Feeding-related motor patterns of the locust suboesophageal ganglion induced by pilocarpine and IBMX

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Abstract

The mandibular motor pattern induced by the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) in isolated locust suboesophageal ganglia (SOG) was investigated and compared with the motor pattern induced by pilocarpine in an already established preparation of the SOG. Motor patterns occurring after bath application of IBMX or pilocarpine were recorded extracellularly from suitable nerves of isolated SOG. For a quantitative evaluation of long (15 min) sequences of rhythmic neural activity containing several hundred cycles, spectral analysis of spike trains was applied. Using a set of characteristic parameters extracted from spectra computed for each individual preparation, quantitative comparisons of the rhythms induced by IBMX and pilocarpine were made. Significant differences in regularity, frequency of oscillation, and intra-burst frequency were found whereas the phase relationships of different motor pools were similar. Differences in the effect of the drugs on the activity recorded extracellularly from mandibular closer motoneurones were investigated further using intracellular recordings. Our findings imply that the IBMX-induced motor pattern is a suitable in vitro model of mandibular central motor control like the pilocarpine induced pattern. The better regularity is an advantageous feature for further experiments on central pattern generation. Information on second messengers involved in central pattern generation provided by the pharmacological profile of IBMX forms a basis for pharmacological and histological investigations on the mandibular central pattern generating network. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Locust feeding has been studied for a long time and from several perspectives. Patterning and sensory control of locust feeding was described quantitatively, especially with respect to numerous intrinsic and extrinsic influences (Simpson 1990, 1996; Simpson et al., 1996), whereas central nervous mechanisms are still poorly understood. Most work has been performed with semi-intact preparations, e.g. the identification of mechanosensory and chemosensory interneurones (Simpson, 1992; Rogers and Simpson, 1999) or monitoring the activity of neurosecretory and visceral motor neurones (Bräunig, 1987; Baines et al., 1989). Later, recordings from these neurones were made in intact locusts (Schachtner and Bräunig 1993, 1995), since their peculiar morphology renders them accessible. However, morphological constraints impair the electrophysiological analysis of the central nervous networks integrating sensory information and controlling efferent neurones during feeding behaviour.

The isolated suboesophageal ganglion (SOG) of the tobacco hornworm spontaneously produces motor patterns similar to chewing rhythms (Rowell and Simpson, 1992). Using this approach, premotor interneurones were identified (Rohrbacher, 1994). In contrast, the locust SOG does not show spontaneous coordinated motor activity. However, the muscarinic agonist pilocarpine induces a motor pattern in the isolated locust SOG (Rast and Bräunig, 1997) which closely resembles naturally occurring motor activity, as is the case in various other reduced arthropod preparations (Chrachri and Clarac, 1990; Elson and Selverston, 1992; Ryckebusch and Laurent, 1993; Büschges et al., 1995; Braun and Mulloney, 1995; Johnston and Levine, 1996; Heinrich et al.,

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1997). The pilocarpine-induced suboesophageal motor pattern involves both motoneurones to power muscles and to muscle receptor organs (Bräunig, 1990; Rast and Bräunig, 1997) representing important elements of putative mandibular sensorimotor reflex loops (Seath, 1977a,b). Pilocarpine also affects the activity of a serotonergic neurohaemal system in the locust head (Bräunig, 1987).

Pharmacologically activated reduced preparations provide good access to the central nervous system for invasive electrophysiological techniques; therefore such preparations proved to be valuable tools for the analysis of pattern generating neural circuits, although batch application of neuroactive substances may also lead to unspecific activation of neurones involved in other functional contexts than the motor pattern under investigation. If a set of different drugs is sufficient to influence rhythmogenesis, a greater range of questions can be covered in experiments using these drugs, since the pattern generating circuitry can be studied in different modes of operation. For example, a large number of neuromodulators has been shown to modify centrally generated motor patterns pointing to modulator-dependent mechanisms of motor pattern reconfiguration (e.g. Simmers et al., 1995; Satterlie and Norekian, 1996; Hashemzadeh-Gargari and Friesen, 1089; Yeoman et al., 1996; Vehovszky et al., 1998; Ramirez and Pearson, 1991; Harris-Warrick and Cohen, 1985; Barthe and Grillner, 1995; Weimann et al., 1997; Sillar et al., 1998; Rossignol et al., 1998).

