Z. vergl. Physiologie 74, 103–120 (1971)
© by Springer-Verlag 1971

The Sensory Control of Motor Output in Fly Proboscis Extension

PETER A. GETTING
Group in Biophysics and Medical Physics, University of California
Berkeley, California

Received June 2, 1971

Summary. Proboscis extension, the initial sequence of feeding behavior in the blowfly, *Phormia regina*, can be induced by sucrose stimulation of a single labellar sensillum. Exploiting the ability to record single unit sensory input from labellar sensilla and single unit motor output from the extensor of the haustellum, I have investigated the degree of control exerted by the sensory spike train from the sugar receptors on motor output. Extension of the proboscis can be triggered by temporal summation of sensory activity during a 20 millisecond period after the first sensory spike (Fig. 1, Tables 1, 2). The duration and number of muscle spikes per motor response are determined in part by the sensory frequency and duration of sensory input (Figs. 2, 3). Habituation of the motor response to repeated stimulation of a single sugar receptor occurs and is independent of activity in other sugar receptors (Fig. 4). A role for receptor adaptation and habituation at central synapses in determining the duration and number of spikes per motor response is postulated. Nonlinear summation between spatially separate sensory inputs was found and is discussed in terms of the findings from stimulation of single receptors. (Figs. 5, 6) A minimal neuronal model to account for motor activity in response to labellar sugar receptor activity is proposed (Fig. 7).

Introduction

In only a few studies has electrophysiological activity in specific sensory receptors been monitored simultaneously with the consequent behavioral responses (Dethier, 1968). Even fewer studies have been conducted in which the activity of more than one receptor has been correlated with motor activity. The feeding behavior of the blowfly, *Phormia regina*, provides an excellent opportunity for an electrophysiological analysis of the processing of specific sensory information from several chemosensory modalities in relation to motor output (Dethier, 1969). This study is an analysis the relation between single and multi-channel sensory input from one of four chemosensory modalities and the specific muscle activity involved in feeding behavior.

A food deprived, water satiated fly can be induced to extend its proboscis, the initial step of feeding behavior, by sucrose stimulation of a single labellar sensillum (Dethier, 1955). Electrophysiological studies

indicate that each labellar taste hair contains five sensory cells; a monovalent cation receptor (Evans and Mellon, 1962; Gillary, 1966), a monovalent anion or fatty acid receptor (Steinhardt, 1965; Dethier and Hanson, 1968), a selective carbohydrate receptor (Hodgson, 1957; Omand and Dethier, 1969), a water receptor (Evans and Mellon, 1962), and a movement receptor (Wolbarsht and Dethier, 1958). Only the carbohydrate and water receptors are active when a sensillum is stimulated with sucrose. Since only a single sensillum need be stimulated to evoke feeding behavior, monitoring of the necessary sensory activity is greatly simplified.

The feeding response of the blowfly consists of extension of the proboscis, spreading of the labellar lobes, and sucking. Complete extension involves the movement of three sections of the proboscis: the rostrum, the haustellum, and the labellar lobes. The rostrum, or more proximal portion of the proboscis, appears to be extended by distension of air sacs in the head. Puncture of these air sacs or ligation of the neck which prevents the passage of air from the thoracic cavity into the head prevents rostrum extension (Dethier, 1959). In contrast, extension of the haustellum, the middle section of the proboscis, is mediated by direct muscular activity in two pairs of muscles, the extensors of the haustellum and its adductors, located in the rostrum (Graham-Smith, 1930; Dethier, 1959). Since these are the only muscles involved in the extension of the haustellum, recording the activity from a single extensor-adductor complex gives an adequate measure of the motor output involved in the extension of the haustellum. This paper deals solely with the sensory control of haustellum extension.

Exploiting the ability to monitor both the sensory input and motor output during feeding behavior, I investigated the degree of control exerted by sensory impulse trains from the sugar receptor on the motor output impulse pattern in the extensor of the haustellum. Sucrose stimulation of a single sensillum showed that (a) initiation of motor activity is closely controlled by phasic sensory input from labellar sensilla, and (b) the duration and the number of muscle spikes per response is a function of both receptor adaptation and habituation at central synapses. Stimulation of two sensilla revealed nonlinear summation between sensory inputs from two sugar receptors; this is discussed in terms of the findings from single sensillum stimulation.

Materials and Methods

Blowflies, *Phormia regina*, age 3–18 days, were starved at room temperature in small plastic boxes containing water saturated filter paper. After a designated starvation period the wings were waxed together to prevent flight and all legs were amputated at the coxotrochanteral joint to eliminate possible sensory input from

the tarsal chemoreceptors. The proboscis was fixed in a semi-extended position by inserting the constriction between the rostrum and the haustellum into a slot in a silver-chlorided plate. The fly was further immobilized by placing a dissecting pin against the ventral surface of the neck and another pin against the dorsal surface of the thorax under the wings. Neither pin penetrated the cuticle. In its final position the fly was lying on a wax surface with the dorsal-ventral plane oriented horizontally. These procedures were carried out at 4° C to facilitate handling. During a post-operative period of one hour at room temperature the fly was fed distilled water until satiation as indicated by the lack of a behavioral response to water stimulation in at least ten labellar sensilla.

