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Differential modulation of chemical and electrical components of mixed synapses in the lobster stomatogastric ganglion

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Abstract 1. Two pairs of neurons in the pyloric network of the spiny lobster, *Panulirus interruptus*, communicate through mixed graded chemical and rectifying electrical synapses. The anterior burster (AB) chemically inhibits and is electrically coupled to the ventricular dilator (VD); the lateral pyloric (LP) and pyloric (PY) neurons show reciprocal chemical inhibition and electrical coupling. We examined the effects of dopamine (DA), serotonin (5HT) and octopamine (Oct) on these mixed synapses to determine the plasticity possible with opposing modes of synaptic interaction.

2. Dopamine increased net inhibition at all three pyloric mixed synapses by both reducing electrical coupling and increasing chemical inhibition. This reversed the sign of the net synaptic interaction when electrotonic coupling dominated some mixed synapses, and activated silent chemical components of other mixed synapses.

3. Serotonin weakly enhanced LP→ PY net inhibition, by reducing electrical coupling without altering chemical inhibition. Serotonin reduced AB→ VD electrical coupling, but variability in its effect on the chemical component made the net effect non-significant.

4. Octopamine enhanced LP→ PY and PY→ LP net inhibition by enhancing the chemical inhibitory component without altering electrical coupling.

5. Differential modulation of chemical and electrical components of mixed synapses markedly changes the net synaptic interactions. This contributes to the flexible outputs that modulators evoke from anatomically defined neural networks.

Key words Electrotonic coupling · Synaptic modulation Stomatogastric ganglion · Amines · Lobster

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Abbreviations AB anterior burster · PD pyloric dilator VD ventral dilator · LP lateral pyloric PY pyloric constrictor · IC inferior cardiac STG stomatogastric ganglion · DA dopamine 5HT serotonin · Oct octopamine · I/O input/output TTX tetrodotoxin · PTX picrotoxin

Introduction

Synaptic contacts with anatomical specializations for both chemical and electrical transmission are widespread in invertebrate and vertebrate nervous systems (reviewed in Bennett 1977; Sotelo and Korn 1978; see also Mugnaini and Maler 1987; Bass and Marchaterre 1989; Bosch 1990; Surchev 1992; Lee and Krasne 1993). In fact, it is thought that most of the anatomically described electrotonic synapses in the vertebrate central nervous system are components of mixed chemical-electrical synapses (Peters et al. 1991). Despite abundant and detailed anatomical descriptions of mixed synapses, we are only beginning to understand the functional significance of having combined chemical and electrical transmission between synaptically coupled neurons (Pereda et al. 1992). The presence of both electrical and chemical transmission at the same junction creates the potential for subtle and complex synaptic interactions (Peters et al. 1991). When chemical inhibition is mixed with electrotonic coupling, the resulting synaptic interaction is the summation of opposing synaptic components (Graubard and Hartline 1987). It has been suggested that differential modulation of these two synaptic components could produce both quantitative and qualitative changes in the net post-synaptic response (Graubard and Hartline 1987).

In the spiny lobster *Panulirus interruptus*, the pyloric network contains 14 neurons of 6 major types (Mulloney 1987). Two pairs of neurons in the pyloric network communicate through mixed synapses that have both a rectifying electrical component (Graubard and Hartline 1987; Johnson et al. 1993a) and a chemical inhibitory component mediated by glutamate (Marder 1987). The anterior

burster (AB), the major pacemaker neuron for the pyloric motor pattern (Bal et al. 1988), makes a mixed synapse with the ventricular dilator (VD) neuron (Eisen and Marder 1982). The lateral pyloric (LP) neuron and neurons of the pyloric (PY) type have reciprocal inhibitory chemical synapses and rectifying electrical coupling (Graubard and Hartline 1987; Johnson et al. 1993a). Mixed synaptic interactions are also occasionally seen between the pyloric dilator (PD) and the inferior cardiac (IC) neurons (Johnson and Harris-Warrick 1990), but since these are not found consistently, they have not been studied in detail. Chemical transmission between the pyloric neurons is both spike-evoked and graded; the pre-synaptic neuron releases transmitter as a continuous function of voltage with a threshold near the resting potential (Hartline and Graubard 1992). Graded synaptic transmission appears to be important for the organization of the network output, while spike -evoked transmission functions to send signals to distant targets, such as muscles or neurons in other ganglia (Russell and Graubard 1987; Hartline et al. 1988).

