

This was in agreement with previous observation made in vivo<sup>12</sup>. Since recording and stimulating electrodes can be applied independently to portions of a neuron identified microscopically, thin sections as used in the present experiments are expected to serve as excellent preparations for studies on chemosensitivity of the neuronal membrane as well as on neuron-neuron and neuron-glia interaction in the brain.

<sup>12</sup> H. KAWAMURA and L. PROVINI, Brain Res. 24, 293 (1970).

<sup>13</sup> I thank Profs. H. MANNEN and K. SASAKI for valuable discussion.

**Zusammenfassung.** In Dünnschnitten vom Kleinhirn des Meerschweinchens wurden in künstlichem Medium Purkinjezellen identifiziert und Spontanentladungen der Zellkörper registriert. Elektrophoretischer Antransport von Glutaminsäure zu Zellkörper oder Dentriten rief jeweils starke Erregung hervor.

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### Neurosecretory Cells in the Hypocerebral Ganglion of *Gryllus bimaculatus* de Geer (Orthoptera: Gryllidae)

The occurrence of various types of neurosecretory cells (NSC) in the brain and the ventral ganglia, which control all the major physiological events in the life cycle of insects, is well known. There is no previous report of the presence of neurosecretory cells in the hypocerebral ganglion beyond the recording of B cells in *Schizodactylus*<sup>1</sup>.

The Bouin-fixed paraffin embedded material was serially cut at 6 µm and stained with Chrome Haematoxylin-Phloxine (CHP), Paraldehyde-Fuchsin (PF) and Heidenhain's Azan (Azan).

The hypocerebral ganglion has 2 types of neurosecretory cells which can be classified as A and B cells on the basis of their tinctorial affinities with PF, CHP and Azan (Figures 1–3). The first type, designated as 'A' cells, is characterized by cytoplasmic inclusions staining deep

purple with PF, blue-black with CHP and red with Azan. The fine homogenous inclusions of the second type of cells, the 'B' cells stain greenish with light green-orange G, red with phloxine of CHP and faint blue with the aniline blue-orange G counterstains of Azan.

Each half of the hypocerebral ganglion has 8 to 10 A cells and 4 to 6 B cells found along with a large number of nerve cells. The anterior part of the ganglion has 2 cells in the median and midventral position, 2 cells in the anterior region and 4 to 6 cells in its posterolateral part (Figure 1). The axons arising from the A cells run towards the lateral border of the ganglion (Figure 3) from where they enter the corpora cardiaca through the nervi hypocerebrii (NH, Figure 1). It is easy to trace the axonal pathways (AX) due to the presence of stained neurosecretory material in them which stains deep purple with PF, red with Azan and blue-black with CHP.

The mid-dorsal region of the anterior half of the hypocerebral ganglion has 4 to 6 B cells which stain greenish with PF, red with CHP and faint blue with Azan. The axons of these cells can be traced only for a short distance due to lack of staining colloids.

A large amount of neurosecretory material (NSM) has been observed laterally in the hypocerebral ganglion of males as the two NCC I pass through its lateral margins, whereas the amount of NSM is comparatively less in females as the NCC I are free and have no connection with hypocerebral ganglion. The small amount of NSM, which is particularly seen before copulation and oviposition in females, is the product of the neurosecretory cells of this ganglion. Thus the greater amount of NSM in males is the product of the neurosecretory cells of the hypocerebral ganglion as well as the pars intercerebralis of the brain.

Thus the present work records for the first time in insects the presence of both A and B cell types and neurosecretory pathways up to the neurohaemal organs (corpora cardiaca).

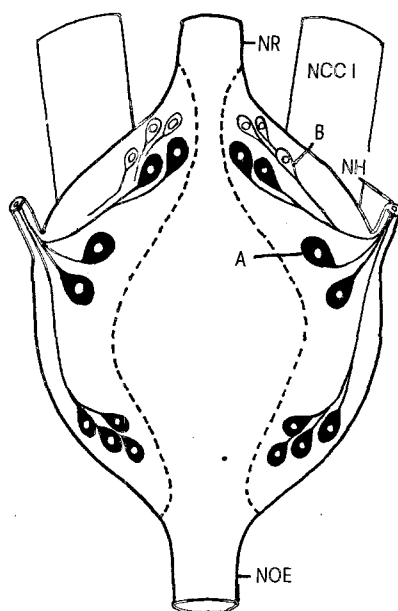


Fig. 1. Diagrammatic representation of the distribution of neurosecretory cells in the hypocerebral ganglion of *Gryllus bimaculatus*. A and B, neurosecretory cell types; NCCI, nervus corporis cardiaci; NH, nervus hypocerebrii; NOE, nervus oesophagei; NR, nervus recurrentus.

<sup>1</sup> N. KHATTAR, Bull. Soc. Zool. fr. 93, 225 (1968).

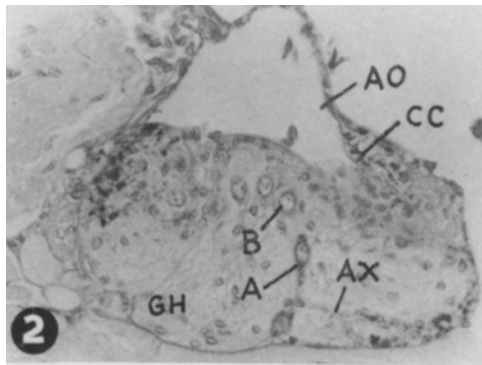


Fig. 2. T. S. through aorta (AO), corpora cardiaca (CC) and hypocerebral ganglion (GH) showing neurosecretory cell types (A, B) in the latter.  $\times 270$ .

