DISHABITUATION AND AROUSAL IN THE RESPONSE OF SINGLE NERVE CELLS IN AN INSECT BRAIN

By C. H. FRASER ROWELL and G. HORN

Department of Zoology, Makerere University College, Uganda, and Department of Anatomy, University of Cambridge

INTRODUCTION

In previous papers (Rowell & Horn, 1967; Horn & Rowell, 1968) the behaviour of certain neurones in the tritocerebrum of the desert locust have been described. Among other characteristics, these cells respond briskly to the movement of small dark objects in the visual field of the contralateral eye, irrespective of the movement of the animal itself. If a given movement is repeated, the response wanes (habituation), though the original level may be regained after a period of rest. Response decrement is not due to changes affecting the recorded cell directly, for it responds at the original level to an identical stimulus presented elsewhere in the visual field.

The time courses of habituation and of recovery from it in these cells are influenced in a complex fashion by various parameters of the stimulus routine. These influences are discussed elsewhere (Horn & Rowell, 1968). In this paper we report on a further group of factors affecting response level of tritocerebral neurones. For example, activity in regions of the central nervous system remote from the recorded cell can produce medium and long-term changes in responsiveness. These influences can be grouped together as arousal phenomena. Arousal has been little studied at the individual neurone level in invertebrate nervous systems (but see Kennedy & Preston, 1963), though there is a large body of work, concerned mainly with the reticular activating system, on arousal in vertebrates.

The events treated here are (1) dishabituation brought about by electrical stimulation of the nerve cord and (2) 'spontaneous' and diurnal changes in responsiveness.

MATERIAL AND METHODS

Mature adult desert locusts (*Schistocerca gregaria* Forskål) of either sex from a crowded laboratory culture were used. Most of the techniques used have been previously described (Horn & Rowell, 1968). The particular arrangements for the present experiments are illustrated in Fig. 1.

The locust was waxed into a special steel holder, and the holder and the micromanipulator carrying the electrode were mounted on two 25 cm. square plates of 1 cm. mild steel set at right angles. This arrangement permitted the whole assembly to be moved round, rotated, or turned over for access to the ventral surface of the animal for dissection of the thoracic nerve cord. No difficulty was experienced in retaining

the same units for recording for 24 hr. or more. Two silver electrodes on a second micromanipulator were connected to an isolated square-pulse generator for stimulation of the ventral nerve cord. In all cases this stimulus consisted of a 10 sec. train of 50 V. pulses with a duration of 0·5 msec. and a frequency of 36/sec.
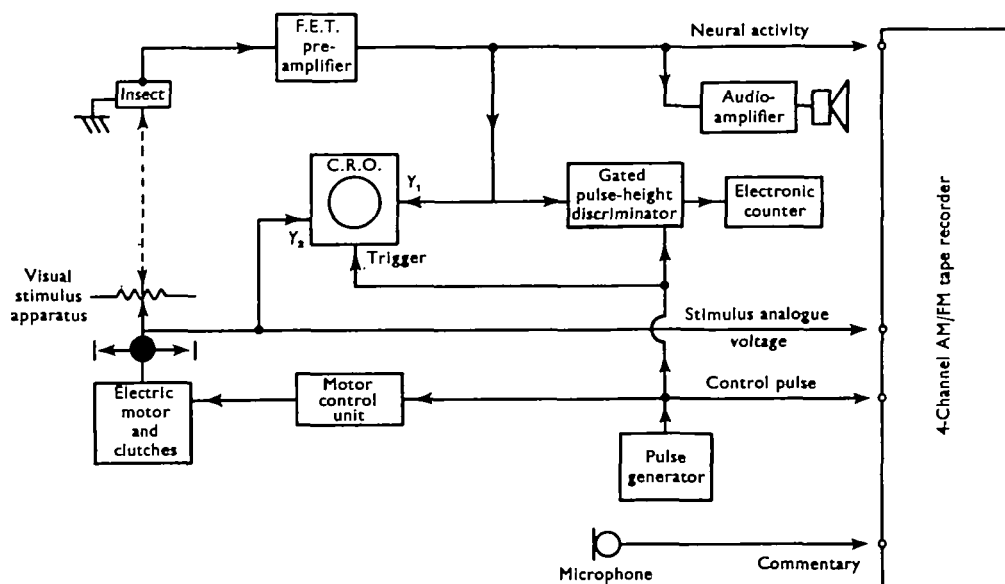

Fig. 1. Block diagram of experimental apparatus.

RESULTS

For these experiments a tritocerebral visual neurone of the appropriate type was recognized on the criteria described by Horn & Rowell (1968) and the electrode was positioned for optimum reception. The unit was then subjected to routine tests to ascertain its characteristics, including its responsiveness to the moving black disk and its background activity. The experimental procedures described below were not commenced until at least 1 hr. had elapsed after the electrode was placed in position.

Legend for Fig. 2.

Fig. 2. Effect of stimulation of the neck connectives on background activity of recorded cell. Data plotted in A and B are from different animals, in which the sequence of stimulation differed. In A the ipsilateral connective was first stimulated (curve 1) followed by the contralateral connective (curve 2), followed by simultaneous stimulation of both connectives (curve 3). In B, first the contralateral connective (curve 1) was stimulated, then the ipsilateral (curve 2). Both connectives (curve 3) were then stimulated, followed by the ipsilateral connective (curve 4) once again. In this figure the open squares indicate the expected number of impulses in a 10 sec. period, based on the mean background firing rate measured prior to stimulation. The large filled triangle gives the time at which the 10 sec. stimulus was applied to the neck connective.

