The Journal of Experimental Biology 214, 757-763

doi:10.1242/jeb.049379

RESEARCH ARTICLE

Evidence of a central pattern generator regulating spermathecal muscle activity in *Locusta migratoria* and its coordination with oviposition

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Accepted 8 November 2010

SUMMARY

Electrophysiological recordings were conducted to determine the control of spermathecal contractions during oviposition of interrupted egg-laying locusts, *Locusta migratoria*. Following transection of the central nervous system below the metathoracic ganglion, rhythmic patterned bursting was detected by extracellular recordings of the nerve N2B2 that innervates the muscles of the spermatheca. Subsequent transections at more posterior regions of the ventral nerve cord revealed more robust rhythmic bursting in N2B2. This rhythmic bursting pattern was found to be coordinated with bursting in the ventral opener nerve (N2B1) that innervates the ventral opener muscle. This muscle controls the ventral ovipositor valves. Electromyographic recordings from the spermathecal muscle and ventral opener muscle confirmed a rhythmic bursting pattern resulting in an increase in muscle activity. Taken together, the results indicate that there is probably a central pattern generator (CPG), which is regulated by descending inhibition, that controls the spermathecal muscle activity. This CPG appears to be located within the VIIth and VIIIth abdominal ganglia, and was found to integrate with the CPG that regulates oviposition digging in locusts. These results provide further insight into the intricate coordination and control of reproductive tissues underlying reproductive behaviours in locusts.

Key words: spermatheca, descending inhibition, muscle contraction, fertilization, reproduction, insect.

INTRODUCTION

Successful oviposition of fertilized eggs requires the integrated coordination of the spermatheca, oviducts and ovipositor valves. This coordination is achieved through signals from both the endocrine and nervous systems (for a review, see Lange, 2009b). Neuropeptides and biogenic amines acting as neurotransmitters and/or neuromodulators and neurohormones play an important role in modulating the physiological activity of these structures (Lange, 2009a; Lange, 2009b). The study reported here investigated the central neural control of the spermatheca and its possible integration with neural activity that controls other reproductive tissues and behaviours.

The spermatheca of the African migratory locust, *Locusta migratoria*, is situated in the posterior region of the female abdomen and consists of a spermathecal sac, coiled duct and straight duct. This sperm repository organ is innervated by branches of the ventral ovipositor nerve (VON), which originates from the sternal nerve (N2) of the VIIIth abdominal ganglion (Fig. 1) (Clark and Lange, 2000). The N2B2 branch of the VON divides into at least three branches that innervate the spermathecal sac, coiled duct and anterior straight duct regions of the spermatheca. Other branches innervate the posterior regions of the straight duct, with the N2B6b nerve continuing posteriorly along the straight duct to the genital chamber (Clark and Lange, 2000). Cell bodies located in the lateral walls of the genital chamber have their axons in N2B6b (Clark and Lange, 2000).

Previous work has suggested that there is a neural loop, or reflex arc, that regulates spermathecal activity (Okelo, 1979; Clark and Lange, 2001). During egg laying, the presence of a mature egg in the genital chamber stimulates sensory cells of this chamber that feedback information to the VIIIth abdominal ganglion, altering motor neuron output to the spermatheca and thereby stimulating spermathecal contractions (Okelo, 1979; Clark and Lange, 2000). As a result, rhythmic contractions of the spermathecal sac are initiated that travel posteriorly along the length of the spermatheca. This leads to the propulsion of seminal fluid into the genital chamber, thereby fertilizing the egg prior to deposition in the soil, a process that is aided by activities of the ovipositor valves (Okelo, 1979). These reproductive tissues are under neural control and must be tightly coordinated so that sperm and egg are in the appropriate location at the appropriate time. Interestingly, the exact location of sperm during reproductive events remains to be determined.

The oviducts of *L. migratoria* are innervated by axons within the sternal oviducal nerve originating from the VIIth abdominal ganglion (Lange and Orchard, 1984; Lange et al., 1984a; Kalogianni and Pflüger, 1992). Neural activity within these motor neurons leads to contractions of the lower lateral and upper common oviducts such that eggs are retained within the oviducts (Lange and Orchard, 1984). This egg-retention behaviour is of importance when the female is searching for a suitable oviposition site, and during digging, so that mature eggs remain in the lateral oviducts before fertilization and deposition (Lange et al., 1984a).

