

Neural mechanisms coordinating the female reproductive system in the locust

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Oviposition of a fertilized egg
 - 3.1 The locust reproductive system
 - 3.2 Integrative aspects of egg-laying
 - 3.2.1. Mating
 - 3.2.2. Digging behavior
 - 3.2.3. Egg-laying
4. Neural control of reproductive structures
 - 4.1. Innervation and neural substrate
 - 4.1.1. Oviducts and ovaries
 - 4.1.2. Spermatheca
 - 4.1.3. Ovipositor valves and associated skeletal muscles
 - 4.2. Functional significance of neural substrate
 - 4.2.1. Oviducts and ovaries
 - 4.2.2. Spermatheca
 - 4.2.3. Ovipositor valves and associated skeletal muscles
 - 4.2.4. Coordinated actions of CPGs and neural input
5. Modulation of behaviors
 - 5.1. Neurochemicals as neuromodulators
 - 5.1.1. Oviducts and ovaries
 - 5.1.2. Spermatheca
 - 5.1.3. Ovipositor valves and associated skeletal muscles
6. Perspectives
7. Acknowledgements
8. References

1. ABSTRACT

The production of viable offspring is a complex task, involving courtship, mating, maturation of eggs, ovulation, fertilization of eggs, and oviposition. With particular regard to the female, the reproductive system must produce eggs at the appropriate time and deposit them after fertilization in an appropriate place. Thus, the various structures of the reproductive system must be tightly coordinated and integrated. This review focuses on the female reproductive system and the neural mechanisms that lead to its integrated control. Central pattern generators, that are linked, control oviposition digging behavior, and contractions of the lower lateral and upper common oviducts that lead to retention of eggs. Sensory neurons also provide information about the presence of an egg in the genital chamber via a feedback loop to coordinate the spermatheca and thereby, fertilization. Neuropeptides and amines can modulate central and peripheral control mechanisms. These neural mechanisms are integrated such as to produce coordinated behavior, leading to the accomplishment of the ultimate task, that of producing viable offspring.

2. INTRODUCTION

Reproductive behavior / physiology in insects is highly controlled and regulated by the nervous and endocrine systems (1-4). The ultimate task appears quite straightforward; that of producing viable offspring. The route towards that task is however, very complex. Elaborate courtship behaviors lead to successful mating, followed by fertilization of the matured eggs as the insect is egg-laying (oviposition). With this latter consideration in mind, the female reproductive system must produce eggs at the appropriate time and then deposit them after fertilization in an appropriate place. There are long-lasting cues of matedness, maturation of eggs (vitellogenesis), ovulation, and the egg-laying process itself, including finely controlled movements of the ovipositor valves for digging, the movement of the oviducts for propulsion of eggs, and the concurrent fertilization of these eggs.

The endocrine control of reproductive structures, involving hormones such as juvenile hormone and ecdysteroids, has been well reviewed over recent years (4 - 8). This paper will review the relevant literature on the

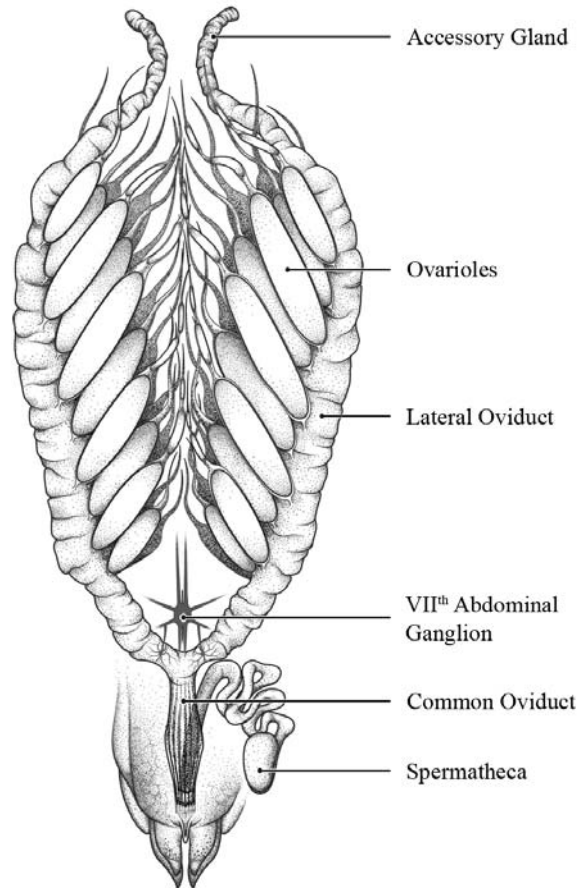


Figure 1. Schematic diagram of the ventral view of the female reproductive system of *L. migratoria*. The spermatheca has been pulled out from behind the common oviduct. Drawing was done by Paul Hong.

neural control of reproductive structures, concentrating on the locust, *Locusta migratoria*, and how these neurons or networks of neurons lead to the coordinated behaviors for digging, sperm release, and egg-laying in the female. As will be seen, coordination of behavior involves many different neuronal mechanisms from simple reflex arcs (9-13) through to complex central pattern generators (CPGs) (9, 10, 14). While the key feature of these CPGs is their ability to generate rhythmic neural output in the absence of rhythmic input, both rhythmic and non-rhythmic input is very important in modifying the motor output so that a final coordinated and adaptable behavior is produced (see 9, 10, 14). It is equally well established that modulation, particularly by neuropeptides and biogenic amines, plays a major role in the organization and generation of many invertebrate behaviors (9, 10, 15 - 21).

3. OVIPOSITION OF A FERTILIZED EGG

3.1. The locust reproductive system

The basic structure of the locust reproductive system is shown in Figure 1. The reproductive system of the locust consists of two ovaries which lie on either side of the mid-line in the dorsal aspect of the abdomen. Each

ovary consists of 50-100 panoistic ovarioles in which the eggs accumulate yolk protein until they are mature. Mature oocytes (eggs) are ovulated into two lateral oviducts and are held there until a suitable oviposition site is found. The lateral oviducts are muscular tubes and at the time of egg-laying, the eggs are propelled posteriorly along the lateral oviducts, passing through the muscular common oviduct on their way to the genital chamber. The passage of eggs is likely due to muscular contractions of the oviducts (see 1, 3). Eggs pass through the genital chamber and exit the animal via the gonophore, situated between the ovipositor valves. As eggs pass through the genital chamber, sperm are deposited on the micropyle region of the egg for fertilization to occur. Sperm are stored within the spermatheca from previous matings until the time of oviposition, and during oviposition, sensory information about the presence of an egg in the genital chamber stimulates motor activity to the spermatheca (22). Sperm are propelled from the spermathecal sac or endbulb (5 mm in length) and move down the spermathecal duct (25 mm in length). This movement of sperm is due to muscular contractions of the spermathecal sac and duct (3, 22 - 24). A pair of female accessory glands is also associated with the reproductive system of the locust (Figure 1). They are situated at the anterior end of the lateral oviducts and function to produce a secretion which surrounds the eggs and forms the egg pod in the soil (see 3). These secretions must also be moved along the oviducts. Eggs are laid only in suitable sites, i.e. soft, damp, sandy soil, at depths of several centimeters (25). Once a suitable oviposition site is found, the locust probes the soil by digging with its ovipositor; a highly specialized structure comprised of heavy cuticular appendages, hinges and large skeletal muscles. The ovipositor valves are then responsible for digging a deep chamber in the ground, for manipulation of eggs and to assist in capping the egg-pod with froth from the accessory glands. During oviposition digging, the valves undergo cyclical opening, closing, retraction, and protraction movements (26 - 28).

