The Journal of Experimental Biology 213, 1182-1194

doi:10.1242/jeb.040378

Octopamine promotes rhythmicity but not synchrony in a bilateral pair of bursting motor neurons in the feeding circuit of *Aplysia*

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Accepted 8 December 2009

SUMMARY

Octopamine-like immunoreactivity was localized to a limited number (<40) of neurons in the *Aplysia* central nervous system, including three neurons in the paired buccal ganglia (BG) that control feeding movements. Application of octopamine (OA) to the BG circuit produced concentration-dependent (10^{-8}–10^{-4} mol l^{-1}) modulatory actions on the spontaneous burst activity of the bilaterally paired B67 pharyngeal motor neurons (MNs). OA increased B67's burst duration and the number of impulses per burst. These effects reflected actions of OA on the intrinsic tetrodotoxin-resistant driver potential (DP) that underlies B67 bursting. In addition to its effects on B67's burst parameters, OA also increased the rate and regularity of burst timing. Although the bilaterally paired B67 MNs both exhibited rhythmic bursting in the presence of OA, they did not become synchronized. In this respect, the response to OA differed from that of dopamine, another modulator of the feeding motor network, which produces both rhythmicity and synchrony of bursting in the paired B67 neurons. It is proposed that modulators can regulate burst synchrony of MNs by exerting a dual control over their intrinsic rhythmicity and their reciprocal capacity to generate membrane potential perturbations. In this simple system, dopaminergic and octopaminergic modulation could influence whether pharyngeal contractions occur in a bilaterally synchronous or asynchronous fashion.

Key words: central pattern generator, driver potential, bursting, B67, conditional synchrony, conditional rhythmicity, buccal ganglion.

INTRODUCTION

The ability of neuromodulators to broadly influence and reconfigure multifunctional motor circuits is well established (Harris-Warrick and Marder, 1991; Calabrese, 1998; Briggman and Kristan, 2008). To accomplish such reorganization of complex motor systems, specific neuromodulators must exert coordinated actions on neurons that possess a broad spectrum of intrinsic properties. For example, the neurons comprising a motor network commonly vary along a continuum with respect to their rhythm-generating and burst-generating capabilities, ranging from cells that completely lack spontaneity to cells that produce spontaneous patterned burst activity. Our understanding of how neuromodulators achieve precise and coherent modifications of motor activity from circuits composed of such diverse neuronal phenotypes remains incomplete.

The biogenic amine octopamine (OA) belongs to the family of mammalian brain 'trace amines' that has been implicated in the pathogenesis of several neurological and psychiatric disorders (Lindemann and Hoener, 2005; Burchett and Hicks, 2006; Berry, 2007). In many invertebrates, OA is well established as a neurotransmitter and modulator of synaptic signaling (Robertson and Jurio, 1976; Evans and O'Shea, 1977; Roeder, 1999; Roeder, 2005). A role for OA as a neurotransmitter in the gastropod mollusk *Aplysia* was originally suggested by microchemical studies demonstrating its localization to specific ganglia (McCaman and McCaman, 1978; McCaman, 1980; Takeda, 1992). Further support for a transmitter function was provided by studies in which application of exogenous OA to *Aplysia* neurons elicited diverse cell-specific responses (Carpenter and Gaubatz, 1974; Swann and Carpenter, 1975; Pellmar, 1981). The role of OA as a neuromodulator was originally suggested by Levitan and Barondes, who demonstrated OA-stimulated protein phosphorylation in the abdominal ganglion of *Aplysia*, and proposed that these effects could reflect sustained second messenger-mediated synaptic actions (Levitan and Barondes, 1974). To date, however, octopaminergic neurons have not been identified in *Aplysia* and the participation of OA signaling in the control of its neural circuits has not been examined.

Octopaminergic regulation of gastropod behavior has been intensively studied in the neural circuits that control feeding in the pond snail *Lymnaea stagnalis*. Three octopaminergic interneurons (termed OCs) were shown to modulate the fictive feeding motor programs generated by the *Lymnaea* buccal ganglion (Elliott and Vehovszky, 2000; Vehovszky and Elliott, 2000; Vehovszky et al., 2000; Vehovszky and Elliott, 2001; Vehovszky et al., 2004). The OCs function as widely acting buccal interneurons that fire during the swallowing phase of feeding motor programs (Vehovszky and Elliott, 2001). OC synaptic actions can influence follower neurons over a range of temporal intervals and can activate and reconfigure motor programs (Vehovszky et al., 2004; Vehovszky et al., 2005). The OCs were therefore proposed to act as intrinsic modulators of the *Lymnaea* feeding central pattern generator (CPG) (Vehovszky and Elliott, 2001; Vehovszky and Elliott, 2002).

In this investigation, octopamine-like immunoreactivity (OA_{li}) was localized to specific neurons in the nervous system of *Aplysia*, and modulatory actions of OA were assessed on the bilaterally paired B67 motor neurons (MNs) that innervate multiple targets involved with swallowing and salivation (Nagahama and Takata, 1987; Park et al., 1999; Park et al., 2000). Previously, the rhythmicity and synchrony of spontaneous burst activity in the B67 MNs were both shown to be enhanced by dopamine (DA), another modulator in the

Aplysia feeding system (Serrano and Miller, 2006). This study thus permitted a comparison of octopaminergic and dopaminergic modulatory effects on specific neuronal attributes that promote spontaneity, rhythmicity and synchrony of patterned motor activity in this system.

MATERIALS AND METHODS

Specimens

Electrophysiology experiments were conducted on *Aplysia californica* Cooper (150–250 g) purchased from the *Aplysia* Resource Facility and Experimental Hatchery (University of Miami, Coral Gables, FL, USA) or from Marinus Inc. (Long Beach, CA, USA). Smaller specimens (20–40 g) were used for anatomical experiments. Animals were maintained in refrigerated aquaria (14–16°C) and fed dried seaweed twice per week.

Whole-mount immunohistochemistry

The central nervous system (CNS) was dissected and placed in fixative solution (0.1 mol l^{-1} sodium cacodylate, 2% paraformaldehyde, 1% glutaraldehyde) for 3 h. Ganglia were washed (5×, room temperature with agitation) in phosphate buffer solution containing 0.2% Triton X-100 [PBS-T; for details of buffer composition, see Díaz-Ríos et al. (Díaz-Ríos et al., 2002)]. Following preincubation with normal goat serum (0.8%), tissues were immersed (48 h, room temperature) in a blocking solution (PBS-T with 1% NGS, 0.25% BSA, 3% fat-free milk powder) containing a 1:1000 primary antibody [mouse monoclonal, Jena Bioscience, Catalog No. ABD-029, Jena, Germany (see Dacks et al., 2005)]. Following repeated PBS-T washes (5×, at least 20 min each, room temperature), ganglia were incubated in blocking solution containing 1:200 Alexa 488 goat anti-mouse IgG (H+L) conjugate (Molecular Probes, Eugene, OR, USA: A-11029).

Preparations were viewed on a Nikon Eclipse fluorescence microscope (Tokyo, Japan) or on a Zeiss Pascal laser scanning confocal microscope (LSCM) (Oberkochen, Germany). Images were captured with the Nikon ACT-1 (version 2.10) software of the Eclipse or the Zeiss LSM 5 Image Browser (version 3.1.0.11) program of the Pascal. They were exported as BMP files to Adobe Photoshop (Adobe Systems, Mountain View, CA, USA) for adjusting overall contrast and brightness and then imported to Corel Draw 10 (Ottowa, Ontario, Canada) for addition of labels and organization of panels.

Electrophysiology

Preparations consisting of the paired buccal and cerebral ganglia were secured to a Sylgard-lined chamber with minutien pins (Fine Science Tools, Foster City, CA, USA). All experiments in this study were performed on B67, a large superficial MN located on the caudal surface of each buccal hemiganglion [designated pharynx burster (PB) in *Aplysia kurodai* (Nagahama and Takata, 1987) and B67 in *A. californica* (Park et al., 1999; Serrano and Miller, 2006)]. Previous studies established the presence of endogenous burst- and rhythm-generating properties in B67, including a tetrodotoxin (TTX)-resistant driver potential (DP), a slowly developing depolarization ('sag' potential) in response to prolonged imposed hyperpolarization, and post-inhibitory rebound (PIR) (Serrano and Miller, 2006; Serrano et al., 2007). Independent microelectrodes were used for recording the membrane potential (10–20 MΩ) and injecting current (5–10 MΩ) into B67.

Extracellular signals were recorded with polyethylene suction electrodes and AC coupled amplifiers (Model 1700, AM Systems, Sequim, WA, USA). Cut-end recordings from buccal nerve 1 were used to confirm the identification of B67, which produces the largest efferent impulse recorded from this nerve (Nagahama and Takata, 1987; Serrano and Miller, 2006). Extracellular recording from the radula nerve was used to monitor the phase of buccal motor programs (BMPs) corresponding to fictive closure of the radula (Morton and Chiel, 1993a; Morton and Chiel, 1993b).

The normal artificial seawater (ASW) contained the following (in mmol l^{-1}): 460 NaCl, 10 KCl, 55 $MgCl_2$, 11 $CaCl_2$ and 10 Hepes. In most experiments, an ASW solution with elevated concentrations of divalent cations [2.2 × [Ca^{2+}] and 2 × [Mg^{2+}] (Liao and Walters 2002)] was used to attenuate polysynaptic activity. Experiments were conducted at room temperature (19–21°C).

Pharmacology

Solutions of drugs were prepared from powder immediately before application. OA and TTX were obtained from Sigma Chemical Co. (St Louis, MO, USA). Preparations were superfused with the ASW solution at a rate of 0.5 ml min^{-1} using a gravity-fed multi-channel system (ALA Scientific Instruments, Farmingdale, NY, USA; Model VM4).