The excavation of the soil by female locusts is also an important component of oviposition in locusts. Three pairs of ovipositor valves are situated at the most posterior end of the female abdomen and are controlled by ten pairs of muscles that are responsible for the rhythmic opening, closing, protracting and retracting of the valves during digging behaviours (Thompson, 1986a; Thompson, 1986b). Two of these muscle pairs include the ventral opener muscles, which are innervated by the N2B1 branch of the VON, and the ventral

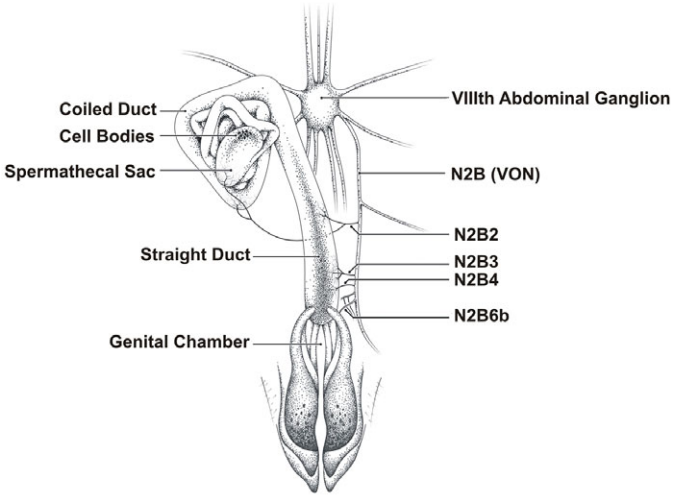

Fig. 1. Schematic diagram of the innervation of the spermathecal sac, coiled duct and anterior straight duct of the spermatheca of *Locusta migratoria,* by the N2B2 branch of the ventral ovipositor nerve (N2B). The innervation of the posterior straight duct by N2B3 and N2B4 is also shown in addition to the innervation to the genital chamber (by N2B6b) (drawing by Paul Hong).

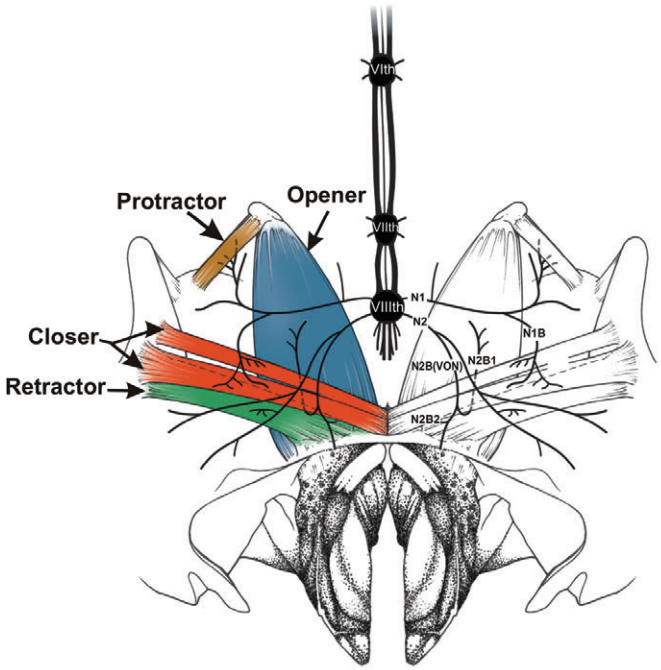

Fig. 2. Schematic diagram of the ventral ovipositor muscles and associated innervation by branches of the tergal (N1) and sternal (N2) nerves of the VIIIth abdominal ganglia of *L. migratoria*. The nerves N1B, N2B1 and N2B2 that respectively innervate the ovipositor closer muscle, ovipositor opener muscle and spermatheca are labelled (drawing by Zach McLaughlin and Paul Hong).

closer muscles, which are innervated by the N1B branch of the tergal nerve (N1; Fig. 2) (Thompson, 1986a; Thompson, 1986b). The rhythmic movements of the ovipositor valves are controlled by neurons within the VIIth and VIIIth abdominal ganglia (Thompson, 1986a; Thompson, 1986b).