Seconds after stimulus
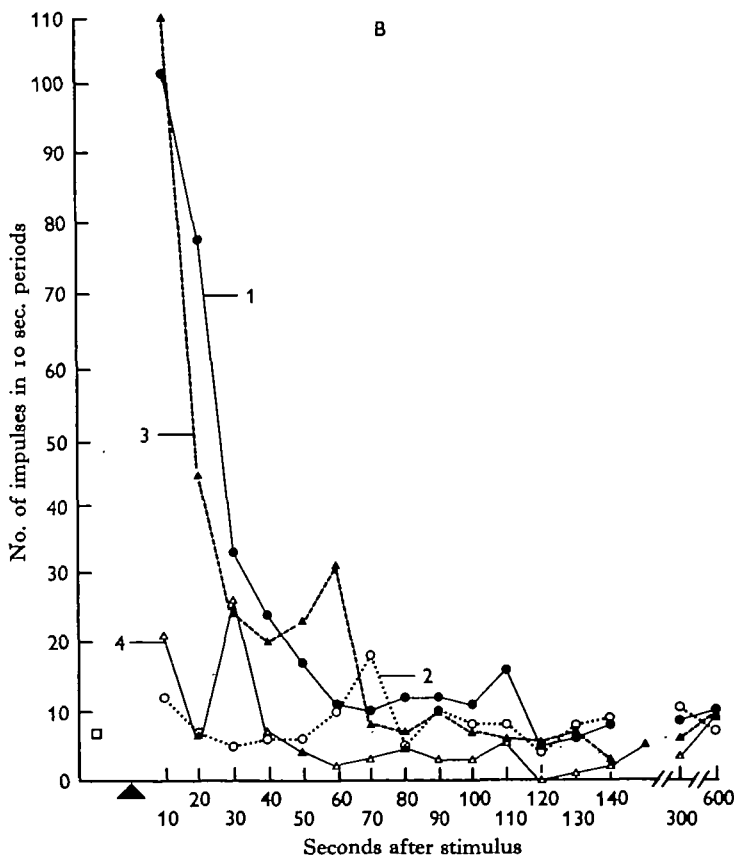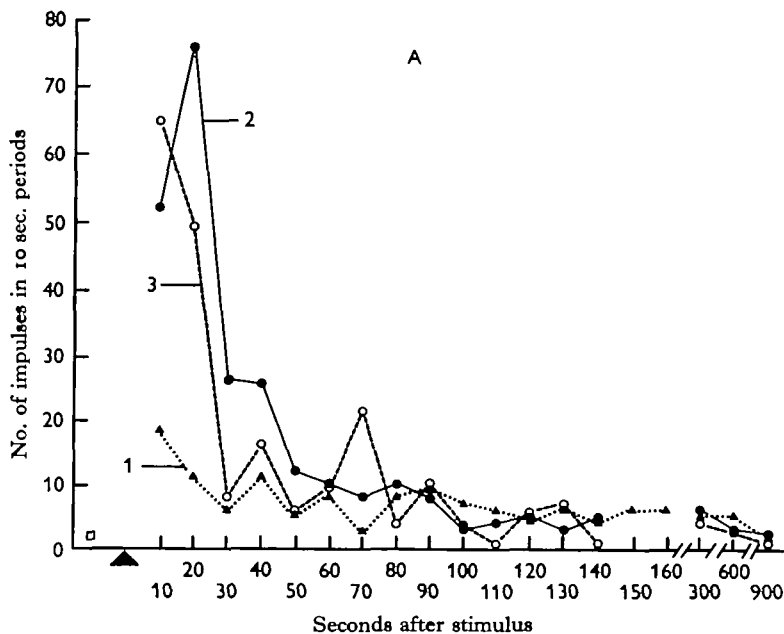
For legend see previous page.

A. *Effect of electrical stimulation on the neck connectives*

(i) *Failure of antidromic invasion.* Stimulation of the neck connectives never gave rise to 1:1 activity in the recorded cell, and any activity that was observed during stimulation was aphasic with respect to the stimulus. However, records have been made from fibres in the neck connectives showing similar properties to the trito-cerebral units; so that the failure to record antidromic activity may represent a failure of the impulse fully to invade the neurone (cf. Miledi, 1967), though the possibilities also remain that the axon in the neck connective was not excited by the stimulating current, and that the tritocerebral unit has no axon in the neck connective.

(ii) *Background activity.* Repetitive stimulation of either neck connective caused a temporary increase in the background level of activity of the recorded cell. This increase was small when the ipsilateral connective was stimulated, but large and long-lasting when the contralateral connective, or the contra- and ipsilateral connectives together, were stimulated.

These effects are shown in Fig. 2, data for Figs. 2A and B being obtained from different animals. Background activity was measured before and after stimulation as the total number of action potentials recorded in successive 10 sec. periods. For a short time after stimulation of the ipsilateral neck connective the background activity of each unit was slightly increased. Stimulation of the contralateral neck connective, by contrast, gave a very large increase in background activity, which fell off exponentially from the initial high level. The original low level of discharge was not regained for some minutes; on occasions 10–15 min. have been required. Simultaneous stimulation of both ipsi- and contralateral connectives gave an increase in background activity similar to that caused by stimulation of the contralateral connective alone (Fig. 2B).