3.2. Integrative aspects of egg-laying

The events leading to successful oviposition of a fertilized egg into a suitable environment involves the coordinated and integrated activities of the reproductive system and associated digging behaviors. It is worth examining in some more detail the processes and behaviors that occur at these times.

3.2.1. Mating

Consideration must be given to the behavior of mating, which might begin with species recognition through an often complex courtship behavior, to the production and composition of male accessory gland secretions, the production of the spermatophore, and the transfer of sperm to the female during mating (see 1, 3 - 8). During mating in *L. migratoria* sperm are transferred from the male to the female via a spermatophore. Sperm, and associated secretions, are deposited in the upper spermathecal duct and must travel the short distance to the spermathecal sac. The sperm are stored in the spermathecal sac until oviposition. There have unfortunately, been very few studies on the physiological control of the spermatheca

Neural mechanisms coordinate the female reproductive system

and the role it plays during mating. More is understood about its role during fertilization. In addition, copulation induces physiological / endocrinological changes in the female concerning matedness, which results in the appropriate maturation of eggs and the subsequent deposition of fertile eggs in a suitable environment (see 3, 8, 29). A number of studies have examined this “matedness” factor and more recently a sex peptide has been identified that appears to decrease female receptivity and stimulate egg production post-mating in virgin *Drosophila melanogaster* (30 - 32).

3.2.2. Digging behavior

During oviposition, female locusts dig a hole in an appropriate location and substrate into which they deposit their eggs (26). The ovipositor valves, opened and closed by contractions of skeletal muscles, are used to dig this hole into a substrate which needs to be damp, sandy in nature and of low salinity (25). The female adopts a body posture such that the tip of the abdomen can be pressed into the soil. Excavation and preparation of a hole is achieved by rhythmic opening and closing of the ovipositor valves (26, 27, 33 – 35). The soil is moved aside by the opening action of the valves and a slight retraction of the abdomen. This is followed by a closing of the ovipositor valves and the abdomen is then further protracted into the substrate. A secured, smooth and robust hole is produced by periodically ceasing the digging, and tamping the hole by rotating the closed ovipositor valves around the long axis while patting the walls. The digging and tamping actions continue as the abdomen is eventually extended up to 10 cm into the substrate and the hole is ready for oviposition. The female locust has elasticized intersegmental membranes that allow for the large abdominal elongation into the substrate. Protracting and retracting movements of the abdomen provides the necessary force for the extension. A CPG within the VIIIth (terminal) abdominal ganglion controls the locust oviposition digging behavior (see later).

3.2.3. Egg-laying

A pair of ovaries lies in the anterior abdomen of the female locust with anterior extensions that result in accessory glands (Figure 1). Eggs develop in the ovarioles of the ovaries and mature eggs pass into one of two muscular lateral oviducts which extend posteriorly and converge at the common oviducts (see 1, 3). The eggs remain in the lateral oviducts during pre-ovipositional activities; a process that involves a CPG in the VIIth abdominal ganglion (see later). Once the hole has been dug, the eggs must progress posteriorly along the lateral oviducts where they have been held, prior to passage into the common oviduct and on into the genital chamber. Here, each egg is individually fertilized prior to deposition. The female locust typically lays 50-100 eggs into the oviposition hole that was made by the digging behavior, which gives an indication of the number of eggs being held in the lateral oviducts. As oviposition proceeds, the abdomen is slowly retracted from the hole in order to make room for the deposition of each egg. During these behaviors, sperm must travel from the spermathecal sac down the spermathecal duct, a distance of 30 mm, to arrive at the genital chamber for fertilization of the eggs (2).

Sperm are deposited onto the micropyle of the egg within the genital chamber, resulting in fertilization. The egg is then released from the female, with a small volume of fluid from the accessory glands accompanying each egg (4). When all 50-100 eggs have been laid, the egg pod is capped with accessory gland secretions which are frothed by rapid opening and closing movements of the valves. The frothed secretion then hardens (27). The abdomen is then fully withdrawn from the substrate. The female then conceals the hole and egg pod, using surrounding material, by scratching the ground with the mesothoracic and metathoracic legs (36).

4. NEURAL CONTROL OF REPRODUCTIVE STRUCTURES

4.1. Innervation and neural substrate

Extensive parts of the female reproductive system receive innervation from the VNC. These have been studied by a variety of techniques in order to identify the neural substrate. These techniques incorporate neurophysiology, backfilling and forward filling techniques, and immunohistochemistry for neuroactive chemicals.

4.1.1. Oviducts and ovaries

The muscles of the oviducts of *L. migratoria* receive polyneuronal innervation from a relatively small group of neurons located in the penultimate (VIIth) abdominal ganglion, that have axons projecting through the oviducal nerve (N2) that branches left and right from the sternal nerves (1, 37) (Figure 2). Backfilling of the branches of the oviducal nerve that innervates the oviducts reveals three pairs of motor neurons (OVN1-3) and two dorsal unpaired median (DUM) neurons referred to as DUMOV1 and 2 in the VIIth abdominal ganglion. These appear to innervate the junctional regions of the lateral and common oviduct. Additional DUM and ventral unpaired median (VUM) neurons appear to project to the lateral oviducts but only two project to the junctional area of the lateral and common oviduct (38). There are extensive neuropilar arborizations from these neurons and one of the motor neurons (OVN 3) in each bilateral cluster has a large contralateral projection ending in a profuse branching pattern of processes (37- 39). The innervation from the neurons of the VIIth abdominal ganglion is confined to the lower lateral and upper common oviducts (38, 39). The upper lateral oviducts, delineated by the attachment of the ovaries and by the accessory glands, appear to receive no innervation (37, 39). Other researchers have described additional neurons within the VIIth abdominal ganglion, but these were filled from the main oviducal nerve and not just from the branches that project to the visceral muscles of the oviducts (40). The main oviducal nerve also projects to skeletal muscles elsewhere in the abdominal segments, including M234, the external ventral protractor muscle of the VIIth abdominal segment, and the anterior and posterior muscles of the common oviduct (M257 and M258) (see below; 41). A CPG with patterned activity within the oviducal nerve has been described in some detail (42). The electrical properties of the visceral muscles of locust oviducts have been examined using intracellular recordings

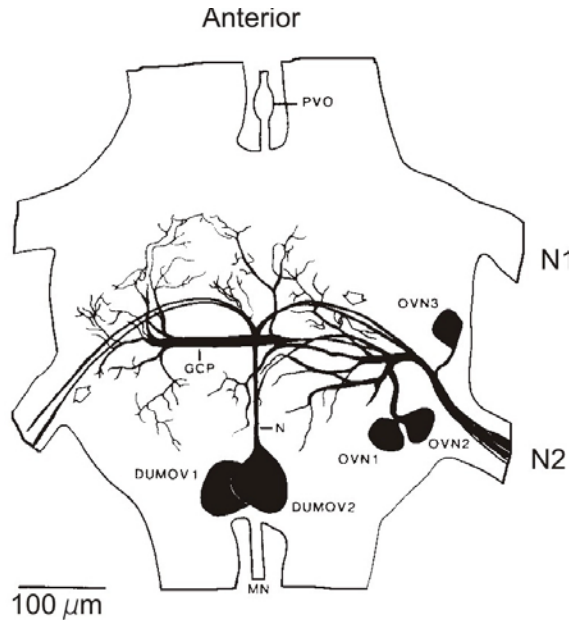


Figure 2. Cell bodies and central processes of neurons with axons passing to the lower lateral and upper common oviducts of *L. migratoria* as determined by cobalt lysine backfills of branches of the oviducal nerve (note: only the nerves innervating one side of the oviducts were filled). DUMOV 1 and 2 are dorsal unpaired median neurons with neurites (N) which pass anteriorly and then bifurcate to produce axons (open arrows) exiting the ganglion through the sternal nerves (N2). Three ventrally located motor neurons are also present (OVN 1 – 3, oviducal neurons 1, 2, and 3). OVN3 produces a giant contralateral process (GCP) that result in a field of processes within the neuropile. PVO; perivisceral organ; MN, median nerve. Redrawn from 37.

