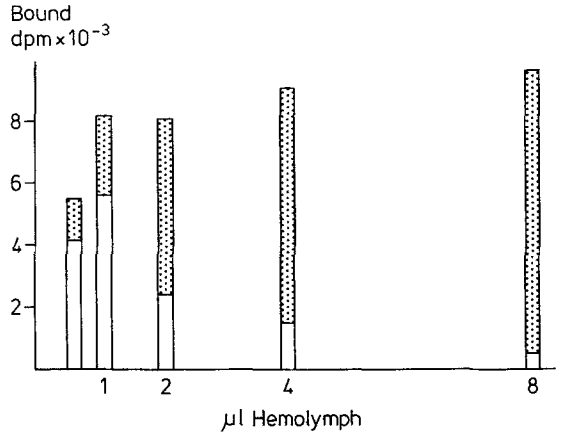

Figure 2. Relation between [^{3}H] EFDA-labeling and the amount of hemolymph from the American cockroach. [^{3}H] EFDA (50,000 dpm, spec. act. 11.0 Ci/mol) was incubated for 1 h with increasing amounts of hemolymph in 200 µl phosphate-buffered saline. After illumination by UV-light as described previously[10], bovine serum (25 µl) was added and the covalently bound [^{3}H] EFDA was precipitated with equal volumes of 10% TCA. After centrifugation, the pellets were washed with 500 µl 5% TCA, 500 µl ether/ethanol 1:1 v/v (2×) and 500 µl ether, dried and the radioactivity counted as described[10]. Whole columns, total amount of covalently bound radioactivity; shaded columns, amount of radioactivity after co-incubation with 10^{-6} M unlabeled JH-III; open columns, amount of [^{3}H] EFDA specifically bound to the JH-III binding sites.

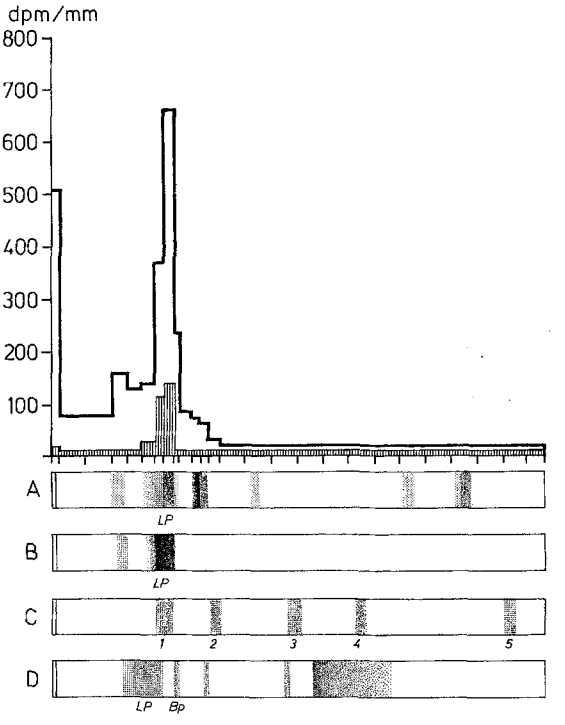

Figure 3. Electrophoretogram (A) and distribution of radioactivity (graph) in a native gradient gel of hemolymph from adult male *Periplaneta* treated with [^{3}H] EFDA (for details see fig. 2). After concentration of the sample, equivalents of 5 µl of hemolymph were applied to each slot, and gels were run and stained as described before[10]. Gels were subsequently sliced as indicated on the horizontal axis of the graph. Slices from two runs were pooled and counted[10]. The shaded graph represents the radioactivity when UV-illumination is omitted. The same slab-gel contained samples of lipophorin from *Periplaneta* (B) isolated by ultracentrifugation[7]; high molecular weight standard proteins (C): 1) Thyroglobulin (669,000 mol. wt), 2) Ferritin (440,000 mol. wt), 3) Catalase (232,000 mol. wt), 4) Lactate dehydrogenase (140,000 mol. wt), 5) Albumin (67,000 mol. wt); and a sample of *Locusta* hemolymph (D). Lp, lipophorin; BP, binding protein.