The different results of stimulating the ipsi- and the contralateral connectives are not artifacts caused by a gradual arousal process built up through repeated stimulation; comparison of Figs. 2A, B, performed on different animals, shows that the differences persist in spite of differences in the sequences of stimulation.

(iii) *Dishabituation of response to moving disk.* A characteristic of the tritocerebral cells is that the response to a moving black disk in the visual field falls off rapidly when the stimulus is repeatedly presented (Horn & Rowell, 1968). If, when the response is weak, the contralateral neck connective is stimulated with a 10 sec. train of pulses as described above, the response to the repeated visual stimulus is greatly increased. This dishabituation effect may last for some minutes. Dishabituation is not obtained by stimulating the ipsilateral neck connective. There thus appears to be a close parallel between the increase of background activity and the dishabituation effect, suggesting the same mechanism is involved.

Dishabituation is illustrated by the specimen response curves plotted in Fig. 3. These data and those plotted in Fig. 2B are derived from the same cell. In the experiments shown in Fig. 3 the animal saw a black disk move forward across its visual field at 10 sec. intervals. Each traversal took 1·4 sec. The number of spikes elicited by each traversal of the disk gradually declined (Fig. 3, curve 1). After a pause of 120 sec., during which counts of background activity were made for successive periods of 1·4 sec., the response (Fig. 3, curve 2) was back to the initial level. After a second pause of 120 sec. (Fig. 3, curve 3) recovery was not complete. In the interval between
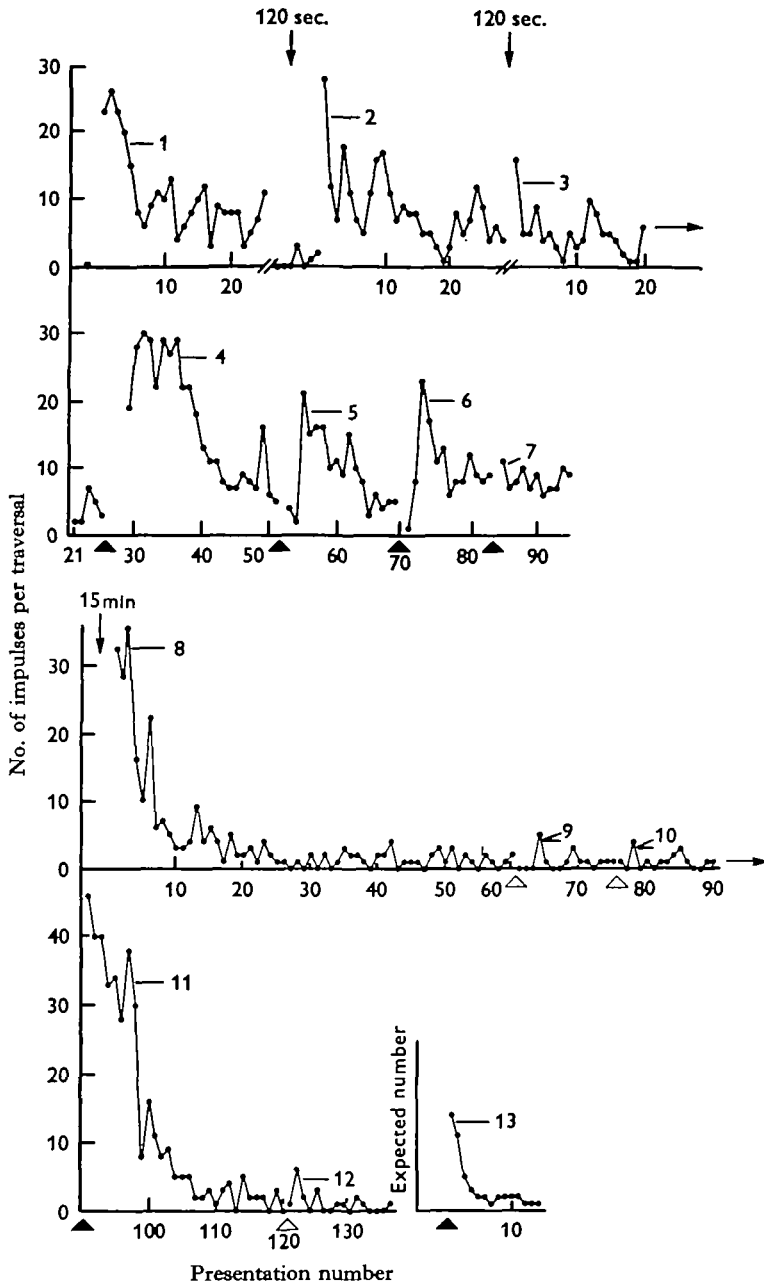
Fig. 3. Habituation to a repeated stimulus and dishabituation following stimulation of the contralateral neck connective, but not following stimulation of the ipsilateral neck connective. The visual stimulus was a disk moved through an angle of 40° in 1·4 sec. The interval between successive movements was 10 sec. In this and in Figs. 4 and 5 the filled black square gives the expected number of impulses per traversal (i.e. in 1·4 sec.) based on the background firing rate; the large filled and open triangles indicate when a train of shocks was applied to the contralateral and ipsilateral connectives respectively; and the numbers above the arrows indicate the interval of time elapsing between successive groups of stimuli. The arrow parallel to the abscissa indicates that stimulation was continued without a break in the sequence of movements. The curve between curves 1 and 2 is a control, giving dummy counts in the absence of stimulation. The values plotted in curve 13 were obtained from curve 1 of Fig. 2 B, by multiplying each of the ordinate values by 0·14. This gives the number of impulses in 1·4 sec. measured in successive 10 sec. intervals after stimulating the contralateral connective and in the absence of visual stimulation. The data obtained in this way are used as controls for the results plotted in curves 4–7 and 11.