In this study, we examined the effects of dopamine (DA), serotonin (5HT) and octopamine (Oct) on the graded, mixed chemical-electrical synapses in the pyloric network of the spiny lobster. Dopamine, 5HT and Oct are endogenous neuromodulators in Crustacea (Kravitz 1988; Fingerman and Nagabhushanam 1992), and are components of the large array of substances modulating the pyloric motor output (Harris-Warrick et al. 1992b; Marder and Weimann 1992). These 3 amines are localized in input fibers projecting to the stomatogastric ganglion (STG) or act as circulatory hormones to affect the pyloric motor pattern (Barker et al. 1979; Kushner and Barker 1983; Beltz et al. 1984). Each amine evokes a distinct pattern from the pyloric network when bath applied to the isolated, quiescent STG (Flamm and Harris-Warrick 1986a). This is accomplished by a unique constellation of effects each amine has on the intrinsic excitability of the pyloric neurons (Marder and Eisen 1984; Flamm and Harris-Warrick 1986b) and on the strength of graded chemical (Johnson and Harris-Warrick 1990; Harris-Warrick et al. 1992a) and electrical synapses (Johnson et al. 1993a) between the pyloric neurons. We are trying to understand how amines affect all the "building blocks" (Getting 1988) that produce a network's output. We previously described amine effects on the isolated electrical component of the pyloric mixed synapses under conditions where the chemical component was blocked with picrotoxin (PTX) (Johnson et al. 1993a). We use these earlier results to help interpret amine effects on the intact mixed synapses, where the chemical component is free to oppose the electrotonic coupling. Here we demonstrate that amines can profoundly modulate communication at mixed synapses, including reversal of the sign of the net synaptic interaction and the functional creation of chemical synapses. Some of these results have been presented in preliminary form (Johnson et al. 1993b).

Materials and methods

Spiny lobsters (*Panulirus interruptus*) were purchased from California suppliers and maintained in marine aquaria at 15°C. The stomatogastric nervous system, consisting of commissural, esophageal and stomatogastric ganglia, their connecting nerves and the motor nerves leaving the STG (Selverston et al. 1976), was dissected and placed in a preparation dish filled with *Panulirus* saline of the following composition (m*M*): NaCl 479, KCl 12.8, $CaCl_2$ 13.7, Na_2SO_4 3.9, $MgSO_4$ 10.0, glucose 2.0, tris base 11.1, maleic acid 5.1, pH 7.4 (Mulloney and Selverston 1974). The STG was desheathed, enclosed in a small (1 ml) pool of saline walled by vaseline, and constantly perfused at 5 ml/min with oxygenated saline at 20–21°C. The experimental temperature was raised above the aquarium temperature to enhance the strength of graded chemical synaptic transmission (Johnson et al. 1991).

Standard intracellular techniques were used for current injection and voltage recording using KCl-filled (3 *M*, 10–25 MΩ) microelectrodes. The cell bodies of the pyloric neurons were identified during rhythmic pyloric activity by: (1) matching action potentials recorded extracellularly from an appropriate motor nerve root and intracellularly from the soma, (2) the timing of spike activity within the pyloric rhythm, (3) the characteristic shape of membrane potential oscillations and action potential amplitudes, and (4) the synaptic connectivity. The PY cell population in these experiments was a mixture of early and late firing PYs (Hartline et al. 1987). After cell identification, we superfused the preparation with 10^{-7} *M* tetrodotoxin (TTX)-saline to block spiking synaptic transmission within the ganglion. The TTX-saline also blocked rhythmic pyloric activity by eliminating all descending (Russell 1979; Nagy and Miller 1987) and ascending (Katz et al. 1989) modulatory inputs to the STG.