The neural networks that control the oviducts and ovipositor valves generate rhythmic neural output in the absence of rhythmic input. These centrally located neural networks are termed central pattern generators (CPGs) (Bässler, 1986). CPGs are of widespread occurrence, underlying diverse activities that include walking in cats, locust flight, turtle scratching and leech swimming (Brown, 1911; Wilson, 1961; Robertson et al., 1985; Stent et al., 1978). Previous studies have shown that two CPGs are involved in egg-laying behaviours in locusts. There is a CPG in the VIIth abdominal ganglion controlling the oviducts and egg retention (Lange et al., 1984b; Facciponte and Lange, 1992; Facciponte and Lange, 1996). This CPG is active during digging and if egg laying is interrupted, but is not active in non-egg-laying locusts (Facciponte and Lange, 1992; Facciponte and Lange, 1996). The other CPG, the digging CPG, is located in the VIIth and VIIIth abdominal ganglia and is responsible for the coordination of the opening–protracting and closing–retracting movements of the ovipositor valves during digging of the oviposition hole (Thompson, 1986a; Thompson, 1986b). Both these CPGs are regulated by descending inhibition (Thompson, 1986b; Facciponte and Lange, 1992) and have been found to integrate with each other (Facciponte and Lange, 1996).

Although the characteristics and coordination of the CPGs that regulate egg retention and digging behaviours have been described, little is known about the coordination of spermathecal activity with these activities. At the time of egg laying, there is evidence that a sensory feedback loop coordinates the arrival of an egg in the genital chamber with contractions of the spermatheca, which results in expulsion of sperm onto the egg prior to deposition (Clark and Lange, 2001). Because the CPG that controls the opening and closing of the ovipositor valves is situated within the VIIth and VIIIth abdominal ganglia, and because the neural substrate that controls the spermatheca is in the same ganglia, the authors predicted that there should be some coordination between oviposition events and spermathecal activity, and set out to test this hypothesis.

MATERIALS AND METHODS

Animals

All experiments were conducted on sexually mature 3- to 4-week-old female locusts of *L. migratoria* L. reared at 30°C under a 12 h:12 h light:dark regime and fed fresh wheat seedlings supplemented with bran and carrots.

Preparations

Female locusts were interrupted 5–15 min after the onset of digging and were used for experiments 0.5–3 h later. Semi-intact locust preparations were prepared by removal of the legs and wings, and the locust was secured, ventral side up in a Sylgard-coated dish, with insect pins. A small mid-ventral incision was made through the posterior three abdominal segments to expose the spermatheca. The cuticle was held back using minuten pins and the common and lateral oviducts were held aside to expose the spermatheca. Physiological locust saline (150 mmol l^{-1} NaCl, 10 mmol l^{-1} KCl, 4 mmol l^{-1} $CaCl_2$, 2 mmol l^{-1} $MgCl_2$, 4 mmol l^{-1} $NaHCO_3$, 5 mmol l^{-1} Hepes (pH 7.2), 90 mmol l^{-1} sucrose, 5 mmol l^{-1} trehalose) was added to the tissues to keep them moist.

Electrophysiological recordings

For extracellular nerve recordings, the spermatheca was flipped posteriorly and held in place by a minuten pin to expose the posterior abdominal ganglia of the central nervous system (CNS). Extracellular recordings from N2B2 that innervates the anterior

region of the spermatheca, the ventral ovipositor opener nerve (N2B1) that innervates the ventral opener muscle, and the ventral closer nerve (branch of the tergal nerve, N1B) that innervates the ventral closer muscle, were made using glass suction electrodes or tungsten hook electrodes. Motor patterns were also recorded from isolated nerve cords. The VIth, VIIth and VIIIth abdominal ganglia were dissected out, pinned in a Sylgard-coated dish and bathed in locust saline. Suction electrodes were used at the N2B2, opener (N2B1) and closer nerves (N1B) so that electrical activity projecting to each muscle could be recorded. To obtain extracellular electrophysiological recordings, the nerves were cut from the closest branch that innervates each respective muscle. All extracellular nerve recordings were pre-amplified and filtered (low band-pass filter 300 Hz and high band-pass filter 1000 Hz) using an AM Systems (Everett, WA, USA) differential AC amplifier (model 1700).

Electromyographic recordings from muscle tissue were obtained using a 40 gauge enamelled nickel wire, stripped at the tip, and then wrapped around and inserted into the muscle. Electromyograms were amplified and filtered with a low pass filter at 100 Hz and a high pass filter at 300 Hz. All electrophysiology recordings were displayed on a Tektronix (Beaverton, OR, USA) dual beam storage oscilloscope (model 5113). Recordings were further displayed, stored and analyzed using an ADI Instruments (Colorodo Springs, CO, USA) Powerlab acquisition system and LabChart 6 Pro.