the responses plotted in curves 3 and 4 a total of 40 sec. elapsed. In the first 10 sec. of this interval the contralateral connective was stimulated. The sequence of disk presentations continued without a break. The unit now (curve 4) responded to the disk more vigorously than on any previous occasion. A high level of response (> 19 impulses per traversal) was maintained for ten presentations—that is, for 100 sec.—before falling to lower levels. When the response had declined the contralateral connective was again stimulated, without interrupting the sequence of presentations of the disk. This procedure was repeated twice and the response curves (5, 6 and 7) were plotted. It may be seen that the dishabituating potency of the connective stimulus gradually waned and that the period of augmented response to visual stimulation was sometimes preceded by a brief period (curves 5 and 6) when the response was low. Four hours elapsed between the sequences of stimuli which generated response curves 7 and 8, and during this time the results described above were repeated and confirmed. Graph 8 is the response curve to the disk following a pause of 15 min, showing the usual recovery following a lapse of time. After sixty presentations the ipsilateral connective was stimulated, and stimulated again after the seventy-fifth presentation. This procedure was not followed by recovery (curves 9 and 10). In the 10 sec. intervening between the 90th and 91st presentations the contralateral connective was stimulated. The unit gave a vigorous and well-maintained response to the disk (curve 11). When the responses plotted in curve 11 had declined the ipsilateral connective was stimulated between presentations 120 and 121. Once again there was no dishabituation (curve 12).

The increased discharge to the disk which follows connective stimulation is a real increase in responsiveness and cannot be accounted for by the increase in background firing rate which also follows this manœuvre (Fig. 2). The evidence is as follows. The data plotted in Figs. 3 and 2B are from the same unit. It is thus possible to calculate from the data contained in Fig. 2B the number of impulses expected in counts of 1·4 sec. duration made at successive 10 sec. intervals after stimulating the contralateral connective and with no moving disk in view. These results are plotted in curve 13, Fig. 3. It may be seen that the counts are much smaller than the values obtained when the moving disk was present in the period following stimulation of the contralateral connective (Fig. 3, e.g. curves 4 and 11).

The influence exerted by structures in the connective on responsiveness of the recorded cell to visual stimulation does not depend on the direction in which the disk is moved. Thus discharges evoked by both forward movements (Fig. 4, curves 1–3) and backward movements (Fig. 4, curves 4–6) were dishabituated by stimulating the contralateral connective. From this figure it is also clear that the initial response following such stimulation (curves 2, 3 and 5, 6) exceeds those responses which are not preceded by this stimulus (curves 1 and 4).

B. 'Spontaneous' change in responsiveness with time

We have occasionally observed units which suddenly commence to give a maintained response to repeated stimulation although previously they had shown typical decremental behaviour. Such a change is illustrated in Fig. 5. The animal saw a black disk move upwards across its visual field at intervals of 10 sec. Following two series of such movements (Fig. 5, curves 1 and 2) this unit suddenly commenced to respond to each

of ten stimuli without decrement (Fig. 5, curve 3) though on the two previous occasions
the response clearly waned in this time. This sustained responsiveness is unlikely to
be due to a recovery following time lapse, since the period of rest was 5 min. compared
with 23 min. and 5 hr. which preceded the decremental responses of curves 1 and 2
respectively. Enhanced responsiveness was not due to any deliberate experimental
manipulation and appeared to be 'spontaneous'. It is worth noting in parenthesis that
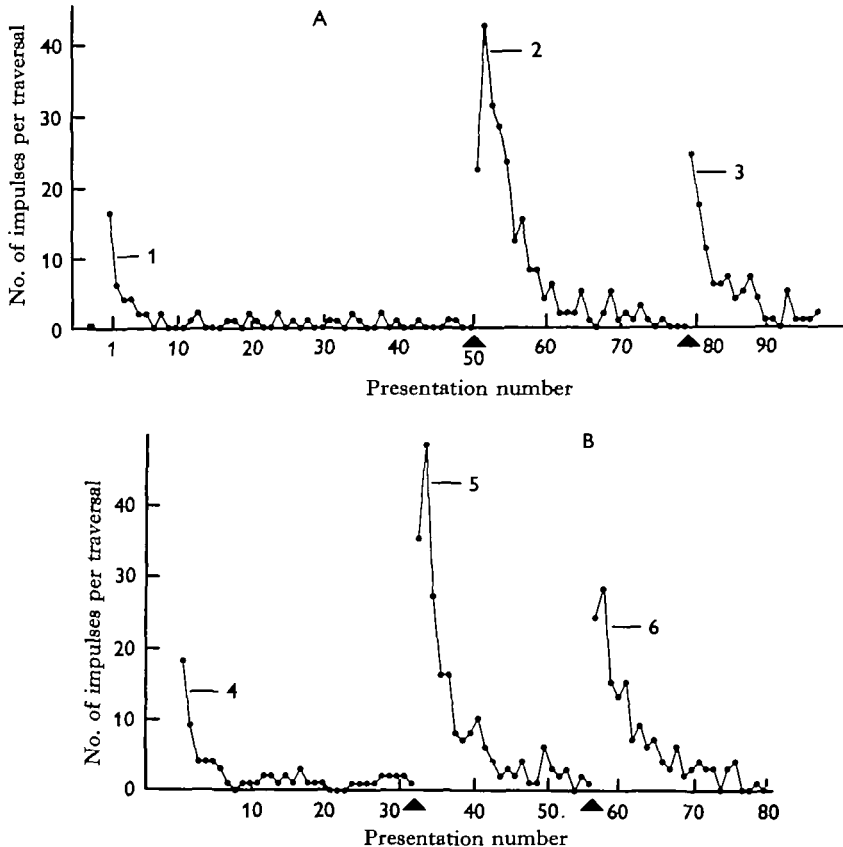

