# HABITUATION, DISHABITUATION, AND TRANSFER OF HABITUATION IN THE LAST ABDOMINAL GANGLION OF THE COCKROACH, P. AMERICANA

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# ABSTRACT

# HABITUATION, DISHABITUATION, AND TRANSFER OF HABITUATION IN THE LAST ABDOMINAL GANGLION OF THE COCKROACH, P. AMERICANA

By

Phillip William Singer

This investigation was performed to demonstrate a model system suitable for the study of habituation. The number of spikes recorded in axons of the connectives anterior to the last abdominal ganglion of the cockroach, *P. Americana*, in response to deflection of the cercus, were counted as a function of both number and frequency of deflections. The number of spikes decreased as the number of deflections increased, but returned to a higher level after a rest period. The decrease in number of spikes was moderated by increasing the frequency of deflection; this decrease was also moderated by deflection of the contralateral cercus. After a series of deflections, deflection of the contralateral cercus yields fewer spikes than if not preceded by a series of deflections. These results are taken as examples, respectively, of habituation, with the usual properties of increased frequency of the habituating

stimulus; dishabituation by a novel stimulus, and transfer of habituation; in a simple neural system.

The preparation was prepared by dissecting out the last abdominal ganglion, cutting the anterior connectives and cercal motor nerves, and attaching a device for mechanically deflecting a cercus medially. Recordings were made with suction electrodes, platinum hook electrodes, and glass insulated tungsten microelectrodes.

Deflection of a cercus results in a train of 15-30 spikes, lasting 40-70 msec., being recorded in the anterior connectives. Repeating the stimulus at a rate of 1.6/sec. results in a reduction of the response in both number of spikes and length of train. The reduction is complete within 10 trials, and consists of a 50% reduction of spikes and a reduction of train length to 20-25 msec. The number of spikes and train length are highly correlated.

Dishabituation was found to require a stimulus upon the contralateral cercus different from that on the ipsilateral cercus. Dishabituation does not result from an increase in intertrial background discharges.

Transfer of habituation is apparent after a single training trial. The degree of habituation transferred was on the order of a 10% decrement from the initial value, as compared to a 50% decrement on the trained cercus.

Close analysis of the spike train revealed that there are three classes of spikes in the train: Giant

spikes, which did not habituate; medium spikes, which did; and small spikes, which are scattered throughout the train. An afterdischarge was found in those axons responsible for the medium spikes. The theory is advanced that the habituation mechanism is a reduction of the afterdischarge, reducing the train to 25 msec.

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# INTRODUCTION

This study is in the area of learning and memory. This problem has many facets, one of which is the difficulty of even formulating the questions. Evidently, before any precise work can be done, the underbrush must be cleared away.

## Definition of Learning

The subject area itself, learning and memory, is poorly defined. Some authors (Glaser 1966) are so restrictive as to exclude all animals, except some mammals, from the capability of learning. Others (Eisenstein 1972) want to generalize it enough to allow protozoa to be said to learn. This distinction can roughly be said to center around the amount of "thought" believed to be used in the learning operation by the animal involved.

Glaser provides a suitable example of a restricted definition of learning. He defines learning as the production of new responses; the animal now responds to a stimulus in a manner which it had never used previously in response to any stimulus. Glaser feels that this forces learning to be a by-product of what he believes to be "thought." This definition excludes phenomena such as Pavlovian conditioning, and Glaser thinks this desireable.

The exact content of "learning" is an epistemological question that each person must ultimately answer for himself. But there are good methodological reasons for defining learning so as to incorporate such phenomena as habituation and conditioning. "Thought" is not a quantity that can be measured, either quantitatively or qualitatively, nor is there any agreement among philosophers as to what "thought" is. Phenomena such as conditioning are the easiest to examine experimentally and interpret theoretically. This is because all possible experimental outcomes can be listed in advance; the system is self-contained. Moreover, the patterns of stimuli are as simple as possible, and the stimuli can be made as simple and explicit as possible.

A general definition is that of Eisenstein. The formal statement is as follows:

> A system is said to demonstrate learning when the output (response) to a given test input (stimulus) is a function of the total previous input-output pattern of which the test input was a part.

Such a formulation includes instrumental learning as well as Pavlovian conditioning and habituation. It applies to any case in which the organism's behavior (output) changes over time, in response to some repeated input (stimulus), and where the change is dependent on the particular context in which the stimulus and response occurred. One advantage is that it allows one to sensibly discuss learning related phenomena in isolated parts of the nervous system, e.g., a single ganglion.

## Habituation

Habituation was chosen as the specific type of learning for this study for the following reasons. It seems to be the most primitive type of learning, as well as one of the most widespread. It is also involved in most other kinds of learning. Finally, the theoretical analysis is straightforward.

The following is the most common definition of habituation:

> A subject (or preparation) is said to exhibit habituation to a stimulus when, after repeated presentations of the stimulus (1) the response shows a decrement, and (2) after a rest period, the response level demonstrates recovery.

However, the theoretical description of habituation is currently unsatisfactory. Theories of habituation are, without exception, *ad hoc* postulates which do little more than shift the unknown quantity into something less amenable to experimentation than habituation itself. The definition is refined in the methods section; elsewhere I attempt further clarification (Singer, in preparation). I therefore digress slightly to show what is wrong.

First, there is no generally accepted definition of habituation. Some workers (Gardner 1968, Baxter 1957, Hughes 1965) use our definition, while others (Kupferman et al. 1970) require all of Thompson and Spencer's (1966) nine criteria to be present before they are willing to call a response decrement "habituation." The root of the problem is ignorance about what is happening theoretically.

Sokolov (1963) and Glaser (1966) illustrate one extreme of the confusion. Their theories explain habituation by postulating a processing of neural information in the brain. Of course, no one knows how the brain processes information, and thus these theories explain nothing. But more importantly, there are too many instances of habituation in brainless preparations for these theories to be the answer.

Stein (1966) has a theory intermediate in confusion. Rather than leave the explanation in terms of brain processing, he reduces habituation to what he takes as a primitive process, Pavlovian conditioning. However, it seems much more likely that Pavlovian conditioning is a more complicated phenomenon. Furthermore, no attempt is made to explain conditioning, so habituation is not truly explained.

Groves and Thompson (1970) illustrate the opposite extreme to Sokolov. Their theory purports to be a molecular mechanism. Close examination reveals that they do nothing but postulate that the neural elements involved act in such a way as to produce the experimentally observed response. Moreover, they have to postulate a neural process, "facilitation," which they admit is not directly observable experimentally.

Dispite the confusion, habituation is worth studying. Instances of habituation are occurring almost constantly in sensory systems of animals. For example, one is not normally aware of temperature, background noises, or the clothes on one's back even though these stimuli are constantly there. Any organism must have a way of subordinating continuous stimuli to novel, and thus potentially dangerous stimuli, and the process is that of habituation.

## How to Study Habituation

Having decided on the problem to study, it is time to decide on the preparation as well as the stimulus, input, and response output to be measured. Many different systems have been exploited in habituation studies, such as the startle response of rats (Korn and Moyer 1966), recognition in infants (Frantz 1964), the escape response of earthworms (Gardner 1968), the wiping response of the frog (Kimble and Ray 1963) and contractility in Protozoa (Osborn et al. 1973).

