sucrose was kept at the K_b value of 70 mM/L. When unmated flies were given a choice between sucrose at its K_b and liver extract at its K_b (1.43% w/v), they ate such a small amount of sucrose solution that, once the average evaporation

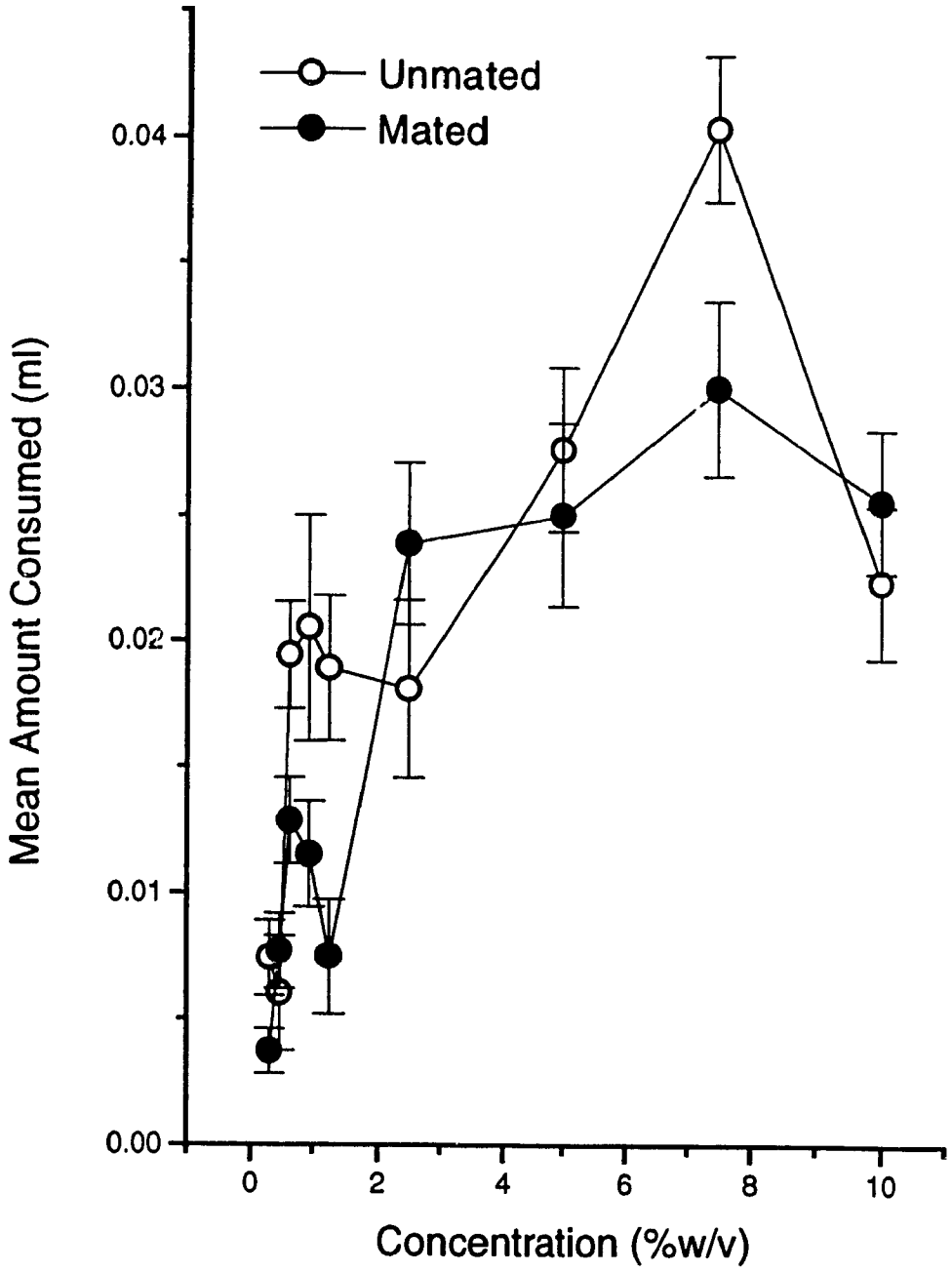

Figure 3. Mean amount consumed (ml ± 95% C.L.) by six-day-old females of a range of concentrations of liver extract solution. The n of animals used for each concentration are as follows. Unmated: 0.3125% (30), 0.46875% (37), 0.625% (29), 0.9375% (14), 1.25% (26), 2.5% (28), 5% (33), 7.5% (28), 10% (27). Mated: 0.3125% (34), 0.46875% (35), 0.625% (36), 0.9375% (35), 1.25% (32), 2.5% (33), 5% (38), 7.5% (31), 10% (31).

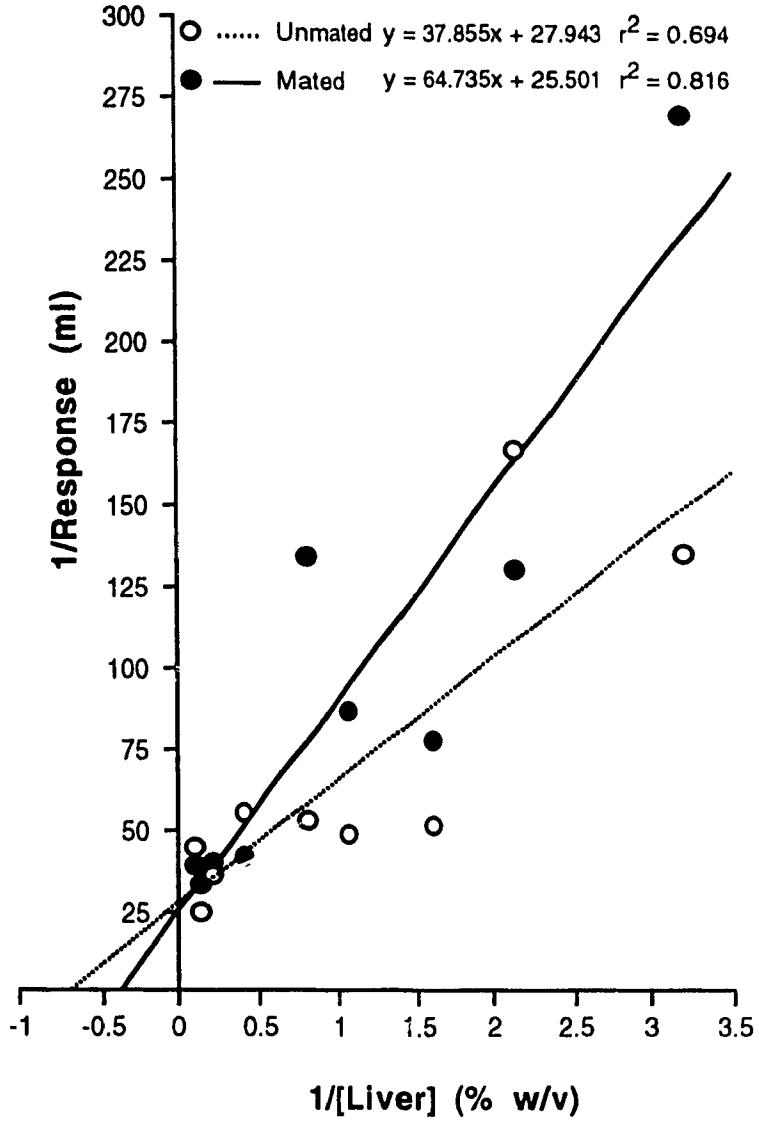

Figure 4. Double-reciprocal plot of the data from Figure 3.