The activity of a single labellar sensillum was recorded through a glass capillary (tip diameter 100 μ) using a technique similar to that employed by Hodgson, Lettvin, and Roeder (1955), and Omand and Dethier (1969). The sugar receptor was stimulated by sucrose solutions containing 50 mM LiCl to provide electrical conductivity. To stimulate the water receptor alone, a 50 mM LiCl solution was used. This concentration of LiCl was insufficient to stimulate either the cation or anion receptors which can mediate withdrawal of the proboscis (Gillary, 1966; Steinhardt, 1965). To control the duration of solution contact with the sensillum, a square wave of current was passed through the coil of a small speaker to which the capillary was fastened. Sensory signals were amplified 4000 times (band-pass 400 cps to 2.5 kc).

Muscle activity was recorded using a glass insulated tungsten electrode (tip 2×5 μ) inserted through the cuticle into the extensor of the haustellum. The electrode was placed visually under the proximal end of the apodeme and in contact with the extensor muscle. A small drop of saline was placed on the rostrum between the insertion of the muscle electrode and the silver-chlorided plate which served as the indifferent electrode. Muscle activity was amplified 1000 times and displayed simultaneously with the sensory activity on an oscilloscope. Consistent motor and sensory responses could be recorded for several hours.

Results

Sucrose stimulation of a single labellar sensillum of a water satiated fly starved 72 hours resulted in motor activity in the extensor-adductor muscle complex. Fig. 1 shows two typical records of sucrose stimulation of a single sensillum at two concentrations. The two spike amplitudes in the sensory traces represent activity in the sugar and water receptors. The resultant motor activity also consists of two amplitude classes; these differed in latency and the number of impulses per response. The larger spike always had a longer latency, and fewer impulses. At lower receptor activity than shown in Fig. 1 the motor response consisted only of the smaller unit indicating that the smaller unit has a lower threshold. It is not clear whether the two classes of impulse amplitude, recorded in the muscle represent activity from two units of the extensor of the haustellum, or one unit each from the extensor and its adductor.

Using sucrose stimulation of a single labellar sensillum, I investigated the effects of sensory interspike interval (ISI) on the initiation of motor activity in the extensor-adductor muscle complex. The sensory ISI was

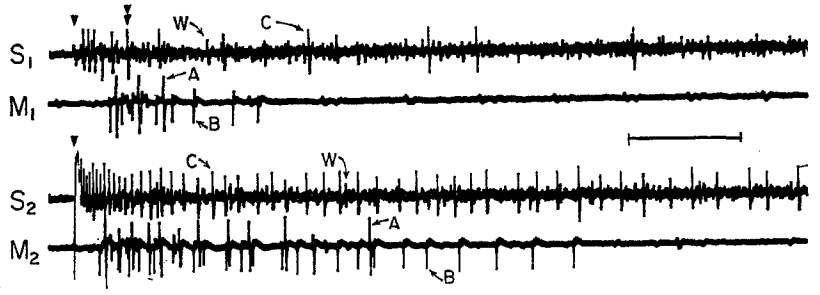

Fig. 1. Electrophysiological records of sucrose stimulation of a single labellar sensillum and the resultant motor activity in the extensor-adductor muscle complex of a water satiated fly starved 72 hours. S_1 and M_1, receptor and motor activity respectively in response to 100 mM sucrose; S_2 and M_2, receptor and motor activity respectively in response 400mM sucrose. The two spike sizes in the sensory records represent activity in the sugar (C) and water (W) receptors. Muscle activity in the extensor-adductor complex also consists of two spike sizes; large (A) and small (B). The single arrow at the beginning of both sensory traces marks the instant of solution contact with the sensillum. The double arrow in S_1 marks the last sugar spike for which the sensory interspike interval (ISI) is less than 20 milliseconds. Note the cessation of motor activity in M_2 despite a constant sensory ISI of less than 20 milliseconds. Time mark, 100 milliseconds

varied by stimulation with various sucrose concentrations. The first sensory ISI and the latency of the motor response from the first, second, third, and fourth sensory spikes are summarized in Table 1 for four flies starved for 70–72 hours. A single sugar spike was never sufficient to trigger a motor response; similarly, two sugar spikes with an ISI greater than 20 milliseconds were inadequate. The value of sensory ISI below 20 milliseconds required to trigger a motor response varied from fly to fly. Generally, a sensory ISI of less than 15 milliseconds always produced a response. As the sensory ISI was decreased, the latency of the motor response from the second sensory spike remained constant. The motor latencies from any of the other sensory spikes varied as the sensory frequency was increased. Examination of Table 1 shows that the motor latency from the second sensory spike has the smallest standard deviation. For flies starved 70–72 hours, motor activity was triggered by the first two sensory spikes if their ISI was less than approximately 20 milliseconds and the motor response followed the second sensory spike with a characteristic latency that was independent of the number of sensory spikes preceding the motor response.

For flies starved for 62–66 hours, motor activity was triggered by the first two or three sensory spikes, depending upon the first sensory ISI. The sensory conditions and motor latencies from the second and third

Table 1. *Mean latencies of the first motor spike from the first, second, third, and fourth sugar spikes in the sensory spike train as a function of the number of sensory spikes before the motor response*

ISI_1 refers to the interspike interval between the first and second sugar spikes. The column numbers under motor latencies refers to the number of the sensory spike from which the latency was measured. All latencies are given in milliseconds. In all cases the motor latency from the second sensory spike showed the least variation with sensory input, that is the smallest standard deviation.