In this study a new tool for the induction of mandibular motor patterns in the locust suboesophageal ganglion is introduced. The aim of the present study is to describe the locust mandibular motor pattern induced by IBMX and to compare it with the pattern known to be induced by pilocarpine (Rast and Bräunig, 1997). The motor patterns induced by IBMX and pilocarpine show similar phase relationships; however, they differ significantly in their cycle period, regularity, and the intra-burst activity of motoneurones. The latter finding is supported by a significantly different amplitude of the synaptic input to these motoneurones.

2. Materials and methods

2.1. Drugs

Drugs were purchased from Sigma-Aldrich Chemie GmbH, Steinheim, Germany. Stock solutions and final dilutions were prepared as follows using locust physiological saline (Clements and May, 1974): 3-isobutyl-1-methylxanthine (IBMX): 4.5 mM (stock solution), 450 µM (final dilution); pilocarpine: 10 mM (stock solution), 40 µM (final dilution).

2.2. Insects and preparation

Experiments were performed on adult African migratory locusts (Locusta migratoria migratorioides R. & F.) taken from a crowded laboratory culture within the first week after final moult. Prior to dissection, locusts were immobilised by chilling to 4°C. Subsequently they were decapitated and the suboesophageal ganglion (SOG) was exposed after the removal of frons, clypeus, labrum, and the remainder of the foregut. Anatomical terms are taken from Snodgrass (1928); for an anatomical description of the mandibular motor system see Bräunig (1990). The peripheral nerves of the SOG were cut at suitable lengths. After severing the circumoesophageal connectives and the tentorium the SOG was excised from the head capsule leaving the main branches of the tracheal system intact and was pinned into a Petri dish coated with Sylgard (Dow Corning Corp., Midland, Michigan, USA); the stumps of the tracheae were opened at the surface of the saline.

2.3. Recordings, data acquisition, and evaluation

For intracellular recordings, the ganglionic sheath was softened with a few crystals of collagenase (type 1A, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) for 90 s and cell somata were impaled with glass micropipettes (5% Lucifer Yellow in 1 M LiCl in the tip (Molecular Probes, Eugene, Oregon, USA) and 1 M LiCl in the shaft; resistance 20–60 MOhms). Cells were identified by iontophoretic injection of Lucifer Yellow and/or correlation of extra- and intracellularly recorded spikes. For extracellular recordings, the endings of the peripheral nerves of the SOG (see Fig. 1) were introduced into the tips of suction electrodes. Spikes were preamplified by Grass P15 amplifiers and stored with a DAT recorder (DTR 1404, Biologic, Grenoble, France). Play back data were converted into TTL pulses by analog window discriminators. The timings of the TTL pulses were stored with a personal computer using the CED 1401 interface and spike2 software (Cambridge Electronic Design, Cambridge, UK). Data analysis was performed using software written by the authors in C running under the LINUX operating system on a personal computer.

Extracellularly recorded spike trains may be represented by the timings of spikes. Lists of spike timings are Fourier transformed yielding spectra which hold information on the frequency contents of the time series and on the relative phase of the particular frequencies with respect to a reference. Coherency spectra computed from two individual time series contain information on the frequencies represented in both time series and on the relative phase of these frequencies with respect to the other time series. Information on frequency contents of a time series is extracted in power spectra, or, in case two time series are to be compared with respect to com-
Fig. 1. Schematic dorsal view of the SOG with its peripheral nerves and primary arborisations of the mandibular nerve. Anterior is to the top. COC: circumoesophageal connectives; N: nerve; Tr: trachea; N1: mandibular nerve; N1B1: motor nerve of dorsal muscle receptor organ; N1C: mandibular opener motor nerve; N1D: mandibular closer motor nerve; N1D1: motor nerve of ventral muscle receptor organ; ST: satellite nerve. For detailed descriptions see Altman and Kien (1979) and Bräunig (1987, 1990).

Mon frequencies, in coherence spectra; relative phase is extracted in phase spectra (for details see Rosenberg et al., 1989; Rigas, 1991; Dahlhaus et al., 1997; Miller and Sigvardt, 1998).