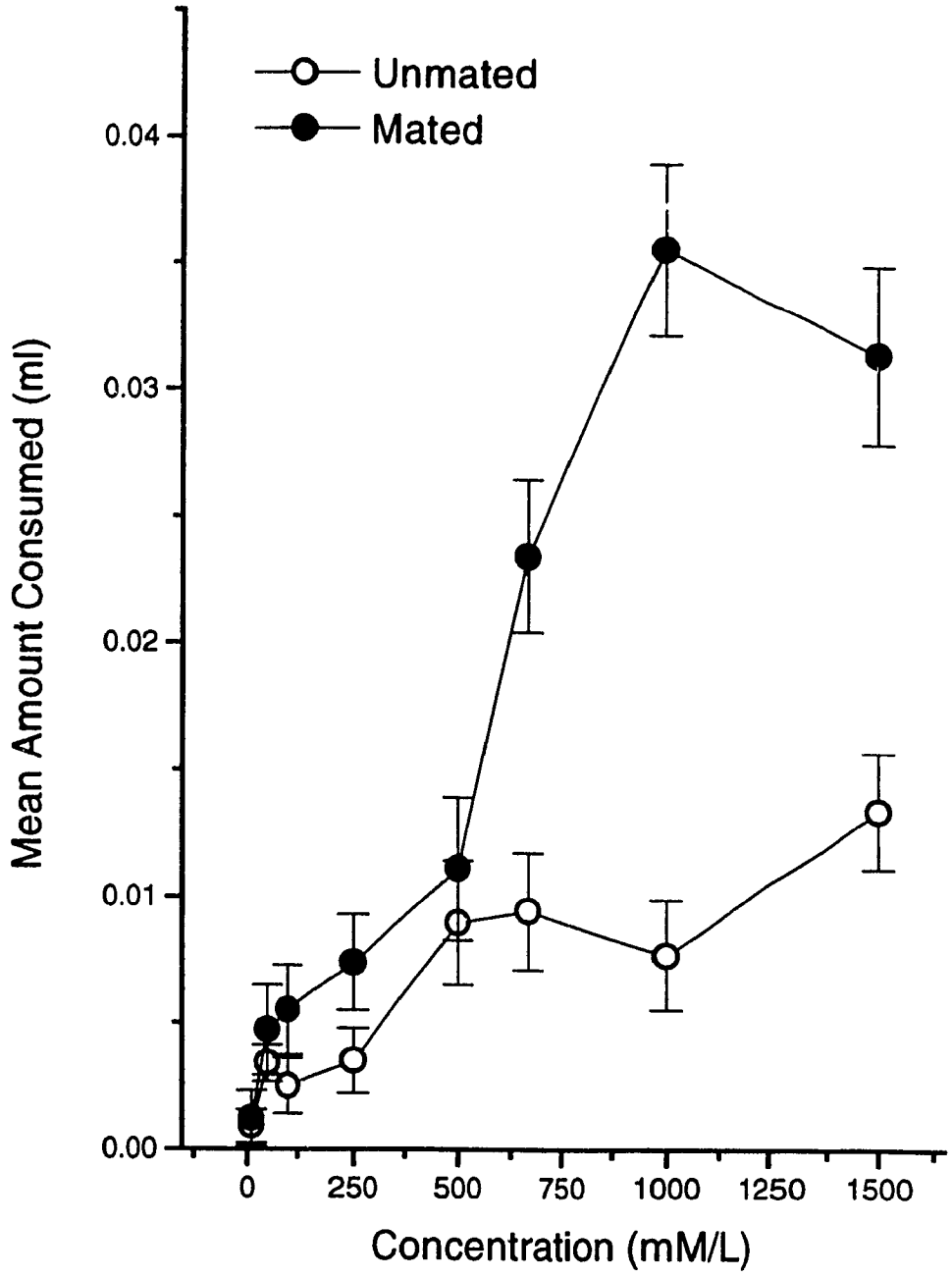

Figure 5. Mean amount consumed (ml ± 95% C.L.) by six-day-old females of a range of concentrations of sucrose solution. The n of animals used for each concentration are as follows. Unmated: 10 mM/l (28), 50 mM/l (46), 100 mM/l (30), 250 mM/l (35), 500 mM/l (30), 667 mM/l (26), 1000 mM/l (30), 1500 mM/l (27). Mated: n = 35 for all concentrations.

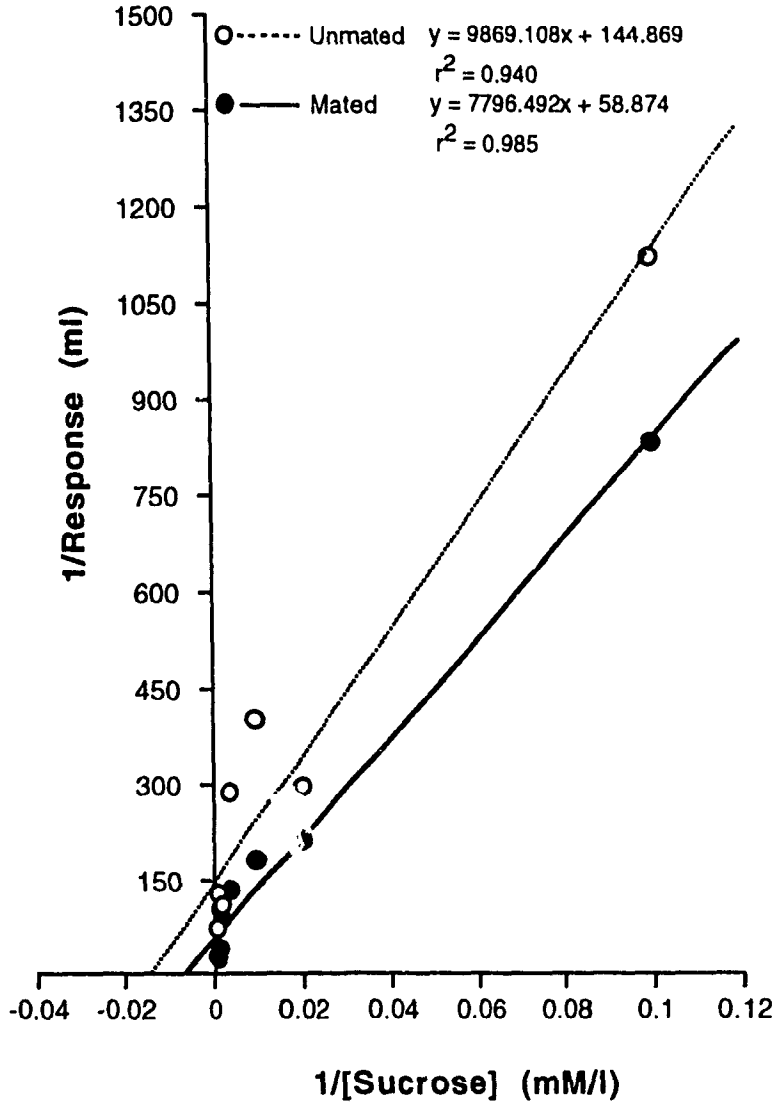

Figure 6. Double-reciprocal plot of the data from Figure 5.

