

MODIFICATION OF THE MOTOR REFLEX RESPONSES DUE TO REPETITION OF THE PERIPHERAL STIMULUS IN THE COCKROACH

I. HABITUATION AT THE LEVEL OF AN ISOLATED ABDOMINAL GANGLION

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INTRODUCTION

All living organisms react to modifications of the external environment in their own generally quite precise manner, the best known example being the reflex reactions of animals to a given stimulus. If the stimulus is repeated, however, the reflex generally undergoes a change which allows the animal to adapt itself to the new environmental conditions. This change can be manifested by an increase or a decrease of the successive reflex responses. If the stimulus is repeated at a frequency lower than about 1/sec, the former can be referred to as a 'sensitization' (cf. e.g. Kandel & Spencer, 1968) and the latter as a 'habituation' (e.g. Horn, 1967), provided the receptors for the reflexogenic stimulus have not changed and the capacity for the response to occur is unaltered. These phenomena are very general and can be found in the whole animal kingdom, from the least-organized beings—protozoa are habituable (Applewhite, 1968)—to the superior organisms.

The final aim of our work was to try to explain observations of this type of behavioural plasticity by the transfer properties of the different synapses involved. The chosen reflex was the forward escape reflex of the cockroach to an air puff applied to small abdominal appendices, the cerci. It is described (Roeder, 1948) as anatomically simple, i.e. having only two central non-cephalic synaptic levels successively involved: the 6th abdominal ganglion (A.G.), followed by the thoracic ganglion innervating the legs of the corresponding segment. In the intact animal receiving successive air puffs a particularly rapid and durable habituation was observed by Roeder (1948); the escape reflex decreases and even disappears after a few stimuli. Occasionally we could observe a sensitization preceding the response diminution; the reflex reactions to the first three or four stimuli then increase progressively before habituation sets in.

Some authors had, before us, tried to relate this habituation to the transfer properties of the synaptic relays participating in the reflex, but they had come to partial and contradictory results which can be summarized as follows: stimulation of the cerci by an air puff or electrical stimulation of a cercal nerve always elicits a response in the abdominal cord, while in the motor nerves the response is much more labile

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(Roeder, 1948). According to Roeder (1948), the habituation of the escape reflex should therefore be mainly related to this lability at the thoracic synapses. The results of Baxter (1957) gave support to this conclusion by eliminating any eventual role of the abdominal ganglia in habituation; stimulating the cerci by air puffs every 10 sec, Baxter could record a habituation of the escape reflex, but no change in the response of the abdominal cord. However, Hughes (1965), in apparently the same conditions, noted a decrease in the response at the abdominal level, implying an eventual role of the 6th A.G. in habituation.

Our work consisted in studying the transmission properties of the two central relays as systematically as possible in order to resolve the discrepancies between the different authors and to see how these properties could explain the sensitization and habituation observed in the intact animal. In this paper an attempt is made to analyse the change of the responses to successive stimuli after the impulses have passed through the synapses of the 6th A.G. The role of the thoracic relay will be analysed in the next paper where a general discussion on the problem stated above will be presented (Zilber-Gachelin & Chartier, 1973). A brief report of these results has appeared previously (Zilber-Gachelin, 1966*b*).

ANATOMICAL DATA

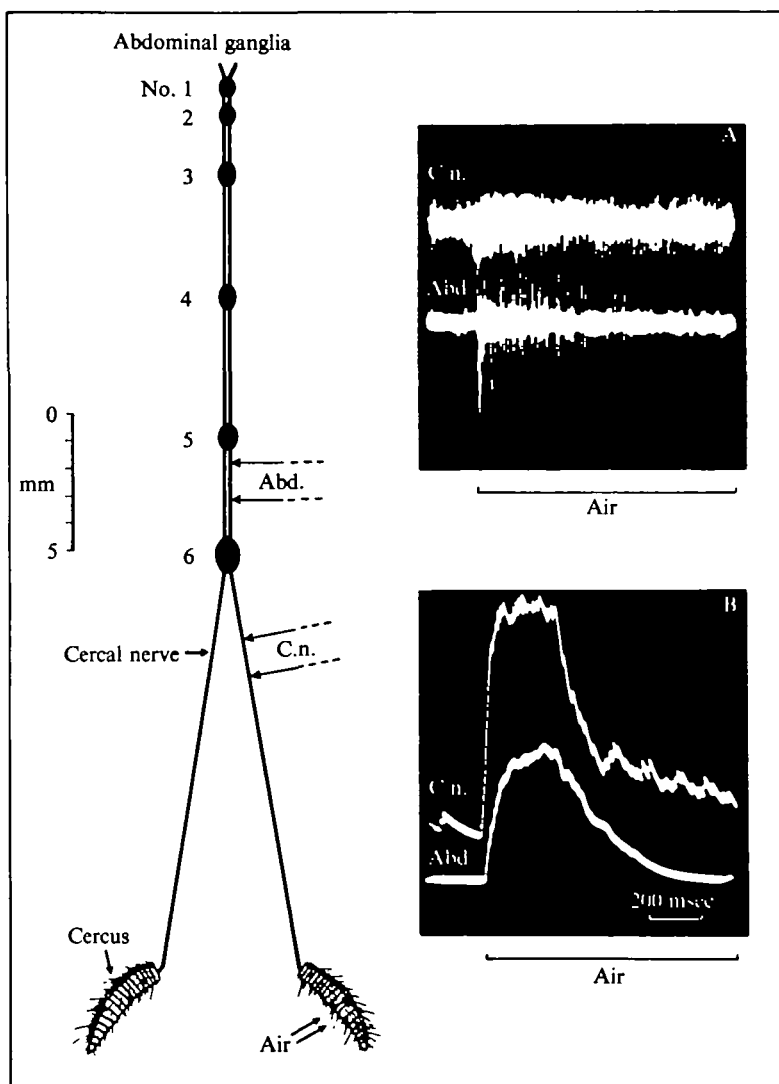
All the experiments were carried out on cockroaches of the species *Blabera craniifer* Burm. 1838, also called *Blabera fusca* Brunner 1865 (Rehn & Hebard, 1927). It was preferred to *Periplaneta americana* used by most authors, for we had to study the activity of the cercal nerves which, in *Blabera*, are about 1.5 cm long while in *Periplaneta* they do not exceed 2–3 mm. However, most anatomical or physiological descriptions relative to the latter appear to be valid also for our preparation.

Let us recall some anatomical data which will make easier the discussion of the results:

In the cockroach the abdominal ventral cord is made up of six ganglia separated from one another by two connectives (Text-fig. 1).

The sensory origin of the reflex studied is on the cerci, which are two jointed appendices situated at the extremity of the abdomen. They are moved as a whole by contraction of muscles attached to their roots. They are covered by a complex array of receptors, of which some mechanoreceptors (Sihler, 1924), consisting of filiform hairs (600–750 μm in length, 6 μm in diameter in *Blabera craniifer*—Le Bris, 1964) located on the ventral side of the cerci are particularly well adapted to work as receptors of weak air movements (Nicklaus, 1965). Each cercal receptor sends one afferent fibre to the 6th A.G. through the homolateral cercal nerve. The 'cercal nerve' (C.n.) in the present work is a branch (NIC, Richard, Zilber-Gachelin & Chartier, 1973) of the larger trunk commonly called N_{XI}. N_{XI} has been shown by this study to be a complex mixed nerve, and only one of its branches is the sensory cercal nerve (C.n.) which can be clearly seen to enter the cercus.

It has been shown by physiological studies (Roeder, 1948) as well as by degeneration experiments (Hess, 1958) that all the fibres of the cercal nerve end in the 6th A.G., some ipsilaterally, some contralaterally, so that stimulation of only one cercus gives postsynaptic responses in both abdominal connectives leaving the 6th A.G. The gang-



Text-fig. 1. General method for the study of habituation at the level of the 6th A.G. The drawing on the left side, drawn to scale, represents the portion of the nervous system kept for the study of habituation in the 6th A.G. The double arrows indicate the position of the recording electrodes on the cercal nerve (C.n.) and on the abdominal cord (Abd.). (A) Type of responses obtained, at these levels, to an air puff ('air') applied to a cercus. (B) Analogous curves giving the instantaneous frequency of the discharges of A. These curves may be used for quantifying the responses.

lionic delay (1.4-1.9 msec according to Roeder, 1948) could suggest intraganglionic interneurons, but recent work (Farley & Milburn, 1969; Milburn & Bentley, 1971) has established the existence of monosynaptic connexions with at least some post-ganglionic neurones—those with 'giant' fibres.

Roeder (1948) showed in *Periplaneta americana* that the connectives at the exit of the 6th A.G. contain 6-8 fibres—the 'giant' fibres—of a diameter (20-45 μm) clearly larger than that of the other fibres. By making transverse cuts in the connectives, we

saw the same to be true in *Blabera craniifer*, but with a maximum diameter slightly smaller ($\leq 30 \mu\text{m}$) (Pl. 1). These giant fibres pass directly through the five other abdominal ganglia without interruption (Hess, 1958; Roeder, 1948) to terminate only in the suboesophageal ganglion (Parnas *et al.* 1969).

According to Hess (1958) the sensory cercal fibres from both cerci converge upon these neurones with giant fibres, as well as upon others with smaller fibres, located in the dorsal part of the connectives.

MATERIALS AND METHODS

Operative techniques

Only male cockroaches were used, for dissection of females is made more difficult by their wealth of fat body; on the other hand, it is an ovo-viviparous species and presence of the oothecae in the females is a supplementary obstacle to dissection in the abdominal region.

The animals were operated under CO_2 anaesthesia. All the experiments were carried out on the isolated abdomen (animal transected at the limit between the thorax and abdomen), so as to suppress any possible influence from the more anterior centres (cerebral, sub-oesophageal and thoracic ganglia). The abdomen was then fixed on its dorsal surface to a cork plate by a few spots of a wax of low melting-point (Adheso sticky wax, S. S. White Company). The cuticle was opened on the ventral side of the abdomen above the selected nervous region and the fat-body was removed. The tracheae and the muscles which masked the cord or nerve were moved aside and the ventral contractile diaphragm around the nervous cord (Wigglesworth, 1950) was opened. As many as possible of the tracheae were kept intact so as to maintain a quasi-normal oxygenation.

