tradecen-1-ol acetate, although each elicited a response above 1×10^{-12} M. Because the emission concentration of the other components is lower than 1×10^{-12}, it is not likely that the NS(a) neuron can detect naturally-emitted concentrations of (*Z*)-7-dodecen-1-ol acetate or any of the other pheromone components.

Consideration of the sensitivity and the qualitative selectivity of the three known pheromone-sensitive neurons on the *T. ni* antenna leads to the question of what compounds comprise the effective pheromone blend perceived by a male *T. ni.* From one perspective, the 'pheromone' may comprise any compound identifiable in the effluent of a virgin calling female which enhances the sexual response. From another, however, the pheromone may comprise only those components detectable by receptor neurons at physiological concentrations. Ideally, these two perspectives should predict the same pheromone blend, but at this time, they yield incomplete answers for *T. ni.* Fourteen compounds have been identified from virgin female cabbage looper sex pheromone gland extracts and seven from their volatile emissions[17]. Wind tunnel bioassays suggest that the seven emitted components affect behavior[8]. Thus, by behavioral criteria, the *T. ni* pheromone is comprised of several components. Electrophysiological methods have revealed the presence of three, highly selective neurons that have the requisite sensitivity to detect naturally-emitted concentrations of three of the pheromone components. No sensilla have been found with the requisite sensitivity for (*Z*)-5-dodecen-1-ol acetate, dodecan-1-ol acetate and 11-dodecen-1-ol acetate. Thus, the electrophysiological evidence implicates three components of the volatile emission in intraspecific chemical communication. The eventual resolution of the differences between the neurobiological record and the behavioral record is an intriguing problem.

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1 Hildebrand, J. G., Matsumoto, S. G., Camazine, S. M., Tolbert, L. P., Blank, S., Ferguson, H., and Ecker, V., in: Insect Neurobiology and Pesticide Action (Neurotox 79), p. 375. Ed. F. E. Rickett. Society for Chemical Industry, London 1980.
2 Christensen, T. A., and Hildebrand, J. G., in: Arthropod Brain: Its Evolution, Development, Structure, and Functions, p. 457. Ed. A. P. Gupta. John Wiley & Sons Inc., New York 1987.
3 Mayer, M. S., and Mankin, R. W., Comprehensive Insect Physiology Biochemistry and Pharmacology, p. 95. Eds G. A. Kerkut and L. I. Gilbert. Pergamon Press, Oxford 1985.
4 O'Connell, R. J., Grant, A. J., Mayer, M. S., and Mankin, R. W., Science *220* (1983) 1408.
5 Grant, A. J., and O'Connell, R. J., J. Insect Physiol. *32* (1986) 503.
6 Grant, A. J., O'Connell, R. J., and Hammond, A. M. Jr, J. Insect Behav. *1* (1988) 75.
7 Van Der Pers, J. N. C., Ent. exp. appl. *31* (1982) 255.
8 Linn, C. E. Jr, Bjostad, L. B., Du, J. W., and Roelofs, W. L., J. chem. Ecol. *10* (1984) 1635.
9 Linn, C. E. Jr, Campbell, M. G., and Roelofs, W. L., J. chem. Ecol. *12* (1986) 659.
10 Linn, C. E. Jr, Hammond, A., Du, J., and Roelofs, W. L., J. chem. Ecol. *14* (1988) 47.
11 Bjostad, L. M., Linn, C., Roelofs, W. L., and Du, J.-W., in: Proc. Am. chem. Soc. Symp., p. 223. Eds T. E. Acree and D. M. Soderlund. De Gruyter, Berlin 1985.
12 Grant, A. J., Mankin, R. W., and Mayer, M. S., Chem. Senses *14* (1989) 499.
13 Mankin, R. W., Grant, A. J., and Mayer, M. S., J. Neurosci. Meth. *20* (1987) 307.
14 Mayer, M. S., Mankin, R. W., and Grant, A. J., J. chem. Ecol. *13* (1987) 509.
15 Kaissling, K.-E., R. H. Wright Lectures on Insect Olfaction, p. 22. Ed. K. Colbow. Simon Fraser Univ., Burnaby 1987.
16 Mayer, M. S., and Mankin, R. W., unpublished.
17 Bjostad, L. B., Linn, C. E., Du, J.-W., and Roelofs, W. L., J. chem. Ecol. *10* (1984) 1309.