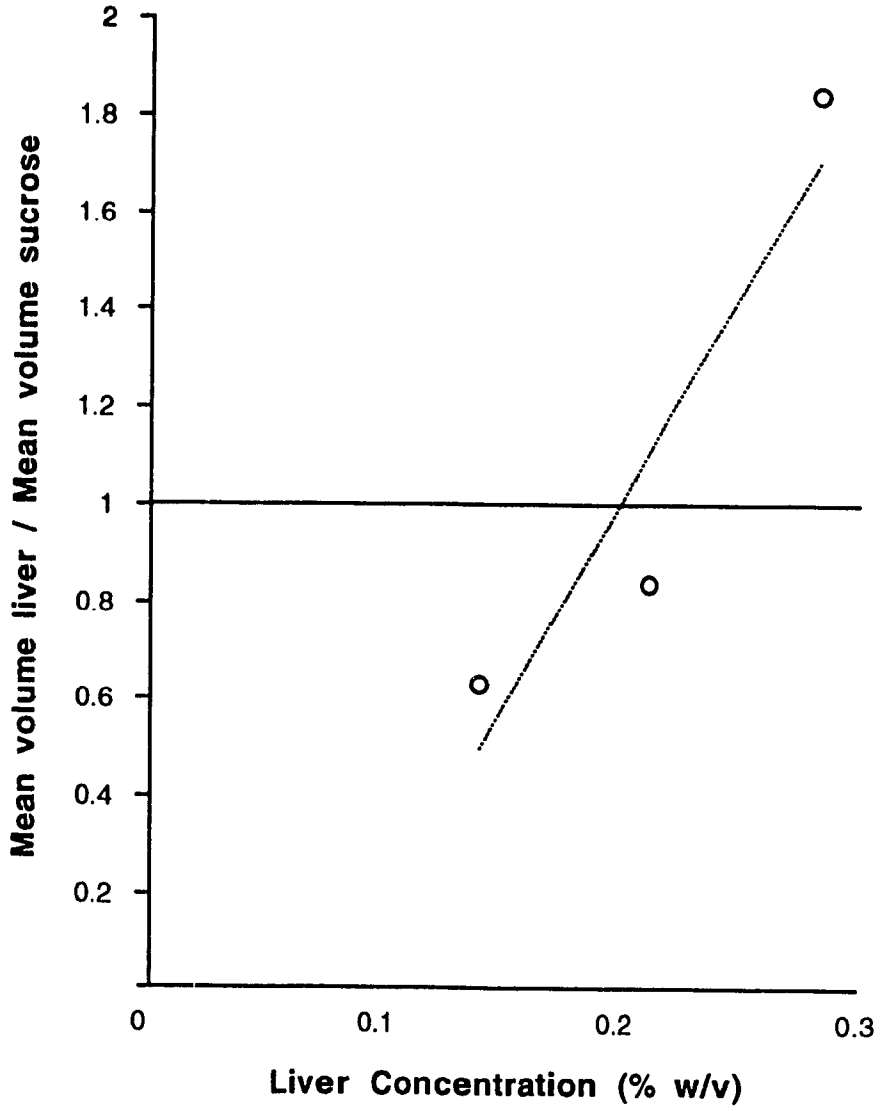

Figure 7. Ratio of amounts consumed by unmated females of liver/sucrose at different concentrations of liver and the K_b concentration of sucrose.

from the control vials was subtracted from the average consumption of sucrose, the result was a negative number. Meanwhile, they consumed an average of 0.037ml of the liver extract solution. The amounts consumed were significantly different (Two Sample Sign Test, $p < 0.001$). Because of the huge difference, the results of this test were excluded from Figure 7.

To find a concentration of liver extract solution at which the flies would eat the same amount as they would of sugar at its K_b, the flies were given a choice of sucrose at its K_b and liver at 10% of its K_b, or 0.143% w/v. After subtracting the average evaporation from the control vials, the flies were found to have eaten an average of 0.0476ml of sucrose and 0.0299ml of the liver extract solution. These amounts were not significantly different ($p > .5$) statistically, but I wanted to find a concentration of liver at which the two solutions were consumed in amounts as similar as possible, so another experiment was performed. Since sucrose at its K_b was preferred over liver extract at 10% of its K_b, in the next set of tests the flies were given a choice of sucrose at its K_b and liver extract solution at 20% of its K_b (0.286% w/v). After subtracting the average evaporation from the controls, the flies were shown to have consumed an average of 0.00518ml of sucrose and 0.00953ml of the liver. These amounts were significantly different ($0.02 < p < 0.05$), and this time the liver extract solution was preferred over the sucrose. Thus, a lower concentration of liver extract was chosen for the next experiment, where flies chose between a sucrose solution at its K_b and a liver extract solution at 15% of its K_b, or 0.215% w/v. The average amounts consumed were 0.00303ml for the sucrose solution and 0.00254ml for the liver extract solution. These values were not significantly different ($p > .5$), and the totals were deemed similar enough so that 0.21% w/v was chosen as the equilibrium concentration for the unmated flies.

Figure 8 shows the results of a set of similar tests conducted on mated

flies. I did not provide the flies with a choice between the two solutions at their respective K_b concentrations, as it seemed likely that the sucrose would be ignored. Instead, the flies were given a choice between sucrose at its K_b and liver extract at 0.625% w/v. The average amounts consumed were 0.00411ml for the sucrose solution and 0.0196ml for the liver extract solution. These values are significantly different (Two Sample Sign Test, $0.001 < p < 0.002$). Once again I wanted to find an equilibrium concentration, at which flies would eat the same amount of liver extract solution as they would of sucrose at its K_b. Since the flies overwhelmingly preferred the liver extract solution in the first test, the concentration of liver extract was lowered in the subsequent test, which was a choice between sucrose at its K_b and liver at 0.0625% w/v. The average amounts consumed were 0.0170ml for the sucrose solution and 0.000341ml for the liver extract solution. These values were significantly different ($0.02 < p < 0.05$). I then conducted a test in which the flies chose between liver extract solution at 0.313% w/v, with the sucrose solution once again held steady at its K_b. The average amounts consumed were 0.0128ml for the sucrose solution and 0.0133ml for the liver extract solution. These values are not significantly different ($p > 0.5$), and were deemed to be similar enough so that 0.313% was chosen as the equilibrium concentration for the mated flies.

Electrophysiology

Once equilibrium concentrations as well as K_b concentrations had been determined for both mated and unmated six-day-old female fleshflies, the next set of experiments involved stimulating flies electrophysiologically with solutions at these concentrations. Figures 9-10 show the results of the control, where sensory hairs were presented with 100 mM/l KCl as the first and last stimulus. Figure 9 shows the average number of action potentials elicited per second from

each of the responding cells in small, medium and large hairs, for the unmated flies. Figure 10 shows the average number of action potentials elicited per second from each of the responding cells in small, medium and large hairs, for the mated flies. In each case, there was no significant difference between the initial and final stimulation in the average number of action potentials elicited from Cell 1 or Cell 3 (T-test, $p = 0.8$). Cell 2 did not respond to KCl at all. There were no significant differences in response between the hair sizes for either the unmated or mated flies (3-way ANOVA, $p = 0.431$). There was a significant difference between the responses of Cells 1 and 3 (3-way ANOVA, $p = 0.000$). There was no significant difference between mated and unmated flies, in their response to KCl (3-way ANOVA, $p = 0.264$).