(39). The muscle cells of the innervated areas are both dye and electrically-coupled indicating the presence of gap junctions, and they possess an array of spontaneous electrical activity ranging from slow oscillations of muscle membrane potential to action potentials. They also generate excitatory junctional potentials (EJPs) in response to nerve activity. These forms of electrical activity explain the rather broad range of spontaneous and induced contractile activity present in the oviducts (39, 43). Slow peristaltic movements may be generated by the slow membrane potential oscillations, whereas phasic contractions may be induced by muscle action potentials, and by neural input. Since the muscle cells are electrically-coupled, a few cells may act as pacemakers for neighboring cells for spontaneous activity.

The upper lateral oviducts, anterior to the attachment of the ovaries, the ovaries, and the female accessory glands do not appear to receive any innervation (38, 39).

4.1.2. Spermatheca

The spermatheca of the locust receives innervation from the fused terminal (VIII) abdominal

ganglion via the bilaterally paired sternal nerves (N2, Figure 3). This innervation has been described for *Schistocerca vaga* (44) and for *L. migratoria* (45). Branching of the VIIIth and IXth segmental nerves results in innervation to various abdominal muscles that are involved in the digging behavior (see later). The anterior portion of the spermatheca receives innervation via the receptaculum seminis nerve (N2B2) and the posterior portion of the spermatheca receives innervation from the ductus seminalis aperture nerve (N2B3), as well as from N2B4. Another small nerve, N2B6b, is associated with the anterior lateral regions of the genital chamber (45).

Retrograde filling of N2B2 and N2B3 reveals a similar neural substrate with 3 bilaterally paired ventro-lateral neurons and 6 posterior DUM neurons in the VIIIth abdominal ganglion, and 2 DUM neurons in the VIIth abdominal ganglion (Figure 4). Neuropilar arborizations from some of these neurons are found in the VIIth and VIIIth abdominal ganglia and also in the VIth abdominal ganglion with some processes extend anteriorly towards the Vth abdominal ganglion. Nerve N2B6b projects along the lateral surface of the posterior straight duct towards the genital chamber. Of some considerable interest is the fact that anterograde filling of this nerve reveals extensive innervation to the spermatheca, but also many bipolar and unipolar neurons lying on the ventral, anteriolateral, portion of the genital chamber (45). Similar neurons have been reported in the posterior genital chamber wall of *Teleogryllus commodus* (46), and suggested to be sensory neurons that monitor the presence of an egg during oviposition (22, 44 - 46). Retrograde filling of N2B6b also reveals 3 bilaterally paired ventro-lateral neurons and 3 DUM neurons in the VIIIth abdominal ganglion; however there are no DUM neurons within the VIIth abdominal ganglion. Numerous processes and arborizations are observed in the VIIth and VIIIth abdominal ganglia with a few processes continuing on into the VIth abdominal ganglion.

4.1.3. Ovipositor valves and associated skeletal muscle

Rhythmic movements of the ovipositor valves are produced by contractions of ten pairs of muscles which receive innervation via the VIIIth and IXth segmental nerves of the fused terminal (VIIIth) abdominal ganglion (Figure 3, 27, 28, 47, 48). These have been studied in detail in *Schistocerca gregaria* and *Schistocerca americana* and in *L. migratoria* (see 27, 28, 34, 47, 48). The muscles that operate the ovipositor valves are attached to the internal surfaces of the three pairs of ovipositor valves, the apodemes, and the abdominal body wall of segments VIII and IX. The ten pairs of ovipositor muscles include 3 opener, 3 closer, 2 protractor, and 2 retractor muscles of which the opener muscles are by far the largest. The ventral closer and protractor muscles are innervated by the VIIIth dorsal (tergal, N1) nerves and the ventral opener and retractor muscles by the VIIIth ventral (sternal, N2) nerves of the VIIIth abdominal ganglion (27, 28, 34, 47, 48). The muscles of the dorsal valves receive innervation from the both the IXth dorsal (tergal) and ventral (sternal) nerves of the VIIIth abdominal ganglion (47, 48).

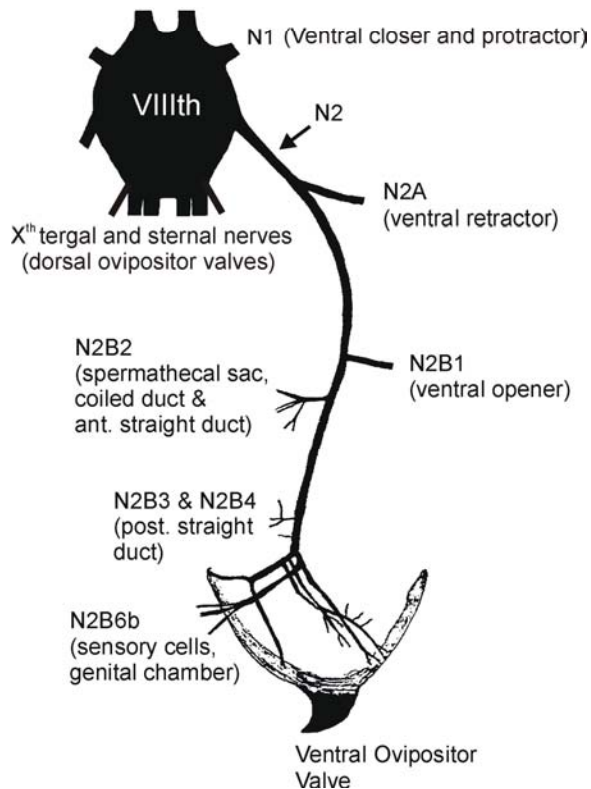


Figure 3. Schematic drawing of the VIIIth abdominal ganglion of *L. migratoria* with segmental nerves. The sternal (ventral or N2) nerve of the VIIIth segment of the ganglion innervates various regions of the spermatheca, as well as the ventral retractor and opener muscles. The tergal (dorsal or N1) nerve of the VIIIth segment of the ganglion innervates the ventral closer and protractor muscles, and muscles of the dorsal ovipositor valves are innervated by the IXth segmental nerves. Target muscles are indicated in brackets. Redrawn from 45, incorporating data from 27, 47, 48.

Cobalt backfilling techniques have been used to determine the location of the 17 ovipositor motoneurons supplying the ventral ovipositor valves of *S. gregaria* (27). Backfilling of branches of the VIIIth sternal nerve reveal that the ventral opener and the ventral retractor muscles are supplied by a group of 5 and 7 motor neurons (respectively) that lie in the VIIIth abdominal ganglion near the origin of the VIIIth tergal nerve. The innervation to the ventral opener muscle is similar in *L. migratoria* although two DUM neurons are also seen (Figure 5A, 34). The two ventral protractor motor neurons are located on the contralateral side of the ganglion from where their axons exit the tergal nerve. A group of 3 closer motor neurons also have axons in the VIIIth tergal nerve but their cell bodies are located in the VIIth abdominal ganglion close to where the sternal nerve originates (Figure 5B). The neural substrate for the dorsal ovipositor valves has not been defined and therefore the specific location of these neurons is unknown at this time. The motor neurons are part of a CPG controlling digging behaviors that has been extensively analyzed and defined (27, 28, 33, 34).

