A METHOD FOR CHRONICALLY IMPLANTING STIMULATING ELECTRODES INTO THE BRAINS OF LOCUSTS, AND SOME RESULTS OF STIMULATION

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INTRODUCTION

The results of electrical stimulation of the insect supraoesophageal ganglion have been reported by Huber (1960a and previous works) from acute preparations of Gryllus. The present study is centred around a technique of cerebral stimulation which augments this work in two ways: first, by using a different species, Schistocerca gregaria, the desert locust, and secondly by providing a method of stimulating virtually unrestrained insects through chronically implanted electrodes. Huber’s method has been especially valuable for studying such functions as respiration, for the movements of spiracles, etc., can only be observed accurately in the restrained animal; the method described below, which allows a free-moving animal to be tested in a familiar environment on many different occasions, is more suited to behavioural studies.

METHOD

Four main obstacles were encountered. These were:

(1) Implanting suitable electrodes, and coupling them to a stimulator.
(2) Localizing the electrode.
(3) Devising a compatible and efficient silver impregnation method for histological examination of the brain.
(4) Evaluating the physical conditions affecting current flow through the electrode during stimulation, and the way they can distort the interpretation of the result.

Solutions to the last two problems have been described elsewhere (Rowell, 1963a, b). The others are treated below.

(1) Implantation of electrodes

Adult locusts were anaesthetized in either nitrogen or carbon dioxide/air mixture, in order to stop the heart and thus the blood flow during the operation. Nitrogen gave a quicker recovery and fewer after-effects. The animal was then clamped into a gas chamber which allowed for further anaesthetic during the operation if necessary, with the head protruding under a binocular microscope. A small window was cut dorsally between the eyes, and the cuticle was hinged back and stuck down with sticky wax (Weis-Fogh, 1956) (Text-fig. 1). The dorsal surface of the brain was exposed, which involved damage to the occipital air sac; this appeared to have no harmful results. A

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small slit was made in the brain membrane with a fine tungsten knife, and the electrode was inserted through this into the brain.

The electrode was a length of 25 μ diameter stainless-steel wire, insulated to the tip in an epoxy resin (‘diamel’ Johnston Matthey). At the implanted end it was cut square across, presenting a stimulating surface of approx. $6 \times 10^{-6} \text{ cm}^2$; at the other end, a length of insulation was scraped off, the steel was electroplated with copper and tinned with solder. The electrode was waxed to a needle in a micro-manipulator, and was sufficiently rigid to penetrate the brain tissues unaided. After the needle had been inserted, the wax was melted with a heating coil, and the electrode was detached without vibration. The cuticle was hinged back into place, and the free end of the electrode was brought to the back of the head, where it is not caught by the front legs in normal cleaning movements; the whole of the operated area was then covered with a layer of sticky wax. The animal was replaced in the stock cage when sufficiently recovered from anaesthesia.

(2) Testing procedure

If necessary, the electrode was left in place for the whole 13 weeks of adult life, and the insect could be tested many times in this period. For testing, two long flexible 48 s.w.g. enamelled copper wires were attached to the pronotum with sticky wax (Pl. 1). One terminated in an ‘indifferent’ stainless-steel electrode of relatively large size, which was inserted into the thoracic haemolymph. The other was tinned and soldered to the free end of the implanted electrode, which was sufficiently long and flexible to allow the head to move freely. The copper wire was sufficiently large, relative to the electrode, to act as a heat sink and prevent lesion due to heating, providing soldering was rapid.