Figures 11-12 show the results of stimulation of the sensory hairs with sucrose at its K_b. Figure 11 shows the average number of action potentials elicited per second from each of the responding cells in small, medium and large hairs, for the unmated flies, with 70 mM/l sucrose. Figure 12 shows the average number of action potentials elicited per second from each of the responding cells in small, medium and large hairs, for the mated flies, when stimulated with 135 mM/L sucrose. For both Cell 2 and Cell 3, there was no significant difference in firing rates between the mated and unmated flies for similar hair sizes (3-way ANOVA, $p = 0.267$). There was a significant difference between firing rates of different hair sizes (3-way ANOVA, $p = 0.003$). There was a significant difference between firing rates of Cells 2 and 3 (3-way ANOVA, $p = 0.000$).

Figures 13-14 show the results of stimulation of the sensory hairs with the K_b and equilibrium concentrations of liver extract solution. Figure 13 shows the average number of action potentials elicited per second from small, medium and large hairs, for unmated flies. In this case the K_b concentration is 1.43% w/v, and the equilibrium concentration is 0.21% w/v. Figure 14 shows the average number

of action potentials elicited per second from small, medium and large hairs, for mated flies. In this case the K_b concentration is 2.6% w/v, and the equilibrium concentration is 0.31% w/v. There was no significant difference in firing rates between the mated and unmated flies for similar hair sizes, for either Cell 2 or Cell 3 (3-way ANOVA, p = 0.938 for K_b; p = 0.976 for Eq.). There was a significant difference in firing rates between hair sizes (3-way ANOVA, p = 0.007 for K_b; p = 0.059 for Eq.). There was a significant difference in firing rates between Cells 2 and 3 (3-way ANOVA, p = 0.000 for both K_b and Eq.).

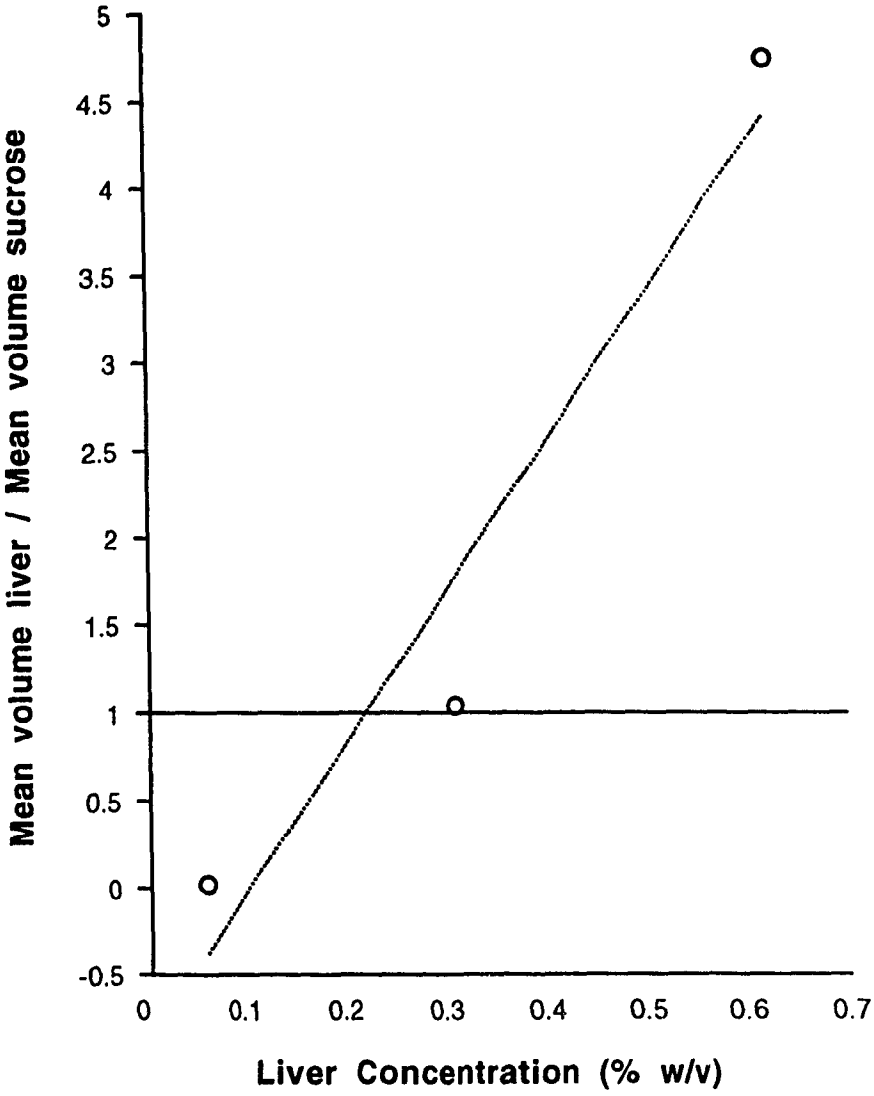

Figure 8. Ratio of amounts consumed by mated females of liver/sucrose at different concentrations of liver and the K_b concentration of sucrose.