Data analysis

All results reported in this study were observed in a minimum of four specimens. Measurements are reported as the mean ± s.d. or ± s.e.m., as noted. Statistical tests (Student's *t*-test, two-tailed) were performed by comparing measurements obtained prior to drug application with those attained at the peak of the response. When multiple treatments were examined, the analysis of variance (ANOVA) was initially applied to test overall significance, and Holm–Sidak *post hoc* tests were used to compare specific treatments. A value of $P<0.05$ was established as the criterion for significance.

For data analysis, each burst was treated as a point event, the timing of which was assigned at the peak of its initial impulse. To enable quantification of synchronization, an operational index of synchrony was defined as the fraction of bursts that exhibited overlap of impulse firing (see Serrano and Miller, 2006). Autocorrelation and cross-correlation functions were generated from occurrences of bursts using the methods applied to spike trains by Perkel et al. (Perkel et al., 1967a; Perkel et al., 1967b).

RESULTS

Localization of OA_{li}

OA_{li} was detected in <40 neurons in the *Aplysia* CNS (Figs 1, 2). The distribution of the OA_{li} cells was limited to the buccal (Fig. 1A), cerebral (Fig. 1B) and pedal (Fig. 2) ganglia. None were detected in the pleural or abdominal ganglia.

Three moderately sized (approximately 80 μm diameter) OA_{li} neurons were present in the paired buccal ganglia (Fig. 1A). Of these, two somata were located in bilaterally symmetrical positions in the medial region near the caudal surface of each hemiganglion (Fig. 1Aii, arrows). The third OA_{li} buccal neuron was usually located within the buccal commissure that joins the two hemiganglia (Fig. 1Aii, arrowhead). There was some variation in the precise position of this third cell, which tended to be skewed toward the left hemiganglion. In no instance was a complementary contralateral cell detected. Although the immunolabeling did not permit extensive tracing of the projections from the three buccal OA_{li} neurons, it was usually possible to follow the major process from each of the bilateral paired cells across the buccal commissure to the contralateral hemiganglion. The unpaired cell had a bipolar profile, with major processes originating from its soma projecting toward the neuropil regions of each buccal hemiganglion. No OA_{li} fibers were observed

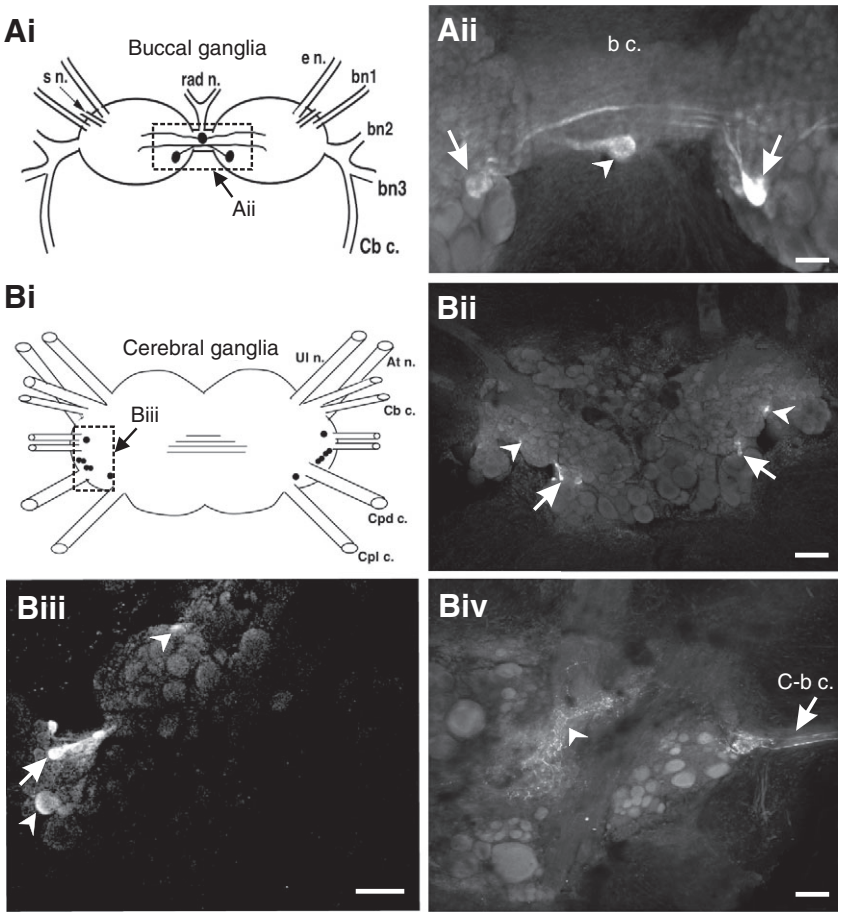

Fig. 1. Octopamine-like immunoreactivity (OA_{li}) in the buccal and cerebral ganglia. (Ai) Schematic summary of OA_{li} in the paired buccal ganglia. Strong OA_{li} was limited to three moderately sized neurons. Broken rectangle indicates region shown in Aii. Abbreviations: s n., salivary nerve; rad n., radula nerve; e n., esophageal nerve; bn1, buccal nerve 1; bn2, buccal nerve 2; bn3, buccal nerve 3; Cb c., cerebral-buccal connective. (Aii) Two of the OA_{li} neurons (arrows) were positioned symmetrically in the medial region of the caudal surface of each hemiganglion. These cells gave rise to fibers that traversed the buccal commissure (b c.). An unpaired cell (arrowhead) was located within the b c. Calibration bar=100 μm. (Bi) Schematic summary of OA_{li} in the cerebral ganglion. Approximately six cells were symmetrically positioned on the dorsal surface of the lateral region of each hemiganglion. Broken rectangle indicates region shown in (Biii). Abbreviations: Ul n., upper labial nerve; At n., anterior tentacular nerve; Cpd c., cerebral-pedal connective; Cpl c., cerebral-pleural connective. (Bii) Low power image of the dorsal surface of the cerebral ganglion. Cluster of approximately four small (~20 μm diameter) cells was located slightly anterior to the origin of each cerebral-pedal connective (arrows). Additional individual bilateral neurons (arrowheads) are present in a more anterior position. Calibration bar=200 μm. (Biii) Higher magnification of OA_{li} neurons in the left cerebral hemiganglion. Two slightly larger (40–50 μm) cells were located separately (arrowheads), one anterior to the cluster and another more posterior, between the origins of the cerebral-pedal and cerebral-pleural connectives. Calibration bar=100 μm. (Biv) Upper quandrant of the ventral surface of the cerebral ganglion. No OA_{li} neurons were detected on the ventral surface but immunoreactive fiber plexuses were observed near the origin of the cerebral-buccal connective (C-b c.) (arrow) and in the region of the M cluster (arrowhead). Calibration bar=100 μm.

in any of the buccal nerves. Moreover, examination of selected peripheral tissues associated with the buccal ganglion, e.g. buccal muscles, pharynx and the esophagus did not reveal OA_{li} fibers or cells.

Approximately 12 OA_{li} neurons were located in the cerebral ganglion (Fig. 1Bi,Bii,Biii). These cell bodies were smaller (ranging from 20 μm to 50 μm in diameter) than the buccal OA_{li} neurons. The cerebral OA_{li} somata (approximately six in each hemiganglion) were distributed in a symmetrical fashion on the ventral surface of the lateral region of the ganglion [E cluster of Jahan-Parwar and Fredman (Jahan-Parwar and Fredman, 1976)]. A tightly grouped cluster of approximately four small (~20 μm diameter) cells was

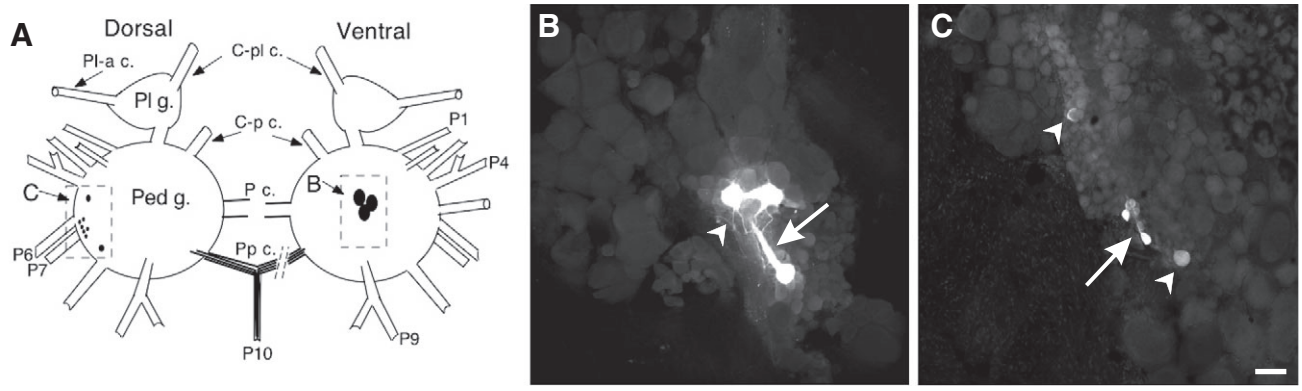

Fig. 2. Octopamine-like immunoreactivity (OA_{li}) in the pedal ganglia. (A) Schematic drawing showing the positions of OA_{li} neurons in the paired pedal ganglia (Ped g.). All of the pedal OA_{li} neurons were bilaterally paired. The cells on the dorsal surface of each ganglion are shown on the left side of this drawing and the neurons on the ventral surface are shown on the right. The parapedal commissure and the unpaired parapedal commissure nerve (P10) contained several strongly staining OA_{li} fibers. Broken rectangles mark the areas of each ganglion shown in panels B and C. Abbreviations: Pl g., pleural ganglion; C-pl c., cerebral-pleural connectives; C-p c., cerebral-pedal connective; P c., pedal commissure; Pp c., parapedal commissure. (B) Three intensely stained cell bodies (approximately 75 μm diameter) were clustered in the central region of the ventral surface of each pedal ganglion. Each soma appeared to give rise to multiple large protrusions (arrow) and fine fibers (arrowhead). (C) Smaller OA_{li} neurons in the lateral region of the dorsal surface of each pedal ganglion. A tightly grouped cluster of approximately six small (20 μm diameter) cells was located slightly anterior to the origins of pedal nerves P6 and P7 (arrow). Two slightly larger (approximately 40 μm) cells were located separately (arrowheads), one anterior to the cluster and another more posterior, near the origin of P8. Scale bar=100 μm, applies to panels B and C.

located slightly anterior to the origin of each cerebral-pedal connective (arrows, Fig. 1Bii,Biii). Two slightly larger (40–50 μm) cells were located separately (arrowheads, Fig. 1Bii,Biii), one anterior to the cluster and another more posterior, between the origins of the cerebral-pedal and cerebral-pleural connectives. No OA_{li} neurons were detected on the dorsal surface of the cerebral ganglion. Several immunoreactive fiber plexuses were observed, however, most notably near the origin of the cerebral-buccal connective (arrow, Fig. 1Biv) and in the region of the M cluster (arrowhead, Fig. 1Biv).