Negative-going rectangular voltage pulses of 0.1–2.0 msec. duration and variable
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Amplitude were applied at rates from 5 to 200 pulses/sec. Current flow was monitored with a suitably connected c.r.o. (Rowell, 1963b). In general, current values were much smaller than is usual with vertebrate preparations, although the current densities were about the same; it was rarely necessary to use more than 20 μA., and frequently the threshold for complete behavioural responses was less than 5 μA. At these levels it is almost certain that the active elements are few in number and near the electrode tip. This was checked if necessary by coagulating the tissue around the electrode by applying a R.F. alternating current, about 6 mA. at 2.5 Mc./sec. for 5–10 sec. Absence of response after this usually means the death of the active elements. This is not absolutely certain, as the electrical conductivity of the tissue will now be changed, and may cause a change in current flow. However, this is unlikely to be important in most cases, as the effect of coagulation is to decrease the tissue resistance. Localization of the stimulated area is easier if bipolar electrodes are used, but to date such electrodes have been of large size (> 50 μ) relative to the brain and thus unsatisfactory.

Animals were tested in a large glass trough, floored with moist sand, and provided with fresh grass, bran, twigs to climb on, and if necessary other locusts. A radiant heater overhead heated this arena, and thermocouples implanted in test insects showed that under these conditions they maintained their blood temperature at 30–31° C.

All the insects used in the tests described below were adult *Schistocerca gregaria* of the gregarious phase, reared in laboratory culture. The method has also been successfully applied to house crickets (*Acheta domestica*) and another large catantopine *Ornithacris turbida,* the nucleus of which culture was kindly supplied by the Anti-locust Research Centre. This insect is on the whole less suitable, being less resistant to operative shock than *Schistocerca,* more sluggish in its habits, and having the dorsal surface of the brain obscured by the relatively larger mandibular muscles.

(3) Localization

There are four main methods of locating an electrode embedded in the nervous system:

1. X-ray.
2. Fixation before removal of the electrode, and examination of the electrode canal.
3. Coagulation of the surrounding area by R.F. microcautery.
4. Electrolytic deposition of marker ions from the electrode, and subsequent precipitation and staining.

Any of these methods can be combined. When it is necessary to locate the site of the electrode microscopically, it is essential that there be visible indication of the electrode on the mounted slide. The last three methods provide this. The electrode canal is a valuable supplementary aid, but is essentially a negative feature, and if the electrodes are small, it is not by itself reliable.

Coagulation is reliable, but has two restrictions. It is only possible with metal electrodes, and its results are visible only if stained with one of two stains. These are osmium tetroxide and azan. Osmium is an excellent fixative and stain of the nervous system, but very poorly penetrant. It can therefore be used only when the tissue is exposed as in acute preparation, or after dissection of a chronic preparation, which means removing the electrode first. This in turn loses the electrode canal, and risks damage to the tissue. Azan allows penetrant fixatives and thus fixation with the
electrode in situ. Coagulated tissue is clearly shown, but unfortunately nervous anatomy is not. Silver impregnation methods, which do show anatomy, are not reliable for small areas of coagulated tissue.

Electrical deposition of marker ions from the electrode is the most accurate and satisfactory method, and has been used in various ways for over 20 years. When the electrode is steel, the final deposit can conveniently be prussian blue, ferric ferrocyanide. This method shows the actual area of electrical contact with the tissue, and therefore also shows up faulty insulation. The small blue speck produced is visible in dissection and staining; silver impregnation merely changes the colour from blue to purple-black. It is not, as is sometimes stated, necessary to use hydrochloric acid to free the ferric ions in the tissue; this avoids a source of difficulty with subsequent silver treatment.

Procedure

(1) Cut the head from the insect with the electrode in place, make some slits in the cuticle, and fix for 3 hr. or more in Carnoy, alcoholic Bouin, or some other penetrant fixative.

(2) Place the head in a drop of fixative in contact with a carbon electrode. Couple this in series with a 120 V. battery, a 2-8 MΩ resistor, a microammeter, and a pair of metal forceps. Grasp the free end of the electrode with the forceps, and pass about 40 μA. for 10–20 sec., with the implanted electrode as anode. The high voltage source and series resistance give a constant current unaffected by polarization.

(3) Return the head to fixative for about an hour. This allows the ferric ions to diffuse from the electrode tip, though the use of a d.c. rather than an a.c. current has already largely done this. Otherwise, there is a danger that when precipitated, the ferric compound will form a clot at the electrode tip and be removed along with it.

