and uranyl acetate and examined in a Phillips 301 electron microscope.

Results and discussion. The coupling between postsynaptic neurones of the dopamine neurone is illustrated in figure 1. Hyperpolarizing and depolarizing current pulses in any one neurone are transmitted to the others. Spikes produce small transmitted potentials (epsps) which may reach threshold individually or more usually by summation. Coupling coefficients for different pairs of neurones were very variable, ranging from nearly 0 up to 0.5 (coupling coefficient is the post-/presynaptic potential[7]). The strength of coupling does not depend on the proximity of the somata. There is little change in the electrotonic epsps when the ganglia are bathed in saline containing no Ca, 6 times normal Mg and 1 mM EGTA; this indicates the absence of any chemical component.

During ipsps produced by stimulating the dopamine neurone the strength of coupling between postsynaptic neurones is considerably reduced. The addition of dopamine (10^{-5} M) to the bath produces the same effect. This is similar to the synaptic decoupling first described in Navanax by Spira and Bennett[8]; the decoupling is thought to be important in enabling the neurones to fire independently of each other[8–10]. It is not known whether decoupling has a similar function in Planorbis.

The postsynaptic neurones often fire spontaneously in couplets of spikes. Maintained depolarization of one of the neurones usually results in longer bursts. Burst formation is a property of a number of electrically coupled groups of neurones and may result from the intrinsic properties of non-rectifying electrotonic synapses rather than endogenous bursting activity of individual neurones[11–14]. For example, Getting and Willows[11,12] have shown that bursts in electrically coupled neurones in Tritonia develop by regenerative excitation within the network and are terminated when a majority of neurones fire in near synchrony; synchronization results in a net hyperpolarization caused by an accentuation and prolongation of the spike after-hyperpolarization due to a reduction in junctional shunting. In Planorbis burst termination is almost always accompanied by the synchronous firing of another recorded neurone in the network (figure 1) suggesting that a similar mechanism is operating.

Electron microscopic examination of the visceral ganglion revealed occasional areas of close apposition of neuronal membranes in the neuropile (figure 2). At high magnification the outer stained layer of each apposing membrane appeared to touch. By analogy with data in other systems[15,16] these points of membrane apposition were considered to be morphological counterparts of electrotonic coupling. They were not observed between neurone somata or in nerve tracts, but it was not possible to determine whether they were axodendritic, axoaxonic or dendrodendritic.

The results indicate that electrotonic coupling between postsynaptic neurones of the dopamine neurone occurs at specialized areas of apposition in the neuropile. The coupling synchronizes the firing of a proportion of the neurones, and may be important for the generation of burst activity.

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Electrophysiological evidence for chemosensitivity to adenosine, adenine and sugars in Spodoptera exempta and related species

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Summary. Electrophysiological studies show that Spodoptera exempta and closely related species possess a receptor with specific sensitivity towards adenosine and adenine. 2 other types of receptors responded to certain sugars. The functional significance of these receptors in controlling chemoresponses of the larvae is discussed.

Adenosine and sucrose, isolated from maize plants, have recently been identified as strong phagostimulants for the African armyworm, Spodoptera exempta[3]. This harmful Noctuid feeds exclusively on grasses (Gramineae, Cyperaceae)[4]. Other studies on this species indicated that the maxillary styloconic sensilla play an important part in the food discriminative behaviour[5,6]. Evidence for the function of the styloconic sensilla in determining the chemosensitivity of S. exempta to adenosine and sugars is presented in this paper.

Materials and methods. Diet-reared 1–2 days old last-instar larvae were used throughout. For electrophysiological recording the tip-recording technique[7] was employed with conventional methods of amplification and registration of signals. A receptor 'response' is defined here as the number of impulses generated in 1 sec beginning 50 msec after the onset of stimulation.

Results and discussion. Adenosine evoked slowly adapting trains of action potentials from a receptor located in the lateral sensilla of S. exempta (figures 1 and 2). Sodium chloride, which had been added to the stimulus solution at 0.1 M in order to improve the conductance properties in the stimulating-recording pipette, elicited an essentially different response pattern in these sensilla (figures 1 and 4). The concentration-response relation of the adenosine-sensitive receptor (figure 3) indicated a sensitivity threshold between 10^{-4} and 10^{-5} M, while saturation was reached at about 10^{-2} M adenosine (figures 2 and 3). The

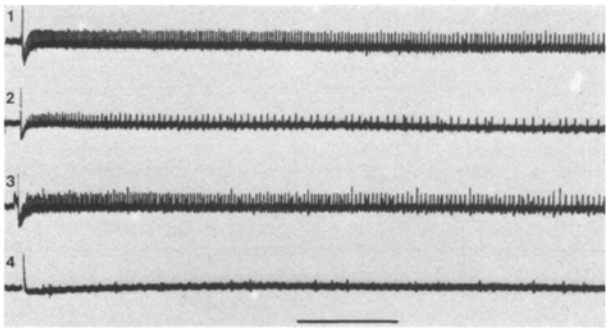

Fig. 1. Responses of a lateral sensillum styloconicum of S. exempta to: *1* 4.0 mM sucrose; *2* 2.0 mM adenosine; *3* mixture of 4.0 mM sucrose and 2.0 mM adenosine; and *4* 100 mM NaCl. Horizontal bar equals 250 msec.

