size of the compound action potential of non-myelinated fibres of the vagus nerve of rabbit in concentrations as low as $10\ \mu M$ it did not affect the Na-K currents in squid giant axon a phenomenon also observed by us for L-*trans*-Δ^8-tetrahydrocannibol in concentrations as great as 0.4 mM (unpublished observations).

Zusammenfassung. Nachweis, dass Lysergsäurediäthylamid^{-25} (LSD) die Ionenströme von Na und K im Riesenaxon des Tintenfisches auch in hohen Konzentrationen nicht beeinflusst.

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Male Sex Pheromone from the Wing Glands of the Indian Meal Moth, *Plodia interpunctella* (Hbn.) (Lepidoptera: Phycitidae)

Several male phycitid moths including the Indian meal moth, *Plodia interpunctella*, possess a characteristic gland located at the base of each forewing[1]. It consists of a membraneous flap overlying specialized scales that are presumed to release a scent that stimulates the female[1,2] but no function has yet been demonstrated. In the case of the related pyralid moth, *Achroia grisella* (Fabr.), the male wing glands (also located at the base of the forewings) release a sex pheromone that, in combination with sound, attracts the female to the male[3]. However, the male scent scales of other moths and butterflies usually emit a sex pheromone during courtship which acts as an aphrodisiac inhibiting flight and rendering the female receptive to the copulatory attempts of the male[4–7]. This report describes the function of the supposed scent glands of the Indian meal moth.

Observations of mating behaviour were made in a glass petri dish (10 cm diam.). The sexually excited male typically approaches the calling female with his wing glands open; that is, the flap covering the scent scales is raised and the scales themselves are erected and splayed. Generally courtship is initiated when the male approaches from the rear and nudges the underside of the female abdomen and wings with his head and antennae. If receptive, the female turns 180° and usually lowers her abdomen from the calling position. She faces the male head on and remains stationary. The male moves forward and puts his head under the head of the female. The female responds by bringing her antennae forward so that they lie close to the wing glands but apparently do not touch them. Following this, the female raises her abdomen so that it projects between her wings in a position similar to calling but the pheromone gland is not exposed. This is termed the acceptance posture and as soon as it is taken up the male makes genital thrusts and copulation usually follows.

If the female cannot perceive the male scent, however, her courtship behaviour is drastically altered and she rejects the courting male. Elimination of female perception of the male scent was accomplished in two ways, either by bilateral antennectomy of the female or by removing both male forewings including the glands. Controls, to assess the effects of amputation, consisted of females which had about $^3/_4$ of their antennae removed leaving small flagellar stumps which contained numerous chemoreceptors or males which had most (about $^7/_8$) of their forewings removed except the glands which were left attached to the male. All amputations were carried out on insects lightly narcotized with carbon dioxide.

Courtship involving a glandless male and a normal female was initiated in the usual manner. However, the female when nudged on the rear of her abdomen by the male turned either less than or more than 180° so that she did not face the male head on. At the same time she backed up and turned her head and body to one side then the other of the male's head and continued to avoid the head-on configuration with the male. The male advanced as the female backed up and attempted to put his head under hers but her manoeuvers generally prevented this. Moreover, the female did not erect her abdomen between her wings in the typical acceptance posture. Consequently, copulatory thrusts were infrequent during rejection behaviour. Eventually contact was lost between the partners and courtship was terminated. If the male's courtship approach was to

Effect of removal of male wing glands or female antennae on mating success of *Plodia interpunctella* during 10 min observation period

Sex and treatment	No. of pairs	Percent mated
Controls		
♂ and ♀ Normal	92	93
♀ 3/4 Antennaeless	30	83
♂ 7/8 Wingless, gland intact	20	80
Experimentals		
♀ Antennaeless	30	27
♂ Glandless	30	37

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[1] R. Barth, Z. wiss. Zool. *150*, 1 (1937).

[2] R. Barth, Zool. Jb., Abt. allg. Zool. Physiol. Tiere *58*, 297 (1937).

[3] K. H. Dahm, D. Meyer, W. E. Finn, V. Reinhold and H. Röller, Naturwissenschaften *58*, 265 (1971).

[4] N. Tinbergen, B. J. D. Meeuse, L. K. Boerema and W. W. Varossieau, Z. Tierpsychol. *5*, 182 (1942).

[5] L. P. Brower, J. van Z. Brower and F. P. Cranston, Zoologica, N.Y. *50*, 1 (1965).

[6] M. Birch, Anim. Behav. *18*, 310 (1970).

[7] J. R. Clearwater, J. Insect Physiol. *18*, 781 (1972).

the front of the female she immediately rejected him by backing up and turning back and forth to avoid the head-on configuration. This rejection behaviour was duplicated exactly in courtships involving antennaeless females and normal males.

The effectiveness of female rejection behaviour is demonstrated in the Table. The mating success of glandless males or antennaeless females during a 10 min observation period was reduced to approximately $^{1}/_{3}$ that of normal moths and only 10 to 13% of the reduction was attributable to the amputation procedures (see controls). Moreover, the mating success of some of the experimentals was an artifact of the test situation. Persistant males, despite female rejection behaviour, were able to trap the females against the walls of the petri dish and achieve the head-under position. Females which were thus trapped and which had been courted repeatedly beforehand responded by raising their abdomen to the acceptance posture. This response elicited copulatory attempts from the male.