Prep. No.	Hours starved	Number of sensory spikes before motor	ISI_1	Motor latencies			
				1 mean (S.D.)	2 mean (S.D.)	3 mean (S.D.)	4 mean (S.D.)
1	72	1		no motor response			
		2	$ISI_1 > 20$ msec	no motor response			
		2–8	$ISI_1 < 20$ msec	25.6 (6.5)	18.3 (1.6)	13.1 (3.0)	10.8 (3.1)
2	72	1		not motor response			
		2	$ISI_1 > 20$ msec	no motor response			
		2–7	$ISI_1 < 8$ msec	31.0 (1.5)	26.6 (1.1)	20.6 (4.6)	14.7 (7.9)
3	72	1		no motor response			
		2	$ISI_1 > 17$ msec	no motor response			
		2–8	$ISI_1 < 17$ msec	35.9 (5.7)	29.2 (2.1)	23.3 (3.9)	19.7 (5.8)
4	70	1		no motor response			
		2	$ISI_1 > 10$ msec	no motor response			
		2–8	$ISI_1 < 10$ msec	26.2 (2.6)	20.7 (0.7)	11.6 (7.2)	8.4 (8.4)

sensory spikes are given in Table 2. If the first sensory ISI was greater than 10 milliseconds, no motor response was triggered. In stimulations in which the first sensory ISI was between 5 and 10 milliseconds, the mean latency from the third sensory spike was identical ($p = 0.05$) with the mean latency from the second sensory spike for stimulations in which the first sensory ISI was less than 5 milliseconds. A "Student's *t*-test" was used to test for differences in mean values. All other latencies were significantly different. Therefore, motor activity was triggered by the first three sensory spikes if the first ISI was between 5 and 10 milliseconds, or by the first two sensory spikes if the ISI was less than 5 milliseconds.

Table 2. *Mean motor latencies measured from the second and third sensory spikes as a function of the first two sensory interspike intervals for flies starved 62–66 hours*

ISI_1 and ISI_2 refer to the sensory interspike intervals. The column numbers under motor latencies refer to the sensory spike number from which the corresponding latency was measured. All latencies given in milliseconds. The starred latencies in each group are not significantly different by a "Student's" t-test ($p = 0.05$). All other latencies are significantly different.

Prep. No.	Hours starved	Sensory conditions ISI_1 and ISI_2	Motor latencies	
			2 mean (S.D.)	3 mean (S.D.)
5	62	$ISI_1 = 8$ msec, $ISI_2 = 15$ msec	no motor response	
		ISI_1 between 5 and 10 msec $ISI_2 < 10$ msec	36.5 (2.1)	28.0* (1.4)
		$ISI_1 < 5$ msec	28.7* (0.5)	22.0 (2.8)
6	66	*Large motor unit*		
		$ISI_1 > 10$ msec	no motor response	
		ISI_1 between 5 and 10 msec $ISI_2 < 10$ msec	35.5 (2.1)	29.5* (2.1)
		$ISI_1 < 4.5$ msec	26.5* (1.9)	23.0 (2.1)
		Small motor unit		
		$ISI_1 > 10$ msec	no motor response	
		ISI_1 between 6 and 10 msec $ISI_2 < 10$ msec	33.0	27.0*
		$ISI_1 < 5$ msec	25.3* (1.1)	21.4 (1.3)

Once triggered, the duration and the number or spikes per motor response were affected by the sensory frequency. The duration of the motor response and the number of motor spikes per response are linearly related; therefore either can be used as a measure of the magnitude of the motor response. Fig. 2 shows the motor response magnitude as a function of the average sensory frequency during the initial second of stimulation for four of seven experiments with flies starved between 60–72 hours. In two of the cases the motor response magnitude increased linearly with the sensory frequency over the range of sensory input used. In the other two cases the motor response attained a maximum response level after which increased sensory frequency did not result in an increase in the motor response.

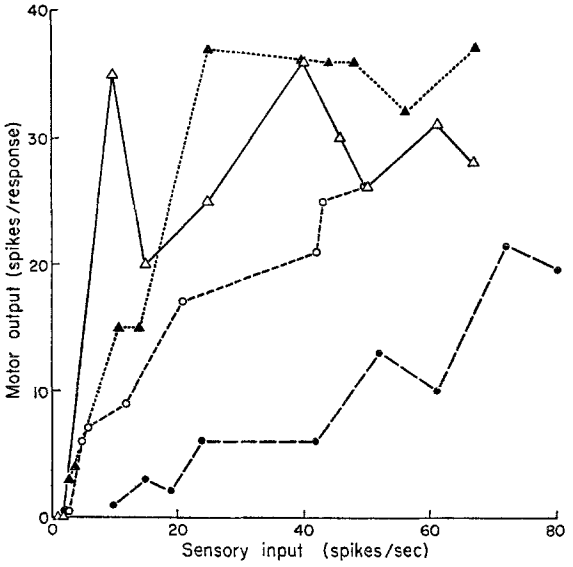

Fig. 2. Magnitude of the motor response as a function of the average sensory frequency during the initial second of stimulation of a single sensillum. In three experiments using flies starved for 62–66 hours the motor response increased linearly with sensory frequency. A representative case from one of these experiments is shown (filled circles). The motor response of a fly starved 72 hours (open circles) also increases linearly with sensory frequency but at a higher response level. The motor responses of two other flies starved for 72 hours (open and filled triangles) increase linearly with sensory frequency until a maximum motor response level is attained (saturation). An increase in the sensory frequency results in no further increase in motor activity. Differences in pre-starvation food intake probably accounts for the differing degrees of responsiveness for the three flies starved for 72 hours. In all cases only the long sensilla contralateral to the motor electrode were used (sensilla numbers 8–11 by the system of Wilczek, 1967)

