The Journal of Experimental Biology 208, 4451-4466
Published by The Company of Biologists 2005
doi:10.1242/jeb.01917

The insecticide pymetrozine selectively affects chordotonal mechanoreceptors

Jessica Ausborn[1], Harald Wolf[1,*], Wolfgang Mader[1] and Hartmut Kayser[2]

[1]*University of Ulm, Neurobiology Department, D 89069 Ulm, Germany* and [2]*Syngenta Crop Protection AG, Research and Technology, WRO-1004.4.46, CH 4002 Basel, Switzerland*

*Author for correspondence (e-mail: harald.wolf@biologie.uni-ulm.de)

Accepted 3 October 2005

Summary

Pymetrozine is a neuroactive insecticide but its site of action in the nervous system is unknown. Based on previous studies of symptoms in the locust, the feedback loop controlling the femur–tibia joint of the middle leg was chosen to examine possible targets of the insecticide. The femoral chordotonal organ, which monitors joint position and movement, turned out to be the primary site of pymetrozine action, while interneurons, motoneurons and central motor control circuitry in general did not noticeably respond to the insecticide. The chordotonal organs associated with the wing hinge stretch receptor and the tegula were influenced by pymetrozine in the same way as the femoral chordotonal organ, indicating that the insecticide affects chordotonal sensillae in general. Pymetrozine at concentrations down to 10^{-8} mol l^{-1} resulted in the loss of stimulus-related responses and either elicited (temporary) tonic discharges or eliminated spike activity altogether. Remarkably, pymetrozine affected the chordotonal organs in an all-or-none fashion, in agreement with previous independent studies. Other examined sense organs did not respond to pymetrozine, namely campaniform sensillae on the tibia and the subcosta vein, hair sensillae of the tegula (type I sensillae), and the wing hinge stretch receptor (type II sensillae).

Key words: insecticide, pymetrozine, chordotonal organ, locust, leg motor control, chemical ablation.

Introduction

Most of the currently used insecticides are neuroactive substances. This fact demonstrates that the nervous system exhibits target sites for chemically diverse compounds, which interfere with proper function of neurons and associated cells, usually with lethal consequences. To date, only a limited number of target sites have been identified for commercially used insecticides, with acetylcholine esterase, voltage-gated sodium channels, nicotinic acetylcholine receptors and GABA receptors representing the most dominant ones (Bloomquist, 1996; Matsuda et al., 2001). The typical symptoms of action of these insecticides may be summarized as a quick knock-down effect, accompanied by paralysis, tremor and related neuromuscular symptoms.

Pymetrozine, a pyridine azomethine (Fig. 1), represents a new chemical class of insecticides with a remarkable selectivity for plant-sucking insects, such as aphids, whiteflies and plant hoppers, due to its systemic action (Kristinsson, 1994; Wyss and Bolsinger, 1997). This fairly narrow biological spectrum seems to be related to a novel mode of action. Observation of feeding behaviour in aphids demonstrates that pymetrozine application results in immediate feeding inhibition, followed by delayed death through starvation (Harrewijn and Kayser, 1997). In detail, sucking aphids immediately withdraw their stylet from the plant vascular system, while probing and stylet insertion are blocked in non-feeding aphids, when pymetrozine is applied by injection or ingestion.

Further work aimed at locating and identifying the cells and cellular target sites that respond to pymetrozine is difficult to perform in aphids. During studies concerned with the basic mode of pymetrozine action, it was observed that the locust *Locusta migratoria*, though not a target pest for the field use of pymetrozine, is also sensitive to this compound. This is illustrated by feeding inhibition, as in aphids, but also by unique intoxication symptoms (Kaufmann et al., 2004). As a response to injected or food-applied pymetrozine, locusts raise their legs with the femur–tibia joints almost fully extended (Fig. 2C). Paralysis or other knock-down effects, while typical for other neuroactive insecticides, are not observed. These effects are not restricted to locusts but also occur in other orthopterans and are particularly clear in cockroaches and stick insects (as we show here).

We thus decided to pursue the mechanism of pymetrozine action in locusts, animals amenable to both intra- and extracellular electrophysiology. In fact, the femur–tibia joint control system (f–t loop) of the middle leg was chosen as the ‘experimental platform’ since its elements have been studied in particular detail (locust data in Burrows, 1996; stick insect data in Ebner and Bässler, 1978; Bässler, 1993) and lend themselves to the analysis of limb motor control in general.

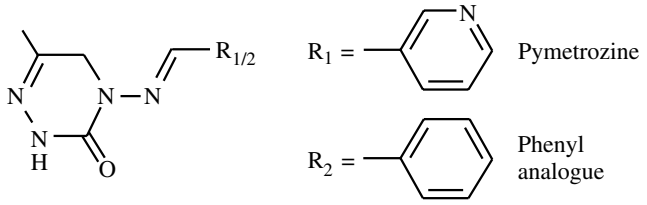

Fig. 1. Chemical structures of pymetrozine and its inactive phenyl analogue.

The hind leg femoral chordotonal organ, although fulfilling the same function, is less accessible than the middle leg receptor (e.g. Burrows, 1996). An outline of the joint control system is provided in Fig. 3. Study of this control network should allow identification of the neuronal elements sensitive to pymetrozine and of possible changes in their response characteristics. Elements that might be affected by pymetrozine are, in principle (see Fig. 3A,B): mechanosensory neurons (1 in Fig. 3A,B); central neurons (3 and 4), including spiking and nonspiking interneurons and motoneurons; synaptic transmission among any of these nerve cells; muscle properties or neuromuscular transmission (6); axonal spike propagation. Accordingly, pymetrozine effects were studied at these stages of signal transmission and processing.

It turns out that pymetrozine affects chordotonal organs, including the femoral chordotonal organ responsible for femur–tibia joint control, with high potency and selectivity (see also Ausborn et al., 2001). It blocks stimulus-related responses and consequently abolishes joint control in the context of postural reflexes. These effects may fully account for the raising and stretching of legs in the locust. The results may have further bearing on the interpretation of the feeding inhibition observed in pymetrozine-treated insects, which is essential for the insecticide action on plant-sucking pests.

Materials and methods

Chemicals

Pymetrozine and its phenyl analogue (pyridyl substituted for phenyl; Fig. 1) were synthesised at Syngenta, Basel, Switzerland and obtained with >97% purity. The compounds were first dissolved in a small volume of DMSO (dimethylsulfoxide) and then diluted with saline (see below) to the desired test concentrations.

Animals

Experiments were carried out under daylight conditions at room temperature (20–22°C). All behavioural and electrophysiological studies were performed with fully mature adult locusts (*Locusta migratoria* L.), both males and females taken from a crowded breeding colony at the University of Ulm. Experiments for Fig. 5 were performed with adult female stick insects, *Cuniculina impigra* (Redtenbacher) (syn. *Baculum impigrum* Brunner) also taken from a breeding colony at the University of Ulm.

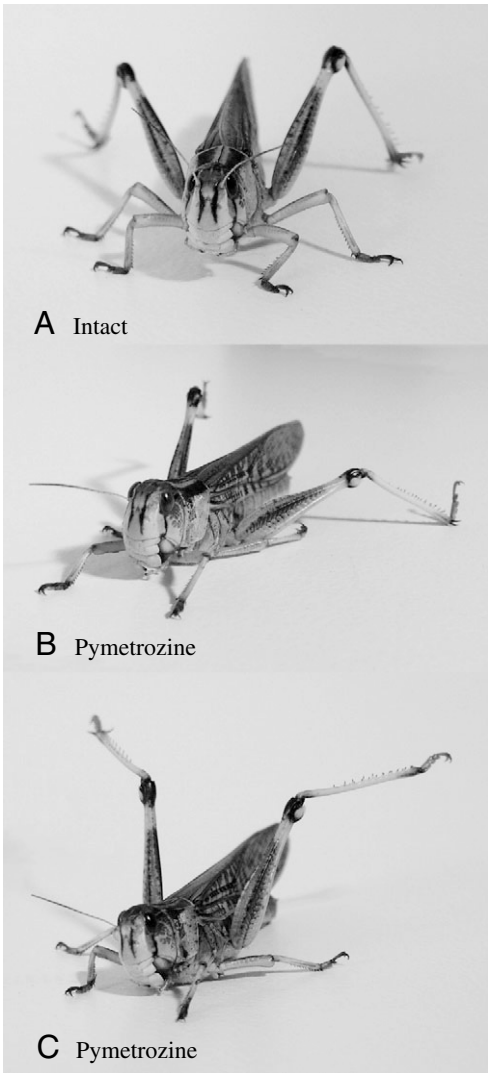

Fig. 2. Locust, *Locusta migratoria*, before (A) and after (B,C) pymetrozine application (0.5 μg g^{-1} body mass). The animal in A assumes a typical resting posture, holding the body elevated above the substrate with all the legs, sometimes except the hind legs, touching the ground. (B,C) Note extended femur–tibia joints, lifted legs (in the coxal joints) and lifted tarsi after pymetrozine application. Leg and body posture appear uncoordinated and the body is not held above the substrate but rather rests on the floor. In C, the conspicuous levitation of both hind legs is evident. Body length of insects is 60 mm.

Preparations and experimental procedures

Locusts (or stick insects; Fig. 5) were immobilised on a cork board with minuten pins (ESPE, Seefeld, Germany) and dentist glue (Pluradent AG, Offenbach, Germany). The femur of the middle leg under examination was glued to the cork board such that the femur–tibia joint extended beyond the edge of the board and the tibia was free to move. Tibia movements were monitored by attaching a semi-circular paper flag to the tibia, in the plane of movement and centred on the femur–tibia joint. The flag cast a shadow on a semi-circular photosensitive diode, the degree of shading, and hence the voltage output of the

