The Journal of Experimental Biology **198**, 435–456 (1995)
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RHYTHMIC PATTERNS IN THE THORACIC NERVE CORD OF THE STICK INSECT INDUCED BY PILOCARPINE

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Accepted 22 September 1994

Summary

Bath application of the muscarinic agonist pilocarpine onto the deafferented stick insect thoracic nerve cord induced long-lasting rhythmic activity in leg motoneurones. Rhythmicity was induced at concentrations as low as 1×10^{-4} mol l^{-1} pilocarpine. The most stable rhythms were reliably elicited at concentrations from 2×10^{-3} mol l^{-1} to 5×10^{-3} mol l^{-1}. Rhythmicity could be completely abolished by application of atropine. The rhythm in antagonistic motoneurone pools of the three proximal leg joints, the subcoxal, the coxo-trochanteral (CT) and the femoro-tibial (FT), was strictly alternating. In the subcoxal motoneurones, the rhythm was characterised by the retractor burst duration being correlated with cycle period, whereas the protractor burst duration was almost independent of it. The cycle periods of the rhythms in the subcoxal and CT motoneurone pools were in a similar range for a given preparation. In contrast, the rhythm exhibited by motoneurones supplying the FT joint often had about half the duration. The pilocarpine-induced rhythm was generated independently in each hemiganglion. There was no strict intersegmental coupling, although the protractor motoneurone pools of the three thoracic ganglia tended to be active in phase.

There was no stereotyped cycle-to-cycle coupling in the activities of the motoneurone pools of the subcoxal joint, the CT joint and the FT joint in an isolated mesothoracic ganglion. However, three distinct 'spontaneous, recurrent patterns' (SRPs) of motoneuronal activity were reliably generated. Within each pattern, there was strong coupling of the activity of the motoneurone pools. The SRPs resembled the motor output during step-phase transitions in walking: for example, the most often generated SRP (SRP1) was exclusively exhibited coincident with a burst of the fast depressor trochanteris motoneurone. During this burst, there was a switch from subcoxal protractor to retractor activity after a constant latency. The activity of the FT joint extensor motoneurones was strongly decreased during SRP1. SRP1 thus qualitatively resembled the motoneuronal activity during the transition from swing to stance of the middle legs in forward walking. Hence, we refer to SRPs as 'fictive step-phase transitions'.

In intact, restrained animals, application of pilocarpine also induced alternating activity in antagonistic motoneurone pools supplying the proximal leg joints. However, there were marked differences from the deafferented preparation. For example, SRP1 was not generated in the latter situation. However, if the ipsilateral main leg nerve was cut, SRP1s reliably occurred. Our results on the rhythmicity in leg motoneurone pools of deafferented preparations demonstrate central coupling in the activity of the leg motoneurones that might be incorporated into the generation of locomotion *in vivo*.

Key words: rhythm generation, walking, central pattern generator, joint coordination, muscarinic agonists, step-phase transition, invertebrate acetylcholine receptor, *Carausius morosus*, stick insect.

Introduction

The neural basis of rhythmic movements has been studied extensively in a variety of systems. It is now generally accepted that rhythmic motor activity used for locomotion is generated by an integration of central and peripheral (sensory) mechanisms. Emphasis has sometimes been placed on the peripheral influences (e.g. Bässler, 1988; Sherrington, 1913) and sometimes on the central influences (e.g. Delcomyn, 1980; von Holst, 1939; Selverston *et al.* 1976; Wilson, 1961; see also Grillner, 1985). Although peripheral influences are well known for most systems, investigations have, for practical reasons, usually focused primarily on the generation of rhythmic motor activity by central pattern generators (CPGs). A CPG can be studied in an isolated part of the nervous system, whereas the investigation of a peripheral influence requires at least a semi-intact preparation. Isolated parts of some central nervous systems (CNS), either *in vivo* or *in vitro*, can produce a motor output, spontaneously or under the influence of certain drugs. In some systems, this motor output is similar to the motor

output during a particular rhythmic movement and can therefore be considered as the output of a CPG (e.g. cat walking, Grillner and Zangger, 1984; crayfish swimmeret system, Ikeda and Wiersma, 1964; lamprey swimming, Grillner, 1985; leech swimming, Kristan and Weeks, 1983; leech heartbeat, Calabrese and Arbas, 1989; Thompson and Stent, 1976; locust flight, Pearson and Ramirez, 1992; Robertson and Pearson, 1985; Stevenson and Kutsch, 1987; Wilson, 1961; mollusc swimming, Arshavsky *et al.* 1993; stomatogastric system, Harris-Warrick *et al.* 1992; Selverston and Moulins, 1987; *Tritonia* swimming, Getting, 1989; *Xenopus* embryo swimming, Arshavsky *et al.* 1993). In motor systems underlying locomotion, such motor output is called 'fictive locomotion'.

Neuronal mechanisms underlying the generation of fictive locomotion can only be considered to be the mechanisms underlying the generation of real locomotion if two requirements are met. (1) The role that the CPG plays in the isolated CNS must not differ from its role in the intact animal (Bässler, 1986; Pearson, 1987). This requirement can only be tested when at least some features of pattern generation in the intact animal are known. (2) The fictive locomotion must show at least the basic characteristics of the motor output of the intact system. Interpretation of this requirement depends greatly upon the depth of knowledge about the intact motor output and on the theoretical standpoint of the investigator.

Until recently, fictive locomotory patterns were unknown in arthropod walking systems. This led to the conclusion that CPGs for walking do not exist in arthropods (Bässler and Wegner, 1983; Pearson, 1985, 1987). It has recently been shown in both the crayfish (Chrachri and Clarac, 1990) and the locust (Ryckebusch and Laurent, 1993) that muscarinic agonists, e.g. pilocarpine, induce 'walking-like' rhythms in leg motoneurones of isolated thoracic ganglia. Because of similarities between these rhythms and the motor outputs during walking, the evoked activity was called 'fictive locomotion'. These results seem to contradict earlier findings on the stick insect.

In the stick insect, the movements of the legs (Bässler, 1993; Graham, 1985; Wendler, 1964), the torques generated at single leg joints (Cruse, 1976) and the motor output of the different motoneuronal pools have been described in great detail (e.g. Büschges *et al.* 1994; Epstein and Graham, 1983; Graham and Epstein, 1985; Graham and Wendler, 1981*a*; Schmitz and Haßfeld, 1989). All these elements depend greatly upon the walking situation (e.g. Cruse *et al.* 1993; Foth and Graham, 1983*a*,*b*; Graham and Wendler, 1981*a*; Schmitz, 1993; for reviews, see Bässler, 1983; Graham, 1985). Much is also known about the sensory influences on the generation of the motor output of the walking stick insect (for reviews, see Bässler, 1983; Cruse, 1990; Graham, 1985). There is no evidence for a CPG in the thoracic ganglia that, by itself, produces a coordinated motor pattern for walking. The proper coordination of several joints requires sensory feedback (Bässler and Wegner, 1983; Bässler, 1993). Present evidence suggests that the neural network that produces the motor activity underlying walking in the stick insect is a modular system composed of both peripheral and central elements (Bässler, 1993). Neither peripheral nor central elements alone can generate coordinated motor output. Only an interactive cooperation of these modules is able to generate an appropriate motor pattern (Bässler, 1993; Bässler and Nothof, 1991). Nonspiking interneurones underlying sensory–motor pathways within the walking system appear to be part of these modules, organising the motor output of leg joints in the standing as well as in the walking animal (Bässler and Büschges, 1990; Büschges, 1990; Büschges *et al.* 1994; Schmitz *et al.* 1991).

In the light of these results, we set out to investigate the effect of pilocarpine on the thoracic nervous system of the stick insect. Because so many details of the walking motor output are known and because some basic principles of pattern generation are also known (e.g. the modular organisation), it should be possible to test the above requirements of fictive locomotion for the stick insect. We mainly focused on rhythms in the middle leg motoneurones supplying the three main leg joints, i.e. the subcoxal joint, the coxa–trochanter (CT) joint and the femur–tibia (FT) joint.

We show that pilocarpine induces long-lasting alternating rhythmicity in the antagonistic motoneurone pools supplying the three proximal leg joints in isolated and in partially deafferented thoracic ganglia. In the stick insect, pilocarpine does not induce a motor pattern that could be termed 'fictive locomotion', as we did not observe a cycle-to-cycle coupling between the rhythms in the motoneurone pools supplying the different leg joints. Nevertheless, pilocarpine reliably induces distinct patterns of coupled motor activity in leg motoneurone pools. These patterns are termed 'fictive step-phase transitions'.

Materials and methods

Experiments were performed on adult female stick insects of the species *Carausius morosus* (Br.) taken from laboratory cultures at the Universities of Kaiserslautern and Bielefeld. All experiments were carried out under daylight conditions at room temperature (20–22 ˚C). The data presented were gathered from 124 experiments.

Preparation

Prior to experiments on the thoracic nerve cord, all legs of the stick insect were amputated at the femur, distal to the trochanteral autotomy point. The animals were then mounted dorsal side up on a foam platform using dental cement. A cut was made along the dorsal midline of the pro-, meso- and metathoracic segments and along the first three abdominal segments. The thorax was opened by bending both tergal halves away from each other to the right and to the left. This procedure moved the coxae of the legs ventrally into cavities of the platform. The left and the right parts of the tergum were fixed with minuten pins onto the foam platform so that the thorax formed a 'bathtub'. Ventilation of the nervous system was maintained through the trachea that remained intact. The

gut was removed. The following lateral nerves (labelled according to Marquardt, 1940) were cut close to the thoracic ganglia: nervus anterior (n_a); nervus posterior (n_p), nervus unparis (n_{up}). All other lateral leg nerves; nervus lateralis 2–5 (nl2–nl5) and nervus cruris (n_{cr}) were cut as well, after positioning an extracellular hook electrode on some of them. In preparations using the isolated mesothoracic ganglion, both anterior and posterior connectives of the mesothoracic ganglion were cut. In preparations concerning the action of pilocarpine on the thoracic nerve cord, the connectives between the prothoracic ganglion and the suboesophageal ganglion were cut (see text). Stick insect saline (Weidler and Diecke, 1969) was then applied dropwise into the body cavity. This preparation remained healthy for at least 120 min, and often up to 180 min. In the experiments on intact, but restrained, animals all legs and leg nerves were left intact. In some experiments, the leg nerves were then severed during the experiments (see Fig. 14 and text).

Stock solutions of 10^{-2} mol l^{-1} pilocarpine and atropine sulphate (Sigma) in saline were prepared in advance and diluted with saline to their final concentrations prior to application. Application of the drugs was performed by removing the saline from the thorax of the experimental animal and replacing it with the drug solution under investigation at a final concentration of 10^{-5} to 10^{-2} mol l^{-1}.

Electrophysiology

Activity of the protractor coxae, retractor coxae, levator trochanteris, depressor trochanteris and extensor tibiae motoneurones was monitored in the appropriate leg nerves by using extracellular hook electrodes (Schmitz *et al.* 1988). The identification of motoneurones in recordings from the lateral nerves was, in all cases, unambiguous, as the recorded nerves either carried only axons of the motoneurones under investigation (e.g. C1, C2, innervating the muscles of the CT joint, see below), or the axons of the motoneurones that were being investigated had the largest diameter and so produced the biggest spikes in the nerve recording (retractor motoneurones in nl5; protractor motoneurones in nl2, Graham and Wendler, 1981*b*; FETi, SETi in nl3; for the relationship between motor axon diameter and extracellularly recorded action potential amplitude, see Pearson *et al.* 1970). In several experiments, intracellular recordings were obtained from neuropilar processes of certain motoneurones in the mesothoracic ganglion. To do this, the ganglion was placed on a wax-coated platform and treated according to established procedures (Büschges, 1989). Intracellular recordings were performed using thin-walled glass microelectrodes with resistances of 40–60 MΩ when the tips were filled with 5 % Lucifer Yellow and the shanks were filled with 1 mol l^{-1} LiCl. The motoneurones were identified either by their extracellularly recorded spikes or by their morphology (Graham and Wendler, 1981*a*,*b*; Storrer *et al.* 1986). Morphology was determined by staining the neurones with Lucifer Yellow according to established procedures. All data were stored either on a seven-channel FM recorder (Racal Store 7) or on an eight-channel digital audio tape recorder (Biologic, DTR 1800 or 1802).

Nomenclature

Motor nerves were named according to Bässler (1983), Graham and Wendler (1981*b*) and Marquardt (1940). The following abbreviations are used in the present paper: CI1, common inhibitor 1 motoneurone; C1, nervus C1, innervating the levator trochanteris muscle; C2, nervus C2, innervating the depressor trochanteris muscle; FDTr, fast depressor trochanteris motoneurone; FETi, fast extensor tibiae motoneurone; nl2, nervus lateralis 2, carrying the protractor coxae motoneurones; nl3, nervus lateralis 3, carrying the extensor tibiae motoneurones; nl5, nervus lateralis 5, carrying the retractor coxae motoneurones; SDTr, slow depressor trochanteris motoneurone; SETi, slow extensor tibiae motoneurone.

Data analysis

The data were either displayed on a Gould ES1000 chart recorder or analysed off-line. Analog-to-digital conversion was performed by using either a DT 2821/f 16 SE AD/DA card and the program Turbolab V4.0 (Stemmer) or a CED 1401plus (Cambridge Electronic Co.). Further analysis was performed with the program Spike2 (Cambridge Electronic Co.). This program was used to create phase histograms of the recorded motoneuronal activity.