(4) Place the head in a solution of potassium ferrocyanide for an hour or more.

(5) Dissect out the brain, withdraw the electrode, noting its position and orientation. Wash, dehydrate and clear. When cleared, or before, the prussian blue will be visible and its position can be noted. Embed and section.

(6) Examine the slides in water before staining. The blue-stained areas are more conspicuous before general staining of the tissue.

(7) Silver-impregnate by a reliable method. A method based on those of Holmes and Samuel (Rowell, 1963a) has been found satisfactory.

RESULTS

It is common knowledge in behavioural studies that any given stimulus is less likely to elicit a response from a constrained animal in an unfamiliar situation than it would from an animal in its normal state; that is, the threshold of the response is increased by the experimental conditions. This process can go to remarkable lengths, causing the animal, in its efforts to escape from the unfamiliar situation, to ignore peripheral stimuli severe enough to cause injury (e.g. Rowell, 1961b). The method of stimulation through chronic electrodes minimizes this sort of disturbance, but a certain amount is inevitably present, and accounts for the general impression that experimental animals are less responsive than controls. The disturbance is considerably
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less, however, than that caused by an acute implantation, where the animal is at best clasped rigidly by the thorax, with the limbs free. A comparison of different measured thresholds from the same electrodes, obtained in the two types of experimental set-up, are given in Table 1. It will be seen that while more complex activities are affected as described, local motor responses are not. This result explains why the thresholds measured from chronically implanted locusts are usually lower than those obtained from acutely implanted crickets (Huber, 1960a, and previous work). The differences are not in the nervous systems nor in the criteria of the observers, but in the state of the animals.

Table 1. Threshold currents of various activities produced by brain stimulation

(The same animal is tested freely moving in an arena, and restrained by the thorax, its feet touching a foam plastic ball. Each figure is the average of five or more determinations.)

<table>
<thead>
<tr>
<th>Animal</th>
<th>Activity</th>
<th>Restrained</th>
<th>Free</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Antennal cleaning</td>
<td>31</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Jump</td>
<td>40</td>
<td>18</td>
</tr>
<tr>
<td>B</td>
<td>Foraging</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>Foraging</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>D</td>
<td>Locomotion</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>E</td>
<td>Antennal flexure</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>F</td>
<td>Vibration of palps</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

The responses obtained from a given electrode are greatly influenced by environmental stimuli, and the method described above is particularly well adapted to study these effects. A further paper will deal with them. Apart from this, and the threshold effects mentioned above, many of the results obtained from locusts are extremely similar to those which Huber (loc. cit.) has reported from crickets. As there are considerable differences both in brain structure and way of life of the two species, it is worth recording the main points of similarity and difference, for they suggest some common organization which may be true of all insects. The remainder of this paper deals mainly with this comparison under the following headings: (1) antennal movements, (2) locomotion, including turning, (3) feeding, (4) sexual behaviour.

(1) Antennal movements

Simple antennal movements, synchronous with the stimulus frequency at rates below the muscle tetanus, are obtained from the sites shown in Text-fig. 2 (compare with fig. 4, Huber (1959)). These lie in the 'antennal motor centre' of the anatomists, posterior to the main antennal glomerulus, in the tracts running laterally round the glomerulus connecting this area with the antennal nerve, in the antennal commissure region, and the tractus olfactorio-globularis. The commissure contains motor fibres to both antennae, for stimulation here gives bilateral synchronous vibration; prolonged stimulation of one of the antennal motor centres will often eventually cause a spread of synchronous vibration to the contralateral antenna, and this is abolished if the commissural region is destroyed by microcautery. The result from the tractus olfactorio-globularis is of interest because it is usually considered on anatomical grounds to be afferent from the antennal sensory areas to the protocerebrum, but the present
result suggests that it may also carry efferent fibres, as suggested by Hanström (1928), the relation between antennal movement and stimulus frequency would be unlikely to be 1:1 if it involved a polysynaptic protocerebral path. With this exception, simple motor effects of the antennae are obtained only from the deuterocerebrum; the same is true of crickets (Huber, 1959).