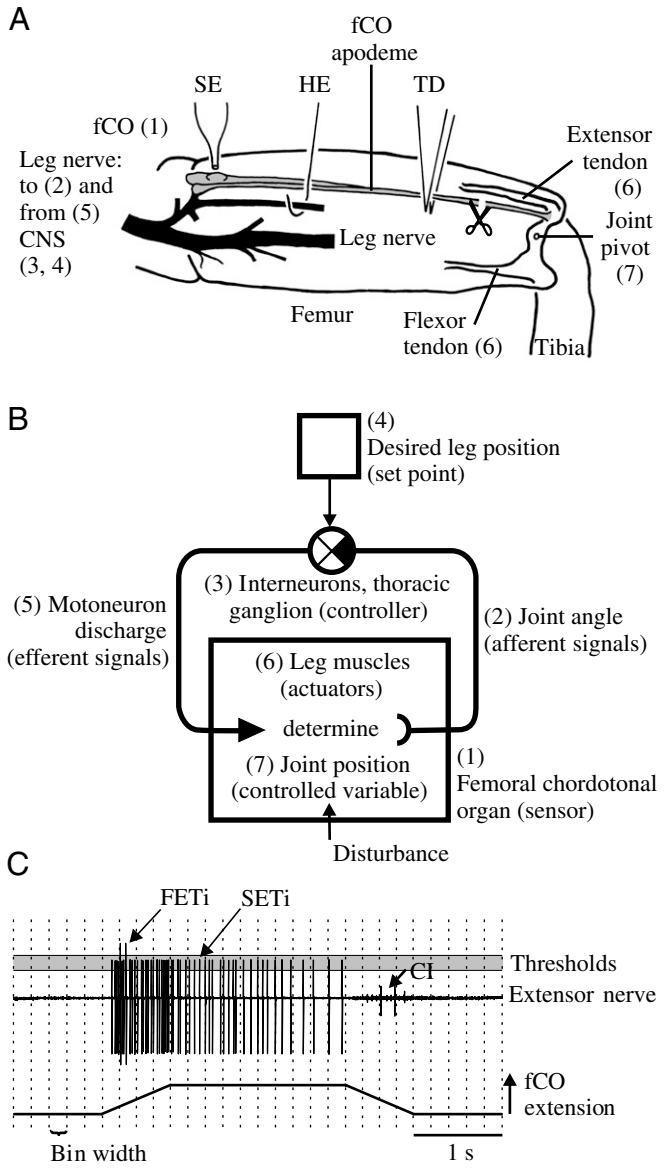

Fig. 3. Feedback loop controlling femur–tibia joint position in insects. (A) Anatomical situation, semi-schematic diagram of a locust middle leg. The means of experimental access to elements of the joint control loop are indicated: a clamp attached to a transducer (TD) allows stimulation of the femoral chordotonal organ (fCO) *via* its apodeme (tendon); a suction electrode (SE) records activity of fCO sensillae; a hook electrode (HE) monitors discharges of motoneurons supplying the extensor (or flexor) tibiae muscle; and movement of the tibia is monitored optically. (B) Cybernetic diagram of the feedback loop for joint control. Anatomical correlates of the elements of the control circuit are indicated (cybernetic terms in brackets). Numbers in A and B indicate corresponding structures (e.g. 1, sensor, fCO). (C) Resistance reflex generated in the quiescent animal. A ramp-and-hold stimulus delivered to the fCO (bottom trace; arrow indicates imposed fCO elongation, mimicking tibia flexion) elicits a spike discharge in the nerve supplying the extensor muscle (top trace; spikes of the three innervating motoneurons are marked: FETi, fast; SETi, slow extensor motoneuron; CI, common inhibitor). Note the pronounced response to movement and the smaller maintained discharge due to altered fCO position. Thresholds and bins for evaluation are indicated; for details, see text.

diode, being dependent on the femur–tibia angle (Weiland et al., 1986). In other experiments, the tibia was immobilized with dentist glue and the reflex response was monitored by means of electromyogram or nerve recordings from leg muscles (see below).

For intracellular recordings from motoneurons and interneurons, the above preparation was modified to allow access to the thoracic ganglia. The body was opened by a dorsal midline incision before setting of the dentists' glue. Gut, fatty tissue, salivary glands and (diaphragm) muscles overlying the nervous system were removed. The thoracic ganglia were supported on a wax-coated steel platform for intracellular recording with an npi SEC-10L amplifier (Tamm, Germany).

Stimulation of the femoral chordotonal organ (fCO)

To stimulate the fCO (Burns, 1974; Field and Pflüger, 1989), a window was cut into the anterior dorsal surface of the femur, sparing the attachment sites of tibia muscles as far as possible. This exposed the apodeme ('tendon') of the fCO, which was clamped into a pair of forceps attached to a transducer (TD in Fig. 3A; modified loudspeaker with feedback system; Hofmann and Koch, 1985; 2 in Fig. 3B) and cut distally (open-loop situation). Sinusoidal or ramp-and-hold stimuli were applied, centred around a joint angle of 110–120°. Stimulus amplitudes ranged from 240 to 480 μm, corresponding to approximately 40–80° tibia movement.

Application of compounds

In intact animals, pymetrozine and its phenyl analogue (Fig. 1) were each applied by injection through the abdominal intersegmental cuticle using a Hamilton microsyringe. Stock solution of insecticide was 10 mmol l^{-1} in DMSO; it was diluted 1:5 in water before injection. The final dose of insecticide in the insect body was 0.5 μg g^{-1} body mass (Kaufmann et al., 2004). In dissected preparations, the saline (Usherwood and Grundfest, 1965) in the body cavity was replaced with saline containing the desired concentration of the compound. Again, both pymetrozine and phenyl analogue were used, the phenyl analogue only being at 10^{-5} mol l^{-1}. Usual pymetrozine concentrations were 10^{-6} to 10^{-7} mol l^{-1}, although the highest and lowest applied concentrations were 10^{-5} mol l^{-1} and 10^{-9} mol l^{-1} when determining threshold concentrations (Fig. 7; see also Kaufmann et al., 2004). Minor dilution may have occurred due to residual saline in the preparation. Pymetrozine application could be restricted to the nervous system in the body cavity by plugging the lumen of the coxa with Vaseline™ and thus isolating the hemolymph space of the leg, avoiding pymetrozine exposure of the fCO (experiments shown in Fig. 11).

In control experiments, the solvent DMSO alone was administered at the highest concentrations used to apply pymetrozine (10^{-5} mol l^{-1}) to intact and dissected animals. This had no effects on the responses of joint reflex or fCO, as

was to be expected from previous literature dealing with neurophysiological effects of DMSO (Theophilidis and Kravari, 1994).

Recording technique

The above preparation not only exposed the fCO apodeme but also the nerve supply of the extensor tibiae muscle (Theophilidis and Burns, 1983). Neurograms of slow and fast extensor tibiae motoneurons (SETi, FETi) were recorded by attaching mono- (Schmitz et al., 1988) or bipolar hook electrodes (HE in Fig. 3A; 5 in Fig. 3B) to a nerve branch entering the extensor muscle. Alternatively, and in particular in the walking preparation described below, bipolar electromyographic recordings of muscle activity were obtained. A pair of 30 μm V2A steel wires, lacquer-coated except for the cut end, was inserted through small holes just through the cuticle and fixed with a beeswax–resin mixture. The holes had been pierced into the attachment sites of the muscles of interest with minuten pins. Amplification and storage of electrophysiological data and movement recordings were conventional [extracellular amplifiers, custom-made by Peter Heinecke, Seewiesen; data recording, DRA-800 analogue–digital converter (CED Cambridge Electronic Devices, Cambridge, UK) and Pioneer PDR-04 CD recorder (Pioneer Electronic, Willich, Germany), SPIKE2 software (CED Cambridge Electronic Devices)].

Extracellular recordings of fCO activity were obtained by a suction electrode (SE in Fig. 3A; 1 in Fig. 3B). The electrode tip was positioned on the distal scoloparium (the sensor of femur–tibia joint position; Field and Pflüger, 1989), and electric contact to cell bodies of sensory neurons was established by application of moderate suction. Different regions of the scoloparium were sampled until satisfactory recordings, of individual sensory neurons where possible, were obtained.

To record discharges of the whole fCO (e.g. Fig. 7) or of the campaniform sensillae on the dorsal tibia (below; see also Fig. 9A), the leg nerve was severed close to its exit from the mesothoracic ganglion and inserted into a suction electrode. Since all sensillae in the middle leg send their axons through this nerve, it was possible to monitor input from several sensors in this way, although together with the background of many other spontaneously active sensory neurons.

For intracellular recordings from motoneurons, thin-walled glass microcapillaries with tip resistances of 30–90 MΩ when filled with a mixture of 2 mol l^{-1} potassium acetate and 0.5 mol l^{-1} KCl (or 5% Lucifer Yellow in 0.5 mol l^{-1} KCl for morphological identification of recorded neurons) were used as electrodes. Processing of ganglia for morphological identification of recorded neurons was conventional.

Stimulation of campaniform sensillae on the subcosta wing vein and the dorsal tibia

Head and posterior abdominal segments of the locust were severed, and gut and salivary glands removed from the thorax. This exposed mesothoracic nerve 1C (nomenclature of nerves according to Campbell, 1961), which supplies the wing and wing hinge. This nerve was later lifted onto hook electrodes for recording spike discharges of the campaniform sensillae, as outlined above for the fCO. Wings, wing base and thoracic dorsum were immobilised on a cork board with dentist glue. The ventral surface of one wing was left free, however, to allow access to the campaniform sensillae on the subcosta vein at the wing base (Gettrup, 1966). This field of sensillae was stimulated by indentation with a minuten pin. The pin was attached to minuten relay contacts, serving as a stimulus monitor. Sometimes, specific stimulation of the campaniform sensillae appeared questionable, for instance due to high spontaneous spike activity in nerve 1C or incomplete inclusion of the wing hinge in the dentist glue. In these cases, the nerve supply to other sensory structures of the wing and wing base was cauterised, namely tegula and all wing veins except the subcosta, and the subcosta distal to the stimulation site.

The group of campaniform sensillae on the proximal dorsal tibia was stimulated in the same way, and its activity recorded through a suction electrode on the severed leg nerve (above). The stimulation needle was aimed at the centre of the medial dorsal group of 6–9 sensillae (Mücke, 1991; Newland and Emptage, 1996). In this way, probably all sensillae in the group were activated by the stimulus. Care was taken, however, not to touch any hair sensillae in the vicinity.

Stimulation of the tegula hair field

The preparation used to examine wing campaniform sensillae was also used to test the sensory hairs on the tegula (Kutsch et al., 1980). However, instead of the subcosta vein, a front wing tegula was left free of dentist glue. Elimination of other wing sensors was not necessary in this case because the tegula is inserted in soft membranous cuticle, allowing specific mechanical stimulation with a minuten probe. The probe was moved by hand or by a modified loudspeaker (above) to achieve controlled bending of the sensory hairs located on the posterior-medial half of the tegula.

As a control, the chordotonal organ associated with the tegula (Kutsch et al., 1980) was stimulated by denting the anterior cuticle of the tegula, which is devoid of hairs, or the adjacent membranous ligament (Fischer et al., 2002).

Stimulation of the wing hinge stretch receptor (SR)

The preparation just described for the examination of subcosta campaniform sensillae and tegula hair field was also employed to study the wing hinge stretch receptor (e.g. Möhl, 1985). However, nerve 1D of the mesothoracic ganglion was recorded with the hook electrodes, since it supplies the wing hinge stretch receptor, wing chordotonal organ (Pearson et al., 1989) and dorsal longitudinal muscles. The nerve was severed proximally to eliminate motoneuron spikes. The thorax was mounted on its ventral surface to leave the wing hinge area free to move. The stretch receptor was stimulated by inserting the

front wing into a brace attached to a mechanical stimulator. The wing was moved through ~100° between a downstroke position, ~30° ventral of the horizontal plane, and an upstroke position, ~70° dorsal of the horizontal.

As a control, the chordotonal organ associated with the wing hinge stretch receptor was stimulated. Since this sensor is most sensitive to wing vibration (Pearson et al., 1989), this was achieved by touching the wing surface with a small paint brush. However, even the wing movement used to stimulate the stretch receptor activated chordotonal sensillae (Figs 9Bi, 10A), which allowed stimulus-related evaluation.

Tethered walking preparation

To examine walking behaviour, locusts were suspended from the arm of a balance by their pronotum. The counterweight of the balance was adjusted such that the animal had to carry approximately its own body weight. The experimental animal was lowered onto a Styrofoam™ ball (diameter 10 cm) rotating around a central steel rod inserted into glass capillaries as bearings. Walking movements of a middle leg were monitored by attaching a small piece of scotch light tape to the tibia. The position of the tape in the anterior–posterior axis was converted into an electric signal by an opto-electronic camera (von Helversen and Elsner, 1977). Activity of selected leg muscles was recorded with electromyogram wires (above).