Pilocarpine-induced activity of the motoneurone pools consisted of bursts of spikes. Fast motoneurones have bigger axon diameters in the motor nerves than do slow motoneurones. Fast and slow motoneurones could therefore be reliably distinguished by their different extracellular spike amplitudes (Bässler, 1983; Graham and Wendler, 1981*b*; Schmitz, 1986; see also Pearson *et al.* 1970). The onset and the offset of a burst of activity in the excitatory motoneurone pools were usually clearly defined by their abruptness. Usually the slow and the fast motoneurones of a motor nerve exhibited clear bursting activity. In a few cases, one or two slow motoneurones (from the retractor and the protractor coxae nerve or the levator trochanteris nerve) showed weakly modulated activity that was tonically increased during pilocarpine application. The only motoneurone that was regularly found not to show bursts, but instead had increased tonic and modulated activity following pilocarpine application, was the common inhibitor 1 (CI1). All cycles of rhythmicity of each motor nerve of an evaluated animal were used to calculate mean values. Circular statistics (see Fig. 9) were performed according to Batschelet (1965); beside the mean vector length (**r**), the test variable $z=n\mathbf{r}^2$ was calculated for testing significance by means of the Rayleigh test.

Results

Pilocarpine-induced rhythms in the subcoxal joint motoneurones of the isolated mesothoracic ganglion

Recordings from nerves that contain the axons of

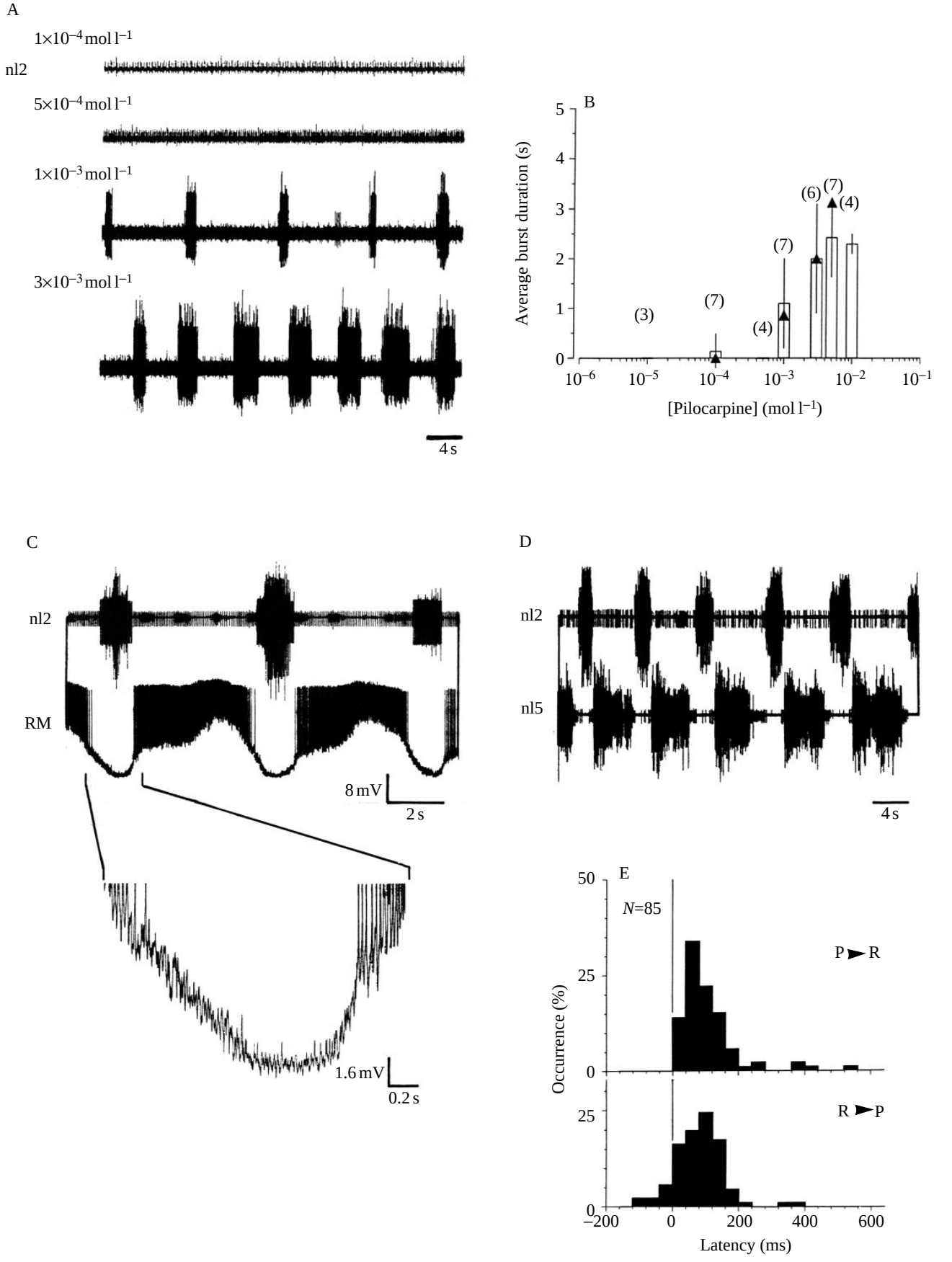
A
1×10⁻⁴ mol l⁻¹
nl2
5×10⁻⁴ mol l⁻¹
1×10⁻³ mol l⁻¹
3×10⁻³ mol l⁻¹
4 s
B
Average burst duration (s)
(3)
(7)
(4)
(7)
(6)
(7)
(4)
[Pilocarpine] (mol l⁻¹)
C
nl2
RM
8 mV
2 s
1.6 mV
0.2 s
D
nl2
nl5
4 s
E
N=85
P ► R
R ► P
Occurrence (%)
Latency (ms)

Fig. 1

Fig. 1. Pilocarpine-induced rhythmicity in motoneurones of the subcoxal joint in isolated mesothoracic ganglia. (A) Recordings from the protractor nerve nl2 during bath application of pilocarpine of increasing concentration in one preparation. (B) Dependence of the average protractor burst duration on pilocarpine concentration. Values are means ± S.D. Data are from seven different preparations. Triangles denote the average protractor burst durations from a single preparation. Numbers in parentheses give the number of preparations tested at each concentration. (C) Alternating rhythms in excitatory protractor coxae motoneurones (nl2, extracellular record) and an intracellularly recorded retractor coxae motoneurone (RM) following bath application of pilocarpine. At the bottom, the end of one retractor motoneurone burst and the beginning of the next are shown on an expanded time scale (see text). The continuously firing small unit in the nl2 record is CI1 (see Fig. 8). (D) Alternating rhythmicity in the excitatory protractor coxae (nl2) and retractor coxae (nl5) motoneurone pools following bath application of 5×10^{-3} mol l^{-1} pilocarpine. (E) Latency between the bursts of slow motoneurones. Data are pooled from three different preparations. P→R, latency between the end of protractor burst activity and the beginning of retractor burst activity; R→P, latency between the end of retractor burst activity and the beginning of protractor burst activity.

motoneurones innervating the leg muscles in an isolated mesothoracic ganglion (i.e. all connectives cut) revealed weak tonic activity in some axons that produced very small action potentials in the extracellular recordings. When pilocarpine was applied at various concentrations onto the isolated mesothoracic ganglion, changes occurred in the activity of the recorded motoneurone pools. This is shown in Fig. 1 for the activity of the motoneurones of the protractor coxae muscle recorded in nerve nl2 (Graham and Wendler, 1981*b*). At a concentration of 5×10^{-4} mol l^{-1} pilocarpine, tonic activity was increased in some small units in this preparation (Fig. 1A). At concentrations of 1×10^{-3} mol l^{-1} and greater, pilocarpine induced clearly defined bursts of activity in the slow and fast motoneurones in the protractor nerve (Fig. 1A). CI1 also fired, but its modulation was different from the modulation of the excitatory motoneurones (see below). The threshold concentration for the induction of rhythmicity in the excitatory motoneurone pool was different in each preparation, ranging from 1×10^{-4} mol l^{-1} to 2×10^{-3} mol l^{-1} pilocarpine. During pilocarpine-induced activity, the burst duration of the excitatory protractor motoneurones varied somewhat within each animal as well as among animals. At 1×10^{-3} mol l^{-1} pilocarpine, the burst duration in one preparation ranged from 0.4 to 2.7 s (0.9±0.4 s; mean ± S.D., N=31). For different animals, the average burst duration in 1×10^{-3} mol l^{-1} pilocarpine solution ranged from 0.9 to 2.4 s (five animals, see Fig. 1B). In all cases, the mean burst duration in the excitatory protractor motoneurones increased with increasing concentrations of pilocarpine (Fig. 1A,B), reaching its maximum at concentrations of about 5×10^{-3} mol l^{-1}.

The cycle period of protractor activity (defined as the time between the onsets of two successive protractor bursts) also depended to some extent on the concentration of pilocarpine, but it was much more variable. At low pilocarpine concentrations, cycle periods were longer than at higher concentrations; for example, in 1×10^{-4} mol l^{-1} pilocarpine, cycle period was on average 17.9 s, and in 3×10^{-3} mol l^{-1}

Table 1. *Temporal characteristics of rhythmicity in different animals treated with pilocarpine at concentrations from 3×10^{-3} to 5×10^{-3} mol l^{-1}*

Animal number	Number of cycles	Mean period (s)	Nerve	Slope	Regression coefficient
1	46	5.6±1.6	nl2	0.033	0.180
			nl5	0.930	0.959
2	32	9.6±4.3	nl2	0.174	0.506
			nl5	0.750	0.944
3	31	4.1±1.7	nl2	0.074	0.450
			nl5	0.670	0.874
4	36	7.8±1.6	nl2	–0.038	–0.199
			nl5	0.997	0.938
5	34	7.0±1.7	nl2	–0.050	–0.200
			nl5	0.350	0.898
6	40	5.8±3.4	nl2	0.096	0.230
			nl5	0.880	0.911
	38	6.4±3.0	C1	0.400	0.643
			C2	0.570	0.687

Temporal characteristics of the pilocarpine-induced rhythmicity in the motoneurone pools of the stick insect mesothoracic ganglion (see also Fig. 2).

Number, number of experiment presented; Cycles, number of cycles evaluated; Mean period, average duration of the cycle period given with standard deviation; Nerve, nerve recorded from; Slope, slope of the regression line.

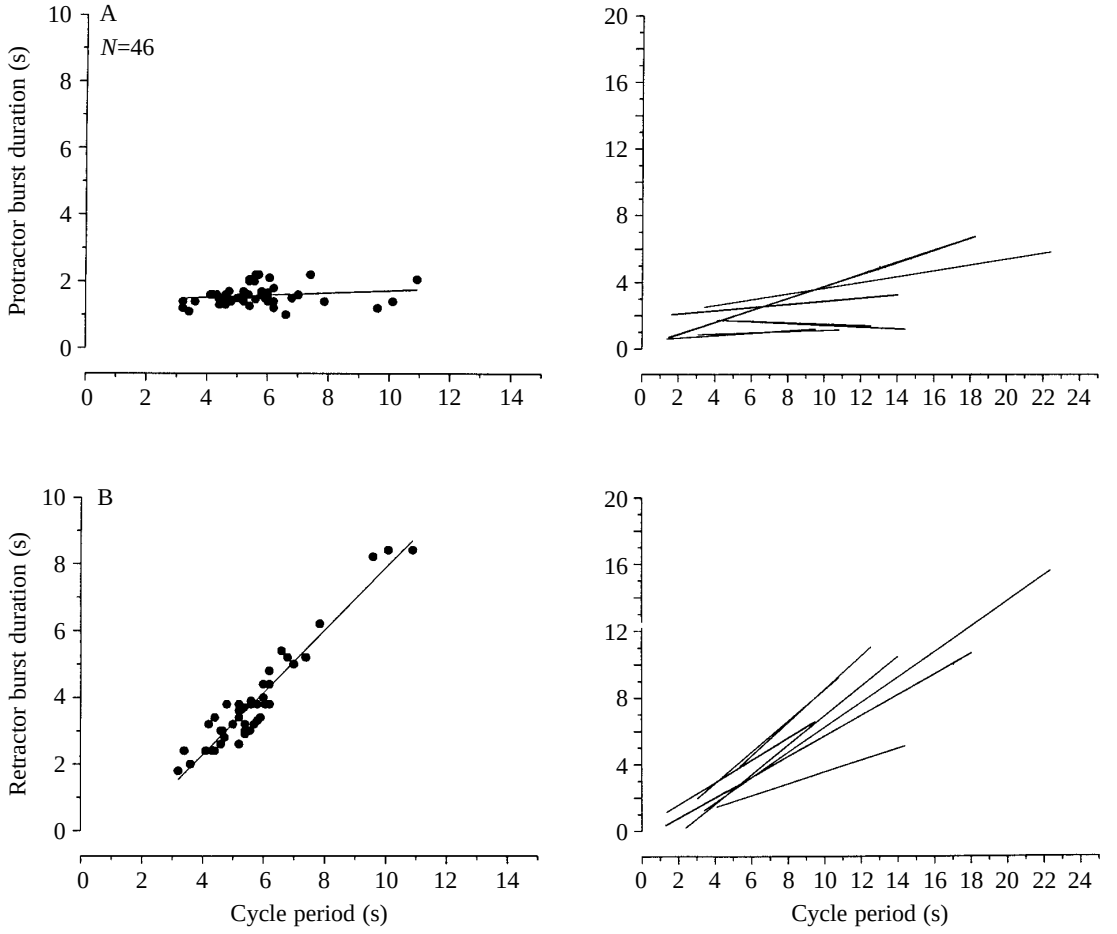

Fig. 2. Dependence of protractor (A) and retractor (B) burst duration on cycle period during rhythmic activity following application of 3×10^{-3} to 5×10^{-3} mol l^{-1} pilocarpine. Left, data points and regression lines for a single experiment; right, regression lines from six other experiments. See also Table 1.

pilocarpine, average cycle periods ranged from 4.9 to 9.4 s in different preparations. The most regular and the longest lasting rhythmicity was found for pilocarpine concentrations in the range 2×10^{-3} mol l^{-1} to 5×10^{-3} mol l^{-1}. In this range, the protractor burst duration was on average 2.4±0.9 s (*N*=283, eight animals) and the average cycle period was 6.4±1.5 s (*N*=283).