Co-ordinated cleaning movements of the antennae by the legs result from stimulation of the anterior part of the antennal glomerulus, the commissural area, and the base of the antennal nerve (there are not separate sensory and motor branches of the antennal nerve in *Schistocerca* (Text-fig. 3). These parts apparently contain fibres from the antennal sensilla, and fine fibres from the antennal nerve can be seen histologically to radiate into this part of the glomerulus.

![Text-fig. 2 and 3](Image)

Text-fig. 2. Sites of electrical stimulation which produce simple motor responses from the antenna. The diagram shows a frontal view of the brain, omitting the optic lobes. The left-hand side of the diagram represents a more posterior part of the brain than the right-hand side. The landmarks which are sketched in include the median ocellar nerve and one lateral ocellar nerve, the mushroom bodies, the bridge, the antennal glomerulus, the corpus cardiacum nerve I, the antennal commissure, and the olfactorio-globularis tract. The black circle representing the site of stimulus indicates by its area relative to the outline of the brain the degree of localization usually achieved by the method described.

Text-fig. 3. Sites of electrical stimulation which produce co-ordinated cleaning of the antenna by the foreleg. Conventions as in Text-fig. 2.

Antennal sensory input and motor activities are involved in many more complex forms of behaviour, such as feeding, sexual behaviour, alerting, and so on. The over-all control of these activities is apparently protocerebral; they do not occur in an organized form without the protocerebrum or important parts of it, and they are elicited by electrical stimulation within the protocerebrum. Excitatory and inhibitory effects of the protocerebrum on antennal movements and cleaning have been described by Huber (1959) in crickets, and are also true of locusts. However, it must not be assumed (Huber, 1960a) that all antennal activities which are associated with motor activities arising outside the deuterocerebrum are mediated through connexions with the protocerebrum. The cleaning responses mentioned, involving complex interaction of antenna and foreleg musculature, can take place with the protocerebrum excised.
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[also true of cockroach (Hoffman, 1933) and cricket (Huber, 1955)]. In the cockroach and cricket the mouthparts as well as the forelegs are involved in cleaning the antennae, and the linkage between the different limbs is complex. In the locust, which normally uses only the foreleg, a lesion to the outer face of the glomerulus below and behind the entrance of the antennal nerve cuts fibres which appear to transmit direct to the thorax. After this operation, the sensory input to the antenna and its coordination with the antennal motor mechanism remains intact; the antenna is lowered for cleaning in the usual way. The leg makes no cleaning movement, however, and the antenna stays down, uncleansed, for many minutes. In the normal animal an attempt will be made to reach the antenna with another leg if the ipsilateral front one is incapacitated, but in the operated animal this does not happen. The leg still cleans readily areas of the head which are innervated by the tegumentary nerves, showing that these areas have a separate connexion with the thoracic ganglia.

(2) Locomotion

Stimulation of the brain commonly produces locomotion. Often it is merely a part of a more complex series of activities, such as searching for food, which follows stimulation; it is also a response to any painful stimulus, and electrodes in afferent tracts often give pain responses (wincing, cleaning an affected part, running away, regurgitation) if the stimulus amplitude is too high. Apart from these instances, where the locomotion component is clearly secondary, sites are found which give locomotion apparently free from any other motivation. These observations are true also of the cricket (Huber, 1959, 1960a).

In the locust there are three distinct levels of arousal associated with this 'pure' locomotion, and they are produced from three broadly different parts of the brain (Text-fig. 4). In the upper protocerebrum the latency is long, often more than 10 sec. at normal stimulus currents, and then a slow arousal is seen. A previously resting insect raises its head a little, its antennae are raised into the upright 'alert' position, the palps start to move, the animal stands rather higher off the ground, and its respiratory movements quicken up a little. As stimulation is continued the animal starts to walk forward, slowly at first, then faster and faster. Continued or increasing stimulation will cause running and eventually jumping, which may lead to flight if the animal does not hit something. In the lower protocerebrum there is little or no slow arousal. The locust starts to walk almost at once, and with continued or increased stimulation, jumps. Finally, in the posterior deuterocerebrum instant jumping can be obtained with very little latency indeed, unaccompanied by walking or other build-up of activity.