Courtship involving either of the controls was qualitatively indistinguishable from normal pairs and was quantitatively similar as well (see Table). Thus, the importance of other stimuli that might involve the male forewings or intact female antennae can be ruled out. For example, mechanical stimulation of the female antennae by the scent scales is not a likely stimulus because females with only small antennal stumps which cannot reach the glands nonetheless behave normally.

These results clearly demonstrate that the wing glands of the male Indian meal moth release a sex pheromone that is essential for successful courtship. Its function is to induce the female to remain stationary and adopt the acceptance posture. The absence of the male sex pheromone leads to a specific pattern of female rejection behaviour rather than avoidance by flight as apparently occurs in other moths[6,7].

Zusammenfassung. Die Flügeldrüsen der Männchen von *Plodia interpunctella* sondern ein Sex-Pheromon aus, das für den erfolgreichen Vollzug des Balzrituals notwendig ist. Das Pheromon bewirkt, dass das Weibchen nicht flieht und die zur Auslösung der Kopulation notwendige Bereitschaftsstellung einnimmt.

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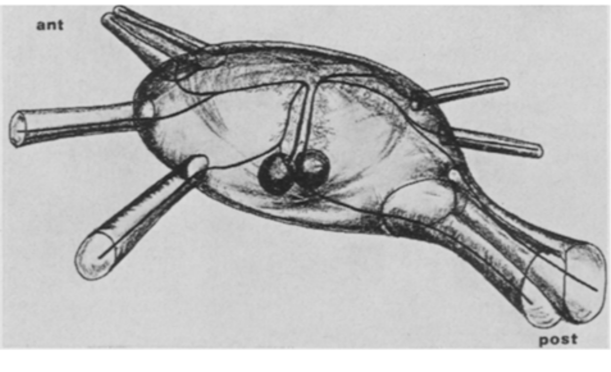
Neuronal Geometry of RETZIUS Cells in *Hirudo medicinalis*

The Retzius cells, the two largest cells in the segmental ganglia of the leech, *Hirudo medicinalis*, have been the subject of many electrophysiological and pharmacological studies[1-5]. It has been suggested that the axons of these cells emerge only from the lateral segmental nerves and are responsible for mediating mucus release from the body wall[6,7]. Lent[6], using an intracellular Procion yellow staining technique, could not find any fibres from the Retzius cells in the intersegmental connectives. This was supported by the observation that high Mg^{2+} ringer blocked excitation of the cell bodies elicited by stimulation of the connectives, suggesting the presence of a synaptic input[8]. In contrast, the original results of Retzius, using methylene blue staining, show a fibre from each cell in the posterior connectives[1].



A 3-D reconstruction of the neuronal geometry of the paired Retzius cells in the segmental ganglion of the leech, *Hirudo medicinalis*. Each Retzius cell sends its major branches ipsilaterally, one down each of the paired lateral nerves and one down both posterior and anterior ipsilateral connectives.

In the present investigation the neuronal geometry has been studied using the technique of Pitman, Tweedle and Cohen[9] which enables specific neurones to be stained black with cobalt sulphide. The electrophysiology of the cells, with special reference to their behaviour in high Mg^{2+} ringer, has confirmed these results.

Materials and methods. Both staining and electrophysiological techniques were carried out using intracellular microelectrodes made from 4 mm O.D. glass tubing.

For the staining method, electrodes were filled with 1.25 M cobalt chloride and had a resistance of 5–20 MΩ. Cobalt was injected into the cells by applying an 18V hyperpolarizing clamp. The injected preparation was bathed in a dilute solution of ammonium sulphide in ringer, whereupon the cobalt sulphide precipitated as a black stain. Preparations were fixed in buffered paraldehyde/glutaraldehyde, and mounted in creosote.

For the electrophysiological studies, microelectrodes were filled with 1 M potassium acetate (buffered to to pH 6.5) and had a resistance of 20–40 MΩ. Potentials were monitored using a conventional cathode follower and bridge circuit, and displayed on an oscilloscope or pen

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[1] S. Retzius, *Biologische Untersuchungen*, Neue Folge II. (1891), p. 13.

[2] S. Hagiwara and H. Morita, J. Neurophys. *25*, 721 (1967).

[3] V. D. Gerasimov, Symp. on Neurobiol. of Inverts (1962), p. 285.

[4] C. A. Marsden and G. A. Kerkut, Comp. Biochem. Physiol. *31*, 851 (1969).

[5] R. J. Walker and P. A. Smith, Comp. Biochem. Physiol. *45* A, 979 (1973).

[6] C. M. Lent, Comp. Biochem. Physiol. *44* A, 35 (1973).

[7] C. M. Lent, Science *179*, 693 (1973).

[8] C. M. Lent, Comp. Biochem. Physiol. *42* A, 857 (1972).

[9] R. M. Pitman, C. D. Tweedle and M. J. Cohen, Science *176*, 412 (1972).