The difficulty with most of these preparations is that the results are not unambiguous. It is difficult to exclude other stimuli from the animal, and most of these other stimuli are capable of causing or modifying the desired behavior. Furthermore, even if the technique of the experiment is correct, the data are difficult to interpret. This is because often the interactions of the entire nervous system are acting on the response.

A more tractable approach then, is to use a phylogenetically simpler or surgically simplified preparation. Such studies run the gamut from single cell studies in isolated mammalian spinal cords to protozoa. Molluscs anthropods are convenient preparations as integrative functions tend to be localized in ganglia near the site innervated by the ganglion (Bullock and Horridge 1965). These ganglia can be isolated from the rest of the nervous system with little apparent loss of integrative capacity. Moreover, the surgery is considerably simpler than with animals having highly developed respiratory and circulatory systems.

## Last Abdominal Ganglion

The preparation we have chosen to exploit is the isolated last abdominal ganglion of the cockroach. Besides the above mentioned technical advantages, this preparation has some methodological advantages. The sensory stimuli on the cerci form a well defined input. Moreover, associated with the cerci is a behavior (escape) seen in the intact animal.

The central nervous system is organized around 11 ganglia joined together by a pair of connectives. These are two ganglia in the head, three in the thorax (one for each pair of legs), and six in the abdomen (Guthrie and Tindall 1968) (for simplicity, we are ignoring the stomatogastric nervous system, which does not seem to be involved in what we will study).

The classical picture of the microanatomy of the last abdominal ganglion and associated structures was drawn by Pumphrey and Rawdon-Smith (1937) and by Roeder (1948a), using extracellular recording. They concluded that each cercal nerve contains hundreds of small nerve fibers. These fibers pass into the last ganglion, where some synapse onto cell bodies in either the isolateral or contralateral side, while others, the so-called through fibers, continue up the connectives uninterrupted, again in either side. However, it has recently been shown (Richard et al. 1973) that this ganglion is more complicated in design and function than this simple picture would suggest, and care must be taken to insure that the output observed is entirely due to the experimental input.

## Giant Fibers

An important class of cell bodies in the ganglion are the ones which give rise to giant fibers. First Roeder (1948a), and later Pipa et al. (1959) showed that there are six to eight of these fibers in each connective, lying in three tracts. They fire reliably to electrical

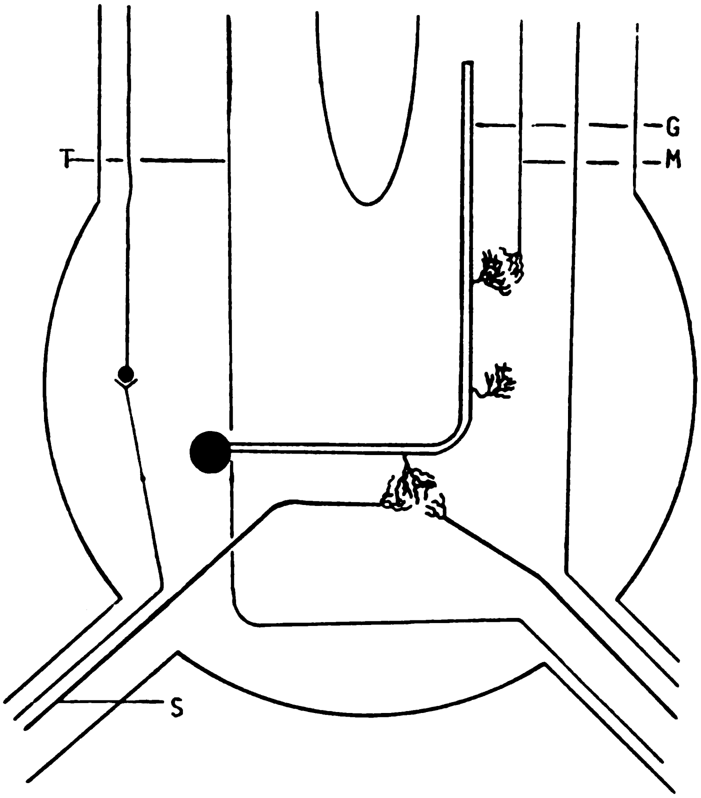

Figure 1.--Diagram of the neuroanatomy of the last abdominal ganglion. S: Sensory fibers; G: Giant fibers; M: Fiber receiving synaptic input from giant fiber, possibly medium fiber of text; T: Through fibers.

stimulation of the cercal nerve at rates up to 20/sec. By histologically examining lesioned specimens at varying degrees of degeneration, it was found (Hess 1958, Farley and Milburn 1969) that each giant fiber had several cell bodies associated with it. However, dye injection studies have only been able to stain one cell per fiber (Harris and Symth 1971, Milburn and Bently 1971).

Giant fibers are important for two reasons. The first is technical; they have a large action potential, easily distinguished from both background noise and other, smaller, axons (Bullock and Horridge 1965). This makes them convenient preparations for electrophysiological studies. The second is behavioral. By tradition, they have been ascribed as mediating escape behavior (but see Appendix 1).

These details can now be related to the preparation used experimentally. For simplicity of analysis, extraneous components, such as input from other ganglia, were removed from the system. Next, a suitable method of stimulation the cerci was derived. Finally, a system for recording from the nervous system was constructed.

# MATERIALS AND METHODS

## Experimental Preparation

Thirty-nine adult, male cockroaches were used. They were anesthetized with $CO_2$ gas, and the legs and wings removed. Next, they were pinned, dorsal side up on a clay block, the dorsal cuticle, the gut, and the fat body removed. The last abdominal ganglion was isolated by cutting the motor nerve to the cercus and also cutting the interganglionic connectives just posterior to the fifth abdominal ganglion. The exposed nervous system was covered with Roeder's saline (1948b). When using suction electrodes the system was next covered with a thin film of Vaseline.

For stimulation, we wanted to stimulate the sensory receptors as naturally as possible. Initially, air puffs on the cerci were tried. This resulted in very few spikes being recorded on the connectives and was difficult to analyze. However, a well defined train of spikes could be generated if a blunt insect pin was attached to the voice coil of a 2" p.m. loudspeaker and fastened to the fifth or sixth joint of a cercus with wax so as to produce a medial deflection of the cercus whenever a pulse was fed to the loudspeaker.

To prevent the deflection from vibrating the entire animal and possibly stimulating other receptors, the base of the cercus was fastened to the block with clay. No spikes were recorded on the connectives when the sensory nerve was cut and the cercus thus stimulated. Occasionally, the wax joint would break. No response was then recorded.

Three msec. square wave pulses provided by a Tektronic 161 & 162 modular pulse generating system were used to drive the loudspeaker, a homebrew amplifier being inserted between them as an impedance metch. For recording, platinum hook electrodes, suction electrodes (consisting of silver wire in a polyethylene tube), and glass insulated tungsten microelectrodes were used, depending on the specific experiment. The preparation and recording procedures are diagrammed in Figure 2.