In most experiments, we isolated AB-VD and LP-PY neuron pairs from the rest of the pyloric network, to ensure that amine-induced changes in a mixed synaptic interaction were not occurring indirectly through neurons either electrically or chemically coupled to the pair under study (Johnson and Harris-Warrick 1990; Johnson et al. 1993a). For this synaptic isolation, we used 5,6-carboxyfluorescein photoinactivation (Miller and Selverston 1979; Flamm and Harris-Warrick 1986b) to kill those pyloric neurons which could mediate indirect actions. The strength of the chemical synaptic connections appeared to weaken after the cell kill procedure and removal of all modulatory inputs to the ganglion. In a few experiments, we left the pyloric network intact or only partially isolated the synaptic pairs. There were no obvious differences in the effects of amines on isolated or non-isolated synaptic pairs. We did not, however, do a thorough comparison that might have revealed more subtle quantitative differences in amine action between isolated and non-isolated synaptic pairs.

We described the mixed synaptic interactions between the LP-PY and the AB-VD neurons with two pre-synaptic and, in most experiments, two post-synaptic electrodes. One of each pair of electrodes was used for current injection and the other for voltage recording in each neuron. We use arrows to indicate the direction of current injection. For example, AB→ VD reflects an experiment where current was injected into the AB (pre-synaptic) and the resulting voltage change was measured in the VD (post-synaptic). Pre-synaptic current injection was used to construct input-output (I/O) curves for the synaptic interactions, with 1 s pre-synaptic peak polarizations (square pulses) of varying amplitude and sign plotted against the peak amplitude of the post-synaptic response. Pre-synaptic depolarization began with the smallest current injection and increased to the largest current injection at a stimulation rate of 0.2 Hz (Johnson and Harris-Warrick 1990). We used tonic current injection to maintain the pre-and post-synaptic resting potentials at −55 mV. This resting potential is within the normal range of pyloric neuron resting potentials in TTX-saline (Johnson et al. 1991).

Post-synaptic responses were measured as integrated (net) responses, and separated into chemical and electrical components. The net response was calculated as the integrated area of the post-synaptic response (relative to the resting potential) during the pre-

synaptic voltage step. We took advantage of the partial temporal distinction of the rapid peak electrotonic and slower chemical components of the AB→VD mixed interaction (Fig. 1) to measure the amplitude of each synaptic component separately. The depolarizing electrical component was measured from its origin on the resting potential to its peak value before the chemical PSP began. The amplitude of the chemical component was measured from its origin on the electrotonic component of the post-synaptic response to its peak hyperpolarization. Net responses were also calculated for the LP→PY and PY→LP synaptic interactions, but at these mixed synapses, the chemical and electrical synaptic components were not temporally distinct (perhaps due to greater electrotonic distance between these synapses and the recording sites in the somata). Usually only one or the other synaptic component was observed in control conditions (see Fig. 6), and we measured the peak chemical or electrotonic response amplitude relative to the resting potential. To verify this separation of electrical and chemical components, experiments were performed in the presence of 5×10^{-6} *M* picrotoxin (PTX) which completely eliminates the glutamatergic chemical component (Bidaut 1980; Eisen and Marder 1982), thus exposing a pure electrical component (Johnson et al. 1993a,b). The pre-synaptic voltage threshold to elicit a detectable post-synaptic chemical response was calculated as the x-intercept point of a simple regression line through data points with measurable post-synaptic responses. Our estimations of this response threshold are not exact because release is not a simple linear function of pre-synaptic voltage above threshold, because pre-synaptic depolarizations evoked in the soma must decrement somewhat en route to the release sites in the neuropil, and because the opposing interaction between electrical and chemical inhibitory synaptic transmission would partially obscure the voltage dependence of transmitter release. However, these values are useful for comparison between control and amine modulated conditions. We calculated the electrical coupling coefficient between a cell pair as the ratio of the post-synaptic voltage change to the pre-synaptic voltage change. This coupling coefficient was the slope of the I/O curve in the preferred direction of synaptic current for these rectifying synapses (Johnson et al. 1993a).