Fig. 4. Habituation and stimulus-induced dishabituation in a neurone responding to (A)
forward movements (curves 1–3) and (B) backward movements (curves 4–6). The contralateral
neck connective was stimulated for 10 sec. at the position in the sequences indicated by the
filled triangles. Each presentation consisted of a 6° black disk traversing 40° of the visual
field in 1·4 sec. The interval between successive presentations was 6 sec.

the pause of 5 hr. between the responses plotted in curves 1 and 2 (Fig. 5) was not
sufficient to restore the response to an upward movement. Obviously it is necessary to
consider whether such a prolonged depression is a result of a general deterioration of
the preparation. This seems unlikely because the cell responded to a forward move-
ment (Fig. 5, open triangle) presented immediately after the second set of up move-
ments (curve 2) almost as vigorously as it had done before the first set of up movements
had been delivered (Fig. 5, closed triangle).

Spontaneous changes in responsiveness could not be correlated with any particular

environmental event, and fortunately for the success of experimentation they were rare. On the few occasions when we followed the responsiveness of a neurone through a day, the following night, and the succeeding day, a more regular change in responsiveness was apparent. We have data on only three animals, but these agree in over-all performance. An illustration of this is given in the response curves of Fig. 6. The animal saw a black disk move across its visual field alternately upwards and downwards; the interstimulus interval (i.s.i.) was approximately 10 sec. Records obtained during daylight hours (graphs 1 and 2, 8, 9 and 10) showed a consistent performance in which the unit exhibited an over-all preference for upward as opposed to downward movement, and also primacy effects (Horn & Rowell, 1968) at the start of each series,
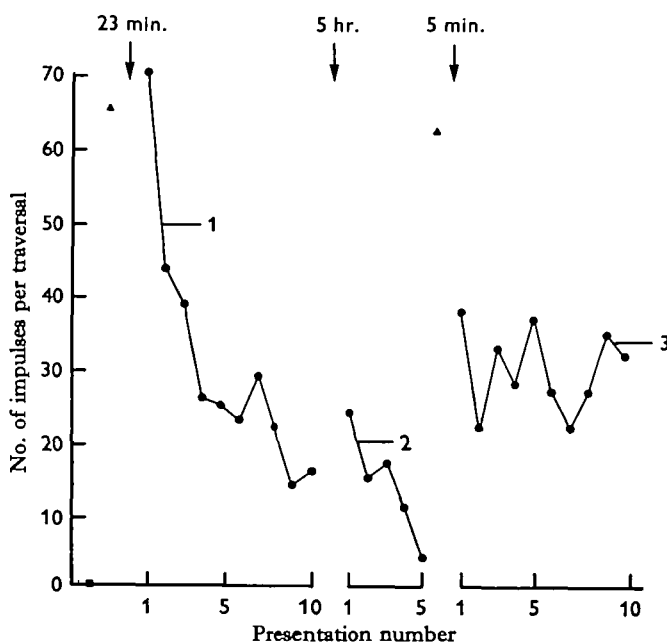

Fig. 5. Unit showing 'spontaneous' change from decremental to non-decremental response to a repeated stimulus. Each filled circle represents the number of impulses elicited by an upward movement of the disk, presented once every 10 sec. An interval of 5 hr. elapsed between the first sequence of movements (responses plotted in curve 1) and the second (curve 2). The initial stimulus of curve 2 evoked only a relatively weak discharge (twenty-five spikes) compared with the initial discharge in curve 1 of 71 spikes. Movement in a forward direction, immediately after the second group of up movements, evoked almost as many spikes (open triangle) as were evoked before the first set of up movements were presented (closed triangle).

resulting in a diminution or reversal of this preference when the downward movement was presented first. These records were obtained on the afternoon of the first day and the morning of the second. At 22.00 hr. in the intervening night, 4 hr. after local nightfall, experiments were resumed; the room lights were switched on, and testing began. For the first 30 min. the responsiveness was extremely low, and there was no clear directional preference (curves 3–5). At the end of this time there was an abrupt change in behaviour; responsiveness became very good, directional preference was more strongly evinced than at any other time, and there was obvious primacy (curves 6–7).