## Experimental Design

The experimental designs were based upon the following considerations. First, they are immediately adapted to a simple statistical test, the sign test. Second, they permit unambiguous definitions of habituation, dishabituation, and transfer of habituation. Finally, they are delicate enough to detect effects hidden within a large amount of scatter. For example, the magnitude of the transferred habituation is less than the normal variation in the initial response. However, the response reduction is consistent enough to be detected by a sign test.

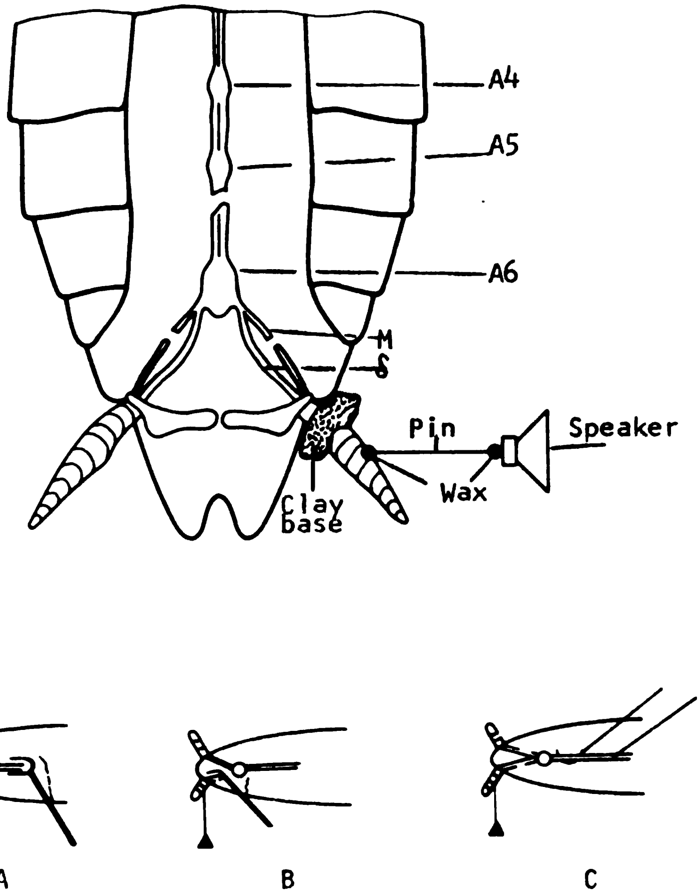

Figure 2.--Top: Diagram of preparation used. Dorsal view. A4 - A6: Last three abdominal ganglia. M: Cercal motor nerve; S: Cercal sensory nerve.

Bottom: Placement of recording electrodes. A: Suction electrode over both cut connectives of A6; B: Suction electrode over cut sensory nerve; C: Hook electrode and microelectrode simultaneously on cut connectives.

## Habituation

The following operational definition was used for habituation in this study. A response decrement was said to occur in a given series of stimulus trials (called runs) whenever the number of spikes recorded on trial 10 was less than that of trial 1; and, after a two minute rest, the response demonstrated at least partial recovery, defined as a response increase over that of trial 10. Habituation was said to have been demonstrated in a given preparation if six or more of the eight runs showed such a decrement. See Bradley (1968) for details of statistics used in this paper.

## Dishabituation

Two different stimuli were used, a single pulse from a stimulator of the contralateral cercus or a brushing of this cercus. The stimulus was applied between trials 10 and 11, with a five minute rest between runs. A response increase was said to occur if the number of spikes on trial 11 (the "test stimulus") was greater than the number on trial 10. A given preparation was said to exhibit dishabituation to a given stimulus if three or more of the five runs made demonstrated a response increase. Both stimuli were used on each animal, one being used in five consecutive runs, and then the other for five runs, the order being varied on different animals.

## Transfer of Habituation

A pulse stimulator was attached to each cercus. An experiment on a given preparation consisted of 10 runs, one each of 1, 2, 4, 6 or 10 trials on either the right or left side, which was then followed by a "test" trial on the contralateral cercus at what would have been the time of the next regular stimulus. Thus there were five runs which began on the right cercus and five which began on the left. Transfer was defined to have been demonstrated in a given run if the number of spikes to the "test" was less than the expected response to the first stimulus on that side. The expected response to the first stimulus on a side was computed as the median number elicited by the first stimulus of the five runs beginning on the side in question. The order of the runs was determined randomly for each experiment. A five minute rest was allowed between runs.

## Data Analysis

The data were analyzed in the following manner. The number of spikes on the first trial of a run was scored as 100 percent. The number of spikes on subsequent trials were scored as a percentage of this first trial. Multiple runs were averaged by medians. Scatter was measured by the interquartile range.

The following variation was used for Figure 6, which compares different initial trials. For a given animal, trial 1, run 1 was scored as 100 percent. Subsequent trials, including trial 1 on subsequent runs, were scored as a percentage of trial 1, run 1.

# RESULTS

## Single Deflection

The deflection of a cercus resulted in a train of spikes being recorded in the preparation (Figure 3A). There were three classes of spikes in the train; large spikes, somewhat smaller (or medium) spikes, and very small spikes. The large spikes, probably from giant fibers, appeared primarily in the first 20 msec., and the medium ones appear primarily thereafter. The small spikes were scattered randomly throughout the trace.

Throughout this paper, when "spike" is mentioned, it is a large or a medium spike that is being considered. The small spikes were scattered randomly throughout the trace, and were easily distinguished from the other spike classes. The spike train, so defined, elicited by a single deflection of a well rested preparation, contains 15-30 spikes and lasts 40-70 msec., depending on the animal.

## Habituation

Repeating the stimulus lead to a progressive decrease in length of train and quantity of spikes (Figure 3B, C). A two minute rest resulted in recovery. This was noted in all 39 preparations, usually in all