Careful dissection and sectioning of the different nerves entering the 6th A.G. and of all the branches, except C.n., of the nerve trunks N_{XI} was necessary for two reasons. First, this made it possible to record an unambiguous C.n. response and to eliminate all other afferents entering the 6th A.G. Secondly, it was found during this study that a habituable local loop reflex existed between the receptors activated by air-puff stimuli and the cercal muscles. Functionally this reflex consists of an adduction of both cerci toward the mid line with air puff applied to one cercus. The response gradually habituates and disappears with repeated stimulation. Electrophysiologically, this may be represented by the following sequence of activity: an afferent volley in response to stimulus, a motor discharge from the ganglion, and finally an eventual sensory response from the cerci as they move through the air. Since this local reflex is habituable and involves only the abdominal ganglia and possibly only the 6th A.G., it could be studied by itself. In the present study it interfered with the process in which we were interested and was therefore suppressed by section of the motor nerves of the cerci.

The Ringer solution was that used by Boistel (1960):

NaCl, 154 mM/l; KCl, 2.7 mM/l; CaCl_2 , 1.8 mM/l; glucose, 22 mM/l. The solution was adjusted to pH 7.2 by addition of NaHCO_3 .

Electrodes (bipolar chlorided silver hooks) were placed under a C.n. and also under both connectives of the abdominal cord linking the 6th and the 5th A.G. In this fashion,

the nervous responses to cercal stimulation could be recorded just before ('cercal responses') and just beyond ('abdominal responses') the synapses of the 6th A.G. The abdominal response consisted of potentials from the closest fine fibres and, because they are much bigger, of potentials from the giant fibres although the latter are located more ventrally (Pl. 1).

During the experiment, desiccation was avoided by covering the exposed surface with pure petroleum jelly (Diathera) applied while warm and liquid.

Histology

The histological sections were made according to the classical techniques for nervous structures (Langeron, 1949). Let us only note here that fixation was effected with alcoholic Bouin; the sections obtained after dehydration and embedding in paraffin were coloured with ferric haematoxylin, and finally differentiation was obtained with iron alum.

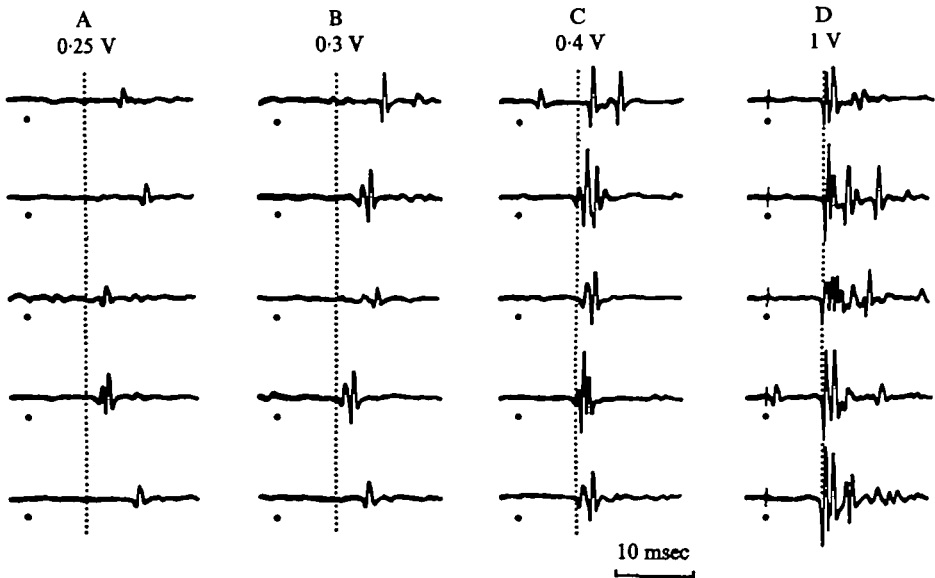
Materials used

Apart from the standard electrophysiological arrangement we used a few special devices made in the laboratory:

(1) The air puff used for stimulating the cerci was supplied by a system of rigid tubes connected to a source of compressed air through a pressure reducer and an electro-magnetic valve controlled by a stimulator which determined the onset and the duration of the air puff. A sensitive electro-mechanical transducer (RCA 5734) in the air stream allowed us to monitor the characteristics of the air puffs. We could thus adjust the air-puff delivery system so that the air velocity was constant during the entire puff period and that the rising and falling phases were as rapid as possible. These parameters were periodically checked with the transducer to be sure that the stimuli were remaining constant. It was particularly important to know that all air puffs were of the same amplitude as the first one; any tendency of the system to have a high-pressure reservoir would give rise to an initial high-amplitude puff followed by puffs of lower amplitude. Such a sequence yields sensory cercal responses which appear to be adapting.

The air puff went out through a tapered glass nozzle on a ball-and-socket joint which could be precisely oriented towards the ventral surface of a cercus, perpendicular to its long axis, which is the most efficient stimulus (Wozniak *et al.* 1967).

(2) A 'threshold circuit' was used to selectively record only those potentials which exceeded a pre-set level (generally chosen as the base-line noise level). Potentials exceeding the selected level gave rise to pulses of fixed amplitude and duration which could be used for subsequent manipulations. In some cases the pulses were fed into a frequency-to-voltage converter (R-C circuit which for small variations in frequency provided an almost linear voltage-to-frequency relation—cf. Text-fig. 1.). The second form of data manipulation was a step generator which produced a stepwise change in output voltage for each input pulse. This generator was started with the onset of the stimulus and stopped after a period which included all of the response after the stimulus, thus yielding a voltage linearly related to the number of spikes during the period of measurement. It is this measurement which, unless otherwise specified, will be referred to as 'the response amplitude'.



Text-fig. 2. Influence of the intensity of stimulation of a cercal nerve on the response of the abdominal cord. The moment of the stimulation is indicated by a dot placed under the corresponding trace. The voltage of stimulation is indicated above each column; for each voltage, 5 stimuli of 0.1 msec duration are delivered every 20 sec. The vertical dotted lines indicate a delay of 7.5 msec after the stimulation; they are intended to facilitate a comparison of the latencies of the responses recorded on the connectives between the 6th and the 5th A.G. In A and B, in addition to the spontaneous low-amplitude activity, a response with a variable latency can be seen. In C and D, where the intensity of stimulation is higher, the latency of the responses is smaller and more constant.

(3) Finally, some of the data have been directly analysed with a numerical computer with fixed programs and a memory of 1024 channels (SAIP, type ART 1000).

RESULTS

The transfer properties of the 6th A.G. for the impulses coming from the cerci has been studied both by electrical stimulation of a cercal nerve and by directing air puffs at the cerci.

Electrical stimulation of a cercal nerve

By electrically stimulating the distal end of a cercal nerve and recording both on the C.n. itself before the ganglion and on the abdominal cord we could bring out the following characteristics of transmission at the level of the 6th A.G. Some of them had already been observed by Pumphrey & Rawdon-Smith (1937), but some important differences from their results have nevertheless appeared, which will be examined in the discussion. This type of stimulation enabled us to simplify the study of transmission through the 6th A.G.; the different fibres of the cercal nerve having very similar diameters (Richard, Zilber-Gachelin & Chartier, 1973) and the length of C.n. between the stimulating and recording electrodes being small, the stimulus produced an intensity-dependent, narrow and unique summated cercal potential—the 'cercal impulse'—so that the 6th A.G. afferent signal was easily quantified by its amplitude. Furthermore it was possible to stimulate the nerve at high frequencies without any modification of this potential.

Description of the abdominal response

The synapses of the 6th A.G. do not transmit the 'cercal impulse' in a constant manner (Text-fig. 2), even at the lowest stimulation rates (e.g. one every 20 seconds in Text-fig. 2), while the cercal impulse remains perfectly constant.

The abdominal response to a liminal stimulation consists usually of a single wave (Text-fig. 2A), recognizable by its amplitude above the background activity. Its latency depends of course on the exact position of the electrodes. The mean latency is usually about 12 msec, but even for a given preparation it is very variable (± 2.5 msec). Moreover, transmission does not occur to every stimulation.

For the same stimulation rate, but with higher stimulus intensity, transmission becomes more effective, i.e. a larger number of stimuli induce a response; from a certain intensity, a post-synaptic response is obtained to each stimulation. Moreover, as the stimulation intensity increases, so does the abdominal potential amplitude through activation of additional abdominal fibres, while its latency decreases. In addition, this initial potential is followed (Text-fig. 2D) by an increasing number of others; the first few are of about the same amplitude (corresponding probably essentially to the giant-fibre discharge - cf. Roeder, 1948), the following are smaller and fire during a period of up to 30 msec and more. This whole response is, like that to a liminal stimulation, very variable as regards its total duration, the response latency, the amplitude of the different potentials and their timing. When the response is more complex, the latency is decreased, and for the highest stimulation intensities it is more constant (compare Text-fig. 2B and D).

So, in all cases, it is impossible to obtain a constant response to successive stimuli. Moreover, this variability seems to be random; we could never establish a regular variation of the response.

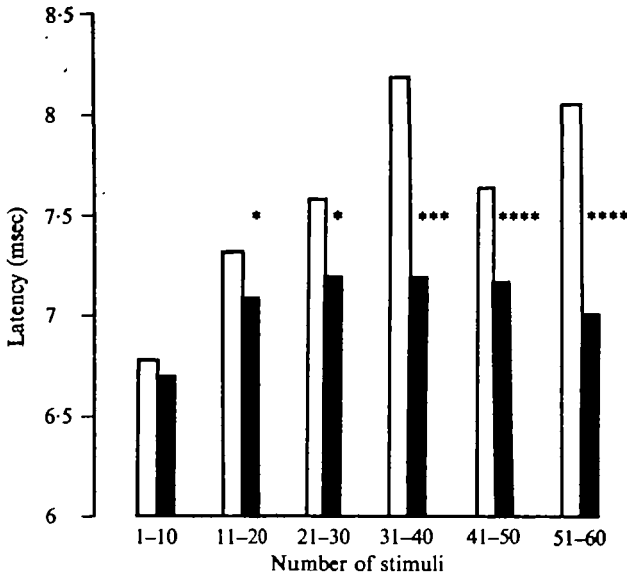
Influence of the stimulation frequency on the abdominal response

At higher stimulation rates than those above, the abdominal response decreases in a consistent way as a function of the number of stimuli. A minimum frequency—the 'limit frequency'—is found which depends on the stimulation intensity, but is in general around 1 stimulus per second. At this frequency the latency of the responses increases progressively from the first trial to reach a steady value (on the mean from 7 to 9 msec—if the complex response (Text-fig. 2D) has been obtained), while generally the amplitude of the initial potentials of the response and the total discharge duration decrease to a plateau. For higher frequencies of stimulation the response decreases to a lower level; the latency increases further (to 10 and even 12 msec), and at the same time the number of failures increases and the response can even disappear completely.