Amine solutions were prepared in TTX-saline just before application to a final concentration of: 10^{-4} *M* DA (DA-HCl), 10^{-5} *M* 5HT (creatinine sulfate complex), and 10^{-5} *M* Oct (D,L-Oct). These amine concentrations cause the maximum qualitative effect on the pyloric motor pattern (Flamm and Harris-Warrick 1986a) and, unlike higher concentrations, have effects on graded chemical synaptic transmission that are easily reversible (Johnson and Harris-Warrick, unpublished observations). In addition, the amine concentrations we chose were used previously to examine amine effects on pyloric neuron excitability and graded synaptic transmission (Marder and Eisen 1984; Flamm and Harris-Warrick 1986b; Johnson and Harris-Warrick 1990; Johnson et al. 1993a). This facilitates achieving our ultimate goal: to develop a complete explanation for the motor patterns induced by each amine based on their cellular and synaptic actions.

The post-synaptic response was measured before amine application, after 5 min perfusion with an amine solution and after a wash period, usually 30 min. When an amine changed a synaptic interaction, we accepted the data only if the effect was reversible. An amine was only applied once to a preparation, and all three amines were not applied to every preparation. Dopamine almost always, and 5HT sometimes caused membrane potential oscillations in the AB neuron in TTX-saline at our experimental temperatures (Johnson et al. 1992), which made it difficult to measure amine effects on the AB→VD synaptic interaction. We blocked or significantly reduced the amplitude and frequency of DA- and 5HT-induced AB oscillations by superfusing the STG with low (50%) Na^+-TTX saline (Na^+ replaced by n-methyl glucamine, Johnson et al. 1992). Although this treatment did not appear to affect electrical or chemical graded transmission, DA and 5HT effects on graded transmission in low Na^+ saline were always compared with control measurements also made in low Na^+ saline. In two experiments, DA caused PY membrane potential oscillations when the PY membrane potential was held at −55 mV. We measured the chemical synaptic amplitude in these experiments during the wash period immediately after these oscillations had stopped (3–6 min after starting the wash). Dopamine enhancement of the chemical synapse was still evident but it may have been underestimated by this procedure.

To make statistical comparisons of post-synaptic responses under control and amine conditions, we compared the post-synaptic responses during pre-synaptic depolarizations to −35 mV, i.e., 20 mV of pre-synaptic depolarization. We considered this level of pre-synaptic depolarization to be within the normal oscillation range of the pyloric neurons and thus relevant for amine effects on pyloric network interactions. For example, the AB neuron rhythmically oscillates between approximately −60 and −30 mV with varied modulatory input (Miller 1987; Johnson et al. 1992). We used the paired Student's *t*-test to determine statistically significant differences in graded synaptic transmission before and during amine application; $P < 0.05$ (two-tailed probability) was used to accept means as statistically different. Mean data are given ± SEM.

Results

Amine effects on the AB→VD synapse

The electrical and chemical components of the AB→VD mixed synapse were easily observed in the VD response to AB polarizations under control conditions in 10^{-7} *M* TTX-saline (Fig. 1). The electrical component of the AB→VD mixed synapse shows marked rectification with a preferred negative current flow in the direction AB→VD. Thus, the electrical coupling is greatest during hyperpolarization of AB and rectifies strongly during AB depolarization (Fig. 1A, control; Johnson et al. 1993a). The mean electrical coupling coefficient during AB hyperpolarization in these experiments was 0.13 ± 0.03 (n = 10), not statistically different from the value of 0.11 ± 0.01 measured when the chemical synapse was abolished with PTX (Johnson et al. 1993a). In 5 of 10 isolated AB→VD preparations, electrotonic coupling in the non-preferred direction (i.e., during AB depolarization) was stronger than graded chemical transmission (Fig. 1A, control), resulting in an I/O curve showing a net VD depolarization when integrated over the AB voltage step throughout the range of AB depolarizations from the holding potential of −55 mV (Fig. 1B, control). In these experiments, AB depolarization evoked a complex post-synaptic potential in the VD, composed of an initial peak depolarizing electrotonic component which declined in amplitude due to the onset of the delayed hyperpolarizing chemical inhibitory component (Fig. 1A, control). The chemical component became progressively stronger with greater AB depolarizations, as indicated by the decrease in net VD depolarization (Fig. 1B). When 5×10^{-6} *M* PTX was applied to block the chemical component, AB depolarization evoked only weak rectifying coupling in the non-preferred direction (Johnson et al. 1993a,b). In the remaining 5 preparations, chemical inhibition was stronger than electrical coupling and dominated the synaptic interaction during AB depolarizations (Fig. 1C, control). In these preparations, a net VD hyperpolarization was seen throughout all or most of the range of AB depolarization (Fig. 1D). The chemical inhibitory com-