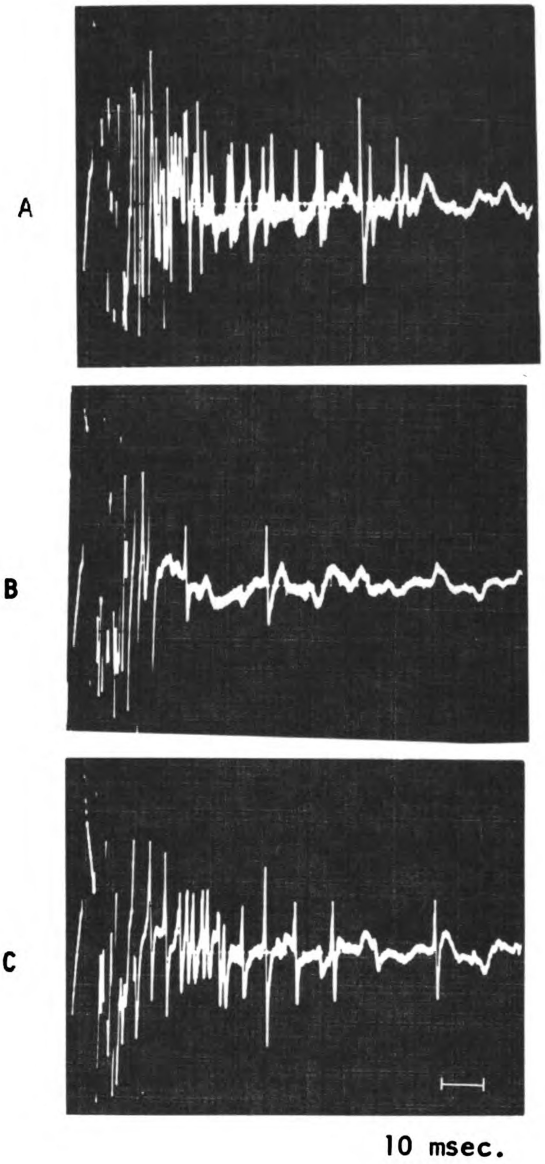

Figure 3.--Results of repetitive stimulation, recording as in Figure 2a. A: Response to first trial; B: Response to trial 10 at a stimulation frequency of 1.6/sec.; C: Response to trial 10 at a stimulation frequency of 1/10 sec.

runs. The spikes did not "drop out" uniformly throughout the train; rather they progressively "dropped out" from the left as is shown by the high correlation between train size and duration (Figure 4). Little, if any change appeared in the first 15-25 msec. of the train. Thus, habituation occurs mainly in the class of "medium size" fibers described above.

Reducing the frequency of stimulation resulted in a reduction of the degree of habituation, the differences appearing in the center portion of the train (Figures 3c and 5). Intervals longer than 30 seconds did not lead to noticeable habituation over a 10 to 20 trial period. Individual variations tended to mask the frequency effects for the early trials; however by trial 10 the different frequencies were seen to fall into clearcut rate classes (Figure 5).

There was no deterioration of the preparation during the experiments; as for the 34 preparations for which spikes were counted, the initial trial on the last run contained more spikes than the initial trial of the first run in 15 cases. Fifteen preparations showed the reverse, and there were four ties.

We had planned to compare subsequent runs with the first run to see if repeated training results in faster habituation. Preliminary work had suggested that recovery was complete within two minutes, following 20 trials at

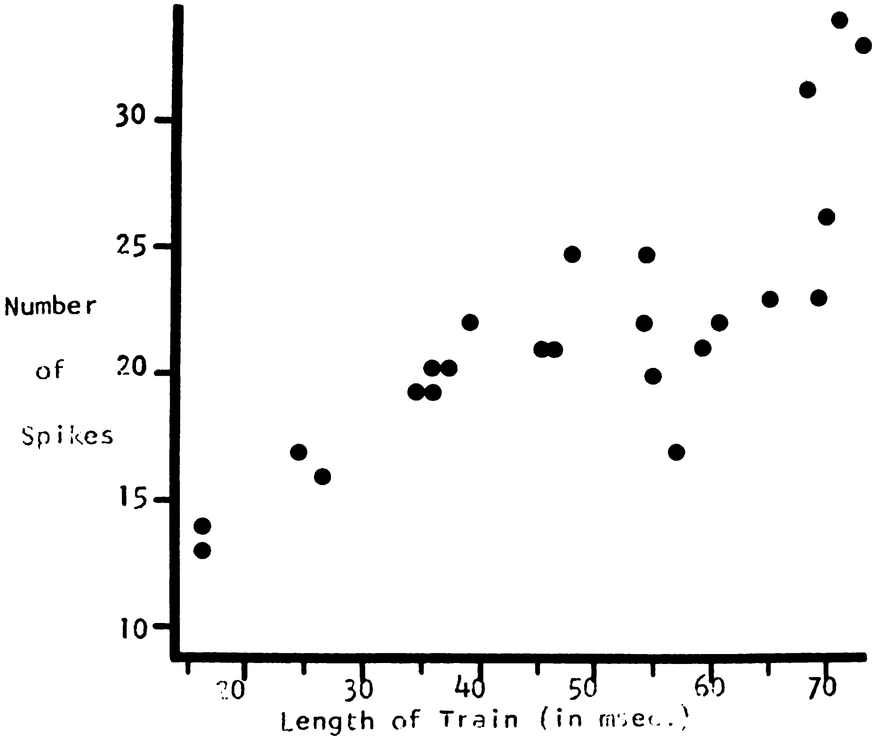

Figure 4.--Correlation between length of spike train and the number of spikes in the train. Study made on 4 runs of one preparation at the rate of 1.6/sec. Correlation significant at .001 level (Hotelling and Pabst's Spearman rank order correlation test).

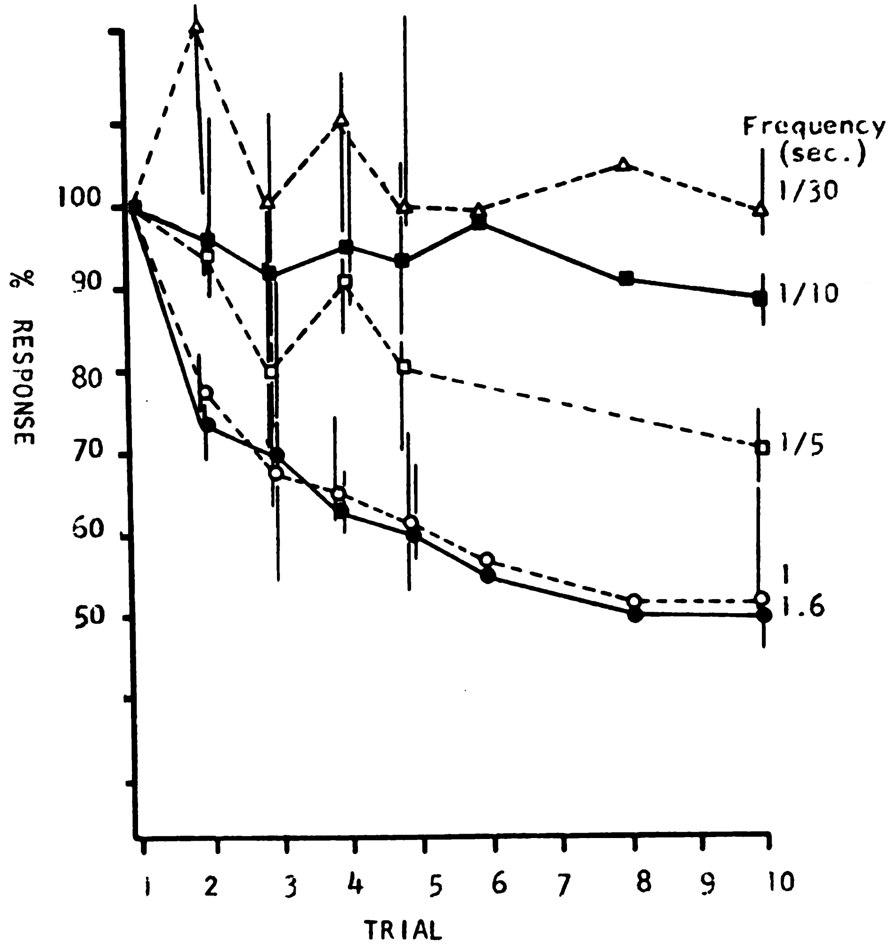

Figure 5.--Habituation at different stimulation rates. The difference between trials 1 and 10 is significant at the .05 level for all runs except 1/30 sec. (two tailed sign test). Error bars are interquartile ranges. The same seven animals used for all frequencies except 1/30 sec., where data on four animals were collected. Median percent response plotted.