Data acquisition and evaluation

The data stored on CD were run out on a chart recorder (Yokogava ORP 1200) or digitised with electrophysiology software for further evaluation (SPIKE2, CED, Cambridge, UK; DATAPAC, RUN Technologies, Lake Oswego, OR, USA), for instance construction of peri-stimulus–time histograms (bin width indicated in Fig. 3C). Window discrimination (thresholds indicated in Fig. 3C) was used to evaluate the spikes of a particular neuron. Throughout the text, *N* represents the number of animals used, and *n* represents the number of measurements carried out in the course of an experiment in a given animal.

Results

As outlined in the Introduction, the feedback loop controlling femur–tibia joint position (Fig. 3A,B) in the locust was chosen to identify the mechanism of pymetrozine action. Since pymetrozine elicits the conspicuous extension and lifting of the legs (Fig. 2), the latter particularly evident in the hind legs (Fig. 2C), we expected to gain information on the target site(s) of the insecticide in this way. Possible neuronal sites of pymetrozine action in the control loop are: mechanosensory neurons (1 in Fig. 3A,B); central neurons (3 and 5 in Fig. 3A,B), including different types of interneurons and motoneurons; synaptic transmission among any of these neurons; muscle properties or neuromuscular transmission (6 in Fig. 3A,B); axonal spike propagation.

Response characteristics of the complete feedback loop

In a first set of experiments, and as a reference and control for subsequent experiments, the function of the complete femur–tibia joint control loop was examined (*N*=20; see below). The sensor in the feedback loop, the fCO, was stimulated by clamping the receptor apodeme into an electromechanical transducer (see Materials and methods; Fig. 3A). The response of the feedback loop was monitored through movement recordings (middle trace in Fig. 5A) and electromyograms (or neurograms) from the flexor or extensor tibiae muscles (or their nerve supplies) (top traces in Figs 3C, 4A,C, 5A).

At pymetrozine concentrations close to threshold (5×10^{-8} mol l^{-1}), most locusts (eight out of 10) responded to insecticide application with complete cessation of the feedback response (Fig. 4Bii; compare with Fig. 4Bi). In the remaining animals, no changes were recorded (Fig. 4Biii); that is, they behaved like untreated controls. At higher pymetrozine concentrations (up to 10^{-5} mol l^{-1}), stimulus-related responses in the recordings of motor activity (top traces in Fig. 4A,C; *N*=10) and leg movement (Fig. 5; *N*=6) were always (i.e. in all preparations) completely abolished.

In summary, the effects of pymetrozine were not concentration dependent but rather exhibited an all-or-none behaviour. That is, pymetrozine effects were either absent or fully present, without intermediate states. Towards lower concentrations, the proportion of unaffected animals increased but the effects, where present, were the same as at higher concentrations (see also dose–response curve below in Fig. 7).

Stimulus-related responses were never restored, neither by rinsing with saline for up to 2 h nor by increasing stimulus amplitude from 40° to 60° or 80° (Fig. 4E) or increasing stimulus velocity from 45 deg. s^{-1} to 455 deg. s^{-1} (Fig. 4F). Tibia extensor motoneurons and muscles were often tonically active just after pymetrozine application. The same held true for the flexor muscle, where recorded. These results are illustrated in Fig. 5A (experiment performed in a stick insect; see below): the receptor apodeme was sinusoidally stimulated throughout but the corresponding leg movement ceased within less than 60 s after pymetrozine application, the leg assuming an extended position. In parallel to the decrease in stimulus-related leg movement, the electromyogram of the flexor tibiae muscle ceased to show stimulus-related spike bursts (top trace in Fig. 5A). Fig. 4 shows corresponding experiments with ramp-and-hold stimulation of the fCO. The upper traces (Fig. 4Ai,Bi,Ci,Di) give sample recordings and peri-stimulus–time histograms of tibia extensor activity in the control situation, while the lower traces (Fig. 4Aii,Bii,Cii,Dii) illustrate the tonic spike discharge and the lack of stimulus-related responses after pymetrozine application.

Cybernetic analysis of the feedback loop after pymetrozine application (e.g. measurement of amplitude and phase relationships for the construction of Bode diagrams) usually proved futile since the effect of pymetrozine was rapid enough to abolish stimulus-related responses within a few stimulus cycles, or even a single cycle at low stimulus

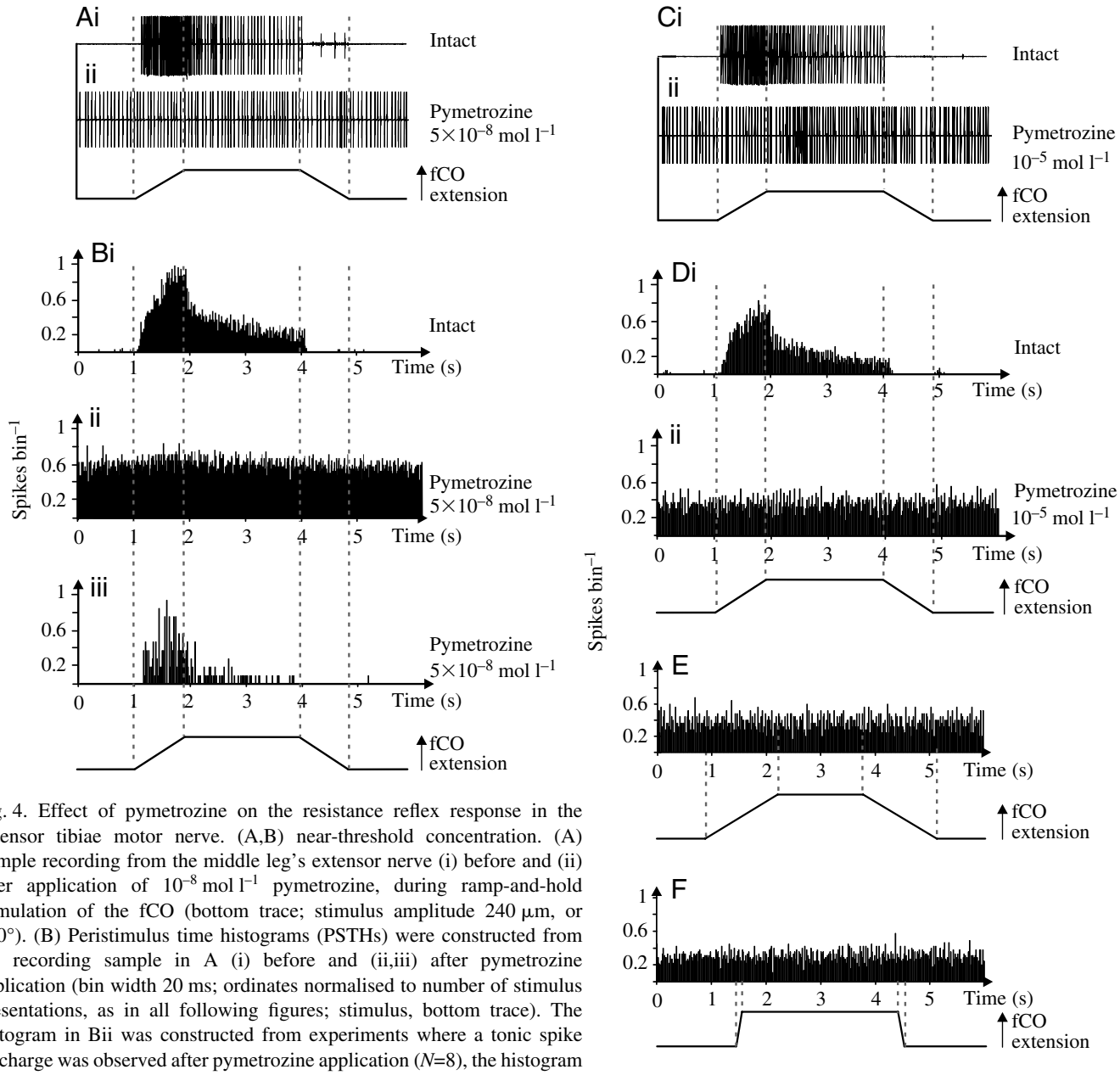

Fig. 4. Effect of pymetrozine on the resistance reflex response in the extensor tibiae motor nerve. (A,B) near-threshold concentration. (A) Sample recording from the middle leg's extensor nerve (i) before and (ii) after application of 10^{-8} mol l^{-1} pymetrozine, during ramp-and-hold stimulation of the fCO (bottom trace; stimulus amplitude 240 μm, or ~40°). (B) Peristimulus time histograms (PSTHs) were constructed from the recording sample in A (i) before and (ii,iii) after pymetrozine application (bin width 20 ms; ordinates normalised to number of stimulus presentations, as in all following figures; stimulus, bottom trace). The histogram in Bii was constructed from experiments where a tonic spike discharge was observed after pymetrozine application (N=8), the histogram in Biii from experiments without such tonic discharge (N=2). (C,D) Effect of pymetrozine at higher concentrations (10^{-5} mol l^{-1}); same presentation as in A and B. (C) Sample recording from the middle leg extensor nerve (i) before and (ii) after pymetrozine application (stimulus, bottom trace). (D) PST histograms constructed from the recordings (i) before and (ii) after pymetrozine application (bin width 20 ms; stimulus, bottom trace). (E) Increased stimulus amplitude [360 μm (~60°); instead of 240 μm] or (F) increased stimulus velocity (455 deg. s^{-1}; instead of 45 deg. s^{-1}) did not restore the feedback response abolished by pymetrozine. Same recordings as in C and D, response before pymetrozine application in Ci and Di.

frequencies. In the stick insect *Cuniculina impigra*, however, the effect of pymetrozine sometimes developed more slowly (Fig. 5A), allowing measurement of a partial Bode diagram (filled circles in Fig. 5B,C). This is perhaps due to the longer and more slender legs of these insects, providing more gradual access of the applied insecticide to the fCO inside the leg. It is evident in these experiments that pymetrozine affected response amplitude but not response phase, indicating that the timing in the reflex pathway, and thus synaptic transmission, is not strongly influenced by pymetrozine, if at all.

It should be noted in this context that, in *C. impigra*, similar responses to pymetrozine were observed as in the locust, although they were examined in less detail (N=2–6). This is true in particular for leg posture, responses and response thresholds of the middle leg fCO, effects on the fCO feedback loop, and walking behaviour.