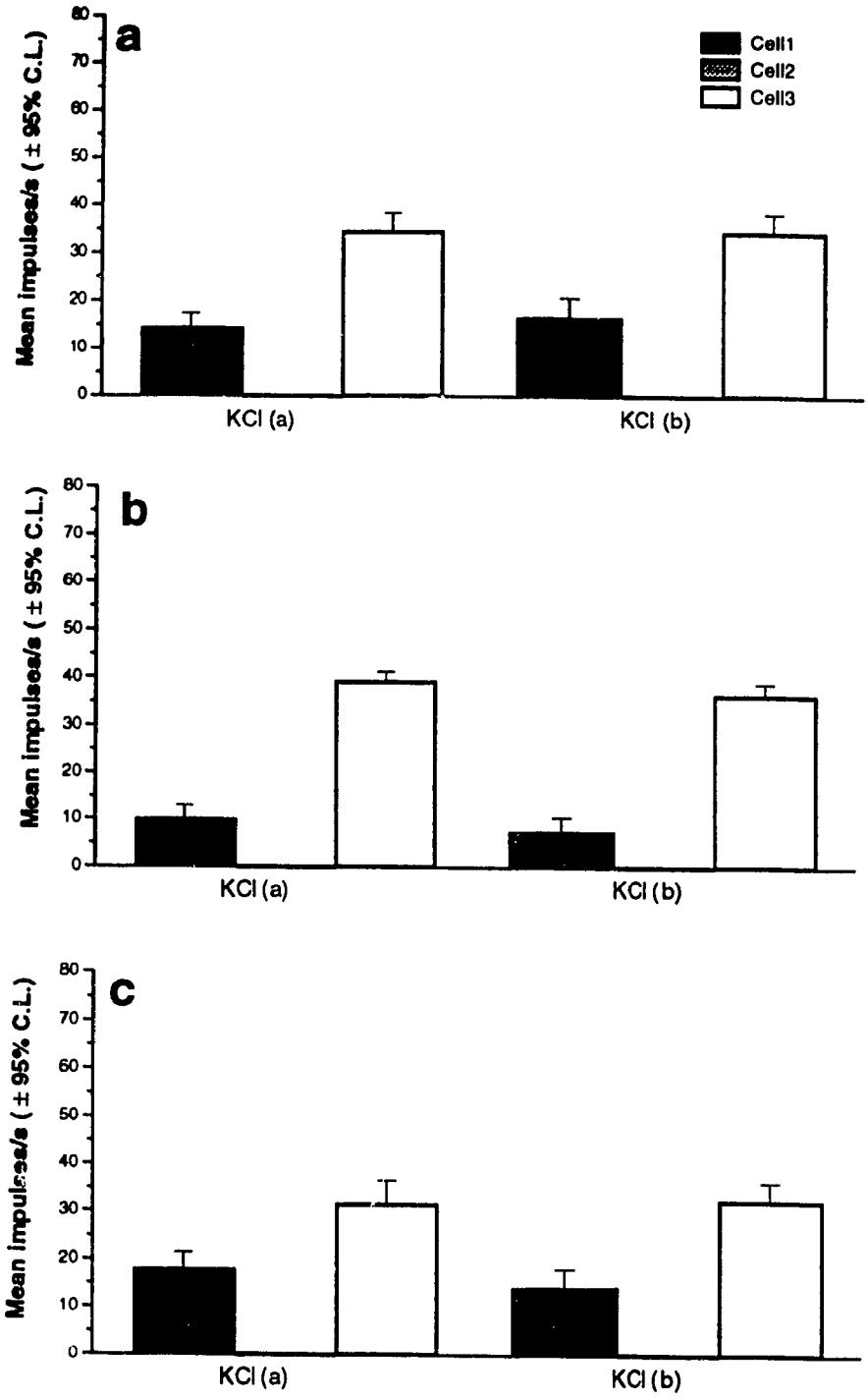

Figure 9. Mean impulses/s (± 95% C.L.) elicited from labellar chemosensilla of unmated flies when stimulated with 100 mM/l KCl as a control for habituation. KCl_a and KCl_b represent hits 1 and 2 of the same solution. a, large hairs, $n(KCl_a)$ = 15, $n(KCL_b)$ = 15; b, medium hairs , $n(KCl_a)$ = 18, $n(KCl_b)$ = 14; c, small hairs, $n(KCl_a)$ = 17, $n(KCl_b)$ = 15.

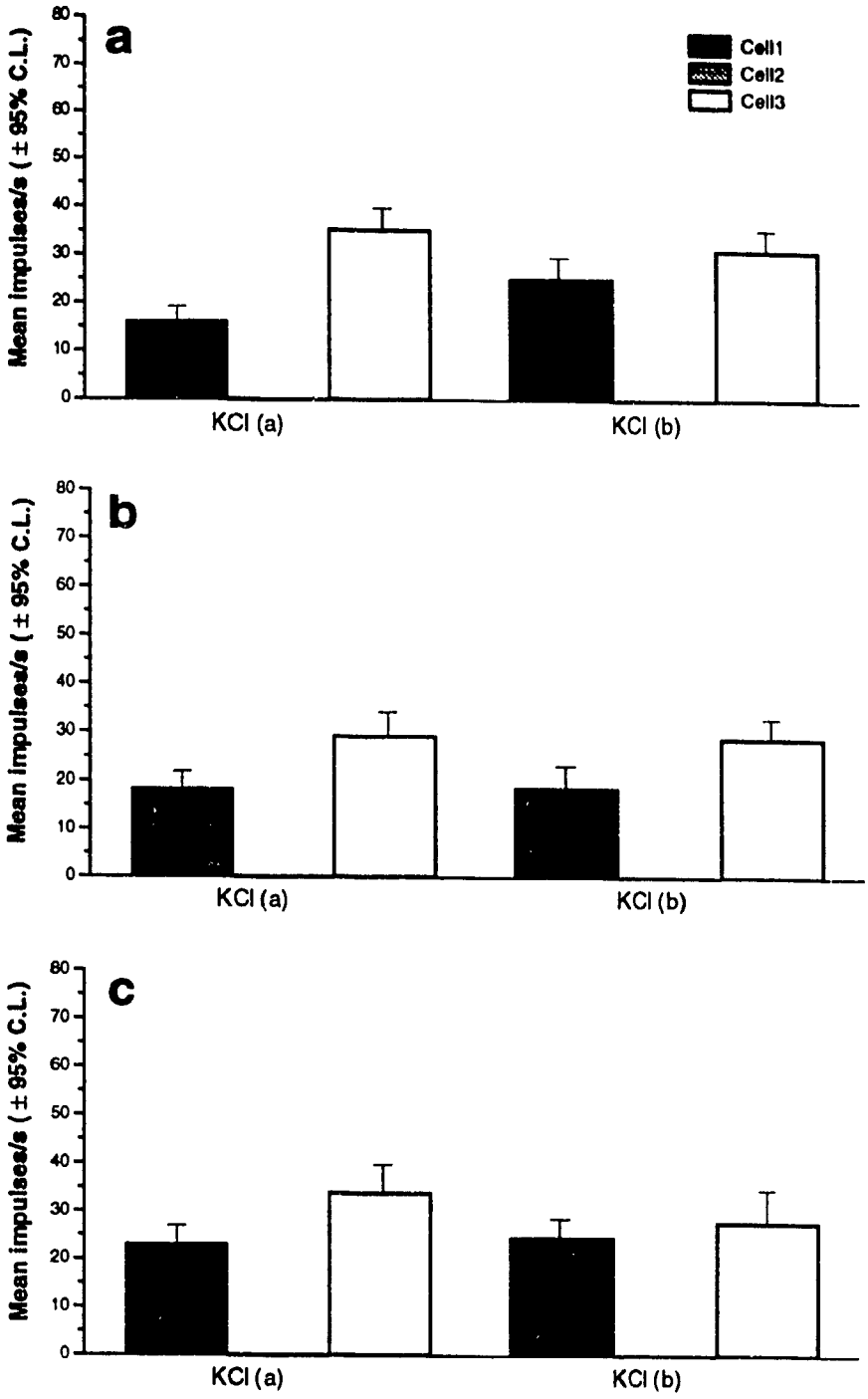

Figure 10. Mean impulses/s (± 95% C.L.) elicited from labellar chemosensilla of mated flies when stimulated with 100 mM/l KCl as a control for habituation. KCl_a and KCl_b represent hits 1 and 2 of the same solution. a, large hairs, $n(KCl_a) = 13$, $n(KCl_b) = 11$; b, medium hairs, $n(KCl_a) = 15$, $n(KCl_b) = 14$; c , small hairs, $n(KCl_a) = 14$, $n(KCl_b) = 12$.