the rate of 1.6/sec. A more detailed examination (Figure 6) revealed that there is still some retention at the start of the second run. There was no change with further training; trial 1 or runs 3, 4 and 5 did not differ significantly from trial 1 of run 2. Also, there is no increase in the degree of habituation, all five curves asymptoting at the same level (N = 12 for all of these statements about Figure 6; $p < .05$, two tailed sign test). Recovery is complete after five minutes, as is shown in the transfer of habituation series. In 40 instances where the same cercus was trained twice in sequence, fifteen times the initial response to the second run was greater than the first, 23 times it was less, and there were two ties. The difference is not even significant at the .10 level.

Habituation was not due to a reduction of the sensory input in the cercal nerve, but rather to a reduction of output from the ganglion. This is seen by looking at the response in the isolated cercal nerve to stimulation of the cercus. It consists of 8-12 large action potentials, which appear to be compound potentials, lasting typically 20-25 msec (Figure 7). That habituation is not due to a reduction of activity in the cercal nerve is inferred from the following two observations: (1) using the above criterion for habituation (6 or more of the 8 runs must show a decrement) four animals out of seven were found to demonstrate a response decrement between trials 1 and 10,

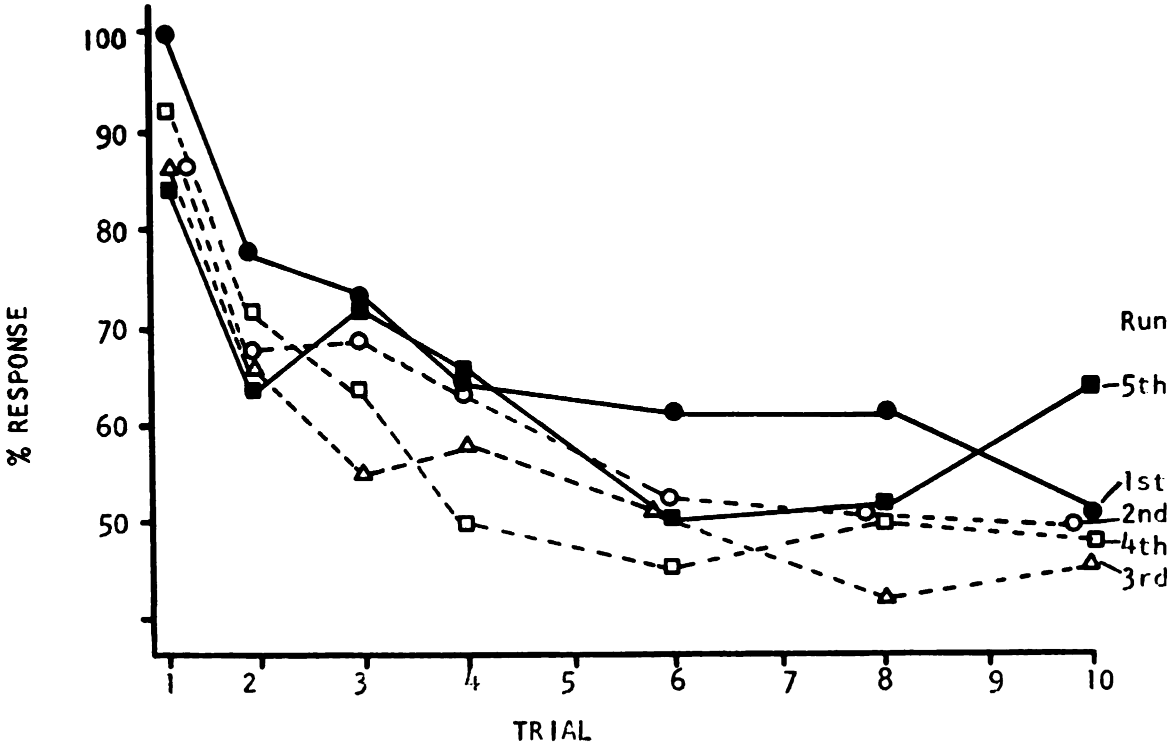

Figure 6.--Effects of a two minute rest upon subsequent habituation runs. 100% = trial 1, run 1. Stimulus rate 1.6/sec. Median percent response plotted.

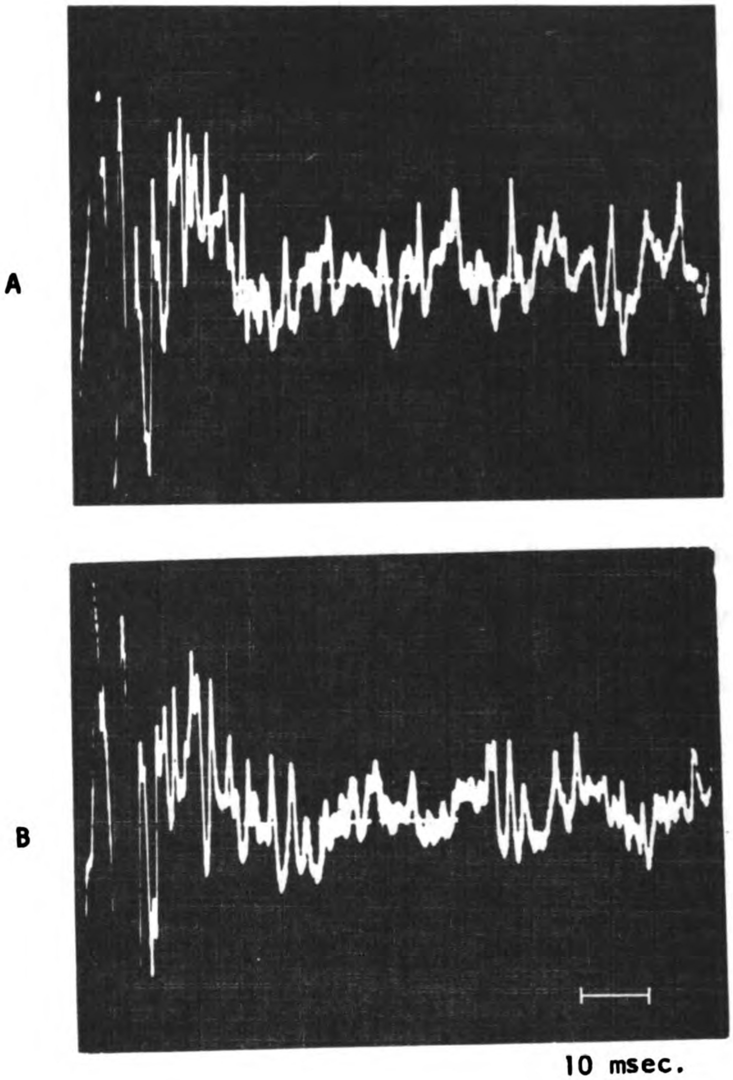

Figure 7.--Typical response in cercal nerve, showing no habituation. Recorded as in Figure 2b. Stimulation rate 1.6/sec. A: Trial 1; B: Trial 10.

two showed an increase, and one showed no change. A two-tailed sign test is not even significant at the .50 level, whereas the decrement from the connectives is highly significant ($p < .001$); (2) 80% of all runs on all animals show a response <u>increase</u> on the sensory nerve between trials 1 and 2, while more than 80% of the recordings from the connectives show a <u>decrease</u> between trials 1 and 2.

