The Effect of Electrical Stimulation on the Cephalic Neurosecretory System in the Last Instar Nymph of the Dragonfly, *Orthetrum chrysis* (Selys) (Odonata; Libellulidae)

WIGGLESWORTH[1] suggested that the abdominal distention due to food intake causes stimulation of stretch receptors which produces nerve impulses and finally induces release of cerebral neurosecretory material (NSM) in *Rhodnius prolixus*. CLARKE and LANGLEY[2-4] reported that the stretch receptors in the wall of pharynx produce impulses which are transmitted to the frontal ganglion and thence to the brain through the frontal connectives and to the corpora cardiaca (CC) through the hypocerebral ganglion. These nerve impulses release NSM from cerebral neurosecretory cells (CNC) and CC respectively in *Locusta migratoria*. In *Schistocerca gregaria* HIGHNAM et al.[5] and HILL et al.[6] confirmed the above results through starvation and feeding experiments.

GOSBEE et al.[7] emphasized that the axons of CNC conduct nerve impulses, and electrical stimulation leads to the release of NSM. Further the nervous control of the release of NSM from the CNS is confirmed by various workers through electrical stimulation[8-10]. Information on dragonfly is not available.

The present investigation is, therefore, undertaken to elucidate the effect of electrical stimulation on the CNS and the neurosecretory pathway in the last instar nymph of the dragonfly *Orthetrum chrysis*.

The last instar nymphs of *O. chrysis* were collected from local ponds in the month of June and kept in aquaria in the laboratory for 1 week. They were not provided with any food. Effect of electrical stimulation on the CNS was studied by giving electrical stimuli of 2 volts, 3 msec, 10 c/sec by fine steel micro-electrodes on the protocerebral region of the brain, at the root of the median ocellus and the compound eyes for 1, 2, 3, 5 and 10 min. 5 insects were used for each experiment. Similar areas of control insects were pricked with electrodes without providing current and sacrificed at the same time. The neuroendocrine organs were dissected out, fixed in Bouin's fluid immediately and processed for whole mount preparations after staining with aldehyde fuchsin (AF) according to the method of DOGRA and TANDAN[11].

In the starved last instar nymphs, 2 groups of AF-positive neurosecretory cells are found on either side of a root of the medial ocellar nerve in the anterodorsal region of the pars intercerebralis medialis (Figure 1). They are heavily loaded with the AF-positive NSM. After 1 min of electrical stimulation (Figure 2), the neurosecretory pathways become clear due to the release of NSM from the cells to their axons. These axons form 2 distinct axonal tracts. Both axonal tracts, loaded with the NSM, run obliquely towards the midline and cross over in-

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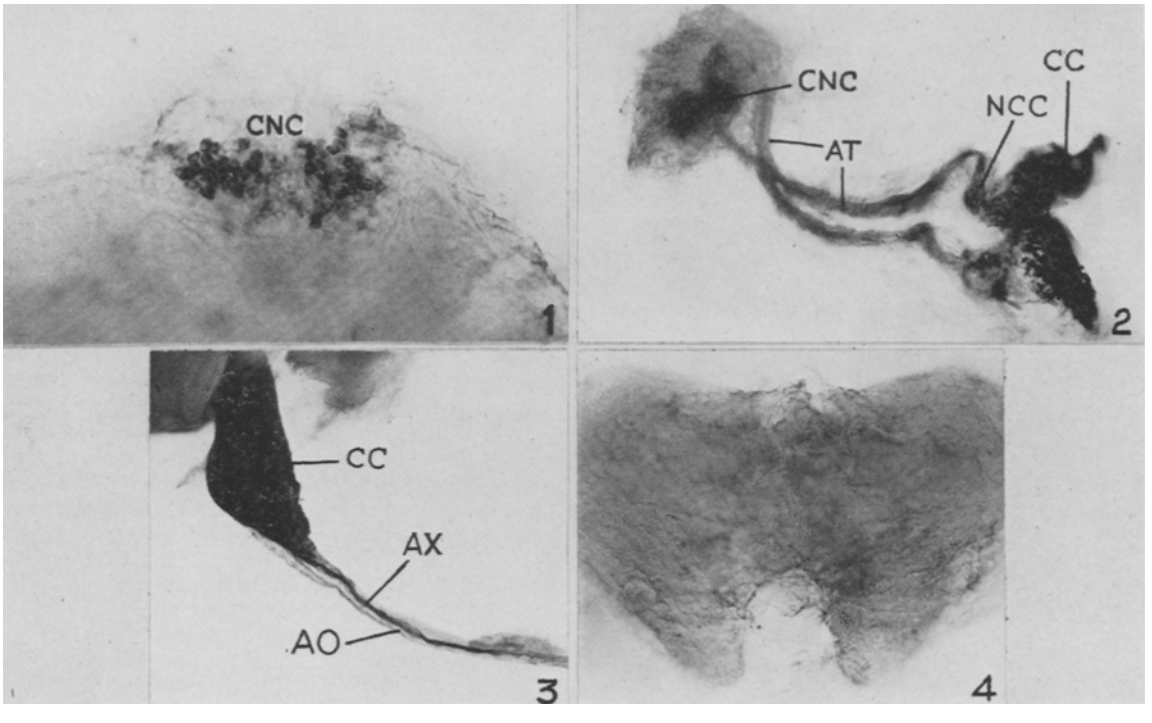