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Location of allatostatic centers in the pars lateralis regions of the brain of the Colorado potato beetle

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Summary. Selective lesions in the pars intercerebralis and pars lateralis areas of the brain of the adult Colorado potato beetle were produced by radiofrequency cautery. The effect of these lesions on the corpus allatum activity, determined by the short-term in vitro radiochemical assay, revealed that gland inhibitory centers are located in the pars lateralis.
Key words. Colorado potato beetle; corpus allatum control; corpus allatum activity; radio-frequency cautery; pars intercerebralis; pars lateralis; lateral neurosecretory cells; allatostatin.

The control of corpora allata by the central nervous system has fascinated insect endocrinologists ever since Wigglesworth's classic experiments with the bug, *Rhodnius prolixus*[1]. It has been proposed that the brain initiates inhibitory (allatostatic) as well as stimulatory (allatotropic) signals which reach the gland either by way of nerve connections (neural pathway) or by way of the hemolymph (humoral pathway), or both[2,3]. Very little is known about the precise source and nature of these signals. The adult Colorado potato beetle, *Leptinotarsa decemlineata* Say, has proved to be a useful model for such studies. The application of an in vitro radiochemical assay, for comparing activities of innervated and denervated corpora allata under various experimental conditions, revealed that in this beetle the glands were controlled by signals arriving along the neural as well as the humoral pathway[4]. Any humoral influence, however, was clearly subordinate to an inhibitory effect sustained by intact nerve connections with the central nervous system. This was observed in several experimental situations e.g. when beetles under long-day conditions were starved[5], when the juvenile hormone titer was experimentally elevated[6], and in young pre-diapause beetles[4].

To trace the neural pathways we back-filled the axons terminating in the corpus allatum with horse radish peroxidase and discovered that each of the corpora allata were innervated, via the nervi corporis cardiaci (NCC) 2, by a compact group of about 8 cells, identified as the lateral neurosecretory cells (L-NSC), lying in the pars lateralis region of the brain[10]. Recently, an electron microscope procedure involving tissue fixation with tannic acid was used for visualizing the release of elementary particles from the axon terminals in the corpora allata[8]. Significantly more elementary particles appeared to be released in restrained glands as compared with active glands, lending further support to our assumption that the L-NSC produce an allatostatin. However, definite proof for this hypothesis was still lacking. Moreover, in the cockroach, *Diploptera punctata*, it has been suggested that the median NSC (M-NSC) lying in the pars intercerebralis are the source of an allatostatin[9]. In the beetle, the axons from the M-NSC, which travel via the NCC 1 and NCC 2, do not seem to terminate in the corpus allatum but in the corpus cardiacum lying adjacently (fig. 2). Theoretically however, the M-NSC could influence the gland indirectly by releasing their products into the hemolymph or by providing input into the L-NSC[7].

Employing radio-frequency (RF) cautery, we attempted to cauterize either the pars lateralis or the pars intercerebralis unilaterally and observed the effect of these lesions on the activity of the ipsilateral corpus allatum, using the contralateral gland as control. Operations were performed on 0-day-old female *L. decemlineata* reared under short-day conditions[10]. After anesthetization using carbondioxide and surface sterilization with ethanol, the dorsal part of the brain was exposed by cutting a rectangular opening in the frons. Localized lesions were produced by applying a high frequency pulse with a RF (50 mHz) microcauterizer (Murphy Developments, Hilversum, Holland). Pulse duration was 400 ms and the amplitude was adjusted between 50–60% of the maximum output (10W). Stainless steel insect pins (size 000) whose tips had been tapered electrolytically[11] to approximately 20 µm were used to deliver the pulse, a newly sharpened tip being employed for each cauterization. Just before current was applied, hemolymph was removed by aspiration. After closing the wound, beetles were kept for another day under short-day conditions on fresh potato foliage. Thereafter the animals were transferred to long-day and simultaneously starved. After 3 days starvation, the animals were sacrificed, brains were fixed for autopsy while activities of individual corpora allata were estimated by using the short-term, in vitro assay procedure as described previously[12,13]. L- (Me- ^{14}C)-methionine (Radiochemical Centre, Amersham, Bucks; final specific activity 34 to 37 mCi/mmol) and L- (Me-^{3}H)-methionine (New England Nuclear, Chicago; final specific activity 115–140 mCi/mmol) were employed as labeled precursors.