Influence of the stimulation intensity on the limit frequency

When the stimulation intensity is increased, the limit frequency required to obtain a diminution in the abdominal response increases; for a sufficiently high intensity, the response will not decrease for frequencies up to 10/second and more.

In addition, it has been observed that, for a given frequency of stimulation chosen above the limit frequencies for the whole range of intensities tested, the changes in



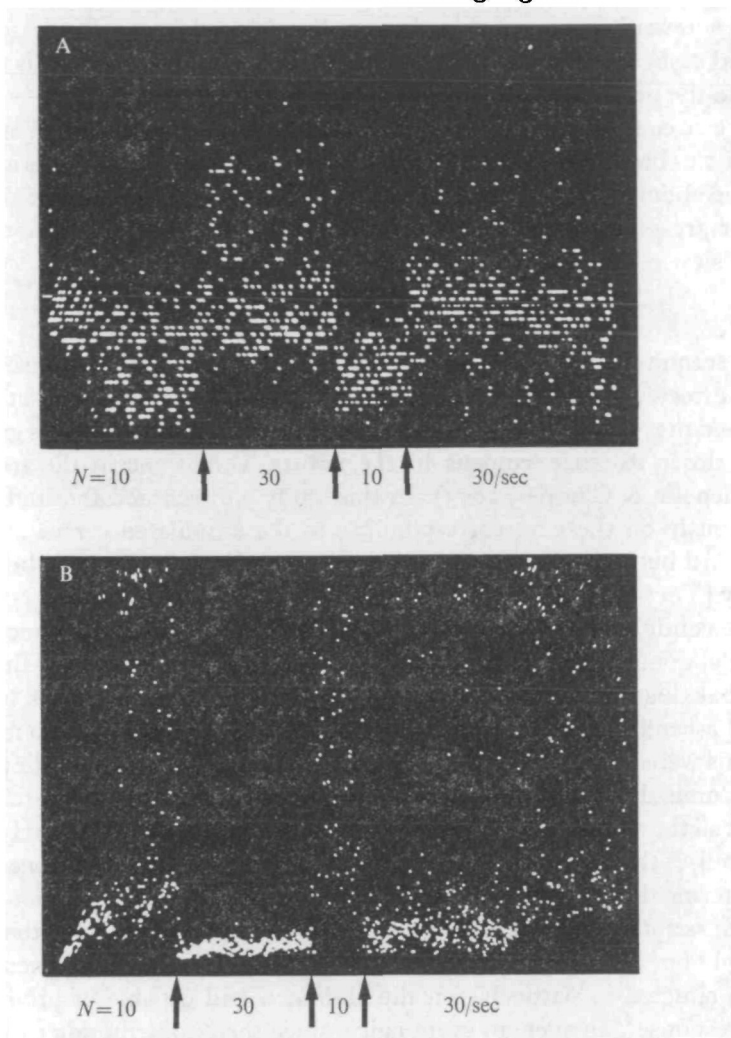
Text-fig. 3. Influence of the intensity of stimulation of a cercal nerve on the abdominal response habituation. The diagram shows the increase with time in the latency of the abdominal cord responses to the stimulation, every second, of a cercal nerve by rectangular shocks having a duration of 0.1 msec and an amplitude of 300 mV (white blocks) or 325 mV (black blocks). Each block represents the mean latency of ten successive responses. The latencies of the responses to the two types of stimuli, which are not significantly different (test of Student-Fisher) at the beginning of the series (cf. first block), become increasingly different as the stimuli are repeated; the responses to the weaker stimuli (white blocks) increase much more than those to the stronger stimuli. The significance of the differences is noted as follows: * < 0.1; ** < 0.05; *** < 0.01; **** < 0.001.

latency are inversely related to stimulus intensity (Text-fig. 3) and the rate and amount of decrease of the response are smaller as the stimulation intensity increases.

Possible restoration of the abdominal response

If, after repetition of the cercal stimulations, the abdominal response disappears completely, its restoration is always possible. Recovery of its initial amplitude is obtained either by interrupting the stimulations entirely, or by changing the stimulation rate from that above the limit frequency to a value below. In this second case the time course of the recovery can be followed and seen to be progressive. The period of the recovery may vary from tens of seconds to 1 min in both cases.

On the other hand, as described by Pumphrey & Rawdon-Smith (1937), it is also possible to restore the abdominal response instantaneously, either by an increase, even very small, of the intensity of stimulation (which increases the cercal response), or by an increase in the frequency of stimulation (which of course leaves the cercal response unchanged). For instance if the abdominal response has disappeared after stimulations at a rate of 10/sec, increasing the rate to 30/sec makes the response reappear (Text-fig. 4); if then it disappears again, it reappears at 50/sec. It is, however, important to observe that in both cases the restoration of the response is due to an



Text-fig. 4. Restoration of the abdominal response by an increase in the frequency of stimulation. The amplitude (A) and the latency (B) of the abdominal responses to successive electrical shocks on a cercal nerve are displayed. The successive dots (from left to right) correspond to the successive stimuli. The frequency of stimulation, N , is noted under each photograph.

(A) The computer measured the amplitude of the first potential appearing between two successive stimuli. The level of activity reached by the middle of the first stimulation period corresponds to the disappearance of the response (the computer was then measuring the amplitude of spontaneous potentials). The response reappears if the frequency of stimulation is switched over to 30/sec, but will disappear again if it is returned to 10/sec.

(B) The computer measured the latency of the first potential appearing between two successive stimuli. A progressive increase in latency can be seen, together with a larger dispersion of the values. When the dots appear on the higher part of the picture, they correspond to the measured 'latency' of spontaneous potentials. After the response has disappeared ($N=10$ /sec), the switching over to $N=30$ /sec leads to an immediate disappearance of the response. The second switching over to $N=30$ /sec is less effective: the responses have longer and more dispersed latencies and finally disappear.

increased summation occurring in the ganglion (spatial in the first case, temporal in the second case) and is in no way a dishabituation since no response is observed after a return to the previous parameters of stimulation (Text-fig. 4).

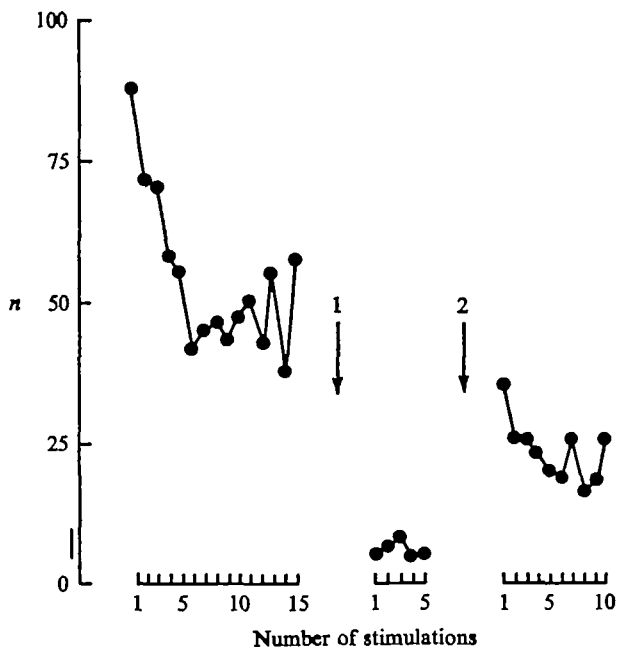
So we can conclude from this study with electrical stimulation that the synapses of the 6th A.G. between cercal fibres and ascending abdominal neurons are particularly labile. The abdominal cord responses are not constant; diminution of these responses occurs for pre-synaptic stimulation frequencies superior or equal to about 1/sec; and there is a slow recuperation of the former reactivity.

Stimulation of the cerci by puffs of air

In the second part of our work we studied the abdominal responses to repetitive air puffs directed at the cerci. Although this second type of stimulation somewhat complicated the analysis as will be seen below, we used it because of its great advantage in being closer to what happens in the natural conditions in the free animal (cf. Zilber-Gachelin & Chartier, 1973). In this study we recorded the traffic of impulses simultaneously on the C.n. corresponding to the stimulated cercus and on the abdominal cord between the 6th and 5th A.G., respectively before and beyond the synaptic relay (Text-fig. 1).

In these conditions, for instance, we could show a decrease in the successive responses of the abdominal cord if the stimuli were repeated every 10 sec. But before concluding that this diminution comes from the lability of the synapses between cercal fibres and ascending abdominal axons at the level of the 6th A.G., two methodological precautions were imperative: first, to be sure that the response to the air puffs obtained on the abdominal cord had no other origin than the excitation of the cerci; secondly, to check that all the modifications observed on the abdominal cord came only from passage of the impulses through the 6th A.G. and not from an equivalent decrease in the activity of C.n. entering the ganglion, through receptor adaptation after repetition of the stimuli. *The excited receptors.* The air puff was directed towards one cercus or the other, but air eddies could not be totally excluded, so that a possibility existed for excitation of other receptors, situated in particular on the abdomen and capable of producing an 'abdominal response'. In such an eventuality, since their contribution to the abdominal response could not be determined, comparison of this response and of the cercal nerve response would of course become meaningless.

