J. exp. Biol. 122, 413–425 (1986)
Printed in Great Britain © *The Company of Biologists Limited 1986*

OVIPOSITION DIGGING IN THE GRASSHOPPER
II. DESCENDING NEURAL CONTROL

BY KAREN J. THOMPSON*
Department of Biology, University of Oregon, Eugene, OR 97403, USA

Accepted 1 January 1986

SUMMARY

1. Transection of the ventral nerve cord of female grasshoppers activates the rhythmical motor programme for oviposition digging.

2. Electrical stimulation of the cut nerve cord had the following effects on elicited oviposition motor activity: (i) short- and long-lasting inhibition of activity, (ii) phase resetting and (iii) modulation of burst frequency.

3. Cold saline applied to the nerve cord reversibly elicited the oviposition motor programme.

4. The effects of transection and stimulation at different levels of the nerve cord indicate that the higher neural control of the motor pattern is not confined to the head ganglia, but includes a thoracic component.

5. In intracellular recordings of ventral opener motoneurones, stimulus-related IPSPs were observed in response to stimulation of the cut nerve cord. Stimulation also abolished slow wave synaptic input to the motoneurones during inhibition of the oviposition motor programme.

6. It is suggested that oviposition digging behaviour is initiated and maintained by a mechanism of 'release' from descending neural inhibition.

INTRODUCTION

In the course of investigations into the neural basis of oviposition digging (see preceding paper: Thompson, 1986) it was noticed that transection of the nerve cord caused the automatic appearance of the behaviour in sexually mature females. In this study the mechanism of descending control of oviposition digging in the grasshopper was examined.

Other investigators have found that specific regions in the central nervous system can initiate and control the level of activity of selected behavioural circuits. For example, a region of the mesencephalon in the cat drives walking upon electrical stimulation, and the speed of locomotion is determined by the strength of the stimulation (Shik, Severin & Orlovsky, 1966). In insects, neurones descending from

* Present address: Department of Zoology, University of California, Berkeley, CA 94720, USA.

Key words: grasshopper, central pattern generator, descending inhibition, command neurones, oviposition.

the brain and suboesophageal ganglion have been found to be important in initiating and maintaining behaviour (song in crickets, Bentley, 1977; walking in locusts, Kien, 1983). In crustaceans, individual neurones have been identified that drive swimmeret beating in crayfish (Wiersma & Ikeda, 1964), lobster walking (Bowerman & Larimer, 1974) and escape tail flips (Wine & Krasne, 1982). From such studies arose the concept of 'command' centres and 'command' neurones (Davis, 1977; Kupfermann & Weiss, 1978; Davis & Kovac, 1981). By definition, command elements are excitatory; their activity is both necessary and sufficient to elicit and maintain 'recognizable behavioural acts', such as those generated by central pattern generators (CPGs).

Obviously, oviposition digging behaviour, which is driven by a CPG and which is activated by nerve cord transection, appears to be controlled differently. Oviposition digging, not unlike several types of reproductive behaviour in insects, may be controlled by a mechanism of 'release' from descending inhibition. For example, decapitation activates copulation in male mantids (Roeder, Tozian & Wieant, 1960) and mosquitoes (McDaniel & Horsfall, 1957). Abdominal isolation elicits oviposition behaviour in female crickets (Carrow, Cabeza & Flores, 1982). Calling songs are activated in larval crickets after selective brain lesions (Bentley & Hoy, 1970). Decapitated female moths (M'Cracken, 1907) and mantids (Chopard, 1914) have been found to be able to produce oviposition behaviour. In addition, studies of the dogfish have shown that lesions of the medulla activate continuous swimming behaviour (Roberts & Williamson, 1983).

To test the hypothesis that oviposition digging was controlled by 'release' from inhibition, the effect of descending activity was studied by several methods in which that activity was removed or stimulated. The effects of these manipulations were monitored by recording from ovipositor nerves and muscles, and from identified motoneurones. In addition, the impact of nerve cord transection during natural oviposition behaviour was studied. The results of transection and stimulation at various levels of the nerve cord were analysed to determine which ganglion or ganglia are involved in the descending control of oviposition activity.

MATERIALS AND METHODS

Preparations

Sexually mature female grasshoppers, *Schistocerca americana*, locally raised in a crowded colony were used in this study. Preparations usually consisted of an intact animal in which the ventral nerve cord was exposed in the abdomen after shallow, mid-ventral incision, deflection of the sternal plates and removal of oviducts and spermatheca. Isolated abdomens were used for some experiments.