Data analysis

Electrophysiological activity was analyzed in 60-s recordings, and included the measurement of four variables: mean number of action potentials per burst, mean duration of burst, mean duration of interburst interval (measured as the time from the end of one burst to the beginning of the next burst) and mean cycle duration (measured from the start of one burst to the start of the subsequent burst). Statistical analyses included one-way ANOVAs with Tukey's *post hoc* test, and unpaired *t*-tests. These statistical analyses were conducted using Graphpad Prism. The significance level was set at $P<0.05$. Confidence interval analysis (95%) was performed to determine variability in mean number of action potentials per burst, mean burst duration and mean duration of interburst interval after transection of the CNS. A polynomial, first order linear regression analysis was performed to determine the relationship between burst duration and cycle period.

RESULTS

Neural activity in N2B2 to the spermatheca

Preliminary electrophysiological recordings from N2B2 (see Figs 1 and 2), which innervates the muscles of the spermatheca, showed variable basal activity depending on the amount of time that the females had been allowed to dig before being interrupted and processed for recordings. Females that had just started digging displayed either tonic high-frequency firing, or tonic firing with intermittent bursting motor patterns. Females that had been digging for longer periods before interruption showed more coordinated bursts of action potentials. As a result, all experiments were conducted on females that were in the later stages of digging.

When the CNS was severed between the metathoracic ganglion and the IVth abdominal ganglion, movements of the ovipositor valves were elicited that were similar to the digging movements observed in intact digging females. Simultaneous extracellular recordings were made from both the left and right N2B2. Observations revealed that these bursts were correlated with spermathecal contractions, and simultaneously with contractions of the ventral opener muscles and the opening of the ovipositor valves. The onset of the motor activity was delayed by 5–30 s following CNS transection. Transections of the CNS anterior to the metathoracic ganglion did not lead to any observed change in motor activity of N2B2 of interrupted egg-laying females. It was difficult to initiate a rhythmic motor pattern in non-egg-laying female locusts (27.3% success rate, N=33) and as a result all experiments were conducted on interrupted egg-laying females (62.5% success rate, N=40).

Upon isolation of the VIIth and VIIIth abdominal ganglia, extracellular nerve recordings from N2B2 revealed a similar motor pattern of bursts of action potentials that were coordinated between left and right N2B2 (Fig. 3).

Coordination of spermathecal activity with the ventral opener muscle

Extracellular nerve recordings from N2B2 and the ventral opener nerve (N2B1; Fig. 2) were obtained from the isolated VIIth and VIIIth abdominal ganglia. The activity patterns recorded from N2B2 coincided with the digging motor program patterns recorded from the ventral opener nerve. There was a sharing of phase relations and coordination of bursts between N2B2 and the ventral opener nerve (Fig. 4A). In contrast, the activity pattern recorded from N2B2 did not have the same phase relation or bursting pattern as the ventral closer nerve (N1B; Fig. 2) innervating the ventral closer muscles (the antagonists of the opener muscles; Fig. 4B). Indeed, the bursting patterns observed in N2B2 were out of phase with those observed in the closer muscle nerve.

Phase relationship of spermathecal activity with digging CPG

Extracellular recordings of N2B2 and the ventral opener nerve were obtained in isolated nerve cord preparations to compare the phase relationship. Phase analysis was performed by measuring the cycle duration following transection below the VIth abdominal ganglion. The activity patterns recorded from the nerves were similar in N2B2, which innervates the muscles of the spermatheca, and N2B1, the ventral opener nerve, which innervates the ventral opener muscles. The spermatheca and the ventral opener muscle were active at the same time, and the spermathecal activity and the ventral closer muscle was under reciprocal activation (Fig. 4C).

The ventral opener muscles are large muscles and therefore it is easy to record electromyographic activity from them. Electromygraphic recordings from the spermatheca muscle while simultaneously observing muscle contractions revealed the timing of muscle activity and the level of neural input to the muscle. Following interruptions during digging, electromyographic recordings demonstrated un-patterned bursts that coincided with spermathecal muscle contractions. When the CNS was transected

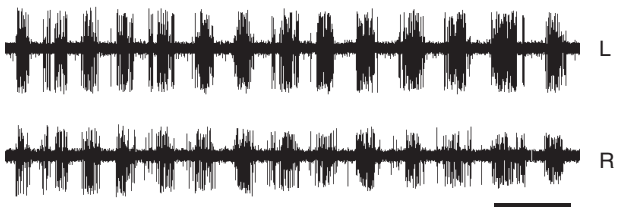

Fig. 3. Simultaneous extracellular recordings from both the left (L) and right (R) N2B2 that innervates the muscles of the spermatheca of *L. migratoria*. The traces represent the coordinated rhythmic motor bursting patterns in an isolated nerve cord (this is a representative trace, N=3; scale bar, 10 s).