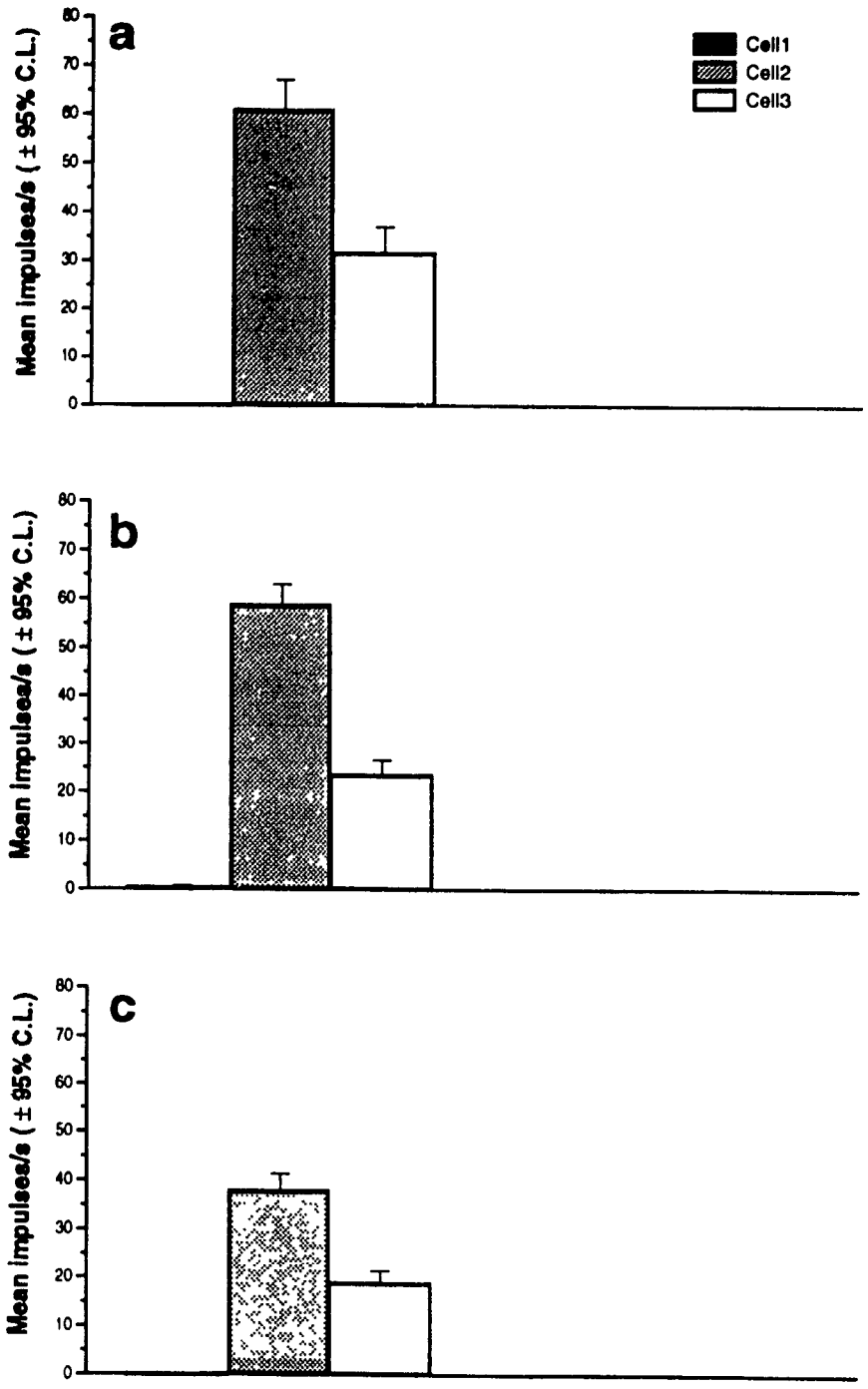

Figure 11. Mean impulses/s (± 95% C.L.) elicited from labellar chemosensilla of unmated flies when stimulated with 70 mM/l sucrose. a, large hairs, n = 12; b, medium hairs, n = 17; c, small hairs, n = 18.

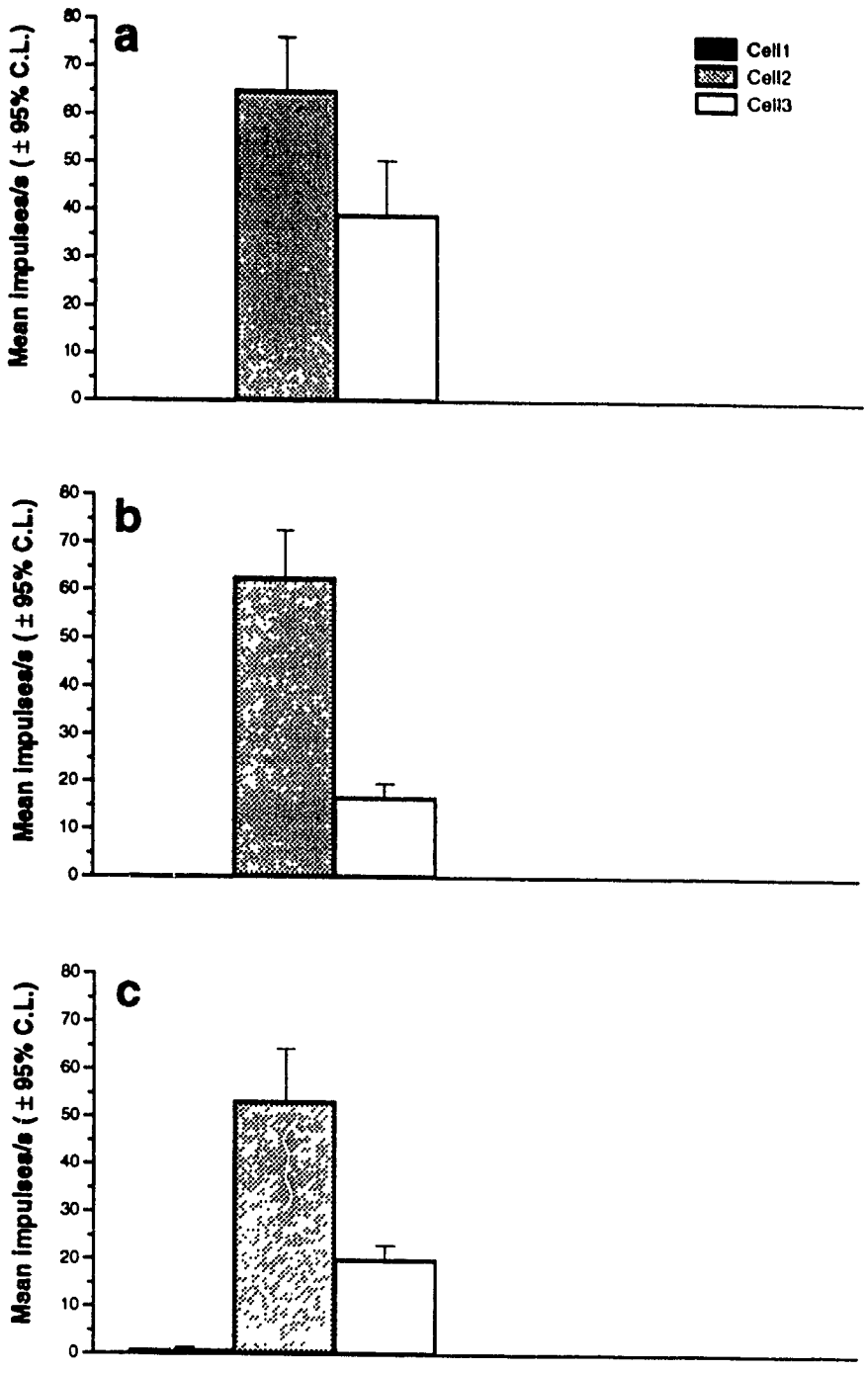

Figure 12. Mean impulses/s (± 95% C.L.) elicited from labellar chemosensilla of mated flies when stimulated with 135 mM/l sucrose. a, large hairs, n = 11; b, medium hairs, n = 15; c, small hairs, n = 12.

