phase, were incubated with dibenzofuran or dibenzo-p-dioxin metabolic products accumulated in the medium. They were extracted, purified by HPLC, and identified by GC-MS and

NMR analysis. The major products formed are shown in Fig. 2. In contrast to the cleavage of the oxygen-containing heterocycles, dibenzothiophen was converted by *Pseudo-*

monas HH69 to the sulfoxide and the sulfone (Fig. 2). The elucidation of the initial reactions of the degradative pathways of dibenzofuran and dibenzo-p-dioxin is in progress.

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Fig. 2. Products formed from dibenzofuran (A), dibenzo-p-dioxin (B), and dibenzothiophen (C) by *Pseudomonas* HH69. Washed cells, pregrown in peptone medium, were incubated in phosphate buffer pH 7.2. Dibenzofuran, dibenzo-p-dioxin, or dibenzothiophen were directly added to the cell suspension which was incubated in an Erlenmeyer flask with baffles on a rotary shaker at 28 °C. Excretion of polar products was monitored by the use of reversed-phase HPLC. The incubation was terminated when the rate of product formation decreased. The cell-free medium was extracted with ethylacetate at neutral pH and reextracted after acidification at pH 2. After evaporation of the solvent the residue was dissolved in methanol and separated by preparative HPLC on a C-18 column using an acidified (pH 2) methanol/water mixture (60:40). Fractions obtained from this procedure were subjected to GC-MS and NMR studies. Gas chromatographic separations were carried out on a 30-m fused-silica column with OV-1 as a stationary phase using a temperature program (80 °C/5 °C). Identifications are based on comparison of 70-eV mass spectra obtained with a VG 70-250 SE GC/MS system. NMR data were obtained with a Bruker WM 400 at 400 MHz. Synthetic samples served as references

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GABA-like Immunoreactivity Suggests an Inhibitory Function of the Thoracic Low-frequency Neuron (TN1) in Acridid Grasshoppers

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In the auditory system of acridid grasshoppers about 40 auditory interneurons have been morphologically and physiologically identified [1–5]. Behavioral tests after surgical interferences on *Chorthippus biguttulus* have shown that the metathoracic ganglion plays an important role in pre-processing auditory information [6]. Our knowledge about the interconnections between metathoracic neurons,

however, is scarce. Double recordings demonstrated an excitatory connection from the BSN1 to the AN1 and TN3 and a – not necessarily monosynaptic – inhibitory influence of SN1 on AN1 in *Locusta migratoria* [4]. Beyond this, only speculations about interactions between auditory interneurons are possible; such speculations are based on the comparison of threshold curves [7], or on the overlapping of dendritic areas

in the frontal neuropil and a comparison of latencies [8] (see also [9]).

Many of the auditory interneurons ascending from the metathoracic ganglion show an intensity-dependent inhibition, starting at 10–20 dB above threshold, and, in several cases, converting tonic into phasic reactions [4, 5]. One question of interest is how this inhibition is achieved: Is it produced by other interneurons or – less likely – do the receptor cells themselves have an inhibitory influence on interneurons (cf. [4, 10])? An interneuron that would be especially suited to exert such inhibitory effects is the TN1 (in the locust: [3] synonymous with thoracic low-frequency neuron, [1]; in *Ch. biguttulus*: [5]). This cell reacts tonically, in a very similar way as low-frequency receptors, and its threshold curve is nearly identical to that of type-1 receptors in the locust [7]. The very same

Naturwissenschaften 76 (1989) © Springer-Verlag 1989

223

threshold curve is also found with the inhibitory postsynaptic potentials (IPSP's) of another interneuron, the AN3 (Fig. 3 in [7]). This suggests an inhibitory connection from TN1 on AN3 (see also [9]). The inhibition of AN3, as well as of other ascending neurons, can be suppressed by applying small amounts of picrotoxin to the ganglion [10]. Since in insects GABA (gamma aminobutyric acid) is known as an inhibitory transmitter that can be blocked by picrotoxin [11], it seemed worthwhile to test whether GABA-like immunoreactivity is found in the TN1-neuron.

A considerable part of the above-mentioned results were obtained with *Locusta migratoria*. In this study, in addition to *L. migratoria*, we used *Chorthippus biguttulus*, an indigenous acridid, whose auditory system has gained interest in the context of acoustic communication [12]. There is little doubt that the TN1-neuron, as well as other auditory interneurons identified in *Locusta*, possess homologues in *Ch. biguttulus*, and the thoracic auditory pathway is organized similarly in both species [5, 8, 13].