Simultaneous recordings of the activity of the excitatory retractor motoneurones in nerve nl5 (Graham and Wendler, 1981*b*) and the protractor motoneurones in nerve nl2 revealed that the retractor motoneurones were also rhythmically active following pilocarpine treatment. Application of pilocarpine induced an increase in the activity of the excitatory retractor motoneurones that led to bursting activity after a few seconds (5–30 s in different preparations). When rhythmicity was established, slow and fast retractor motoneurones displayed strong bursts of activity in antiphase with the activity of excitatory protractor motoneurones (Fig. 1C,D). This is particularly obvious from intracellular recordings of individual retractor motoneurones (Fig. 1C). The bursts of excitatory protractor and retractor motoneurones almost never overlapped (Fig. 1C–E). In the few cases when overlapping burst activity was observed, it was restricted to a few spikes exhibited by slow motoneurones in the retractor nerve at the end of retractor activity and the beginning of protractor activity. We never found protractor and retractor bursts to overlap at the end of a protractor burst (Fig. 1D,E), and we did not find fast motoneurones in both motor nerves to be active at the same time. These observations indicate that these antagonistic motoneurone pools did not burst independently. At pilocarpine concentrations that induced the most regular rhythmic activity (2×10^{-3} to 5×10^{-3} mol l^{-1}), retractor burst duration was on average 3.7±1.3 s (*N*=283, eight animals). At a given concentration of pilocarpine, the burst duration of the protractor motoneurones was independent of cycle period in most preparations (Fig. 2A; Table 1). In those few cases (numbers 2, 3 in Table 1) when there was a significant correlation between cycle period and protractor burst duration, the slopes were very close to zero. In contrast, retractor burst duration was always found to depend on cycle period (Fig. 2B; Table 1). This means that in the subcoxal motoneurone pools each individual cycle in pilocarpine-induced rhythms is

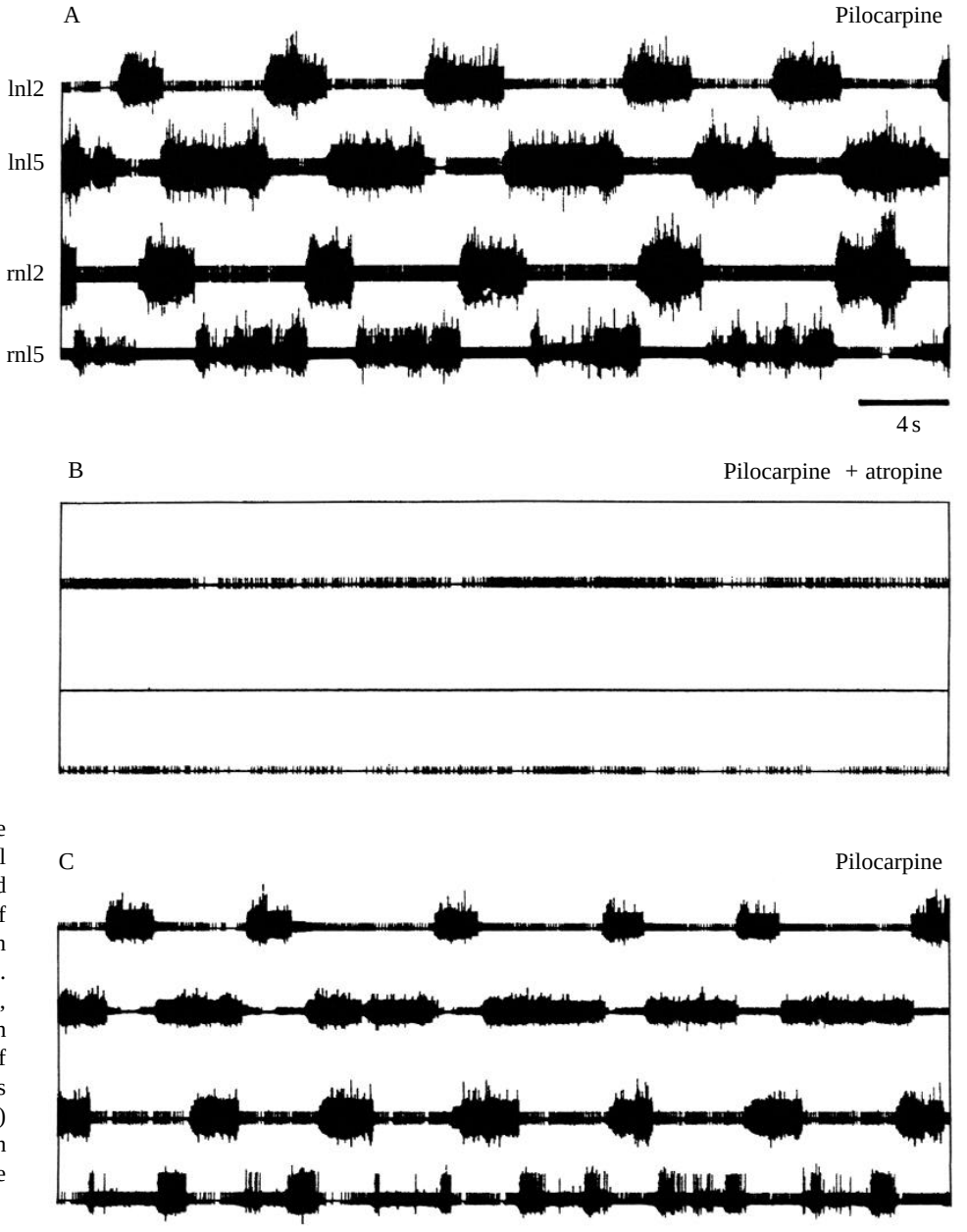

Fig. 3. Atropine blocks rhythms in the motoneurone pools supplying the subcoxal joint. (A) Rhythmicity in the protractor and retractor motoneurone pools of both sides of the mesothoracic ganglion following bath application of 2×10^{-3} mol l^{-1} pilocarpine. nl2, protractor nerve; nl5, retractor nerve; r, right; l, left. (B) Rhythmicity ceased within 1 min following bath application of 2×10^{-3} mol l^{-1} atropine plus 2×10^{-3} mol l^{-1} pilocarpine. (C) Rhythmicity was re-established after bath application of pilocarpine alone (2×10^{-3} mol l^{-1}).

composed of protractor bursts at rather constant duration and retractor bursts of changing duration, a characteristic that is typical for the tetrapod gait in stick insects (Graham, 1972, and see Discussion).

Addition of the muscarinic acetylcholine antagonist atropine at concentrations of 2×10^{-3} mol l^{-1} to the pilocarpine solution (1×10^{-3} to 5×10^{-3} mol l^{-1}) led to a cessation of the observed rhythm in the recorded motoneurone pools (Fig. 3A,B). After replacing the bath solution of pilocarpine and atropine with pilocarpine alone, rhythmicity was re-established (Fig. 3C).

Each hemiganglion is able to produce the observed rhythm

In most preparations (10 out of 11 preparations), both sides of the isolated mesothoracic ganglion exhibited rhythms simultaneously (Figs 3 and 4). Only once (i.e. in less than 10 % of all experiments) did rhythmic activity occur on only one side of the ganglion. In most cases of simultaneous rhythmicity on both sides, we found no strict cycle-to-cycle coupling between the opposite sides of the mesothoracic ganglion. In 7 out of 10 preparations, however, left and right excitatory protractor motoneurone pools tended to be active in phase (Fig. 4A,Bi). We never observed the two opposite excitatory protractor motoneurone pools showing rhythms in clear antiphase over several consecutive cycles.

Bisecting a mesothoracic hemiganglion along its midline using microscissors did not affect its ability to produce rhythms (Fig. 5; *N*=4). We did not find major differences in the activity of the recorded motoneurones after the operation. The same was true for the rhythmicity that was induced by pilocarpine

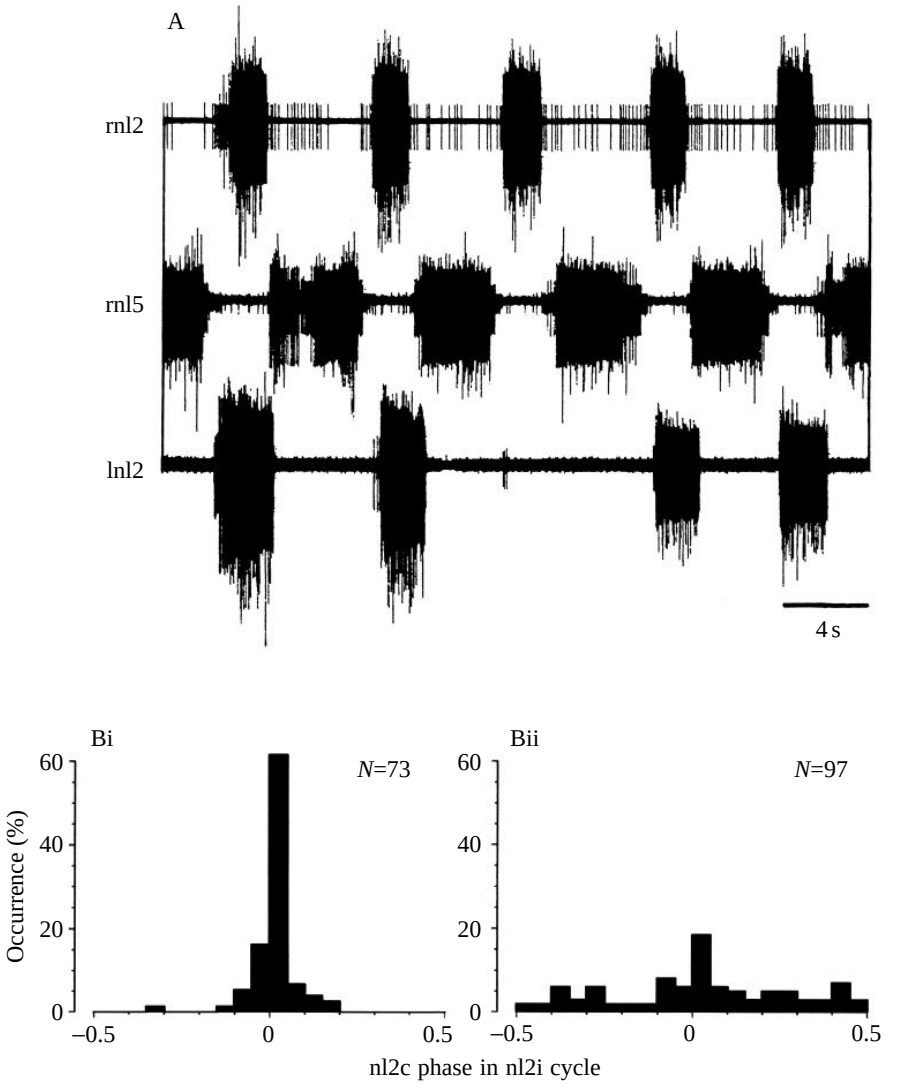

Fig. 4. Coupling between the rhythmic activity in the two mesothoracic hemiganglia. (A) Recordings of the rhythms in the motoneurones supplying the left (lnl2) and right (rnl2, rnl5) subcoxal joints. (Bi,ii) Phase histograms of the onset of activity of the contralateral protractor motoneurones (nl2c) in the ipsilateral protractor motoneurone cycle (nl2i) for two experiments (data for Bi derive from the experiment shown in A). *N* values refer to the number of cycles evaluated.

in the isolated prothoracic (not shown) and metathoracic ganglia (Fig. 5). Even in hemiganglia that had been cut prior to pilocarpine application, rhythmicity was established following pilocarpine application (not shown).