The longer latencies obtained from the dorsal protocerebrum suggests that in life it is sensory input to these areas which causes arousal and eventually locomotion. The shorter latency areas nearer to the circumoesophageal connectives seem lower down the command sequence, and the sites which give instant jumping lie in areas traversed by large descending fibres which run straight to the connectives. Maynard (1956) has shown that an electric shock applied to a sensory channel (the antenna of the cockroach) can be traced up into the protocerebrum and then down again as large-scale activity in the mushroom bodies. This agrees with the present observations.

Frequently a stimulated insect has a turning component superimposed on its loco-
motion. This can be due to differential locomotion on the two sides, but more usually it is accompanied by a difference in tonus affecting the whole body. In some cases this can be obtained independently of locomotion, causing a curving of the body to one side. At threshold, only the antennae of the moving insect show a deflexion towards the affected side; increase in stimulus amplitude then increases the tonus, resulting in smaller and smaller circles.

Such enforced turning, an interference with the normal ability of the animal to control the tonus of the two sides of the body and the fine co-ordination of locomotion, is one of the commonest results of any sort of interference with the C.N.S., either by direct lesion or stimulation, or by interference with the sensory input, and there is a very large literature on the subject. It is virtually the only wound effect seen after implantation of electrodes. Often an insect recovering from anaesthesia after implantation shows such an impairment. It almost always quickly adapts, and no more circus movements are seen. When, however, an electrical stimulus is applied to the electrode, the circus movement often reappears, usually with reversed sign from that produced by the original lesion. This suggests that lesion damage unbalances the normal bilateral machinery of the brain, but that the animal soon compensates for the injury. Electrical stimulation is likely to cause activity of neurones which have been silent since the implantation, and the new compensation now results in an over-correction, and thus turning in the opposite direction.

It is clear that this control of orientation is one of the most basic functions of the brain, and involves virtually all its afferent and a large part of its efferent channels. It is therefore not surprising to find that the sites of stimulation which produce turning make no sort of anatomical sense when plotted, but are scattered all over the brain. The same is true of crickets; see fig. 20 of Huber (1960a).

Occasionally stimulation makes the insect walk backward. In nature this is a response to painful or unpleasant stimuli at the head, such as can be seen if dilute acid
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is applied to the antenna. This is probably the explanation when backward walking is produced from sites which have shown in other ways to be in afferent tracts. Sometimes, however, the animal walks backwards slowly and deliberately, very differently from the hasty retreat caused in the other cases. This has only been obtained to date from the upper protocerebrum. A turning component can be superimposed.

Stimulation of parts of the protocerebrum, particularly the mushroom bodies, regularly inhibits locomotion. Sometimes the inhibition is more general, and also affects respiratory movements. The latter as soon as or before locomotion is affected, showing that this effect is not merely a secondary consequence of the reduced activity. Huber (1960a, b) has reported identical results from crickets. These sites do not, however, inhibit all forms of behaviour. When halted, by stimulation, the animal will often feed or groom itself, or assume a sun-bathing posture; at the end of the stimulation, this new activity stops and the animal starts to walk about again as before. Suppression of grooming and similar activities by locomotion has also been observed in birds, and used as the basis of an explanation of the so-called 'displacement activity' occurrence of the former in conflict situations (e.g. Rowell, 1961a). The present observations suggest that the same hypothesis might fit similar behaviour in insects.