Each pedal ganglion contained approximately 11 OA_{li} neurons (Fig. 2). Three large cell bodies (~75 μm diameter) were clustered in the central region of the ventral surface of each pedal ganglion (Fig. 2A,B). These were the most reliably and intensely stained OA_{li} neurons in the CNS. Each soma appeared to give rise to multiple large processes and fine fibers that formed a local network. No further projections were detected.

Approximately eight smaller OA_{li} neurons were present in the lateral region of the dorsal surface of each pedal ganglion. A tightly grouped cluster of approximately six small (20 μm diameter) cells was located slightly anterior to the origins of pedal nerves P6 and P7 (arrow, Fig. 2A,C). Two slightly larger (~40 μm) cells were located separately (arrowheads, Fig. 2C), one anterior to the cluster and another more posterior, near the origin of P8. The parapedal commissure and the unpaired parapedal commissure nerve (P10) contained several strongly staining OA_{li} fibers (Fig. 2A). It was not possible to trace these fibers to their central or peripheral origins or destinations. In separate experiments, the region of the foot that is innervated by P10 was examined but no OA_{li} cells or fibers were detected.

Effects of exogenous OA on feeding-related motor activity

The effects of OA on feeding-related motor activity were assessed with recordings from the pharyngeal motor neuron B67 (see Nagahama and Takata, 1987; Park et al., 1999; Serrano and Miller, 2006). Initial experiments examined the actions of OA in an ionic milieu that supports chemical synaptic signaling (normal ASW, see Materials and methods). Under these conditions, B67 exhibits two forms of patterned activity, multiphasic buccal motor programs (BMPs) and monophasic endogenous bursts [Fig. 3A (see also Serrano et al., 2007)]. BMPs consist of a stereotyped protraction–retraction sequence that reflects the coordinated activity of a large number of buccal neurons (see Morton and Chiel, 1993a; Morton and Chiel, 1993b; Elliott and Susswein, 2002; Murphy, 2001; Cropper et al., 2004). BMPs occur very infrequently in normal ASW in *Aplysia* (<0.01 Hz) (Nargeot et al., 1997; Lechner et al., 2000; Mozzachiodi et al., 2003). The bilaterally paired B67 MNs receive strong synaptic excitation during the initial phase of BMPs, which corresponds to fictive protraction of the radula–odontophore complex. B67 firing is completely eliminated by strong synaptic inhibition during the subsequent BMP retraction phase. By contrast, the monophasic bursts of B67 (Fig. 3A, top record) were previously shown to reflect its endogenous cellular properties (Serrano and Miller, 2006). Their spontaneous occurrence is more frequent (0.05–0.1 Hz) than the BMPs. Under control conditions, this spontaneous bursting is irregular and the two B67s (left and right hemiganglia) are not highly synchronized (see Serrano and Miller, 2006).

Addition of OA (10^{-7} mol l^{-1} to 10^{-4} mol l^{-1}, Fig. 3Bi,Bii) to the bathing medium had multiple effects on B67's intrinsic burst activity (N=4). The frequency and rhythmicity of bursting were increased, as was the number of impulses comprising each burst. At these concentrations, OA also increased the amplitude of the slow potentials underlying each burst and the overall level of synaptic activity impinging on B67. Recording from additional buccal neurons and

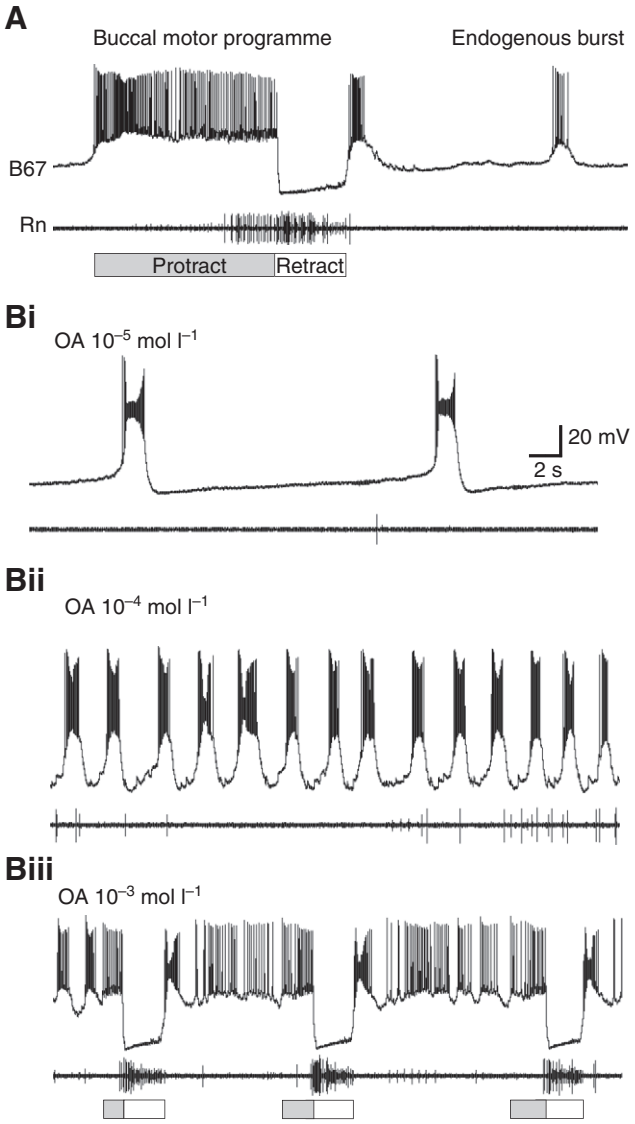

Fig. 3. Effects of octopamine (OA) on B67 in normal artificial seawater. (A) Control recordings. Upper trace: intracellular recording from B67. Lower trace: extracellular recording from the radula nerve (Rn). B67 exhibited two modes of patterned activity in the isolated cerebral-buccal preparation. It was recruited into multi-phasic buccal motor programs (BMPs) where it fired during the phase of fictive radula protraction (shaded bar below recording) and was inhibited during retraction (white bar). B67 also exhibited a monophasic endogenous burst pattern that was previously shown to reflect the intrinsic properties of this cell (Serrano and Miller, 2006). In contrast to the spontaneous bursts, which were detected only in B67, the BMPs involved a large number of buccal neurons (compare radula nerve recording below each pattern; B67 does not project to the Rn). The BMPs occurred less frequently (<0.01 Hz) than the spontaneous intrinsic bursts (<0.1 Hz). (B) Actions of OA. Addition of a range of OA concentrations (10^{-5} mol l^{-1}, Bi; 10^{-4} mol l^{-1}, Bii) produced increases in the frequency of the spontaneous B67 bursts. (Biii) At millimolar concentrations, OA also increased the frequency of BMPs (10^{-3} mol l^{-1} shown). Note that Rn activity is predominantly associated with the retraction phase of the OA-induced BMPs (unfilled bars below recordings), supporting their qualitative classification as ingestive-like programs (see Morton and Chiel, 1993a; Morton and Chiel, 1993b).