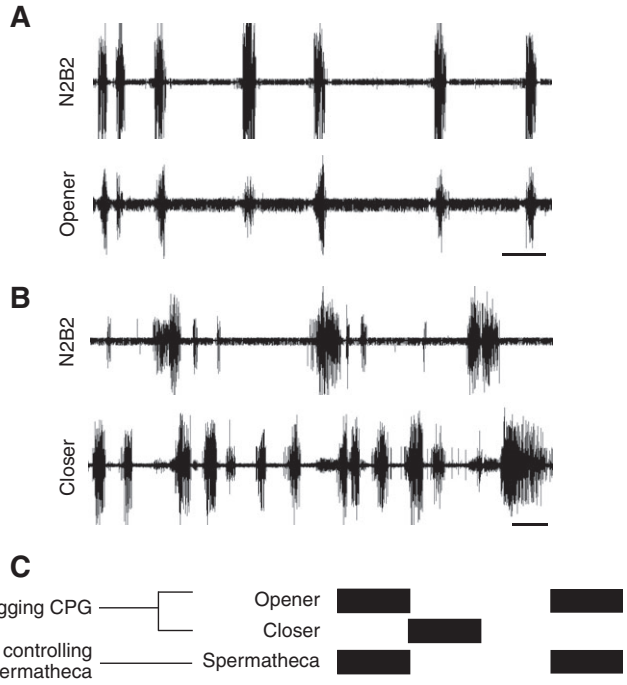

Fig. 4. Extracellular nerve recordings from N2B2 and the ventral opener nerve (A) and the ventral closer nerve (B) of the ovipositor valves of *L. migratoria*. Recordings were obtained from the isolated VIIth and VIIIth abdominal ganglia. Note in this preparation that N2B2 is never in phase with the neural activity of the ventral closer nerve (this is a representative trace, *N*=6; scale bar, 10 s). (C) A phase diagram showing the motor program for oviposition digging and spermathecal activity in *L. migratoria*. The ventral protractor and opener muscles are active at the same time, with reciprocal activation of the ventral closer muscles. The spermatheca is active at the same time as the opener muscle.

Fig. 5. Electromyographs from the spermathecal muscle (at the region of the coiled ducts) and the opener muscle following transection of the CNS of *L. migratoria*. Arrows denote individual bursts in the electromyographic recordings (this is a representative trace, *N*=7; scale bar, 10 s).

below the metathoracic ganglion, the spermathecal bursting pattern became more regular. Electromyographic recordings from the spermathecal muscle (at the region of the coiled ducts) and from the opener muscle confirmed the coordination of muscle activity (and by inference neural activity) between the spermatheca and the opener valve muscles (Fig. 5). These electromyograms coincided with visually observed muscle contractions of the spermatheca and opener muscles.

Source of neural inhibition, and localization of the spermathecal neural substrate

To identify the neural substrate generating the rhythmic motor pattern to the spermatheca, transections were made along the length of isolated CNS. The rhythmic motor pattern to the spermatheca was different depending on the location of transection of the CNS. When compared with the spermathecal electromyographic activity obtained from recordings with an intact CNS (Fig. 6A) of interrupted egg-laying females, the transection below the metathoracic ganglion was essential in obtaining a rhythmic bursting pattern (Fig. 6B). The rhythmic motor pattern persisted upon transection below the Vth abdominal ganglion. Upon transection of the CNS between the VIth and VIIth abdominal ganglia, the bursting pattern became even more regular (Fig. 6C). When the CNS was transected between the VIIth and VIIIth abdominal ganglia, the induced rhythmic motor pattern either disappeared or there was a much longer interburst duration (Fig. 6D).

Locusts that had their CNS transected between the VIth and VIIth abdominal ganglia showed a decrease in the mean number of action potentials per burst (29.35±4.46; mean ± s.e.m., *N*=6–10) (Fig. 7A) compared with locusts with an intact CNS (40.14±10.3; *N*=6–10). The rhythmic burst durations (Fig. 7B) and interburst interval durations (Fig. 7C) of these locusts were also shorter than those of locusts with an intact CNS. With transection between the VIIth and VIIIth abdominal ganglia there was an increase in the number of action potentials per burst, burst duration and interburst interval duration. Confidence interval analysis determined that there is less variability in these three measured variables in locusts that have their CNS transected below the metathoracic ganglion and between the VIth and VIIth abdominal ganglion than in locusts with an intact CNS or following transection between the VIIth and VIIIth abdominal ganglia. This indicates that the motor pattern is more regular (i.e. less variable) in preparations where the VIIth and VIIIth abdominal ganglia remain intact. Linear regression analysis shows the relationship between burst duration and cycle period, where shorter burst duration corresponds to shorter cycle period (Fig. 7D).