Physiological methods

Extracellular muscle recordings were obtained with paired silver wires, insulated except at their tips, implanted into ovipositor muscles. Silver hook electrodes

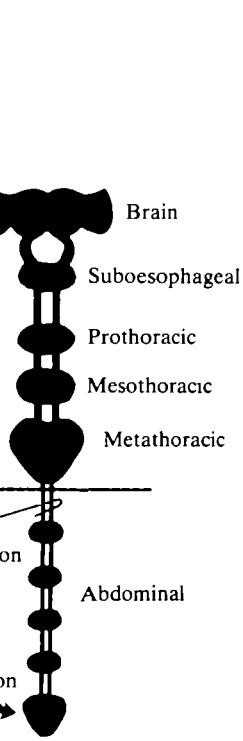
were used for extracellular recording of ovipositor nerves and stimulation of the ventral nerve cord. Intracellular recordings from ovipositor motoneurones were obtained with 2 mol l^{-1} potassium acetate microelectrodes of 30–90 MΩ resistance. Motoneurones were identified using the method of Hoyle & Burrows (1973), by matching intracellular spikes with electromyogram spikes. Physiological recordings were observed on an oscilloscope and transcribed by a chart recorder (Gould 220). In the cold-block experiments the cold saline was at 3 °C and the warm saline was at 35 °C. Routine experiments were done at room temperature, approximately 23 °C. Small amounts of insect saline (Hoyle, 1953) were added to keep the preparations moist.

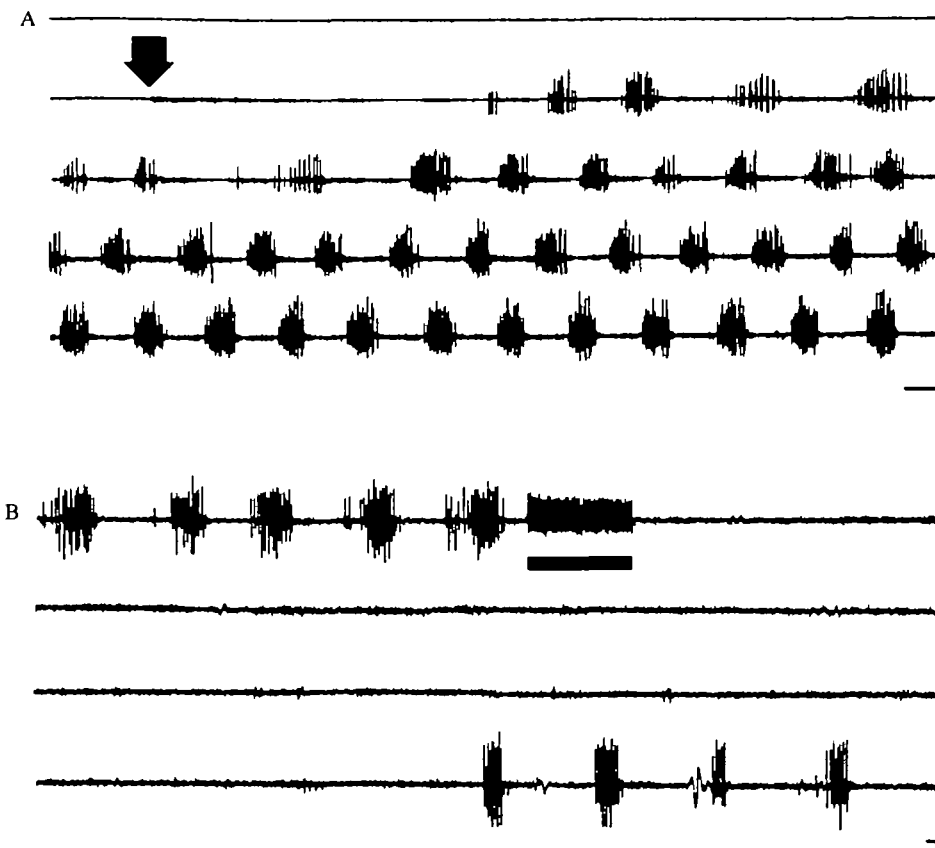
RESULTS

Effect of nerve cord transection and stimulation

The stable oviposition digging motor programme is conveniently monitored by recording extracellularly from the ventral opener muscle, or from the branch of the eighth ventral nerve (8vn) to that muscle. During expression of the motor programme, rhythmical bursts of activity are recorded in the opener muscles and nerves (see Thompson, 1986). The state of the motor programme in response to various experimental manipulations was assessed by observing the presence or absence of the rhythmical discharges, and observing changes in their phase, frequency or intensity. Transection of the ventral nerve cord just caudal to the metathoracic ganglion elicited the motor programme for oviposition digging (Fig. 1A). The onset of motor activity displayed a characteristic delay of several seconds after the nerve cord had been cut (arrow). Such nerve cord transections always elicited rhythmical ovipositor movements in sexually mature female grasshoppers. In general, severing the nerve cord anywhere in the abdomen between the metathoracic ganglion and seventh abdominal ganglion had the same effect. Transections at other levels also activated ovipositor movements, but other transections usually resulted in a preparation with reduced motor activity (see below, and Thompson, 1986). Transection was also effective in activating oviposition digging in other acridid species, including *Romalea microptera, Barytettix psolus* and *Schistocerca gregaria*.

