Effect of certain environmental factors on the cerebral neurosecretory cells of American cockroach *Periplaneta americana* (L.)

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Summary. Under illumination, the cerebral neurosecretory cells (NSC) of *Periplaneta americana* were found to be depleted of neurosecretory material (NSM). It is suggested that, due to prolonged exposure to illumination, the synthesis of NSM was checked. The NSC were found densely packed with NSM after keeping the insects in total darkness. Clumping of NSC and their axons was brought about by 15 days of starvation. Diet played no significant role in the neurosecretory activity.

In recent years, attention has been focussed on the effects of environmental factors on the neurosecretory cells (NSC) of certain insects[2-8]; but very little is known about the changes in the activity of cerebral neurosecretory cells of *Periplaneta americana* (L.) due to certain environmental factors. In the present investigation, an attempt is made to study the effect of light, darkness, starvation and protein-, fat- and carbohydrate-rich diets on the cerebral neurosecretory activity of the American cockroach, *Periplaneta americana*.

The experiments were carried out on 1-month-old adult female cockroaches. In the first experiment, the insects were divided into 3 groups, and were kept in small insect cages. They were supplied with food consisting of wet bread pieces and water at regular intervals. 1 group was exposed to the illumination of 200 W incandescent lamp from a distance of 30 cm for a period of 12 days. The 2nd group was kept in total darkness for the same length of period with provision for free passage of air. The 3rd group was maintained as control for the same length of period. For the 2nd experiment, the insects were divided into 2 groups. 1 group was fed on the normal diet of wet bread pieces, while the 2nd group was not given any food for 15 days except some water. In the 3rd experiment, insects were separated into 3 groups. The first, 2nd and 3rd groups of insects were fed on protein-, fat- and carbohydrate-rich diets respectively for 15 days. The brains were removed from all the experimental and control in-

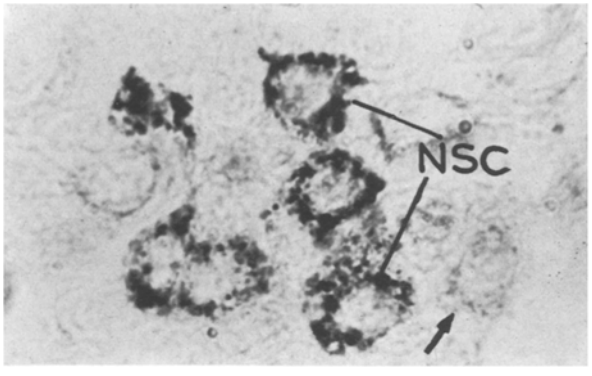

Fig. 1. Transverse section of brain passing through the pars intercerebralis showing neurosecretory cells (NSC) with depletion of neurosecretory material (NSM). Note a single cell marked with → showing no traces of neurosecretory material (NSM). The individual was exposed to illumination for a period of 12 days. PF-stain, ×1000.

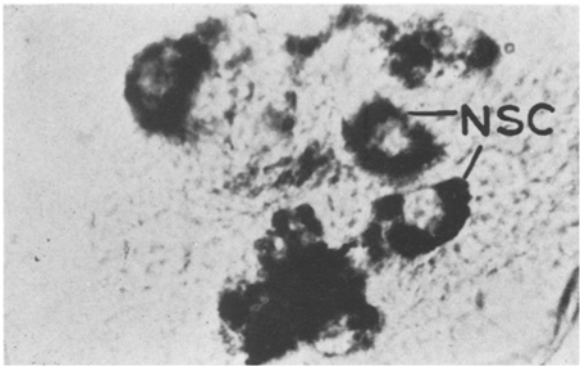

Fig. 3. Transverse section of brain passing through the pars intercerebralis exhibiting neurosecretory cells (NSC) with deformity in their structure and clumping of neurosecretory material (NSM). The individual was starved for a period of 15 days. PF-stain, ×1000.

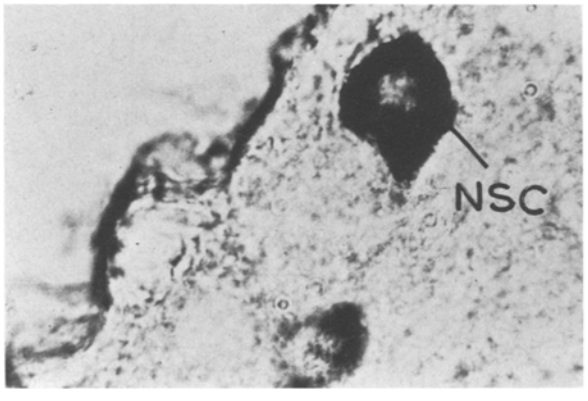

Fig. 2. Transverse section of brain passing through the pars intercerebralis showing neurosecretory cells (NSC) with abundant neurosecretory material (NSM). The individual was kept in darkness for a period of 15 days. PF-stain, ×1000.