The mean number of action potentials per burst (59.38±18.29, 81.62±22.95; *N*=6), burst duration (2.31±0.52 s, 2.05±0.44 s; *N*=6), interburst interval durations (7.17±3.87 s, 7.74±3.96 s; *N*=6) and cycle period (9.53±4.46 s, 9.83±4.40 s; *N*=6) were compared for N2B2 and the ventral opener nerve respectively upon transection below the VIth abdominal ganglion. There was no statistically significant difference in the measured variables, indicating that the rhythmic motor pattern to the spermatheca is similar to the rhythmic motor pattern to the ventral opener muscle.

DISCUSSION

Previous studies have examined the innervation and neural control of spermathecal contractions in *L. migratoria* (Clark and Lange, 2000; Clark and Lange, 2001). Stimulation of peripherally located sensory cells demonstrated a neural loop stimulating motor activity to the spermatheca (Clark and Lange, 2001). The coordination of reproductive activities that include hole digging and egg laying have also been examined (Facciponte and Lange, 1996); however, their coordination with sperm release during oviposition has, until now, not been described. Previous studies have shown that digging and egg retention are coordinated such that eggs are only released during appropriate times (Facciponte and Lange, 1996). One can imagine that the control of spermathecal contractions and hence the release of sperm requires coordination with other reproductive tissues and

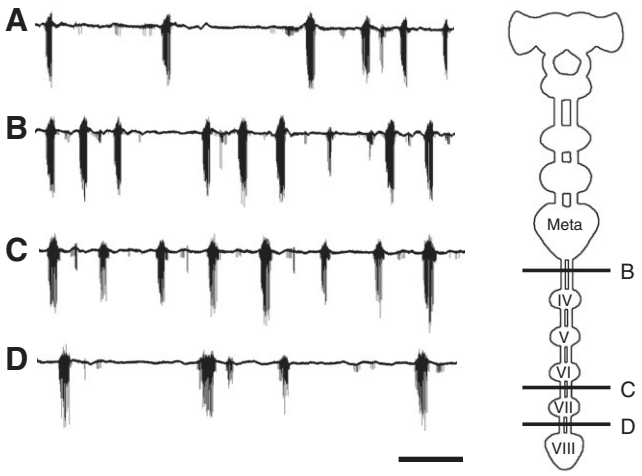

Fig. 6. Electromyographs identifying the inhibitory centre controlling spermathecal muscle activity of *L. migratoria*. Electromyograms of the spermatheca were obtained with an intact CNS (A), and following transection below the metathoracic ganglion (B), VIth abdominal ganglion (C) and VIIth abdominal ganglion (D) of interrupted egg-laying females (this is a representative trace, *N*=6–10; scale bar, 10 s).

events. It is postulated that the spermatozoa remain in the spermathecal sac until a time when the contractions of the anterior regions of the spermatheca lead to the movement of the stored sperm towards the posterior straight duct. Sensory cell activity, stimulated by the presence of an egg in the genital chamber then leads to the release of sperm onto the micropyle of the egg (Lange and da Silva, 2007). The similar rhythmic motor pattern observed between the left and right N2B2, as seen in the present study, suggest a coordinated motor rhythmic output to regulate spermathecal muscle activity. Similar coordination between the left and right N2B2 nerves was observed when measuring N2B2 activity in response to sensory cell stimulation in the genital chamber of female locusts (Clark and Lange, 2001). The fact that these bursts were observed during spermathecal contractions, and during the opening and closing of the ovipositor valves, suggests that the spermathecal contractions are essentially priming the system, leading to the movement of sperm along the 30 mm length of the spermathecal duct towards the most posterior region of the straight duct.

