STUDIES ON CROP FUNCTION IN THE COCKROACH
(PERIPLANETA AMERICANA L.)

II. THE NERVOUS CONTROL OF CROP-EMPTYING

BY K. G. DAVEY* AND J. E. TREHERNE†

Department of Zoology, University of Cambridge

(Received 29 May 1963)

INTRODUCTION

A previous paper described some of the factors which govern the rate of emptying of the crop in Periplaneta (Davey & Treherne, 1963). The rate of emptying was shown to be related to the frequency of opening of the proventricular valve. Higher concentrations of glucose, for example, resulted in lower frequencies of opening of the valve. This frequency was, however, not the only factor involved in the release of the fluid into the midgut, for, except at concentrations of glucose near 0.1 M/l. the decline in frequency was not paralleled by the decline in volume of the fluid in the crop. It was necessary to postulate that additional control was exercised by other more complex changes in the behaviour of the valve and its associated pressure gradient.

The more rapid emptying of the crop observed in dilute solutions of glucose appeared to result from changes in the osmotic pressure of the ingested solution, for it was demonstrated that the release of fluid into the midgut remained constant when widely different substances were tested at the same osmotic concentrations (Treherne, 1957). Such a phenomenon implies the existence of a receptor mechanism to detect differences in osmotic pressure together with a system capable of controlling the proventricular valve and the pressure gradient between the crop and the midgut. The present paper in the series describes one of the nervous pathways which takes part in the control of the behaviour of the valve.

MATERIALS AND METHODS

The general approach to the analysis of the nervous pathway involved surgical intervention, usually nerve section. Operated animals were fed on a 0.5 M/l. solution of glucose containing 8.0 mM/l. of the dye Amaranth. Two hours after feeding, the cockroaches were killed and the percentage of the meal remaining in the crop was estimated by the technique which was described in an earlier paper (Treherne, 1957).

RESULTS

The stomodeal nervous system. Willey (1961) has described the innervation of the foregut in several cockroaches, and this account draws heavily on his results.

* Research Fellow of Gonville and Caius College. Present address: Institute of Parasitology, McGill University, Macdonald College, P.Q., Canada.
† A.R.C. Unit of Insect Physiology.
Anteriorly, the paired frontal connectives from the tritocerebrum unite in the mid line to form the frontal ganglion. Fibres from the frontal ganglion continue posteriorly in the single recurrent nerve which gives off branches to the pharynx and which also supplies the retrocerebral complex of endocrine organs. At this level the recurrent nerve is joined by fibres from the diffuse hypocerebral ganglion and continues posteriorly as the oesophageal nerve which ends midway along the crop in the ingluvial ganglion. From the ingluvial ganglion there arises a pair of ingluvial nerves each of which ramifies over the surface of the crop and terminates in the proventricular ganglion. Each of the two proventricular ganglia sends branches into the musculature of the proventriculus (Text-fig. 1).

The frontal ganglion is also connected to the central nervous system by the single nervus connectivus which enters the protocerebrum (Text-fig. 2). Several other nerves also arise from the frontal ganglion and supply various parts of the pharynx or buccal cavity. Four pairs arise from the antero-lateral borders of the ganglion and are closely associated with the frontal connectives. Nerves 1 and 2 arise in the same root; \( N_1 \) branches and serves various muscles associated with the buccal cavity while \( N_2 \)
unites with its opposite partner to form a medial nerve supplying the labrum and buccal epithelium. Fibres of $N_3$ extend as far as the labrum, while those of $N_4$ innervate the pharynx in the immediate vicinity of the frontal ganglion. The last pair of nerves, $N_5$, pass ventrally and posteriorly towards the lining of the pharynx. In addition to these nerves described by Willey, a single median nerve which passes posteriorly from the ventral surface of the frontal ganglion to the heavy muscular coat of the pharynx was apparent in our sections.