Coupling of the rhythmic activity in the isolated thoracic nerve cord

So far we have shown that pilocarpine application induced rhythms in both hemiganglia of each isolated thoracic ganglion. Application of pilocarpine onto a ventral nerve cord consisting of the deafferented pro-, meso- and metathoracic ganglia and the intact abdominal cord elicited rhythms in all thoracic ganglia in most of the preparations (9 out of 11; Fig. 6). In the remaining two experiments, rhythmicity was only observed in the meso- and metathoracic ganglia. The frequencies of the observed rhythms could be different in different ganglia. Nevertheless, in most cases, the excitatory protractor motoneurone pools of all three thoracic ganglia burst at similar frequencies (Fig. 6A). In general, it appeared that there was no stereotyped or fixed coupling between the rhythms in different ganglia, but in some preparations there were long periods of in-phase activity in the excitatory protractor motoneurone pools of the three ganglia, ranging from 5 to 25 consecutive cycles (Fig. 6A). In each of the three ganglia in the preparation consisting of only the thoracic nerve cord, the burst duration of the protractor motoneurone pools was only weakly correlated with cycle period, as in the isolated mesothoracic ganglion. Retractor burst duration showed a strong dependence on the cycle period in the mesothoracic ganglion of this preparation (Fig. 6B).

Pilocarpine-induced rhythms in the excitatory motoneurones of the coxa–trochanter and femur–tibia joints in the isolated ganglion

Application of pilocarpine also induced alternating rhythmicity in the excitatory motoneurone pools innervating the muscles of the CT and FT joints. Fig. 7Ai shows a typical rhythm induced by $3\times10^{-3}\,\mathrm{mol\,l^{-1}}$ pilocarpine in the excitatory levator trochanteris (nerve C1) and depressor trochanteris (nerve C2) motoneurones of the isolated mesothoracic ganglion. In nerve C1, some small units (in general 1–3 slow levator motoneurones; for a discussion of

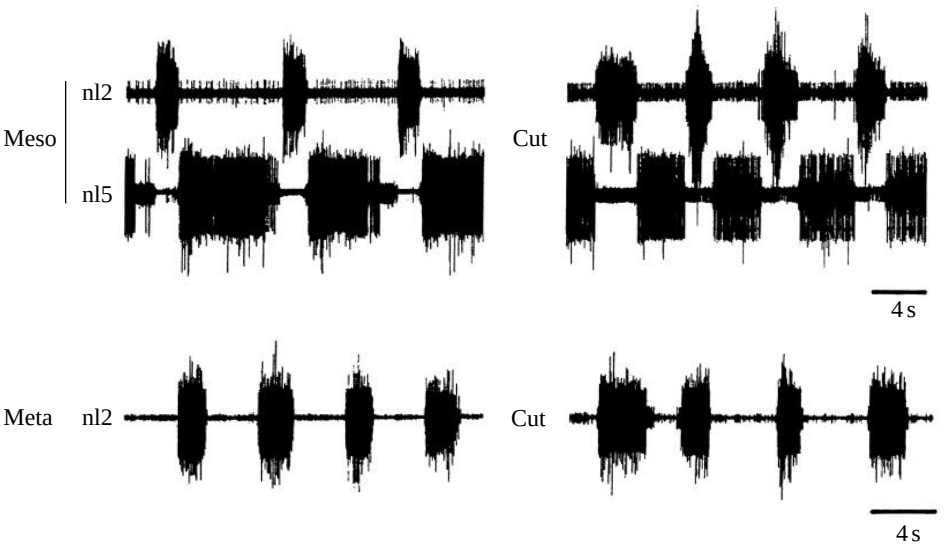

Fig. 5. Persistence of the rhythm in the isolated mesothoracic and metathoracic ganglia following a cut along the midline. Upper traces: rhythm in the isolated mesothoracic ganglion before and after the cut. nl2, protractor nerve; nl5, retractor nerve. Lower traces: rhythm in the isolated metathoracic ganglion before and after the cut.

the activity of CI1, see below) became tonically active following pilocarpine application, whereas all larger units (semi-fast and fast motoneurones) in the C1 nerve showed synchronised bursts of activity. The two excitatory depressor motoneurones (slow depressor trochanteris, SDTr; fast depressor trochanteris, FDTr) in nerve C2 also showed strong burst activity, which was in clear antiphase with the bursts of the levator motoneurones (Fig. 7A). However, the FDTr was not activated above its action potential threshold within each cycle of activity (see below). The burst durations of both levator and depressor trochanteris motoneurones depended on cycle period (Fig. 7Aii,iii; Table 1). The cycle period of the rhythm in the motoneurone pools supplying the CT joint was more variable than that in the motoneurone pools of the subcoxal joint. In most experiments, however, the average cycle periods of the motoneurone pools were in a similar range. For example, in the preparation shown in Fig. 6, the average cycle period of the retractor and protractor coxae motoneurone pools was 7.4±2.2 s (N=53) and the average cycle period of the levator and depressor trochanteris motoneurones was 6.9±4.6 s (N=55). The values for another animal are given in Table 1.

Pilocarpine application also induced rhythmicity in motoneurones supplying the FT joint. This is shown in Fig. 7Bi,ii for the effects of 3×10^{-3} mol l^{-1} pilocarpine on the activity of the two excitatory extensor motoneurones, the SETi (slow extensor tibiae) and the FETi (fast extensor tibiae), recorded in nerve nl3. In general, the burst duration of the excitatory extensor motoneurones during the pilocarpine-induced rhythms depended on the cycle period (Fig. 7Biii). In most preparations, the cycle period of the observed rhythm in the excitatory extensor motoneurones was less than half the cycle period observed in the motoneurone pools of the other two leg joints. The average cycle period for SETi and FETi in a typical preparation (5×10^{-3} mol l^{-1} pilocarpine) was 2.0±1.1 s (N=51), whereas it was 4.2±2.9 s (N=31) for the motoneurone pools of the CT joint and 5.1±3.7 s (N=30) for the motoneurone pools of the subcoxal joint (see also Fig. 10B). On average, the cycle period for the rhythm in the FT joint extensor motoneurones was 2.3±0.2 s (four animals, 308 cycles). Intracellular recordings from FT joint flexor motoneurones showed them to be active in antiphase with the excitatory extensor motoneurones (not shown). As described for the slow and fast CT joint depressor motoneurones, not every burst in the SETi was accompanied by a suprathreshold activation of the FETi (Fig. 7Bi). Nevertheless, intracellular recordings of FETi showed that it received synaptic drive in time with the observed SETi rhythm (Fig. 7Bi). The observed rhythm in the extensor motoneurones was repeatedly interrupted by pauses lasting for 2–5 cycles. Intracellular recordings from the excitatory extensor motoneurones showed that this was due to a strong inhibition superimposed on the rhythmic synaptic drive in the motoneurones (Fig. 7Bi; see below).

Pilocarpine-induced activity in the common inhibitor 1 motoneurone

Application of pilocarpine also affected the activity of the common inhibitor 1 motoneurone (CI1). In the untreated isolated mesothoracic ganglion, CI1 spiked rarely (0.1–0.2 Hz), as in the inactive or restrained intact animal (see Bässler, 1983). Pilocarpine application increased the activity of CI1 prior to any rhythmicity exhibited by the other motoneurones. The activity of CI1 could be recognized in recordings from nerves nl2 (protractor nerve) and nl5 (retractor nerve) because its action potentials occur with constant latency in both recordings. As rhythmicity was established, CI1 activity was modulated with the existing rhythm of the subcoxal motoneurones. CI1 showed a clear minimum of activity at the beginning of a protractor burst (Fig. 8A) and was maximally activated at the end of a protractor burst and the beginning of a retractor burst (Fig. 8B). We confirmed this result in seven other preparations.

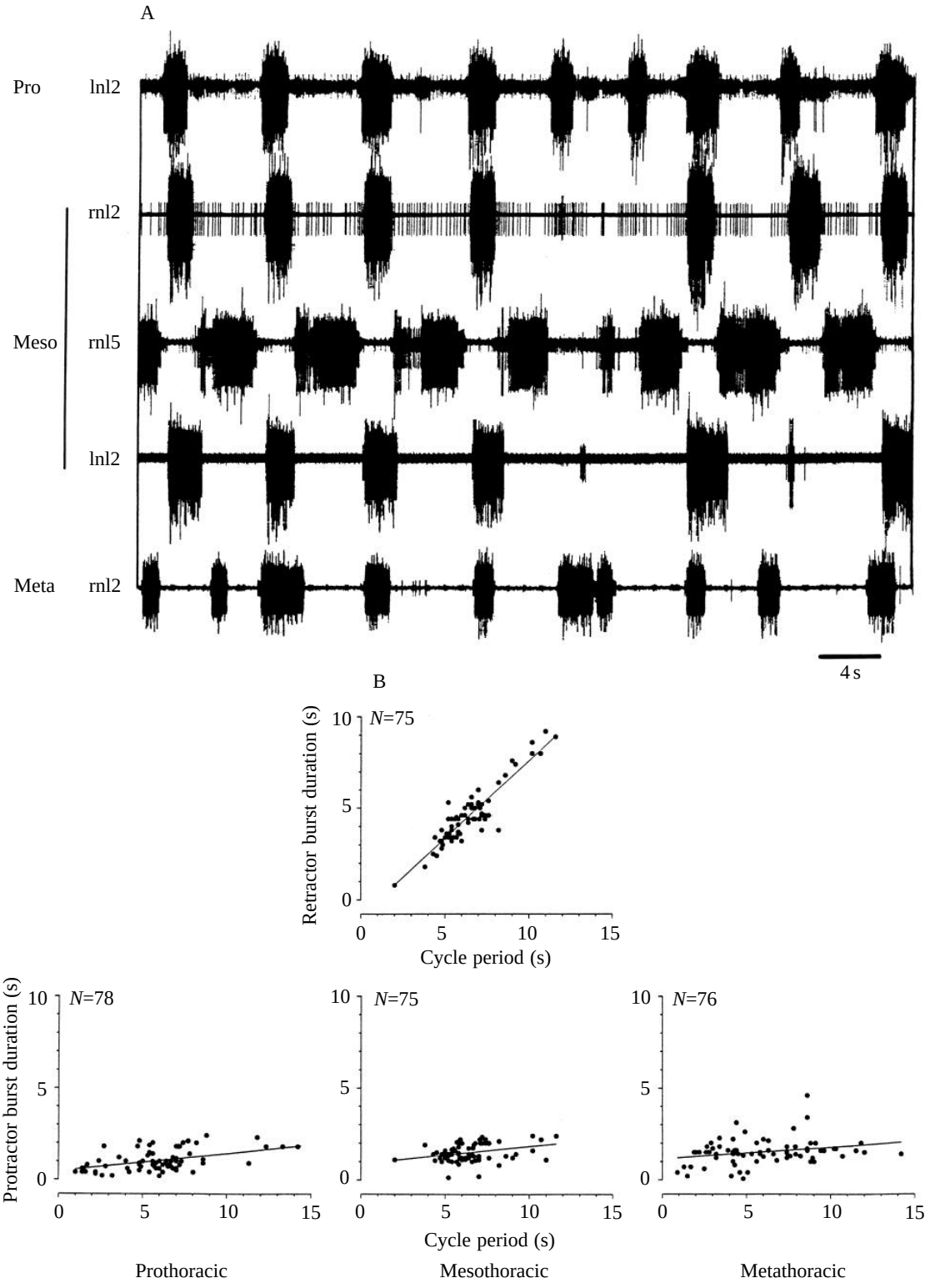

Fig. 6. Intersegmental coordination of the activity of the excitatory protractor motoneurones in the deafferented chain of thoracic ganglia. (A) Bursts of activity in the protractor nerves of the pro-, meso- and metathoracic ganglia. The activity of the right mesothoracic retractor nerve is given as a reference. Pro lnl2, left prothoracic nl2; Meso rnl2, rnl5, right mesothoracic nl2 and nl5; meso lnl2, left mesothoracic nl2; Meta rnl2, right metathoracic nl2. (B) Plots of the protractor and retractor (mesothoracic only) burst duration *versus* the cycle period for the pro-, meso- and metathoracic rhythms. *N* gives the number of cycles evaluated.