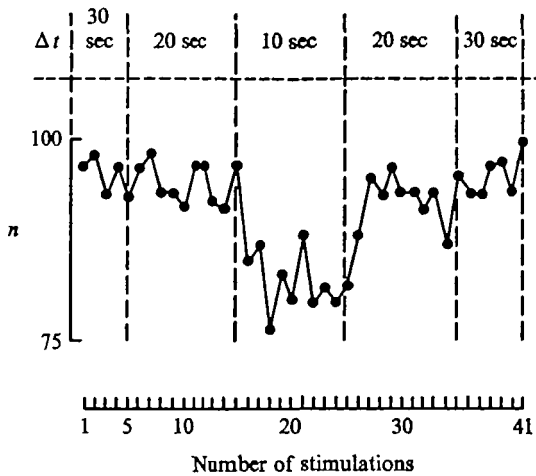
Some control experiments allowed us to determine the importance of, and eliminate, this cause of error. After section of the cercal nerves the abdominal response generally disappeared, provided that the stimulus remained oriented towards the cerci. But in a few cases a small abdominal response persisted, and in all cases if the direction of the air puff was shifted slightly towards the front of the animal a response reappeared. This response due to abdominal receptors other than those of the cerci was shorter but not negligible; worse, it appeared habituable (Text-fig. 5). We made the same experiment after successive sections of the nerves corresponding to the 5th, 4th, 3rd, 2nd and 1st abdominal ganglia. The responses persisted until the nerves of all the abdominal ganglia had been cut. In all the experiments described below, where we intended to study habituation of the reflex of cercal origin, we therefore always took the precaution of effecting such a de-afferentation, leaving finally only the two C.n. intact.



Text-fig. 5. Diminution, through repetition of the air puffs, of the abdominal response of a non-cercal origin. n = number of action potentials of the abdominal cord response to an air puff applied to a cercus, counted during 1.6 sec after the beginning of the stimulus and expressed as a percentage of the number of action potentials of the first response in the experiment. Between the stimuli the number of spontaneous action potentials during 1.6 sec periods is measured; it varies between the values indicated by the line located on the left side of the Y axis. The air puffs, of 1 sec duration, are delivered every 12 seconds, and the curves indicate the corresponding variations of the abdominal response. In 1: section of both cercal nerves: the response disappears (compare to the spontaneous activity). In 2: the tube for air puff delivery is directed slightly more towards the front of the animal. An habituable response reappears.

Adaptation of the cercal receptors. A decrease in the abdominal response after repetition of the puffs can be concluded to be a habituation phenomenon only if, at the same time, the invariance of the sensory message (the 'cercal response') is assured. We therefore examined the question of receptor adaptation and its duration. For puffs of 1 sec duration and intervals between stimuli less than 10–20 sec, the cercal nerve response to the successive stimuli does decrease down to a plateau (Text-fig. 6), i.e. there occurs a receptor adaptation which lasts at least 10–20 sec. With higher frequencies of stimulation the responses decrease to a plateau of lower level.

Thus the observed decrease in the successive responses of the abdominal cord might well have been due only to this long-lasting adaptation leading to a decrease in the afferent messages to successive stimuli. But, as a matter of fact, the plateau of the cercal responses is often reached while the decrease of the abdominal responses goes on (Text-fig. 7A); the latter is then due uniquely to habituation by passage through the 6th A.G. The same conclusion that a habituation process takes place in this ganglion holds even in the cases when the two responses, cercal and abdominal, reach their plateau after the same number of stimuli; if subsequently the intervals between stimuli are increased progressively, the cercal response always recovers before the abdominal response (Text-fig. 7B). So in all the cases when the successive cercal responses



Text-fig. 6. Determination of the minimum time interval between the stimulations allowing to eliminate any influence of cercal adaptation on the waning of the abdominal response to successive air puffs. n = number of action potentials of the cercal response to air puffs applied to the corresponding cercus (expressed as a percentage of the number of action potentials of the maximum response). Δt = time intervals (sec) between successive stimulations. The cercal responses to successive puffs markedly decrease for inter-stimulus intervals of 10 sec.

decrease, it can be shown that a ganglionic phenomenon of habituation is superimposed over this long-lasting adaptation.

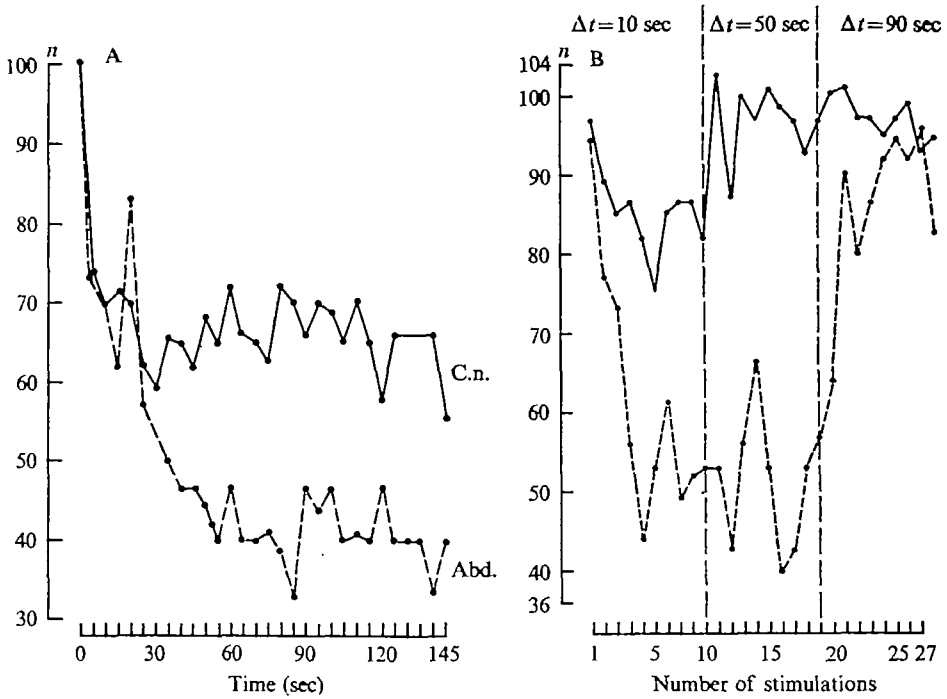
Moreover, it is always possible to determine a range of frequencies of puff repetition for which the response of the cercal nerve remains constant, and that of the cord decreases. This diminution can then only be attributed to a habituation at the level of the 6th A.G.

The following sections of this paper will be devoted to an analysis of the main characteristics of this habituation process. So as to study habituation *per se* and to eliminate all possible interfering sensory adaptation, the minimum stimulus interval for constant cercal response was determined for each animal, and the experiments were carried out with longer intervals.

Decrease of the response

We established that if air puffs are delivered to the cerci more often than every 3-5 min, in the conditions stated above, the abdominal response decreases regularly until it reaches a plateau above the zero level. This phenomenon is illustrated by Text-fig. 8A, where the stimuli are presented every 10 sec and the responses are displayed as variations in the frequency of the discharges recorded on the cercal nerve and on the abdominal cord. Text-fig. 8B is an example of the curves obtained by plotting the instantaneous frequency of the discharge at a selected time after the onset of the air puff* as a function of the number of stimuli applied. The curves are

* This type of measure has been done before the step tension generator was designed in our laboratory (cf. Materials and Methods). The amplitude of a response was then onwards-evaluated by the total number of spikes appearing during the response; this latter case is that of all the curves where the ordinate measuring the amplitude of the response is denoted by n .



Text-fig. 7. Possibility, in spite of a cercal adaptation, to demonstrate a habituation phenomenon at the level of the 6th A.G.

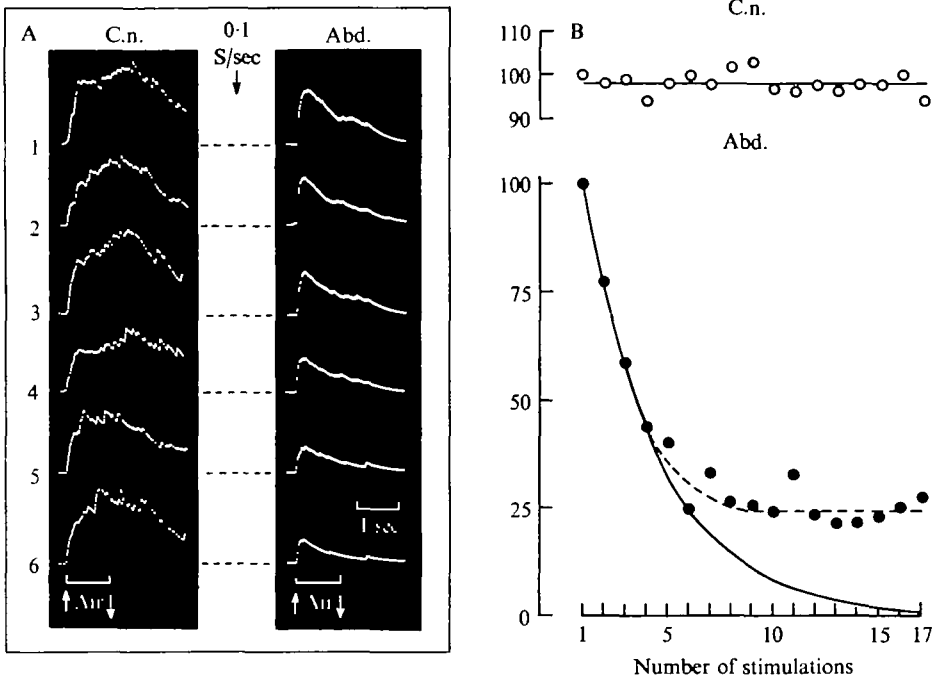
(A) n = number of action potentials of the response of a cercal nerve (C.n.) and of the abdominal cord (Abd.) (expressed as a percentage of the corresponding number of action potentials of the first response). The air puffs are delivered every 5 sec. The abdominal response continues to decrease while the cercal response has already reached a plateau.

(B) n = number of action potentials of the responses of a cercal nerve (continuous curve) and of the abdominal cord (dotted curve), expressed as a percent of the number of corresponding action potentials of the first response in the experiment.