For example, the largest peak in the power spectrum of the spectral coherence shown in Fig. 5(Ai) (lower panel) indicates that a frequency of about 0.8 Hz is most common in both time series. This corresponds with the bursting frequency of the preparation (Fig. 2(Ai)). The intra-burst frequencies are distributed over too wide a range to form a single pronounced peak. The phase spectrum shown in Fig. 5(Ai) (upper panel) indicates that the relative phase at this frequency (see dashed line) is about $-\pi$, i.e. an alternation of bursts (Fig. 2(Ai)). Phase relationships are always given with the mandibular opener activity as reference. Power and phase of the spectral coherence are displayed simultaneously as polar plots with the length of the vectors representing the coherence, and the angle of the vectors representing the relative phase (Fig. 5(Bii)).

Spectra were computed from 900 s of spiking activity sampled at a rate of 1 kHz. A windowed Cooley–Tukey fast Fourier transform algorithm was used to transform data with 262144 data points per window yielding spectra of the frequency range 0–500 Hz at a resolution of 262,144 data points per Hz.

Statistical tests on linear data were performed using the $U$-test, or, when more than two samples were tested, using the $H$-test (Statistical Package for the Social Sciences (SPSS)); coherency data representing bivariate samples with a linear variable (coherence) and a circular variable (phase) were tested with a program written by the authors in C applying data transformation according to Mardia’s bivariate two sample test and a subsequent circular rank sum test as suggested in Batschelet (1981).

3. Results

3.1. Dose–response relationships

Both the phosphodiesterase inhibitor IBMX and the muscarinic agonist pilocarpine induce a mandibular motor pattern from isolated locust SOG (Fig. 2(Ai, Aii)). For both drugs the dose dependence of the cycle period was determined (Fig. 2(Bi, Bii)). Four individual preparations that had not been exposed to any drug before were tested for each dose. In each preparation the motor activity of the mandibular opener motoneurones was monitored for 15 min beginning approximately 5 min after application. From the resulting time series power spectra were computed and the location of the principal peak (the highest peak in the spectrum, see Fig. 2(Bi, Bii), insets), representing the bursting frequency, was plotted against the dose. In case no spike occurred in the observation period, the bursting frequency was set to zero. Both for IBMX and pilocarpine, the null hypothesis that samples drawn for the different doses do not differ can be rejected ($H$-test: $p<0.05$). Thus, the dosage has a significant effect on the frequency of oscillation despite the large inter-individual variance. The concentrations used as standards for the subsequent experiments (450 $\mu$M IBMX and 40 $\mu$M pilocarpine) were chosen in the saturation range (IBMX) or close to the peak frequency (pilocarpine).

3.2. IBMX induces less activity in the mandibular closer motoneurones

According to the results of backfills (P. Bräunig, unpublished observations), both mandibular opener and closer muscles are controlled by two pools of motoneurones. There is a minimum of 7 and a maximum of 12 motoneurones for each muscle. Uncertainties arise due to a variable number of serotonergic neurosecretory cells filled together with the motoneurones. The somata of these cells have similar locations to the motoneurones Bräunig (1987, 1988). The number of opener motoneurones recruited (5–7) is similar after application of pilocarpine and IBMX, as revealed by inspection of spike shapes and spike sizes in the extracellular recordings (data not shown).

In contrast, mandibular closer motoneurones are recruited at different numbers (7–10 with pilocarpine and 0–4 with IBMX; for sub-threshold activity, see below) and they spike less frequently during a burst. Thus, the activity of the mandibular closer motoneurones
is significantly lower in preparations treated with IBMX (Fig. 2(Ai, Aii)). The spike frequency in the mandibular closer motor pools was averaged over a period of 500–900 s yielding a measure of the overall activity of the neurones after application of either of the drugs (median values of 10 preparations per drug; IBMX: 0.77 Hz; pilocarpine: 58.2 Hz; \( p < 0.05; N=10 \)). In five out of ten IBMX-treated preparations less than one spike per cycle on average was recorded from the mandibular closer motor nerve.

Intracellular recordings from the somata of mandibular closer motoneurones show that the reduced activity after application of IBMX is due to reduced synaptic input (Fig. 3(A, B)). The oscillation of the membrane potential differs significantly in its peak-to-peak amplitude after application of IBMX and pilocarpine (median values of 9 preparations per drug; 10 cycles evaluated each: IBMX: 4.6 mV; pilocarpine: 8.0 mV; \( p < 0.05; N=9 \)). Nevertheless, at least subthreshold oscillatory activity was present in all motoneurones recorded. This suggests that almost all motoneurones receive rhythmic synaptic input at varying strength.