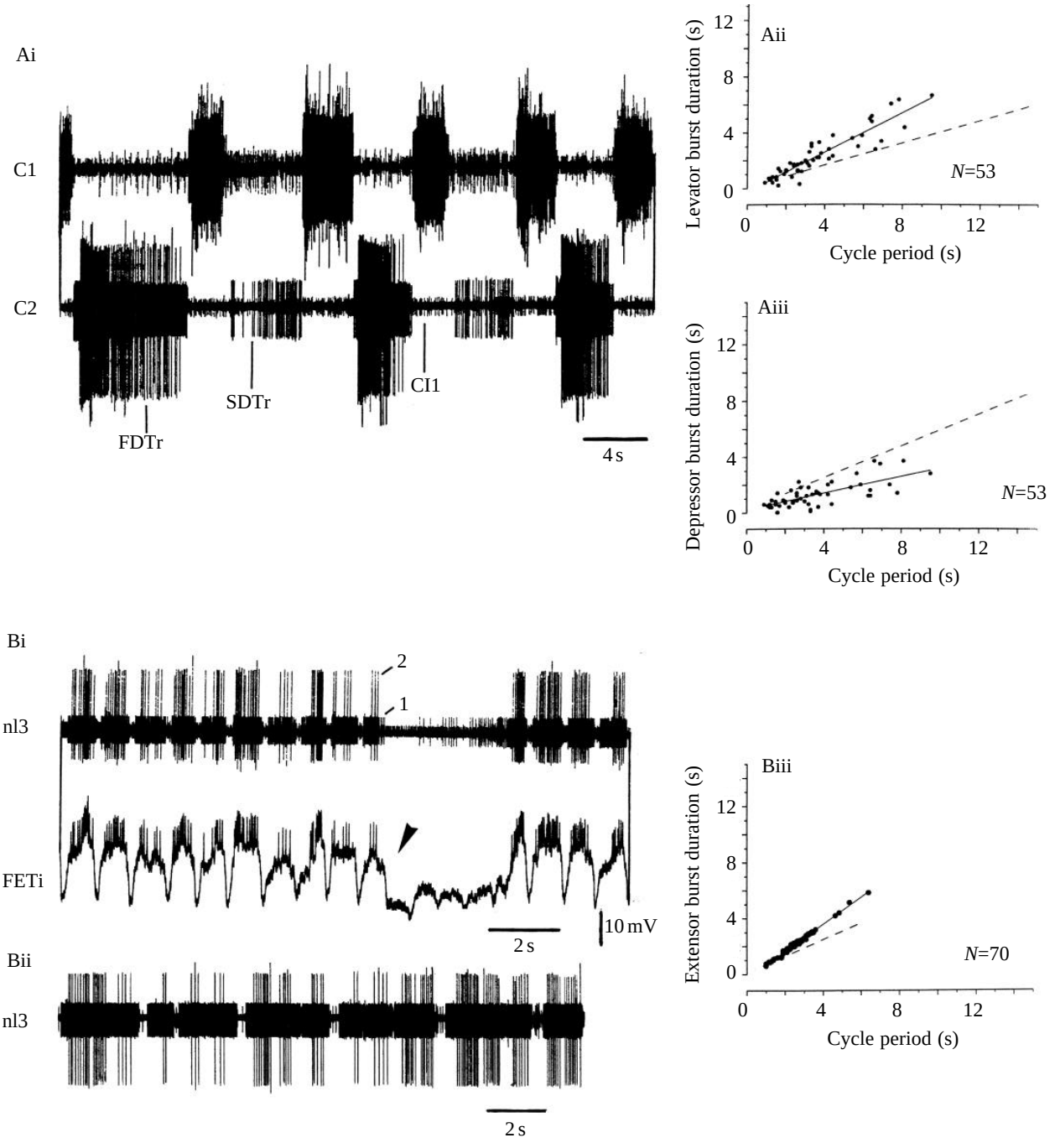

Fig. 7. (A) Rhythmicity in the excitatory motoneurone pools supplying the coxa–trochanter joint. (Ai) Extracellular recordings from the levator trochanteris nerve (C1) and the depressor trochanteris nerve (C2) in the isolated mesothoracic ganglion following application of 5×10^{-3} mol l^{-1} pilocarpine. Note action potentials with three different amplitudes in nerve C2: these correspond to FDTr, SDTr and CI1 (Schmitz, 1986). The dependence of burst duration of excitatory levator and depressor motoneurones on cycle period is given in Aii and Aiii, respectively. The solid lines are the regressions for the data points. The dashed lines are regressions from another animal. (B) Rhythmicity in the extensor motoneurones of two preparations (i,ii) following pilocarpine application. Extracellular recordings from nerve nl3, showing burst activity of SETi (1) and FETi (2). In Bi, FETi motoneurone was recorded simultaneously intracellularly. Note the similarity between the modulation of membrane potential in FETi and the modulation of SETi activity (see text). The arrowhead marks a spontaneously occurring inhibition superimposed on the observed rhythmicity (see text for further details). (Biii) Dependence of the burst duration in SETi on cycle period. The solid line is the regression for the data given in Bii. The dashed line is the regression from a different animal.

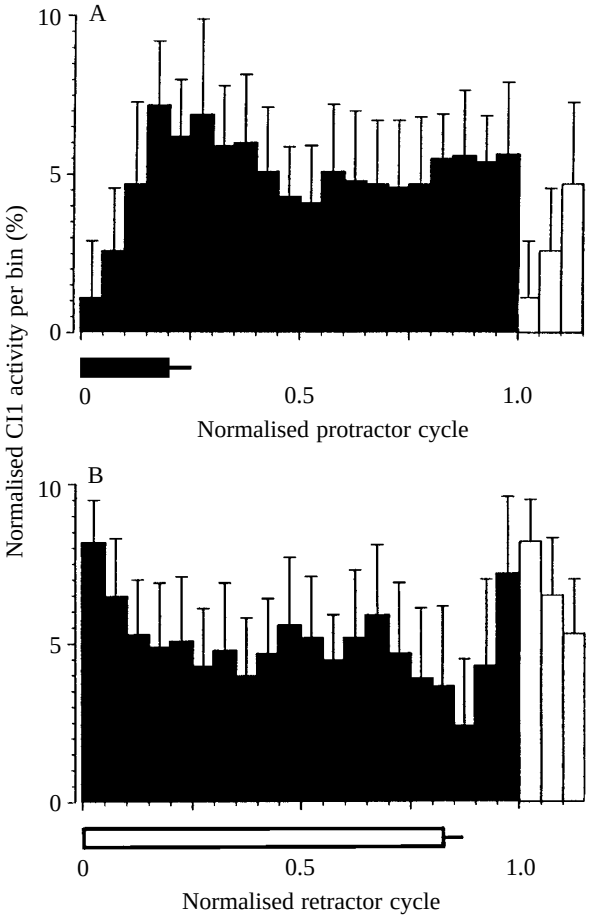

Fig. 8. Modulation of activity in the CI1 motoneurone following bath application of pilocarpine (4×10^{-3} mol l^{-1}). Normalised averaged activity (+S.D.) of CI1 in the normalised protractor (A) and retractor (B) cycle (data derive from 23 consecutive cycles of a typical experiment). For clarity, the first three histogram bins are repeated at the end of the normalised cycle (open bars). Horizontal bars denote the average (+S.D.) normalised protractor (filled bar) and retractor (open bar) burst durations.

Coordination of the motoneurones supplying muscles of different leg joints: recurrent patterns of activity

In the isolated mesothoracic ganglion, we recorded simultaneously from different motor nerves. These were the nerves supplying the muscles of the subcoxal, CT and FT joints. The rhythms exhibited by motoneurones supplying each of these joints have been described above. We did not find significant coupling in the burst activity between the rhythms of the motoneurones supplying the three different joints. This is most obvious from simultaneous recordings of the activity of the protractor motoneurones supplying the subcoxal joint and extensor motoneurones supplying the femur–tibia joint (Fig. 9A). The rhythms exhibited by the motoneurone pools of leg joints were in general not coupled to each other from cycle to cycle (see below). For example, the onsets of bursts in SETi and FETi did not show a significantly preferred phase in the cycle of subcoxal motoneurones (Fig. 9B,C; circular statistics for extensor bursts in protractor cycle: **r**=0.07; *z*=0.58; *N*=134; *P*>0.1). The same was true for the onsets of bursts in protractor motoneurones occurring during the extensor cycle (Fig. 9D; circular statistics for protractor bursts in extensor cycle: **r**=0.19; *z*=2.6; *N*=71; *P*>0.1). Similar results were obtained from the evaluation of the burst activity between the subcoxal and the CT joint (circular statistics for the experiment shown in Fig. 9: protractor bursts in depressor cycle: **r**=0.03; *z*=0.05; *N*=71; *P*>0.1; depressor bursts in protractor cycle: **r**=0.1; *z*=0.66; *N*=66; *P*>0.1), as well as the CT and the FT joint (e.g. circular statistics for the experiment shown in Fig. 9: depressor bursts in extensor cycle: **r**=0.18; *z*=2.71; *N*=79; *P*>0.1; extensor bursts in depressor cycle: **r**=0.14; *z*=2.58; *N*=134; *P*>0.1). We occasionally observed that the activity of motoneurone pools of one leg joint changed in time with changes in the activity of motoneurone pools of other leg joints. This is demonstrated in Fig. 10A, where retractor activity during a burst was sometimes increased with activity in the SDTr motoneurone.

Although there was no cycle-to-cycle coupling between the rhythms of the motoneurones of different joints, we found spontaneous, recurrent patterns of activity (SRPs) in about 90 % of the experiments (30 animals). These patterns strongly interfered with the existing rhythm in the motoneurone pools supplying an individual joint. In contrast to the ‘normal’ rhythms in the antagonistic motoneurone pools of each leg joint, these recurrent patterns showed a strong coupling between the activity of the different motoneurone pools (see below). We found three different types of SRPs.

The most common SRP, called SRP1, was characterised as follows (Fig. 10A). During a combined burst of the depressor motoneurones SDTr and FDTr, protractor motoneurones were active at first. In many cases, their activity increased as the depressor burst started (Fig. 10A, arrowheads). During the existing SDTr/FDTr burst, the protractor burst stopped and a burst occurred in the retractor motoneurones. In the majority of occurrences of SRP1 (about 78 %, *N*=155 SRPs for six animals), there was one switch from protractor to retractor motoneurone activity during the FDTr/SDTr burst (Fig. 10A,B and see below). The activity of the simultaneously active subcoxal motoneurone pool, usually the retractor motoneurones, stopped near the end of the FDTr/SDTr burst (Fig. 10). However, in about 22 % of occurrences of SRP1 (*N*=155 for six animals), there was more than one switch between protractor and retractor activity (see below), mostly (about 15 %) attributable to the fact that the retractor activity ended earlier than the depressor burst (see below).

The activity of the motoneurones supplying the FT joint was also incorporated into SRP1 (Fig. 10B). Prior to the SDTr/FDTr burst, SETi and, usually, FETi were active. Their activity was dramatically reduced or even stopped when the burst in the depressor motoneurones started. In general, no activity occurred in the extensor motoneurones during the burst in the depressor motoneurones. At the end of the burst in the depressor motoneurones, FETi and SETi became active again.

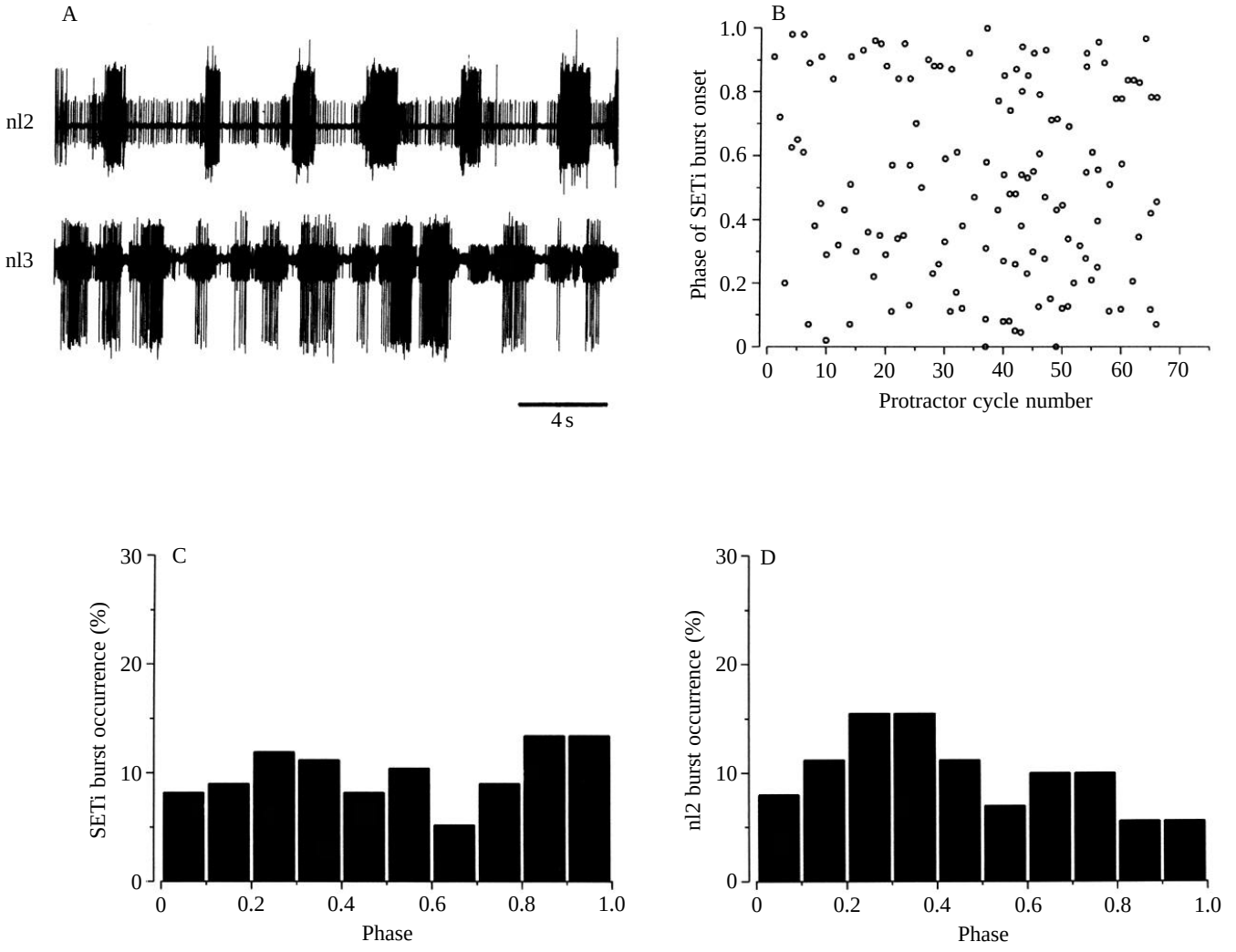

Fig. 9. Simultaneous recordings of the activity of motoneurones supplying the subcoxal joint and motoneurones supplying the femur–tibia joint in an isolated mesothoracic ganglion. (A) Original recording showing the activity of the motoneurones innervating the protractor coxae (nl2) and the extensor tibiae (nl3). (B) Plot of the phase of the onset of extensor bursts in the protractor cycle as a function of the number of the protractor cycle. (C) Phase histogram for the onset of extensor bursts in the protractor cycle (134 extensor cycles were evaluated; see text for circular statistics and Materials and methods for explanation). (D) Phase histogram of the onset of protractor bursts in the extensor cycle (71 protractor cycles were evaluated; see text for circular statistics). Data for A–D derive from the same preparation.