Δt = time interval between two successive stimulations (sec). When this inter-stimulus interval is increased from 10 to 50 sec, the receptor adaptation disappears while habituation persists. The abdominal response will recover only for inter-stimulus intervals of 90 sec.

the same, irrespective of the time selected for the measurement. While the cercal response is seen to be constant, with the exception of the variations due to spontaneous activity, the abdominal response shows a progressive decrease down to a plateau level; in this case the puffs were delivered every 10 sec and resulted in a decrease of about 75%.

The frequency of the abdominal discharges usually decreases exponentially as a function of the number of presentations of the stimuli during the first four or five puffs; then the diminution is slower and finally the response remains practically constant (Text-fig. 8B). Since, independently of habituation, the response strength may fluctuate markedly, the waning is not often smooth (e.g. Text-fig. 9), and this tendency to an exponential waning over time is usually better displayed with grouped data.



Text-fig. 8. Demonstration of a habituation phenomenon taking place in the 6th A.G.

(A) The analogous curves represent the instantaneous frequency of the discharges of the cercal nerve (C.n.) and of the abdominal cord (Abd.) recorded simultaneously, in response to six successive air puffs (S) delivered every 10 sec. The base line (zero firing rate) is indicated by a dotted line. The only abdominal response is seen to decrease consistently when the stimuli are repeated.

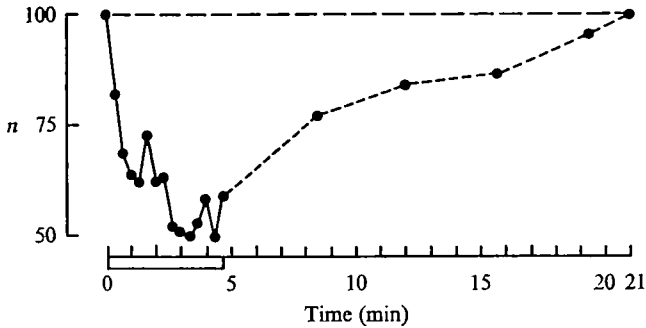
(B) The amplitude of the responses of a cercal nerve (C.n., white circles) and of the abdominal cord (Abd., black circles) were expressed as a percentage of the amplitude of the response to the first stimulation and plotted as a function of the number of stimulations. The stimulations were applied every 10 sec. The amplitudes have been evaluated by measuring the instantaneous firing frequency (cf. A) 435 msec after the beginning of the stimulation (this number corresponds to an integer of mm on the record).

The curves display clearly the statistical constancy of the cercal response as opposed to the gradual decrease of the abdominal response down to a non-zero plateau. The successive abdominal response amplitudes have been plotted on a semi-log scale, and the first values found to be on a straight line. The corresponding exponential curve has been drawn in (B) as a continuous line. The broken line corresponds to the experimental mean decrease of the abdominal response when it deviates from the exponential curve.

Spontaneous recovery

For this decrease of the abdominal response to be attributed to a phenomenon of habituation (adaptation being excluded) and not to an artifact such as for instance deterioration of the preparation, it has to be followed by a spontaneous return of the response to its initial size after the stimulations have stopped. We always observed this spontaneous recovery over time.

It is possible to study the time course of this restoration by delivering, when habituation is established, test-stimuli at a frequency low enough not to superimpose habituation. It can thus be established that the response recovery is progressive (Text-fig. 9); after habituation of the abdominal response by about 15 air puffs delivered every 20 sec, an exact return of the response to its initial amplitude is ob-



Text-fig. 9. Habituation and recovery with time of the abdominal cord response to air puffs applied to the cerci. n = number of action potentials of the abdominal response to air puffs applied to a cercus (expressed as a percentage of the number of action potentials of the first response). Habituation is brought about by a series of 15 stimulations delivered every 20 sec, i.e. during the length of time indicated by a line under the X-axis. The abdominal cord response recovers its initial size 16 min after the end of the stimulations; to follow the time course of this recovery, test-stimuli are delivered every 220 sec.

tained after 3–16 min, depending on the preparation. This delay is still longer (30 min or more) if the number of stimuli of the series bringing about the habituation has been higher, although the plateau of amplitude reached in both cases has been the same. Thus the recovery time is not a linear function of the response decrement.

This dependence of the recovery time on the recent history of the preparation will also be illustrated in the next paragraph.

Potentiation of the habituation

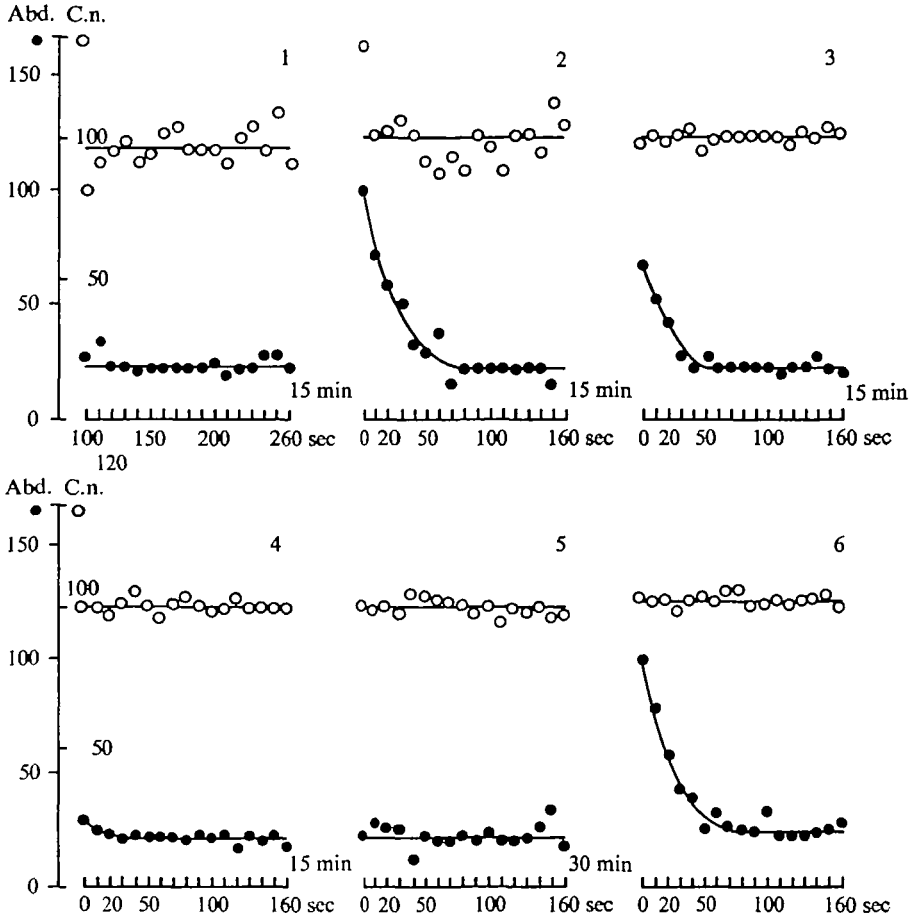
If successive series of habituations and spontaneous recoveries are given, the recuperation time becomes longer and longer; or, to put it another way, the same recovery period between series of stimuli becomes less and less effective; there is a cumulative effect, i.e. a potentiation of habituation. This fact is illustrated in Text-fig. 10, where a pause of 15 min is less and less effective in restoring the response, and after five series of 17 stimuli separated by 15 min a pause near to 30 min is necessary for a restoration of the response to its initial level.

Influence of the frequency of stimulation

We tried to compare the habituation obtained by delivering air puffs to the cerci at different frequencies. In order to prevent habituation it is necessary to stimulate at a maximum frequency of about 1 every 5 min. For higher frequencies of stimulation habituation sets in. A close relationship exists between response amplitude and frequency of stimulation: the higher the frequency of repetition of the stimuli, the more pronounced is the habituation, as indicated by a decreased abdominal response amplitude at the plateau (Text-fig. 11, filled circles). For the highest stimulation frequencies a receptor adaptation is superimposed (Text-fig. 11, open circles).

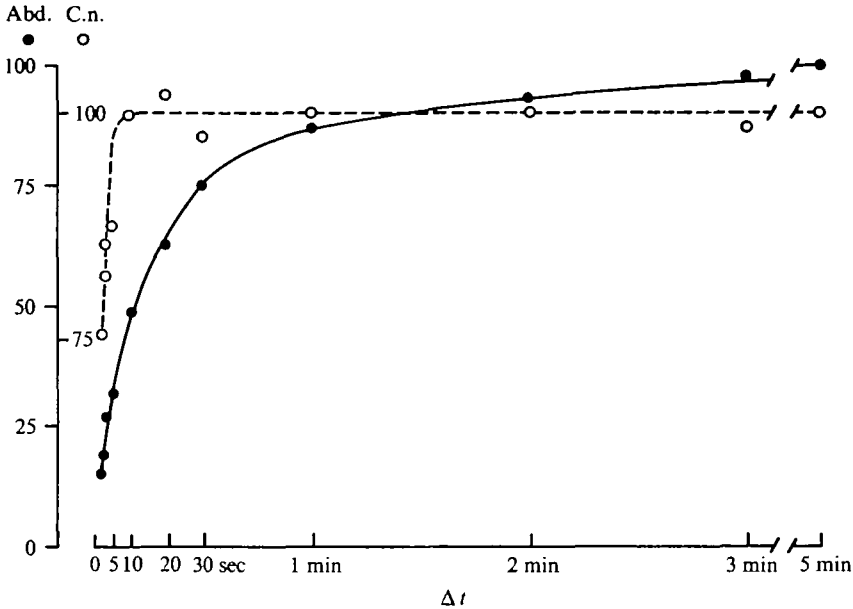
Specificity of habituation

Some authors (e.g. Coombs, 1938; Porter, 1938; Crampton & Schwann, 1961; Thompson & Spencer, 1966) have described on other preparations a phenomenon of generalization of habituation, according to which, after habituation of the response to



Text-fig. 10. Potentiation of habituation. The curves represent the amplitude of the successive responses of the cercal nerve (C.n., white circles) and the abdominal cord (Abd., black circles) as a function of time (air puffs applied to the cerci every 10 sec). The mode of quantification of the responses is that used for Text-fig. 8B. No long-lasting adaptation occurs in this case. In 1: plateau level of the responses after 100 sec of stimulation. Fifteen minutes elapse between two successive series of stimulation, and lead to a smaller and smaller recovery of the abdominal response amplitude. Between series 5 and 6 a pause of 30 min is given and allows a total recovery of the initial size of this response.