The duration of the sensory input also modulated the magnitude of the motor response. For a fly starved for 60–72 hours, a 200 mM sucrose stimulation of only 50 milliseconds duration could trigger a motor response which continued for a hundred milliseconds after the end of stimulation (Fig. 3). Electrophysiological studies have shown that activity in the labellar receptors ceases within a few milliseconds after the stimulus solution is removed (Tateda and Morita, 1959). Therefore, continued but unmonitored receptor activity could not account for the extension of the motor response beyond the end of stimulation. As the duration of stimulation was increased, the motor response attained a maximum duration which was independent of continued sensory input. From short stimulation experiments and plots of both the sensory and

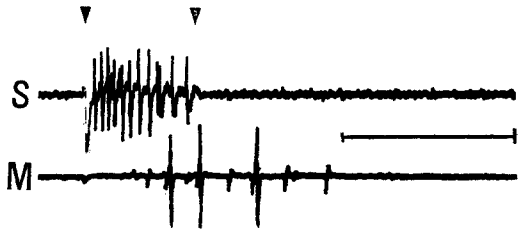

Fig. 3. Short 200 mM sucrose stimulation (*S*) of a single labellar sensillum and th resultant motor response (*M*) from a fly starved 65 hours. The two arrows mark th duration of the sensory stimulation. All sensory ISI's are less than 20 millisecondse The motor response continues for 84 milliseconds after the last sugar spike. Time mark, 100 milliseconds

motor interspike intervals, I have been unable to find a simple correlation between the cessation of motor activity and the sensory input. The cessation of motor activity did not depend solely upon the adaptation of the receptor response to an interspike interval above some critical value. Motor activity terminated over a wide range of sensory interspike intervals and there could be no change in the sensory interspike interval immediately preceding or following the cessation of the motor response. After triggering a motor response, sensory input could help maintain continued motor activity but was neither a necessary nor a sufficient condition for prolonged motor output.

At the sucrose concentrations used, both the sugar and water receptors were active to varying degrees depending upon the sucrose concentration. The preceding analysis of the fine structure of the sensory spike train depends critically upon the assumption that water receptor activity does not participate in triggering and maintaining a motor response. At one molar higher sucrose concentrations only sugar receptor activity was recorded, indicating that a motor response could be triggered by activity in a single sugar receptor. To test the possibility of summation between sensory spikes from the water and sugar receptors, one sensillum was stimulated with sucrose and an adjacent sensillum with water. The motor response during water and sucrose stimulation was identical in latency and magnitude to stimulation with sucrose alone. This result indicates that water receptor activity did not affect the triggering or maintenance of a sugar-stimulated motor response in a food-deprived, water-satiated fly, and that water receptor activity can be ignored in an analysis of the sensory impulse train. For a food-deprived, water-satiated fly the motor activity in response to sucrose stimulation of a single labellar sensillum was due to activity in a single receptor neuron, the carbohydrate receptor.

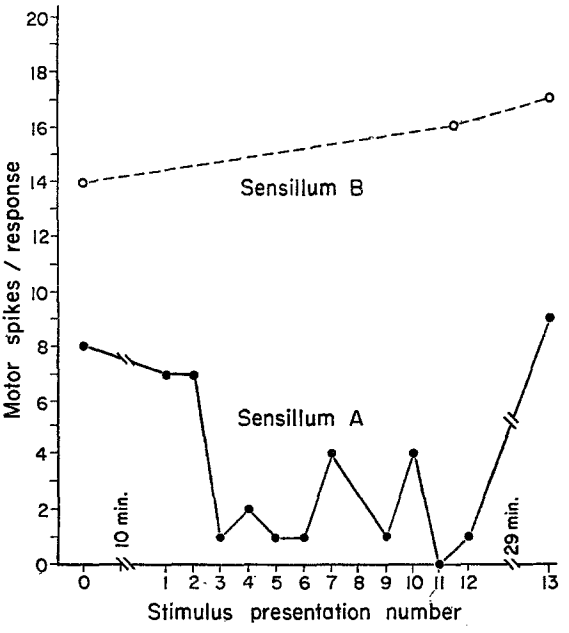

Fig. 4. Repeated one second, one molar sucrose stimulation of a labellar sensillum at one minute intervals on a fly starved 72 hours. Solid and dotted lines show the motor response magnitude to stimulation of two adjacent sensilla A and B respectively. Stimulations 0 and 1 set the pre-habituation response level. Stimulations 1 to 12 show the decline in motor output due to repeated stimulation of sensillum A at one minute intervals. The sensory frequency was identical in all stimulations. Between stimulations 11 and 12 sensillum B was restimulated resulting in a full motor response. Restimulation of sensillum B did not have any dishabituating effect on the motor response to sensillum A (stimulation number 12). The motor response to sensillum A showed spontaneous recovery to the pre-habituated response level after a period of 29 minutes without stimulation. Theslight increase in the motor response to stimulation of sensillum B with time is not significant; it is within the normal response range

Some deviations in the motor response magnitude over short periods, on the order of minutes, can be explained by habituation at central synapses. With repeated sucrose stimulations of a single sensillum at one-minute intervals, the motor response declined to a low level after a few stimulations although the sensory input frequency was unchanged (Fig. 4). However, stimulation of an adjacent sensillum always resulted in a normal motor response. Since receptor adaptation and motor cell fatigue could be ruled out, the habituation must have occurred at an interneuronal synapse, before the convergence of sensory inputs from different sensilla. No dishabituating effect of sucrose stimulation on the

adjacent sensillum was observed. Once the motor response had habituated to a one-half initial response level, a period of twenty minutes without stimulation was required for recovery to its initial level. To avoid habituation a period of ten minutes was necessary between successive stimulations of the same sensillum, and this interval was used in all experiments.