There are other skeletal muscles associated with the ovipositor muscles that play a role in the protraction and retraction of the ovipositor valves. One such muscle is the external ventral protractor muscle, M234 of the VIIth abdominal segment (41, 42, 47, 49). This muscle receives innervation from a branch of the oviducal nerve (N2B) of the VIIth abdominal ganglion. Cobalt backfilling of the branches of the sternal nerve that innervate M234 and neurophysiological techniques revealed a group of 2 bilaterally paired ventro-lateral neurons with neurites that bifurcate and send an axon contralaterally across the ganglion ending in a field of arborizations. Extracellular stimulation of the oviducal nerve, while gradually increasing the stimulation intensity, results in two different amplitudes of EJPs indicative of the recruitment of two motor neurons. These two neurons are the same neurons that innervate the muscles of the lower lateral and common oviducts (37, 41, 42, 49) and therefore share common rhythmic neural activity from the CPG in the VIIth abdominal ganglion, referred to as OVN 1 and 3 (38).

4.2. Functional significance of neural substrate

It is clear from the previous description that the reproductive tissues of *L. migratoria* receive extensive input from the CNS. Some of this input consists of motor neurons directly controlling contraction of skeletal and visceral muscles, and resulting in patterned activity and behavior, while others might be modulatory in nature.

4.2.1. Oviducts and ovaries

The ovaries and upper lateral oviducts in *L. migratoria* are not innervated and it has been postulated that there is neurohormonal control during oviposition. Thus, a myotropic neurohormone might be released from the CNS during pre-ovipositional activities which stimulates contractions of the lateral oviducts resulting in ovulated eggs being swept along the oviducts towards the common oviduct (see 1, 50, 51). With this in mind then, what might be the functional significance of the innervation to the lower lateral and common oviducts? The muscles in these areas receive polynervous innervation from neurons in the VIIth abdominal ganglion. Electrical activity in the motor neurons results in contraction of the muscles of the lower lateral and common oviduct (37). One might immediately assume that these contractions are used to expel the eggs through the common oviduct and into the genital chamber. In fact, the reverse is true. Contractions of these muscles results in a constriction of the lumen of the oviducts such that when the oviducal nerve of an oviduct with ovulated eggs is stimulated *in vitro*, the sustained contractions of the oviducts actually propel eggs back towards the ovaries (52). It would appear that the neural activity prevents egg progression and thereby prevents oviposition. This would be particularly relevant during the time when the myotropic neurohormone is present and the eggs are being propelled along the lateral oviducts. Egg progression could be inhibited, and ovulated eggs retained, until a suitable oviposition site is found and the digging behavior has ceased. Direct evidence for this has been shown using implanted electrodes. These electrodes have been used to monitor the *in situ* electrical

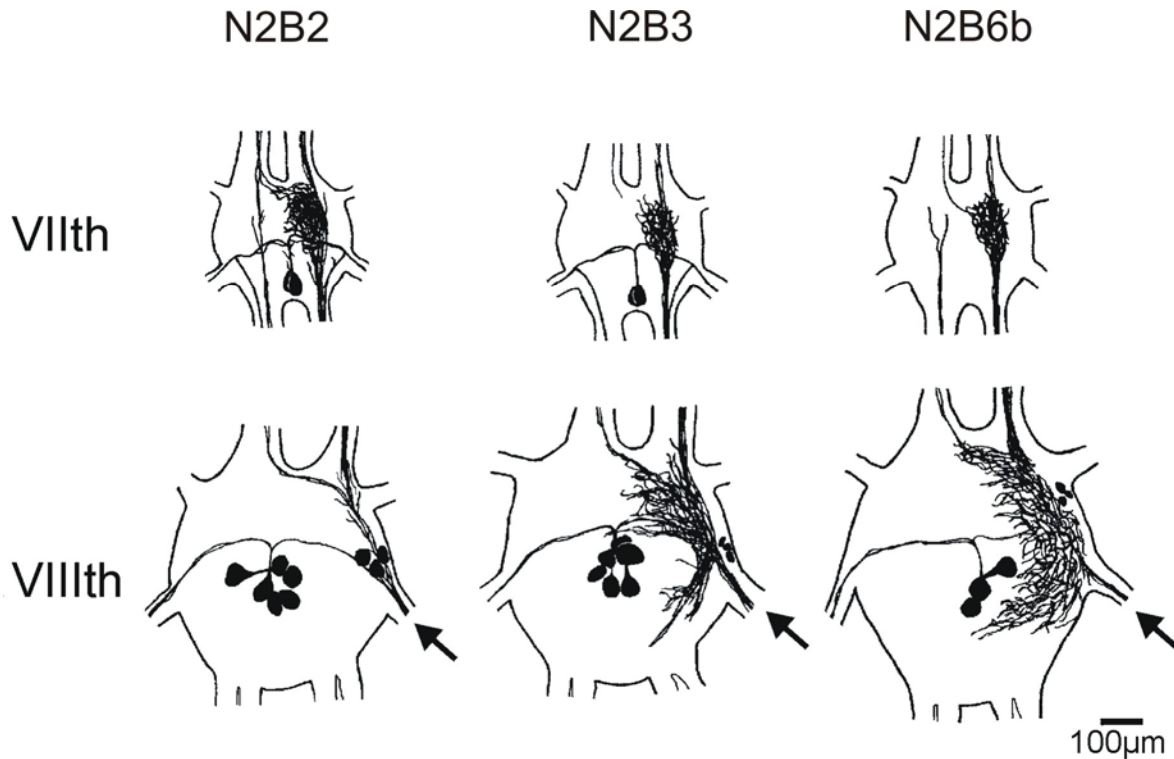


Figure 4. Drawings of cobalt lysine and neurobiotin backfills of the individual nerves innervating the spermatheca of *L. migratoria*. N2B2 innervates the spermathecal sac and coiled duct, N2B3 innervates the posterior straight duct and N2B6b innervates the genital chamber. Only the nerve on one side of the ganglion was filled (arrows). Cells and processes were filled in both the VIIth and VIIIth abdominal ganglia. Processes were seen to extend into the VIth abdominal ganglion although no cell bodies were present (not shown). Redrawn from 45.

activity of the oviducal nerves during times of digging and egg-laying (52). The patterned electrical activity is only present when egg-laying must be prevented; that is, during digging behavior or following interruption of egg-laying. The nerves are essentially silent when an egg is actually being deposited into the soil (52). Indirect evidence is also available since spontaneous rhythmic activity can be recorded from the oviducal nerves in dissected locusts that have been interrupted during oviposition (38, 42, 52). Apparently, this pattern can also be recorded when copulation is interrupted (38) although the functional significance of this is not known. The rhythmic activity recorded from the oviducal nerves is a result of a CPG located in the VIIth abdominal ganglion (38, 42, 49, 52). Interestingly, as with the digging CPG (see later), the CPG in the VIIth abdominal ganglion that controls contractions of the lower lateral and common oviduct is under the control of descending neural inhibition (28, 49). Thus, sucrose gap block of the neural activity within the VNC anterior to the VIIth abdominal ganglion of non-egg-laying locusts initiates the CPG, resulting in the rhythmic pattern of action potentials in the oviducal nerves (49). When the sucrose gap block is removed, the rhythmic pattern ceases. The apparent descending inhibition can be mimicked by electrical stimulation of the VNC in which the rhythm has been induced by transecting the VNC. The source of inhibition appears to be localized to the brain,

subesophageal ganglion and thoracic ganglia. The CPG is predominantly active in disturbed egg-laying locusts, and interestingly, also produces a rhythmic motor pattern which results in the reciprocal contractions of the external ventral protractor (M234) of the VIIth abdominal segment and of the lower lateral and upper common oviduct (42, 49). This discovery has led to a modification and refining of the notion that the CPG is used to prevent egg-laying at inappropriate time, and links this pattern to ovipositional digging activities also (see later). The DUM neurons contain and release octopamine that inhibits contractions of the lateral oviducts via a cAMP-dependent mechanism (53, 54). It is possible that the DUM neurons are also active during times of egg retention in order to counteract the activities of the myotropic hormones. Alternatively, or in addition, the two DUM neurons that project to the junctional regions of the lower lateral and upper common oviducts might be active at the time of egg-laying in order to relax the visceral muscles of the junction for passage of eggs.