a given stimulus, the response to other stimuli (not repeated frequently) is also smaller. We tried to see whether this phenomenon can be found in our preparation, by using as a habituating stimulus and as a test stimulus respectively two independent air puffs on one and then on the other cercus. Since the ventral face of the cercus is covered with very fine hairs, sensitive to the least movement of air (for instance, a small movement of the hand at a distance of 1 m from the preparation), air eddies produced by the air puff to one of the cerci had to be prevented from reaching the other by a wall made of low melting-point wax, attached to the abdomen and placed between the cerci. This wall had to extend from the cork plate to 1-1.5 cm above the abdomen and beyond the end of the abdomen by at least 1.5 cm. Undesired air movements around a cercus could be detected with the binocular microscope by the movements of the fine hairs; as a final test, we ascertained that no response appeared in the cercal nerve



Text-fig. 11. Variation of habituation as a function of the inter-stimulus interval (Δt). Ordinate: amplitude of the cercal responses (C.n., white circles) and of the abdominal responses (Abd., black circles) to air puffs applied to a cercus. Each circle represents an average of five measurements made at the plateau established after the eventual decrease in the response. The amplitude is evaluated by the number of action potentials in the response and expressed as a percentage of that obtained for $\Delta t = 5$ min, when no decay in the responses occurs in either record. While a long-lasting adaptation appears only for Δt less than 10 sec, a ganglionic habituation is already manifested for $\Delta t = 3$ min and is all the more pronounced as Δt is smaller.

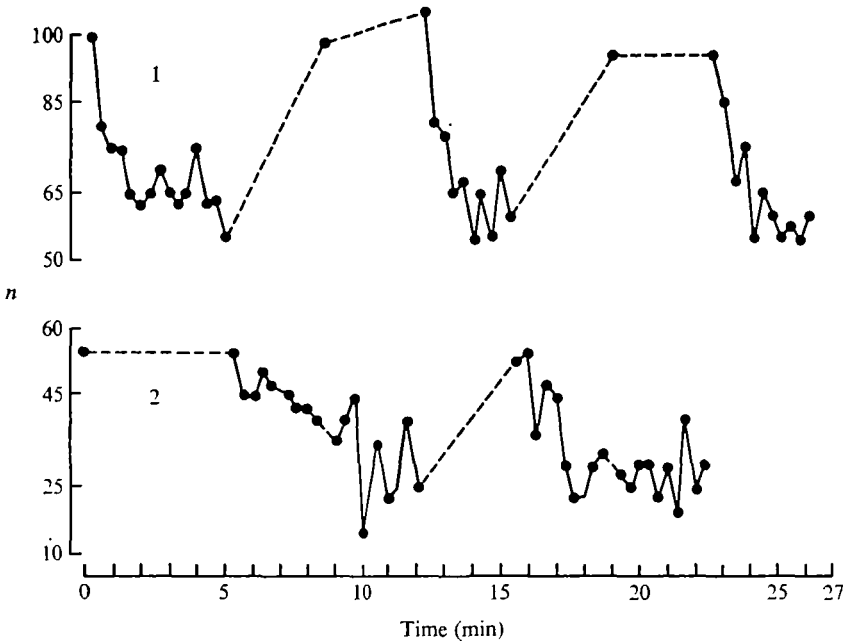
In these conditions we observed (Text-fig. 12) that habituation of the abdominal response (R_1) to air puffs applied to one of the cerci did not lead to any decrease in the size of the abdominal response (R_2) to a test stimulus of the other cercus; and if a habituation of R_2 was then induced, this did not prevent simultaneous spontaneous dishabituation of R_1 , and vice versa. So there is no generalization of habituation in this case.

Attempts to produce dishabituation by an extra stimulus

In isolated abdomen preparations with all nerves intact we tested the possibility of a dishabituation of the response to an air puff by different extra-stimuli: an air puff oriented differently or of a different velocity, a sound, a volley of electrical shocks on the abdomen cord or on a cercal nerve, and a tactile stimulus applied at various levels (external or internal) of the abdomen. We have never been able to obtain a dishabituation of the abdominal response by any of these stimuli.

DISCUSSION

In this paper the term of habituation has been used in its widest acceptance, i.e. as (sensory adaptation and effector fatigue being excluded) the decrease in the amplitude of a response to a stimulus, due to repeated presentation of this stimulus at



Text-fig. 12. Specificity of habituation. n = number of action potentials of the abdominal cord response to an air puff applied to one cercus (1) or the other (2). This number is in both cases expressed as a percentage of the number of action potentials of the response to the first puff delivered to cercus 1 (which, in the experimental conditions of stimulation and recording, turns out to be superior to the first response to stimulation of cercus 2). In both cases, the habituating stimuli are delivered every 20 sec, and dishabituation is tested every 220 sec. No generalization of habituation is observed.

intervals of 1 sec or more, provided recovery of the initial response amplitude can be obtained. The abdominal response to electrical stimulations of a C.n. is thus habituable. It has been recently shown (Callec, Guillet, Pichon & Boistel, 1971) that the amplitude of the unitary e.p.s.p. recorded in a giant fibre after stimulation of a single receptor may vary at random by a factor of three and will consistently decrease if the receptor is repeatedly stimulated. These properties seem adequate to explain the different observations we have reported in the electrical stimulation section, the action potentials we recorded being due to a summation of such e.p.s.p.'s up to the firing level. Thus the variability we noticed would reflect that of the unitary e.p.s.p.'s. The decrement of the global response through repetition of the stimuli would be due to the disappearance of the action potentials in some fibres as a result of the failure of the decreased compound e.p.s.p.'s to reach threshold for spike initiation. The simultaneous increase in the response latency would result from the decline in the slope of the e.p.s.p.'s, leading to a longer time to reach the firing threshold (cf. also Horn & Wright, 1970). Moreover, there is a high degree of convergence upon the ganglion cells (Pumphrey & Rawdon-Smith, 1937; Roeder, 1948; Callec *et al.* 1971). When the intensity of stimulation is increased, so is the number of presynaptic elements activated; thus a compound e.p.s.p. of higher amplitude and faster rise time will be elicited and the number of failures and the latency of the response will decrease. The fact that the diminution of the global response with time by repetition

of the stimuli is slower when stimuli of a higher intensity are delivered can be also explained by the higher amplitude of the compound e.p.s.p.'s, which implies that a same percent of decrease will less easily drive them below the firing level. Finally, the recovery by an increase in the frequency of stimulation would be also due to a higher summation of the e.p.s.p.'s; this would explain that no dishabituation remains when the frequency reverts to the habituating frequency.

Before discussing the possible mechanism responsible for such a type of labile response, it is worth while pointing out the limit frequency we found for a stimulation to lead to habituation. While Pumphrey & Rawdon-Smith (1937) noted a decrease of the abdominal cord responses for stimulation frequencies higher than 40–50/sec for a 'maximal stimulus' (i.e. giving a maximum response) and of 15/sec for an infra-maximal stimulus, we observed a decrease in responses from a stimulation frequency of about 1/sec. This discrepancy can be due to the fact that, unlike these authors who recorded only the giant-fibre activity, we recorded also from fibres of smaller diameter which appear to receive their cercal afferents through more labile synapses (unpublished results).

On the other hand, the fact that Pumphrey & Rawdon-Smith had observed a decrease in the abdominal responses through repetition of electrical stimuli on a cercal nerve did not imply—since no mention was made of the recovery time—an intervention of the 6th A.G. in the escape-reflex habituation. But we observed that actually this diminution of reactivity of the abdominal synapses could persist after the end of the stimulations for several tens of seconds, and sometimes for over 1 min. Thus an air puff to the cerci, which produces on the cercal nerves a de-synchronized volley of impulses firing at a frequency much higher than 1/sec (up to 360/sec according to Callec *et al.* 1971) could lead to a decrease in the efficacy of transmission across the 6th A.G., which could last until the next air puff. This therefore implies the existence of a habituation of the abdominal responses to cercal air puffs. Actually, if air puffs to the cerci of a cockroach are repeated with time intervals smaller than 3–5 min, a decrease in the abdominal cord response is always observed, which confirms Hughes' observations (1965). Moreover, this decrease has been shown to have the main parametric characteristics of habituation as listed in vertebrates by Thompson & Spencer (1966): spontaneous recovery of the response amplitude, potentiation of habituation through repetition of series of stimuli, habituation more pronounced for higher stimulation frequencies or for weaker stimuli.

To our knowledge, in the cases where such a central habituation has been described, a possible role of sensory adaptation has not been considered or has been excluded (e.g. Hinde, 1970; Kandel, Castelluci, Pinsker & Kupfermann, 1970; Rowell, 1970). This possibility had also been dismissed for the cockroach cerci (Hughes, 1965; Eisenstein, 1972). On the contrary, in our study, for intervals of stimulation inferior or equal to about 10 sec, we have shown, in addition to the ganglionic habituation, a long-lasting adaptation of the cercal receptors which certainly plays a functional role. This difference may be due to the fact that we recorded from the sensory cercal nerves N 11c and not from the whole so called 'cercal nerves'. The cercal receptors may well be more adaptable than the para-cercal receptors whose response was probably recorded in the 'cercal nerves' and could mask the cercal adaptation.

Since a monosynaptic connexion has been demonstrated between the cercal fibres

and the abdominal giant fibres (Farley & Milburn, 1969; Milburn & Bentley, 1971) and, as discussed above, the giant fibre unitary e.p.s.p. due to the activation of a single cercal fibre is labile (Callec, Guillet, Pichon & Boistel, 1971), it seems reasonable to assume that the habituation of the abdominal response to cercal air-puff stimulation results from a post-excitatory synaptic depression intrinsic to the reflex's excitatory synapses themselves (cf. also Horn, 1965; Spencer, Thompson & Neilson, 1966; Thompson & Spencer, 1966; Horn, 1967; Segundo, Takenaka & Encabo, 1967; Kandel *et al.* 1970). As pointed out by Rowell (1970), this mechanism appears particularly satisfactory in the case of insects, which have complex behaviour and a small number of cells (e.g. 663 in an abdominal ganglion of *Carausius* (Becker, 1965). Although an hyperpolarization component has been seen in a few cases in the giant fibres (Callec *et al.* 1971) it does not seem necessary in the present state of our knowledge to involve an inhibitory neural pathway running parallel to the reflex pathway and strengthening with repetition of the stimulus through post-tetanic potentiation (Wickelgren, 1967 *a, b*; Wall, 1970) or to postulate a mechanism such as that proposed by Sokolov (1960) and Hernandez-Peon and his colleagues (1962, 1965, 1966), implying the formation of a 'model' of the stimulus, a circuit for comparison with incoming signals, and an active feed-back blocking of transmission.