This sequence of changes in behaviour strongly suggests the presence of two different arousal states by day and night, and a transition from the night state to something resembling the day state following an interval of illumination in the middle of the night.
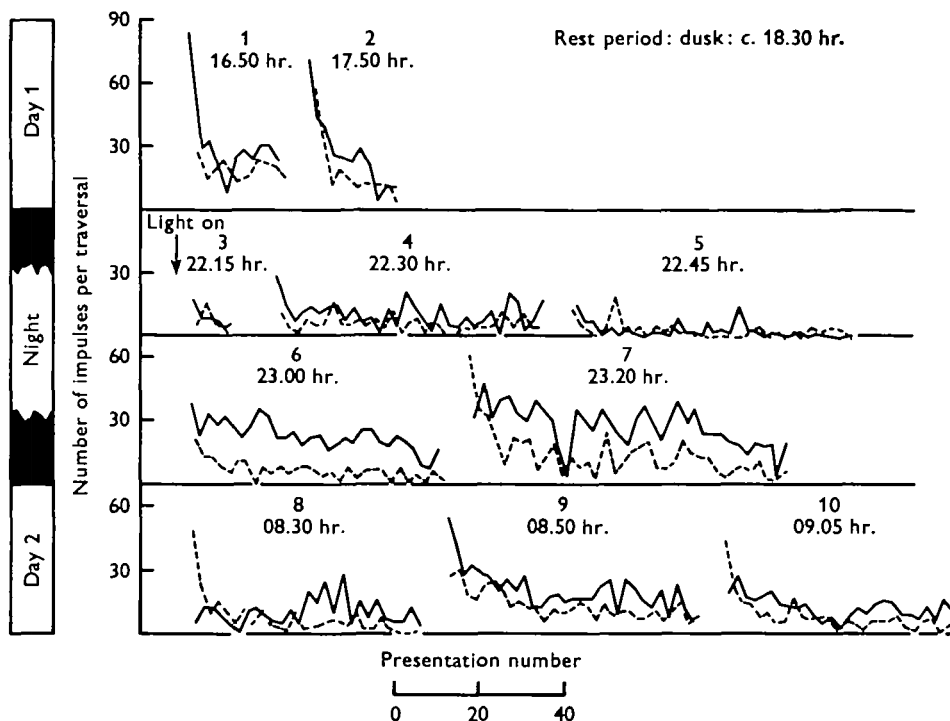

Fig. 6. Change in responsiveness to groups of identical stimuli presented at intervals over a 17 hr. period. In each group of presentations the animal saw alternate up and down traversals of a black disk across 40° of its visual field, each traversal taking approximately 1 sec. The i.s.i. was 10 sec. The number of impulses evoked by each traversal is plotted (*Y* axis) against stimulus number (*X* axis). Up responses are joined by a solid line, and down responses by a broken one. Graph 1 represents the first sequence of traversals in the up–down direction which the animal saw, but follows some 7 hr. of tests with the disk moving in other directions. Note that the first responses obtained in graphs 1 and 2 are higher than any subsequently obtained, even after intervals of > 8 hr. In tests in daylight hours (07.00–19.00 hr.) this unit had an over-all preference for up over down, and initially good responsiveness with fairly rapid habituation to repeated stimulation; there are also primacy effects which modify the response to the first few stimuli of each series (further explanation in text). The tests carried out in the first 30 min. after switching on the room lights at 22.15 hr. show very low responsiveness, and no directional preference. In the next ½ hr. an abrupt change in behaviour is seen, and responsiveness, directional preference, and primacy are all well marked. Scale is of the abscissa (presentation numbers).

DISCUSSION

Dishabituation

Once the discharge evoked by a moving disk has waned through repetition this stimulus may become effective again in eliciting a response if the contralateral neck connective is shocked. Presumably this dishabituation is brought about through the activity of fibres in the connective, though we do not know which fibres. It may be that axons of the tritocerebral units are directly stimulated when shocking the cord,

as we have not excluded the possibility that the axons descend into the thoracic cord (see section A(i) of Results). Dishabituation may also be brought about trans-synaptically by the activity of other units stimulated in the connectives. No primary sensory fibres are known to occur in the connectives, though they may in fact do so (cf. Rowell & Dorey, 1967). However, it is probable that much of the sensory input to the thoracic and abdominal ganglia is reported to the brain via interneurones, and this is known to occur for at least some tactile (Rowell, 1963a) and auditory (e.g. Horridge, 1961; Yanagisawa, Hashimoto & Katsuki, 1967; Adam & Schwartzkopf, 1967) inputs to the thorax. Neither tactile nor auditory stimulation was shown to have any effect on these neurones, either on the background firing rate (Horn & Rowell, 1968) or on the responsiveness to a moving disk. Dishabituation may be brought about in nature by activity in ascending proprioceptive fibres, though we have not excluded the possibility that it may be a result of antidromic stimulation of descending fibres present in the neck connective. In either case there exists the possibility that motor functions bring about the effect—either through collaterals of descending fibres or through proprioceptive feedback. It is therefore of interest to find (Wiersma, 1967) that the response to visual stimulation of certain fibres in the crayfish optic nerve is increased while the preparation is making leg and other movements compared with the response when the animal is at rest. Whether or not the excitation of the recorded cell is accomplished trans-synaptically, it is interesting to compare these results with those of Kennedy & Preston (1963), who stimulated neurones of the central nervous system of the crayfish both directly and trans-synaptically. The effects so obtained included prolonged after-discharge and long-term enhancement of spontaneous activity. Kennedy & Preston (1963) did not test for dishabituation, but there are clearly close parallels with the present results.