During normal feeding it is very unlikely that only one labellar sensillum will come in contact with the substrate. As the proboscis is extended more and more hairs are normally stimulated. By measuring thresholds for proboscis extension to sucrose stimulation, summation between receptor inputs has been demonstrated behaviorally (Dethier, 1953, 1955). Using a second sensory capillary and associated amplifiers, I analysed quantitatively the effects of two simultaneous sensory spike trains upon the motor output pattern. Fig. 5 shows five typical records of individual and simultaneous stimulation of two labellar sensilla and the resulting motor responses. Two aspects of the motor output in response to stimulation of two sensilla are shown in Fig. 6, taken from one of five experiments. First, the motor response to simultaneous stimulation was larger than that to stimulation of either sensillum alone. For example, stimulation of sensillum A at 30 sugar spikes per second yielded a motor response of 10 spikes. Stimulation of sensillum B at 43 sugar spikes per second yielded 12 motor spikes. However, stimulation of both sensilla simultaneously such that the sum of their frequencies was 26 spikes per second (sensillum A at 11 spikes/second; sensillum B at 15 spikes/second) resulted in a much larger motor response of 25 spikes. Dividing the total sensory input between two input channels was more effective in maintaining a motor response than the same or higher sensory frequency on a single input channel.

The second aspect of two channel input demonstrated in Fig. 6 is nonlinear summation of the sensory inputs. Simultaneous stimulation of two sensilla elicited motor responses which could be from one to six times the sum of the motor responses elicited by stimulation of both sensilla individually. For example, the motor response to 100 mM sucrose applied to both sensilla simultaneously was larger than the sum of the motor responses to 100 mM applied to each sensillum individually (Fig. 5). Summation between receptor activities was thus nonlinear, with a variable increase in the response above that predicted by the sensory frequencies alone.

The possibility exists that the increased motor response to simultaneous stimulation of two sensilla was not due entirely to spatial summation, but in part to a central excitatory state induced by the sucrose stimulation of another sugar receptor (Dethier, 1955; Dethier, Solomon, and Turner, 1965). To evaluate the effects of a possible change in central

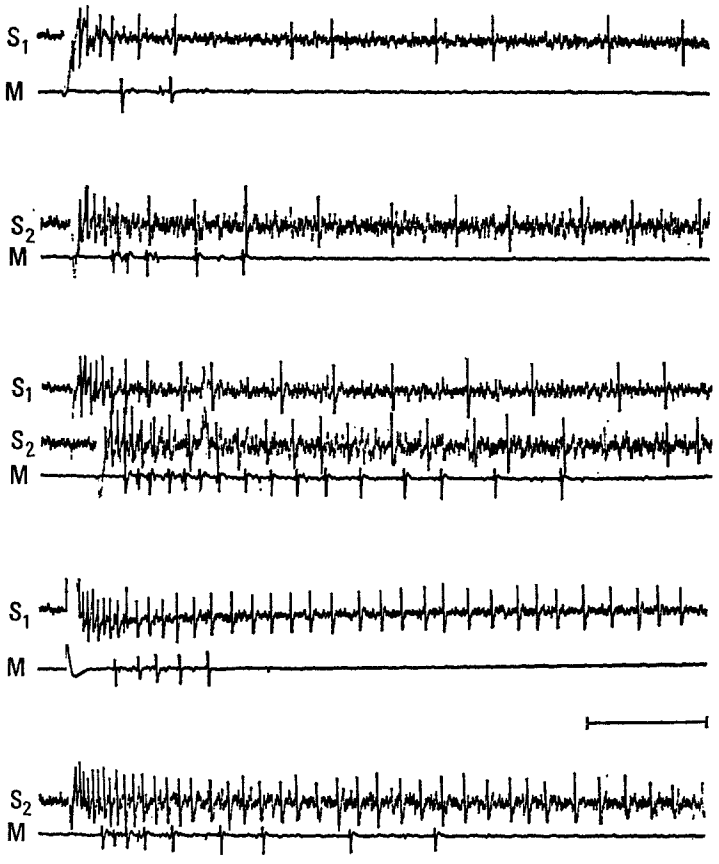

Fig. 5. Individual and simultaneous sucrose stimulation of two adjacent sensilla and the resultant motor activity in the extensor-adductor muscle complex of a fly starved 73 hours. Top two traces (S_1 and M), 100 mM sucrose stimulation of sensillum 1 and the resultant motor response. Second two traces (S_2 and M), 100 mM sucrose stimulation of sensillum 2 and motor response. Middle three traces (S_1, S_2, and M), simultaneous stimulation of both sensilla with 100 mM sucrose and motor output. The sensory frequencies in S_1 and S_2 during simultaneous stimulation of both sensilla are higher than the individual stimulations due to evaporation at the tip of the stimulus electrode. However, the increased sensory frequency in both sensilla can not account for the large motor response. Sucrose stimulation of either sensillum 1 or 2 alone at a sensory frequency equal to or greater than the sum of the sensory frequencies during simultaneous stimulation yields a smaller motor response (bottom two sets of traces). The summation of receptor activities is nonlinear. Time mark, 100 milliseconds