The actual mechanism of the decrease in synaptic efficacy can be either pre-synaptic (decrease in the release of transmitter, whatever its origin may be) or post-synaptic (some de-sensitization of the subsynaptic receptors). The results obtained on the specificity of habituation can give some clue to this problem. We have shown that, unlike the escape reflex of the crayfish which shows an important generalization of habituation (Krasne & Woodsmall, 1969), the response of the cockroach abdominal cord to stimulation of one of the cerci is not modified when the response from the contralateral cercus is habituated. Such a specificity of habituation has also been shown in the locust (Horn & Rowell, 1968), the crayfish (Bruner & Kehoe, 1970) and *Aplysia* (Kehoe & Bruner, 1966; Bruner & Kehoe, 1970; Kandel & Spencer, 1968). In these different examples it has been taken as an indication that the decrement leading to habituation occurs 'upstream' of the recorded cell. Now, the giant fibres of the cockroach are monosynaptically coupled to the C.n. fibres (Farley & Milburn, 1969; Milburn & Bentley, 1971), and if we observe in the abdominal responses only the large-amplitude potentials which are supposed to correspond to activity in these giant fibres, the same specificity of habituation is found. Since the fibres of the two cercal nerves converge upon the same abdominal neurones (Hess, 1958), the fact that habituation occurs for one cercus independently of the other would indicate that the process is pre-synaptic (cf. also Bruner & Tauc, 1966; Horn, 1970). However, while any gross postsynaptic change can certainly be ruled out, it must be remembered that each giant fibre bears numerous ramifying axonal processes equivalent to a dendritic tree and on which most of the afferent cercal fibres seem to make synaptic contacts (Farley & Milburn, 1969), so that a possibility remains of a highly localized post-synaptic change. The present results do not permit more specific interpretations as regards the actual mechanism of habituation in the 6th A.G.

One of the classical characteristics of habituation, which is generally considered as fundamental for distinguishing between habituation and 'fatigue' (e.g. Pavlov, 1927; Humphrey, 1933; Prosser & Hunter, 1936; Harris, 1943; Konorski, 1967;

Thompson & Spencer, 1966; Hinde, 1970) is the possibility of dishabituation by presentation of another stimulus; this phenomenon consists in an immediate restoration of the initial amplitude of the response by interpolation of a stimulus different from the one used to produce habituation. While we have observed such a dishabituation for the escape reflex to cercal air puffs (Zilber-Gachelin, 1966*a*), it apparently cannot be obtained at the level of the 6th A.G., and has been shown to take place only in the second relay of the reflex loop (Zilber-Gachelin, 1970). So there exist habituable synapses which cannot be dishabituated by an extraneous stimulus. Moreover, Zilber-Gachelin (1966*a*, 1970) showed that the increase in the reflex response produced in the cockroach by an extra stimulus (increase equivalent to a dishabituation) is in fact due to a transient increment in the general level of excitation of the animal; it is not a neutralization of the habituation process but an enhancement of responsiveness which is superimposed on habituation without suppressing it (cf. also Sharpless & Jasper, 1956; Thompson & Spencer, 1966). For these reasons, it seemed to us that this identification criterion of habituation should not be retained.

Finally, since we have shown in this paper that repetition of air puffs to the cerci establishes a habituation at the first synaptic relay, the problem was then to know whether this habituation was sufficient to explain that of the reflex or whether the thoracic ganglia also contributed to the habituation. This will be considered in the next paper (Zilber-Gachelin & Chartier, 1973). Let us just underline that this abdominal habituation appeared essential for the habituation of the escape-reflex response.

SUMMARY

1. In the cockroach *Blaberus craniifer* repeated air puffs to the cerci induce a habituation of the corresponding escape reflex, which we have tried to relate to the transfer properties of the two central relays of the reflex. In this paper transmission through the first relay, the 6th abdominal ganglion (A.G.), was studied both by electrical stimulation of the sensory cercal nerve and by application of natural stimuli (air puffs) on the cerci, while recording simultaneously on the cercal nerve and the abdominal cord, i.e. respectively before and beyond the synaptic relay.

2. The electrical stimulation study shows transmission to be relatively labile. This property is still more apparent if air puffs are delivered. If they are separated by less than about 10 sec the cercal nerve response decreases, which is a sign of a receptor adaptation. For longer intervals, although this adaptation disappears, the response nevertheless decreases on the cord, implicating the ganglionic relay as the centre of a habituation phenomenon.

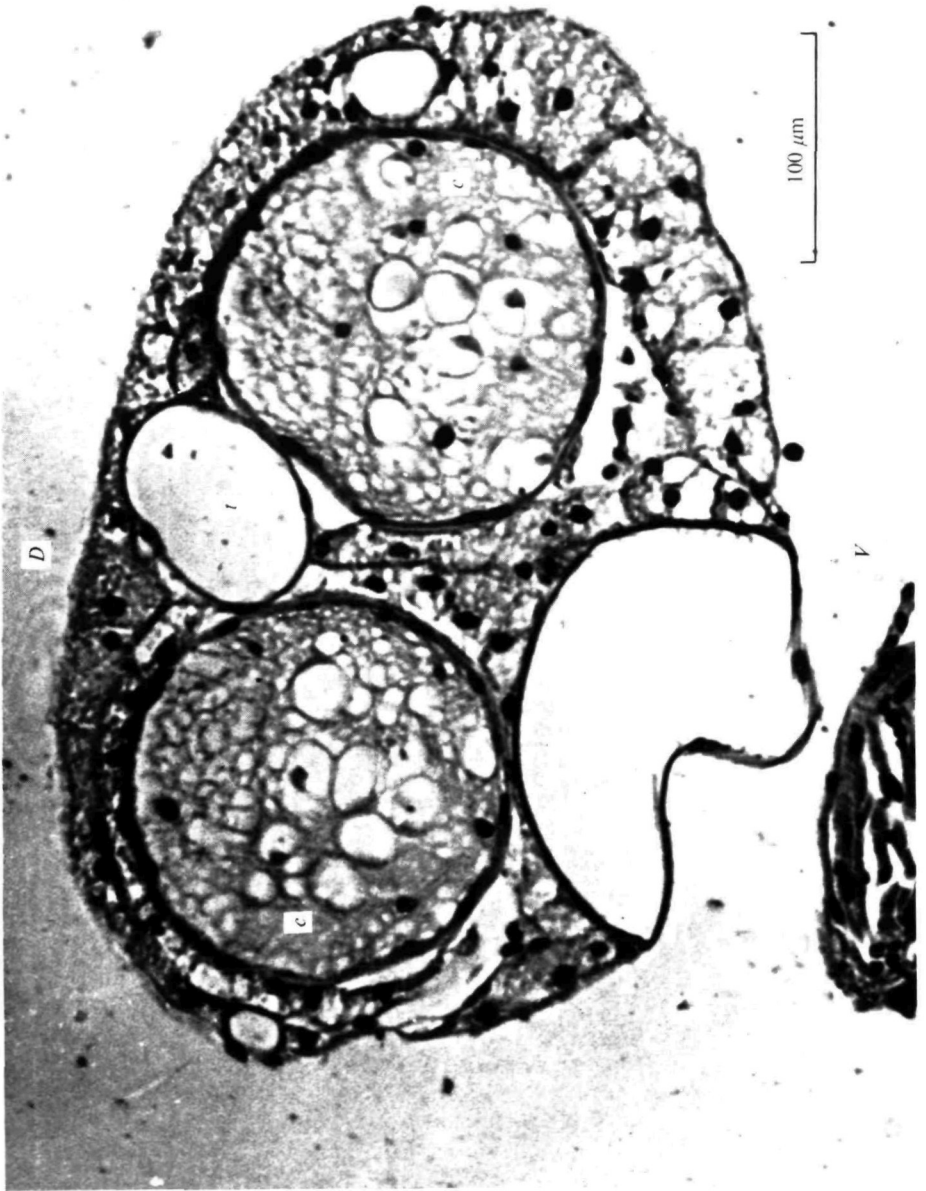
3. The characteristics of this habituation are the following: with repetition of the stimuli, the response decreases down to a non-zero plateau (decrease of about 50% if a puff is delivered every 20 sec); it recovers spontaneously if stimulations are suppressed; habituation is potentiated when successive series of habituations followed by spontaneous restorations are accumulated; it is more pronounced as the stimulation frequency is increased; finally, it does not show generalization. These results confirm that the 6th A.G. independently exhibits a real habituation process.

4. The mechanism of this habituation process is discussed. A post-excitatory presynaptic depression intrinsic to the reflex's excitatory synapses themselves is tentatively proposed.