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Suboesophageal DUM neurones are part of the antennal motor system of locusts and crickets

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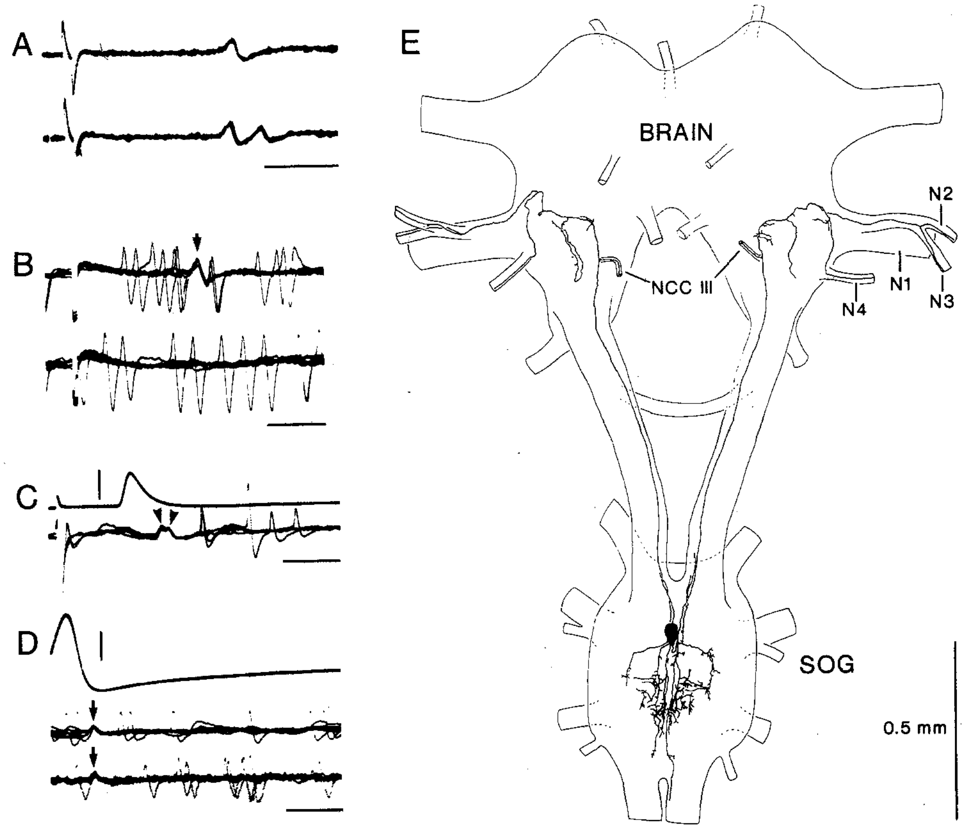
Summary. The muscles which move the antennae of locusts and crickets are innervated by motoneurones of the deutocerebral part of the brain. In addition, these muscles receive axon collaterals of two dorsal, unpaired, median (DUM) neurones which are located in the suboesophageal ganglion. These DUM neurones also send axons towards the retrocerebral glandular complex.

Key words. Insects; antennae; nervous system; suboesophageal ganglion; DUM neurones.

The dorsal, unpaired, median (DUM) neurons of insects have received much attention from invertebrate neurobiologists because of their unique morphological, physiological and developmental properties. In contrast to the great majority of insect neurones, they derive from unpaired precursor cells of the neuroectoderm, develop into bilaterally-symmetrical neurones[1], and have spiking somata[2-4]. DUM neurones have been shown to modulate neuromuscular transmission[5,6], and it appears that each insect neuromuscular system is supplied by at least one