The time course of the membrane potential in FETi during SRP1 is shown in Fig. 7Bi (arrowhead).

This pattern of strictly coupled activity in the motoneurone pools of the three proximal leg joints occurred exclusively during bursts performed by both the SDTr and FDTr (Figs 10, 11A). We hardly ever (Fig. 11A) observed this pattern in the absence of FDTr activity (Figs 7, 10). The frequency of occurrence of SRP1 is shown in Fig. 11B as a function of depressor burst number for three different preparations. From this evaluation, it is obvious that the frequency of occurrence of SRP1 was variable and unpredictable within each preparation.

The duration of the FDTr/SDTr burst in SRP1 could vary within a single preparation and from one preparation to another, ranging from 1.5 to 8 s (Fig. 11C). In contrast, the latency from the start of the depressor burst until the first, usually the only, switch from protractor to retractor activity was less variable (Fig. 11C). A quantitative evaluation for four animals showed that this switch occurred on average 1.2±0.14 s (N=126) after the onset of the depressor burst, irrespective of its duration (Fig. 11C). For a single animal, the standard deviation for this latency measurement could be as low as 0.1 s (see Fig. 13A). Whenever a FDTr/SDTr burst occurred with no SRP (Fig. 11A), the FDTr/SDTr burst had a duration of 1 s or less.

In about one-fifth of the occurrences of SRP1 evaluated, protractor and retractor motoneurones produced more than one switch in their activity during the FDTr/SDTr burst (Fig. 12, see above). Two switches occurred mostly when retractor activity ended a bit earlier than the depressor burst, and thus protractor activity began again before the end of the depressor burst. Less frequently (5.2 % of occurrences) protractor and

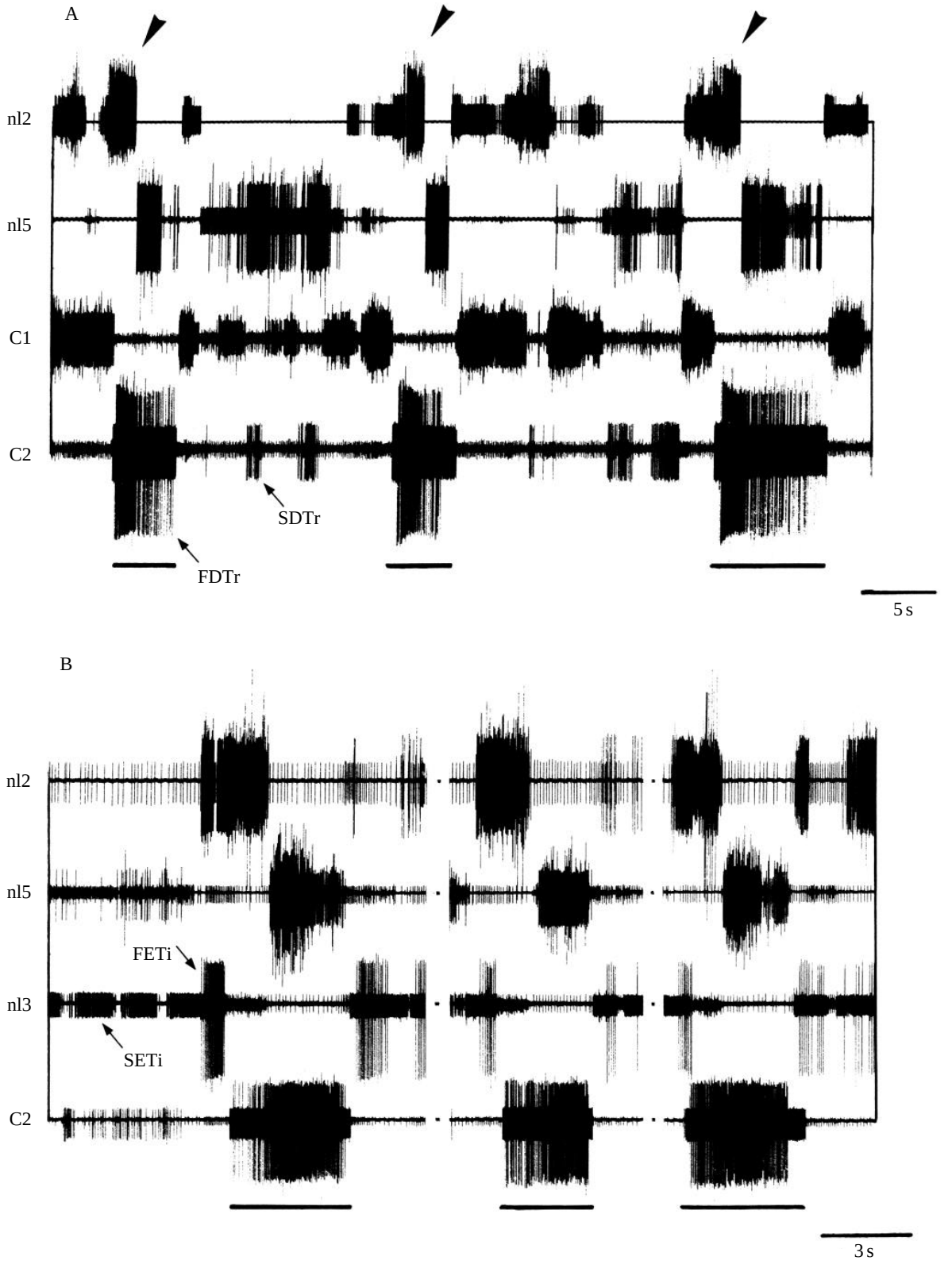

Fig. 10

retractor motoneurone pools showed more than two switches in their activity during the FDTr/SDTr burst (Fig. 12).

In summary, Fig. 13A shows a schematic representation of the motoneuronal activity in the recorded motoneurone pools of the three proximal leg joints during SRP1 in the isolated mesothoracic ganglion.

Fig. 10. A spontaneous, recurrent pattern of activity (called SRP1, black bars, see text) in the isolated mesothoracic ganglion. (A) Simultaneous recordings of the activity of the subcoxal (protractor coxae, nl2; retractor coxae, nl5) and the coxa–trochanter (levator trochanteris, C1; depressor trochanteris, C2) motoneurone pools following application of 5×10^{-3} mol l^{-1} pilocarpine. Note the rhythmic activity in the antagonistic motoneurone pools and the activity pattern that only occurs with a combined FDTr/SDTr burst. The arrowheads mark protractor activity during the first part of the depressor burst (see text). (B) Simultaneous recordings of the activity of the subcoxal motoneurone pools (nl2, nl5), the extensor tibiae motoneurones (nl3) and the depressor trochanteris motoneurones (C2). Note that no SETi and FETi activity is visible during the FDTr/SDTr bursts. The CI1 motoneurone is the only neurone in nl3 that is active during the depressor bursts.

We found two other types of SRPs in the motoneurone pool activity during pilocarpine-induced rhythmicity in the mesothoracic ganglion. These SRPs were much less frequent than SRP1 (5–8 % of all occurrences of an SRP). As for SRP1, SRP2 occurred with a burst in the FDTr/SDTr motoneurones (Fig. 13B). SRP2 started with a depressor burst coincident with retractor activity. During the depressor burst, retractor activity stopped and protractor activity started. As described for SRP1, tibial extensor motoneurones were inhibited during the depressor burst. Thus, this pattern resembled a 'reversed SRP1'. SRP3, the third type of SRP, involved a burst in the FETi and SETi motoneurones. During this burst, first the retractor motoneurones were active and then activity switched to the protractor motoneurones (Fig. 13C). During the burst in the extensor motoneurones, activity in the depressor motoneurones was strongly decreased, i.e. FDTr was never found to be active and SDTr generated few action potentials (not shown). Because of the low frequency of occurrence of SRP2 and SRP3, they were not quantified in more detail.

Pilocarpine-induced rhythms in intact, restrained animals

The results presented above demonstrate the ability of pilocarpine to induce rhythms in the motoneurone pools of

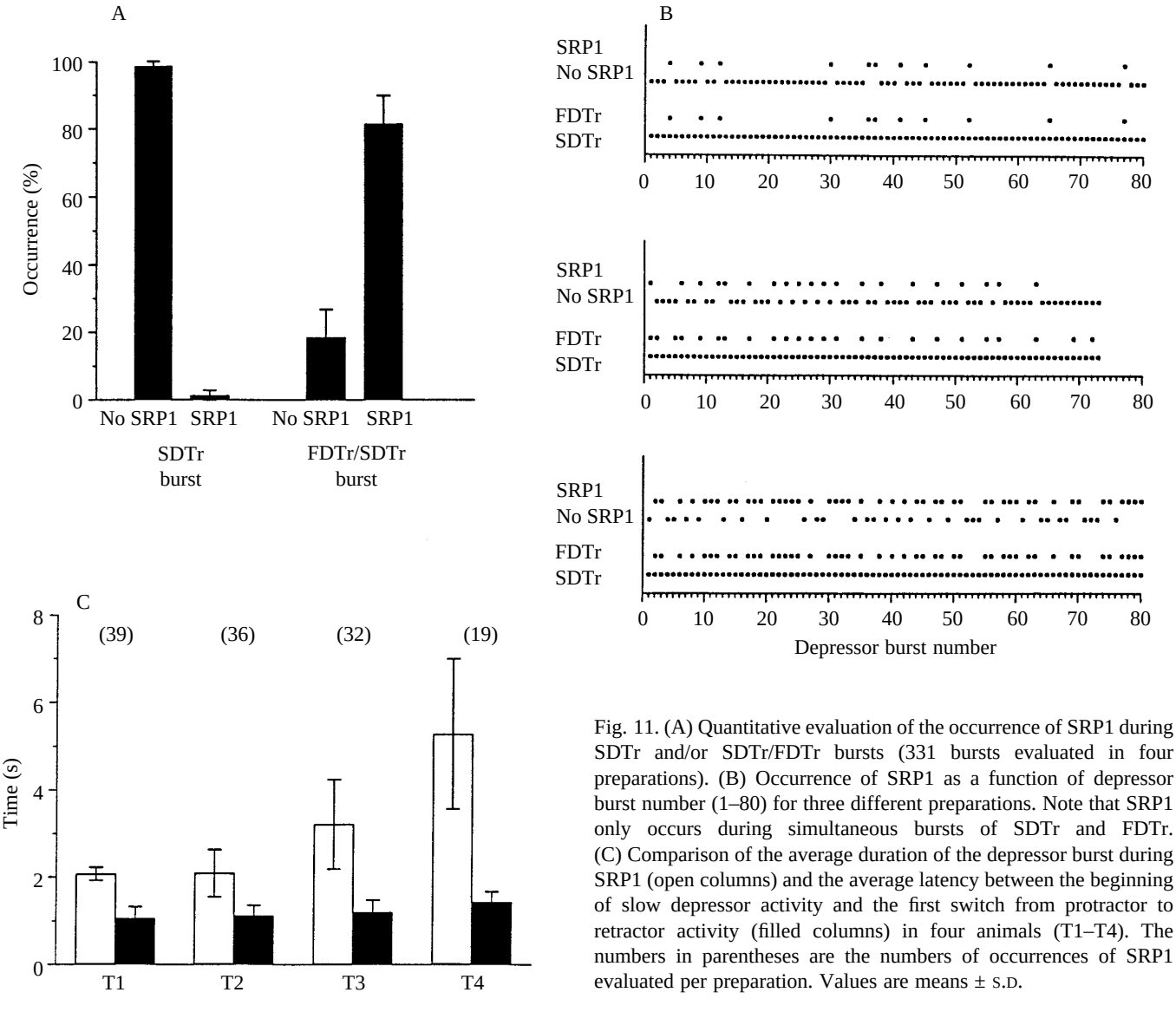

Fig. 11. (A) Quantitative evaluation of the occurrence of SRP1 during SDTr and/or SDTr/FDTr bursts (331 bursts evaluated in four preparations). (B) Occurrence of SRP1 as a function of depressor burst number (1–80) for three different preparations. Note that SRP1 only occurs during simultaneous bursts of SDTr and FDTr. (C) Comparison of the average duration of the depressor burst during SRP1 (open columns) and the average latency between the beginning of slow depressor activity and the first switch from protractor to retractor activity (filled columns) in four animals (T1–T4). The numbers in parentheses are the numbers of occurrences of SRP1 evaluated per preparation. Values are means ± S.D.