A rhythmic CPG regulates spermathecal activity and integrates with the digging CPG following release from descending inhibition

The present study demonstrates that transection below the metathoracic ganglion of interrupted egg-laying locusts results in the production of a rhythmic motor pattern in N2B2 that innervates the most anterior regions of the spermatheca, and that stimulates spermathecal contractions. The most robust rhythmic pattern with the least amount of variability in measured action potentials per burst, burst duration and duration of interburst interval was found to occur with transections between the VIth and VIIth abdominal ganglia. This is particularly interesting given that similar alterations in rhythmic motor patterns have been previously seen in the nerves that innervate the oviducts (Facciponte and Lange, 1992; Facciponte

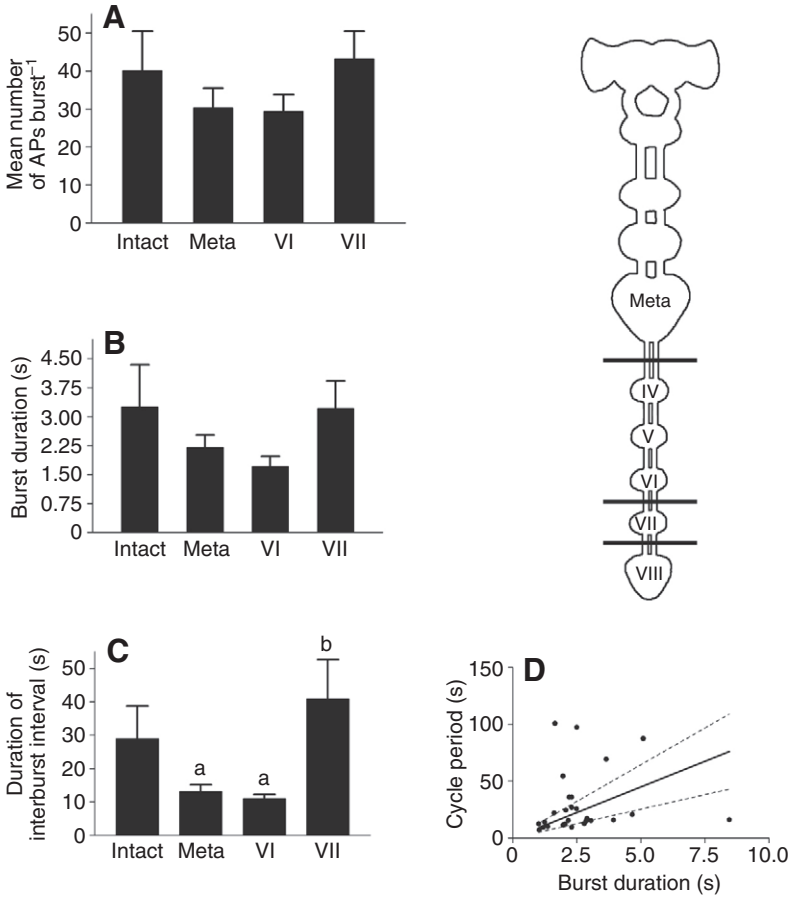

Fig. 7. Number of action potentials (APs) per burst (A), burst duration (B) and interburst interval duration (C) as determined from electromyographic recordings of female locusts following transection of the CNS below the metathoracic ganglion (Meta), VIth abdominal ganglion (VI) or the VIIth abdominal ganglion (VII) compared with those from the electromyographic recordings of females with an intact CNS (Intact). (Data are mean ± s.e.m., different letters above the bars denote significant difference between the treatments, $P<0.05$ ANOVA; $P<0.05$ Tukey's test; $N=6–10$.) (D) The relationship between burst duration and cycle period. Each point represents the burst duration *vs* the cycle period duration for all recordings taken from interrupted egg-laying females. A polynomial first order linear regression line with 95% confidence limit (dotted lines) is shown.

and Lange, 1996) and ovipositor muscles (Thompson, 1986b) upon transection below the metathoracic ganglion. In addition, the rhythmic motor pattern was readily apparent in interrupted egg-laying females unlike non-egg-laying females, which supports the findings of Lange et al. and Facciponte and Lange (Lange et al., 1984b; Facciponte and Lange, 1992).

Despite the fact that there are ten pairs of muscles involved in digging (Thompson, 1986a), the opener and closer muscles provide a good proxy of the motor program that coordinates with spermathecal activity. The ventral opener nerve contains axons from five opener motoneurons and one or two dorsal unpaired median neurons within the VIIIth abdominal ganglion (Belanger and Orchard, 1992). The N2B2 nerve that innervates the anterior regions of the spermatheca contains axons from three bilaterally paired motoneurons and dorsal unpaired median neurons within the VIIIth abdominal ganglion (Clark and Lange, 2000). The similar burst duration and interburst intervals observed in the rhythmic motor patterns of N2B2 and the opener nerve indicate that there may be a common neural basis regulating the activity of the spermatheca and the opener muscle during digging. This is supported by the overlap between the neural substrate that provides innervation to the spermatheca and ovipositor valve muscles (Clark and Lange, 2000; Thompson, 1986a). Locusts finish oviposition digging before they lay their eggs: however, ovipositor valves participate in other important behaviours during the deposition of eggs. Because fertilized eggs are deposited one-by-one during a maintained open gaping posture of the ovipositor valves (Thompson, 1986a), this may be the basis for the coordination between the spermatheca and opener muscle activity.