In the recording of Fig. 1B, the motor programme had previously been activated by severing the nerve cord. When the rostral end of the nerve cord was electrically stimulated, rhythmical activity was immediately suppressed, and the patterned muscle activity did not return for the equivalent of 30 cycle's duration. Because electrical stimulation of the nerve cord was effective in suppressing the motor programme it was utilized in further experiments to mimic the activity of descending fibres. Clearly, gross stimulation of the nerve cord is of questionable relevance to the use of descending neurones by the animal. However, the levels of stimulation necessary were low. At the stimulation levels used in this study, the rest of the abdomen did not produce muscle contractions, and shocks as infrequent as 1 Hz were adequate to produce the effect.





rve cord transection elicits oviposition activity and stimulation inhibits it. A diagram of the nervous system is shown on
s of transection and stimulation are indicated. The continuous recordings are electromyographic activity of the ventral
A, before the nerve cord was cut the muscle was silent. After transection at the arrow, and a delay of approximately 1
oviposition digging motor programme was expressed continuously. In B, the nerve cord had been transected prior
f the recording and oviposition digging activity was occurring. At the bar, the cut nerve cord was stimulated (10 Hz, 0
inhibition of motor activity for over 2 min, although the stimulation of the connectives only lasted 2 s. Resumption of
bserved at the end of the last trace.

Cold-block

The oviposition digging motor programme was also elicited by application of cold saline to a portion of the intact ventral nerve cord rostral to the terminal abdominal ganglion (Fig. 2A,B). Approximately 30–45 s after cold saline was applied to a length of the nerve cord in a quiescent preparation small jerking movements of the ovipositor valves were observed. The valves produced rhythmical oviposition digging movements and corresponding rhythmical electromyographic patterns by approximately midway through the third trace in the record shown. These movements continued throughout the remainder of the cold period. Replacing the cold saline with warm saline, to restore activity in the nerve, deactivated the motor programme immediately.

Differences between decapitated and severed abdomen preparations

Decapitation of female grasshoppers resulted in the appearance of ovipositor movements in 37 % of animals tested ($N=54$). In decapitated animals recordings of oviposition activity from opener muscles revealed bursts with similar duration and intensity to those associated with digging, but the bursts were usually not rhythmical nor were they continuous beyond approximately the first 10 min in most preparations. However, the erratic bursting activity or lack of activity was immediately replaced by regular, rhythmical oviposition digging discharges when the connectives were given a second transection behind the metathoracic ganglion (Fig. 3A). The opener burst durations and oviposition digging cycle length were measured and compared for three animals that had been decapitated and then later received a second nerve cord transection behind the metathoracic ganglion (Fig. 3B). The burst durations showed little difference before or after the second transection (black bars), although bursts tended to be slightly longer in decapitated preparations. In contrast, the cycle durations showed pronounced differences, being much longer on average and subject to much greater variability in decapitated as compared to isolated abdomen preparations; 9–10 s+3–4 s for decapitated animals *versus* 4–5 s+0·2 s for isolated abdomens. Transection behind the prothoracic and mesothoracic ganglia did not change the pattern as compared to that produced by decapitated animals. The transection needed to be behind the metathoracic ganglion for the change to be observed. The type of motor activity exhibited by the decapitated females did not resemble any form of 'real' oviposition behaviour in the animal.

A further distinction between the effects of decapitation and nerve cord transection in the abdomen was found when the transections were repeated in grasshoppers during natural oviposition behaviour. Transection caused an initial pause, perhaps due to inhibition from injury discharges in descending neurones, but then digging movements continued in abdomens that were severed while grasshoppers were digging in sand. In contrast, and somewhat unexpectedly, all digging movements ceased when a digging grasshopper was decapitated. In these animals, digging movements were restored by subsequent abdomen isolation.