(3) Feeding

Feeding is one of the commonest results of stimulating the locust brain. Usually this behaviour is complete and integrated, but stimulation in the tritocerebrum has occasionally produced mandibulation in the absence of any other food-seeking behaviour. This is probably a secondary consequence of stimulating afferent tracts from the mouthparts, for increased stimulation causes cleaning of these areas with the foot of the front limb. The mandibles are of course innervated from the suboesophageal ganglion, and not from the brain direct; this result suggests that there is some sensory projection into the brain. Stimulation in other parts of the brain often gives foraging behaviour. The insect walks around, palps and antenna testing the ground, tasting likely objects, picking up, even digging up, pieces of material from the ground with the front legs and taking them to the mouth. When something is found to taste, eating commences. Such foraging behaviour, leading to eating, has also been produced by stimulating the brains of crickets (Huber, 1960a).

These results might be taken to mean direct cerebral control of feeding. Further observations, however, show that this is unlikely. The points of evidence against this view are presented below.

(1) Only foraging, not feeding, is produced by stimulation. Feeding only follows it if something suitable is found, and never occurs in vacuo.

(2) Feeding declines with performance. The insect can be induced to feed over and over again, but for shorter and shorter periods, and eventually stops altogether. After a period adequate for digestion, it will become responsive again. Similarly, an insect which by stimulation has been induced to feed to satiation on one form of food, e.g. wheat, will become responsive again if presented with a different tasting food stuff, such as bran or bamboo.

(3) Little or no foraging behaviour can be produced by stimulation if the animal is
Points (1)–(3) indicate that feeding produced by cerebral stimulation is controlled by the same environmental and internal factors as normal feeding.

(4) The sites producing foraging behaviour can be found virtually anywhere in the brain (see Text-fig. 5 and also Huber (1960 a, fig. 10). This suggests that brain stimulation merely serves to arouse the animal, or at most to inhibit some other activity which itself prevents feeding, such as resting or attempting to escape from the experimental situation.

(5) This hypothesis is supported by observations of control, non-implanted animals. Foraging and feeding is the commonest response of resting animals to non-specific stimulation, such as being momentarily jerked off the ground by a thread. Further the probability of this response increases with food deprivation ('hunger'), and increases faster than the total probability of feeding during the test period.

(6) One or two cases occurred in which cerebral stimulation produced gross hyperphagy. All but one of these (in which the electrode could not be localized) were shown to have the electrode situated in the region innervated by the nerves connecting the brain to the frontal ganglion and hence to the recurrent nerve. In the blowfly (Dethier & Bodenstein, 1958) section of the recurrent nerve produces hyperphagy by removing feedback from the gut.

The effect of section of the recurrent nerve in the locust was tested in the following experiment. Three groups each of five adult males about 6 weeks old were set up: these are referred to as 'operates', 'dummy operates', and 'normals'. The animals were taken from the stock cage, and the first two groups were operated. In the operates, a window was cut in the frons, the frontal ganglion was exposed, and the recurrent nerve was cut immediately behind the ganglion; the wound was then closed with wax. In the dummy operates the nerve and ganglion were exposed but not damaged. All the animals were kept singly without food for 24 hr., and then provided with
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excess food and observed during a 30 min. test period, when the time spent eating was noted. As a check, the animals were then isolated for a further 36 hr., and the collected faeces were dried and weighed. It is possible that the efficiency of feeding in the operated animals is reduced, and that the time spent feeding is not therefore a fair measure of feeding. The collected faeces control this possibility.

Results. The time spent feeding and the weight of dried faeces are positively \((r = +0.8)\) and significantly \((P < 0.1)\) correlated. This is the same correlation as was previously obtained for a large number of normal animals, and it is therefore concluded that time spent feeding is a valid measure of feeding behaviour.

Table 2. Time spent feeding during a half hour trial of locusts with recurrent nerve section, operated controls, and normal animals

(Further explanation in text.)