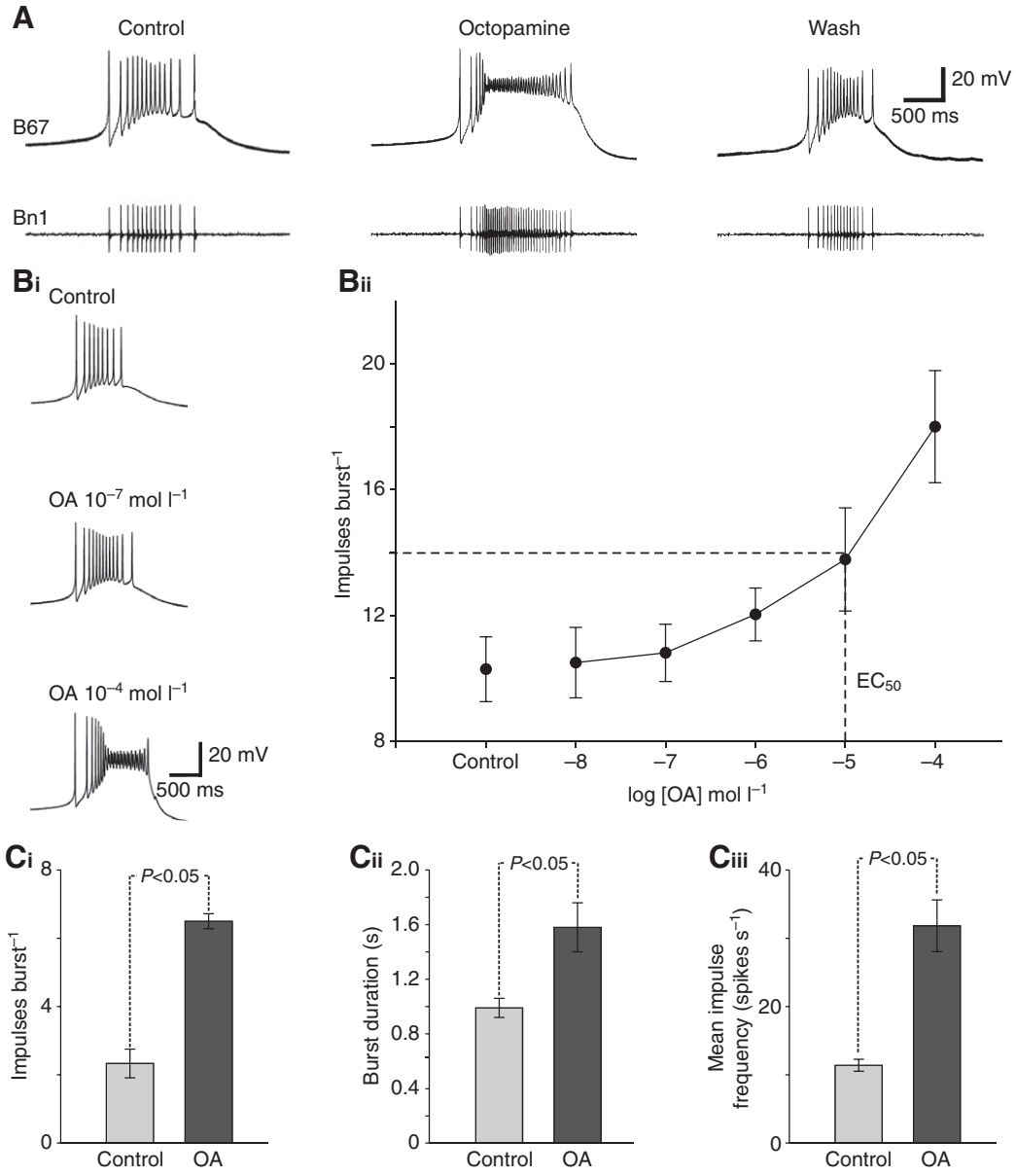

Fig. 4. Concentration-dependent actions of octopamine (OA) on B67 burst parameters. Experiments were conducted in a medium (raised concentrations of Ca^{++} and Mg^{++}) that attenuates chemical synaptic signaling. (A) OA produced multiple effects on B67 burst parameters that were reversible with wash. Bath OA application (1×10^{-5} mol l^{-1}) resulted in an increase in the number of impulses per burst, burst duration and intraburst spike frequency of B67. All burst parameters returned to control levels following wash (30 min) with OA-free solution. (B) Concentration-dependence of OA actions on the B67 burst parameters. (Bi) Recordings show representative control, OA 10^{-7} mol l^{-1} and OA 10^{-4} mol l^{-1} bursts. (Bii) Dose–response curve based on pooled experiments (N=5) in which OA was applied in increasing concentrations over four orders of magnitude. The EC_{50} for this effect was estimated to be approximately 1×10^{-5} mol l^{-1} (broken lines). (C) Group data demonstrating effects of OA on B67 burst parameters. Comparison of parameters measured prior to OA application with those observed in the presence of the EC_{50}, 1×10^{-5} mol l^{-1}. Significant increases were observed in (Ci) the number of bursts per minute (control: 2.3±0.4; OA: 6.5±0.2; P<0.05; N=6), (Cii) the burst duration (control: 0.99±0.07 s; OA: 1.58±0.18 s; P<0.05; N=6) and (Ciii) the spikes per burst (control: 11.4±0.9 impulses s^{-1}; OA: 31.8±3.8 impulses s^{-1}; t=5.27; N=6; P<0.05).

nerves confirmed that the spontaneous B67 bursts produced in the presence of 10^{-7} mol l^{-1} to 10^{-4} mol l^{-1} OA were not associated with the coordinated BMPs that generate consummatory feeding actions. Only at the highest concentration tested (1×10^{-3} mol l^{-1}; Fig. 3Biii) did OA elicit BMPs that periodically interrupted B67's regular bursting (see also Vehovszky et al., 1998). The motor programs evoked by OA were always classified as ingestive using the criteria of Morton and Chiel (Morton and Chiel, 1993a; Morton and Chiel, 1993b) (see also Serrano et al., 2007).

Actions of OA on B67 burst properties

The experiments conducted in normal ASW indicated that OA exerts multiple distributed actions on the buccal system. Experiments were therefore conducted to assess which actions of OA could be attributed to its direct effects on B67. These tests were conducted in a high divalent medium that eliminates spontaneous synaptic activity in *Aplysia* (Liao and Walters, 2002) (see Materials and methods). Under these conditions, BMPs were completely eliminated and B67 produced infrequent (<0.1 Hz) arrhythmic

monophasic bursts (see Serrano and Miller, 2006). Addition of OA to the bath produced multiple actions on the B67 burst that were reversed with 10–20 min wash (Fig. 4A). These effects included increases in the number of impulses per burst and the overall duration of each burst. The effects of OA on the burst properties of B67 were concentration-dependent (Fig. 4B). Threshold effects on the spontaneous bursting were detected with bath application of OA at concentrations between 10^{-8} mol l^{-1} and 10^{-7} mol l^{-1}. These actions increased over the four orders of magnitude of OA concentrations that were examined (Fig. 4Bii). The EC_{50} was estimated to be 1×10^{-5} mol l^{-1} (Fig. 4Bii, broken lines).

The effects of OA on several parameters of B67 bursting were assessed by comparing bursts recorded prior to OA application to those observed at the peak of responses to the EC_{50} (1×10^{-5} mol l^{-1}). Significant increases were observed in the number of bursts per minute (control: 2.3±0.4; OA: 6.5±0.2; $P<0.05$; Fig. 4Ci), the burst duration (control: 0.99±0.07 s; OA: 1.58±0.18 s; $P<0.05$; Fig. 4Cii) and the number of spikes per burst (control: 11.4±0.9; OA: 31.8±3.8; $P<0.05$; Fig. 4Ciii).

Actions of OA on intrinsic membrane properties of B67

B67 possesses an endogenous TTX-resistant DP (Tazaki and Cooke, 1979) that contributes to shaping its impulse bursts (Serrano and Miller, 2006). In the presence of TTX (1×10^{-5} mol l^{-1}), DPs continued to occur in a spontaneous and irregular manner (see following text). Bath application of OA prolonged the DP duration in a reversible fashion (Fig. 5A). Group data showed that DP durations were increased from control values of 0.99±0.08 s to 3.49±0.44 s by 1×10^{-5} mol l^{-1} OA ($P<0.01$; $N=4$; Fig. 5Bi). OA also produced a small increase the amplitude of spontaneous DPs from control values of 21.0±2.1 mV to 24.6±1.6 mV (Fig. 5Bii). Although this effect did not reach our criterion for significance ($t=1.35$; $N=4$; $P>0.05$), a small increase in DP amplitude could greatly impact B67's intraburst impulse firing rate. It appears, therefore, that the modulatory actions of OA on B67 bursting reflect, at least in part, its effects on the properties of the B67 DP.

Bath application of OA at concentrations ranging from 10^{-8} mol l^{-1} to 10^{-4} mol l^{-1} did not affect the basal interburst membrane potential of B67 (Fig. 6A). Moreover, at these concentrations no effects of OA were detected on the input resistance of B67 measured with hyperpolarizing current pulses (1 s, 5 nA) delivered during the interburst interval (IBI) (Fig. 6A). As B67 exhibits a time-dependent depolarizing relaxation or 'sag' when hyperpolarized (Serrano and Miller, 2006) (see below), input resistance measurements were determined at the peaks of the voltage deflections produced by these pulses.

Although OA did not produce detectable effects on the interburst membrane potential or input resistance of B67, its responses to hyperpolarizing current pulses were markedly altered in the presence of OA (Fig. 6B,C). To facilitate comparisons of these responses, test current pulses were adjusted to produce peak hyperpolarizing potentials of –90 mV (Fig. 6B). The depolarizing relaxation from –90 mV was measured at the termination of the current pulse (5 s) (broken lines in Fig. 6B). When compared using this protocol, relaxation potentials were significantly increased in the presence of OA (control: 10.1±0.7 mV; OA: 16.4±2.1 mV; $P<0.05$; Fig. 6B,Ci).

The responses of B67 following the termination of hyperpolarizing current pulses were also affected by OA (Fig. 6B). Two measurements of B67's tendency to exhibit PIR were determined following current pulses (5 s) to –90 mV; the total number of impulses generated following the pulse, and the latency to the initial impulse. Both parameters were affected by OA (10^{-5} mol l^{-1}) in a significant fashion, with the number of impulses increasing (control: 7.6±2.6; OA: 15.7±2.5; $P<0.05$; Fig. 6B,Cii) and the latency to the initial spike decreasing (control: 1.30±0.37 s; OA: 0.42±0.07 s; $P<0.05$; Fig. 6B,Ciii).