### 3.3. IBMX induces a faster and more regular pattern than pilocarpine

Patterns have a significantly shorter cycle period when induced with IBMX (Fig. 4(Ai, Aii, C); \( p < 0.05; N=10 \)). The bursting frequency was assessed for both drugs in ten individual preparations using the location of the principal peak in the power spectrum of the mandibular opener motor activity (Fig. 4(Bi, Bii)). In addition, IBMX induces a more regular pattern which was quantified by counting the number of peaks larger than 10% of the principal peak size (dashed line) in the power spectra obtained from the activity of the mandibular opener motoneurones (Fig. 4(Ai, Aii, Bi, Bii, D); \( p < 0.05; N=10 \)).

### 3.4. Similar phase relationships induced by IBMX and pilocarpine

#### 3.4.1. Closer and opener motor activity

Mandibular closer and opener bursts alternate both in preparations superfused with IBMX and pilocarpine (Fig. 2(Ai, Aii)). Since in IBMX-treated preparations mandibular closer motor activity is greatly reduced (see above), for the assessment of closer/opener coupling recordings were selected whose closer activity exceeded one spike per cycle on average. Typical example spectra are given in Fig. 5(Ai, Aii). The peak values of the power spectra and the corresponding relative phases are summarised in the polar plots shown in Fig. 5(Bi, Bii). Significant differences in the coherence of closer/opener motor activity after application of IBMX and pilocarpine could not be established \( (p > 0.05; N=5) \). A relative phase of less than \( \pi/2 \) at powers close to 1 (Fig. 5(Bi)) occurring only after application of IBMX indicates that in these cases the mandibular closer activity is consistently
3.4.2. Contralateral coupling

We assume that contralateral coupling of the mandibular opener motor activity has the same relative phase and strength in preparations treated with IBMX and pilocarpine (Fig. 6(Bi, Bii); $p>0.05; N=5$). Contralateral coupling of opener motoneurones is stronger and its phase is more constant among individual preparations than coupling of ipsilateral closer/opener motoneurones (Figs. 5 and 6). As ipsi/contralateral opener motoneurones are active synchronously whereas ipsilateral closer/opener motoneurones alternate, the spectral coherences of ipsi/contralateral opener motoneurones and ipsilateral closer/opener motoneurones differ significantly ($p<0.05; N=5$).

3.4.3. Motor output to muscle receptor organs

Both dorsal and ventral mandibular muscle receptor organs (dmro and vmro) couple to the mandibular motor pattern induced by IBMX or pilocarpine (Fig. 7). For the motor output to both muscle receptor organs no significant difference in the coherence after application of IBMX and pilocarpine could be established ($p>0.05; N=5$) whereas there is a significant difference to the coherence of ipsi/contralateral opener motoneurones ($p<0.05; N=5$) both after application of IBMX and pilocarpine. Although phase relationships vary considerably between individual preparations they are very stable within these preparations as can be seen from the length of the vectors in the polar plots (Fig. 7(B, D)). The spectral coherences of muscle receptor organ/opener motoneurones and closer/opener motoneurones do not differ significantly except for the pair dmro/opener and closer/opener in the preparations treated with IBMX ($p<0.05; N=5$). Upon inspection of the polar plots in Fig. 5(Ci) and Fig. 7(Ci) we conclude that this difference is not due to a different median relative phase but due to the high variability of the pair closer/opener motoneurones after application of IBMX (see above).

3.4.4. Coupling of neurosecretory cells

The spikes of the three paired serotonergic satellite neurones are distinguishable as three different units in extracellular recordings from the main branches of the satellite nervous system (Fig. 8(A); Bräunig, 1987). The satellite neurones synchronise with the mandibular motor pattern with varying phase relationship and coupling strength (Fig. 8(B–D)). However, the mean relative phase turns out to range quite consistently between 0 and $-\pi/2$ (thick arrows in Fig. 8(B–D)). This means that satellite activity can be expected during and shortly after the opener bursts. A comparison of the spectral coherences of the different satellite units/opener motoneurones computed for preparations treated with IBMX and pilocarpine did not yield significant differences ($p>0.05; N=5$). In contrast, in comparing the coherence of satellite units/opener activity with the coherence of ipsi/contralateral opener activity a significant difference was found for the small and the large unit but not for the intermediate unit ($p<0.05; N=5$).