In a similar manner in the stick insects, *Carausius morosus* and *Carausius extradentatus*, the progression of eggs into the common oviduct is regulated by neural input (55, 56). Contractions of the muscles of the common oviduct prevent the progression of eggs from the lateral oviduct into the common oviduct. *Carausius*

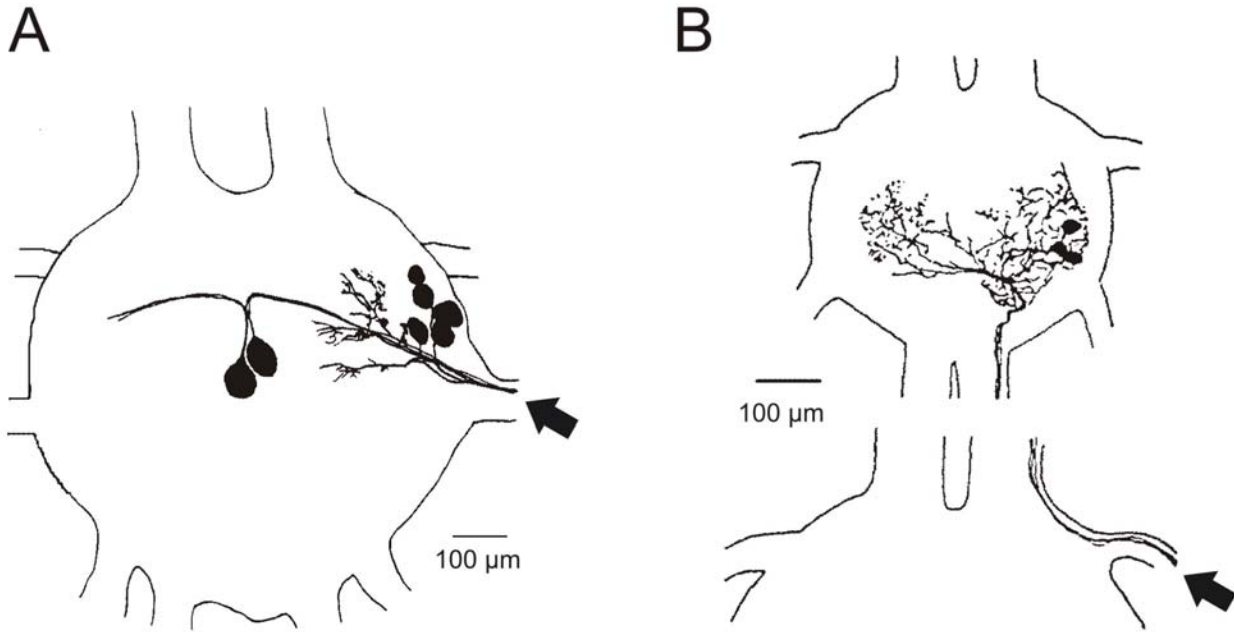


Figure 5. Innervation of the ventral opener and closer muscle. (A) Camera lucida drawing of a cobalt lysine backfill preparation showing cells in the terminal (VIIIth) abdominal ganglion which project to the ventral opener muscle (arrow denotes nerve that was filled). Note a group of five bilaterally paired motor neurons with two central DUM neurons. Redrawn from 34. (B) Camera lucida drawing of the cobalt backfill preparations of the nerve which projects to the ventral closer muscle (arrow denotes nerve that was filled). Note that axons pass through the VIIIth abdominal ganglion and form a group of 3 bilaterally paired cell bodies in the VIIth abdominal ganglion. Redrawn from 27.

morosus lays eggs at night, and so if an egg is ovulated during the photophase it remains in a chamber located between the genital valves until scotophase. It is believed that sense organs in the wall of this chamber initiate a reflex loop which results in contractions of the common oviduct (55). Thus, an egg can only pass into the common oviduct when the chamber is empty.

It is also interesting to note that in the grasshopper, *Chorthippus curtipennis*, it was observed that artificial eggs injected into the lateral oviducts of females which had recently laid eggs were pushed anteriorly up the lateral oviducts (57). Presumably the muscular contractions that moved the eggs could be under neural control in a similar manner to that observed in *L. migratoria*, resulting in the retention of eggs until oviposition is required.

4.2.2. Spermatheca

At mating, sperm of *L. migratoria* are deposited directly into the preapical diverticulum of the spermathecal sac in the female (58), where they are stored until fertilization is required. Prior to egg deposition and fertilization, these sperm must travel 30 mm from the storage area (spermathecal sac) to the posterior straight duct of the spermatheca. In other insect species, spermathecal muscle contraction has been shown to be essential for this transport (23, 24, 59). In *L. migratoria*, the peristaltic contractions of the sac, coiled duct and anterior straight duct are probably involved in the movement of sperm to the posterior straight duct where

they await the arrival of an egg. As described below, it seems clear that a sensory feedback loop coordinates the arrival of an egg in the genital chamber and the subsequent contractions of the posterior straight duct to eject the sperm onto the egg. It is unclear at present what controls the sperm moving from the spermathecal sac to the posterior straight duct. Also unknown, are the factors involved in initiating the initial transfer of sperm out of the spermathecal sac.

As mentioned earlier, spermathecal muscle contraction has been shown to be essential for successful fertilization (23, 24) and it would seem essential, and obvious, that the contractions of the spermathecal muscle must be integrated with oviposition. Thus, it was hypothesized that in *S. vaga*, sensory receptors in the genital chamber, integrated with the spermatheca, could be activated by the passage of an egg, thereby physiologically linking these two reproductive tissues (44). These sensory receptors might reflexively activate motor neurons leading to rhythmically generated spermathecal contractions, and sperm release (44). With the discovery of sensory neurons in the anterior lateral portions of the genital chamber of *L. migratoria*, this hypothesis of a neural loop was tested using electrophysiological techniques (22). Mechanical stimulation of the genital chamber sensory neurons by way of a glass probe, representing the shape and size of an egg, inserted into the genital chamber increases the frequency and alters the pattern of motor activity projecting to the spermatheca. Removal of the probe results in the motor activity returning to its pre-stimulated pattern. In addition,

activation of the genital chamber sensory neurons by use of the probe also results in an increase in the frequency of contractions of the spermatheca, as would be predicted by the change in motor pattern. It seems logical that sperm, stored in the spermathecal sac, are propelled towards the posterior straight duct by contractions of the anterior portion of the spermatheca. These sperm are held in the sac until the sensory neurons are activated by an egg passing through the genital chamber. The sensory neuron stimulation results in a feedback loop, with sensory information passing to the CNS, activating spermathecal motor neurons that lead to contractions of the posterior straight duct of the spermatheca, and thereby ejection of sperm onto the micropyle of the egg. The egg would then pass out of the genital chamber, removing the sensory stimulus, and the contractions of the posterior straight duct of the spermatheca might cease.