<table>
<thead>
<tr>
<th>Animal</th>
<th>Normals</th>
<th>Dummy operates</th>
<th>Operates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.1</td>
<td>9.3</td>
<td>19.3</td>
</tr>
<tr>
<td>2</td>
<td>14.5</td>
<td>5.1</td>
<td>21.0</td>
</tr>
<tr>
<td>3</td>
<td>2.4</td>
<td>9.7</td>
<td>29.0</td>
</tr>
<tr>
<td>4</td>
<td>3.0</td>
<td>17.8</td>
<td>27.7</td>
</tr>
<tr>
<td>5</td>
<td>12.5</td>
<td>11.5</td>
<td>20.5</td>
</tr>
<tr>
<td>Average</td>
<td>7.3 ± 4.8</td>
<td>10.7 ± 4.1</td>
<td>24.7 ± 3.9</td>
</tr>
</tbody>
</table>

Comparison between columns:

<table>
<thead>
<tr>
<th>s.e. of difference</th>
<th>Probability</th>
<th>Difference</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.8</td>
<td>0.23</td>
<td>not significant</td>
<td>very significant</td>
</tr>
</tbody>
</table>

The data concerning feeding time are presented in Table 2. From this it can be seen that dummy operates feed slightly more than the normal animals; the difference is not statistically significant, but if it is real it is probably due to blood loss, etc., caused by the operation. The operates feed nearly two and a half times as much as the dummy operates, and this difference is highly significant. It can therefore be concluded that section of the recurrent nerve causes hyperphagy in adult male locusts.

In view of this result, it is probable that hyperphagy produced by cerebral stimulation was caused by a blockage of the normal feedback from the gut, and not by the activation of any specific nervous mechanism producing feeding.

To sum up, it seems that electrical stimulation only produces feeding in locusts that are already to some degree hungry, or which have been made hungry by stimulation, by some such mechanism as blocking of the recurrent nerve. The feeding behaviour that is produced in this manner is subject to the same changes consequent on performance as normal feeding, and cannot be seen to differ from it in any way. Brain stimulation is probably therefore permissive or disinhibitory, rather than excitatory. It is possible that many of the effects of brain stimulation, especially when they consist of elaborate integrated behaviour, are produced in this way. It is much easier to imagine that the effect of artificially injecting electric current into the complex nervous
mechanism of the brain is to cause something to go wrong (thus releasing a previously inhibited response) than to set going a programmed mechanism or a ‘feeding centre’.

Feeding behaviour is then a common result of brain stimulation but illustrates well the difficulties of interpreting the results. To conclude from this sort of result merely that it causes an ‘activation of the eating drive’ (e.g. Huber, 1960a) is to oversimplify, and does not throw any new light on the problem.

(4) Sexual behaviour

The greatest differences between the results obtained from the cricket and from the locust are in sexual behaviour. The crickets have a complex sexual behaviour, involving territory-holding, song of several different sorts, a tactile stage in courtship before the transfer of the spermatophore, a post-copulatory display and fairly elaborate parental behaviour (see Zippelius, 1949; Huber, 1955, 1960a; Alexander, 1960, 1961).

*Schistocerca*, at least in the gregarious phase, has a much less differentiated sexual behaviour, and being less striking, has remained until recently very poorly described for so well known an insect. Norris (1952, 1954) and Loher (1959, 1960) have now filled much of this gap.

The female has a passive role in courtship, though after copulation she commences complex oviposition behaviour. A sexually mature and receptive female does not run from a sexually motivated male, as an immature one does, and does not resist attempts at copulation. A sexually excited male, on the other hand, has a very characteristic stance and gait, holding himself high off the ground, with the head raised, the antennae vertical or reflexed, and a quick high-stepping walk. In this condition he approaches any object of about the right size and shape, usually from the side, and often makes a few preliminary touches with his antennae. A moving object is especially attractive. Particularly if sexually deprived, the male is not at all specific. Within a certain size range, any insect will do, and if the large and sluggish *Ornithacris* are kept in the same cage as sexually active *Schistocerca*, they will be almost always surmounted by a male locust, irrespective of their own sex. I have even had a deprived male mount and attempt copulation with my forefinger. Apparently the criterion of the male is the resistance he meets; a male or unreceptive female locust kicks furiously with the hind femora until he is dislodged. The male also stridulates, rubbing the forewings together to produce a whirring noise; Loher (1959) distinguishes a ‘short’ and a ‘long’ stridulation, but the two forms intergrade to a large extent. Stridulation occurs chiefly immediately before copulation, and during copulation if the pair are disturbed by, say, another male. A deprived male locust will often stridulate even if no other insect is near.