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REFERENCES

- APPLEWHITE, P. B. (1968). Non-local nature of habituation in a rotifer and protozoan. *Nature, Lond.* **217**, 287-88.
- BAXTER, C. (1957). Habituation of the roach to puffs of air. *Anat. Rec.* **128**, 521.
- BECKER, H. W. (1955). The number of neurons, glial and perineurium cells in an insect ganglion. *Experientia* **21**, 719.
- BOISTEL, J. (1960). *Caractéristiques fonctionnelles des fibres nerveuses et des récepteurs tactiles et olfactifs des Insectes*. Librairie Arnette, Paris, 147 pp.
- BRUNER, J. & KEHOE, J. S. (1970). Long-term decrements in the efficacy of synaptic transmission in molluscs and crustaceans. In *Short-term Changes in Neural Activity and Behaviour* (ed. G. Horn and R. A. Hinde), pp. 323-59. Cambridge University Press.
- BRUNER, J. & TAUC, L. (1966). Habituation at the synaptic level in *Aplysia*. *Nature, Lond.* **210**, 37-9.
- CALLEC, J. J., GUILLET, J. C., PICHON, Y. & BOISTEL, J. (1971). Further studies on synaptic transmission in Insecta. II. Relations between sensory information and its synaptic integration at the level of a single giant axon in the cockroach. *J. exp. Biol.* **55**, 123-49.
- COOMBS, C. H. (1938). Adaptation of the galvanic response to auditory stimuli. *J. exp. Psychol.* **22**, 244-68.
- CRAMPTON, G. H. & SCHWAM, W. J. (1961). Effects of arousal reaction on nystagmus habituation in the cat. *Am. J. Physiol.* **200**, 29-33.
- EISENSTEIN, E. M. (1972). Learning and memory in isolated insect ganglia. *Adv. Insect Physiol.* **9**, 111-81.
- FARLEY, R. D. & MILBURN, N. S. (1969). Structure and function of the giant fibre system in the cockroach, *Periplaneta americana*. *J. Insect Physiol.* **15**, 457-76.
- HARRIS, J. D. (1943). Habitatory response decrement in the intact organism. *Psychol. Bull.* **40**, 385-422.
- HERNANDEZ-PEON, R., DAVIDOVICH, A. & MIRANDA, M. (1962). Habituation to tactile stimuli at the spinal trigeminal sensory nucleus. *Acta neurol. latinoamer.* **8**, 180-6.
- HERNANDEZ-PEON, R., O'FLAHERTY, J. J. & MAZZUCHELLY-O'FLAHERTY, A. L. (1965). Modifications of tactile evoked potentials at the spinal trigeminal sensory nucleus during wakefulness and sleep. *Expl Neurol.* **13**, 40-57.
- HERNANDEZ-PEON, R. & STERMAN, M. B. (1966). Brain functions. *Ann. Rev. Psychol.* **17**, 363-99.
- HESS, A. (1958). Experimental anatomical studies of pathways in the severed central nerve cord of the cockroach. *J. Morph.* **103**, 479-501.
- HINDE, R. A. (1970). Behavioural habituation. In *Short-term Changes in Neural Activity and Behaviour* (ed. G. Horn and R. A. Hinde), pp. 3-40. Cambridge University Press.
- HORN, G. (1965). Physiological and psychological aspects of selective perception. *Adv. Study Behav.* **1**, 155-215.
- HORN, G. (1967). Neuronal mechanisms of habituation. *Nature, Lond.* **215**, 707-11.
- HORN, G. (1970). Changes in neuronal activity and their relationship to behaviour. In *Short-term Changes in Neural Activity and Behaviour* (ed. G. Horn and R. A. Hinde), pp. 567-606. Cambridge University Press.
- HORN, G. & ROWELL, C. H. F. (1968). Medium and long-term changes in the behaviour of visual neurones in the tritocerebrum of locusts. *J. exp. Biol.* **49**, 143-69.
- HORN, G. & WRIGHT, M. J. (1970). Characteristics of transmission failure in the squid stellate ganglion: a study of a simple habituating system. *J. exp. Biol.* **51**, 217-31.
- HUGHES, G. M. (1965). Neuronal pathways in the insect central nervous system. In *The Physiology of the Insect Central Nervous System* (ed. J. E. Treherne and J. W. L. Beament), pp. 79-112. London: Academic Press.
- HUMPHREY, G. (1933). *The Nature of Learning in Its Relation to the Living System*. N.Y.: Harcourt Brace.
- KANDEL, E., CASTELLUCCI, V., PINSKER, H. & KUPFERMANN, I. (1970). The role of synaptic plasticity in the short-term modification of behaviour. In *Short-term Changes in Neural Activity and Behaviour* (ed. G. Horn and R. A. Hinde), pp. 281-322. Cambridge University Press.
- KANDEL, E. R. & SPENCER, W. A. (1968). Cellular neurophysiological approaches in the study of learning. *Physiol. Rev.* **48**, 65-134.
- KEHOE, J. S. & BRUNER, J. (1966). Spécificité de l'habituation de la voie activée étudiée au niveau des neurones centraux de l'*Aplysia*. *J. Physiol., Paris* **58**, 542-3.
- KONORSKI, J. (1967). *Integrative Activity of the Brain*. University of Chicago Press.



- KRASNE, F. B. & WOODSMALL, K. S. (1969). Waning of the crayfish escape response as a result of repeated stimulation. *Anim. Behav.* **17**, 416-24.
- LANGERON, M. (1949). *Précis de microscopie*. Paris: Masson.
- LE BRIS, R. (1964). Contribution à l'étude de la morphogénèse sensori-nerveuse des palpes labiaux, maxillaires et des cerques chez *Blabera craniifer*, D.E.S., University of Rennes.
- MILBURN, N. S. & BENTLEY, D. R. (1971). On the dendritic topology and activation of cockroach giant interneurons. *J. Insect Physiol.* **17**, 607-23.
- NICKLAUS, E. D. (1965). Die Erregung einzelner Fadenhaare von *Periplaneta americana* in Abhängigkeit von der Grösse und Richtung der Auslenkung. *Z. vergl. Physiol.* **50**, 331-62.
- PARNAS, I., SPIRA, M. E., WERMAN, R. & BERGMANN, F. (1969). Non-homogeneous conduction in giant axons of the nerve cord of *Periplaneta americana*. *J. exp. Biol.* **50**, 635-49.
- PAVLOV, I. P. (1927). *Conditioned Reflexes* (trans. by G. V. Anrep). New York: Dover Publications.
- PORTER, J. M. (1938). Adaptation of the galvanic skin response. *J. exp. Psychol.* **23**, 403-10.
- PROSSER, C. L. & HUNTER, W. S. (1936). The extinction of startle responses and spinal reflexes in the white rat. *Am. J. Physiol.* **117**, 609-18.
- PUMPHREY, R. J. & RAWDON-SMITH, A. F. (1937). Synaptic transmission of nervous impulses through the last abdominal ganglion of the cockroach. *Proc. R. Soc. Lond. B* **122**, 106-18.
- REHN, J. A. G. & HEBARD, M. (1927). The Orthoptera of the West Indies. 1. Blattidae. *Bull. Am. Mus. nat. Hist.* **54**, 1-320.
- RICHARD, G., ZILBER-GACHELIN, N. F. & CHARTIER, M. P. C. (1973). What are the so-called 'cercal nerves' in the cockroach *Blabera craniifer*? *J. Insect Physiol.* **19** (in the Press).
- ROEDER, K. D. (1948). Organization of the ascending giant fibre system in the cockroach (*Periplaneta americana*). *J. exp. Zool.* **108**, 243-261.
- ROWELL, C. H. F. (1970). Incremental and decremental processes in the insect central nervous system. In *Short-term Changes in Neural Activity and Behaviour* (ed. G. Horn and R. A. Hinde), pp. 237-80. Cambridge University Press.
- SEGUNDO, J. P., TAKENADA, T. & ENCABO, H. (1967). Electrophysiology of bulbar reticular neurons. *J. Neurophysiol.* **30**, 1194-1220.
- SHARPLESS, S. K. & JASPER, H. (1956). Habituation of the arousal reaction. *Brain* **79**, 655-80.
- SIHLER, H. (1924). Die Sinnesorgane an den Cerci der Insekten. *Zool. Jb. (Anat.)* **45**, 519-80.
- SOKOLOV, E. N. (1960). Neuronal models and the orienting reflex. In *The Central Nervous System and Behavior* (ed. M. A. B. Brazier), pp. 187-276. New York: Josiah Macy, Jun. Foundation.
- SPENCER, W. A. M., THOMPSON, R. F. & NEILSON, D. R. JR. (1966). Decrement of ventral root electrotonus and intracellularly recorded PSP's produced by iterated cutaneous afferent volleys. *J. Neurophysiol.* **29**, 253-74.
- THOMPSON, R. F. & SPENCER, W. A. (1966). Habituation: a model phenomenon for the study of neuronal substrates of behavior. *Psychol. Rev.* **73**, 16-43.
- WALL, P. D. (1970). Habituation and post-tetanic potentiation in the spinal cord. In *Short-term Changes in Neural Activity and Behaviour* (ed. G. Horn and R. A. Hinde), pp. 181-210. Cambridge University Press.
- WICKELGREN, B. G. (1967a). Habituation of spinal motoneurons. *J. Neurophysiol.* **30**, 1404-23.
- WICKELGREN, B. G. (1967b). Habituation of spinal interneurons. *J. Neurophysiol.* **30**, 1424-38.
- WIGGLESWORTH, V. B. (1950). *The Principles of Insect Physiology*. London: Methuen.
- WOZNIAK, A., ALVAREZ, R., WILSON, E. & AUSTT, G. E. (1967). Cercal potential in the *Periplaneta americana*. *Acta Physiol. Lat-Am.* **17**, 102-11.
- ZILBER-GACHELIN, N. F. (1966a). Expériences de sensibilisation chez la Blatte. *J. Physiol., Paris* **58**, 276.
- ZILBER-GACHELIN, N. F. (1966b). Habituation au niveau d'un ganglion abdominal chez la Blatte, *Blabera fusca*. *J. Physiol., Paris* **58**, 643.
- ZILBER-GACHELIN, N. F. (1970). Etude comportementale et électrophysiologique de quelques facteurs responsables de la variabilité des réponses motrices réflexes de la Blatte. Thesis, University of Paris.
- ZILBER-GACHELIN, N. F. & CHARTIER, M. P. (1973). Modification of the motor reflex responses due to repetition of the peripheral stimulus in the cockroach. II. Conditions of activation of the motoneurons. *J. exp. Biol.* **59**, 383-403.

EXPLANATION OF PLATE

PLATE I

Histological section of the abdominal cord of *Blabera craniifer*. Section effected in the connectives joining the 5th and the 6th A.G. In each connective, giant fibres can be distinguished by their diameter (about 30 μm) clearly greater than that of most of the other fibres; they are clustered mainly in the ventral half of the connectives.

c, connectives; t, trachea; V, ventral side; D, dorsal side.