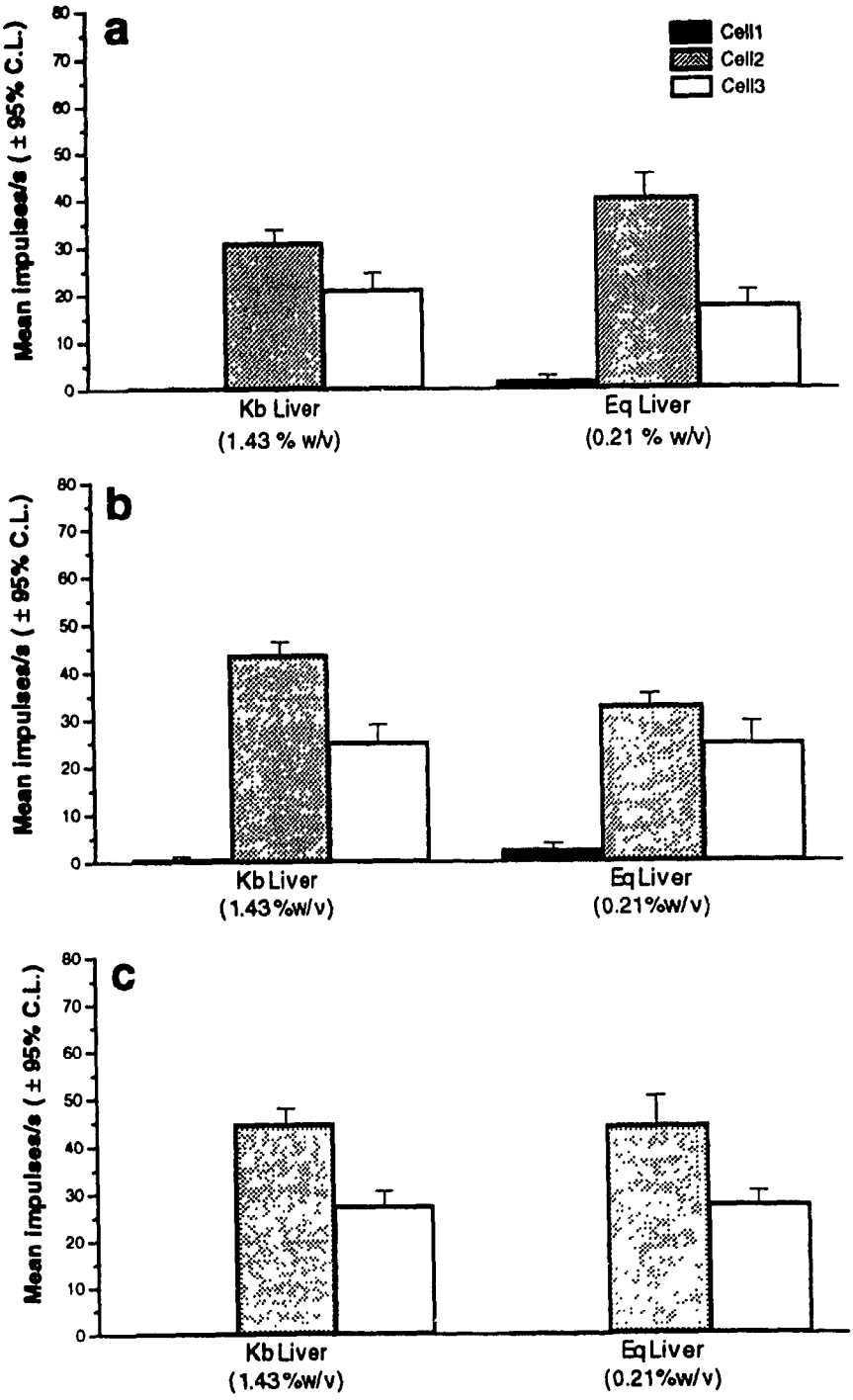

Figure 13. Mean impulses/s (± 95% C.L.) elicited from labellar chemosensilla of unmated flies when stimulated with 1.43% w/v and 0.21% w/v liver extract. a, large hairs, $n(K_b) = 15$, $n(Eq) = 17$; b, medium hairs, $n(K_b) = 15$, $n(Eq) = 16$; c, small hairs, $n(K_b) = 17$, $n(Eq) = 18$.