An involvement of genital chamber mechanoreceptors with changes in spermathecal contractions has previously been postulated in a variety of insect species (60, 61, 62). In the bush cricket, *Metaplastes ornatus*, the presence of an egg in the genital chamber has been shown to result in sperm release (63). Parenthetically, males have been shown to take advantage of this system in order to expel sperm deposited by other males during previous copulations, in an attempt to increase inclusive fitness. Thus, the sub-genital plate of male *M. ornatus* can imitate an egg in the genital chamber and induces sperm release (63). The male then injects his own sperm. A similar mechanism of sperm displacement has been proposed for the dragonfly *Orthetrum coerulescens* (61). In *L. migratoria*, it is primarily sperm from the last successful mating that are released onto the egg for fertilization (64). However, since the penis (or aedeagus) of the male *L. migratoria* does contact the genital chamber during mating (58) it is possible that the male could also make use of this neural loop to induce contractions of the spermatheca and thereby remove rival sperm. This appears not to have been tested, but clearly the mechanism and possibility exists.

4.2.3. Ovipositor valves and associated skeletal muscles

The neural control over oviposition digging behavior has been fully described in *S. gregaria* (27, 28) and confirmed as being similar in *L. migratoria* (33, 34). The oviposition digging behavior is driven by a CPG that can be activated by transection of the VNC in sexually mature females, but not in young females (27, 28, 33, 34). The ovipositional digging behavior is initiated and maintained by the absence of descending neural inhibition in a manner similar to that of the CPG in the VIIth abdominal ganglion. Thus, transection of the VNC or 'cold' block of neural activity activates the digging CPG (28). Again, the source of inhibition could be localized to the brain, subesophageal ganglion and thoracic ganglia. Analysis of the neural substrate reveals that the CPG produces rhythmic activity from the fused VIIIth abdominal ganglion that activates ovipositor muscles in an orderly sequence. The neurons responsible for this have been identified by cobalt backfilling and electrophysiological tracing (see earlier). The activity

produced by the deafferented ganglion has similar phase relationships, burst duration, and cycle frequency as that shown by the ganglion *in situ*. However, this pattern in deafferented ganglia of *L. migratoria* shows a significant decrease in frequency over time, which is not evident in intact or semi-intact locusts (33). The frequency of this pattern can be restored by tonically stimulating the sensory axons from mechanosensory hairs covering the ovipositor valves. Thus, tonic input from mechanosensory hairs of the valves that would be stimulated during the process of digging, is necessary for maintenance of the digging rhythm (33).

The external ventral protractor muscle, M234, in the VIIth abdominal segment is also involved in protraction and retraction of the valves, and as mentioned earlier, this muscle is also under the influence of a CPG in the VIIth abdominal ganglion controlling contraction of the lower lateral and common oviduct (49). It should be noted that M234 has been investigated in some detail (41, 42, 49) but it is only one of many segmentally repeating muscles that are likely involved in the oviposition digging behavior. These other muscles have not been examined.

4.2.4. Coordinated actions of CPG and neural input

A CPG in the terminal abdominal ganglion and a CPG in the VIIth abdominal ganglion control digging behavior, and contractions of M234 and the lower lateral and upper common oviduct respectively (27, 42, 52). The CPG in the VIIth abdominal ganglion is active during digging, and, as mentioned earlier, is needed in part to prevent the passage of eggs along the oviducts at a time when the oviducts are stretched but the oviposition hole has not yet been completed (42, 52). Thus the CPG results in a constriction of the lower lateral and upper common oviducts, thereby impeding the flow of eggs into the common oviduct and the genital chamber. The CPGs for digging behavior and for impeding the passage of eggs are coordinated and integrated, and involve overlapping locations of neural substrates (Figure 2, 4, 5). In addition, both are under descending neural inhibition and can be initiated by transection of the nerve cord anteriorly to their neural substrate (28, 49). It has been further suggested that the two rhythms (digging and oviductal contractions) may be driven by the same central system, since when the oviductal rhythm is initiated the ovipositor valves undergo continuous digging movements (38). Cutting the connectives between the VIIth and VIIIth abdominal ganglion dramatically alters the oviductal rhythm (38). During digging, the reciprocal neural pattern of activity that drives contractions of the lower lateral and upper common oviduct and the external ventral protractors (M234) would appear to act in concert with the retractor and protractor muscles of the ovipositor valves to aid in digging (42). Thus, the lower lateral and common oviducts may also pull the ovipositor valves anteriorly and ventrally since there are some attachment sites of the common oviduct to the ventral ovipositor valves (42). Contractions might therefore aid in the anchoring of the lower ovipositor valve in the substrate such that retraction of the ovipositor valves could help extend the abdomen. Also, it has been suggested that digging relies on the thrusts of the upper ovipositor valves

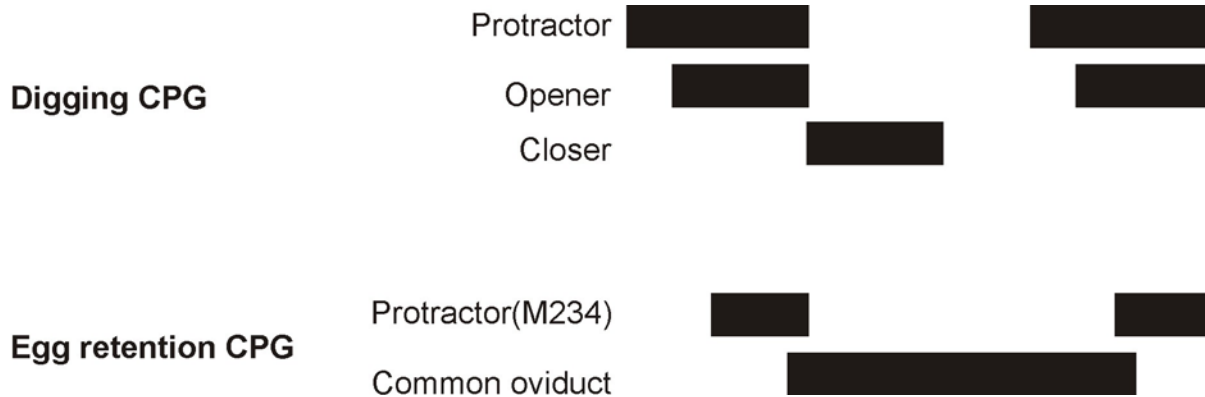


Figure 6. Phase diagram of the motor program for oviposition digging and egg-retention. In the digging CPG of the VIIIth abdominal ganglion of *Schistocerca gregaria*, the ventral protractor and opener muscles are active at the same time with reciprocal activation of the ventral closer muscles. Comparison with the egg-retention CPG in the VIIth abdominal ganglion of *Locusta migratoria* indicates that the ventral protractor muscle (M234) of the VIIth abdominal segment is active along with the ventral protractor muscles in the VIIIth abdominal segment and the common oviduct is active at the same time as the closer muscle of the VIIIth abdominal segment, and overlapping the beginning of the next cycle. Reinterpreted from 27, 42, and 49.

(26) and so the external ventral protractors (M234) of the VIIth abdominal segment which lie in line with the thrusting motion and the tip and hinge of the upper ovipositor valves might play a key role in protraction. This of course requires coordination between the digging CPG in the VIIIth abdominal ganglion and the CPG in the VIIth abdominal ganglion such that the protractor activity is in phase. Such coordination is evident (49). Thus, the CPG in the VIIth abdominal ganglion and the digging CPG in the VIIIth abdominal ganglion share an overall phase relationship and a coordination of burst frequency and intra-cycle events (Figure 6). There is thus a simultaneous activation of M234 and the ventral protractor and opener muscles of the ovipositor valves orchestrating digging, with the rhythmic neural activity in the oviducal nerves having the additional function of retaining the eggs until the digging is complete.