No effect on the joint control loop was observed after

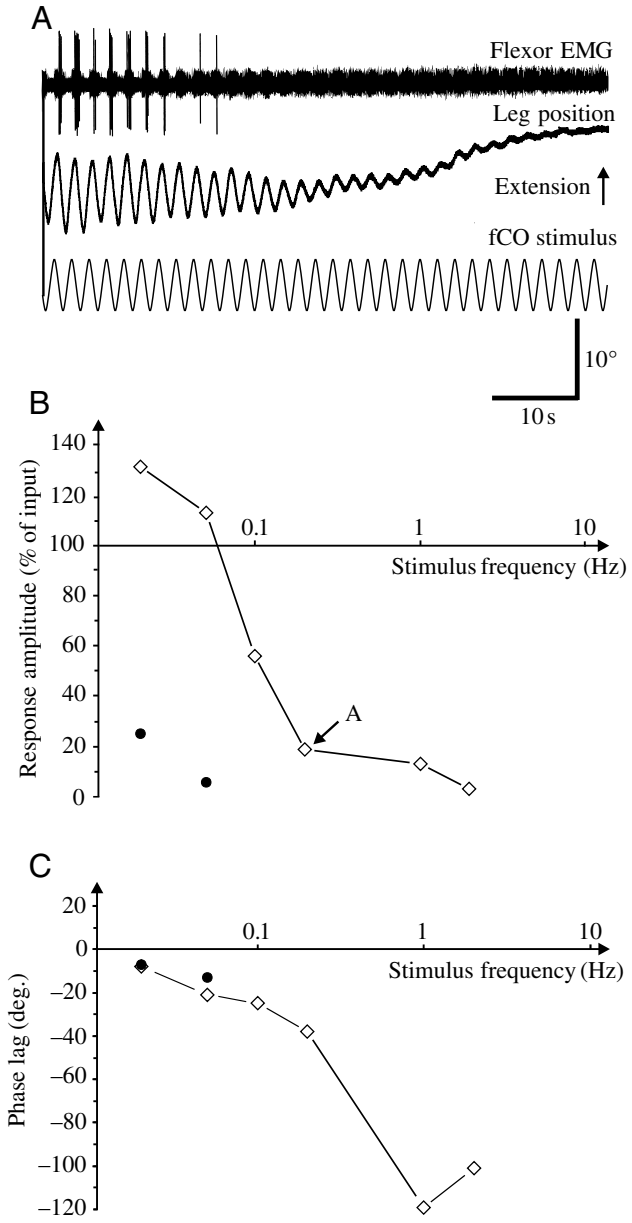

Fig. 5. Effect of pymetrozine on the resistance reflex in the middle leg femur–tibia joint of the stick insect *Cuniculina impigra*. (A) Tibia position (middle trace) in response to sinusoidal stimulation of the femoral chordotonal organ (bottom trace; stimulus frequency 0.5 Hz; amplitude 400 μm, corresponding to ~80° tibia movement; arrow indicates tibia extension and fCO elongation). Top trace shows a flexor tibiae electromyogram. Application of 10^{-6} mol l^{-1} (probably diluted before actually reaching the fCO) pymetrozine was just before the beginning of the sample shown. (B,C) In this animal, movement response declined slowly within 1–2 mins of pymetrozine application. This allowed measurement at two stimulus frequencies (0.02 and 0.05 Hz; filled circles). The modified Bode diagram plots response amplitude (B) and phase lag (C) *versus* stimulus frequency (diamonds, before pymetrozine). Note the decrease in response amplitude and unaltered phase lag after pymetrozine application. Arrow in B indicates stimulus situation depicted in A (0.5 Hz, different animal).

application of the biologically inactive phenyl analogue of pymetrozine (at 10^{-5} mol l^{-1}, including 0.1% DMSO; see Fig. 1). This held true for both locusts and stick insects.

Sensory coding by the fCO

The response of the sensor in the femur–tibia joint control loop, the fCO, was studied by recording from individual receptor cells with suction electrodes (Fig. 6A) while stimulating the receptor apodeme with ramp-and-hold movements. Rather unexpectedly, the receptor cells of the fCO were affected by pymetrozine, basically in the same way as the complete feedback loop (Fig. 6A,B). Stimulus-related responses of fCO receptor cells were abolished within a few seconds following 10^{-5} mol l^{-1} pymetrozine application (N=4; 5×10^{-8} mol l^{-1} pymetrozine had the same effects, although fewer animals were affected; see above). All physiological types of receptor cells were affected, those responding to joint position, to the velocity of joint movement (exemplified in Fig. 6) or to a combination of these parameters (e.g. Burns, 1974; Field and Pflüger, 1989; detailed analyses for the stick insect in Hofmann et al., 1985; Büschges, 1994). This held true for stimulus velocities between 108 and 2730 μm s^{-1} (18–455 deg. s^{-1}) and stimulus amplitudes between 240 and 480 μm (40–80°). Acceleration sensitivity was not examined specifically, although the response to vibration stimuli (Field and Pflüger, 1989; Stein and Sauer, 1999) was affected in the same way as that to ramp-and-hold stimuli (see also Fig. 10A). The receptor cells were either silent or, sometimes, spontaneously active after pymetrozine application. Spontaneous discharge is seen in the original recording (Fig. 6Aii) and the corresponding time histogram (Fig. 6Bii), together with the clear absence of stimulus-related activity. Spontaneous discharges, if present, usually occurred during the first few minutes after pymetrozine application.

The threshold for pymetrozine effects had been determined in previous studies on thoracic ganglia *in situ* to be ~10^{-8} mol l^{-1} (e.g. Kaufmann et al., 2004; see also Fig. 4A,B). We therefore wanted to determine whether or not this same threshold is valid for the effects on the fCO alone – which, if true, would support the interpretation that fCO effects are an important cause of the behavioural consequences of pymetrozine application (e.g. leg posture in Fig. 2). The threshold of pymetrozine action on the fCO was determined by recording total fCO activity in the main leg nerve (see Materials and methods). Fig. 7 illustrates that, indeed, the threshold was ~10^{-8} mol l^{-1} pymetrozine. The responses to stimulation of other sense organs on the leg (hair sensillae, pulvilli receptors, tibial spines) remained completely unaffected in the course of determining these dose–response characteristics (see below).

Intracellular motoneuron recordings

The recordings from the fCO receptor cells suggested that pymetrozine acts on these sensory neurons to abolish stimulus-related responses of the joint control loop. These experiments

could not clarify, though, whether or not *all* of the 80 or so fCO neurons in the distal scoloparium are affected, and thus the sensory effects of pymetrozine might be sufficient to explain the observed cessation of feedback responses. In the locust, intracellular recordings from motoneurons, and particularly from the fast (FETi) and slow (SETi) (N=9) extensor tibiae motoneurons were made to detect any possible remaining subthreshold feedback responses. Mechanoreceptors of the fCO, in addition to their projections onto spiking and nonspiking interneurons, contact leg motoneurons monosynaptically, although usually with low efficacy (Field and Burrows, 1982; review in Burrows, 1996). Therefore, residual activity of receptor cells after pymetrozine application should be visible in intracellular motoneuron recordings, at least after stimulus-related averaging.

Two notable observations were made in the intracellular motoneuron recordings in addition to the electromyograms. First, not even the smallest sub-threshold stimulus-related synaptic potentials were detected in leg motoneurons after pymetrozine application (Fig. 8B). This is evident in original recordings (middle trace in Fig. 8A) as well as in stimulus-related averages (middle trace in Fig. 8B; control before pymetrozine application in top trace), which should reveal even the smallest stimulus-related synaptic input. Accordingly, stimulus-related spikes were also absent, even in flexor tibiae units that were often tonically active (middle trace in Fig. 8C), at least initially after pymetrozine application. This indicates that, indeed, all receptor cells of the fCO's distal scoloparium, i.e. all mechanoreceptor cells involved in joint control, are affected by pymetrozine. This holds for all three parameters of fCO signalling, joint position, movement velocity, and acceleration or vibration stimuli. Second, when the animals

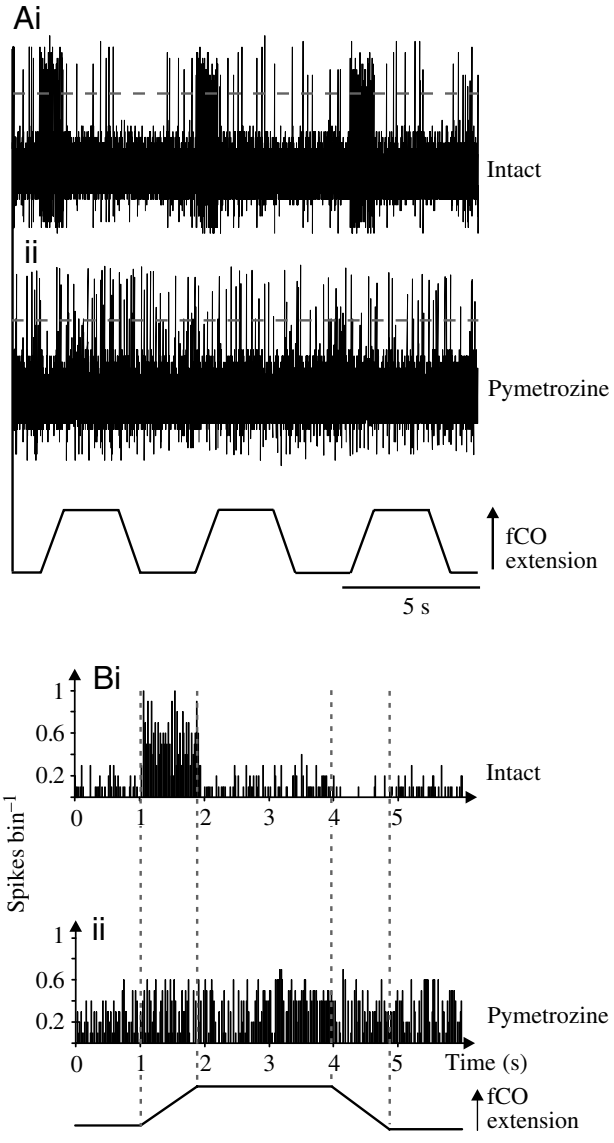

Fig. 6. Effect of pymetrozine on individual femoral chordotonal organ (fCO) receptor cells. (A) Sample recording from the soma of an fCO receptor cell (distal scoloparium) in the middle leg (i) before and (ii) after 10^{-5} mol l^{-1} pymetrozine application. Stimulus shown in bottom trace (amplitude 240 μm, ~40°). Note response of this receptor cell to the elongation phase of the ramp-and-hold stimulus, mimicking flexion of the femur–tibia joint. Scale bar, 5 s. (B) PST histograms constructed from the recording depicted in A, as described for Fig. 4. Broken line in A marks the threshold used for spike selection in the construction of the time histograms in B.

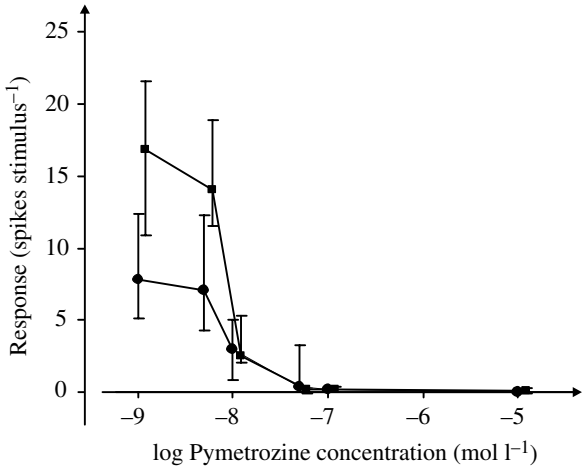

Fig. 7. The threshold for pymetrozine action on the femoral chordotonal organ is 10^{-8} mol l^{-1}. Response of the middle leg femoral chordotonal organ (fCO) was monitored during ramp-and-hold stimulation of the receptor apodeme [ordinate; spike counts during elongation (filled circles) and relaxation (filled squares) ramps, baseline activity subtracted]. Different concentrations of pymetrozine were applied, starting with 10^{-9} mol l^{-1} and increasing in steps of 5-fold or 2-fold (abscissa) every ~25 min until stimulus-related responses had completely vanished. 10^{-5} mol l^{-1} was applied as the final control concentration. Numbers of measurements per data point (n=N) range from 6 (above-threshold concentrations) to 12 (threshold area). Median values and 25% percentiles are shown. Graph for relaxation stimuli is offset to the right by 20% for better visibility of percentiles. Note that calculation of medians from data obtained in several individuals obscures the fact that in any given animal pymetrozine had an all-or-none effect, although at slightly different concentrations; for details, see text.

performed *active* movements (for example, in response to stimulation of the abdomen; heavy arrow in Fig. 8Aii; see also Fig. 12), the motoneurons received synaptic input and discharged spikes, even with insecticide present. Similarly, spike activity in the motoneurons remained unaffected by pymetrozine when elicited by current injection into the intracellularly recorded cell. This demonstrates that pathways involved in the initiation and control of active movement components are probably not, or not directly, affected by pymetrozine.