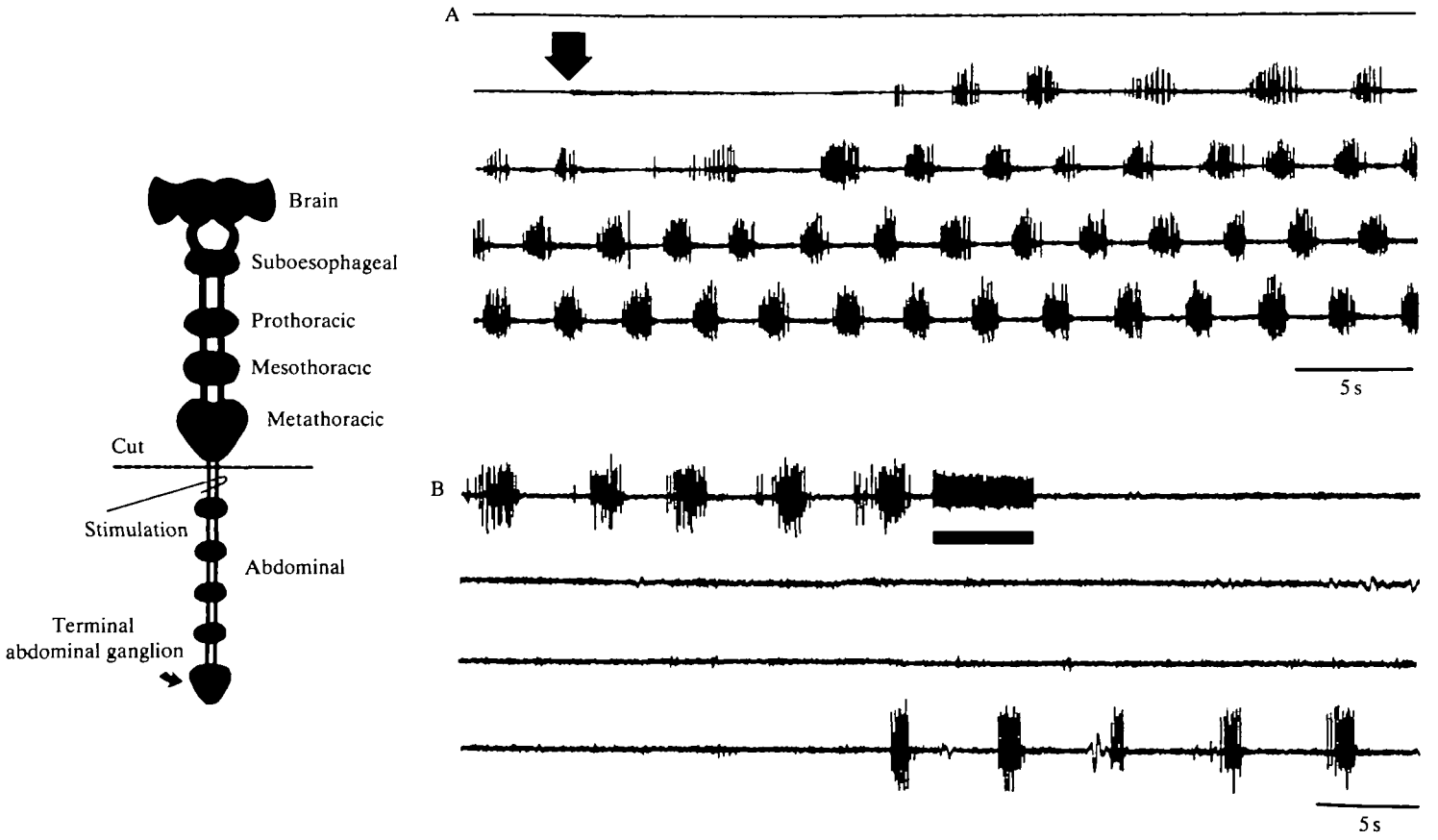


Fig. 1. Nerve cord transection elicits oviposition activity and stimulation inhibits it. A diagram of the nervous system is shown on the left and the sites of transection and stimulation are indicated. The continuous recordings are electromyographic activity of the ventral opener muscle. In A, before the nerve cord was cut the muscle was silent. After transection at the arrow, and a delay of approximately 12 s, the rhythmical oviposition digging motor programme was expressed continuously. In B, the nerve cord had been transected prior to the beginning of the recording and oviposition digging activity was occurring. At the bar, the cut nerve cord was stimulated (10 Hz, 0·1 ms). This led to inhibition of motor activity for over 2 min, although the stimulation of the connectives only lasted 2 s. Resumption of motor activity is observed at the end of the last trace.

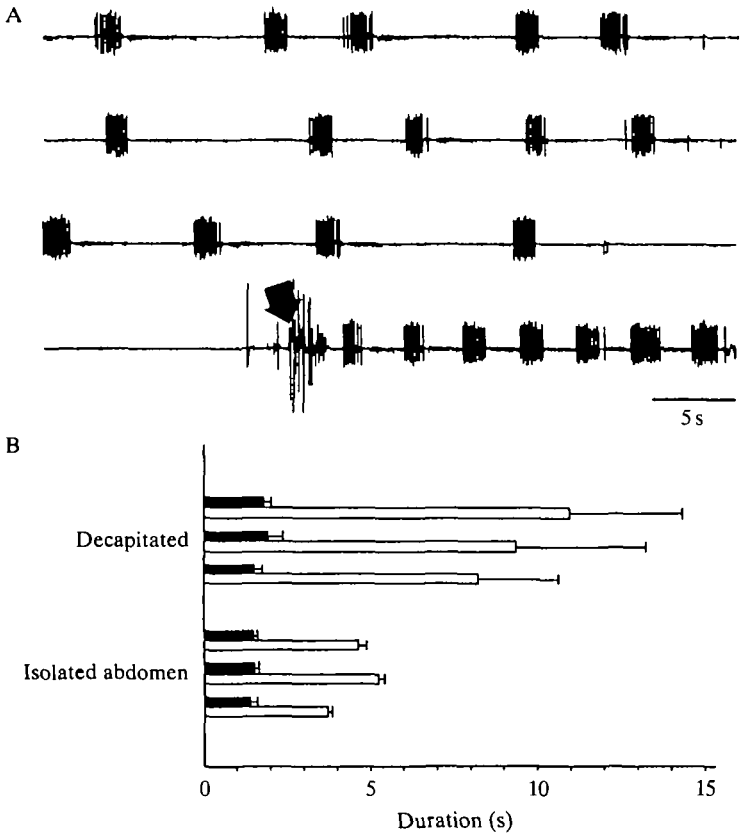


Fig. 3. Comparison of motor activity in decapitated females and severed abdomens. In A, which is a continuous electromyographic recording from a ventral opener muscle, the animal had been decapitated before the start of the record. At the arrow, the nerve cord was severed caudal to the metathoracic ganglion. Before the arrow, the bursts occurred at variable intervals, but after the arrow they occurred rhythmically. The electromyographic data for three preparations that were decapitated and then cut at the abdomen are represented graphically in B. Ventral opener burst durations are indicated by black bars, cycle durations are indicated by white bars and standard deviations are shown as error bars. The burst durations are not statistically different between the decapitated and isolated abdomen conditions. Cycle length or period is significantly different between the two conditions with a P value, calculated from the student's t-test, of less than 0·001.