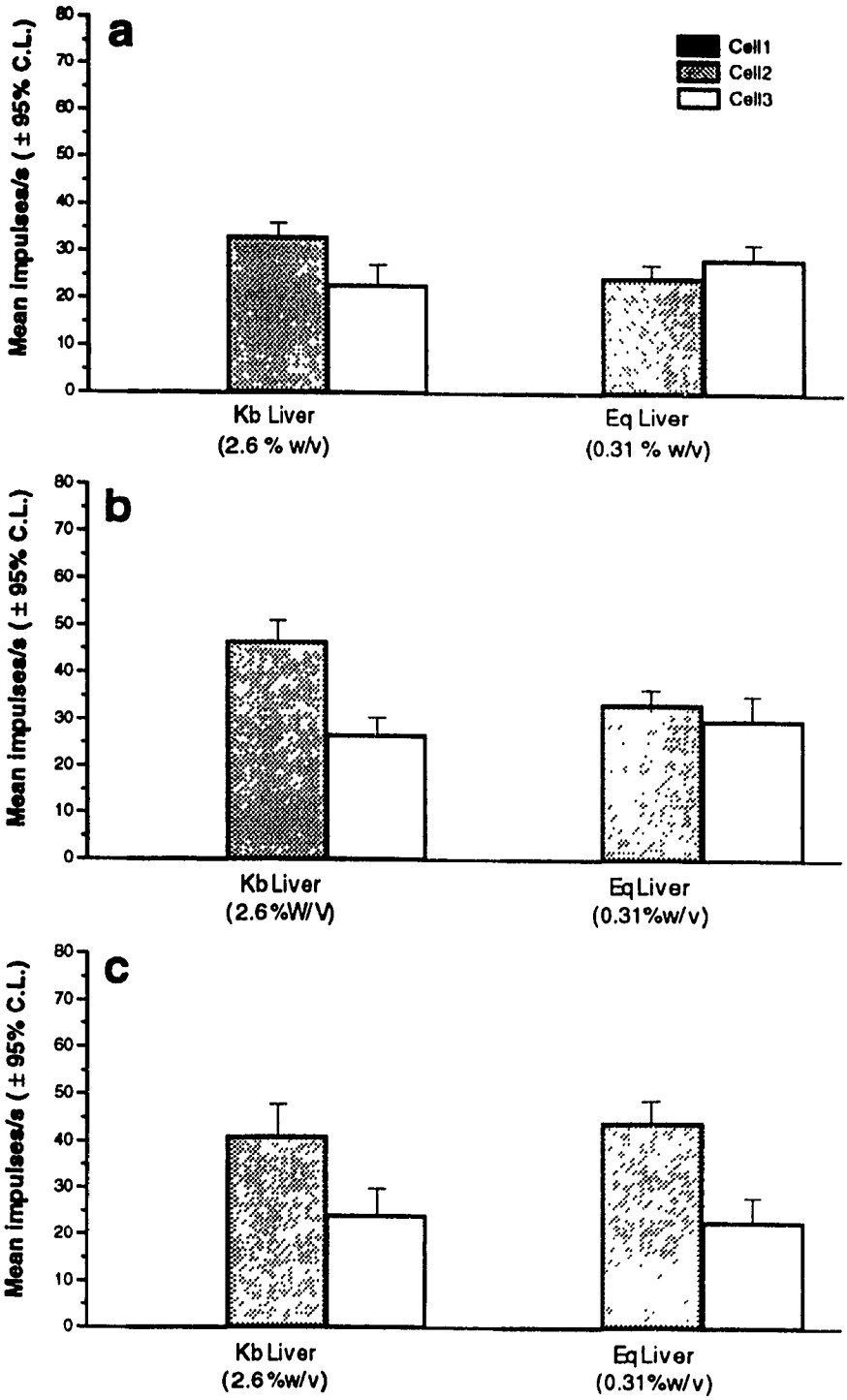

Figure 14. Mean impulses/s (± 95% C.L.) elicited from labellar chemosensilla of mated flies when stimulated with 2.6% w/v and 0.31% w/v liver extract. a, large hairs, $n(K_b) = 12$, $n(Eq) = 12$; b, medium hairs, $n(K_b) = 15$, $n(Eq) = 16$; c, small hairs, $n(K_b) = 12$, $n(Eq) = 15$.

DISCUSSION

Although the differences are not statistically significant, there is a trend indicating that unmated 6-day-old females consume larger quantities of liver solutions at lower concentrations than do mated flies. This suggests that the threshold level of stimulation required for the insect to eat is somehow increased in the mated flies. This change in threshold could be triggered by a male factor transferred with the sperm, or by a hormonal change associated with mating or oogenesis. Mating can cause behavioural changes in insects. Rivet and Albert (1990) demonstrated that mating triggers the search for an oviposition site in *Choristoneura fumiferana* females. Yamaoka and Hirao (1977) stimulated oviposition in virgin *Bombyx mori* females with an extract of whole male reproductive tract.

A comparison of electrophysiological responses reveals further differences between mated and unmated flies. When stimulated with the equilibrium concentration of liver extract, Cell 2 of the large hairs fires at a significantly higher rate and Cell 3 at a lower rate in unmated flies than in mated flies, but there were no differences between mated and unmated flies for the other hair sizes. There were no significant differences for any hair size between mated and unmated flies when stimulated with the K_b concentration of liver extract and sucrose solution. These results are unexpected considering that the concentrations of K_b and sucrose solutions presented to the mated flies were almost double those of the solutions presented to the unmated flies. The only exception is the firing rate of large hairs when stimulated with the equilibrium solution of liver, which is 50% higher for mated than for unmated flies. The reason for these results once again involves a change in the threshold concentration at which a solution becomes an effective behavioural stimulus. We

now have added evidence that the threshold has been increased by the act of mating or by oogenesis. Thus the change in threshold is shown to function at the level of the peripheral receptors, but it must be controlled centrally, possibly by a male factor transferred with the sperm, or by a hormonal change brought about indirectly by the act of mating. A precedent for the idea of a hormone affecting peripheral chemoreceptors is found in Angioy *et al.* (1983). They found cyclic variations of chemosensory function in the blowfly, *Phormia regina*, and showed that these variations are time-related to ovarian cycles. Vitellogenesis is triggered by the release of juvenile hormone from the corpus allatum (Pappas and Fraenkel, 1978). The Angioy study demonstrated that an increase in sensitivity of the sensilla on the labellum occurs at the beginning of vitellogenesis. They suggest that juvenile hormone is the endocrine factor responsible for changing chemosensillar sensitivity. In the present study, a decrease, rather than an increase in sensitivity, is related to the reproductive cycle. However, this decrease in sensitivity is expressed towards the attractive solutions of liver extract and sucrose that both stimulate the "sugar" cell (Cell 2), while the Angioy study found an increase in sensitivity to NaCl, a deterrent that stimulates the "salt" cell (Cell 1). Since the present study used KCl as a control stimulus, a look at those recordings (Figs 9 & 10) shows that in all of the cases studied, Cell 1 (the "salt" cell) shows a slight increase in response for mated flies. Although this is not statistically significant, the trend is consistently in the same direction as that observed by Angioy *et al.*. (1983). Thus, an increase in response to deterrents could go along with a decrease in response to attractants, with the mechanism for causing this change having different effects on Cell 1 and Cell 2.

The results of this study are important in that they both support and expand upon the conclusions made by Angioy *et al.*. (1983). I provide evidence of

the same phenomenon revealed in the earlier experiment, but in a different species of fly. This indicates the effect is likely to be widespread among insects. I also suggest that a shift in sensitivity can alter the behavioural response of an insect in more than one direction to more than one type of stimulus, and to varying degrees. The effect is more complex than would be suggested by the Angioy study.

Thus, when stimulated with an attractive solution, the central nervous system of the mated fly receives less input in impulses/s than an unmated fly receives from the same stimulus. The effect of this is that mated flies prefer more concentrated food sources and end up providing more nutrition for their larvae. At the time of ingestion of the protein meal, the mated fly prefers a stronger protein source, and is thus better able to produce larvae more quickly, or to produce greater numbers of larvae. A similar effect is noted in the behaviour of the fly towards a carbohydrate meal. When ingesting a carbohydrate meal, mated flies not only prefer stronger solutions, but consume significantly greater amounts of strong solutions (Figure 3). This indicates that they are compensating for the increased demands placed on their internal metabolism by the developing larvae, by ingesting larger quantities of their staple food source.

CONCLUSION

The use of unfiltered signals and analysis of firing pattern as well as spike shape, allowed me to establish that a different cell was responsible for the majority of the spikes in response to the salt deterrent than was responsible for the majority of the spikes in response to the attractive solutions.

Both the behavioural tests and the electrophysiology show that mated flies are less sensitive than unmated flies in their response to nutritive solutions of either carbohydrate or protein. This could indicate that the threshold concentration at which flies begin to ingest the food has been raised somehow by the act of mating. The end result is likely to be that mated flies become more efficient at acquiring meals that provide sufficient nutrients for them to develop their larvae. The change in sensitivity is likely to function at the level of the peripheral chemoreceptors, and to be controlled centrally, either directly or indirectly as a result of mating. The results are in agreement with those found earlier with *Phormia regina*. The present study shows possible evidence of a change in sensitivity to a deterrent salt stimulus, and we show that concurrently there is a change in sensitivity in the opposite direction, to attractive protein and carbohydrate stimuli. These differential sensitivity shifts are associated with two different cells in the same sensillum.

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