To date, none of this behaviour has ever been elicited by stimulation of the brain. This is true even if the insect is tested in the company of other insects. A deprived male locust, which normally shows sexual behaviour when put with others, can be inhibited in this by brain stimulation; sites which normally produce grooming of the antenna, or locomotion, or feeding behaviour, or no positive motor activity at all, have been seen to inhibit sexual behaviour, but the latter has never been positively elicited.

In this the locust differs markedly from the cricket. Huber (1960a) has shown that stimulation of the mushroom bodies produces both normal and abnormal song, and related behaviour.
A technique of cerebral stimulation

The reasons for this difference are probably to be found in the different ways of life of the two species. Doubtless the sexual activity of the cricket is influenced by maturational processes and their associated changes in hormone balance, but at least part of the control of courtship is known to be directly nervous. The male cricket does not court in the absence of a ripe spermatophore in the genitalia, and can be instantly 'switched off' by cutting the nerve cord between the genital ganglion and the rest of the nervous system (Huber, 1955). The cricket is a solitary, territory-holding animal, and the female is attracted to the male by the song; it is reasonable to think that under these circumstances it is necessary to have the very important stridulation and courtship under direct control of the higher nervous centres.

The gregarious locust, on the other hand, has relatively little problem in finding a mate. When it is sexually mature, a simple reflex causing attempted copulation with insects moving alongside of it is likely to produce successful mating in nearly half of all attempts. This reflex apart, the rest of the timing and control can safely be left to less sensitive mechanisms than the highest centres of the C.N.S. Loher (1960) has shown the importance of the corpora allata in sexual behaviour in *Schistocerca* males, and the pheromonal control of maturation. If, as seems likely, the sexual behaviour of the locust is mainly hormonally controlled, short-term stimulation of the brain is much less likely to produce spectacular sexual display than it is in the solitary crickets.

The only exception to the general rule that brain stimulation does not cause sexual behaviour in locusts is that frequently it causes apparently autonomous activity of the genital segments, characteristically copulatory movements in the male, and preliminary oviposition movements in the female. This is probably merely a case of disruption of normal cerebral inhibitory control, for it never happens in immature insects, and similar effects can often be seen in decapitated insects. In some insects, e.g. the mantis (Roeder, 1935), the head ganglia are known to inhibit the reflexes of the genital segments.

SUMMARY

1. Methods are described for implanting permanent stainless-steel electrodes into the brains of locusts, for stimulating the brain under near-normal conditions, and for localizing the electrode subsequently.
2. Threshold currents measured under these conditions are lower than those required in acute preparations, or if the animal is restrained.
3. The results of stimulation are described for four common aspects of behaviour. These are antennal movement, locomotion, feeding and sexual behaviour.
4. The effect of stimulation on antennal and locomotory movements largely confirms previous work on crickets.
5. Feeding and foraging behaviour, which is a very common result, is shown to be almost completely determined by peripheral stimuli at the time of brain stimulation. The role of the latter is permissive or disinhibitory rather than causal or excitatory.
6. Integrated sexual behaviour is occasionally inhibited, but never elicited, by stimulation. This contrasts with observations on crickets, and its implications are discussed.
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REFERENCES


EXPLANATION OF PLATE

Plate 1. A locust with a chronically implanted brain electrode connected up to stimulating leads. The fine electrode wire running between the end of the lead and the top of the head can just be seen at this magnification. The insect's posture is due to it being in the act of turning around on a narrow edge.