A Stimulation of antennal motor nerve 2 on one side causes one (upper trace) or two (lower trace) phase-locked spikes recorded from contralateral nerve 2 (5 sweeps pretriggered by stimulus; stimulus intensity 6 V in upper, 7 V in lower trace; time scale 5 ms); *B* Upon stimulation of antennal motor nerve 4 on one side a compound spike (arrow) appears in corresponding contralateral nerve with constant latency (upper trace). Cutting one of the circumoesophageal connectives abolishes this response (lower trace), 10 sweeps each, time scale 10 ms; *C* Stimulation of antennal motor nerve 4 causes attenuated spikes in the cell body of a suboesophageal DUM neurone (upper trace), and two axon spikes (arrowheads) in the contralateral nerve 4 (lower trace), 5 sweeps, time scale: 10 ms, vert. cal. 20 mV; *D* When the neurone from (*C*) is slightly depolarized (1 nA current injected) it produces large amplitude soma spikes of long duration (upper trace) which are followed by axon spikes (arrows) in both right (middle trace) and left (lower trace) antennal nerves 4 (5 sweeps, calibrations as in (*C*); *E* Camera lucida drawing of an antennal DUM neurone as revealed by intracellular injection of cobalt hexammine chloride and subsequent wholemount silver intensification. SOG, suboesophageal ganglion; N1–N4, antennal nerves 1–4; NCC III, Nervus corporis cardiaci III ((*A*) and (*E*) are from cricket, *B–D* are from locust preparations, but all experiments were carried out in both species).

DUM neurone[7]. With few exceptions[8, 9], the motor systems of the insect thorax and abdomen are innervated by segmental DUM neurones. As will be shown here, the muscles moving the antennae of locusts and crickets are innervated by intersegmentally-projecting DUM neurones with somata in the suboesophageal ganglion.

In both locusts and crickets there is one major antennal nerve (N1 in fig., E) which innervates flagellum and pedicel, and 2 (locust[10]) or 3 (cricket; N2–N4 in fig., E) smaller nerves, which innervate the muscles moving scape and pedicel (and some scapal proprioceptors). Cobalt backfills of these smaller antennal nerves showed that all motor neurones of the antennal muscles reside in the deutocerebral neuromere of the brain[11–13]. No DUM neurones appeared in these preparations, but they are generally difficult to stain because of their small axons[14]. We therefore searched for such neurones using electrophysiological techniques.

When stimulating a particular antennal motor nerve on one side of the nervous system, we recorded one (fig., A; B) or two spikes (fig., A; C) from the corresponding contralateral nerve, as would be expected when bilaterally-symmetrical neurones are present. In most experiments it was impossible to clearly separate two spikes, since they had nearly identical thresholds and conduction velocities. Contralateral spikes could only be observed in preparations where the suboesophageal ganglion was still attached to the brain. Cutting one of the circumoesophageal connectives abolished these spikes (fig., B). Next we impaled cell bodies along the dorsal midline of the suboesophageal ganglion with microelectrodes. Some of these cell bodies responded to stimulation of antennal motor nerves with spikes of constant latency (fig., C). When depolarized, these cell bodies produced soma spikes which were followed in a one-to-one fashion by axon spikes recorded from corresponding antennal nerves on both sides (fig., D). Finally, staining these neurones individually by intracellular dye injection revealed their typical DUM neurone structure (dorsal, median position of soma, bifurcating neurite, bilateral symmetry; fig., E). All neurones stained (locust: 11; cricket: 6) had, in principle, the same morphology. In one exceptionally good fill-preparation of a locust neurone, an axon collateral could be traced as far as its terminals on one of the antennal muscles.

A striking feature common to both locust and cricket antennal DUM neurones is that they send axon collaterals not only into each antennal motor nerve, but also into the 'Nervus corporis cardiaci III' (NCC III). So far these collaterals could only be followed into the main trunk of this nerve, where staining faded. Work is in progress to investigate the complex peripheral branching pattern of the NCC III in the locust, and to define the targets of the DUM neurone collaterals in this nerve. In other insect species, the nerve has been shown to innervate the retrocerebral glandular complex, the pharyngeal dilator muscles, and the antennal heart[15,16].