Spermathecal contractions might be initiated during digging behavior; a behavior that is under the control of the VIIIth abdominal ganglion CPG and feedback from mechanosensory hairs on the ovipositor valves (33). It is possible that this same sensory feedback might also act directly and/or indirectly on the neural substrate that controls spermathecal muscle contractions; contractions that are necessary for the passage of sperm from the spermathecal sac along the spermathecal duct for fertilization of the egg in the genital chamber. A second possibility is that the digging CPG might also coordinate the motor neurons projecting to the spermatheca, in a manner similar to the coordination of the oviposition digging and egg-retention CPGs, as referenced earlier. Appropriately, the neural substrate controlling the spermatheca lies within the same abdominal ganglia that are involved in these CPGs (27, 28, 33, 45, 49). Feedback from sensory neurons located in the genital chamber, and stimulated by the arrival of an egg, also control contractions of the spermathecal posterior duct in order to eject sperm onto the egg.

5. MODULATION OF BEHAVIORS

Flexibility and plasticity in the nervous system can be achieved by modulating ongoing activities that are generated either centrally or peripherally. Centrally this might involve directly or indirectly influencing the CPG. Peripherally this might involve influencing the output of the tissue under control, eg. tension of muscle contraction. Such effects have been well described in other invertebrates, such as in the stomatogastric nervous system of crustaceans (9, 10, 17 - 19). One should not be surprised, therefore, that the behavior of egg-laying is also under the influence of neuromodulators.

5.1. Neurochemicals as neuromodulators

5.1.1. Oviducts and ovaries

Only one study has examined the effects of neuroactive chemicals on the VIIth abdominal ganglion CPG that control the oviducts and M234. Perfusion of the VIIth abdominal ganglion with SchistoFLRFamide results in an increase in action potential frequency of the CPG recorded from the oviducal nerve, which results in a concomitant increase in the frequency of contractions of the oviduct muscle (65). Proctolin is also capable of altering the rhythmic bursting of action potentials in the oviducal nerve when applied to the VIIIth abdominal ganglion. This change in the CPG also results in changes in oviducal contractions (65). In contrast to the limited number of studies on the CPG, spontaneous and neurally-evoked contractions of the oviducts of *L. migratoria* have provided model preparations for investigating the influence of neuroactive chemicals on insect visceral muscle (1). Proctolin is a cotransmitter with glutamate in at least one of the motor neurons driving contraction (66 - 69) and octopamine, present and released from the DUM cells, inhibits contraction of the oviducts via a cAMP dependent mechanism (53, 54). SchistoFLRFamide is also associated with the innervation to the oviducts and also inhibits contraction, but not via cAMP (70). Indeed, there is

evidence to suggest that tyramine released from DUM neurons might inhibit contractions of the oviducts via an involvement of cGMP (71, 72). The ovaries are not innervated, but a myotropic neurohormone has been postulated to control movement of eggs along the lateral oviducts. This neurohormone has not been identified (see 4, 50, 51). Presumably the presence of different neuroactive chemicals, either acting as neurotransmitters / neuromodulators or neurohormones (1, 73), leads to a fine tuning of the contractions of the oviducts, balancing retention of eggs until a suitable oviposition site is found, with deposition of the eggs through the common oviduct and genital chamber.

5.1.2. Spermatheca

The extensive neural innervation, from multiple branches of the sternal nerve, and including multiple ganglia of the VNC, implies that fine neural control is required over the various regions of the spermatheca. The presence of putative sensory neurons within the lateral walls of the genital chamber is also suggestive of a controlled feedback loop associated with the arrival of an egg in the genital chamber. A variety of neuroactive chemicals are associated with the innervation of the spermatheca of *L. migratoria*. These have been fully reviewed recently (2) and only a brief description will be provided here. Neuropeptides belonging to various families including FMRFamide-related peptides (FaRPs), proctolin, crustacean cardioacceleratory peptide (CCAP), locustatachykinin and allatostatins, as well as amines, are associated with the neural input to the spermatheca (2, 74 - 79). In addition, and likely via the DUM neurons, is input from octopamine and tyramine (2, 78, 79). All of these neuroactive chemicals are myoactive on the spermatheca except for the FaRPs which are inhibitory and the allatostatins which appear to have no effect on muscle contraction. This wide array of neuroactive chemicals certainly implies a fine tuning of the contractions of the spermatheca which might be expected given the fact that the spermatheca must not only receive and store the sperm at mating, but then reverse the progression of the sperm for fertilization. An intricate series of contractions of the various areas of the spermatheca might be needed in order to perform these complex behaviors. An interesting observation was recently made with cell bodies (most likely sensory neurons) present on the spermathecal sac revealing immunoreactivity against tyramine. It would seem possible that there might be a sensory feedback loop associated with the behaviors of the spermathecal sac that might be mediated by tyramine and / or tyramine might be locally released from these cells to modulate muscle contraction (79).

5.1.3. Ovipositor valves and associated skeletal muscle

The pentapeptide proctolin influences locust ovipositional behavior at a number of locations (35). Peripherally, proctolin is a cotransmitter in motor neurons of the ventral opener muscle, where it has a variety of concentration-dependent effects. At low concentrations (10^{-10} M) proctolin increases the frequency of miniature EJPs. At a higher concentration of 10^{-9} M, proctolin enhances neurally-evoked twitch tension and then at 10^{-8} M,

10^{-8} M, proctolin results in a large increase in basal tension with superimposed slow myogenic rhythms. In support of a true physiological role for proctolin in this preparation is the observation that electrical stimulation of the motor neurons and activity of the digging CPG releases proctolin. Furthermore, proctolin appears to be required for the normal functioning of the muscle. Thus, muscle contraction tension that is produced during the oviposition digging CPG actually decreases over time *in vitro*; a time at which there is a decrease in the amount of proctolin released. Superfusion with 10^{-9} M proctolin can restore the muscle contractions back to their original amplitude. It was suggested that proctolin is not merely aiding contraction but is an absolute requirement for contraction (35). Centrally the digging pattern CPG declines in frequency in a totally isolated VIIIth abdominal ganglion (35). Perfusion of the ganglion with proctolin restores the pattern to its original level of activity. It is not known if the effects of proctolin are direct on the neurons of the CPG or indirect via interneurons. Thus, proctolin appears to maintain the "functional integrity" of the central digging pattern.

Proctolin is also associated with M234 (80). Examination of the axons in the external ventral protractor muscle, M234, and nerve N2b_{2b1} by electron microscopy reveals two axon types; one containing 100 nm diameter electron-dense granules (Type 1) that reveal immunogold staining for proctolin, and the other containing 135 nm diameter granules of high electron-density (Type 2) (80). The Type 1 terminals are most likely derived from the two motor axons in the nerve and produce typical synaptic sites indicative of motor innervation in insect skeletal muscle (81). The Type 2 axons also produce typical synaptic sites, but in addition generate neurohemal sites covering the perineural sheath of the nerve that innervates M234. This suggests an ability of the same neuron to release its contents from neurohemal sites along the nerve and directly onto the target tissue. A similar situation has been shown in *Rhodnius prolixus* where axons innervating the ventral abdominal intersegmental muscles appear to release at both neurohemal sites along the nerve and locally onto the muscle (82). The neuroactive chemicals might therefore act as neurohormones released into the hemolymph or as neuromodulators, released more directly onto the muscle. A number of motor neurons in insects have been shown to co-localize glutamate, as the excitatory neuromuscular transmitter, and neuropeptides as a modulator (69). Bath application of glutamate to M234 rapidly increases the basal tonus indicating that it may also be the excitatory transmitter at this muscle. The activity of the muscle is, however, modified by a number of neuropeptides, including proctolin and SchistoFLRFamide, and the biogenic amine, octopamine (80). These modulators are capable of enhancing the twitch amplitude. This modulation of signaling occurs peripherally, at the level of the muscle and muscle contraction.