Although others (Hughes 1965) have also noted that the cercal nerve response is independent of the number of stimuli, Zilber-Gachelin and Chartier (1973a) dissent. They find a partial reduction in cercal activity, in degree amounting to about half of that found on the connectives. This discrepancy may be partly explained by our stimulating primarily one type of receptor (joint receptors) while Zilber-Gachelin and Chartiers and Hughes stimulated hairs. At the rates Hughes used, 1/30 second and slower, Zilber-Gachelin and Chartier found no decrement in the cercal response either.

In comparing this work to that of Hughes, Constantine (1972), and Zilber-Gachelin and Chartier, it is found that they did not remove the upper abdominal ganglia. When we repeated our habituation experiments, but sectioned the nerve cord at the level of the first, rather than the last, abdominal ganglion it resulted in well over sixty spikes being recorded at the level of the last ganglion in a fifty msec. time frame (as compared with thirty in the isolated ganglion). Neither Constantine nor Zilber-Gachelin and Chartier were able to find evidence for transfer of habituation, most likely because the change is now too small relative to the total response, to measure. Similar remarks pertain to Zilber-Gachelin and Chartier's failure to find dishabituation.

## Transfer of Habituation and Dishabituation

The number of spikes elicited was reduced if the contralateral cercus was first stimulated. This transfer of habituation was evident after the very first ipsilateral stimulus (Figure 8). In transfer of habituation, no difference was observed between the left and right sides, and the results are pooled in the following discussion.

The decrement was significant at the .01 level for test trials 4 and 10, the .05 level for trials 1 and 2 and the .10 level for trial 6, using a two-tailed sign test on the 15 animals. The extent of habituation (on the "test" side) is less than that of the fully habituated side. Indeed, the test side is habituated only to the extent of 8-15%, compared to approximately 50% for the trained side. No trend is seen in the magnitude of transfer as a function of level of habituation training.

Interpolating a suitable stimulus on the contralateral cercus during a run resulted in a response increase to the next regular stimulus trial (dishabituation). The

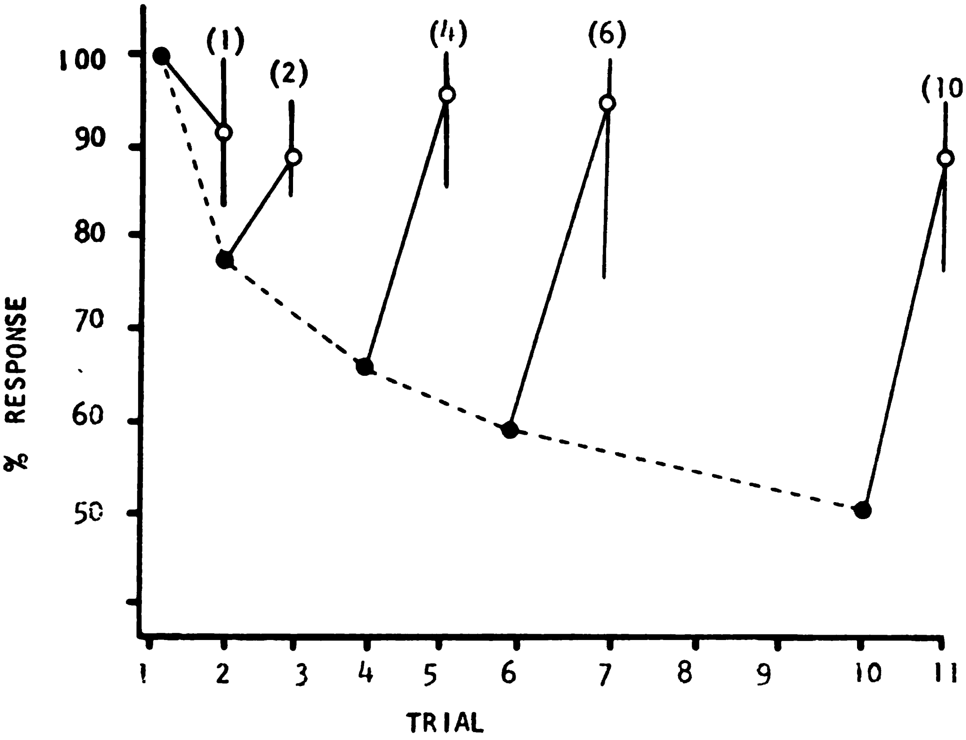

Figure 8.--Relation of transfer of habituation to habituation. Dotted line is the habituation curve for the 15 preparations; solid line signifies transfer to the contralateral cercus. The point at the end of the solid line is the median response to the "test" at that point. Numbers in parentheses refer to the number of prior training trials before the test for transfer. Median response plotted. Error bars are interquartile ranges.

typical increase in responsiveness was two spikes (Table 1 and Figure 9). Unlike habituation, dishabituation was not present on every run. Critical to the mechanism of the dishabituation is the determination of whether the increased portion of the response occurs to the test stimulus, or is due to an increase in background discharges as a result of the dishabituating stimulus. No correlation whatever was found between background activity before trial 11 and the presence or absence of dishabituation on trial 11 ($n = 51$, $p < .95$, $\chi^2$ test). Indeed, on many runs no background activity whatever was noted. Figure 10 is an example.

TABLE 1.--Data on dishabituation. The results with the pulse stimuli are not significant; those with the brush are significant ($p < .05$, two-tailed sign test).

| Dishabituating Stimulus | No. of Experiments with Dishabituation | No. of Experiments without Dishabituation |
|---|---|---|
| Brush | 10 | 0 |
| Pulse Stimulator | 7 | 3 |