For histological examination brains were fixed in Bouin Hollande sublime for 6 h. Transverse serial sections were cut at 5 µm and stained with paraldehyde fuchsin according to Gabe[14]. Prior to application of Orange G, the sections were submerged for 10 min in a 14 mM solution of tungstophosphoric acid. The effect of this histological procedure was that most of the brain tissue (neuropil) was stained light green. The purple colored M-NSC were prominent while the L-NSC could be occasionally recognized by their dark green appearance. The coagulated and damaged areas were distinctly tinted orange (fig. 1). After histological assessment of the lesions, the samples were divided into the following 4 categories (fig. 2):

I the pars lateralis harboring the L-NSC was cauterized, but the M-NSC lying in the pars intercerebralis appeared undamaged

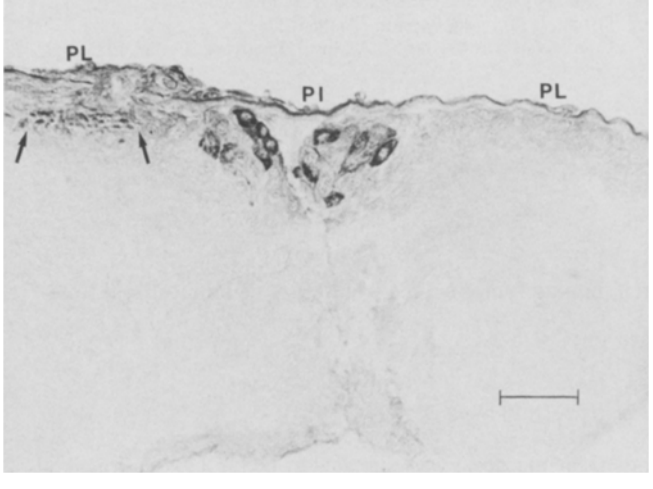

Figure 1. Photomicrograph of a histological section of the brain showing the effect of unilateral cauterization of the pars lateralis (PL). The lesion is indicated by arrows. The darkly stained cells in the pars intercerebralis (PI) belong to the median neurosecretory cells. Scale line represents 50 µm.

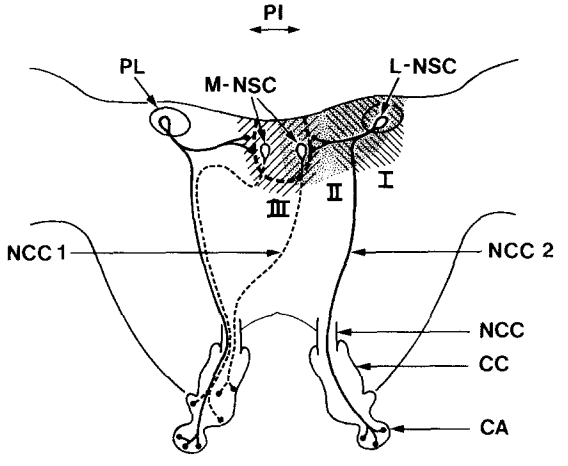

Figure 2. Schematic representation of the locations of the various brain lesions and projections of the neurosecretory cells associated with the retrocerebral complex. The categories of lesions (I, II, and III) are described in the text. CA, corpus allatum; CC, corpus cardiacum; L-NSC, lateral NSC; PI, pars intercerebralis; PL, pars lateralis.