Results from the present study suggest that the spermatheca is probably controlled by a CPG that is centralized in the VIIth and VIIIth abdominal ganglia. Much like the CPGs that regulates digging (Thompson, 1986a; Thompson, 1986b), transection between the VIth and VIIth abdominal ganglia leads to the rhythmic and patterned activation of a CPG regulating spermathecal activity. As a result, the CPG controlling spermathecal activity runs with the oviposition digging CPG in a phase-locked manner and is activated following release from descending inhibition. These similarities are so great that it would be worth examining whether there are separate CPGs that control spermathecal activity and digging, or whether there are separate sets of motoneurons that are driven by the same CPG. The digging and egg-retention CPGs are also activated following release from descending inhibition, but the control of these tissues is through separate CPGs (Thompson, 1986b; Facciponte and Lange, 1992). These findings suggest that locust reproductive events include the timely passage and release of not only eggs, but also sperm at the appropriate times during digging and oviposition to ensure the coordinated fertilization and eventual laying of viable eggs into the oviposition hole.

CPGs in animals are complex regulators of behaviours. When contemplating a CPG that regulates spermathecal activity and other reproductive tissues, one cannot exclude the importance of sensory cues that may act to modulate them. Previous studies have shown that although the oviposition digging CPG produces a rhythmic output in the absence of any external cues, deafferenated animals were not able to maintain the digging CPG for long periods without sensory input, and that in a natural situation, a great number of afferent inputs are involved in maintaining a CPG (Belanger and Orchard, 1992). Another example was observed in the present study when application of saline at or around the ovipositor valves resulted in the inability to record the CPG output. This may be related to the fact that female locusts have sensory hairs that cover the abdomen, and females will not oviposition into substrates that are too wet or have high salinity levels (Kennedy, 1949). The present study also demonstrated a shorter cycle period when comparing isolated CNS recordings (fictive recordings) with those taken from semi-intact animals. Previous work (Thompson, 1986a) has identified that sensory input may be important for cycle period duration, because holding the ovipositor valves closed caused a lengthening in opener muscle cycle period. In the present study, longer cycle periods were obtained while recording in the semi-intact animal where sensory input was available to the motor pattern. In contrast, the isolated CNS recordings were void of sensory input and therefore, as a result, the difference in cycle period may be attributable to sensory feedback provided to the motor pattern.

The neuronal composition of the CPG regulating spermathecal contractions is unknown. This is in contrast to the neural loop involved in controlling spermathecal contractions in *L. migratoria*. The neural loop communicating sensory information from the genital chamber does not have a motor substrate that extends anterior of the VIIIth abdominal ganglion, suggesting that the sensory information from the genital chamber is processed in the VIIIth abdominal ganglion (Clark and Lange, 2001). One can also not exclude the influence of hormones, neurohormones and neuromodulators in modulating the ongoing activities that are generated centrally by these CPGs. The coordination between the digging and egg retention CPG and the coordination between the digging CPG and a potential CPG regulating spermathecal activity provides an indication that reproductive tissues in *L. migratoria* are highly coordinated during oviposition. Little is known about the control of the spermatheca at the time of mating or other reproductive events (such as production of secretions, tamping and retraction of the abdomen from the oviposition hole). Future work needs to explore the control of these behaviours and how they are coordinated *via* the CNS during reproductive events.

LIST OF SYMBOLS AND ABBREVIATIONS

CNS	central nervous system
CPG	central pattern generator
N1	nerve 1 of the VIIIth abdominal ganglion
N1B	nerve 1B that innervates the ventral closer muscle
N2	nerve 2 of the VIIIth abdominal ganglion
N2B1	nerve 2B1 that innervates the ventral opener muscle
N2B2	nerve 2B2 that innervates the anterior region of the spermatheca
N2B6b	nerve 2B6b that innervates the genital chamber
VON	ventral ovipositor nerve

ACKNOWLEDGEMENTS

The authors would like to thank Dr Ian Orchard for his revisions of the manuscript and valuable input. This work was supported by a grant and postgraduate fellowship from the Natural Sciences and Engineering Research Council (NSERC) to A.B.L. and R.d.S., respectively.

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