Journal of Insect Physiology 63 (2014) 1–8

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Octopamine modulates a central pattern generator associated with egg-laying in the locust, *Locusta migratoria*

Raymond Wong [1], Angela B. Lange *

University of Toronto Mississauga, Department of Biology, 3359 Mississauga Rd., Mississauga, ON L5L 1C6, Canada

ARTICLE INFO

Article history:
Received 5 November 2013
Received in revised form 30 January 2014
Accepted 5 February 2014
Available online 13 February 2014

Keywords:
Amine
Egg-laying
Digging
Oviposition
Protractor muscle
Oviduct

ABSTRACT

Egg-laying in *Locusta migratoria* involves the control of a variety of complex behavioural patterns including those that regulate digging of the oviposition hole and retention of eggs during digging. These two behavioural patterns are under the control of central pattern generators (CPGs). The digging and egg-retention CPGs are coordinated and integrated with overlapping locations of neural substrate within the VIIth and VIIIth abdominal ganglia of the central nervous system (CNS). In fact, the egg-retention CPG of the VIIth abdominal ganglion is involved in both egg-retention and protraction of the abdomen during digging. The biogenic amine, octopamine, has peripheral effects on oviduct muscle, relaxing basal tension of the lateral and upper common oviduct and enabling egg passage. Here we show that octopamine also modulates the pattern of the egg-retention CPG by altering the motor pattern that controls the external ventral protractor of the VIIth abdominal segment. There is no change in the motor pattern that goes to the oviducts. Octopamine decreased the frequency of the largest amplitude action potential and decreased burst duration while leading to an increase in cycle duration and interburst interval. The effects of octopamine were greatly reduced in the presence of the α-adrenergic blocker, phentolamine, indicating that the action of octopamine was via a receptor. Thus, octopamine orchestrates events that can lead to oviposition, centrally inhibiting the digging behavior and peripherally relaxing the lateral and common oviducts to enable egg-laying.

1. Introduction

Insects have proven valuable experimental models for studying the neural basis of behaviour (Grillner, 1975; Delcomyn, 1980); in particular, neurophysiological studies of rhythmic behaviour patterns such as walking, flight, and oviposition have been particularly rewarding (Ayali and Lange, 2010; Pearson, 2000). Many of these rhythmic patterns are generated and controlled by the central nervous system (CNS) using discrete neural circuits/networks. These central pattern generators (CPGs) have been extensively studied at the molecular, cellular and circuit levels and shown to generate rhythms in the absence of any patterned sensory or descending input (Ayali and Lange, 2010; Marder et al., 2005; Marder and Bucher, 2007; Pearson, 2000). Having said that, however, it is clear that sensory information/feedback is necessary for making the CPG network fully functional and context dependent and that these networks are targets for modulation by amines and neuropeptides (Kravitz, 1988; Bicker and Menzel, 1989; Marder, 2012). Thus, the CPGs are not static but show plasticity and are able to generate varying outputs (Marder, 2012; Marder et al., 2005). In addition, CPGs may not be entirely independent of each other and may even be coupled to each other (see Rillich et al., 2013).

The female locust, *Locusta migratoria*, lays fertilized eggs in an oviposition site (hole). Digging this hole involves rhythmic movements of the ovipositor valves that are under the control of a CPG whose circuit is located within the VIIth and VIIIth abdominal ganglia (Thompson, 1986a,b). During digging, the mature eggs are held in the lateral oviducts by rhythmic constriction of the lower lateral oviducts, whilst contractions of the upper lateral oviducts tend to propel them posteriorly towards the common oviduct (Lange et al., 1984; Facciponte and Lange, 1992, 1996). An egg-retention CPG, located in the VIIth abdominal ganglion, provides motor output that constricts the lower lateral oviducts and stimulates protraction of the abdomen during digging (Lange et al., 1984; Lange, 2009a,b; Ayali and Lange, 2010). This CPG is necessary and essential to ensure that the eggs are not deposited before the oviposition hole is fully excavated or in case the female is disturbed during egg-laying and needs to find another suitable oviposition site. Both the

* Corresponding author. Tel.: +1 905 828 3972; fax: +1 905 828 3792.
E-mail address: angela.lange@utoronto.ca (A.B. Lange).
[1] Present address: Toronto Western Research Institute, University Health Network, Department of Physiology, University of Toronto, Toronto, ON, Canada.

http://dx.doi.org/10.1016/j.jinsphys.2014.02.002
0022-1910/

digging CPG and the egg retention CPG are under descending neural inhibition and can be activated by transecting the nerve cord anteriorly to the neural substrate (Thompson, 1986a,b; Lange et al., 1984; Facciponte et al., 1995) and both of these CPGs are integrated and coordinated to allow for the retention of eggs during digging of the oviposition hole (Facciponte et al., 1995).