Category of lesion	n	Right CA	Left CA	Level of significance
I Unilateral PL only	27	1.33 ± 0.91	0.31 ± 0.29	$p < 0.005$
II Unilateral PL part of PI	13	1.27 ± 0.76	0.50 ± 0.35	$p < 0.01$
III Unilateral or bilateral PI	12	0.34 ± 0.27	0.41 ± 0.31	NS
IV No apparent abnormality	29	0.53 ± 0.56	0.50 ± 0.54	NS
V Unoperated controls	20	0.66 ± 0.51	0.68 ± 0.47	NS

The effect of selective lesions in the brain on corpus allatum activity. Gland activity is expressed in pmol juvenile hormone synthesized per hour (means ± standard deviation). The right gland was always on the operated side, while the left gland was used as control. The activities of the two glands in each individual were statistically analyzed using the Wilcoxon matched pairs signed ranks test. CA, corpus allatum; NS, not significant; PI, pars intercerebralis; PL, pars lateralis.

II the pars lateralis plus part of the pars intercerebralis were impaired
III more than half or the total number of M-NSC were destroyed but the L-NSC appeared intact
IV no abnormality was apparent, or the damage was restricted to the perineurium.

The results are displayed in the table where values from unoperated controls are also included. In categories I and II a highly significant increase in the mean rate of juvenile hormone biosynthesis by one of the glands of a pair was observed. In categories III and IV, and in the unoperated controls, the mean activities of both glands of a pair remained similar and relatively low. In previous studies, we observed that corpora allata from beetles reared under short-day conditions displayed relatively low rates of juvenile hormone biosynthesis at emergence, and were gradually inactivated just before diapause[13]. However, when the corpora allata of short-day beetles at emergence were denervated, and the animals transferred to long-day and simultaneously starved for 3 days, the glands became activated[4]. Intact glands remained restrained in their activity under these conditions. In the present paper, a similar experimental protocol has been followed to investigate whether selective cauterization could lead to gland activation. The results in the table show that with lesions whereby the pars lateralis area was unilaterally damaged (categories I and II), the gland on the ipsilateral side became activated while the contralateral corpus allatum remained restrained in its activity. The implication of this finding is that a corpus allatum inhibitory center is located in the pars lateralis, since gland inhibition is removed by selective cauterization of this region. Taking the innervation of the corpus allatum into consideration, this evidence provides strong support for our previous suggestions that the L-NSC produce an allatostatin in the Colorado beetle[7].

Surprisingly, removal of the M-NSC did not appear to affect corpus allatum activity. However, we cannot exclude the possibility that under different experimental conditions these cells may indirectly influence gland activity. In *D. punctata*, cauterization of the L or M-NSC led to gland activation[9]. In this cockroach, it has been postulated that the L-NSC do not inhibit the gland directly but provide input into the contralateral M-NSC which produce and release an allatostatin through the neural pathway. Whether this essential difference between the beetle and the cockroach is related to the different pattern of corpus allatum innervation and activity in the two species remains to be seen. Another intriguing aspect is that the precise role of the L-NSC, whether inhibitory or stimulatory, also appears to be species dependent. For example, in the locusts *Schistocerca paranensis*[15] and *Locusta migratoria*[16], the evidence implies that an allatotropin is produced by the L-NSC. A satisfactory explanation for such diverse observations in different species must await further experimentation, including isolation and identification of the substance(s) produced by the L-NSC.

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Hormone and forskolin-stimulated cyclic AMP accumulation in human lymphocytes: reliability of longitudinal time measurements[1]

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Summary. Reliability of measurement of lymphocyte cyclic AMP synthesis in intact cells was estimated by taking 3 successive blood samples during a one-month period from 11 healthy volunteers. Isoproterenol and prostaglandin E_1-stimulated cyclic AMP accumulation were used to evaluate the activity of these two receptor activities in human lymphocytes. Forskolin-stimulated cyclic AMP accumulation was used to evaluate the activity of the Ns/catalytic subunit. Only for forskolin was significant reliability observed. For isoproterenol and prostaglandin E_1 significant reliability was observed only for male subjects.
Key words. Cyclic AMP synthesis; measurement in lymphocytes; forskolin; isoproterenol; prostaglandin E_1.