4. Discussion

We showed that the phosphodiesterase inhibitor IBMX induces coordinated rhythmic activity in motoneurones and neurosecretory cells of the locust mandibular system. This activity is similar to, but not identical with, the rhythms induced by the muscarinic agonist pilocarpine (Rast and Bräunig, 1997) which supports the idea that efferent neurones are driven by the same premotor network after application of IBMX and pilocarpine.
Fig. 4. The IBMX-induced motor pattern is significantly faster and more regular than the pilocarpine-induced pattern. (A): Recordings from the mandibular opener motor nerves of preparations superfused with IBMX (Ai) and pilocarpine (Aii). (B): Power spectra of 900 s of mandibular opener motor activity after application of IBMX (Bi) and pilocarpine (Bii); stippled line: 10% of the principal peak size. (C): Box-and-whisker plot comparing the bursting frequencies after application of IBMX and pilocarpine ($p<0.01; N=10$). (D): Box-and-whisker plot comparing the number of peaks larger than 10% of the principal peak size after application of IBMX and pilocarpine ($p<0.01; N=10$).

However, differences in regularity, frequency, and spiking activity show that the two drugs act differently on the pattern generating network.

4.1. Dose–response curves

For IBMX, a saturation dose seems to exist above which the variance of the frequency of oscillation only depends on individual differences between the preparations and not on the dose (Fig. 2(Bi)). IBMX has been used in a wide range of doses with different preparations (Evans, 1984; Whim and Evans, 1991; Pannabecker and Orchard, 1987; Morton and Truman, 1985; Shibanaka et al., 1991). A possible explanation for the relatively high doses required in our preparation is the diffusion barrier of the intact ganglion sheath, as in work performed with lower doses ganglia were desheathed (Lundquist and Nüssel, 1997). To prevent degradation of cyclic guanylyl monophosphate for immunohistochemical stainings, concentrations of the same order of magnitude as in the present study were used (Truman et al., 1996; Ball and Truman, 1998).

For pilocarpine a dose between 40 μM and 80 μM is most likely to induce the highest frequencies of oscillation (Fig. 2(Bii)). At higher doses the frequency decreases which is mainly due to fusion of bursts into prolonged sequences of tonic activity. The standard dose was therefore chosen conservatively at 40 μM. The dose–response relationship determined for pilocarpine is in good concordance with results from other insect systems (Ryckebusch and Laurent, 1993).

4.2. New features of the IBMX-induced pattern

The differences of the motor patterns induced by IBMX and pilocarpine are not effects of dosage since
Fig. 5. Coherency of mandibular closer and opener motor activity. (A): Representative coherence and phase spectra of 900 s of opener and closer motor activity in preparations treated with IBMX (Ai) and pilocarpine (Aii). Stippled line: frequency at principal peak. (B): Polar plots of the coherency of closer and opener motor activity for preparations treated with IBMX (Bi) and pilocarpine (Bii). The length of the vector indicates the coherence and the angle indicates the relative phase. Thin arrows: individual preparations; thick arrow: mean vector; circle: unit circle with radius=1; angles are to be read counter-clockwise from $-\pi$ to $\pi$, 0 is to the right.

Fig. 6. Coherency of left and right mandibular opener motor activity. (A): Representative traces from preparations treated with IBMX (Ai) and pilocarpine (Aii). (B): Polar plots of the coherency of contralateral opener motor activity at the principal peak for preparations treated with IBMX (Bi) and pilocarpine (Bii). For further explanations see Fig. 5.
the standard concentrations of the drugs were chosen in a range where maximum activity can be expected (Fig. 2(B)). Thus, the differences found for the IBMX- and pilocarpine-treated preparations are due to specific properties of the motor patterns which are peculiar to the drug used. One reason for the reduced activity of the mandibular closer motoneurones in the IBMX-treated preparation is a reduced synaptic input to these neurones (Fig. 3) which leads to the interesting question whether the synaptic currents to motoneurones also differ qualitatively in IBMX- and pilocarpine-treated preparations.