OA actions on repetitive bursting

In addition to its actions on the parameters of individual bursts, effects of OA were also observed on the timing of B67's repetitive bursting. Under control conditions (raised divalent solution), the recurrent bursting of B67 was infrequent (<0.01 Hz) and irregular (Fig. 7A,Bi) (see also Serrano and Miller, 2006). Addition of OA increased the frequency of B67 bursting (Fig. 7A). Although group data (Fig. 7Bi, $N=5$) suggested that the mean IBI was reduced by OA (1×10^{-4} mol l^{-1}) this effect did not reach our criterion for statistical significance due to the large dispersion of control IBI values (control IBI: 93.3±45.3 s; OA: 11.6±0.6 s; $t=1.803$; $N=6$; $P=0.102$). The actions of OA on the timing of repetitive bursting were more evident when the dispersions of the IBIs were compared by calculating the coefficient of variation [C_v=(s.d./m)×100], an

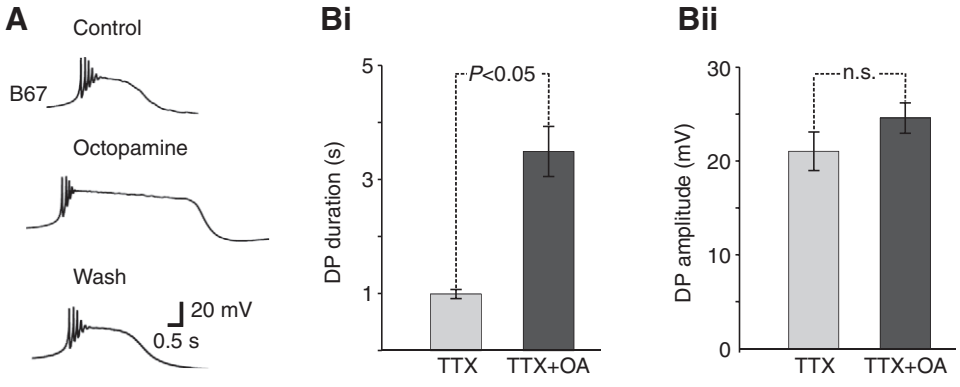

Fig. 5. Effects of octopamine (OA) on the B67 driver potential (DP). Experiments were performed in raised divalent solution and tetrodotoxin (TTX) (1×10^{-5} mol l^{-1}). (A) Representative B67 recordings show spontaneous DPs. Note that three to four impulses persisted during the rising phase of the control DP in TTX (see Serrano and Miller, 2006). Bath application of OA (1×10^{-5} mol l^{-1}) in the presence of TTX prolonged the DP (middle record). This action was reversed following 30 min wash with OA-free solution (bottom record). (Bi) Group data show that DP durations increased from control values of 0.99±0.08 s to 3.49±0.44 s by 1×10^{-5} mol l^{-1} OA ($P<0.01$; $N=4$). (Bii) OA produced a small increase the amplitude of spontaneous DPs from control values of 21.0±2.1 mV to 24.6±1.6 mV. This increase was not statistically significant (see text).

attribute that normalizes their variability and expresses it as a percentage (Fig. 7Bii). The C_v was reduced by OA (control: 88.4±8.9%; OA: 21.1±4.7%; t=6.680 N=6; P<0.001), supporting the conclusion that OA increases the regularity of B67 bursting.

The decrease in IBI and increase in burst duration (Fig. 4A,Cii) indicated that OA acted to increase the B67 duty cycle, i.e. fraction of each period occupied by B67 activity. The OA-induced increase in duty cycle (expressed as per cent) was quantified using group data (Fig. 7Biii). The duty cycle was increased from 2.4±0.2% to 11.4±2.3% (P<0.05; N=5) in the presence of OA (1×10^{-5} mol l^{-1}).

Experiments were conducted to determine whether the conversion to rhythmicity imposed upon B67 by OA was dependent upon impulse signaling. In the presence of TTX (in high divalent solution), spontaneous B67 driver potentials occurred in an irregular and infrequent fashion (see Serrano and Miller, 2006) (see Fig. 5). When OA was applied in the presence of TTX, the spontaneous driver potentials became more frequent and rhythmic (Fig. 7C). These observations indicate that the rhythmic bursting produced in B67 by OA reflects its actions on intrinsic TTX-resistant properties.

OA effects on B67 burst synchrony

In a previous study we reported that, under control conditions, the spontaneous bursting of the two B67s rarely occurred synchronously (Serrano and Miller, 2006). In the presence of the aminergic modulator DA, however, their bursting became both rhythmic and synchronized. In view of the finding that OA also promoted rhythmic B67 bursting (Fig. 7) we explored whether it affected bilateral burst synchrony.

Simultaneous control recordings from the two B67s confirmed their asynchronous bursting, as reported previously (Fig. 8Ai, control) (see Serrano and Miller, 2006). Addition of OA (1×10^{-5} mol l^{-1}) caused both B67s to burst rhythmically (Fig. 8Ai, OA). Although the two B67s tended to burst at comparable rates, close examination of their IBIs disclosed slight differences in their cadence (Fig. 8A, OA, Fig. 8Bi). The two B67s therefore maintained

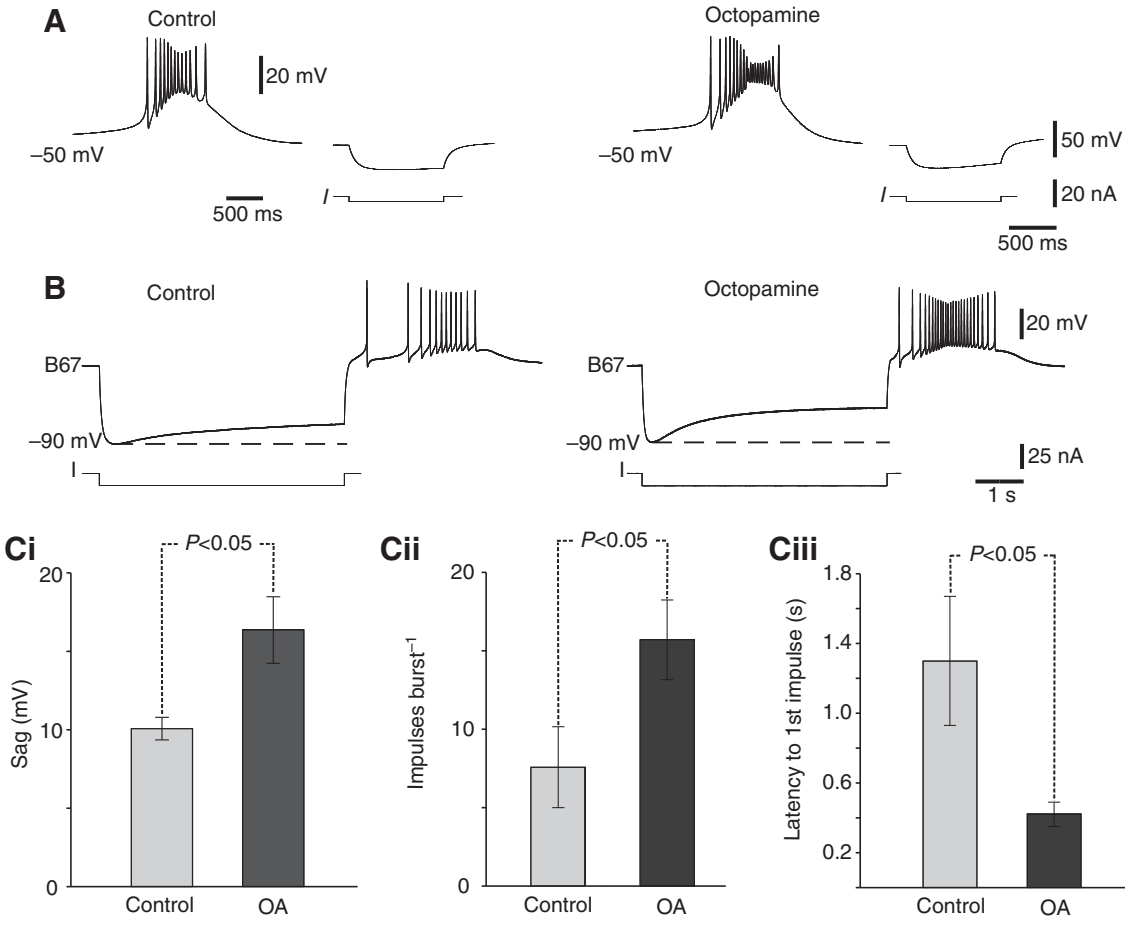

Fig. 6. Effects of octopamine (OA) on membrane properties of B67. (A) Application of OA (1×10^{-5} mol l^{-1}; right panel) did not produce detectable effects on the interburst membrane potential or input resistance of B67. At this concentration, OA produced significant increases in the burst duration and number of impulses per burst (compare with control burst, left panel; see also Fig. 4) but did not alter the basal interburst membrane potential (V_m) (–50 mV in this experiment). Similarly, measurements of the membrane input resistance, tested with hyperpolarizing pulses (5 nA, 1 s) did not disclose effects of OA. (B) The depolarizing relaxation, or 'sag', observed in the response of B67 to larger (10–20 nA) and longer (5 s) pulses were increased by OA (compare OA and control panels). To facilitate comparisons, the V_m was stepped to a fixed level (–90 mV; indicated by broken lines), and the sag was measured as the depolarization from this V_m at the termination of the 5 s pulse. The response of B67 immediately following such pulses was also influenced by OA. The number of spikes following the pulse was increased by OA, and the latency to the first impulse was decreased. (C) Quantification of each of these effects with group data demonstrated that (Ci) sag potentials were significantly increased by 10^{-5} mol l^{-1} OA (control: 10.1±0.7 mV; OA: 16.4±2.1 mV; P<0.05; N=5); (Cii) the number of impulses following pulses was increased (control: 7.6±2.6; OA: 15.7±2.5; P<0.05; N=5); and (Ciii) the latency to the initial spike following the pulses was decreased in OA (control: 1.30±0.37 s; OA: 0.42±0.07 s; P<0.05; N=5)

independent rhythms, with no evident tendency toward becoming synchronized. An operational index of synchrony, defined as the proportion of bursts in which at least one impulse overlapped with the contralateral B67 burst (see Serrano and Miller, 2006), was calculated to quantify the effects of OA on burst synchrony. Although OA produced a small increase in the index of synchrony (control: 0.10±0.04; OA: 0.19±0.04) this change did not reach statistical significance (t=1.536; N=4; P=0.175). This increased synchrony could largely be attributable to the more rapid burst rates (Fig. 7Bi) and augmented duty cycles (Fig. 7Biii) imposed on the two B67s by OA (see Discussion). Cross-correllograms generated with the timing of bursting in the two B67s demonstrated that the occurrence of bursting in one B67 provided little predictive power regarding the timing of bursting in the other (Fig. 8Biii).