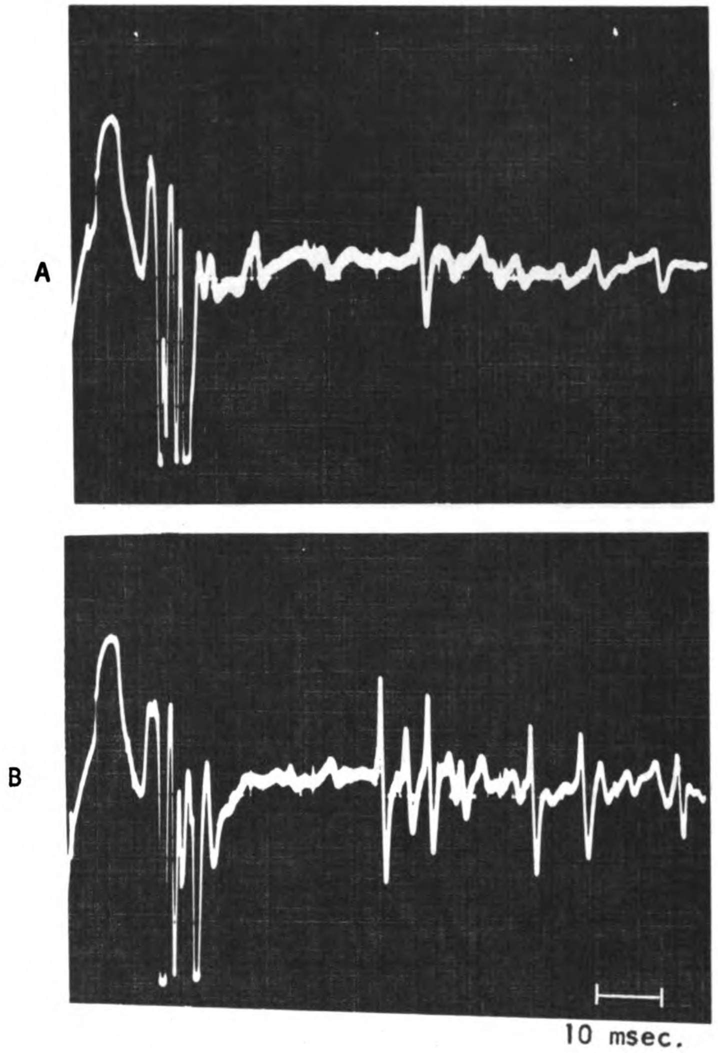

Figure 9.--Example of dishabituation with a brush stimulus.
A: Trial 10 (before dishabituating stimulus);
B: Trial 11 (after dishabituating stimulus).

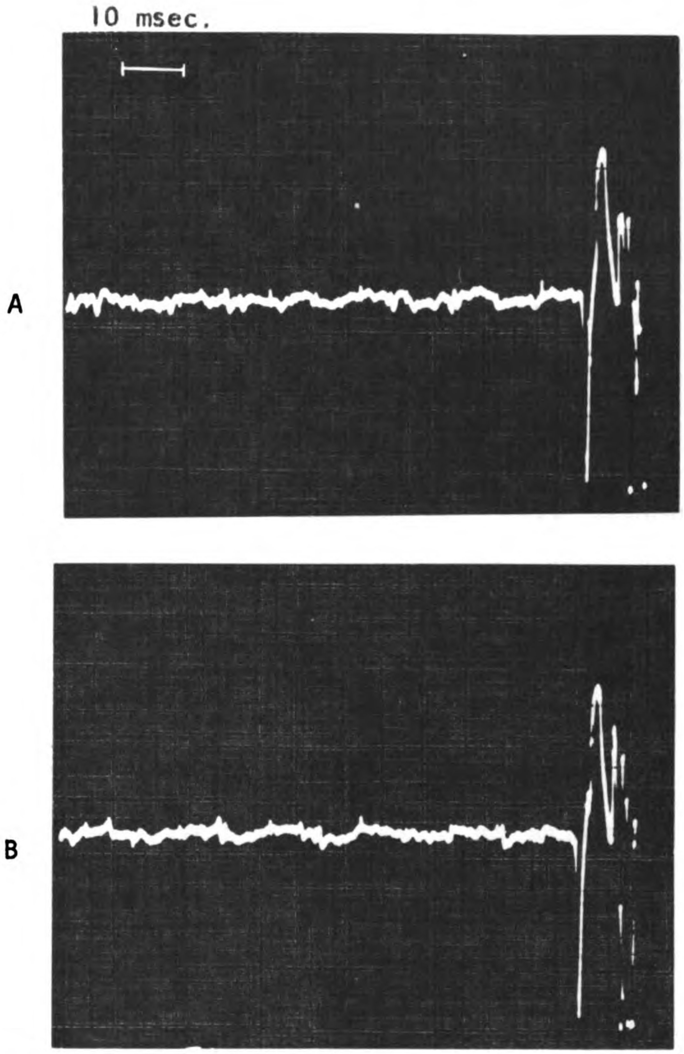

Figure 10.--Examples of background activity on connectives prior to testing for dishabituation. The start of the response to the test stimulus is visible at the far right. A: Background prior to last habituation trial (10); B: Background prior to (successful) test for dishabituation (11).

## Microelectrode Studies

Harris (1969) stimulated the cercal nerve with brief electrical pulses and recorded after-discharges from single fibers in the anterior connectives of the last ganglion. This after-discharge was located in fibers 10-15 microns in diameter. This probably corresponds to the size range of our medium sized spikes, the class of fibers which showed habituation.

The significance of Harris' work can be seen by considering the following question: What triggers the final 35 msec, of spikes in our spike train? The response in the cercal never lasts only 25 msec. The known synaptic delays and conduction velocities can account for almost another 5-10 msec. However, the final 35 msec. of the spike train is left without a direct stimulus. An after-discharge would explain the presence of spikes without a stimulus.

Since the problem was to record from single axons, but not necessarily to make intracellular potential measurements, intracellular electrodes were not necessary. It was found that glass microelectrodes did not penetrate readily, nor were they easily inserted at a specific place in the nerve cord. The tungsten electrodes avoided these problems. The small tip size ($\sim 10\mu$) insured that only a one or two axons would be recorded from (Figure 11).

The results of the recordings confirm that there is an after-discharge present in the ganglion, and that indeed