Connective stimulation can lead to phase reset and frequency modulation

Following experimentally induced inhibition of oviposition activity by stimulation of the nerve cord, the return of rhythmic motor discharges could be accompanied by a change in phase or a slowing of rate. Because the ovipositor stopped moving and closed upon electrical stimulation of the nerve cord, it was necessary to eliminate sensory feedback as a source of resetting in these experiments. It had been previously found that the deafferented nerve cord was capable of generating the oviposition digging motor programme (Thompson, 1986). In the deafferented preparation, after two shocks had been delivered to the cut ventral nerve cord, a phase shift of 0·71

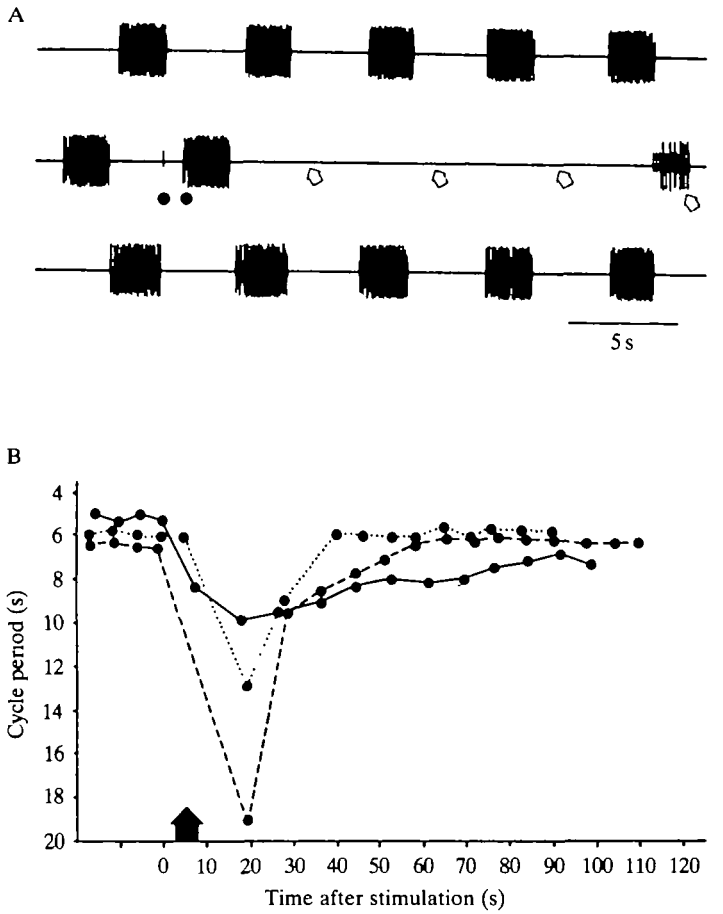


Fig. 4. The timing of the oviposition digging rhythm can be reset and slowed by connective stimulation. In A, oviposition digging activity was recorded from the branch of the eighth ventral nerve to the ventral opener muscle in an isolated nerve cord preparation. When the connectives were stimulated at 1 Hz, indicated by dots under the trace, the phase of the motor bursts was reset. The timing of expected bursts is indicated by open arrows. In B, modulation of cycle frequency resulting from stimulation of the nerve cord is represented graphically. Stimulation took place at the large arrow; 5, 25 or 75 equal shocks were delivered in 5 s to the same preparation at 10-min intervals, by using stimulus frequencies of 1 Hz, 5 Hz and 15 Hz. All caused inhibition of the motor programme, as recorded electromyographically from the ventral opener muscle, but varied in the duration and magnitude of the slowing effects.

occurred, considered as a delay (Fig. 4A). After some stimulations, the pattern returned at its original frequency (as shown in Fig. 4A) but a more frequent result was that the bursting discharges returned at a slower rate after the period of inhibition. This slowing was usually temporary and then the pattern progressively returned to the initial frequency. In Fig. 4B modulation in cycle frequency with time was plotted after three different frequencies of nerve cord stimulation. In these experiments, the nerve cord was stimulated for a duration of 5 s at a rate of 1, 5 or 15 Hz. The slowing of cycles lasted longer, the greater the rate of nerve cord

stimulation, such that modulation persisted for 30 s after 1 Hz stimulation, for 65 s after 5 Hz stimulation, and for longer than 2 min after stimulation at 15 Hz. The magnitude of the inhibitory effect, that is the maximum slowing of rate, increased between the 1-Hz and the 5-Hz tests, but was less at 15 Hz although the time of maximal effect in all three cases was the same, at approximately 18 s. The decrease in magnitude of inhibition at 15 Hz suggests the possibility of excitation descending to the terminal ganglion and interacting with the inhibitory effects. At the lowest level of stimulation, the onset of the inhibition was delayed after one more cycle at the prestimulus rate. Resetting and modulation were also observed in afferented preparations.