The presence of motoneuron discharges and muscle activity during active movement demonstrates that neuromuscular transmission and muscle function remained basically unimpaired by pymetrozine, in agreement with previous results (Kaufmann et al., 2004). This had also been illustrated by our electromyographic (extensor and flexor tibiae muscles), motor nerve (nerves 3B2c, 5B1c) and movement recordings (see above).

Response characteristics of other sense organs

The action of pymetrozine on locust mechanoreceptors other than the fCO was examined in order to test whether or not pymetrozine exerts similar effects on other sensory cells, and perhaps on mechanoreceptors in general. Campaniform sensillae on the dorsal tibia (e.g. Pringle, 1938) and on the subcosta wing vein (Gettrup, 1966) and hair sensillae on the tegula organ of the wing base (Kutsch et al., 1980) belong to the same class of cuticular mechanoreceptors as the chordotonal sensillae (type I sensillae; e.g. Ramirez and Pearson, 1990). The front wing stretch receptor (Möhl, 1985) was studied as a representative of a different group of mechanoreceptors (type II sensillae; Ramirez and Pearson, 1990). It was a favourable coincidence that chordotonal organs are associated with both tegula and stretch receptor, allowing to test the – admittedly unlikely – possibility that pymetrozine effects are specific just for the *femoral* chordotonal organ.

Contrary to our expectations, neither campaniform sensillae (Fig. 9A,C) nor hair sensillae (Fig. 9D) were influenced in their response characteristics by pymetrozine [note similarity of top and middle traces, corresponding to control situation and pymetrozine application, in both original records (i) and time histograms (ii)]. Indentation of the campaniform sensillae on the tibia or on the subcosta wing vein elicited the same response with and without pymetrozine at 10^{-6} mol l^{-1} (N=12 and N=9, respectively). While details of the response characteristic of the tibial campaniform sensillae were not readily discernible in original recordings due to background

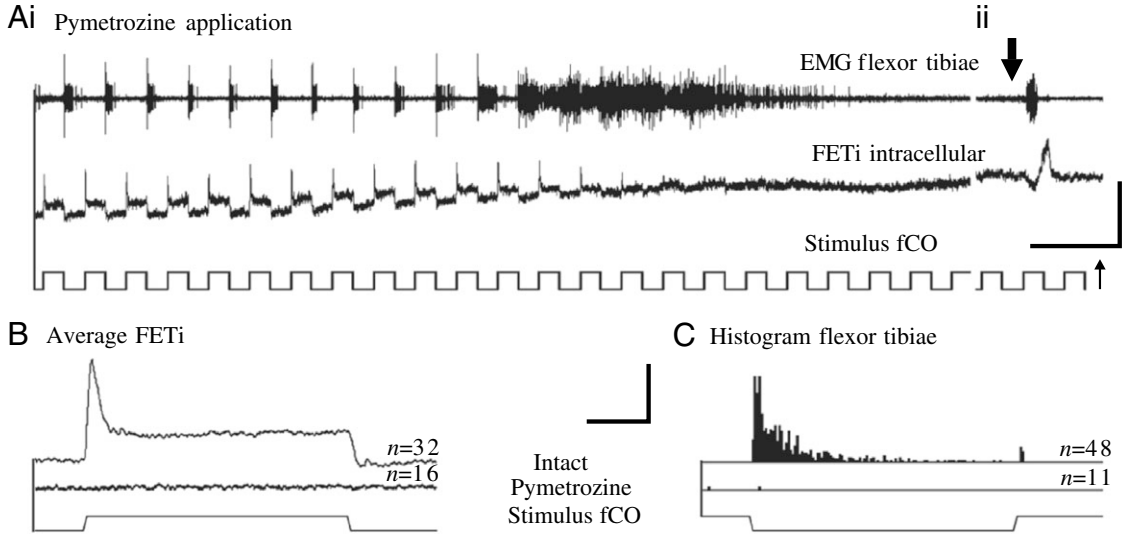

Fig. 8. Intracellular motoneuron recording does not reveal residual stimulus-related responses after pymetrozine application. (A) Recording taken during pymetrozine application (10^{-7} mol l^{-1}, application started ~30 s before sample shown in Ai). Bottom trace, ramp-and-hold stimulation of femoral chordotonal organ (fCO) (240 μm, arrow indicates fCO elongation); middle trace, intracellular recording from FETi soma; top trace, EMG from flexor tibiae muscle. (Aii) Brushing the abdomen (heavy arrow) leads to active movements; concomitant synaptic input to FETi proves that the intracellular recording had not been lost during the previous pymetrozine application. Note that the pronounced depolarisation of FETi was transient, probably caused by the initial tonic fCO discharge sometimes observed after pymetrozine application, and disappeared after a few minutes. FETi membrane potential was approximately –50 mV at rest and increased by 5.5 mV in the course of the sample recording. This depolarisation apparently reduced and eventually prevented spike discharges towards the end of the sample shown. (B) Stimulus-related averaging of the intracellular FETi record before (top trace) and after (middle trace) pymetrozine application demonstrates the absence of residual stimulus-related input after pymetrozine. (C) Histogram of flexor tibiae activity before (top trace) and after (middle trace) pymetrozine application illustrates the absence of stimulus-related discharge in FETi's antagonist. B and C show data from the same recording as in A; bottom traces as in A; note response of FETI to fCO elongation and of flexor tibiae muscle to fCO relaxation. Scale bars: (A) 10 mV, 5 s; (B) 5 mV, 250 ms; (C) 36 spikes bin^{-1} (bin width 10 ms), 250 ms.

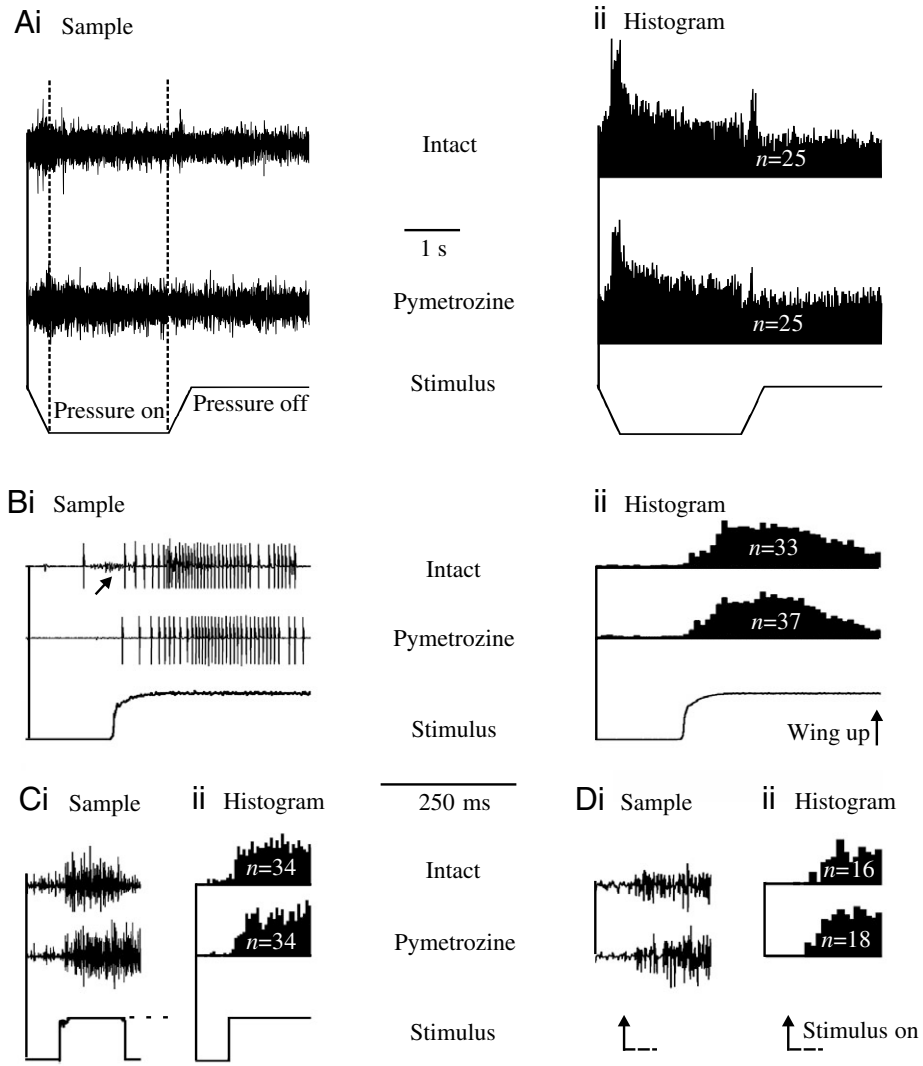

Fig. 9. Pymetrozine was not observed to have effects on the campaniform sensillae of the dorsal tibia (A), the wing hinge stretch receptor (B), the campaniform sensillae on the subcosta vein (C) or the hair sensillae on the tegula (D). Data before (top traces) and after (middle traces) pymetrozine application (10^{-6} mol l^{-1}) are shown for comparison; stimulus application is shown in the lower traces [pressure on sensillae in A, upward movement of the wing in B, pressure on the subcosta vein in C (note that stimulus duration outlasted length of sample trace for lower recording), stroking of tegula hairs in D (only stimulus onset shown here due to variable stimulus durations)]. (i) Sample recordings; (ii) time histograms. Ordinates of histograms normalised to number of stimulus presentations, bin widths 25 ms in A, 10 ms in B and D, 5 ms in C. Note that in A the campaniform sensillae respond strongly to the onset of cuticular pressure but just slightly to maintained pressure and to termination of cuticle indentation. Arrow in B indicates response of wing chordotonal organ sensillae to the stimulus.

activity in the leg nerve (Fig. 9Ai), the results were very clear in histograms (Fig. 9Aii), particularly for the campaniform sensillae on the wing base (Fig. 9C). The same observations were made with stimulation of the hair sensillae (Fig. 9D) on the tegula organ (*N*=7). The response of the wing hinge stretch receptor (Figs 9B, 10A, large spikes) remained similarly unaffected (*N*=10).

By contrast, the chordotonal organs associated with the wing hinge stretch receptor and the tegula were affected by pymetrozine in the same way as the fCO (Fig. 10; *N*=16). No stimulus-related responses are discernible in the middle traces in Fig. 10, in both original records (i) and time histograms (ii). This demonstrates, first, that the experimental situation was adequate, i.e. that pymetrozine indeed reached the wing hinge area and tegula. Second, it also demonstrates that pymetrozine affects several, and by inference perhaps all, chordotonal sensillae.

The above results are corroborated by preliminary observations on the (unaffected) responses of hair fields on the head, signalled by the tritocerebral commissure giant neuron (Bacon and Tyrer, 1978), and of cercal hairs, signalled by giant neurons in the abdominal connectives (Boyan and Ball, 1990). As noted above, mechanosensors on the leg other than the fCO also did not show noticeable deficits after pymetrozine application.