We reported previously that each B67 burst produces a small (0.5–2 mV) TTX-resistant depolarizing response in its contralateral counterpart (Serrano and Miller, 2006). It was proposed that the augmentation of these signals by DA could account, at least in part, for the ability of DA to convert bilateral B67 bursting from an asynchronous to a synchronous mode (Serrano and Miller, 2006). In this study, we measured these contralateral depolarizations in OA (Fig. 9A) and compared their amplitude with those recorded under control conditions and in DA (Fig. 9B). In these experiments (total N=7), the order of addition of the modulators was randomized (OA first, N=4; DA first, N=3). The one-way ANOVA showed an overall effect of the modulators on contralateral depolarizations ($F_{2,18}$=22.4; P<0.05). Holm–Sidak *post hoc* comparisons of groups showed that although the contralateral depolarizing perturbations were increased by OA (control: 0.46±0.05 mV; OA: 1.22±0.11 mV; P<0.05), this increase was significantly less than that produced by DA (1.64±0.18 mV; P<0.05). We propose that the differential capacity of OA and DA to promote burst synchrony in the bilateral B67 MNs reflects this difference in membrane potential perturbations (see Discussion).

Finally, we examined whether the effects of OA on the relative timing of bursting in the B67 pair is dependent on impulse-mediated signaling (Fig. 10). In the presence of TTX (1×10^{-5} mol l^{-1}), OA (1×10^{-4} mol l^{-1}) produced similar increases in the frequency and regularity of spontaneous driver potentials of the two B67s. However, in agreement with its actions on spontaneous bursting, OA did not impose synchrony between the DPs of the left and right B67s.

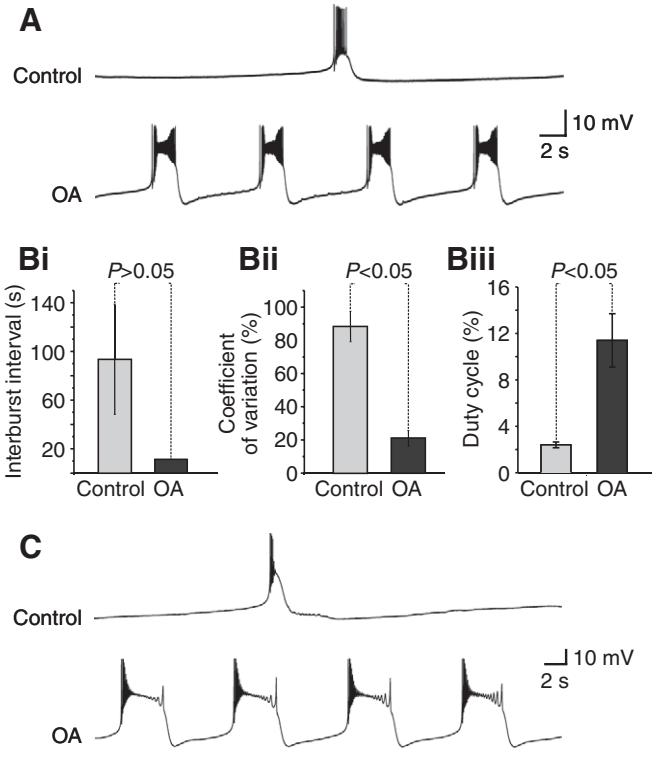

Fig. 7. Octopamine (OA) increases the frequency and regularity of B67 bursting. (A) Under control conditions (control, raised divalent solution), B67 bursting was infrequent and irregular. In addition to its actions on parameters of each burst, OA also increased the frequency and regularity of the B67 bursting. (Bi) Quantification with group data suggested that OA could decrease in the interburst interval (IBI) (mean IBI control: 93.3 s, OA: 11.6 s) but this effect (P=0.23) did not did not satisfy our criterion for statistical significance due to the large dispersion of control values (±45.3 s). (Bii) Normalizing the IBI dispersions by calculating their coefficients of variation revealed a significant reduction by OA (control: 88.4±8.9%; OA: 21.1±4.7%; P<0.001; N=6). (Biii) The decreased IBIs and increased burst durations contributed to increasing the B67 duty cycle in the presence of OA (control: 2.4±0.2%; OA: 11.4±2.3%; P<0.05; N=5). (C) OA-induced rhythmicity persisted in the presence of tetrodotoxin (TTX). In TTX (1×10^{-5} mol l^{-1}; high divalent solution; control), spontaneous B67 driver potentials (DPs) occurred in an irregular and infrequent fashion. Application of OA (1×10^{-4} mol l^{-1}; OA) caused the DPs to occur rhythmically.

DISCUSSION

Localization of OA_{li} in *Aplysia*

This study localized OA_{li} to a limited number (<40) of neurons in the CNS of *A. californica*. These findings support previous studies in which biochemical methods demonstrated the presence of OA in the *Aplysia* CNS. In the pioneering studies of McCaman and McCaman (McCaman and McCaman, 1978) the amount of OA found in the buccal, cerebral and pedal ganglia was 21.2±2.9, 31.8±3.5 and 67.7±6.0 pmoles ganglion^{-1}, respectively, and no OA was detected in the pleural or abdominal ganglia (<0.3 pmoles ganglion^{-1}) (see also McCaman, 1980).

The distribution of neurons containing OA_{li} was similar to that observed in the pond snail *L. stagnalis* (Elekes et al., 1993; Elekes et al., 1996; Hiripi et al., 1998; Vehovszky et al., 1998). In both species, OA_{li} is present in small numbers of neurons (<50) that are located in the buccal, cerebral and pedal ganglia (see also Ormshaw and Elliott, 2006). Consistent with observations in *Lymnaea*, our findings suggest that the octopaminergic neurons in *Aplysia* act predominantly as interneurons. With the exception of the parapedal commissure and P9, no evidence was found for projections of OA_{li} neurons beyond their ganglion of origin. The commonalities between OA localization in *Aplysia* and *Lymnaea* suggest that this neurotransmitter system is ancient and highly conserved, as evidence from the fossil record indicates that the pulmonate and opisthobranch gastropod subclasses diverged about 350 million years ago (Moore and Pitrat, 1960).

In *Lymnaea*, the OC cells fire during the third phase of tripartite BMPs and are thought to participate in the swallowing phase of ingestive behaviors (Vehovszky and Elliott, 2000; Vehovszky et al., 1998). Similarly, a pair of interneurons designated N3a (Quinlan and Murphy, 1996) that control the third phase (termed hyperretraction) of feeding programs in the snail *Helisoma trivolvis* also exhibit OA_{li} (A. D. Murphy, personal communication). Although the feeding motor patterns of *Aplysia* are commonly described as consisting of two phases (radula protraction followed by retraction) (Fig. 3), numerous behavioral, biomechanical and

physiological observations suggest more complex action sequences and neural programs (e.g. Weiss et al., 1986; Murphy, 2001; Neustadter et al., 2002; Serrano et al., 2007). Further studies should explore whether the OA_{li} cells of *Aplysia* function in a capacity that is similar to the OC cells of *Lymnaea* and N3a cells of *Helisoma*, i.e. as CPG elements that drive a hyper-retraction phase of motor activity (see Murphy, 2001; Wentzell et al., 2009). Alternatively, distinct roles of the *Aplysia* OA_{li} neurons could reflect their contributions to modifications of circuit performance that have occurred in response to evolutionary pressures on