Effects of nerve cord stimulation on ovipositor motoneurones

As described previously, the intracellular activity of ventral opener motoneurones during oviposition digging activity is characterized by rhythmical, slow depolarizing waves leading to bursts of impulses (Thompson, 1986, fig. 13). The activity of ventral opener motoneurones in response to nerve cord stimulation was examined by observing stimulus-related, postsynaptic responses and changes in the spontaneous rhythmical activity of these neurones in isolated abdomen preparations. At levels of stimulation below the threshold required to inhibit oviposition digging, stimulus-related inhibitory postsynaptic potentials (IPSPs) nevertheless occurred in the motoneurones (Fig. 5Ai,ii). In response to nerve cord stimulation at higher strengths, the stimulus-related IPSPs continued to be present, but also the slow waves of depolarization were eliminated. The duration of this inhibition long outlasted the duration of the nerve cord stimulation (Fig. 5B). When motor programme activity resumed, the first depolarizing wave to appear was of low amplitude so that it caused fewer action potentials to be generated in the first cycle after recovery. This gradual recovery of burst intensity was also observed in several extracellular recordings of the effects of nerve cord stimulation (e.g. Fig. 4A).

DISCUSSION

The initial observation which prompted this investigation, that digging movements invariably occurred following nerve cord transection, suggested that release from inhibition activated the oviposition digging motor programme. However, another possible explanation was that injury discharge from the cut actually excited the motor programme either by triggering motor activity or by causing tonic release of transmitter from damaged axons descending to the terminal ganglion. For example, the well-studied lobster pyloric CPG in the stomatogastric ganglion was thought to be spontaneously active in isolation until a perfused sucrose gap was applied to the desheathed input nerve to completely block conduction (Moulins & Cournil, 1982). It is now believed that activating transmitters 'dribble' out of input axon terminals to activate conditional bursting neurones that are components of the CPG. In the lobster, electrical stimulation of the input nerve leads to pronounced

and long lasting stimulation of the pyloric system. By contrast, in the female grasshopper, the bursting activity in the terminal abdominal ganglion is not enhanced by electrical stimulation of the descending neurones. Electrical stimulation did not lead to increased rate or enhanced burst intensity nor did the bursting exhibit any such effect after rebound from inhibition. The results of nerve cord stimulation in this study failed to provide evidence of excitation except at the highest levels, and that evidence was merely a diminution of the inhibitory effect. Furthermore, when a cold-block was applied to the intact nerve cord of females, the motor programme was also elicited. Low temperature can be an effective block of nerve conduction (Hodgkin & Katz, 1949), and it was used in these experiments to remove the influence of descending neural systems without cutting the nerve cord. The reversibility of the cold-block, in that the pattern was 'reinhibited' by warm saline, suggests that the motor programme was elicited by a block of conduction and not by damage to the nerve. The results of the stimulation and cold-block experiments are consistent with the suggestion that release from neural inhibition rather than injury-induced excitation elicits oviposition activity.

In this study, stimulation of the cut nerve cord was used to begin to examine the influence of activity in the input nerve on the digging circuit. It was unexpected that electrical stimulation of the nerve cord would exert such specific effects on the motor programme, but since stimulation did not produce abnormal contractions of the abdomen and usable levels were low, the method provided an operational strategy for mimicking the electrical activity in descending fibres. In addition to inhibiting

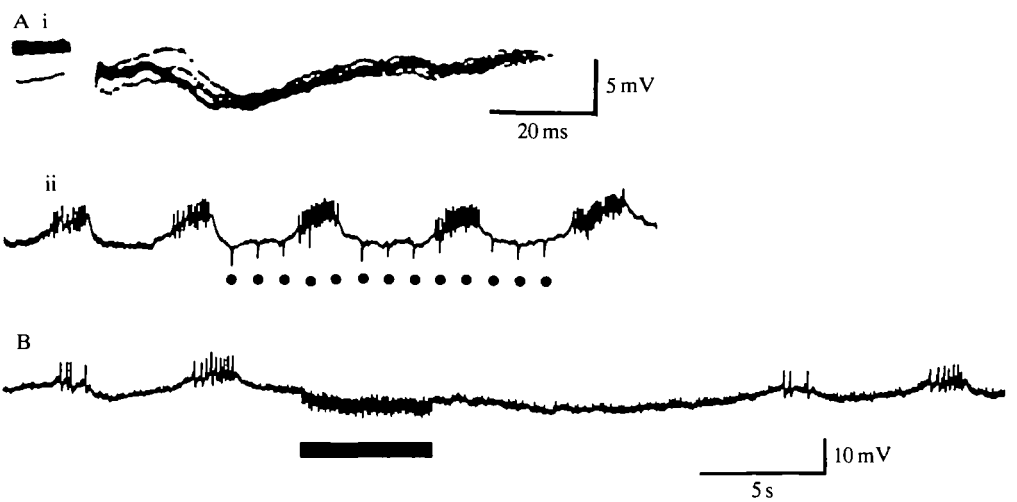


Fig. 5. Intracellular ovipositor motoneurone recordings during nerve cord stimulation. In A, the nerve cord was stimulated weakly to produce IPSPs in the ventral opener motoneurone (i, overlapping sweeps during stimulation at 5 Hz, 0·1 ms), but not inhibition of the digging motor programme (ii, stimuli delivered at dots). In B, the strength and frequency of the stimulation were increased (now 10 Hz). This produced IPSPs in the motoneurones and long-lasting suppression of the slow oscillations in membrane potential.

activity, nerve cord stimulation also led to changes in the timing of the oviposition digging rhythm. Changes in phase and frequency were observed that could not be accounted for by sensory feedback, thus suggesting that the descending systems may inhibit the rhythm generator itself. Effects 'downstream', such as on motoneurones or on pre-motor (non CPG) interneurones, would not be expected to affect the timing of the rhythm.