Pymetrozine effects on central neurons and on higher control of local reflex pathways

Possible effects of pymetrozine on central neurons in the mesothoracic ganglion, and on higher control of the local mesothoracic motor control pathways, were assessed by restricting pymetrozine application to body cavity and ventral nerve cord. The leg and fCO were isolated from the insecticide with a Vaseline™ plug in the coxa of the respective leg. In this way, pymetrozine action on the sensory cells of the fCO was excluded, and major effects on the central nervous circuitry for joint control in the mesothoracic ganglion and on higher motor control centres in subesophageal ganglion or brain (e.g. speculations by Kien, 1983), as far as they affect local processing in the mesothorax, should become evident.

Fig. 11 illustrates that the resistance reflex in the mesothoracic femur–tibia joint remained completely

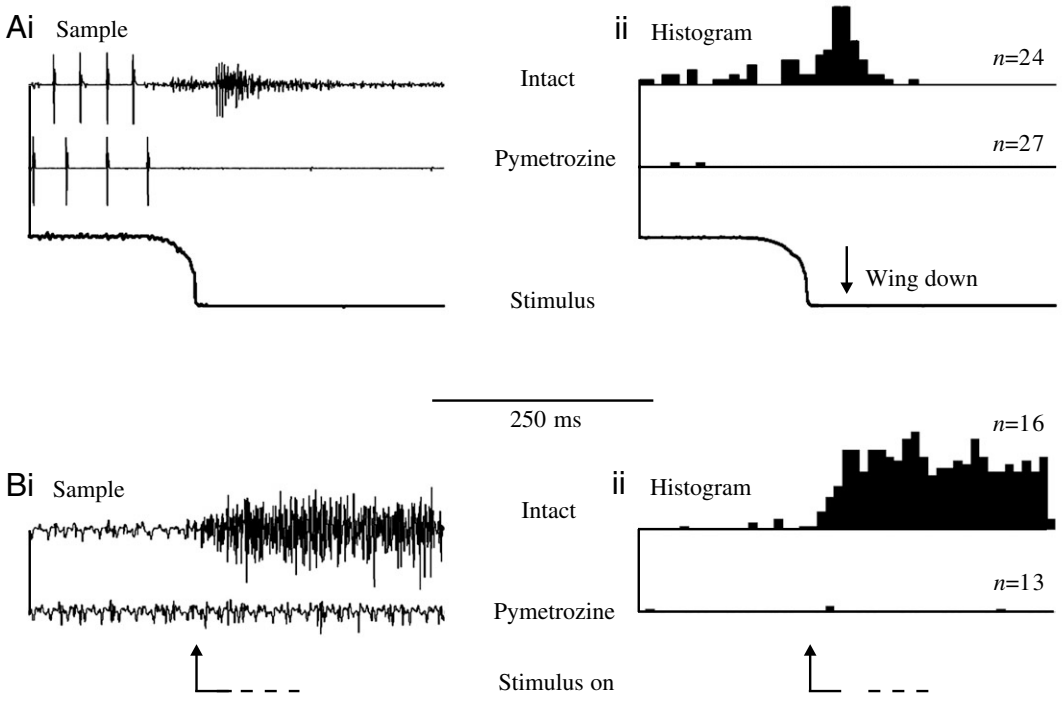

Fig. 10. The chordotonal organs associated with the wing hinge stretch receptor (A) and the tegula (B) are affected by pymetrozine like the femoral chordotonal organ (fCO). Data before (top traces) and after (middle traces) pymetrozine application (10^{-6} mol l^{-1}) are shown for comparison; stimulus (lower trace in A; downward movement of wing); arrows in B (onset of pressure on tegula base). (i) Sample recordings; (ii) time histograms. Ordinates of histograms normalised to number of stimulus presentations, bin widths 10 ms.

unaffected by pymetrozine application to the central nervous system (*N*=6) on the level of the present analysis. The reflex responses of extensor (Fig. 11A) and flexor (Fig. 11B) tibiae nerves were virtually identical before and after pymetrozine application. In original records (i and ii) as well as in peri-stimulus–time histograms (iii and iv) no difference is discernible between the control situation (i and iii) and pymetrozine application (ii and iv). This is particularly remarkable in the case of the flexor tibiae nerve recording, since there were always several motor units present in flexor nerve recordings. And the reflex responses in all motor units remained unchanged, even in presumed slow motor units with a tendency to be tonically active at rest (the sample histograms in Fig. 11Biii,iv comprise all spike amplitudes just above background activity). This attests to the absence of even small pymetrozine effects.

Control application of pymetrozine to the fCO following these experiments reproduced the above result, i.e. those without isolation of the leg hemolymph space (see Results above).

Walking behaviour

Movements of the middle legs were recorded during tethered walking in *Locusta migratoria* in order to examine, in more detail, possible behavioural effects of pymetrozine. So far, only very coarse behavioural observations had been made (e.g. Fig. 2; Kaufmann et al., 2004). Although analysis of feeding behaviour would appear more desirable, the neuronal basis of leg motor control is known in much more detail, allowing more meaningful interpretation. Leg movements in walking were compared before and after pymetrozine application (0.5 μg g^{-1} body mass) (Fig. 12). Walking movements in locust front and hind legs were affected in ways similar to those observed in the middle legs, although the hind legs were more often and more clearly lifted and extended and usually did not participate in walking. This behaviour had initially been observed in *Locusta migratoria* and *Periplaneta americana* by Kayser and co-workers (Kaufmann et al., 2004). The following general observations were made (*N*=11). The femur–tibia joints were extended, the legs were lifted and the body weight was, therefore, usually not supported; rather, the body was dragged across the ground. Sometimes, front and middle legs did not even touch the substrate. Leg coordination was impaired; in particular, there were often almost or completely synchronous swing and stance movements of both segmental legs, contrasting with the alternating stepping pattern in most normal walking situations (e.g. Burns, 1973). Fig. 2B,C shows photographs of a locust taken ~10 min after injection with pymetrozine at 0.5 μg g^{-1} body mass. Locomotion was almost impossible for these animals.

Fig. 12 provides recordings of middle leg movement and muscle activity before (Fig. 12A) and after (Fig. 12B) injection of pymetrozine. Both movement and muscle activity were altered after pymetrozine application (compare, for example, Burns, 1973; Burns and Usherwood, 1979; Theophilidis and Burns, 1990; Wolf, 1990, 1992). This was evident in the brief walking sequences elicited by stroking the animal's abdomen (spontaneous walking was much reduced or absent after pymetrozine). Starting from a lateral to slightly posterior position, the leg was rapidly moved posteriorly just before the swing, reaching the normal posterior extreme position, or a slightly more posterior attitude (by an average 0.4 mm), before the swing movement commenced. The swing movement itself and the subsequent beginning of the stance were sometimes of normal speed, though usually also more rapid [taking an average of 76 ms instead of 143 ms in the

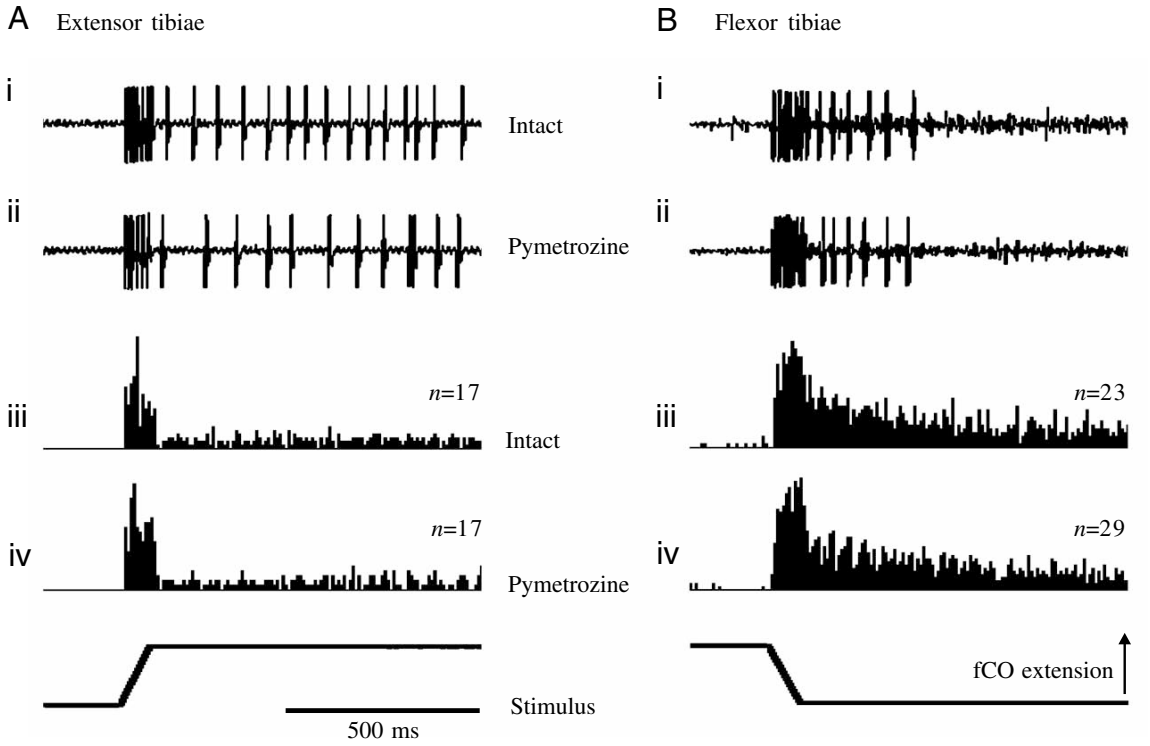

Fig. 11. Pymetrozine was not observed to have effects on central mesothoracic motor control circuits, or on higher control centres, e.g. in the subesophageal ganglion. The response of the extensor (A) and flexor (B) tibiae nerves to ramp-and-hold stimulation of the femoral chordotonal organ (fCO; bottom traces, 240 μm or ~40° fCO movement) was examined before and after application of 10^{-6} mol l^{-1} pymetrozine to the ventral nerve cord, but not to the fCO (hemolymph space of the leg isolated by Vaseline™ plug (see text). Sample recordings (i and ii) and time histograms (iii and iv) are shown for the intact (control) situation (i and iii) and after pymetrozine application (ii and iv). Bin widths of histograms 10 ms; ordinates normalized to number of stimulus presentations. Different individuals were used in A and B.