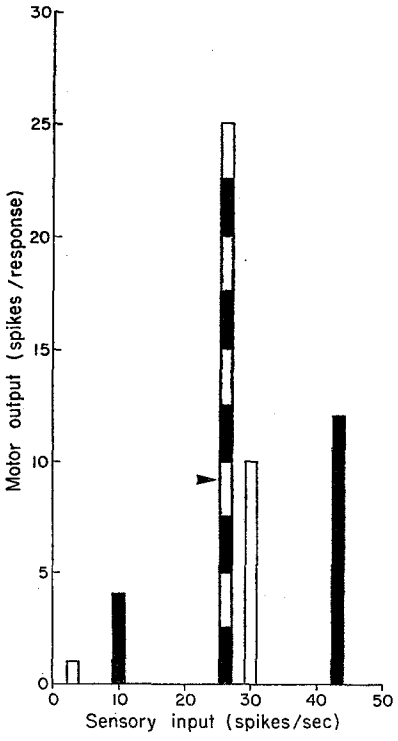

Fig. 6. Two effects of simultaneous stimulation of two sensilla versus individual stimulation of either sensillum is shown. Open bars are the motor response magnitude to stimulation of sensillum A alone at two receptor frequencies. Solid bars show the same except for sensillum B alone. Dashed bar shows the motor response to stimulation of both sensilla simultaneously such that the sum of their activities was 26 spikes per second (sensillum A at 11 spikes/second, sensillum B at 15 spikes/second). The motor response to simultaneous stimultaion of both sensilla is larger than the response to individual stimulation of either sensillum alone. This is true even when the receptor frequency is higher during individual stimulation than the sum of the activities during simultaneous stimulation of both sensilla. The arrow on the dashed bar indicates the purely additive sum of the motor responses to stimulation of both sensilla individually at the same sensory frequency per channel as during simultaneous stimulation of both sensilla

excitatory level, I stimulated sensillum A for one second. Immediately following the sensory spike train of sensillum A, sensillum B was stimulated. The motor response to stimulation of sensillum B immediately following stimulation of sensillum A was compared to stimulation of sensillum B alone. Spatial summation was not possible in this case because both receptors were never active at the same time. There was

no significant difference in the motor response to stimulation of sensillum B in the two cases, and the fine structure of the sensory spike trains were identical. Prior sucrose stimulation did not induce a change in central excitatory level with regard to activity of the extensor-adductor muscle group. This result indicates that the increased motor activity elicited by the simultaneous stimulation of two sugar receptors was probably due to spatial summation between sensory inputs.

Discussion

Behavioral Comparisons

Direct comparison of these results with behavioral studies can be at best of a qualitative nature due to the difficulty in quantifying behavioral responses. However, a few comparisons are possible. From 223 sucrose stimulations of single labellar sensilla on flies starved between 60 to 72 hours the average latency from the moment of solution contact with the hair to the first motor spike was 43 milliseconds for the small motor unit and 54 milliseconds for the large. These values are about one-half the latency of 100 milliseconds recorded cinematographically for tarsal sugar receptors (Dethier, 1955) and electrophysiologically for labellar receptors (Dethier, Solomon, and Turner, 1965). The fact that the electrophysiological latencies recorded in this study are considerably less than those recorded cinematographically is not surprising. The additional delay can be attributed to the longer conduction pathway for sensory information from tarsal receptors through the thoracic ganglion to the brain, and to delays between the occurrence of a muscle impulse and the actual movement of the proboscis due to elastic and inertial properties of the musculature and proboscis. The motor activity recorded by Dethier, Solomon, and Turner (1965) can not be attributed to any specific muscles, so these latencies are not directly comparable.

Comparison of the magnitude of extensor-adductor activity with behaviorally observed extension of the proboscis is again difficult. The only published data on the duration of proboscis extension as a function of stimulus intensity were obtained by stimulating a large number of tarsal receptors (Dethier, 1952). Due to the nonlinear aspects of the summation between receptor activities, the results of tarsal stimulation of many receptors cannot be compared to stimulation of a single labellar sensillum.

A Model

The minimal neuronal model shown in Fig. 7 is proposed to facilitate discussion of the results. The following simple assumptions were made: each sugar receptor impulse results in an excitatory post-synaptic potential (EPSP) in interneuron A; these EPSP's can summate temporally

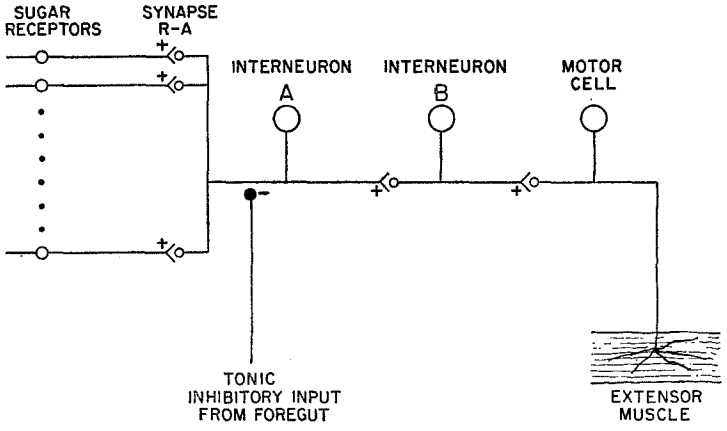

Fig. 7. A minimal neuronal model to account for motor activity in the extensor of the haustellum in response to sucrose stimulation of labellar sensilla (similar to that proposed by Dethier *et al.*, 1965). Each labellar sugar receptor sends its axon to the suboesophagial ganglion without synapsing (Sturckow *et al.*, 1967). Since summation between sugar receptor activities occurs, the receptor inputs converge postsynaptically at Interneuron A through the synapse R-A. Each spike in the receptor axon results in an excitatory post-synaptic potential (EPSP) in Interneuron A. These EPSP's summate temporally and spatially resulting in spike activity in Interneuron A which is transmitted to the motor cell via Interneuron B. A single spike in Interneuron A is sufficient to initiate motor activity. Central habituation occurs on a single input channel before the convergence of the sensory inputs and therefore is probably due to processes occurring presynaptically at the synapse R-A. Behavioral experiments suggest that the responsiveness to sugar stimuli is controlled by tonic inhibitory input from stretch receptors in the foregut which is transmitted to the brain via the recurrent nerve (Evans and Browne, 1960; Dethier and Gelperin, 1967; Gelperin, 1967). This inhibitory input is shown impinging on Interneuron A. Interneuron B is not absolutely necessary, however, the interplay of this system with other sensory modalities suggests its presence (Dethier *et al.*, 1965). Excitatory synapses are indicated by (+) sign; inhibitory by (−) sign