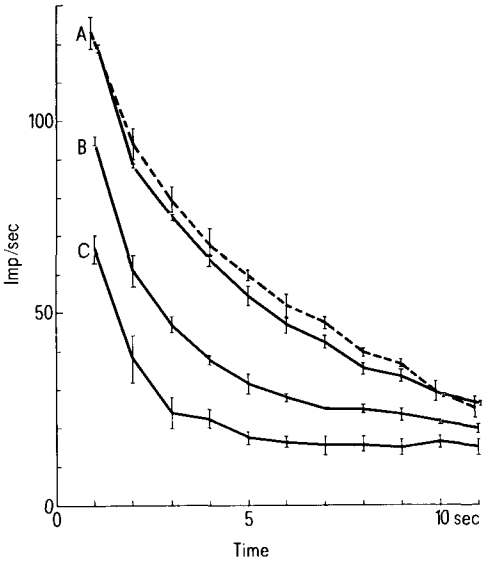

Fig. 2. Time course of adaptation of the adenosine-sensitive receptor. *A*, broken line, 30 mM; *A*, solid line, 10 mM; *B* 3 mM; *C* 1 mM. Average values with the extremes (vertical bars) of 3 series of stimulations are shown.

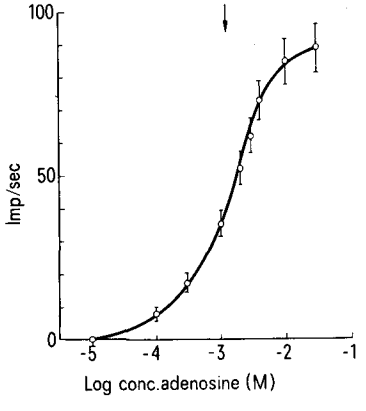

Fig. 3. The dependence of the response of the adenosine sensitive receptor upon the stimulus concentration. Average values ± 2. SE (n = 7). Arrowhead indicates approximate concentration of adenosine in fresh maize leaf.

reaction spectrum of the adenosine-sensitive receptor appeared as rather restricted: apart from adenine no other purine or pyrimidine compound or derivative, including nucleotides and nucleosides, provided effective stimuli. Caffeine was anomalous in that it excited several cells simultaneously and eventually seemed to damage their receptor functions[8].

The lower receptor sensitivity to adenine as compared to adenosine (table) agrees with the behaviour observation that the feeding activity induced by adenine is only 63% of the feeding caused by adenosine[3]. Furthermore, the finding that only adenine and adenosine, but not other purine or pyrimidine compounds, are able to elicit a feeding response in the larvae[3] is in agreement with the reaction spectrum of the adenosine-sensitive receptor. The addition of a ribose group to the N6 position of the adenine molecule apparently greatly enhances its effectiveness as a stimulus. D-ribose itself, however, failed to excite any receptor cell at all in the lateral sensilla, but

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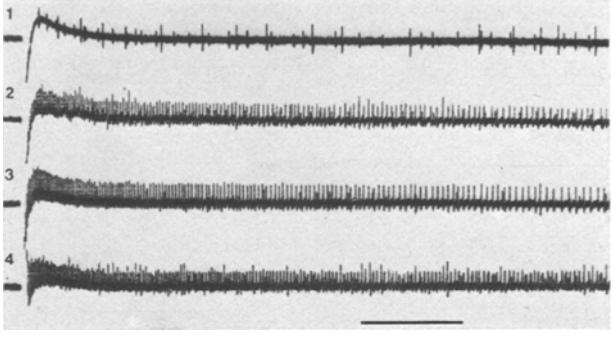

Fig. 4. Responses of a lateral sensillum to: *1* 500 mM NaCl; *2* maize leaf extract, diluted 1.5 times with distilled water; *3* 5.0 mM sucrose; and *4* maize leaf extract, undiluted.

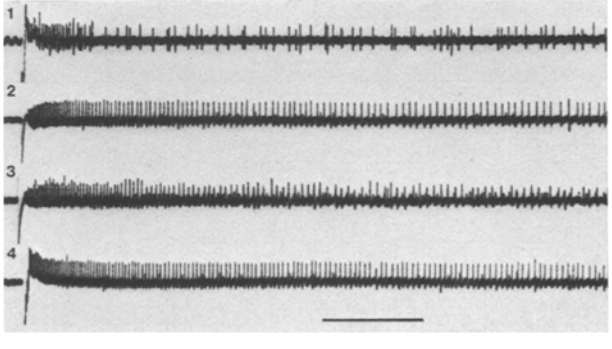

Fig. 5. Responses of a medial sensillum to: *1* 500 mM NaCl; *2* 100 mM m-inositol; *3* maize leaf extract, diluted 1.5 times; and *4* 100 mM D-ribose.