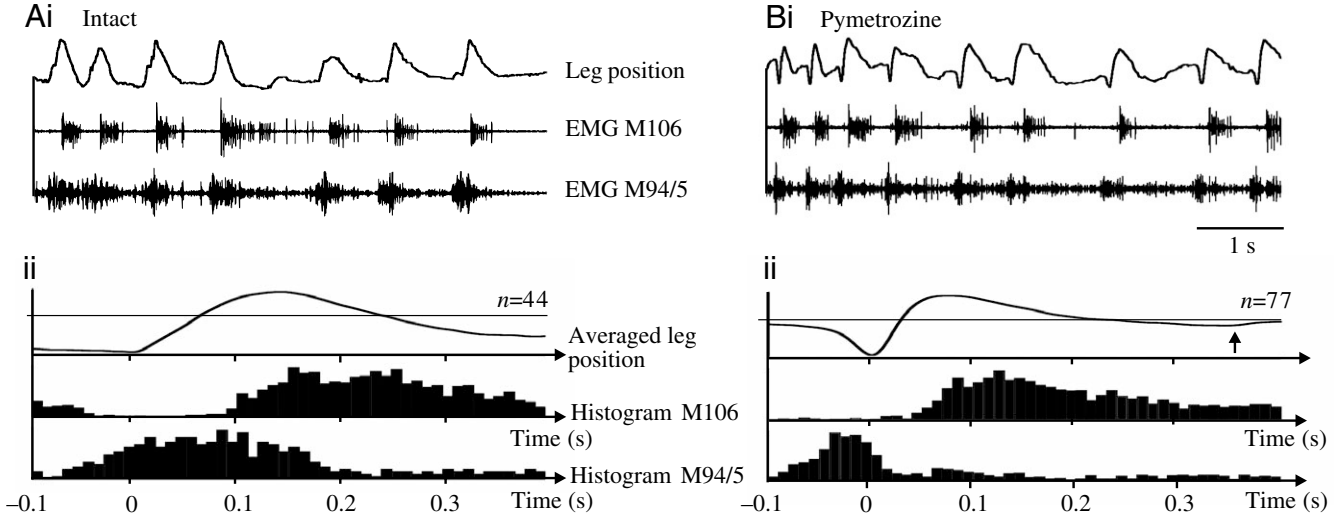

Fig. 12. Patterned motoneuron activity during walking-like movements persists after pymetrozine treatment. Data before (A) and after (B) pymetrozine application (0.5 μg g^{-1} body mass) are shown for comparison. Ai and Bi show sample recordings; leg position, top trace (anterior is to the top, i.e. swing phases are represented by rapid upward strokes); electromyograms (EMGs) of tibia extensor (M106) and coxa levator/protractor (M94/95) muscles, middle and bottom traces, respectively. Aii and Bii show corresponding averages of leg movement (top) and time histograms of EMG discharges (middle and bottom; bin widths 10 ms, ordinate scales normalised to number of steps evaluated). Zero on the abscissa marks the start of swing movement; reference lines in top traces mark lateral leg position. Note the differences in leg movement and timing of levator activity after pymetrozine application; for details, see text.

intact (control) animals]. The stance phase usually did not reach the normal posterior extreme, and often a small anteriorly directed movement occurred during its later part (arrow in Fig. 12Bii), after which the rapid posteriorly directed movement just mentioned heralded the next swing. These altered leg movements were reflected in leg muscle activity, for example in the much shortened discharges of the levator/promotor muscle (M94/5 according to Snodgrass, 1929; middle and bottom traces in Fig. 12A,B). Overall, the impression of leg movements was that of a brief 'paddling' back-and-forth movement around a laterally extended resting position.

Discussion

The present study identifies chordotonal sensillae as highly sensitive targets of pymetrozine, probably responsible for the conspicuous lifting and extension of walking legs in the locust (Fig. 2B,C; Kaufmann et al., 2004), which is particularly obvious in the hind legs. Comparable observations were made previously in cockroaches (e.g. Kaufmann et al., 2004) and stick insects. Strong and specific effects of pymetrozine on chordotonal sensillae are indicated:

(1) by the elimination of stimulus-related responses in the fCO of the middle leg (Fig. 6) above 10^{-8} mol l^{-1} (Fig. 7), in the tegula chordotonal organ (Fig. 10B) and in the wing hinge chordotonal organ (Fig. 10A);

(2) by the resulting abolition of the resistance reflex in femur–tibia joint control (Figs 4, 5);

(3) by the apparent absence of effects on central stations of leg motor control (Figs 11, 12);

(4) by the absence of effects on other types of mechanoreceptors, including both type I and type II receptors (Fig. 9);

(5) by the persistence of active leg movements (Fig. 12), impaired in a manner apparently consistent with the effects on the fCO.

The response of the fCO to joint movement, including position- and velocity-sensitivity (Burns, 1974; Hofmann et al., 1985), is abolished by pymetrozine. A tonic spike discharge may be present, although usually transiently just after pymetrozine application. It is, however, not related to mechanical stimulation. Apparently all chordotonal sensillae are affected since no trace of residual stimulus-related activity could be detected in any of our experiments, and particularly not in intracellular motoneuron recordings (Fig. 8), which should reflect even sub-threshold activation of the feedback loop.

In view of the effects of DMSO on insect neurons, as described by Theophilidis and Kravari (1994), it is important to note that the effects attributed to pymetrozine in the present study were not caused, nor even influenced, by the DMSO present in the applied dilutions of the insecticide in saline (see Materials and methods). There are three lines of evidence to support this interpretation. First, the maximum concentration of DMSO in the animals was 0.1% (by volume), and was probably slightly lower due to some dilution by residual hemolymph. It was thus below the lower limit for neural effects reported by Theophilidis and Kravari (1994). Second, we explicitly tested 0.1% DMSO in saline in control experiments without pymetrozine and did not find any effects (Ausborn, 2001). Finally, the inactive phenyl analogue of pymetrozine was applied in the same solvent as pymetrozine, that is with the same DMSO concentrations. Application of the phenyl analogue had no effect, even at the highest concentrations (10^{-5} mol l^{-1} and 0.1%, respectively). This experiment served as a control for both pymetrozine and DMSO.

Pymetrozine has an all-or-none effect on the fCO and on the reflex responses elicited by it. That is, either the full effect is present or there is no effect at all. Dose dependency manifests itself only in an increased number of unaffected animals at lower insecticide concentrations, while there is no qualitative change in pymetrozine effects, when they are present. The threshold for pymetrozine effects on the fCO is ~10^{-8} mol l^{-1}. This agrees well with previous observations on isolated locust ganglia and foregut (Kaufmann et al., 2004), which exhibited similar all-or-none characteristics with the same concentration threshold. This similarity in threshold supports our present interpretation that the chordotonal organs are the primary site of pymetrozine action.

Postural control in the femur–tibia joint is, of course, impossible without sensory feedback from the fCO. This may explain the altered position of the femur–tibia joint after pymetrozine injection (Fig. 2) and the apparent impairment of postural control. In particular, the tonic spike discharge often observed initially after pymetrozine application will signal to the central nervous system a certain, fixed leg position, which is translated into corresponding muscle activity and resulting joint posture. The fact that the legs are usually hyperextended after pymetrozine application argues for a predominance of tonic discharges in fCO afferents that signal flexed joint positions. And when the fCO falls silent after some time, the feedback loop appears to show some bias into the same direction.

Inactivation of femur–tibia joint control may also affect neighbouring leg joints, namely the tarsal and subcoxal joints, in the context of interjoint reflexes (e.g. Field and Rind, 1981; Heß and Büschges, 1997; Bucher et al., 2003). It is not clear, at present, whether the behavioural effects of pymetrozine application can be attributed entirely to the absence of fCO input and its ramifications through reflex pathways or whether additional effects have to be considered. The present data do not provide more specific suggestions for any additional effects.

Effects on central interneurons are one possibility here. They would have to be minor by comparison with the impact on the fCO but might still have behavioural consequences through amplification in motor control networks. No effects on the central processing of femur–tibia joint control signals were observed in the present study, nor were there effects on sensory receptors other than chordotonal organs. However, the present experiments cannot rule out more inconspicuous effects, for

instance on membrane properties and spike shape. In fact, Riewe (2001) observed slightly increased membrane resistance and broadened action potentials after pymetrozine application in the fast extensor tibiae motoneuron of the locust *Schistocerca gregaria*. Studies by Kayser and co-workers (Kaufmann et al., 2004) suggested effects on serotonergic control (see below).

During active, walking-like movements, severe deficits are evident, much like in postural control. This concerns not just coordination between legs – often almost synchronous movements in the legs of one body segment – but also movements and joint positions in individual legs (Fig. 12; see also Kaufmann et al., 2004). These are in general agreement with the deficits observed in postural control, for instance more-extended femur–tibia joints, lifted subcoxal joints and lifted tarsi. More detailed interpretations are, again, not possible. Most remarkable is the fact that after pymetrozine application, and with the resulting complete absence of sensory feedback from the fCO, walking-like movements are possible at all. The fCO plays a crucial role in current models of leg motor control (reviews, for example, in Büschges, 2005; Cruse et al., 1995), for instance the transition from swing to stance phases (see also review in Pearson, 1993). The present results suggest that our current picture of insect leg motor control may not be complete yet. In particular, fast walking, as in the escape situations elicited here, and the interaction of centrally programmed movement modules and shaping through sensory feedback, especially from the fCO, may merit further scrutiny.

In a preceding study (Kaufmann et al., 2004), pymetrozine enhanced spontaneous spike discharges of the metathoracic and subesophageal ganglia *in situ*. Moreover, pymetrozine effects were mimicked by serotonin, and pymetrozine and serotonin potentiated each other's effects. These findings may be interpreted as indications for central nervous effects of the insecticide. Considering the present results, however, it appears equally possible that the sensory effects of pymetrozine had been translated into altered spike discharges in the motor nerves of the metathoracic and subesophageal ganglia. For instance, in a reduced preparation that is not deafferented, the raising and stretching of the legs observed in the intact animal (Fig. 2) may well be reflected by an increased discharge of levator and extensor motoneurons. Similarly, the serotonergic effects might result from the modulation of chordotonal sensillae, or of other sensory cells, by serotonin (e.g. Ramirez and Orchard, 1990; Kloppenburg et al., 1999) in a way mimicking and potentiating pymetrozine effects.

Pymetrozine may well have other target sites in addition to the chordotonal sensillae demonstrated here. This is suggested by the effects of the insecticide on the motility of isolated foregut preparations, which is again blocked by serotonin antagonists (Kaufmann et al., 2004). It is completely unclear at present whether or not these effects may be produced by similar cellular mechanisms, perhaps sensory structures associated with the ingluvial ganglia.

Irrespective of the occurrence of – by comparison, minor – effects on pathways in the central nervous system transmitting sensory information from the fCO, it should be possible to predict deficits elicited by pymetrozine through its action on chordotonal organs not studied here (review in Field and Matheson, 1998). These are, for instance, Johnston's organs in the bases of the antennae. Maintenance of antenna position and perception of air movement should be impaired by pymetrozine when extrapolating the present findings. Such experiments will provide critical tests for the conclusions outlined here.

Having identified the primary mechanism of pymetrozine action in the locust, it will be interesting to scrutinise the present results in the main pest species affected by the insecticide, namely aphids, white flies and rice hoppers. Even more significant will be the study of the cellular basis of pymetrozine action, which is possible now with chordotonal sensillae identified as the target neurons. This is all the more important in view of the apparent specificity of pymetrozine for chordotonal sensillae, as opposed to other mechanoreceptors. One prediction is possible already when comparing our present results with pharmacological data on locust mechanoreceptors from the literature, particularly Ramirez and Pearson (1990). Fast sodium ion channels responsive to phentolamine are ubiquitous in sensory neurons and can be safely excluded as the site of action of pymetrozine, like all compounds acting on spike propagation. What appears more promising is the fact that chordotonal organs are the only insect sense organs with a fully developed ciliary apparatus, the molecular components of which have recently received notable attention (review in Field, 2005). This may allow identification of the cellular processes underlying pymetrozine action.

Considering this specificity, pymetrozine may prove useful as a tool for the specific elimination of chordotonal organ input in insect neurobiology in general. On the one hand, it is often difficult or impossible to remove a particular sensory input in neurobiological experiments. On the other hand, such experiments are essential, for example, when examining the roles of sense organs and central nervous system in motor control (e.g. Wolf and Pearson, 1988). At least in the case of chordotonal organs, these problems may be outdated due to the specific action of pymetrozine, in particular if this should prove true for a broader spectrum of insect species.