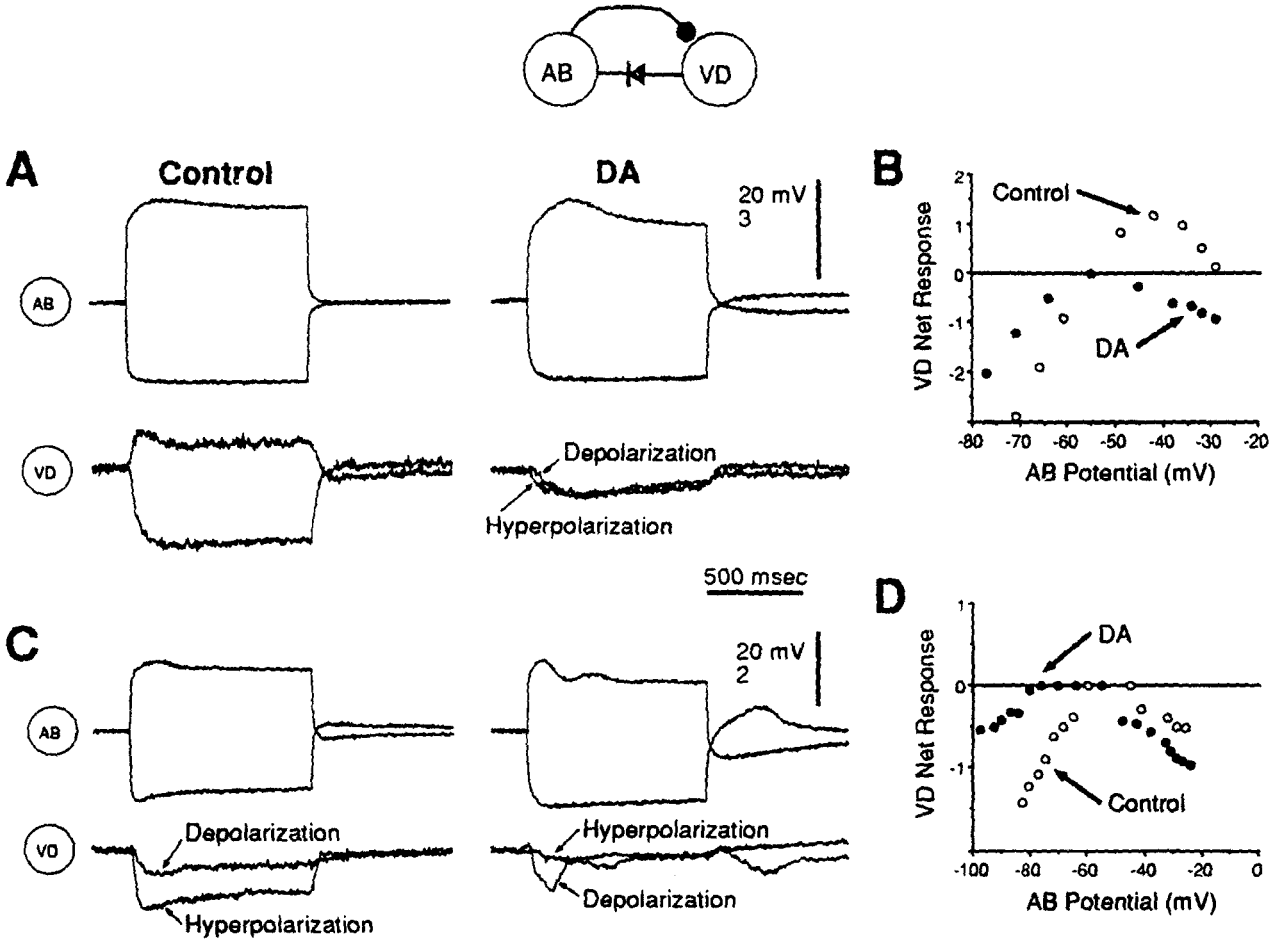

Fig. 1A–D Dopamine enhancement of net inhibition at isolated AB→VD mixed synapses. The digitized traces show the AB response to 1 s depolarizing and hyperpolarizing current injections and the resulting VD synaptic responses from two different preparations in control conditions (10^{-7} *M* TTX-saline) and during 10^{-4} *M* DA superfusion. Depolarization and hyperpolarization labels in the VD traces indicate the VD responses to the appropriate AB current injections. **A** A preparation demonstrating a dominant VD electrotonic response to AB depolarization and hyperpolarization in control conditions. Dopamine reverses the sign of the VD response to AB depolarization and weakens the VD electronic response to AB hyperpolarization. **B** Input-output (I/O) curve from the experiment in **A** showing DA reversal of the net VD response during AB depolarizations and reduction of the net VD responses during AB hyperpolarizations. The AB peak voltage is plotted against the VD net response, calculated as the integrated voltage response during the AB depolarization in mV·s. **C** A preparation demonstrating a dominant VD chemical inhibitory response during AB depolarization and a VD electrotonic response during AB hyperpolarization in control conditions. Dopamine enhances the VD chemical inhibitory response and weakens the VD electronic response. **D** I/O curve from the experiment in **C** showing DA enhancement of the net VD inhibitory response during AB depolarizations and reduction of the net VD responses during AB hyperpolarization. The resting potentials of the AB and VD neurons were held at −55 mV. The time calibration bar applies to the traces in **A** and **C**