Average (± SD) number of impulses in the first sec of counting (50 msec after onset of stimulation) generated by a receptor cell in S. exempta larvae. Stimulation with 4 purine compounds (0.03 M) and 3 sugars (0.1 M)

Lateral sensilla (N = 6)			
Adenosine	Adenine	Guanosine	Hypoxanthin
84.2 ± 39.4	50.6 ± 24.4	0.0 ± 0.0	0.0 ± 0.0
Medial sensilla (N = 12)			
D-ribose	Sucrose	m-Inositol	
36.9 ± 17.0	21.2 ± 14.3	35.5 ± 16.3	

instead stimulated a neuron in the medial sensilla (figure 5). This cell was also very sensitive to sucrose and meso-inositol (table). However, in contrast to sucrose, neither D-ribose nor meso-inositol or any other sugar acts as a strong phagostimulant for S. exempta[3,5]. A possible explanation for this apparent discrepancy is that the larval feeding reactions to sugars are not primarily governed by signals from this particular receptor type, but rather by inputs from other sugar-sensitive neurons. This argument is supported by the observation that another type of sugar-sensitive receptor showing a specific sensitivity towards sucrose is located in the lateral sensilla (figures 1 and 5). It remains, possible that the sugar-sensitive neuron in the medial sensilla participates in the total gustation coding via a subtle across-sensillum patterning, which, however, remains undetectable with the currently employed behaviour methods[3,5]. Experiments comparing the response patterns of the lateral sensilla to sucrose and adenosine showed that either 1 or 2 cells fired depending on whether the compounds were applied singly or in a mixture (figure 1). Thus, 2 separate receptor types are inferred, one sensitive to sucrose and the other to adenosine. Both receptors are sufficiently sensitive to monitor the amounts of sucrose and adenosine as they occur in the natural food[3]. In fact, the aqueous extract of fresh maize leaves, from which adenosine and sucrose were isolated at 2 and 6 mM/1000 g (fresh wt) respectively[3], elicited vigorous response patterns in both the medial and lateral sensilla (figures 4 and 5). If one considers the limited number of 4 chemosensory neurons in each sensillum[5] it seems reasonable to suggest that part if not most of the recorded impulse activity originated in the adenosine- and sugar-sensitive neurons.

Experiments with S. frugiperda, S. littoralis and S. litura have shown that other species of the genus Spodoptera possess a similar arrangement of chemoreceptor cells as described above for S. exempta. Although S. exempta is the only species with an oligophagous behaviour, it is interesting to note that grasses have been recorded as part of the food plant range for all these Noctuids[4].

A comparative study of the differentiation of dissociated nerve cells under different culture conditions

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Summary. In vitro differentiation of chick embryo brain cells was compared under several culture conditions. Morphological observations and acetylcholinesterase histochemical staining revealed that the development was similar in all conditions tested if cells have been derived from 7 days embryos. Considering the cultures from 11 days embryos, the cell dissociation by trypsin and the plastic surface proved to be the most favourable conditions in contrast to mechanical dissection and collagen surface.

Several studies have suggested that primary cultures of dissociated nervous tissues are very good model systems to investigate morphological and biochemical aspects of nerve and glial cell differentiation [3-6]. However, culture conditions can modify the pattern of the differentiation process[7-9]. Therefore, it was considered to be worthwhile to study the stages of differentiation as a function of 1. the age of the embryo yielding the brain cells, 2. the dissociation method of the brain tissue and 3. the surface substrate on which the cells were cultivated. In the present investigation, we have undertaken such a comparative study of the morphological differentiation, combined with the presence of acetylcholinesterase (AChE) activities in chick embryonic brain cells.

Materials and methods. Chick embryos of either 7 or 11 days of age were used for cell preparation. The cells were prepared according to one of the 2 following methods. In the first group of experiments, the cerebral hemispheres were passed through a nylon sieve of 48 µm pore size to dissociate cells, as was described before[8,10]. These cultures were compared to those obtained by stepwise trypsin digestion using 0.05% of trypsin (1 : 250) dissolved in Ca-Mg free BSS (balanced salt solution) supplemented with 0.3% of glucose. 3 times 5 min of incubation at 37 °C combined with gentle pipetting was enough to dissociate the tissue pieces. All the steps were stopped by adding ice-cold serum to the samples. The cells were collected by 5 min centrifugation at 250 g ×g.

Each experimental group was then subdivided again for using one half on a plastic surface and the other half on a surface previously coated by 1% collagen[11]. The different

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