The observations of a 10–15 s latency to experimental activation of the motor programme (Fig. 1A; Thompson, 1982) and post-stimulation slowing effects that could last for some minutes, suggest the possibility of neuromodulator involvement in the inhibitory control of oviposition. In this regard, it is interesting to compare the time course and suggestion of dose-dependent suppression of oviposition activity due to electrical stimulation of the nerve cord (present study) with similar effects on the motor pattern found to result from iontophoretic application of octopamine into the terminal abdominal ganglion of female grasshoppers (Sombati, 1983; Sombati & Hoyle, 1984). These authors also found long-lasting and dose-dependent, but excitatory, effects on flight and walking behaviour when the substance was iontophoresed into the metathoracic ganglion (Sombati & Hoyle, 1984).

When intracellular recordings from ventral opener motoneurones were obtained during connective stimulation, IPSPs were observed in response to the stimulation, even when the stimulation levels were below those required for inhibition of the motor programme. In natural digging behaviour, the valves periodically stop opening while the animal tamps and smoothes the sides of the hole. In recordings of tamping, opener activity was found to be selectively eliminated while the rhythm generator continued to be active (Thompson, 1986). Descending inhibition of the opener motoneurones without inhibiting the CPG may be involved in the regulation of tamping. Intracellular recordings of responses to higher levels of connective stimulation showed that the normal oviposition-related synaptic input to the motoneurones was eliminated during suppression of the motor programme, providing additional evidence that some of the inhibition may take place at levels upstream from the motoneurones.

The inhibitory control centre appears to be distributed in both the head (brain and suboesophageal ganglion complex) and in the metathoracic ganglion. Roeder *et al.* (1960) found the suboesophageal ganglion to provide the inhibitory control of copulation behaviour, but Carrow *et al.* (1982) found the thoracic ganglia to control cricket oviposition activity. Rowell (1964) showed inhibitory effects of both the metathoracic and suboesophageal ganglia on reflex responsiveness of the prothoracic segment. These observations provide precedents for both locations that seem to be used by the grasshopper.

Why should the grasshopper control this behaviour by release from inhibition? At first it seems unreasonable for the animal to continually expend the energy required to inhibit the motor network rather than simply to excite it at the appropriate times. That such a mechanism may have selective value was suggested by the work of Roeder (1935) and Roeder *et al.* (1960) who found that decapitation produced copulation in male mantids. Because female mantids sometimes cannibalize the

heads of their male partners, this mechanism would ensure that copulation proceeded. They also found that cockroaches expressed copulation-like movements after decapitation, but when placed in contact with females were not able to transfer sperm successfully (Roeder *et al.* 1960). The selective value of descending inhibitory neural control, so obvious in the mantid, is difficult to discern for the cockroach. Similarly, the practical result of descending inhibitory control of oviposition digging behaviour for grasshoppers may be of little selective value since the eggs are not laid. Transection of the nerve cord in grasshoppers elicited digging, but not egg-emission behaviour in hundreds of experiments (Thompson, 1982).

The possibility remains that a female which has finished digging and has begun to deposit eggs could continue to do so after a nerve cord damaging attack by a predator. The hormone environment may place the nervous system in a different *milieu* during the time after digging when eggs are emitted. It is known that neurosecretory products are released into the blood from the corpora cardiaca in association with oviposition (Highnam, 1962). However, it is likely that the neurosecretory products are important in the regulation of egg-emission, and not digging, because egg-emission, unlike digging, is obligatory once it has begun (see Popov, 1958). Such a situation would be characteristic, generally, of hormonally controlled behaviour (Truman, 1978). A laying female can be removed from the ground without significant interference in egg-emissions. After females had begun to lay eggs, severing the nerve cord did not necessarily interrupt future egg-emissions, although some animals were observed to revert to digging movements (Thompson, 1982). It is possible that the descending inhibitory system for digging is also involved in the egg-emission phase of oviposition, and that it may be of selective value in that context.

In the grasshopper, the neural elements responsible for control of oviposition digging behaviour have yet to be identified. However, the evidence suggests that the *inactivity* of certain descending neurones is both necessary and sufficient for production of oviposition digging behaviour. Thus, although 'inhibitory command' mechanisms for behaviour may not be nearly so widespread as excitatory ones, they need to be considered within the command concept and included in ideas about the activation of CPGs.

This paper is fondly dedicated to the memory of Professor Graham Hoyle, in whose laboratory the work was done. Dr Graham Hoyle, Dr Edmund Arbas and Gail Robertson provided helpful comments on an earlier version of this manuscript. This work was supported by NIH training grant GMO 7257 to KT and NSF grant BNS 79/23785 to G. Hoyle.