Fig. 1. In situ preparation of the control showing deeply stained NSM in the cerebral neurosecretory cells (CNC). ×160.
Fig. 2. In situ preparation showing NSM in CNC, axonal tracts (AT), NCC and CC after stimulation for 1 min. ×100.
Fig. 3. In situ preparation showing deeply stained axons (AX) running to the aorta from the CC, after stimulation for 3 min. ×100.
Fig. 4. In situ preparation showing lack of NSM in neurosecretory cells and pathways, after stimulation for 5 min. ×80.

between the mushroom bodies. After forming chiasmata, they separate from one another and sweep downwards postero-ventrally, and finally emerge out of the brain in the form of 2 fine nerves, the nervi corpori cardiaci (NCC). The NCC having the NSM enter the CC from their anterior end. 3 min after stimulation very little quantity of NSM is observed in the CNC, axonal tracts and NCC, but the axons running to the aorta from posterior end of the CC, stain intensely (Figure 3). The NSM does not release into the wall of aorta but directly into its lumen. The NSM is lacking from the entire CNS after 5 or 10 min of stimulation (Figure 4).

Electrical stimulation, either through optic lobes, median ocellus or directly on the pars intercerebralis does not show any difference; practically all have the same result.

The electrical stimulation on the brain demonstrates several interesting points worthy of discussion. The electrical stimulus arizing at any part of the brain causes release of the NSM from neurosecretory cells of the brain. It thus confirms the observations of earlier workers that the release of NSM from the CNC is under the control of nervous electrical stimuli.

In most of the insects, the CC are main neurohaemal organs[12–14], while in some Hemiptera[15–18] only the aorta functions as a neurohaemal organ. In these insects, the axons of cerebral NSC terminate not in the CC but in the aorta wall directly and NSM stores in the aorta wall. On the other hand, in some orthopteroid insects[19] and *Calliphora*[20], the aorta functions as a secondary neurohaemal organ. In the present study, it has been observed that some axons from the CC pass into the aorta and there is no discharge of NSM in its wall, but it is directly discharged into the lumen of the aorta. Thus, the aorta here serves as only a releasing site for the cerebral NSM and not as a storage organ, and therefore the function of the aorta in the last instar nymph of *Orthetrum chrysis* differs from that of other insects.

Résumé. L'effet de la stimulation électrique sur le système neurosécrétoire cérébral de la larve d'*Orthetrum chrysis* révèle que la neurosécrétion cérébrale est emmagasinée dans les corps cardiaques et qu'elle est déchargée directement dans l'aorte.

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PRO EXPERIMENTIS

Artificial Feeding of Simuliids (*Simulium venustum*): Factors Associated with Probing and Gorging

Simuliids (Diptera, Simuliidae) are of consequence to human welfare as noxious pests and as vectors of human and animal disease agents, notably the blinding filarial worm, *Onchocerca volvulus*. Despite their importance, knowledge of the mechanisms and factors associated with blood feeding in simuliids is wanting. Other workers have fed simuliids on cotton balls soaked with dextrose and defibrinated blood[1] and on whole blood through skins of young rats and chicks[2,3]. These procedures, however, do not lend themselves to studies with chemically defined media and the skin membranes are difficult to prepare. Herein, we describe a simple and convenient technique for feeding simuliids through inexpensive, commercially available, latex membranes and present preliminary evidence that heat is an essential factor in inducing probing and that adenosine triphosphate and adenosine diphosphate are gorging stimulants.

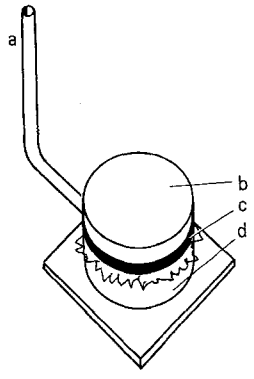

Diagram of artificial feeding chamber. a) right angle glass tube; b) membrane surface; c) rubber ring for attachment of membrane; d) side of plexiglass well.

The feeding chambers used are a modification of those developed by FRIEND and CARTWRIGHT[4] for feeding *Rhodnius prolixus*. Each chamber consists of a plexiglass well with an attached right angle glass tube through which test solutions are introduced (Figure). A latex membrane (Sheik® regular prophylactics) is fitted over the well and secured by a rubber ring. The chambers are heated on a slide warmer. Thermocouples are inserted into the well through the glass tube and the temperature of the test solution monitored with a Leeds and Northrup® temperature potentiometer.

In our experiments female *Simulium venustum* Say were caught individually in vials as they landed on a human subject. Each vial was inverted on the membrane for 5 min during which time the flies were observed as to whether they probed and gorged. Studies were conducted at the Wildlife Research Station, Algonquin Park, Ontario, Canada. Significance of results was determined using the *Z*-test.

The response of simuliids to hosts can be divided into 4 general phases a) activation, b) orientation to and

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