ponent, characteristic of graded synaptic potentials between pyloric neurons, had an initial peak hyperpolarizing response which decayed to a plateau during the presynaptic depolarization (Fig. 1C; Graubard et al. 1983). Thus, the integrated, net VD response depended upon the relative strengths of the electrical coupling and chemical inhibition, which varied between preparations. In all cases the electrical component could be monitored in isolation during AB hyperpolarization.

Dopamine changed the relative strengths of the electrical and chemical components of the AB→VD mixed synapse, leading to increased inhibition of the VD (n = 5). This resulted in an actual reversal in the sign of the net synaptic response during AB depolarization in 4 preparations where the control AB depolarization had evoked a net VD depolarizing response (Fig. 1A,B; Johnson et al. 1993b). In a fifth experiment, characteristic of half of the control AB→VD interactions we examined, the chemical inhibitory VD response was predominant during control AB depolarizations, and this chemical inhibition was enhanced by DA (Fig. 1C,D). Thus, in all 5 experiments, DA caused a net VD hyperpolarization during both AB hyperpolarization (due to the electrical synapse) and AB depolarization (due to enhanced chemical inhibition). The increased AB→VD inhibition is expressed in Fig. 2A as a statistically significant negative change in the net synaptic interaction.

We have verified our preliminary results (Johnson et al. 1993b) that DA acts by both reducing AB→VD electrical coupling and increasing the strength of AB→VD chemical inhibition. Dopamine's reduction of electrical