The neuronal mechanisms responsible for dishabituation are obscure and any hypothesis about them will have to take into account the physiological basis of habituation. If this habituation results from an imbalance between transmitter mobilization and utilization, as proposed by Bruner & Tauc (1966), then dishabituation would occur if the process of transmitter mobilization were accelerated or if the post-synaptic membranes were facilitated (Horn, 1967). The former could be brought about by hyperpolarization of the pre-synaptic terminals (del Castillo & Katz, 1954; Takeuchi & Takeuchi, 1962; Hubbard & Willis, 1962; Mendel & Wall, 1964) and the latter by subthreshold depolarization of the post-synaptic membrane. One or other or both effects may be involved following activation of fibres in the contralateral neck connective and presumably as a result of a spontaneous burst of activity in these or other neurones.

<div align="center">Long-term change in responsiveness</div>

Change in the over-all level of nervous activity in specific parts of the insect nervous system has previously been shown to be correlated with two different classes of events.

(1) *Hormonal environment.* Milburn, Weiant & Roeder (1960) showed that increase of activity in the last abdominal ganglion of cockroach and mantis after lesions to the anterior nerve cord were due not only to disruption of inhibitory nerve connexions but also to the release of corpus cardiacum hormone brought about by injury stress. Haskell & Moorhouse (1963) reported differences in the activity of both central

nervous system connectives and motor nerves between mid-instar locusts and those at ecdysis, and showed that similar effects could be reproduced by appropriate blood transfusions between the two classes; they concluded that hormonal influences on the central nervous system were present.

Many aspects of metabolism and behaviour in insects exhibit well-marked circadian rhythm or periodicity of some other nature. At least some of these fluctuations, as in the case of variation in motor activity, must reflect parallel changes in nervous activity. Harker (1960) has shown that rhythmicity in the activity of cockroach is related to similar rhythmicity in the activity of neural and neurendocrine elements in the suboesophageal ganglion and brain, and has demonstrated by parabiosis and implant that humoral influences can be decisive in programming the motor activity of the central nervous system (but see reference to Brady, 1967, below).

(2) *Specific neural connexions.* There is an extensive older literature on the activating effects on the cord nervous system produced by lesions to the head ganglia. The generally accepted view arising from these experiments was that the brain exerted a depressant effect on locomotor activity arising 'endogenously' in the cord system, and that the suboesophageal ganglion exerted a balanced excitatory effect. Milburn *et al.* (1960) now feel that this evidence must be re-interpreted with caution in view of the interaction between lesion and hormone production, or merely between stress and hormone production (e.g. Highnam, 1961; Brady, 1967).

However, there is other evidence suggesting that activity of relatively large parts of the nervous system can be neurally regulated. Direct stimulation of the brain produces clear activating and depressant effects on a variety of behaviour patterns (Huber, e.g. 1960; Rowell, 1963 *b*) with a latency not consistent with hormonal participation. Highly specific lesions, which can be made after an extensive series of previously ineffective lesions, can have rapid and predictable effects on the activity of various ganglionic neuropiles (Weiant, 1958; Roeder, Tozian & Weiant, 1960; Rowell, 1965).

The long-term change in responsiveness described in this paper which is correlated with darkness and light, suggesting interaction with a circadian rhythm of the animal, may well be due to changes brought about, at least indirectly, by hormonal events. The relatively long interval of 30 min. between an environmental perturbation (switching on the lights) and a change in responsiveness is also compatible with humoral action. However, in spite of the suggestive analogies with the work on cockroaches, it must be borne in mind that the circadian rhythmic activity on the 'parabolic burster' cells of the *Aplysia* parieto-visceral ganglion (Strumwasser, 1965) is apparently endogenous, and Brady (1967) has suggested that the endocrine rhythmicity described by Harker in cockroaches is in fact subordinate to a neural (electrical) rhythmicity elsewhere in the central nervous system. At the level of the activity of a single neurone it is perhaps unimportant whether it receives its input from a generally circulating neurendocrine chemical, or synaptically from rhythmically discharging neurones. The point we make here is that the activity of our recorded cells is influenced in a complex manner by events occurring at least initially in elements of the nervous system far removed both anatomically and functionally from these cells. In the case of the dishabituating cord input there seems no doubt that the connexion is nervous, in view of its short latency, while responsiveness may also be modified by circadian events in the general hormonal environment.

It is noteworthy on the one hand that changes in responsiveness which occur over day and night are not obviously paralleled by changes in the background activity of the cell. This suggests that the site of action of the agents of change is some distance removed from the recorded cell itself. On the other hand the dishabituating input to the nerve cord causes closely correlated changes in both responsiveness and in background activity. These two facts together suggest that responsiveness of the recorded cell and the peripheral system that feeds it can be controlled at more than one level.

SUMMARY

1. The background activity of neurones in the tritocerebrum of the locust responding to objects moved in the contralateral visual field can be influenced, possibly trans-synaptically, by electrical stimulation of the contralateral neck connective. These changes in background activity are always excitatory and may outlast the period of stimulation by several minutes. The effect of stimulating the ipsilateral cord on the discharge is weak or non-existent.