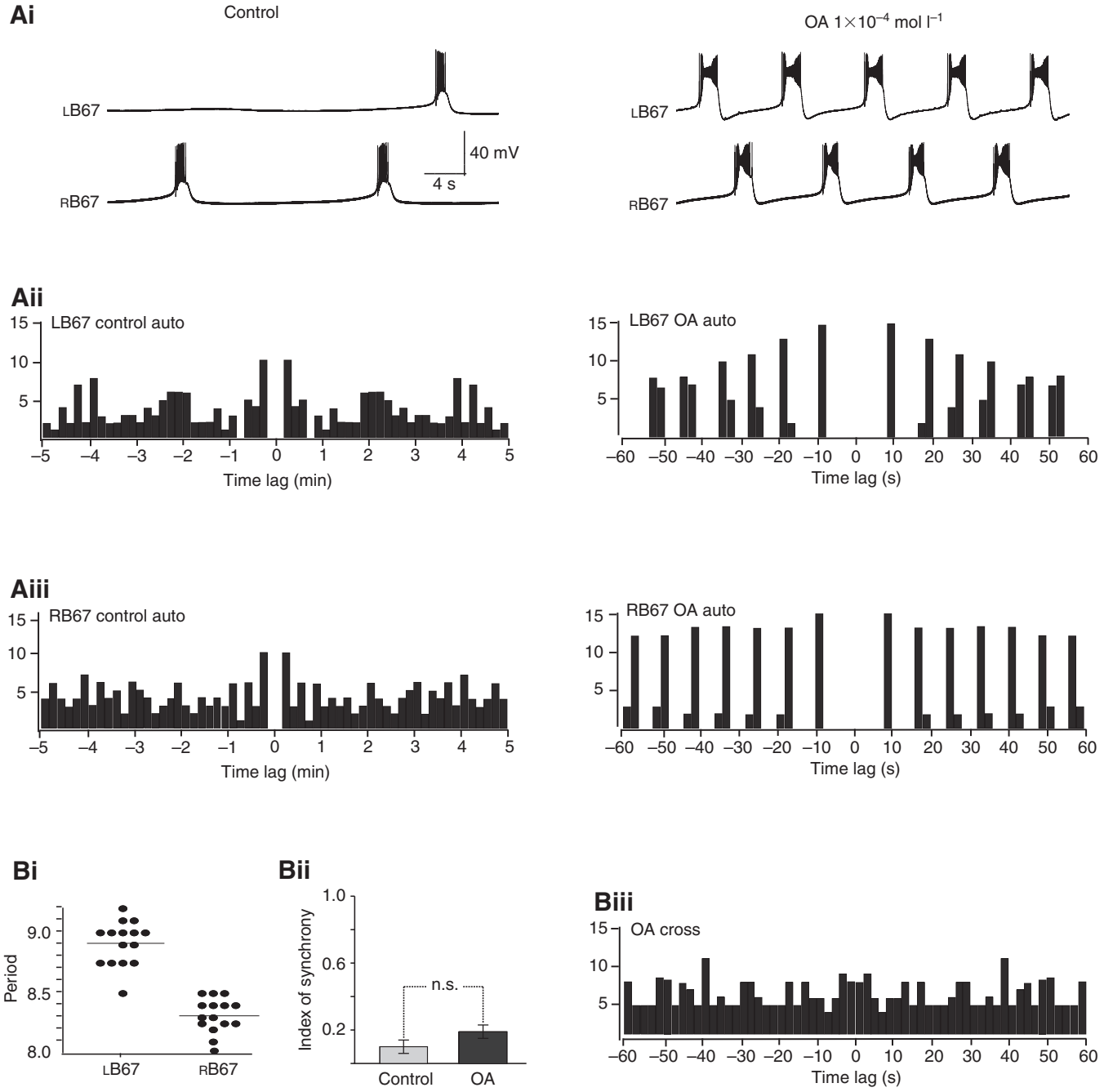

Fig. 8. Octopamine (OA) increases the rhythmicity of bursting in the two B67s but does not increase their synchrony. (Ai) Left panel; simultaneous recordings from the B67 neuron in the left buccal hemiganglion (LB67) and in the right hemiganglion (RB67) under control conditions (raised divalent medium). Right panel: addition of OA (1×10^{-4} mol l^{-1}) resulted in both B67s becoming highly rhythmic. (Aii, Aiii) Left panels: autocorrelograms generated with data from the two B67s exhibited very weak rhythmicity within the time window examined (±5 min; bin width: 10 s). Right panels: in OA, the autocorrelograms for both B67s showed very strong rhythmicity with periodicities in the range of 8–10 s. Note that the time window of the OA autocorrelograms was only ±1 min due to the high frequency of burst activity (bin width: 2 s). (Bi) Comparison of IBIs (15 from each B67) from experiment shown in A. Although bursting of both B67s was highly rhythmic in OA, their rates were different, with the left B67 (range of IBIs: 8.5–9.2 s) bursting slightly slower than the right B67 (range of IBIs: 8.0–8.5 s). (Bii) The index of synchrony, calculated as the proportion of B67 bursts that exhibited overlap with contralateral firing (see Materials and methods) was slightly, but not significantly, increased by OA (control: 0.10±0.04; OA: 0.19±0.04; P=0.175; N=4). (Biii) The cross-correlogram of LB67 and RB67 bursting confirmed their lack of synchrony (time window: ±1 min; bin width: 2 s).

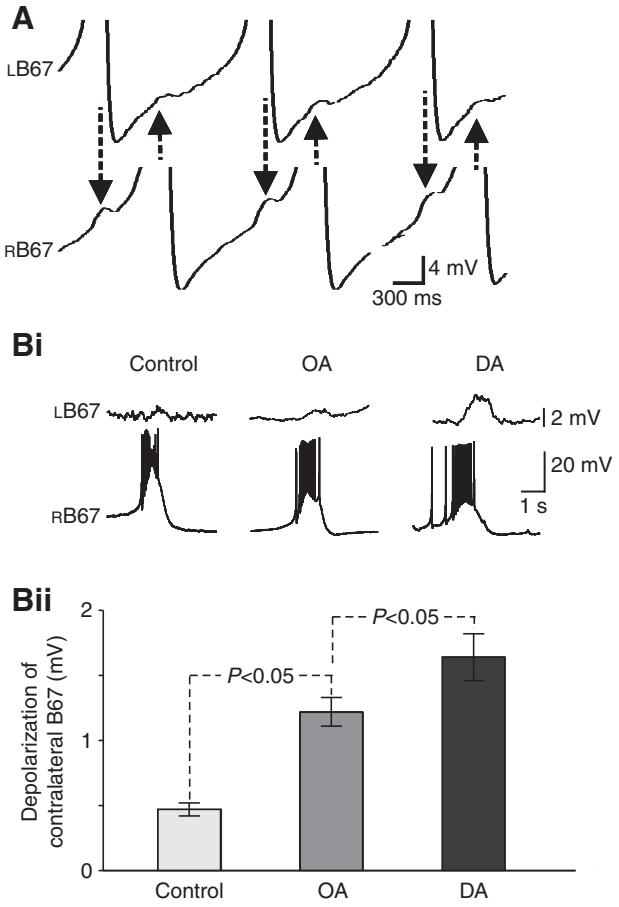

Fig. 9. Modulators regulate perturbations of membrane potential between contralateral B67s. (A) Recordings from the left (LB67, upper record) and right (RB67, lower record) shown at high gain [octopamine (OA), 1×10^{-4} mol l^{-1}]. In both recordings, bursts were truncated (note discontinuous records) to enable viewing small (approximately 1 mV) depolarizations in contralateral cells (arrows). (B) Comparison of the slow depolarizations produced in B67 during bursts in its contralateral counterpart. (Bi) Augmentation of the depolarization in RB67 produced by OA (1×10^{-4} mol l^{-1}) was less than that produced by dopamine (DA; 1×10^{-4} mol l^{-1}). (Bii) Group data comparing the effects of OA and DA on the contralateral depolarization produced by B67 bursting. All experiments (N=7; OA applied first in four experiments, DA in three experiments) were conducted in high divalent solution. While the depolarizations measured in the presence of OA (1.22±0.11 mV) were larger than control values (0.47±0.05 mV; t=4.237; N=7; P<0.05), those observed during application of DA were significantly larger than in OA (1.64±0.18 mV; t=2.373; N=7; P<0.05).

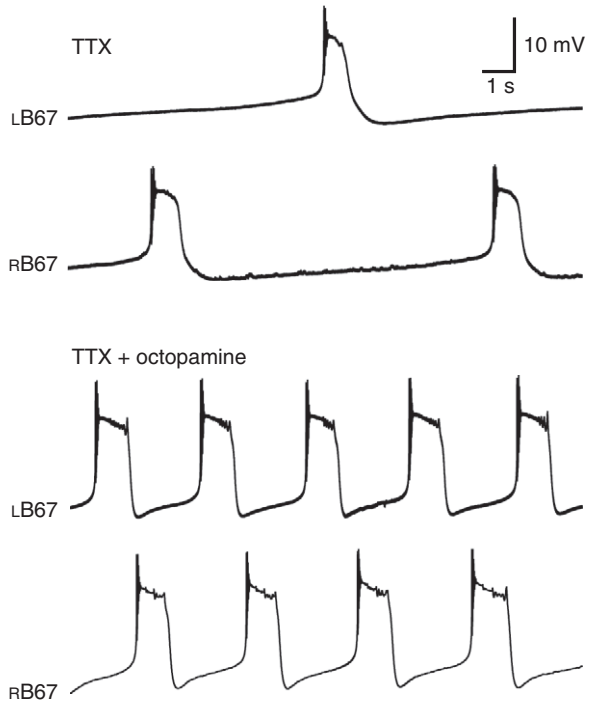

Fig. 10. Effects of octopamine (OA) on B67 relative burst timing are not dependent on impulse-mediated signaling. Under control conditions [tetrodotoxin (TTX), 1×10^{-5} mol l^{-1}], the left B67 (LB67) and right B67 (RB67) neurons produced spontaneous arrhythmic and asynchronous driver potentials (DPs). Addition of OA (TTX + OA, 1×10^{-4} mol l^{-1}) increased the frequency and regularity of spontaneous DPs. OA did not impose synchrony between the DPs of the left and right B67s.

feeding behaviors (Elliott and Susswein, 2002; Katz and Harris-Warrick, 1999).

Actions of OA on the buccal ganglion

The multifunctional central pattern generator network that controls *Aplysia* consummatory behaviors can generate motor patterns that implement both ingestive (biting and swallowing) and egestive (rejection) actions (Weiss et al., 1986; Morton and Chiel, 1993a; Morton and Chiel, 1993b; Kupfermann and Weiss, 2001). The observation that high concentrations of OA (1 mmol l^{-1}) promoted ingestive BMPs when applied to the feeding network is in agreement with findings in *Lymnaea*, where comparable concentrations (0.5 mmol l^{-1}) evoked feeding motor programs when applied to isolated CNS preparations (Vehovszky et al., 1998). These investigators also showed that the feeding behavior of *Lymnaea* was decreased following injection of OA antagonists into freely behaving specimens.

The effects of bath-applied OA on the *Aplysia* feeding network are likely to reflect the activation of multiple receptors that vary in their sensitivity to the agonist and in the time course of their responses. Cloning studies have identified three molluscan G protein-coupled OA receptors (Gerhardt et al., 1997a; Gerhardt et al., 1997b; Chang et al., 2000). These receptors are coupled to distinct second messenger systems and are activated over broadly ranging OA concentrations. One OA receptor that is expressed in the *Aplysia* CNS (designated Ap oa_1) had an EC_{50} of 30 nmol l^{-1} for production of cAMP (Chang et al., 2000). By contrast, the activation of second messenger systems by two *Lymnaea* OA receptors (Lym oa_1 and Lym oa_2) required micromolar or higher agonist concentrations (Gerhardt et al., 1997a; Gerhardt et al., 1997b). It is likely, therefore, that the network effects observed at high (mmol l^{-1}) OA concentrations and the cellular effects observed with lower (μmol l^{-1}) doses in this study both reflect the activation of multiple receptor types and second messenger cascades.