Another conspicuous feature of the antennal DUM neurones is that most of their dendritic ramifications are found in the suboesophageal ganglion, and only a few within the brain (fig., E). This suggests that their activity is mostly determined by integrative processes within the suboesophageal ganglion. There is increasing evidence that this ganglion participates in the coordination of locomotory activity during flight[17] and walking[18]. The antennae participate actively in both behaviours: during flight they act as wind gauges[19], during walking they probe the substrate by movements correlated with the walking rhythm. It is possible that suboesophageal neurones influence the antennal motor system via the activation of the antennal DUM neurones, provided that these neurones also modulate neuromuscular transmission as do other DUM neurones.

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1 Goodman, C. S., and Spitzer, N. C., Nature *280* (1979) 208.
2 Crossman, A. R., Kerkut, G. A., Pitman, R. M., and Walker, J., Comp. Biochem. Physiol. *40A* (1971) 579.
3 Hoyle, G., and Dagan, D., J. Neurobiol. *9* (1978) 59.
4 Heitler, W. J., and Goodman, C. S., J. exp. Biol. *76* (1978) 63.
5 Evans, P. D., and O'Shea, M., Nature *270* (1977) 257.
6 Brookes, S. J. H., J. exp. Biol. *136* (1988) 333.
7 Brookes, S. J. H., and Weevers, R. de G., J. exp. Biol. *136* (1988) 311.
8 Pflüger, H.-J., and Watson, A. H. D., J. comp. Neurol. *268* (1988) 329.
9 Bräunig, P., J. Comp. Physiol. A *163* (1988) 835.
10 Gewecke, M., Z. Morph. Tiere *71* (1972) 128.
11 Klepsch, U. E., Allgäuer, C., and Honegger, H.-W., in: New Frontiers in Brain Research. Proc. 15th Göttingen Neurobiol. Conf., p. 74. Eds N. Elsner and O. Creutzfeldt. Thieme, Stuttgart 1987.
12 Honegger, H.-W., Allgäuer, C., Klepsch, U. E., and Welker, J., J. comp. Neurol. (1990) in press.
13 Bauer, C. K., in: New Frontiers in Brain Research. Proc. 15th Göttingen Neurobiol. Conf., p. 77. Eds N. Elsner and O. Creutzfeldt. Thieme, Stuttgart 1987.
14 Hoyle, G., J. Neurobiol. *9* (1978) 43.
15 Copenhaver, P. F., and Truman, J. W., J. comp. Neurol. *249* (1986) 186.
16 Pass, G., Agricola, H., Birkenbeil, H., and Penzlin, H., Cell Tissue Res. *253* (1988) 319.
17 Ramirez, J. M., J. comp. Physiol. A *162* (1988) 669.
18 Kien, J., and Altman, J. S., J. Insect Physiol. *30* (1984) 59.
19 Gewecke, M., and Heinzel, H. G., J. comp. Physiol. *139* (1980) 357.

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Role of endopeptidase in motility induction in apyrene silkworm spermatozoa; micropore formation in the flagellar membrane

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Summary. Immotile apyrene spermatozoa of the silkworm have a thick electron-dense flagellar membrane. On activation of apyrene spermatozoa by initiatorin, a prostatic endopeptidase of the silkworm, or by trypsin, the flagellar membrane becomes thinner, with the formation of many micropores. It then resembles that of motile apyrene spermatozoa in vivo.

Key words. Apyrene spermatozoa; sperm motility; micropore formation; endopeptidase; initiatorin; *Bombyx mori.*

The flagellar membrane of insect spermatozoa is not a classical unit membrane, but has a complicated ultrastructure[1]. Lepidoptera produce both anucleated apyrene spermatozoa, and nucleated eupyrene spermatozoa that fertilize eggs[2]. In the silkworm, *Bombyx mori,* a specific energy-yielding system for spermatozoa, an arginine degradation cascade coupled with protein degradation[3-6] and extracellular glycolysis[3,7] is present in the spermatophore. At the time of ejaculation, initiatorin, an endopeptidase of the serine protease type secreted from the glandula (g.) prostatica, triggers these reactions[8,9] and the induction of motility of apyrene spermatozoa[7,8,10]. Thus the spermatophore formed in the female bursa copulatrix[11] is the site of sperm maturation as well as being a reactor, and the apyrene sperm stir its viscous contents to promote the dissociation of eupyrene bundles[3,8].