We are indebted to Syngenta and the University of Ulm for financial support. Steffen Harzsch, Uwe Rose and Wolfgang Stein were important partners for discussion and planning of experiments. Thanks are due to Cornelia Beck, Kerstin Braunstein, Alexandra Lipp, Birgit Müller, Thomas Niesner, Carmen Smarandache and Matthias Wittlinger. They all contributed to experiments in the contexts of lab courses, often the crucial initial tests that allowed planning of the experiments reported here. Tove Heller provided excellent technical assistance and Ursula Seifert perfected figures and English text.

References

Ausborn, J. (2001). *Der Angriffsort eines neuroaktiven Insektizids, untersucht am Gelenkstellungsregelkreis der Wanderheuschrecke*. Ulm: Staatsexamensarbeit Universität Ulm.

Ausborn, J., Wolf, H. and Kayser, H. (2001). A neuroactive insecticide disables mechanoreceptor neurons. In *Proceedings of the 4th Meeting of the German Neuroscience Society*, vol. II, *28th Göttingen Neurobiology Conference* (ed. N. Elsner and G. W. Kreutzberg), p. 885. Stuttgart: Thieme-Verlag.

Bacon, J. and Tyrer, M. (1978). The tritocerebral commissure giant (TCG): a bimodal interneurone in the locust, *Schistocerca gregaria*. *J. Comp. Physiol.* **126**, 317-325.

Bässler, U. (1993). The femur–tibia control system of stick insects: a model system for the study of neural basis of joint control. *Brain Res. Revs*. **18**, 207-226.

Bloomquist, J. R. (1996). Ion channels as targets for insecticides. *Annu. Rev. Entomol.* **41**, 163-190.

Boyan, G. S. and Ball, E. E. (1990). Neuronal organization and information processing in the wind-sensitive cercal receptor/giant interneurone system of the locust and other orthopteroid insects. *Prog. Neurobiol*. **35**, 217-243.

Bucher, D., Akay, T., DiCaprio, R. D. and Büschges, A. (2003). Interjoint coordination in the stick insect leg control system: the role of positional signalling. *J. Neurophysiol*. **89**, 1245-1255.

Burns, M. D. (1973). The control of walking in Orthoptera. I. Leg movements in normal walking. *J. Exp. Biol.* **58**, 45-58.

Burns, M. D. (1974). Structure and physiology of the locust femoral chordotonal organ. *J. Insect Physiol.* **20**, 1319-1339.

Burns, M. D. and Usherwood, P. N. R. (1979). The control of walking in Orthoptera. II. Motor neurone activity in normal free-walking animals. *J. Exp. Biol.* **79**, 69-98.

Burrows, M. (1996). *The Neurobiology of an Insect Brain*. Oxford: Oxford University Press.

Büschges, A. (1994). The physiology of sensory cells in the ventral scoloparium of the stick insect femoral chordotonal organ. *J. Exp. Biol.* **189**, 285-292.

Büschges, A. (2005). Sensory control and organization of neuronal networks mediating coordination of multisegmental organs for locomotion. *J. Neurophysiol*. **93**, 1127-1135.

Campbell, J. I. (1961). The anatomy of the nervous system of the mesothorax of *Locusta migratoria migratorioides* (R. and F.). *Proc. Zool. Soc. Lond*. **137**, 403-432.

Cruse, H., Brunn, D. E., Bartling, Ch., Dean, J., Dreifert, M., Kindermann, T. and Schmitz, J. (1995). Walking: a complex behavior controlled by simple networks. *Adapt. Behav*. **3**, 385-418.

Ebner, I. and Bässler, U. (1978). Zur Regelung der Stellung des Femur–tibia-Gelenks im Mesothorax der Wanderheuschrecke *Schistocerca gregaria* (Forskål). *Biol. Cybern*. **29**, 83-96.

Field, L. H. (2005). The chordotonal organ: A unique invertebrate mechanoreceptor. In *Methods in Insect Sensory Neuroscience* (ed. T. A. Christensen), pp. 61-105. Boca Raton, Florida: CRC Press.

Field, L. H. and Burrows, M. (1982). Reflex effects of the femoral chordotonal organ upon leg motor neurons of the locust. *J. Exp. Biol.* **101**, 265-285.

Field, L. H. and Matheson, T. (1998). Chordotonal organs of insects. *Adv. Insect Physiol.* **27**, 1-228.

Field, L. H. and Pflüger, H.-J. (1989). The femoral chordotonal organ: a bifunctional orthopteran (*Locusta migratoria)* sense organ? *Comp. Biochem. Physiol.* **93A**, 729-743.

Field, L. H. and Rind, F. C. (1981). A single insect chordotonal organ mediates inter- and intrasegmental leg reflexes. *Comp. Biochem. Physiol.* **68A**, 99-102.

Fischer, H., Wolf, H. and Büschges, A. (2002). The locust tegula: kinematic parameters and activity pattern during the wing stroke. *J. Exp. Biol.* **205**, 1531-1545.

Gettrup, E. (1966). Sensory regulation of wing twisting in locusts. *J. Exp. Biol.* **44**, 1-16.

Harrewijn, P. and Kayser, H. (1997). Pymetrozine, a fast-acting and selective inhibitor of aphid feeding. In-situ studies with electronic monitoring of feeding behaviour. *Pestic. Sci.* **49**, 130-140.

Heß, D. and Büschges, A. (1997). Sensorimotor pathways involved in interjoint reflex action of an insect leg. *J. Neurobiol.* **33**, 891-913.

Hofmann, T. and Koch, U. T. (1985). Acceleration receptors in the femoral chordotonal organ of the stick insect, *Cuniculina impigra*. *J. Exp. Biol.* **114**, 225-237.

Hofmann, T., Koch. U. T. and Bässler, U. (1985). Physiology of the femoral chordotonal organ in the stick insect, *Cuniculina impigra*. *J. Exp. Biol.* **114**, 207-223.

Kaufmann, L., Schürmann, F., Yiallouros, M., Harrewijn, P. and Kayser, H. (2004). The serotonergic system is involved in feeding inhibition by pymetrozine. Studies on a locust (*Locusta migratoria*) and an aphid (*Myzus persicae*). *Comp. Biochem. Physiol.* **138C**, 469-483.

Kien, J. (1983). The initiation and maintenance of walking in the locust: an alternative to the command neuron concept. *Proc. R. Soc. Lond. B* **219**, 137-174.

Kloppenburg, P., Ferns, D. and Mercer, A. (1999). Serotonin enhances central olfactory neuron responses to female sex pheromone in the male sphinx moth *Manduca sexta. J. Neurosci.* **19**, 8172-8181.

Kristinsson, H. (1994). Pymetrozine: A new insecticide. In *Advances in the Chemistry of Insect Control III*, pp. 85-102. Cambridge: The Royal Society of Chemistry.

Kutsch, W., Hanloser, H. and Reinecke, M. (1980). Light and electron-microscopic analysis of a complex sensory organ: the tegula of *Locusta migratoria*. *Cell Tissue Res.* **210**, 461-478.

Matsuda, K., Buckingham, S. D., Kleier, D., Rauh, J. J., Grauso, M. and Sattelle, D. B. (2001). Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. *Trends Pharmacol. Sci.* **22**, 573-580.

Möhl, B. (1985). The role of proprioception in locust flight control. II. Information signalled by the forewing stretch receptors during flight. *J. Comp. Physiol. A* **156**, 103-116.

Mücke, A. (1991). Innervation pattern and sensory supply of the midleg of *Schistocerca gregaria* (Insecta, Orthopteroidea). *Zoomorphology* **110**, 175-187.

Newland, P. L. and Emptage, N. J. (1996). The central connections and actions during walking of tibial campaniform sensilla in the locust. *J. Comp. Physiol. A* **178**, 749-762.

Pearson, K. G. (1993). Common principles of motor control in vertebrates and invertebrates. *Annu. Rev. Neurosci.* **16**, 265-297.

Pearson, K. G., Hedwig, B. and Wolf, H. (1989). Are the hindwing chordotonal organs elements of the locust flight pattern generator? *J. Exp. Biol.* **144**, 235-255.

Pringle, J. W. S. (1938). Proprioception in insects. II. The action of campaniform sensilla on the legs. *J. Exp. Biol.* **15**, 114-131.

Ramirez, J. M. and Orchard, I. (1990). Octopaminergic modulation of the forewing stretch receptor in the locust *Locusta migratoria*. *J. Exp. Biol.* **149**, 255-279.

Ramirez, J. M. and Pearson, K. G. (1990). Local anesthetic action of phentolamine on insect mechanoreceptors. *J. Comp. Physiol. A* **167**, 475-483.

Riewe, P. (2001). Pymetrozine changes the membrane properties of an insect motoneuron. In *Proceedings of the 4th Meeting of the German Neuroscience Society*, vol. II, *28th Göttingen Neurobiology Conference* (ed. N. Elsner and G. W. Kreutzberg), p. 895. Stuttgart: Thieme Verlag.

Schmitz, J., Büschges, A. and Delcomyn, F. (1988). An improved electrode design for *en passant* recording from small nerves. *Comp. Biochem. Physiol*. **91A**, 769-722.

Snodgrass, R. E. (1929). The thoracic mechanism of a grasshopper and its antecedents. *Smithson. Misc. Collns*. **82**, 1-111.

Stein, W. and Sauer, A. E. (1999). Physiology of vibration-sensitive afferents in the femoral chordotonal organ of the stick insect. *J. Comp. Physiol. A* **184**, 253-263.

Theophilidis, G. and Burns, M. D. (1983). The innervation of the mesothoracic flexor tibiae muscle of the locust. *J. Exp. Biol.* **105**, 373-388.

Theophilidis, G. and Burns, M. D. (1990). The firing pattern of the locust (*Schistocerca gregaria americana*) mesothoracic femoral motor axons in resistance reflexes and during walking on a treadmill. *J. Insect Physiol.* **36**, 513-522.

Theophilidis, G. and Kravari, K. (1994). Dimethylsulfoxide (DMSO) eliminates the response of the sensory neurones of an insect mechanoreceptor, the femoral chordotonal organ of *Locusta migratoria*, but blocks conduction of their sensory axons at much higher concentration: a possible mechanism of analgesia. *Neurosci. Lett.* **181**, 91-94.

Usherwood, P. N. R. and Grundfest, H. (1965). Peripheral inhibition in skeletal muscle of insects. *J. Neurophysiol*. **28**, 497-518.

von Helversen, O. and Elsner, N. (1977). The stridulatory movements of acridid grasshoppers recorded with an opto-electronic device. *J. Comp. Physiol. A* **122**, 53-64.

Weiland, G., Bässler, U. and Brunner, M. (1986). A biological feedback control system with electronic input: the artificially closed femur–tibia control system of stick insects. *J. Exp. Biol.* **120**, 369-385.

Wolf, H. (1990). Activity patterns of inhibitory motoneurons and their impact on leg movement in tethered walking locusts. *J. Exp. Biol.* **152**, 281-304.

Wolf, H. (1992). Reflex modulation in locusts walking on a treadwheel – intracellular recordings from motoneurons. *J. Comp. Physiol. A* **170**, 443-462.

Wolf, H. and Pearson, K. G. (1988). Proprioceptive input patterns elevator activity in the locust flight system. *J. Neurophysiol.* **59**, 1831-1853.

Wyss, P. and Bolinger, M. (1997). Translocation of pymetrozine in plants. *Pestic. Sci.* **50**, 195-202.