6. PERSPECTIVES

As would be expected for such an essential behavior as oviposition of a fertilized egg in an appropriate location, the reproductive tissues of *L. migratoria* are finely

Neural mechanisms coordinate the female reproductive system

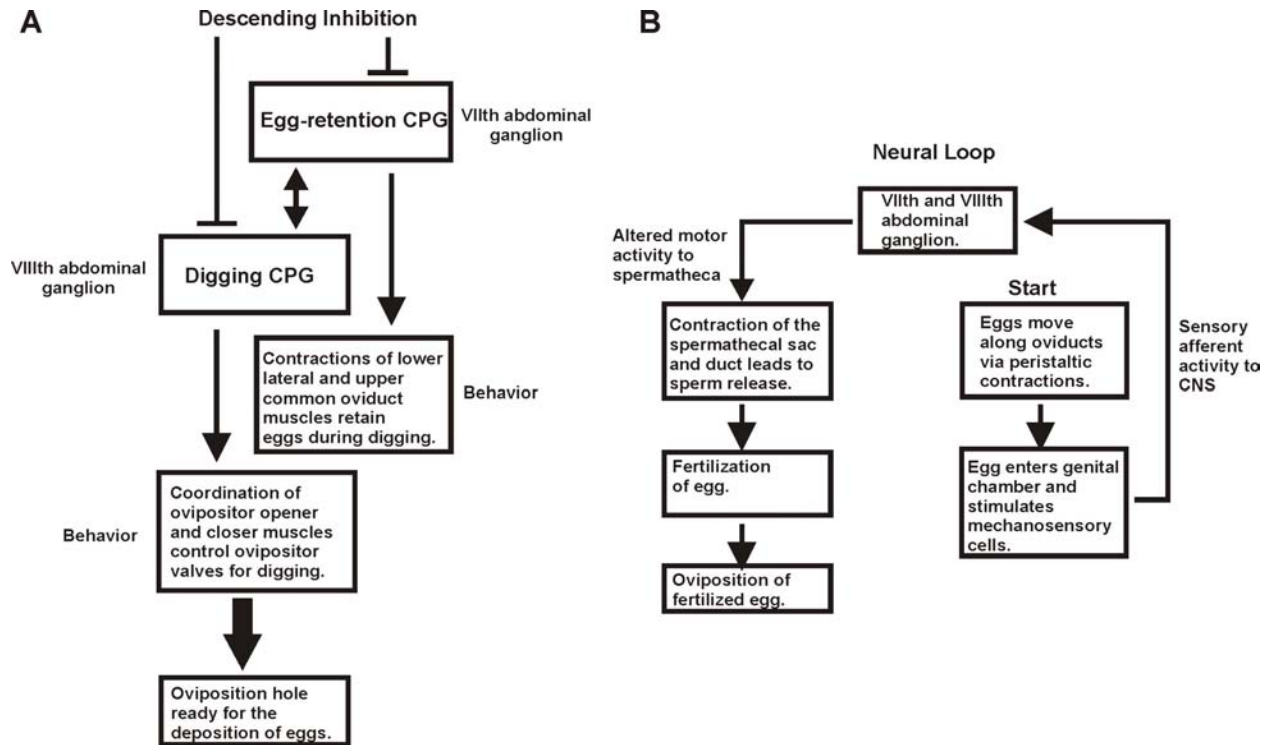


Figure 7. Models of (A) the coordination of the egg-retention central pattern generator (CPG) with the oviposition hole digging CPG leading to the preparation of a hole for egg-laying and (B) the sensory feedback loop responsible for coordinating sperm release with egg release leading to the deposition of a fertilized egg into the oviposition hole.

tuned and coordinated. This review has concentrated on the neural integration of these tissues, but the reader should not lose site of the endocrine control that results in the initial maturation of the reproductive tissue, aspects of matedness, and maturation of eggs and ovulation (4, 51, 83 – 85).

Oviposition within the female requires the coordination of skeletal muscles that control ovipositor valves and other aspects of abdominal movement, and visceral muscles controlling the oviducts and spermatheca (see summary diagram, Figure 7). In addition, prior to oviposition, the spermatheca must receive the sperm at mating, and store the sperm until a mature egg arrives at the genital chamber. Little information is available on the control of the spermatheca at the time of mating, and this is clearly an area in need of study.

The reproductive tissues in *L. migratoria* are under the control of neurons located in the last few abdominal ganglia. The oviposition digging behavior and oviduct egg-retention behavior are both controlled by CPGs that appear to be centrally integrated (Figure 7). Certainly both are under the control of descending inhibition, although the source or integration of this central “higher” oversight is not known. Both CPGs appear to be centrally coordinated within the last few abdominal ganglia, but again there is little information available on the

central neurons involved in this coordination. It is unknown if the coordination is direct or via interneurons. Similarly, there is little information about the neuroactive chemicals mediating this integration. It is possible that neuropeptides may be involved (eg. proctolin, SchistoFLRFamide), but no work on identifiable synaptic connections has been reported. The digging CPG appears to receive feedback from sensory hairs on the ovipositor valves but it has yet to be tested to see if this sensory feedback also impinges upon the oviduct egg-retention CPG. Furthermore, for the ovipositor valves, abdomen and skeletal muscles, only the digging CPG has been examined in detail, and yet there are other behaviors that occur during and after digging, such as rotation of the abdomen, tamping of the walls, and frothing of the secretions. In addition, the abdomen is slowly withdrawn during oviposition. These behaviors have not been studied but might involve CPGs that are spread throughout the abdominal ganglia.

The spermathecal contractions are also under neural control although the presence of a CPG has not been investigated. Spermathecal contractions are necessary for sperm progression along the 30 mm from the spermathecal sac to the posterior straight duct, where they await the arrival of an egg into the genital chamber. It is unclear what controls the timing of the sperm movement to the posterior

straight duct, or indeed, what initiates the original transfer of sperm out of the spermathecal sac. It would seem logical that these activities are coordinated with the ovulation/oviposition of mature eggs and the digging behavior, such that the sperm are in the appropriate location for fertilization. One might anticipate therefore, that the neural substrate controlling contractions of the spermathecal muscle would be coordinated with the CPGs controlling these other activities. This is yet to be investigated. However, there does appear to be a feedback loop, initiated by an egg entering the genital chamber, which activates stretch sensitive sensory neurons in the genital chamber (Figure 7). The sensory information projects back to the CNS where it initiates a reflex activation of motor neurons controlling spermathecal muscle. The central neurons / synapses mediating this feedback loop have not been identified, and nor have the neurochemicals that might be involved. Similarly, it is not known, although possibly might be anticipated, if the sensory feedback might also feedback onto the CNS to inhibit the CPGs associated with digging and egg retention, and / or be feeding back to coordinate abdominal withdrawal from the oviposition hole.

Clearly, the reproductive tissues in *L. migratoria* are finely tuned for the intricate behaviors in which they participate. These have provided model preparations for the study of neural integration and coordination; with a focus on neural substrate, CPGs, sensory feedback loops, and neurochemicals controlling visceral / skeletal muscle. Much is still unknown about this integration and coordination of reproductive tissue. In addition, an area that requires particular attention involves the integration of the neural substrate controlling reproductive tissues with the neuroendocrine and endocrine systems that are also involved in the overall control of reproductive activities. An holistic approach, involving endocrinology, neuroendocrinology, neurophysiology, and neuropharmacology, with whole animal behavior, is necessary for a complete picture of this crucial behavior necessary for inclusive fitness.

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