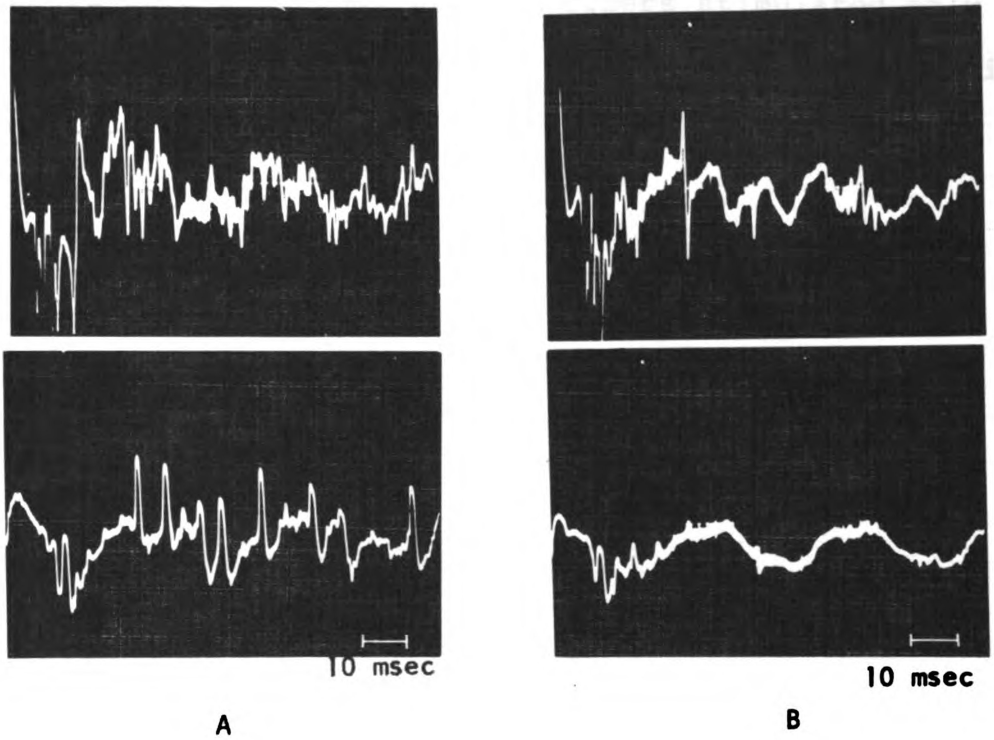

Figure 11.--Typical recordings with micro- and hook electrodes, as in Figure 2c. Stimulus rate 1/sec. Upper trace is the hook electrode, lower trace microelectrode. A: Trial 1; B: Trial 10.

it is responsible for the spike train. No giant fiber was recorded from in this study, so comparisons between giant and medium fibers cannot be made. Other classes of fibers were observed, such as fibers stimulated only by the contralateral cercus, but they were not studied in detail.

# DISCUSSION

The results just described show that the isolated last abdominal ganglion is thus a suitable model system for the study of habituation. This is important, not because of any relationship the model phenomena may have to a behavior in the intact animal (see Appendix 1 for more discussion on this point) but rather because of the possibility for understanding the details of a neural circuit capable of producing habituation. This is because the ganglion is highly amenable to single cell studies.

For example, the work of Callec and Boisted (1971) demonstrated habituation of the post synaptic potential in giant cells of the last abdominal ganglion. These results cannot presently be applied to the spike train, as the cell bodies involved are unknown; however, the difficulties in advancing the work to the point where these results could be applied is much less than in mamalian systems.

Dye injection studies seem to be the quickest way to determine the microanatomy of the circuit involved in the habituation. Fortunately, the cockroach is one of the favorite animals used in such studies. Thus far, efforts have concentrated on injecting the giant axons, possibly because they were the only ones thought to be important. After this study, there should be some effort made to inject the medium axons.

The advantages of the definitions employed are shown by this study having found transfer of habituation, whereas Constantine reported no evidence of it. Applying our criteria to her data would indicate that she did obtain it. The difficulty probably was that in Constantine's preparation the transfer is not dramatic and, having no clear-cut decision procedure, she preferred to err on the side of caution. To avoid ambiguities on this point, it is best to give a detailed, explicit statement of the criteria one is using, as was done in the Methods section of this paper.

## Model

It seems paradoxical that both transfer of habituation and dishabituation can be induced by the same input channel. However, if one postulates that the two cercal inputs converge and effectively overlap on the same postsynaptic fiber, and that habituation occurs post synaptically, then habituation and transfer of habituation are easily explained. Transfer of habituation is explained by the overlap as the contralateral input finds its postsynaptic membrane already partially depressed. Dishabituation might be due to temporal summation. That is, the extra dishabituating stimulus may produce some postsynaptic depolarization which is not sufficient to produce spiking. However, the depolarization produced by the next regularly occurring stimulus in the habituation sequence adds to the first and produces a larger afterdischarge than would have otherwise occurred (mechanism of dishabituation suggested by F. Krasne to E. Eisenstein).

# SUMMARY

1. Brief mechanical deflection of a Cockroach's cercus results in a spike train in the anterior connectives of the isolated last abdominal ganglion which consists of 15-30 spikes and lasts 40-70 msec.

2. Habituation of the spike train is complete within ten trials at a stimulation range of 1.6/sec., and is retained for at least two minutes.

3. The extent of habituation decreases as the frequency of stimulation is decreased.

4. Both transfer of habituation and dishabituation have been observed.

5. The habituation process is hypothesized as the progressive reduction of after-discharges in "medium" sized fibers.

6. A possible model for the neural elements involved is discussed.

# APPENDIX

# APPENDIX

## GIANT FIBERS AND ESCAPE

For a great many years it was believed that the giant fiber system in the cockroach mediated the escape response. Consequently, any work on the last abdominal ganglion contained a discourse on escape behavior. Very recent developments have changed the picture, however, and these events are sketched here for the sake of completeness.

A puff of air to the roaches' cerci results in escape behavior (Baxter 1957). The receptors on the cerci are extremely sensitive to air vibrations (Wozniak et al. 1967), and produce responses in the giant fibers. By the mid 1960's the correlation between behavior and physiology seemed complete, and the search was on to trace the giant fiber response to the legs. The fibers were found to extend to the brain (Spira et al. 1969a, b; Parnas et al. 1969). But a funny thing happened on the way to the forebrain; the bug turned out not to act like the book said it was supposed to.

Dagan and Parnas (1970) investigated the role of giant fibers in escape behavior with various techniques.

First, they stimulated a single giant axon intracellularly, with no response in the leg. Next they blocked conduction of giants; first by cutting the nerve cord at the last ganglion, allowing the giants to degenerate, and stimulating anterior to the lesion; secondly by applying nicotine sulfate to the ganglion which blocks ACH medistal synapses, and stimulating a cercal nerve. In both cases they were able to obtain a response in the leg. Finally, they checked the threshold of fibers in the connectives and found the threshold for leg activity higher than that of giants. This has been confirmed in the roach by Iles (1972) and in the locust by Seabrook (1970). Thus giants are not the sole factor in escape behavior.

Exactly which fiber class is responsible for escape behavior is presently an open question. (Indeed, it may be that many classes of fibers are involved, making it a meaningless question). The best guess seems to be that a class of "medium fibers," intermediate in size between the gianst and the small through fibers is responsible for conducting the escape message (Iles 1972; Seabrook 1970; Harris 1969).

Surprisingly, there are apparently giant fibers which synapse onto other ascending axons in the last ganglion in addition to sending their axons upwards (Harris and Smyth 1971; Milburn and Bently 1971). Obviously, stimulating a giant fiber in the connectives need not affect these fibers. Nor will they degenerate when the connectives are transected. Indeed, if these synapses are not blocked by nicotine sulfate then these fibers could be the medium fibers of this paper. It would be of interest to know whether nicotine sulfate blocks spike train we have observed in the last ganglion. If it did, then escape behavior must be mediated in the thoracic ganglia, and the spike train and escape behavior must be handled in different neural circuits.

It is interesting to speculate about the relationship of the spike train to escape behavior, but little is known about the neurophysiology of the last abdominal ganglion in the intact animal. Most studies done so far (Zilber-Gachelin and Chartier 1973b; Dagan and Parnas 1970; Illes 1972) have been on restrained animals, which have also frequently been surgically modified. However, it has been found that both the behavior and the neurophysiology of such a preparation differs significantly from the intact animal. The leg movements observed vary, depending on how free the animal is, and similar stimuli produce different giant fiber responses depending on whether the animal is intact or surgically modified (V. Chen, personal communication).

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