**Table 1. The percentage of a meal of 0.5 M glucose containing 8.0 mM l. Amaranth remaining in the crop 2 hr. after feeding cockroaches which had been subjected to various treatments**

The final column expresses the probability that the difference between the means of the treated and untreated cockroaches is due to chance.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Individuals</th>
<th>Mean</th>
<th>s.e.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Untreated</td>
<td>6</td>
<td>63.4</td>
<td>1.94</td>
<td>—</td>
</tr>
<tr>
<td>2. Corpora cardiaca removed</td>
<td>6</td>
<td>94.3</td>
<td>1.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3. Corpora cardiaca controls</td>
<td>5</td>
<td>60.6</td>
<td>2.42</td>
<td>—</td>
</tr>
<tr>
<td>4. Labrum burnt</td>
<td>11</td>
<td>65.7</td>
<td>3.43</td>
<td>&gt;0.80</td>
</tr>
<tr>
<td>5. Oesophageal nerve cut</td>
<td>6</td>
<td>92.1</td>
<td>4.21</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>6. Ingluvial nerves cut</td>
<td>6</td>
<td>96.7</td>
<td>0.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>7. Frontal connectives cut</td>
<td>8</td>
<td>42.3</td>
<td>5.04</td>
<td>&gt;0.20</td>
</tr>
<tr>
<td>8. Nerve cord severed</td>
<td>6</td>
<td>80.0</td>
<td>3.10</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>9. Nervus connectivus cut</td>
<td>8</td>
<td>58.1</td>
<td>4.89</td>
<td>&gt;0.70</td>
</tr>
<tr>
<td>10. Nerves 1-6 cut</td>
<td>7</td>
<td>90.0</td>
<td>1.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>11. Nerves 1-3 cut</td>
<td>5</td>
<td>59.5</td>
<td>1.51</td>
<td>&gt;0.50</td>
</tr>
<tr>
<td>12. Nerve 4 cut</td>
<td>6</td>
<td>62.2</td>
<td>3.03</td>
<td>&gt;0.70</td>
</tr>
<tr>
<td>13. Nerve 5 cut</td>
<td>6</td>
<td>90.0</td>
<td>1.19</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The role of the corpus cardiacum. The results of all of the experiments are summarized in Table 1. Unoperated animals retained 63.4% of the meal in their crops after 2 hr. Because a hormone which affects various visceral muscles is known to be released from the corpus cardiacum after feeding (Davey, 1962a), the possibility that these glands might be involved in the control of the crop was investigated. From treatments 2 and 3 in Table 1 it is evident that removal of the corpus cardiacum very markedly retards the movement of the meal from the crop. Animals which had been subjected to a control operation which consisted of exposing the corpora cardiaca without removing them, did not differ significantly from unoperated individuals. On the other hand, burning the labral organs which mediate the release of the hormone from the corpus cardiacum when the animal feeds on sugar (Davey, 1962b) did not alter the rate of emptying. Removal of the corpus cardiacum also involves breaking the recurrent nerve. Since cutting the oesophageal nerve prevents normal emptying (treatment 5 in Table 1), it is unlikely that the corpus cardiacum is involved in the control of emptying of the crop.

The nervous pathway controlling crop-emptying. It is clear from treatments 5 and 6 in the table that cutting the nerves which lead to the proventriculus all but stops emptying of the crop. Furthermore, cutting these nerves in a preparation in which the proventriculus is contracting stops the movements of the valve. The pathway comprising recurrent nerve-oesophageal nerve-ingluvial nerve probably represents the motor end of the system.
Treatments 7 and 9 demonstrate that a connexion between the frontal ganglion and the central nervous system is not essential to the normal emptying process although severing the nerve cord immediately behind the brain did produce some effect (treatment 8). It is therefore likely that the sensory side of the system is represented by some of the nerves entering the frontal ganglion from the pharyngeal region. Cutting all of these nerves interfered with the normal emptying process (treatment 10). On the other hand, cutting nerves 1, 2 and 3 or nerve 4 (treatments 11 and 12) had no effect on the rate of emptying. Nerve 5 appeared to carry the sensory information to the ganglion; severing it prevented the emptying of the crop.