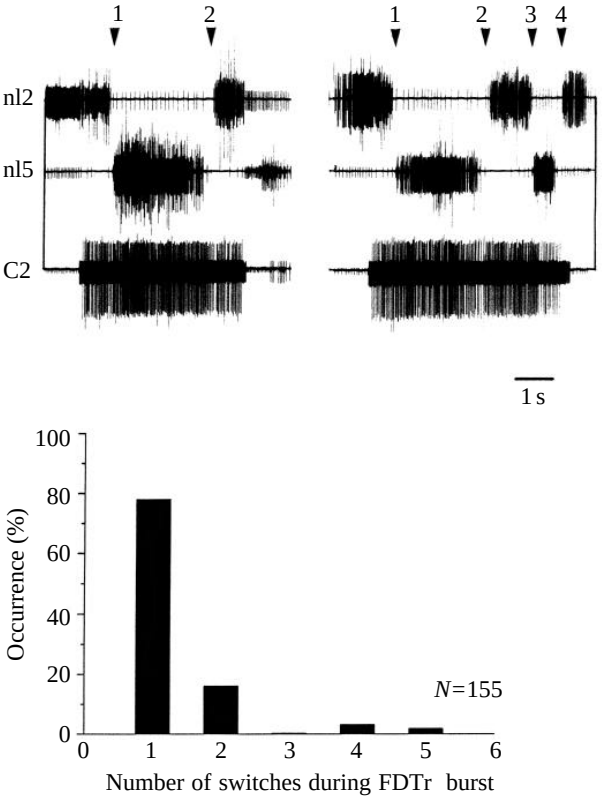

Fig. 12. Frequency of one or multiple switches in protractor and retractor activity during an SDTr/FDTr burst of SRP1. At the top are two examples of SRP1 that show two and four switches (marked by arrowheads) in activity between protractor and retractor activity. The plot at the bottom shows the percentage of each number of switches between protractor and retractor activity during occurrences of SRP1 for six preparations.

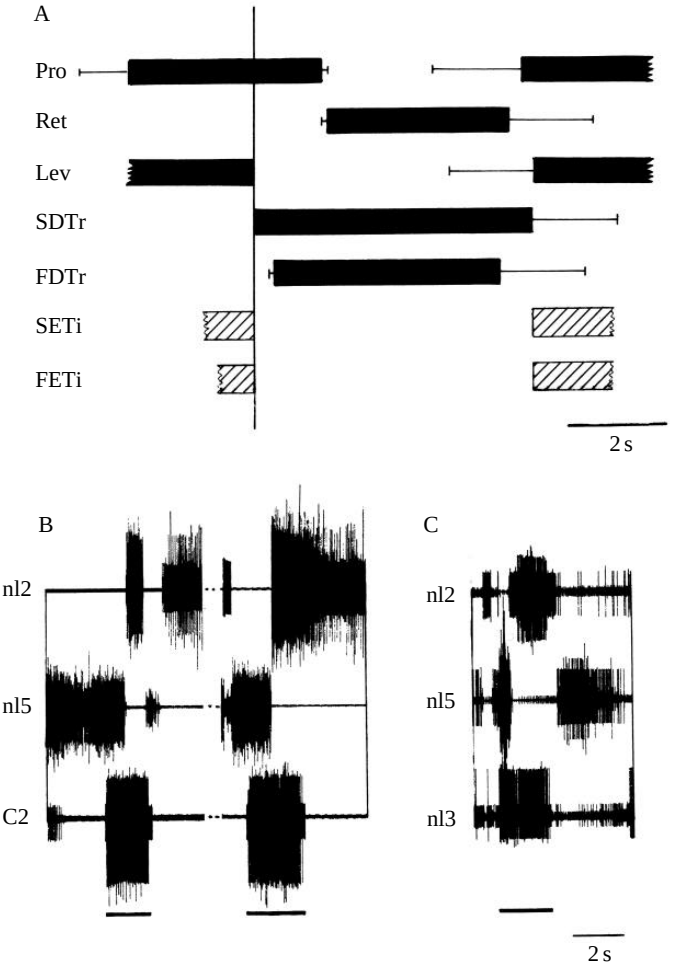

Fig. 13. (A) Schematic drawing of the average (+S.D.) motoneuronal activity during SRP1 in the middle leg motoneurone pools. Data for subcoxal and coxa–trochanter joint motoneurone activity (filled bars) derive from the experiment shown in Fig. 10A and were averaged for 11 subsequent occurrences of SRP1. The vertical line indicates the start of SDTr activity, which was the reference point of the evaluation. The activity of the excitatory extensor motoneurones (hatched bars) was added according to their established activity. (B,C) Examples for the two other observed SRPs (see text). B, SRP2; C, SRP3.

totally denervated ganglia. We repeated all the experiments described above using 42 intact, but totally immobilised, animals. In these animals, all position-sensitive proprioceptors would indicate constant values, and those proprioceptors sensitive to velocity would be inactive. Proprioceptors signalling muscle tension or cuticular stress might still be phasically stimulated as a result of isometric muscle contraction during the induced rhythmic motor activity.

Application of pilocarpine at concentrations higher than 1×10^{-4} mol l^{-1} reliably induced rhythms in the motoneurone pools of the subcoxal joint. Quantitative evaluation of the data showed no difference in the characteristics of the rhythms from those presented in Figs 1–6. Rhythmic activity was also induced in the motoneurone pools supplying levator and depressor trochanteris muscles, but there was one clear difference from the results obtained with denervated animals: the rhythm was above threshold only for the SDTr; the FDTr rarely produced any action potentials. Nevertheless, the rhythm in the CT joint consisted of strictly alternating bursts of activity of the motoneurones belonging to the levator pool and the SDTr. The timing was more variable than that described for the denervated animal (see Fig. 7). For example, the ratio of levator to depressor burst duration changed over time, irrespective of cycle period. The levator/depressor cycle period was also more variable and differed from the cycle period of the subcoxal motoneurones more than in the denervated situation: for example, in six animals tested with 2×10^{-3} mol l^{-1} pilocarpine, the cycle period of the motoneurones supplying the CT joint was 4.3±2.4 s (N=254), whereas it was 5.7± 1.9 s (N=254) for the motoneurones supplying the subcoxal joint.

The most striking difference, however, concerns the occurrence of SRPs. In 14 animals, each tested for several hours, neither SRP1 nor SRP2 was ever observed (Fig. 14A). SRP3 was seldom observed. However, SRP1 reliably occurred after total denervation of the hemiganglion under investigation. After the main leg nerve had been cut, the FDTr in each animal

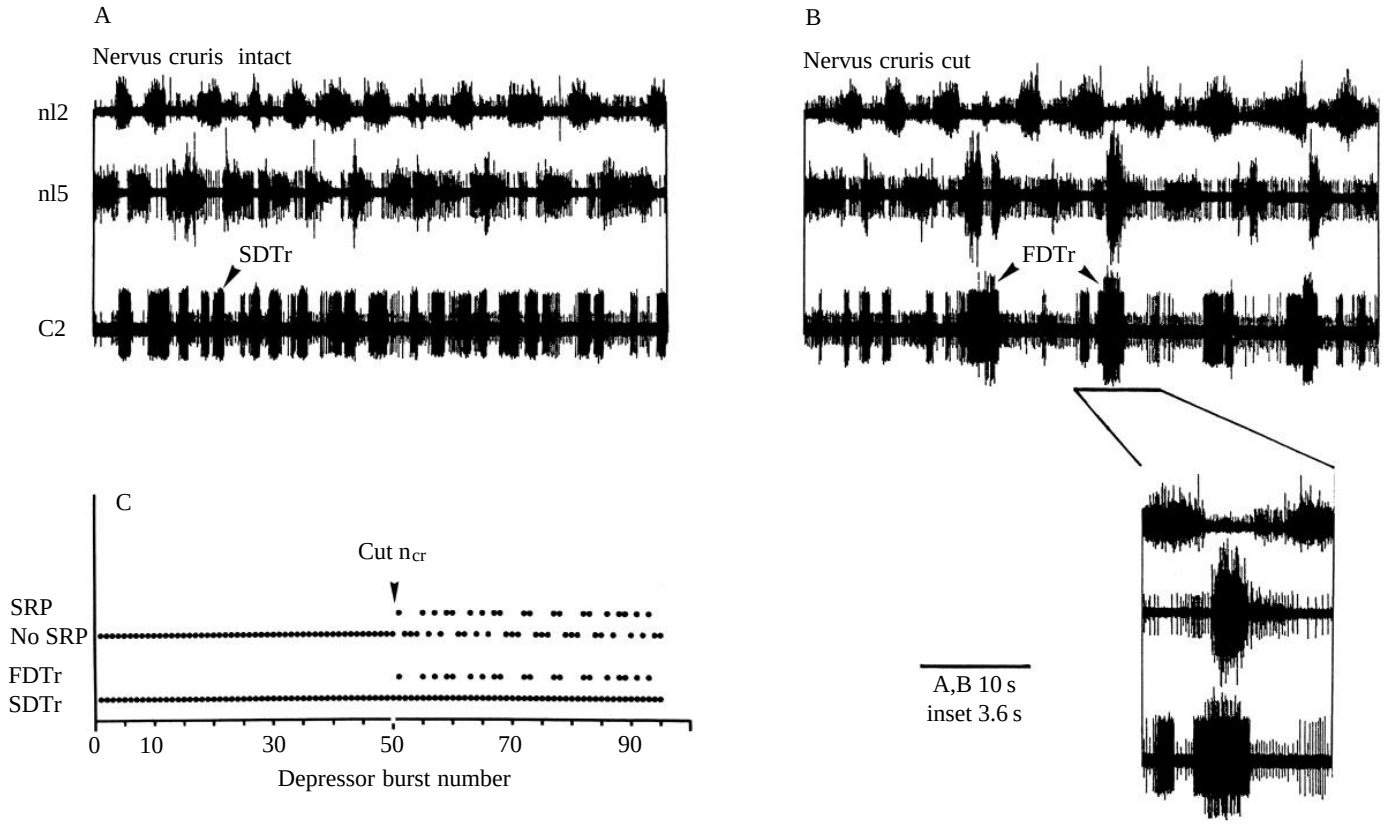

Fig. 14. Rhythmicity of the subcoxal (nl2, protractor nerve; nl5, retractor nerve) and coxa–trochanter motoneurone pools (C2, depressor trochanteris) in the restrained preparation. (A) Pilocarpine-induced rhythms of a mesothoracic ganglion denervated except for the nervus cruris (n_{cr}). Note that the SDTr (arrowhead) is the only one of the depressor motoneurones activated. (B) Rhythmicity in the same preparation after cutting the ipsilateral main leg nerve, the nervus cruris. Note the occurrence of SRP1 together with suprathreshold activation of the FDTr. The enlarged inset shows one occurrence of SRP1 in more detail. (C) Occurrence of SRP1 as a function of depressor burst number (1–95) for another preparation before and after cutting the ipsilateral nervus cruris.

occasionally became activated above threshold. During those depressor bursts involving both SDTr *and* FDTr, switching from protractor to retractor activity occurred reliably (Fig. 14). Further enhancement of the frequency of occurrence of SRP1s was observed following denervation of the contralateral hemiganglion and after cutting the anterior and the posterior connectives.

Discussion

Pilocarpine-induced rhythmicity

In the isolated thoracic nervous system of the stick insect, bath application of the muscarinic cholinergic agonist pilocarpine induced long-lasting strictly alternating rhythms in antagonistic leg motoneurone pools (slow, semi-fast and fast motoneurones) (Figs 1–7). A similar rhythmicity could be induced in intact and in partially deafferented preparations (Fig. 14). Pilocarpine reliably induced rhythmic activity at concentrations higher than $1\times10^{-4}\,\mathrm{mol\,l^{-1}}$. Experiments with desheathed ganglia in the phasmid species *Extatosoma tiaratum* (see Debrodt and Bässler, 1989) revealed threshold concentrations of $1\times10^{-6}\,\mathrm{mol\,l^{-1}}$ pilocarpine for the induction of rhythmicity (O. Dittberner and A. Büschges, unpublished results). This concentration is in the range of threshold concentrations reported for the locust (Ryckebusch and Laurent, 1993) and crayfish (Chrachri and Clarac, 1987). Perhaps the ganglion sheath of the stick insect is less permeable to pilocarpine than are those of the locust and the crayfish.

Application of atropine immediately abolished the rhythms, indicating that muscarinic cholinergic receptors underlie the pilocarpine-induced rhythm. We have no information about the detailed neuronal mechanisms by which pilocarpine induces the particular rhythms observed. There is generally little information about the distribution of muscarinic cholinergic receptors in invertebrates (Breer and Sattelle, 1987; Leitch *et al.* 1993) and there is even less information on their physiological role in nervous systems (Davis and Pitman, 1991; Trimmer and Weeks, 1991). However, the present investigation again suggests that muscarinic agonists are involved in the generation of rhythmic motor patterns (see also Chrachri and Clarac, 1987; Elson and Selverston, 1992; Ryckebusch and Laurent, 1993).

Some characteristics of the induced rhythms depend on

pilocarpine concentration. Increasing pilocarpine concentration decreased the cycle period of the rhythm, as shown for the subcoxal motoneurone pools. For the protractor motoneurone pool, burst duration increased with increasing pilocarpine concentration. The ratio of burst duration to cycle period at a given pilocarpine concentration was rather variable in the motoneurones supplying the muscles of the CT and the FT joints. With the exception of the excitatory protractor coxae motoneurones, the burst activity of the recorded motoneurone pools (retractor coxae, levator trochanteris, depressor trochanteris, extensor tibiae) depended on the cycle period. In contrast, the protractor burst duration in each preparation was fairly constant at a given pilocarpine concentration. Nearly all of the remaining cycle period was filled with retractor burst activity of variable duration; hence, the retractor burst duration strongly depended on the cycle period. The protractor burst duration was therefore the most consistent. The activity of the common inhibitor 1 motoneurone (CI1) was also affected by pilocarpine. CI1 activity was modulated with the existing subcoxal rhythm even though it also innervates the muscles of the CT and FT joints.