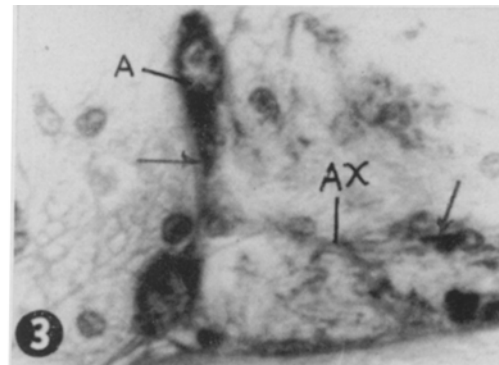


Fig. 3. A part of Figure 2 enlarged to show the A cells (A) and axons (AX) filled with neurosecretory material (arrows).  $\times 520$ .

**Zusammenfassung.** Neurosekretorische Zellen der Typen A und B werden im Hypocerebralganglion von *Gryllus bimaculatus* beschrieben. Die Axone der A-Zellen führen

das Neurosekret durch die Nervi hypocerebri und enden im Corpus cardiacum. Die Ganglien des reifen Männchens enthalten mehr Neurosekret als die des reifen Weibchens.

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### Arousal as a Cyclic Phenomenon During Sleep and Hibernation in the Hedgehog (*Erinaceus europaeus*)

The length of total sleep time (TST) has been claimed to be related to the degree of cortical development, the more primitive the cortex the greater the period of TST<sup>1</sup>. If this is so, the TST of the European hedgehog should be high, and 80% per day has in fact been reported by direct observation<sup>2</sup>. The sleep cycle duration, i.e. the interval between 2 successive paradoxical sleep periods uninterrupted by a waking period of longer than 1 min, has been suggested to be related to basic metabolic rate<sup>3</sup>, animals with a low rate having a long sleep cycle. The hedgehog ( $53.1 \text{ kCal/kg}^{0.75}/\text{day}$ )<sup>4</sup> would be expected to have a longer sleep cycle than the guinea-pig, an animal of similar body weight ( $69 \text{ kCal/kg}^{0.75}/\text{day}$ )<sup>5</sup>. For the majority of the year, the European hedgehog has a body temperature of  $34^\circ\text{C}$ . During winter, when the surrounding temperature drops below  $13^\circ\text{C}$ , it enters hibernation, and its temperature is maintained  $1\text{--}2^\circ\text{C}$  above that of the external environment, with which it varies.

This work was carried out to establish the sleep-wakeful pattern by EEG techniques and to evaluate the influence of metabolic rate on sleep cycle duration.

**Methods.** 9 hedgehogs, captured in autumn, were housed in small individual transparent enclosures and supplied ad libitum with a standard diet of canine prepared food. Under ether anaesthesia a pair of silvered screws was positioned on either side of the sagittal suture over the frontal lobes for recording ECoG. Electrodes were fixed in the neck muscle and subcutaneously on either side of the thorax for EMG and ECG respectively. A strain-gauge attached to the animal's back served as a pneumograph. Body temperature (BT) was measured from thermocouples placed within the abdominal cavity. A polygraph (REEGA VIII, ALVAR) was used for recordings at speeds of  $2.5 \text{ mm/sec}$  and  $60 \text{ mm/sec}$ .

Heart and respiratory rates were counted over periods of 12 sec and checked every 2 min. The response (jerk reflex) of the animals to an auditory stimulus (click) involved a general contraction of the orbicularis dorsi muscle and was recorded for all experimental situations. Continuous recordings for periods of 1 week were made at 3 different BT: in autumn at  $\text{BT} = 34^\circ\text{C}$ , next in winter after the animals had spontaneously entered hibernation at  $\text{BT} = 13^\circ\text{C}$  and finally the hibernating animals were placed in a refrigerator reducing their BT to  $8^\circ\text{C}$ .

**Results.** At  $\text{BT} = 34^\circ\text{C}$ , the following states were identified: alert wakefulness (AW), drowsiness (DR), slow wave (SWS) and paradoxical sleep (PS). An attitude of lateral recumbency was assumed for the last 3 states. AW was characterized by high voltage ( $> 100 \mu\text{V}$ ) fast activity ( $> 25 \text{ c/sec}$ ) and usually corresponded to feeding or exploratory behaviour it was the only state for which the cervical muscular tone was high. During DR the ECoG evolved progressively and the spindles ( $100 \mu\text{V}$ ,  $40 \text{ c/sec}$ ) occurred systematically at each respiratory pause (Figure 1). Towards the end of this period the occurrence of the spindles was inconsistent and their morphology had altered ( $50 \mu\text{V}$ ,  $16 \text{ c/sec}$ ). The ECoG during SWS showed typical high voltage ( $100 \mu\text{V}$ ) low frequency ( $2\text{--}4 \text{ c/sec}$ ) waves with microwaves superimposed. PS was recognized by low voltage ( $50 \mu\text{V}$ ) high frequency ( $> 20 \text{ c/sec}$ )

<sup>1</sup> F. SNYDER, in *Physiology and Pathology of Sleep* (Ed. A. KALES; Lippincott, Los Angeles 1968), p. 266.

<sup>2</sup> P. SUOMALAINEN, in *The Nature of Sleep* (Eds. G. E. W. WOLSTENHOLME and M. O'CONNOR; Churchill, London 1961), p. 307.

<sup>3</sup> T. WEISS and E. ROLDAN, *Experientia* 20, 1 (1964).

<sup>4</sup> G. HILDWEIN and A. MALAN, *Arch. Sci. Physiol.* 24, 133 (1970).

<sup>5</sup> J. PELLET and G. BÉRAUD, *Physiol. Behav.* 2, 131 (1967).