2. If the contralateral connective is shocked when the response to a moving disk has waned, the recorded cell responds to the disk again. The response continues at a high level for many presentations following stimulation of the connective. Such dishabituation does not follow stimulation of the ipsilateral neck connective.

3. Dishabituation sometimes occurs 'spontaneously' and cannot be accounted for as a recovery following a lapse of time.

4. Some units show diurnal variations in their responsiveness to visual stimulation.

REFERENCES

ADAM, L.-J. & SCHWARTZKOPF, J. (1967). Getrennte nervöse Representation für verschiedene Tonbereiche im Protocerebrum von *Locusta migratoria. Z. vergl. Physiol.* **54,** 246–55.

BRADY, J. (1967). Control of the circadian rhythm of activity in the cockroach. II. The role of the sub-oesophageal ganglion and ventral nerve cord. *J. Exp. Biol.* **47,** 165–78.

BRUNER, J. & TAUC, L. (1966). Habituation at the synaptic level in *Aplysia. Nature, Lond.* **210,** 37–9.

DEL CASTILLO, J. & KATZ, B. (1954). Changes in end-plate activity produced by presynaptic polarization. *J. Physiol., Lond.* **124,** 586–684.

HARKER, J. (1960). Endocrine and nervous factors in insect circadian rhythms. *Cold Spring Harb. Symp. quant. Biol.* **25,** 279–87.

HASKELL, P. T. & MOORHOUSE, J. E. (1963). A blood-borne factor influencing the activity of the central nervous system of the desert locust. *Nature, Lond.* **197,** 56–8.

HIGHNAM, K. C. (1961). Induced changes in the amounts of material in the neurosecretory system of the desert locust. *Nature, Lond.* **191,** 199–200.

HORN, G. (1967). Neuronal mechanisms of habituation. *Nature, Lond.* **215,** 707–11.

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,** 143–169.

HORRIDGE, G. A. (1961). Pitch discrimination in locusts. *Proc. R. Soc.* B **155,** 218–31.

HUBBARD, J. I. & WILLIS, W. D. (1962). Hyperpolarization of mammalian motor nerve terminals. *J. Physiol., Lond.* **163,** 115–37.

HUBER, F. (1960). Untersuchungen über die Funktion des Zentralnerven-systems und inbesondere des Gehirnes bei der Fortbewegung und der Lauterzeugung der Grillen. *Z. vergl. Physiol.* **44,** 60–132.

KENNEDY, D. & PRESTON, J. B. (1963). Post-activation changes in excitability and spontaneous firing of crustacean interneurones. *Comp. Biochem. Physiol.* **8**, 173–9.

MENDEL, L. M. & WALL, P. D. (1964). Presynaptic hyperpolarization: a role for fine afferent fibres. *J. Physiol., Lond.* **172**, 274–94.

MILBURN, N., WEIANT, E. A. & ROEDER, K. D. (1960). The release of efferent nerve activity in the cockroach, *Periplaneta americana*, by extracts of the corpus cardiacum. *Biol. Bull. mar. biol. Lab., Woods Hole* **118**, 111–19.

MILEDI, R. (1967). Spontaneous synaptic potentials and quantal release of transmitter in the stellate ganglion of the squid. *J. Physiol., Lond.* **192**, 379–406.

ROEDER, K. D., TOZIAN, L. & WEIANT, E. A. (1960). Endogenous nerve activity and behaviour in the mantis and cockroach. *J. Insect Physiol.* **4**, 45–62.

ROWELL, C. H. F. (1963a). The structure and function of the prothoracic spine of the desert locust, *Schistocerca gregaria* Forskål. *J. Exp. Biol.* **38**, 457–69.

ROWELL, C. H. F. (1963b). A method of chronically implanting stimulating electrodes into the brains of insects, and some results of stimulation. *J. Exp. Biol.* **40**, 271–84.

ROWELL, C. H. F. (1964). Central control of an insect segmental reflex. I. Inhibition by different parts of the nervous system. *J. Exp. Biol.* **41**, 559–72.

ROWELL, C. H. F. & DOREY, A. E. (1967). The number and size of axons in the thoracic connectives of the desert locust, *Schistocerca gregaria* Forskål. *Z. Zellforsch. mikrosk. Anat.* **83**, 288–94.

ROWELL, C. H. F. & HORN, G. (1967). Response characteristics of neurones in an insect brain. *Nature, Lond.* **216**, 702–3.

STRUMWASSER, F. (1965). The demonstration and manipulation of a circadian rhythm in a single neuron. In *Circadian Clocks*, ed. J. Aschoff. Amsterdam: North Holland Publishing Co.

TAKEUCHI, A. & TAKEUCHI, N. (1962). Electrical changes in pre- and post-synaptic axons of the giant synapse of *Loligo*. *J. gen. Physiol.* **45**, 1181–93.

WEIANT, E. A. (1958). Control of spontaneous efferent activity in certain efferent nerve fibres from the metathoracic ganglion of the cockroach. *Proc. 10th Int. Congr. Ent.* **2**, 81–2.

WIERSMA, C. A. G. (1967). Visual central processing in crustaceans. In *Invertebrate Nervous Systems*, pp. 269–84. Ed. C. A. G. Wiersma. Chicago and London: University of Chicago Press.

YANAGISAWA, K., HASHIMOTO, T. & KATSUKI, Y. (1967). Frequency discrimination in the central nerve cords of locusts. *J. Insect Physiol.* **13**, 635–43.