Intima Tubule Sac
Hypodermis
Sense cell

Text-fig. 3. Drawing of a section through the sense organ in the wall of the pharynx.

Nerve 5 was followed in serial sections; it proved to end near the intima in two pockets in the dorsal part of the pharynx. Sections of this area prepared by the osmium-ethyl gallate method (Wigglesworth, 1957) revealed an unusual sense organ (Text-fig. 3; Pl. 1). The sense cells tended to be organized into groups or cysts enclosed in a more-or-less well-defined sheath. The groups of sensory cells appeared as a definite organ, as shown in the Text-fig. 3, or as having a more diffuse organization, as in the photograph in Pl. 1. The intima over the sense organ is very much reduced, and is penetrated by a number of thin tubules which end blindly as small pegs on the surface of the intima lining the pharynx. Internally the tubules end in diffuse spongy structures, which in some cases appear to consist of a single cell. The tubules do not enter the sense cells.
DISCUSSION

The results presented here demonstrate that the nervous pathway involved in the control of crop emptying originates in the pharynx, passes into the frontal ganglion, along the recurrent nerve, oesophageal nerve, through the ingluvial ganglion and along the ingluvial nerves to the proventriculus.

One of the most important factors which determines the rate of emptying is the osmotic pressure of the ingested fluid (Treherne, 1957). The sense organs in the region of the pharynx innervated by nerve 5 from the frontal ganglion appear to be the only structures which could conceivably respond to changes in osmotic concentration.

The present experiments demonstrate that severing the nervous pathway between the pharynx and the proventriculus results in a drastic reduction in the rate of emptying of the crop. This operation also resulted in the cessation of functioning of the proventricular valve, while preliminary experiments with electrical stimulation showed that this resulted in opening of the valve. These results do not, however, elucidate the precise mechanism by which the integrated nervous control of the emptying process is achieved. The flow of fluid through the constricted region of the proventriculus has been shown to depend not only on the frequency of opening of the valve but also on other parameters which change in some complex way during the emptying of the crop (Davey & Treherne, 1963). The changes in the rate of transit of solutions of differing osmotic concentrations must, therefore, also depend on the control of such factors as the rate of change of dimensions of the effective orifice in the proventriculus, the period of opening of the proventricular valve and the gradient in pressure between the crop and the mid gut.

The osmotic pressure of the ingested fluid is not the only factor influencing the rate of emptying of the crop. We have already shown that the behaviour of the proventriculus and its associated pressure gradient are capable of modification to compensate for differences in the viscosity of the ingested fluid. Furthermore, the crop is also controlled in such a way that a constant proportion of the test meal is released into the mid gut in a given time irrespective of the initial volume of the meal (Davey & Treherne, 1963). The precise nervous mechanism capable of controlling such complex factors is obscure. It is hoped that a future investigation involving measurements of hydrostatic pressure may help to throw some light on this problem.

SUMMARY

1. The nervous pathway involved in the control of crop-emptying has been worked out by the method of nerve section.
2. This pathway involves nerve 5 from the pharynx to the frontal ganglion, the recurrent nerve, the oesophageal nerve and ganglion, the ingluvial nerve and ganglion.
3. Function is not abolished by nerve section between the brain and the frontal ganglion.
4. The sensory end of the pathway is in the pharynx where there is a sense organ which, it is suggested, is an osmoreceptor. A preliminary account of its structure is given.
REFERENCES


EXPLANATION OF PLATE

(a) Photomicrograph of part of the sense organ in the wall of the pharynx showing part of the tubule (T) leading to the sac-like structure (S) at its proximal end. (b) Photomicrograph of part of the sense organ showing a group of sense cells. The arrow points to a bipolar sense cell.