Neuromodulators influence CPGs centrally, and/or influence peripheral sensory feedback onto the CPGs (see Marder, 2012). Recently the biogenic amine, octopamine, was found to evoke fictive flight in locusts and it was suggested that octopamine biases the motor network towards flight (Rillich et al., 2013). This is interesting since octopamine is also associated with egg-laying in *L. migratoria* and in *Drosophila melanogaster* (see Lange, 2009b; Rubinstein and Wolfner, 2013). Thus, octopamine, released from dorsal unpaired median (DUM) neurons inhibits contractions of the lateral oviducts in *L. migratoria* which relaxes the muscles enabling egg passage and oviposition (see Lange, 2009b; Cheung et al., 1994). A similar suggestion has been made in *D. melanogaster* where octopamine stimulates ovulation, and relaxes oviduct muscle tonus (Rubinstein and Wolfner, 2013). In light of octopamine's action on peripheral tissues such as the oviducts during egg-laying, we questioned whether octopamine might centrally modulate the egg-retention CPG in the locust, *L. migratoria*. This would be appropriate at the time when octopamine is released peripherally to relax the oviducts during egg-laying allowing the passage of eggs – a time when digging is completed and the egg-retention CPG is no longer required. Here we describe the effects of octopamine on the egg-retention CPG of the VIIth abdominal ganglion, and find that it does indeed modulate this CPG, by inhibiting several of its parameters.

2. Materials and methods

2.1. Animals

Experiments were carried out on 7-week-old sexually mature female *L. migratoria* reared under crowded conditions at 30 °C and 50% humidity. Locusts were kept on a 12 h:12 h light:dark regime, and were given a diet of fresh wheat seedlings supplemented with bran.

2.2. Preparation

Preparations were dissected and maintained in locust physiological saline (150 mM NaCl; 10 mM KCl; 4 mM $CaCl_2$; 2 mM $MgCl_2$; 3 mM $NaHCO_3$; 5 mM HEPES, pH 7.2; 90 mM sucrose; 5 mM trehalose). The dissection consisted of the last three abdominal ganglia with the oviducts (both common oviduct and lateral oviducts) connected to the VIIth abdominal ganglion via the oviducal nerves (sternal nerve branches N2B of VIIth abdominal

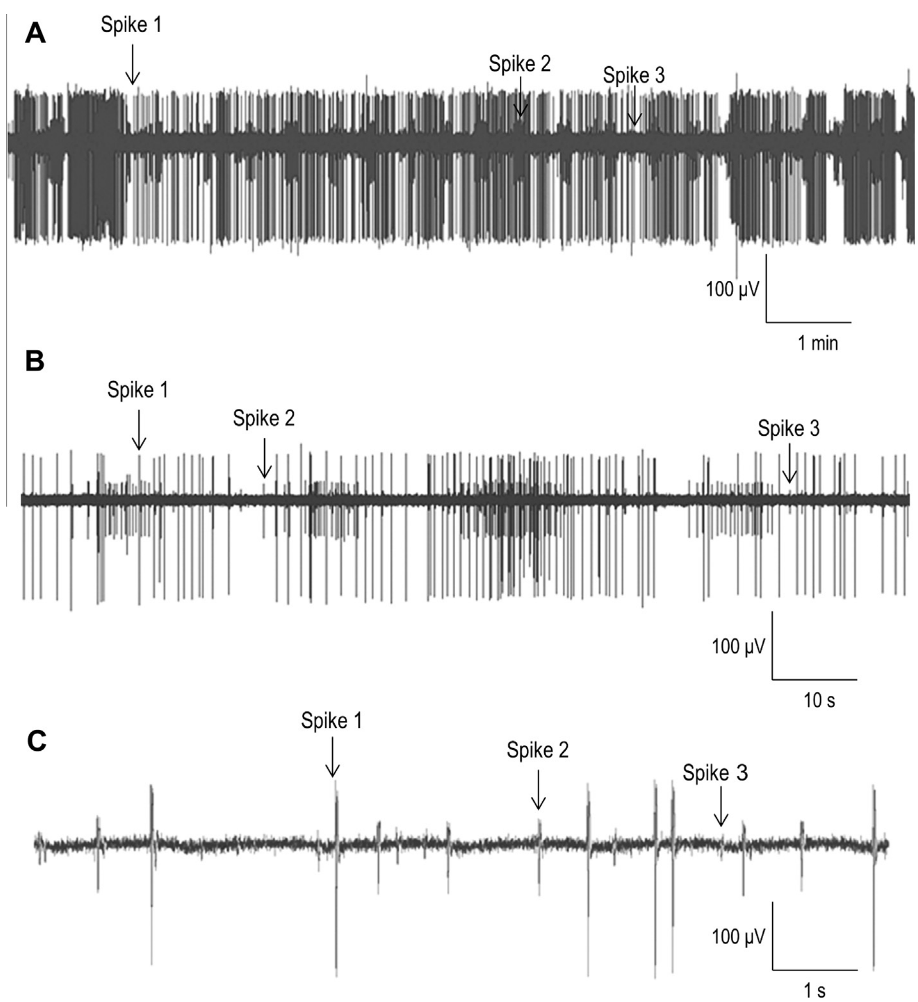

Fig. 1. Extracellular recordings of neural activity in the oviducal nerve of *L. migratoria* from an isolated preparation of the VIIth and VIIIth abdominal ganglia in saline. (A) Characteristic rhythmic motor bursting pattern. Expanded portion of the upper trace is shown in (B) and (C) to show individual spikes and bursting pattern. Note three discernable sizes of action potentials; spike 1, 2, and 3 refer to the large, medium, and small amplitude action potentials respectively ($n = 10$).