The motoneurone pools of each leg joint are driven by discrete rhythm generators

Pilocarpine induced alternating rhythms in the antagonistic motoneurone pools of each leg joint. However, there was no stereotyped cycle-to-cycle coupling in the activity of the motoneurone pools supplying the different leg joints. Each pair of antagonistic motoneurone pools of each leg joint exhibited strictly alternating activity, thus functioning as a discrete oscillating module (joint module). The cycle periods of each module differed: the cycle period of the burst activity in the tibial extensor motoneurones (SETi and FETi) was approximately half that of the motoneurone pools supplying the CT or the subcoxal joints. It has to be taken into account that our investigation was mainly concerned with the activity in the motoneurone pools during rhythmic activity. Thus, from our current knowledge and from the choosen approach, we cannot exclude the existence of more subtle couplings between the joint modules during pilocarpine-induced rhythms. A subsequent investigation of the neuronal mechanisms of the generation of pilocarpine-induced rhythmicity will attempt to investigate this.

Rhythms in the investigated joint modules were not totally independent of each other. In the majority of the preparations, we found SRPs (spontaneous recurrent patterns) involving reproducible coupling between the joint modules. We have no information yet on the neural mechanisms of this coupling. There are at least two alternative explanations: (i) that the observed SRPs are produced by another discrete oscillating neural system that has access to the joint modules and that is able to override their existing activity; (ii) that the neurones within each joint module (interneurones or motoneurones) themselves exhibit variable coupling to neurones within other joint modules, so that there is a state in which there is no coupling between the modules, as well as other states in which certain modules are coupled. Intact, restrained animals only showed SRP3s, indicating an important role of sensory pathways in suppressing SRPs (at least for SRP1 and SRP2), i.e. strictly coordinated activity patterns of different leg joints. Such sensory pathways might either inhibit the SRP-producing network (hypothesis I) or change the coupling strengths among the joint modules (hypothesis II). The observation that the FDTr motoneurone is never activated above threshold in restrained animals shows that sensory information, in this case, decreases the amplitude of pilocarpine-induced activation of FDTr. This might support hypothesis II, as lower-amplitude oscillations could cause weaker coupling strengths among the joint modules and hence prevent occurrences of SRP1 and SRP2 in these animals. In other rhythm-generating systems, such changing interactions between different rhythm-generating networks have been investigated in detail; for example, in the stomatogastric system (Dickinson and Moulins, 1992).

Intersegmental coupling of the rhythms

Our results show that each hemiganglion is able to produce its own rhythms. When the hemiganglia were left connected to each other, the rhythms generated in each hemiganglion tended to synchronise. This was especially true for the protractor–retractor rhythms, which showed a weak but significant preference for in-phase coupling. This phenomenon is best described by the *Magnet-Effekt* introduced by von Holst (1936). In our experiments, there was no evidence for antiphase coupling, because in no preparation did sequences of antiphase coupling exceed three consecutive cycles. These findings again show that the rhythm-generating networks are to some extent independent (see also Foth and Bässler, 1985).

Comparison of the induced rhythmicity with motor output in different behaviours

A leg of a stick insect is able to perform the following rhythmic movements that have been analysed in great detail: walking (for summaries, see Bässler, 1983, 1988; Cruse, 1990; Graham, 1985), searching (Bässler, 1993; Bässler *et al.* 1991; Karg *et al.* 1991) and rocking (Pflüger, 1977). Although there is a great variability in the motor outputs during each of these behaviours, mainly due to variations in external variables (see also Cruse, 1976; Graham and Bässler, 1981), some general features can be recognised and compared with the pilocarpine-induced rhythm.

First, in all these behaviours, the excitatory motoneurones of antagonistic muscles are active in antiphase. This is not trivial, because strong co-contractions may occur under certain circumstances (e.g. Graham, 1979). Pilocarpine-induced rhythms in antagonistic motoneurones also exhibited only antiphase activity.

Second, coordination between the motoneurones of different joints of a particular leg is relatively fixed in each of these behaviours, but this coordination differs for the different behaviours. Coordination such as that of the pilocarpine-induced rhythmicity when there was no SRP (no fixed phase

relationships from cycle to cycle, oscillation frequency different for different joints) does not occur in intact animals. However, it does resemble the vigorous motor output of a denervated thoracic ventral nerve cord (Bässler and Wegner, 1983).

Third, walking can be performed in two different gaits. In the tripod gait, swing and stance duration each depend on cycle period. In the tetrapod gait, swing duration is independent of cycle period (Graham, 1972; Wendler, 1964), but may be affected by step amplitude and load (Dean and Wendler, 1983; Dean, 1991). In tetrapod coordination, under normal conditions, the burst duration of several stance muscles depends strongly upon cycle period, whereas the burst duration of the swing muscles is constant (Bässler, 1986). In the pilocarpine-induced rhythms, the activity of one swing muscle (protractor) is independent of cycle period, and the activity of another swing muscle (levator) is dependent upon cycle period at the same time. This does not occur in any known behaviour.

When the suboesophageal ganglion is separated from the thoracic ventral nerve cord (as in the isolated preparation), the hindlegs walk backwards while the forelegs walk forwards (Bässler *et al.* 1985). One would therefore expect that, in a fictive walking pattern under these conditions, the retractor swing burst duration in a hindleg would be independent of cycle period. In contrast, the protractor burst duration is independent of cycle period in the pilocarpine-induced rhythm.

Fourth, during forward walking, the common inhibitory motoneurone 1 (CI1) is maximally active at the transition from retraction to protraction (Büschges *et al.* 1994; Graham, 1985). In the pilocarpine-induced rhythm, it is minimally active at this point. During rocking, CI1 rarely fires (Bässler and Wegner, 1983; Pflüger, 1977). The activity of CI1 during searching movements has not yet been recorded but, from observations of leg movements during searching (stereotyped movements mainly in the CT and FT joints; Karg *et al.* 1991), one would expect modulation of CI1 activity to be linked to the CT rhythm rather than to the subcoxal rhythm, as during pilocarpine-induced rhythms.

Fifth, there is strict coordination between different legs in walking and rocking and no coordination during searching movements (for a summary, see Bässler, 1983). Coordination comparable to that exhibited by the pilocarpine-induced rhythm (all legs often in phase) only occurs in the intact animal at the beginning of walking prior to the first swing. During this short period, all the legs retract simultaneously (Dean and Wendler, 1984). Several consecutive cycles of in-phase activity of all legs are never observed in intact animals. In animals without a suboesophageal ganglion, in-phase activity of all the legs does not occur even at the beginning of walking.

In summary, except for the anti-phase activity of antagonistic excitatory motoneurones, no feature of the pilocarpine-induced rhythmicity (in the absence of SRPs) appears to correspond to any motor output observed in the intact animal. This is especially noteworthy for rocking, because rocking seems to be mainly generated by a central pattern generator (Pflüger, 1977) and, consequently, the deafferented thoracic ventral nerve cord is able to generate a rocking-like motor output (Bässler and Wegner, 1983). One could speculate that the relatively fast extensor rhythm in the pilocarpine-stimulated preparation corresponds to rocking, even though its cycle period is significantly longer than in the 'rocking-motor output' of the deafferented thoracic ventral nerve cord, but recordings from extensor nerves on opposite sides showed no regular antiphase coordination as during rocking (O. Dittberner and A. Büschges, unpublished observations). Therefore, the pilocarpine-induced rhythm in stick insects (in the absence of SRPs cannot be termed 'fictive locomotion'.

Similarities of SRPs with the motor output during walking

The observed SRPs bear some similarities to distinct portions of the motor output during walking. (1) The most often observed SRP, SRP1, was characterised by a switch from protractor to retractor activity during a concurrent burst of both depressor motoneurones and by an inhibition of FETi and SETi firing. At first, this seems to resemble the transition from swing to stance of the middle legs in normal forward walking, but there are also some differences. When a middle leg touches the ground at the end of a swing, the existing depressor burst is often briefly interrupted, and a shorter levator burst is apparent. At the same time, the protractor is briefly activated (Cruse, 1976; Godden and Graham, 1984; Graham and Wendler, 1981*a*). The same is true for the end of searching, when the leg grasps an object (Bässler *et al.* 1991). Touching the ground inhibits extensor and excites flexor motoneurones (Bässler, 1993). There is some probability that the levator and protractor activation is triggered by sense organs (Cruse, 1985; Epstein and Graham, 1983). In the light of these results, one could argue that SRP1 is the centrally generated portion of the switch from swing to stance or the switch from searching to walking (Graham, 1985; Karg *et al.* 1991). (2) SRP2 bears some similarities to the switch from swing to stance in a backward-walking stick insect, although the motor output during backward walking is variable (Graham and Epstein, 1985; Schmitz and Haßfeld, 1989) and often shows co-contraction of antagonistic muscles (Graham and Epstein, 1985). (3) SRP3 was characterised by a switch from retractor to protractor activity in the subcoxal motoneurones during concurrent bursts of both extensor motoneurones, thus resembling the middle leg motor activity at the transition from stance to swing in the forward-walking middle leg (Büschges *et al.* 1994; Epstein and Graham, 1983; Schmitz and Haßfeld, 1989).

Our data show that, in the absence of sensory input and following the application of pilocarpine, a motor output may occur which shows some similarities to the transition between swing and stance or between stance and swing in walking animals. This result is particularly interesting, because previous investigations of walking in virtually intact preparations have shown that the transitions between stance and swing and between swing and stance depend on sensory feedback (Bässler, 1983; Cruse, 1990; Graham, 1985). Our present findings do not contradict these results. In contrast,

they give rise to the hypothesis that the basic structure of the motor output during the transitions from stance to swing and *vice versa* might be to some extent centrally programmed. The role of sensory input is then to trigger and modify the appropriate transition. This hypothesis is supported by the finding that, in intact restrained animals, the SRP most commonly observed in the isolated preparation, i.e. SRP1, was not generated, but seemed to be suppressed, because it occurred with denervation. We are testing this hypothesis by investigating the effects of afferent stimulation on the pharmacologically induced rhythm. If this hypothesis holds, one can call the SRPs 'fictive step-phase transitions'.

Comparison with other systems

Pharmacologically induced rhythms occur in a number of deafferented locomotory preparations (e.g. cat walking, Grillner and Zangger, 1979, 1984; crayfish walking, Chrachri and Clarac, 1990; lamprey swimming, Wallén and Williams, 1984; locust flight, Stevenson and Kutsch, 1987; locust walking, Ryckebusch and Laurent, 1993; Wolf and Laurent, 1994). In all these systems, the similarities between real motor output and pharmacologically induced rhythms were clear enough for the authors to speak of fictive locomotion. In contrast, the motor output elicited by pharmacological treatment of the stick insect did not resemble the basic features of the motor outputs of continuous forward or backward walking, searching or rocking. This might be due to differences in the neuronal mechanisms activated by pilocarpine in different species. We were, however, able to induce motor patterns that share some similarities with the step-phase transitions during locomotion. On the basis of these results, it is conceivable that the neural networks governing the motor output of the individual leg joints in the walking system of the stick insect are more loosely coupled than they are in the locust or the crayfish, because in the stick insect only the periods of step-phase transition, rather than of an ongoing, stereotyped and tightly coupled rhythm, are exhibited. Perhaps this is because stick insects are slowly climbing animals.

It is noteworthy that pilocarpine induces only fictive locomotion (locust and crayfish) or fictive step-phase transitions (stick insect), i.e. behaviours or parts of behaviours that are thought to be mainly peripherally controlled (e.g. Bässler, 1988, 1993; Cruse, 1990). Behaviours that are known to be mainly centrally generated or centrally organised, such as locust stridulation (Elsner, 1983), locust jumping (Gynther and Pearson, 1986) or stick insect rocking (Pflüger, 1977), are not expressed in the motor output following pilocarpine treatment.

In conclusion, the results of the present paper are consistent with the current understanding of the construction principles of the stick insect walking pattern generator (Bässler, 1993). This generator consists of modules, most of which have sense organs as integral parts. Purely central mechanisms are able to produce an alternating firing of antagonistic motoneurones in each proximal leg joint. Furthermore, we found that pilocarpine induced spontaneous, recurrent patterns (SRPs) of coupled motoneuronal activity in the motoneurone pools of different leg joints. If further investigations verify that the SRPs are fictive step-phase transitions, another type of central module has to be added. These modules would produce centrally programmed short-lasting coordination between motoneurone pools for different joints, of types appropriate for particular step-phase transitions. These centrally programmed motor patterns may be peripherally triggered under natural circumstances.

We would like to thank Drs S. Gramoll, A. E. Sauer and J. Schmidt for critically reading the manuscript and T. Heller for technical assistance. We thank two anonymous referees for their valuable remarks on the manuscript. This work was supported by DFG grants to A.B. (Bu 857), J.S. (Cr 58/2) and U.B. (Ba 578).

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