Actions of OA on B67

OA has been extensively studied in arthropods, where it originates from limited sets of central neurons and is thought to act in a 'gain-setting' fashion that broadly biases neural circuits (Kravitz, 1988; Roeder, 2005). In several well-characterized arthropod motor networks, OA acts to augment motor patterns produced by circuits composed of endogenously bursting neurons (Benson, 1984; Flamm and Harris-Warrick, 1986; Ramirez and Pearson, 1989; Wood,

1995). The actions of OA on the B67 bursting were excitatory, producing bursts with longer durations and increased numbers of impulses. These effects can be attributed, at least in part, to the effects of OA on the TTX-resistant DP that underlies bursting, as the DP duration was also prolonged by OA. Endogenous burst-forming mechanisms are found in many motor systems, and the currents that contribute to their generation are regulated by neuromodulators (reviewed by Harris-Warrick, 2002; Grillner, 2006). Although the majority of neurons that comprise the feeding motor circuits of *Aplysia* do not exhibit sustained responses to brief stimuli, this property is present in certain influential interneurons and MNs that contribute to initiating and coordinating the phases of motor patterns (Susswein and Byrne, 1988; Plummer and Kirk, 1990; Hurwitz and Susswein, 1996).

Under control conditions, the duration of the B67 burst (0.99±0.07 s; measured from the first to the last impulse) was nearly equivalent to the duration of the DP recorded in TTX (0.99±0.08 s; measured from the $t_{1/2}$ of the rise time to the $t_{1/2}$ of decay). Interestingly, the duration of the DP in OA (3.49±0.44 s) was substantially greater than the B67 burst duration (1.58±0.18 s) observed with the same OA concentration (see also Serrano and Millar, 2006). These observations suggest that impulse firing may contribute to termination of bursting in B67, and that this contribution is more prominent when B67 is subject to modulation.

The OA-induced increase of burst frequency may reflect its modulatory actions on two additional intrinsic properties of B67, i.e. the depolarizing relaxation or 'sag' observed in its response to sustained hyperpolarization and its tendency to exhibit PIR. Similar actions on B67 produced by DA were proposed to reflect its enhancement of a hyperpolarization-activated inward current (Serrano and Miller, 2006). Hyperpolarization-activated inward currents (I_h) contribute to oscillatory activity in numerous motor systems (e.g. Arbas and Calabrese, 1987; Angstadt and Calabrese, 1989; Golowasch and Marder, 1992; Thoby-Brisson et al., 2000), and their modulation by biogenic amines can regulate rhythmic motor patterns (Kiehn and Harris-Warrick, 1992; Kjaerulff and Kiehn, 2001; Peck et al., 2006). In mollusks, the presence of an I_h-like current was demonstrated in a specific motor neuron (B4) of the *Lymnaea* feeding network (Straub and Benjamin, 2001) (but see Vehovszky et al., 2005). In *Aplysia*, Kuzyk and coworkers cloned a hyperpolarization-activated, cyclic nucleotide-gated cation channel (designated acHCN) from CNS cDNA (Kuzyk, 2007). In the buccal ganglion, abundant expression of acHCN transcripts was demonstrated with *in situ* hybridization in the ventral motor neuron cluster that includes B67 (Kuzyk, 2007).

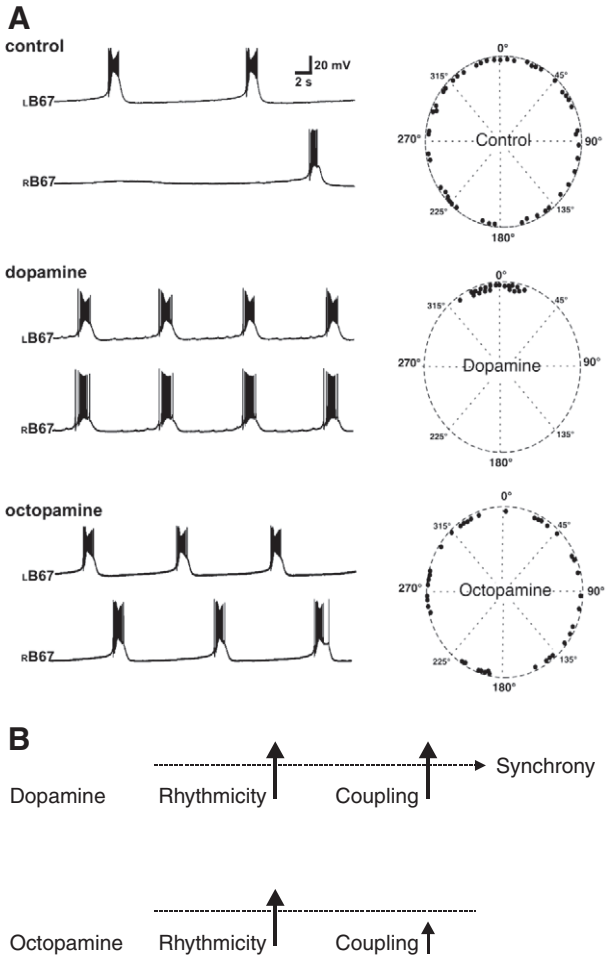

Fig. 11. Common and distinct modulatory actions of octopamine (OA) and dopamine (DA) on B67 bursting. (A) Sequential application of DA and OA illustrate their common and distinct actions on B67 burst activity. Left panels: while DA and OA both produce increases in the duration, frequency and rhythmicity of B67 bursting, only DA also imposes synchronous bilateral B67 bursting. Right panels: circular plots illustrate phase relations between the two B67 neurons under control conditions (top), in DA (middle) and in OA (bottom). (B) Schematic interpretation of proposed modulator actions on B67. It is hypothesized that synchrony of bursting between the two B67s requires that their rhythmicity and mutual coupling both exceed a critical level (horizontal broken lines). Although DA and OA both exert comparable increases in the rhythmicity of B67 bursting (compare left vertical arrows), only DA enhances their coupling sufficiently to achieve burst synchrony (compare right vertical arrows).

Convergent and divergent actions of OA and DA on B67 bursting

The neuromodulator DA was shown previously to increase the duration, frequency and rhythmicity of B67 bursts (Serrano and Miller, 2006). The predominant action of DA on the B67 DP was also a prolongation of its duration, with little augmentation of its amplitude. Moreover, like OA, DA increased the slowly developing depolarization observed during hyperpolarizing pulses in B67 and augmented the PIR that followed such hyperpolarizing pulses (Serrano and Miller, 2006). This common constellation of effects between OA and DA may result from shared or convergent second messenger pathways, a common design of motor network modulation (Swenson and Marder, 2000; Swenson and Marder, 2001; Peck et al., 2006). Convergent actions of modulators have been well documented in neuromuscular elements of the *Aplysia* feeding system (Brezina et al., 1994; Brezina et al., 1995). One proposed adaptive rationale for such apparent redundancy suggests that it permits the functional uncoupling of each modulator's divergent actions that would otherwise be constrained to a relatively fixed ratio (Brezina et al., 1996; Brezina and Weiss, 1997a; Brezina and Weiss, 1997b).

In addition to their common actions, the effects of DA and OA were found to diverge in at least one respect. While DA promotes burst synchrony between the bilateral B67s, OA does not cause them to burst in unison (Fig. 11). These findings further support the proposal that the conditional synchrony produced by DA in the B67 pair is not an obligatory consequence of their conditional rhythmicity (see Serrano and Miller, 2006). We hypothesize that synchrony of bursting between the two B67's requires that their rhythmicity and mutual coupling both exceed critical values (Fig. 11B, broken lines).

In many biological systems, the propensity for pulse-coupled oscillators to become synchronized reflects two factors, their relative frequencies and the amplitude of their mutual coupling (Winfree, 1980; Cohen, 1987; Collins and Stewart, 1993; Strogatz and Stewart, 1993). As OA and DA produce similar effects on the rhythmicity of bursting in the B67 pair (Fig. 11A and B), we propose that their lack of synchrony in OA could be attributable to its weaker augmentation of the depolarizing influence between them (Fig. 11B). Although the nature of this mutual influence remains unknown, its persistence in the presence of TTX indicates that it is not impulse mediated. Moreover, dye injection experiments provide no evidence for contiguity of processes between the bilateral 67s. We hypothesize that their mutual influence reflects the widespread electrical coupling among MNs and interneurons that are active during the protraction phase of BMPs (Borovikov et al., 2000). Although this coupling will be attenuated due to its indirect route between the two B67s, it has been shown to exhibit low-pass filtering characteristics that would favor the transmission of slow events like the TTX-resistant DP (Martínez-Rubio et al., 2007). DA and OA have previously been shown to differentially modulate electrical coupling between identified MNs in the lobster stomatogastric ganglion (Johnson et al., 1993).

Together, these studies indicate that one adaptive benefit conferred upon motor systems by the convergent and divergent actions of neuromodulators could be to regulate the extent to which the rhythmicity and synchrony of specific actions are coupled (Serrano and Miller, 2006). In the *Aplysia* feeding system, such modulation could enable rhythmic bilateral activation of the pharyngeal and salivary targets of B67 to occur in either a synchronous or alternating fashion. Although the adaptive significance of this flexibility is presently unknown, it may be readily appreciated how it could enhance movement of material of varying size and shape through the pharynx during either ingestive or egestive behaviors. In both cases, any advantage afforded by the capacity to exert bilaterally synchronous *versus* bilaterally alternating forces could significantly impact an animal's survival.

LIST OF ABBREVIATIONS

ASW	artificial seawater
BG	buccal ganglia
BMPs	buccal motor programs
CNS	central nervous system
DA	dopamine
DP	driver potential
IBI	interburst interval
MNs	motor neurons
OA	octopamine
OA_{li}	octopamine-like immunoreactivity
OCs	octopaminergic interneurons
PIR	post-inhibitory rebound
TTX	tetrodotoxin

ACKNOWLEDGEMENTS

Supported by the National Institutes of Health: RCMI RR-03051, NIGMS MBRS: GM-08224, 5SC3GM087200 and GM-061838; National Science Foundation DBI-0115825; US ARO Grant W911NF-07-1-0555; University of Puerto Rico Medical Sciences Campus Deanship of Biomedical Sciences, and a Society for Neuroscience MNFP fellowship (NIMH and NINDS). Deposited in PMC for release after 12 months.

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