and spatially to produce a spike in interneuron A which initiates proboscis extension. A single sugar spike was never sufficient to initiate a response. Acceptance of a solution applied to a single labellar sensillum was mediated by the temporal summation of sugar receptor activity within approximately 20 milliseconds after the first sugar spike. Since receptor adaptation occurred quickly in these receptors this period contained the maximum spike activity. Only two spikes within a 20 millisecond period were required to trigger a response for flies starved for 70–72 hours, whereas the threshold in flies starved between 62–66 hours was higher and three spikes were necessary. (Tables 1 and 2). The number of EPSP's needed to reach threshold in interneuron A, and thus initiate extension,

is probably controlled by tonic inhibitory input from stretch receptors in the foregut known to control sugar acceptance thresholds (Evans and Browne, 1960; Dethier and Gelperin, 1967; Gelperin, 1967) and possibly from stretch receptors in other parts of the gut.

Spatial summation between chemosensory inputs has been shown behaviorally (Dethier, 1953, 1955). In order for spatial summation to occur the sensory inputs must converge. In the model the convergence occurs post-synaptically in interneuron A. However, habituation must be due to processes occurring before the convergence of sensory inputs and appears independent of sensory activity of other sugar receptors. The presumed site of the observed habituation is presynaptically at the receptor-interneuron A synapse. Habituation due to presynaptic processes has been observed directly in the *Aplysia* gill withdrawal reflex (Castellucci *et al.*, 1970).

A Role for Receptor Adaptation and Central Habituation

The magnitude of the motor response appeared to be determined in part by receptor adaptation and habituation at central synapses. The effects of these two phenomena can best be seen by analysis of the motor response to stimulation of a single sensillum with low and high sucrose concentrations (Fig. 1). Two sugar spikes with an ISI of less than 20 milliseconds were found to be sufficient to initiate motor activity. In the following analysis, it is assumed that a sensory ISI of less than 20 milliseconds was also sufficient to maintain the motor response. Therefore, the magnitude of the motor response will be determined by the time integral of the sensory input for which the ISI was less than 20 milliseconds. Examination of the sensory and motor responses to 100 mM sucrose stimulation (Fig. 1, S_1 and M_1) shows that receptor adaptation to a sensory ISI greater than 20 milliseconds occurred within the first 50 milliseconds of the sensory stimulation. However, the motor response continued for about 100 milliseconds. Brief stimulation of a single hair showed that sensory input with an ISI less than 20 milliseconds was sufficient to produce a prolonged motor output (Fig. 3). Continued sensory input at an ISI greater than 20 milliseconds probably did not contribute to the maintenance of the motor output. Due to receptor adaption the sensory ISI quickly attained a value greater than 20 milliseconds and therefore could not maintain the motor response.

At higher sucrose concentrations the motor response terminated despite sensory input with an ISI less than 20 milliseconds (Fig. 1, S_2 and M_2). The cause must therefore be a form of central habituation. Motor activity probably terminated due to a decrease in the amplitude of successive EPSP's, which decreased the ability of a given EPSP to summate with previous activity. A similar decrease in the amplitude of

successive EPSP's has been observed directly in frog motor neurons (Fadiga and Broockhart, 1962) and *Aplysia* motor neurons (Kupfermann *et al.*, 1970).

Mechanism of Nonlinear Summation

The functional organization of the fly nervous system that might underlie nonlinear summation of sensory inputs is of particular interest. From single and multiple stimulations of labellar sensilla, five characteristics of the neuronal network can be identified which lead to nonlinear summation. First, the sensory inputs converge post-synaptically at interneuron A allowing for spatial summation of the EPSP's due to activity in different sugar receptors. Second, central habituation occurs before the convergence of the sensory inputs. Third, habituation appears to occur on a single input channel independently of activity in other sugar receptors. Fourth, the sensory spike trains from different sugar receptors are temporally independent. Fifth, the motor response is triggered and appears to be maintained by the temporal summation of two sensory spikes impinging upon interneuron A with an ISI less than some critical value.

To ellucidate the contribution of these five system properties to nonlinea, summation, the motor response to simultaneous stimulation of two sensilla was compared to stimulation of a single sensillum at a frequency equal to the sum of the activities during simultaneous stimulation (Fig. 5, lower three sets of traces). Since habituation occurs before the convergence of sensory inputs and appears independent of other sugar receptor activity, the rate of central habituation is lower for the two channel input due to a lower spike frequency per channel. This is true despite the same total sensory activity impinging upon interneuron A.