The motor pattern induced by IBMX is more stable and faster compared to the motor pattern induced by pilocarpine (Fig. 4). A highly regular motor pattern can thus be centrally generated also in deafferented preparations and does not necessarily require rhythmic sensory input when the appropriate drug for the induction of the motor pattern is used. Questions on central mechanisms stabilising the motor pattern can therefore be addressed using the preparation treated with IBMX.

4.3. Similar phase relationships

As shown in Figs. 5–8 the activity patterns of motor and secretory neurones induced by IBMX and pilocarpine have similar phase relationships. In both IBMX- and pilocarpine-treated ganglia ipsi/contralateral coupling of mandibular opener activity appears to be strongest and most stable in the relative phase (Fig. 6). This corroborates the hypothesis stated in Rast and Braünig.
(1997) that there may exist only a single central pattern generator driving both left and right mandibles in contrast to thoracic motor rhythms which seem to be driven by separate coupled pattern generators for each joint (Ryckebusch and Laurent 1993, 1994; Büschges et al., 1995). This is also in concordance with the observation that contralateral opener activity is strictly coupled to ipsilateral imposed movements in intact preparations (Seath, 1977b).

Coupling of closer and opener motoneurones is less strong and the relative phase varies to a greater extent between individual preparations. Closer activity might therefore rely more on proprioceptive feedback than on opener activity, which could be provided by sensory cues provided by the mandibular muscle receptor organs and other proprioceptors (Bräunig, 1990).

A comparison of the spectral coherences of opener/muscle receptor organ motoneurones shows that these proprioceptors receive motor input alternating with the mandibular opener motor bursts both in preparations treated with IBMX and pilocarpine. The importance of synchronized drive to power muscles and muscle receptor organs and the functional implications made in Rast and Bräunig (1997) become more prominent under the new aspect that this drive is equally obvious in preparations activated with different agents.

The phase relationship of satellite neurosecretory cells and mandibular opener motoneurones varies considerably among individual ganglia both in preparations treated with IBMX and pilocarpine. Within individual preparations relative phase is quite stable. As the satellite neurones have neurosecretory function in the context of

Fig. 8. Coherency of satellite secretory and mandibular opener motor activity. (A): Representative traces from preparations treated with IBMX (Ai) and pilocarpine (Aii). (B)–(D): Polar plots of the coherency of satellite and opener activity for preparations treated with IBMX (Bi, Ci, Di) and pilocarpine (Bii, Cii, Dii). For further explanations see Fig. 5. sat: satellite.
feeding behaviour (Schachtner and Bräunig, 1993), the cells may be coupled to the motor pattern in order to activate them but the exact timing within the short-term mandibular pattern might play a minor role and is thus not controlled strictly by the presynaptic network.

4.4. Second messengers in mandibular pattern generation

IBMX elevates the level of both cyclic adenylyl monophosphate (cAMP) and cyclic guanylyl monophosphate (cGMP) in various tissues by blocking phosphodiesterases which degrade cyclic nucleotide monophosphates. For example, IBMX was used to elevate the level of cAMP in insect muscles (Evans, 1984; Whim and Evans, 1991; Baines et al., 1990) and in locust neuroendocrine tissue (Pannabecker and Orchard 1986, 1987). On the other hand, IBMX mimicks cGMP-mediated hormonal action like the induction of ecysis behaviour by eclosion hormone in the hawkmoth and the silkworm (Morton and Truman, 1985; Shibanaka et al., 1991).

The question whether muscarinic receptors are directly or indirectly coupled to an elevation of cGMP levels is not solved in the locust; however, it was shown in Manduca sexta larvae that such coupling is possible (Qazi and Trimmer, 1999). Though, it was also shown in this system that in some identified neurones the effects of muscarinic agonists are not necessarily mediated by an elevation of cGMP (Qazi and Trimmer, 1999).

A goal for future experiments is to separate the central pattern generator into pharmacologically distinct parts and to identify individual neurones within this network. Cells with elevated levels of cAMP and cGMP might be detectable using immunocytochemical techniques. As mentioned in the introduction, muscarinic agonists are known to induce motor patterns in various invertebrate preparations, perhaps suggesting common mechanisms of pattern generation. Therefore it seems worthwhile to investigate whether cyclic nucleotide-mediated processes represent a mechanism as common as muscarinic activation in other arthropod pattern generating networks.

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