The first step of the procedure consisted of intracellular recordings from TN1-neurons, which were identified on the basis of their physiological properties and stained by injection of Lucifer Yellow. The metathoracic ganglia were then prepared using conventional methods [13] for drawing the neuron in a whole-mount preparation (with a Zeiss fluorescence microscope). Ganglia containing stainings of TN1 were then embedded in paraffin wax and cut in serial sections (8 μm). The somata (Fig. 1) and other typical structures of the TN1 were drawn from the sections and photographed (Kodachrome 64). For immunocytochemical staining we used the indirect peroxidase-antiperoxidase (PAP) technique, slightly modified according to [14] (cf. also [15]). The sections were dewaxed with xylene and rehydrated. To reduce unspecific staining, the slides were covered for 5 min with H_2O_2 (3 %) and afterwards for 30 min with 1 M ethanolamine. After 30 min preincubation in 10 % NGS (normal goat serum, in tris-buffer, 0.1 M + 0.3 M NaCl), the sections were incubated at 4 °C in a moist chamber with GABA-antiserum (raised in rabbits by Immunotech, Mar-

seille) diluted 1:500. They were then washed in tris-buffer, incubated with the secondary antibody (GAR = goat-anti-rabbit, diluted 1:40, 1 h at room temperature), and thereafter with rabbit-PAP (rabbit-peroxidase-antiperoxidase, diluted 1:100, 1 h at room temperature). Afterwards, the sections were treated with 3,3'-diaminobenzidine (DAB, 30 mg per 100 ml + 45 μl 30 % H_2O_2), dehydrated, cleared in xylene and mounted in canadabalsam or fluoromount. As a control, some sections were processed without the primary antibody, which prevented specific labeling. Although the intensity of the PAP staining was variable in different preparations, the TN1-soma was clearly stained by the GABA-antibody in all preparations ($n=8$, two examples are shown in Fig. 1). To demonstrate the identity of the cells filled with

Lucifer and afterwards stained with GABA-antibody, slides of the two stainings (in the same serial section) were superimposed on a screen.

The results shown in Fig. 1 unequivocally demonstrate GABA-like immunoreactivity in the TN1 of both *Ch. biguttulus* and *L. migratoria*, and are thus further evidence of an inhibitory function of the TN1-neuron. The TN1 is an interneuron which receives excitatory input from low-frequency receptors of both ears [4] and is about 15–20 dB less sensitive than the most sensitive receptors (type 2) and interneurons [4, 5]; furthermore TN1 shows extensive branching in the frontal auditory neuropil with a major output function on both sides of the ganglion [9]. Accordingly, the TN1 may be sufficient to explain the intensity-dependent inhibition of many ascending interneurons (see

Fig. 1. The auditory TN1-neurons of *Ch. biguttulus* (a) and of *L. migratoria* (b) show GABA-like immunoreactivity. Transverse sections of the metathoracic ganglia. Left: Lucifer-filled cell body of TN1 in epifluorescence illumination, right: same sections as on the left, after application of the PAP-staining procedure. Cell bodies were identified on the basis of their location and shape. Although some other cell bodies show a more intense staining, the TN1 cell bodies are clearly stained in both preparations (arrowheads). Scale bars 50 μm

above and [5]). Thus, there is no need to postulate direct inhibition from receptor cells.

On the contrary, direction-dependent inhibition in two identified interneurons (AN1, AN2) seems to be caused mainly by high-frequency units [16]. In behavioral experiments with males of *Ch. biguttulus*, however, the accuracy of the lateralization behavior tested with low-frequency bands (4–8 kHz) was not lower than with broader signals [17].

The demonstration of GABA-like immunoreactivity in the TN1-neurons of two grasshopper species provides further support for the assumption that the TN1-neurons are homologous in both species, and indicates a far-reaching similarity in the organization of the auditory pathways within the ventral nerve cord of these grasshoppers.

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Social Stress and Activity of the Immune System in Guinea Pigs

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Changes in the social environment are frequently associated with alterations in the behavior and physiological states of mammals. Stress reactions occur in the neuroendocrine as well as in the immune system [1]. So far, only a few studies indicate that immune competence – and in particular the activity of complement – can be modulated by psychological stress [2]. Complement, a multimolecular system of plasma proteins, acts on its own and in cooperation with other parts of the immune system in vertebrate defense reactions. Inactive complement proteins are sequentially activated in an amplifying reaction cascade by the classical pathway, which is triggered by antibodies. However, in the absence of antibodies,

the alternative pathway of complement can be activated by lipopolysaccharides from, for example, the bacterial cell membrane. The alternative pathway therefore provides the organism with a first line of defense against infections before the immune response is fully activated [3].

This study was undertaken to explore the possible influences of social behavior on the activity of the alternative pathway of complement. The social relations and behavior of guinea pigs (*Cavia aperea f. porcellus*) have been carefully investigated and can be reliably assessed by behavioral observation [4, 5].

Thirty-two guinea pigs (16 males, 16 females) were kept in eight groups,

each enclosure (1.35 × 0.95 m) containing one subadult male (8 months), one adult male (about 1 year), and two females. In particular, the agonistic and courtship behavior of each individual was evaluated by behavioral observation. Each group was observed over a period of 12 weeks, twice weekly at 60–120-min intervals between 8:00 and 10:00 a.m. and 4:00–6:00 p.m. The total observation time was about 300 h for the eight groups (L:D-shift 12 h, photoperiod 8:00 a.m. – 8:00 p.m.; food: fresh vegetables at 12:00 a.m., water, hay, and commercial diet *ad libitum*). Blood samples were taken from each animal over a period of 4 weeks once a week between 10:00 and 11:00 a.m. From the animals of three groups blood samples were taken over 12 weeks. At each sampling time 0.5 ml blood was taken by puncturing the marginal ear vein [6]. The blood was collected in plastic vessels, kept for 1 h at room temperature and 2 h at 4 °C for coagglutination. The serum was separated by centrifugation and stored until assayed for a max. of 2 weeks. The cytotoxic activity of the blood sera was determined in a cytolytic algae assay