REFERENCES

BENTLEY, D. R. (1977). Control of cricket song patterns by descending interneurons. *J. comp. Physiol.* **116**, 19–38.

BENTLEY, D. R. & HOY, R. (1970). Post-embryonic development of adult motor patterns in crickets: a neural analysis. *Science* **170**, 1409–1411.

BOWERMAN. R. F. & LARIMER, J. L. (1974). Command fibres in the circumoesophageal connectives of crayfish. *J. exp. Biol.* **60**, 119–134.

CARROW, G. M., CABEZA, R. J. & FLORES, G. (1982). Isolation of the abdomen releases oviposition behavior in females of the cricket *Acheta domesticus*. *J. Insect Physiol.* **28**, 401–404.

CHOPARD, L. (1914). Sur la vitalité de *Mantis religiosa L.*, ponte après décapitation. *Bull. Soc. ent. Fr.* **19**, 481–482.

DAVIS, W. J. (1977). The command neuron. In *Identified Neurons and the Behavior of Arthropods* (ed. G. Hoyle), pp. 293–305. New York: Plenum Press.

DAVIS, W. J. & KOVAC, M. P. (1981). The command neurone and the organization of movement. *Trends Neurosci.* **4**, 73–76.

HIGHNAM, K. C. (1962). Neurosecretory control of ovarian development in the adult female desert locust, *Schistocerca gregaria*. *Q. J. microsc. Sci.* **103**, 57–72.

HODGKIN, A. L. & KATZ, B. (1949). The effect of temperature on the electrical activity of the giant axon of the squid. *J. Physiol., Lond.* **109**, 240–249.

HOYLE, G. (1953). Potassium ions and insect nerve muscle. *J. exp. Biol.* **30**, 121–135.

HOYLE, G. & BURROWS, M. (1973). Neural mechanisms underlying behavior in the locust *Schistocerca gregaria*. I. Physiology of identified neurons in the metathoracic ganglion. *J. Neurobiol.* **4**, 3–41.

KIEN, J. (1983). The initiation and maintenance of walking in the locust: an alternative to the command concept. *Proc. R. Soc.* B **219**, 137–174.

KUPFERMANN, I. & WEISS, K. R. (1978). The command neuron concept. *Behav. Brain Sci.* **1**, 3–39.

M'CRACKEN, I. (1907). The egg-laying apparatus in the silkwork (*Bombyx mori*) as a reflex apparatus. *J. comp. Neurol.* **17**, 262–285.

MCDANIEL, I. N. & HORSFALL, W. R. (1957). Induced copulation of aedine mosquitoes. *Science* **125**, 745–747.

MOULINS, M. & COURNIL, I. (1982). All-or-none control of the bursting properties of the pacemaker neurons of the lobster pyloric pattern generator. *J. Neurobiol.* **13**, 447–458.

POPOV, G. B. (1958). Ecological studies on oviposition by swarms of the desert locust in eastern Africa. *Anti-locust Bull.* **31**, 1–67.

ROBERTS, B. L. & WILLIAMSON, R. M. (1983). Motor pattern formation in the dogfish spinal cord. In *Neural Origin of Rhythmic Movements* (ed. A. Roberts & B. L. Roberts), pp. 331–350. *Symp. Soc. exp. Biol.* XXXVII. Cambridge: Cambridge University Press.

ROEDER, K. D. (1935). An experimental analysis of the sexual behavior of the praying mantis. *Biol. Bull. mar. biol. Lab., Woods Hole* **69**, 203–220.

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

ROWELL, C. H. F. (1964). Central control of an insect segmental reflex. I. Inhibition by different parts of the central nervous system. *J. exp. Biol.* **41**, 559–572.

SHIK, M. L., SEVERIN, F. V. & ORLOVSKY, G. N. (1966). Control of walking and running by means of electrical stimulation of the midbrain. *Biophysics* **11**, 756–765.

SOMBATI, S. (1983). Neuroethological pharmacology of octopamine in the locust. Doctoral dissertation, University of Oregon.

SOMBATI, S. & HOYLE, G. (1984). Generation of specific behaviors in a locust by local release into neuropil of the natural neuromodulator octapamine. *J. Neurobiol.* **15**, 481–506.

THOMPSON, K. J. (1982). Neural basis of the motor pattern for oviposition digging in the grasshopper. Ph.D. dissertation, University of Oregon.

THOMPSON, K. J. (1986). Oviposition digging in the grasshopper. I. Functional anatomy and the motor programme. *J. exp. Biol.* **122**, 387–411.

TRUMAN, J. W. (1978). Hormonal control of invertebrate behaviour. *Horm. Behav.* **10**, 214–234.

WEIRSMA, C. A. G. & IKEDA, K. (1964). Interneurons commanding swimmeret movements in the crayfish, *Procambarus clarkii*. *J. comp. Biochem. Physiol.* **26**, 1–16.

WINE, J. J. & KRASNE, F. B. (1982). The cellular organization of crayfish escape behavior. In *The Biology of Crustacea* (ed. D. E. Bliss), *Neural Integration and Behaviour* (ed. D. C. Sandeman & H. L. Atwood), pp. 241. New York: Academic Press.