For the two channel input case, EPSP's can occur with any interval due to the convergence of the temporally independent sensory spike trains. Thus two EPSP's able to summate and trigger a spike in interneuron A can occur at any time during the sensory stimulation. This is not true for the single input case. Due to receptor adaptation the ISI of sensory spikes arriving at interneuron A increases with time, thus decreasing the ability of successive EPSP's to summate. A further indication of the importance of cross-channel interspike intervals is demonstrated by the fact that the motor response to stimulation of two sensilla could be from one to six times the sum of the motor responses to individual stimulation. Each simultaneous stimulation resulted in a different cross-channel interspike interval pattern which gave rise to different motor outputs for each input pattern. However, in the long run habituation dominates and the motor response terminates despite on-going sensory activity. Summation of this type has the behavioral

effect of regeneratively directing the proboscis towards the source of stimulation as more sensilla become stimulated.

This work was submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in biophysics, University of California, Berkeley, California. I wish to thank Dr. Richard A. Steinhardt for his continual advice, guidance, ideas, and heartening encouragement in his role as thesis advisor. Funded in part by the following grants: a predoctoral training grant to P.A.G. USPHS grant No. 5-T01-GM00829, and Biomedical Science Support Grant and USPHS grant No. GM1021-08 to Dr. R. Steinhardt. Dr. C. H. F. Rowell, Dr. D. Bentley, and Dr. D. Kennedy kindly criticized the manuscript. I also thank my wife, Marna, for her help in the preparation of the manuscript.

References

Castellucci, V., Pinsker, H., Kupfermann, I., Kandel, E.: Neuronal mechanisms of habituation and dishabituation of the gill-withdrawal reflex in *Aplysia*. Science **167**, 1745–1748 (1970).

Dethier, V. G.: Summation and inhibition following contalateral stimulation of the tarsal chemoreceptors of the blowfly. Biol. Bull. **105**, 257–268 (1953).

— The physiology and histology of the contact chemoreceptors of the blowfly. Quart. Rev. Biol. **30**, 348–371 (1955).

— The nerves and muscles of the proboscis of the blowfly *Phormia regina* Meigen in relation to feeding responses. Smithson. Inst. misc. Coll. **137**, 157–174 (1959).

— Chemosensory input and taste discrimination in the blowfly. Science **161**, 389–391 (1968).

— Feeding behavior of the blowfly. Adv. study Behav. **2**, 111–266 (1969).

— Gelperin, A.: Hyperphagia in the blowfly. J. exp. Biol. **47**, 191–200 (1967).

— Hanson, F. E.: Electrophysiological responses of the chemoreceptors of the blowfly to sodium salts of fatty acids. Proc. nat. Acad. Sci. (Wash.) **60**, 1296–1303 (1968).

— Solomon, R. L., Turner, L. H.: Sensory input and central excitation and inhibition in the blowfly. J. comp. physiol. Psychol. **60**, 303–313 (1965).

Evans, D. R., Barton Browne, L.: Physiology of hunger in the blowfly. Amer. Midland Naturalist **64**, 282–300 (1960).

— Mellon, De F.: Stimulation of a primary taste receptor by salt. J. gen. Physiol. **45**, 651–661 (1962a).

— Electrophysiological studies of a water receptor associated with the taste sensilla of the blowfly. J. gen. Physiol. **45**, 487–500 (1962b).

Fadiga, E., Brookhart, J. M.: Interactions of excitatory postsynaptic potentials generated at different sites on the frog motoneuron. J. Neurophysiol. **25**, 790–804 (1962).

Gelperin, A.: Stretch receptors in the foregut of the blowfly. Science **157**, 208–210 (1967).

Gillary, H. L.: Stimulation of the salt receptor of the blowfly. III. The alkali halides. J. gen. Physiol. **50**, 359–368 (1966).

Graham-Smith, G. S.: Further observations on the anatomy and function of the proboscis of the blowfly, *Calliphora erythrocephala* L. Parasitology **22**, 47–115 (1930).

Hodgson, E. S.: Electrophysiological studies of arthropod chemoreception. II. Responses of labellar chemoreceptors of the blowfly to stimulation by carbohydrates. J. Insect Physiol. **1**, 240–247 (1957).

— Lettvin, J. Y., Roeder, R. D.: Physiology of a primary chemoreceptor unit. Science **122**, 417–418 (1955).

Kupfermann, I., Castellucci, V., Pinsker, H., Kandel, E.: Neuronal correlates of habituation and dishabituation of the gill-withdrawal reflex in *Aplysia*. Science **167**, 1743–1745 (1970).

Omand, E., Dethier, V. G.: An electrophysiological analysis of the action of carbohydrates on the sugar receptors of the blowfly. Proc. natl. Acad. Sci. (Wash.) **62**, 136–143 (1969).

Steinhardt, R. A.: Cation and anion stimulation of electrolyte receptors of the blowfly, *Phormia regina*. Amer. Zoologist **5**, 651–652 (1965).

Stürckow, B., Adams, J. R., Wilcox, T. A.: The neurons in the labellar nerve of the blowfly. Z. vergl. Physiol. **54**, 268–289 (1967).

Tateda, H., Morita, H.: Initiation of spike potentials in contact chemo-sensory hairs of insects. I. The generation site of the recorded spike potentials. J. cell. comp. Physiol. **54**, 171–176 (1959).

Wilczek, M.: The distribution and neuroanatomy of the labellar sense organs of the blowfly, *Phormia regina* Meigen. J. Morph. **122**, 175–201 (1967).

Wolbarsht, M. L., Dethier, V. G.: Electrical activity in the chemoreceptors of the blowfly. I. Responses to chemical and mechanical stimulation. J. gen. Physiol. **42**, 393–412 (1958).

Dr. Peter A. Getting
Department of Zoology
University of Washington
Seattle, Washington, U.S.A.