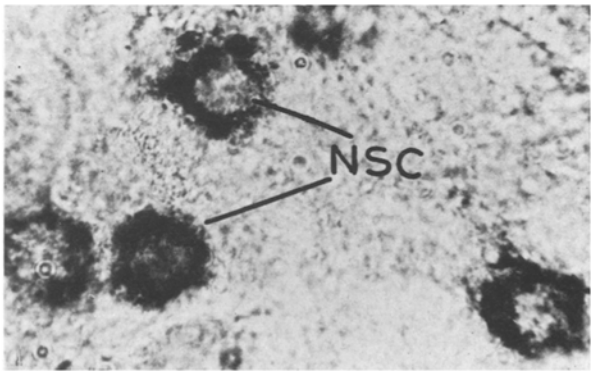

Fig. 4. Transverse section of brain passing through the pars intercerebralis of a normal individual. Note the presence of uniformly distributed neurosecretory material (NSM) in the perikarya of the neurosecretory cells (NSC). PF-stain, ×1000.

sects and were fixed immediately in aqueous Bouin's fluid. The material was processed in the usual way and paraffin blocks were prepared. 6–8 μm thick sections were serially cut and stained with FF-technique of Ewen[9].

The effect of light on the neurosecretory activity was shown by the autoradiographic studies on grasshopper, wherein a higher degree of S^{35}-cysteine was incorporated into the brain neurosecretory cells of the long day insects, as compared with the short day insects[5]. Recently, the site of photoperiodic reception was believed to be the brain itself by some workers[2,3]. In the present study, a few significant changes were observed in the cerebral NSC after exposing the insects to illumination of longer duration. Under illumination, the NSC were found to be depleted of neurosecretory material (NSM) (figure 1). It is suggested that, due to prolonged exposure to illumination, the synthesis of NSM is checked. The NSC were found densely packed with NSM after keeping the insects in total darkness (figure 2). Due to darkness, the NSM remains undischarged and thus accumulates as a densely packed material in the perikarya of the NSC. If these insects are again exposed to illumination, the NSC are deplated of their colloids. Similar observations were made by Gundevia and Ramamurty[10], who reported that light plays a role in the process of neurosecretory material production and its release, because in darkness the cells were densely packed with NSM, while under illumination the cells themselves are depleted of their colloids but in the axons there was an abundance of the neurosecretory granules.

Starvation brought about the inhibition of neurosecretory activity in red cotton bug[6], and clumping of NSC in *Orthetrum chrysis*[11]. In the present investigation, deformity in the NSC and clumping of the NSM in the cells and axons were brought about by 15 days of starvation in *P. americana* (figure 3). Similar observations were made by Bassurmanova and Panov[4]. No change was observed in the neurosecretory activity of brain cells due to different diets as compared with controls. Presumably diet plays no significant role in the secretory activity of NSC of this insect. In all the control insects, the cerebral NSC showed uniformly distributed NSM in their perikarya (figure 4).

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Distribution of γ-aminobutyric acid (GABA) in the ganglia of *Aplysia kurodai*

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Summary. The concentration of GABA was determined in each ganglion of *Aplysia kurodai* by microassay. Highest concentration was observed in buccal ganglia.

GABA has been established as an inhibitory neurotransmitter at the crustacean neuromuscular junction[2–4] and there is now good evidence that the substance is the inhibitory neurotransmitter in vertebrate[5,6] as well as in invertebrate nervous systems[2–4]. Morphological and physiological properties of *Aplysia* ganglia have been investigated for many years. The relatively large size and ready accessibility of the neurons facilitate neurochemical investigation including the determination of putative neurotransmitters; acetylcholine[7], dopamine and serotonin[8,9], glutamate and glutamine[10] and their related enzymes in the individual neurons of the ganglia.

However too little attention has been paid to GABA in the *Aplysia* ganglia. Presence of GABA in the ganglia has not been determined[3], probably because of the technical limitation of GABA assay system in such a small sample as single ganglion or neuron. By the iontophoretic study, Gerschenfeld et al.[11] found that GABA has an exitatory on H-cells and an inhibitory action on D-cells of *Aplysia* nervous system.

In the present experiment, the concentration of GABA was determined in each ganglion of *Aplysia kurodai*, by a sensitive method which combines the GABase system of Scott and Jacoby[12] with the technique of enzymatic cycling of NADPH of Lowry et al.[13]. This method enabled us to measure GABA in the order of 10^{-12} moles.

Aplysia kurodai (220–270 g b.wt) were collected at the Miura Peninsula, Kanagawa Prefecture, in January and February 1974. They were kept in a sea-water tank at

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