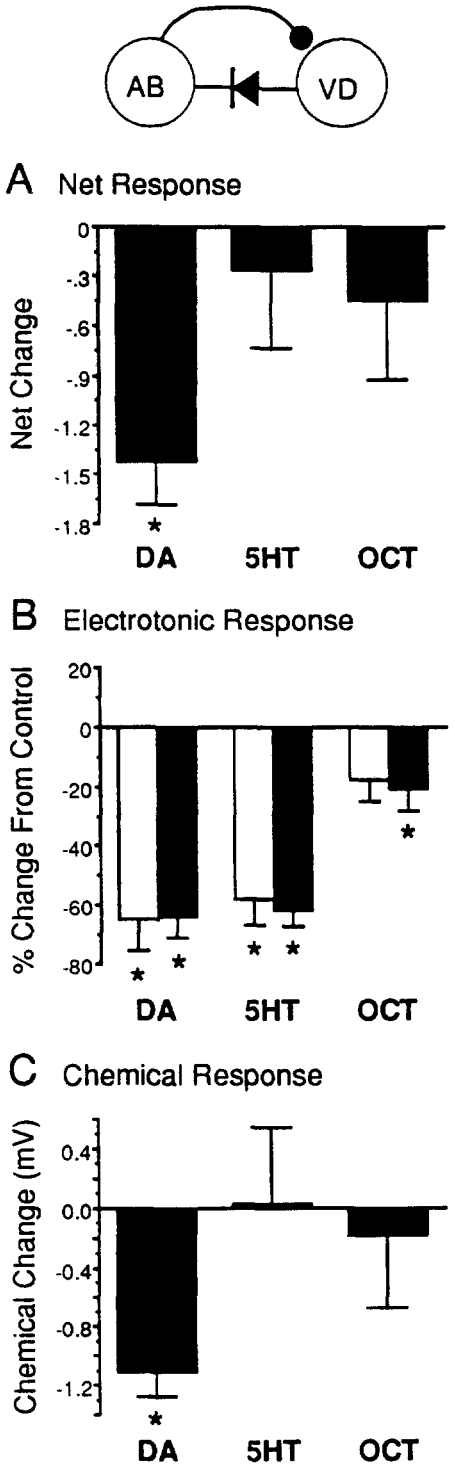

Fig. 2A–C Summary of amine effects on the AB→VD mixed synapse. **A** Mean changes from control values evoked by amines in the VD net response to AB depolarization. Net change units are mV·s. **B** Mean% change from the control electrical coupling coefficient evoked by amines in the stronger (hyperpolarizing) direction. The *open bars* indicate mean changes when the chemical component is blocked with PTX (Johnson et al. 1993a) and the *closed bars* indicate mean changes during AB hyperpolarization when the chemical component is intact. **C** Mean changes from control values evoked by amines in the chemical component of the mixed synapse. * indicates a statistically significant change from the mean control value

coupling was seen here in the reduced VD electrotonic response during AB hyperpolarization (compare VD responses to AB hyperpolarization in the traces of Fig. 1 A,C and in the I/O curves of Fig. 1B,D). An alternative method of analyzing the electrotonic component of the mixed AB→VD synapse is to apply PTX to eliminate the glutamatergic chemical synapse. We found that DA eliminated the weak VD depolarizing electrotonic response to AB depolarization and weakened its hyperpolarizing response when measured in this way (Johnson et al. 1993b). Dopamine's reduction of AB→VD electrical coupling was similar when measured by both methods and was statistically significant in both cases (Fig. 2B). Dopamine significantly increased the mean amplitude of the AB→VD peak chemical response (Fig. 2C). This increase of chemical synaptic strength was caused by a 14 mV hyperpolarizing shift in the threshold for detectable VD chemical inhibitory response to AB depolarizations (Johnson et al. 1993b).

Serotonin had no significant effect on the mean net AB→VD interaction from 10 different preparations (Fig. 2A). This amine did, however, cause a large and statistically significant reduction in AB→VD electrical coupling when calculated from the initial rapid peak response to AB voltage steps (Figs. 2B, 3). A quantitatively similar reduction in isolated electrotonic coupling was seen when the chemical synapse was eliminated with PTX (Fig. 2B; Johnson et al. 1993a). The lack of a statistically significant 5HT effect on the net interaction despite this reduction in electrical coupling is explained by an extreme variability of 5HT's effect on the delayed inhibitory chemical component of this mixed synapse. In some preparations, 5HT caused a reversible enhancement of chemical inhibition (Fig. 3A), but in other preparations, it caused a reversible reduction of chemical inhibition (Fig. 3B). The range of 5HT effects at different AB→VD synapses did not show a bimodal distribution, but instead a continuum from large decreases to large increases in chemical synaptic strength, which averaged to no significant mean effect (Fig. 2C). At synapses where 5HT enhanced chemical synaptic strength, a sign reversal of the net synaptic interaction occurred that resembled the DA-induced sign reversal (Fig. 3A).

Octopamine had no significant effect ($n = 5$) on the AB→VD net synaptic interaction (Fig. 2A). This amine did cause a statistically significant but small reduction in the mean electrical coupling that was quantitatively similar when measured either as the initial peak response in TTX-saline or when PTX was added to eliminate the chemical component of this synapse (Fig. 2B; Johnson et al. 1993a). Octopamine did not significantly alter the AB→VD chemical component (Fig. 2C); it did not cause the range of effects that 5HT showed at this synapse.

Amine effects on the LP→PY mixed synapse

The rectifying electrotonic junction between the LP and PY neurons allows preferential flow of positive current

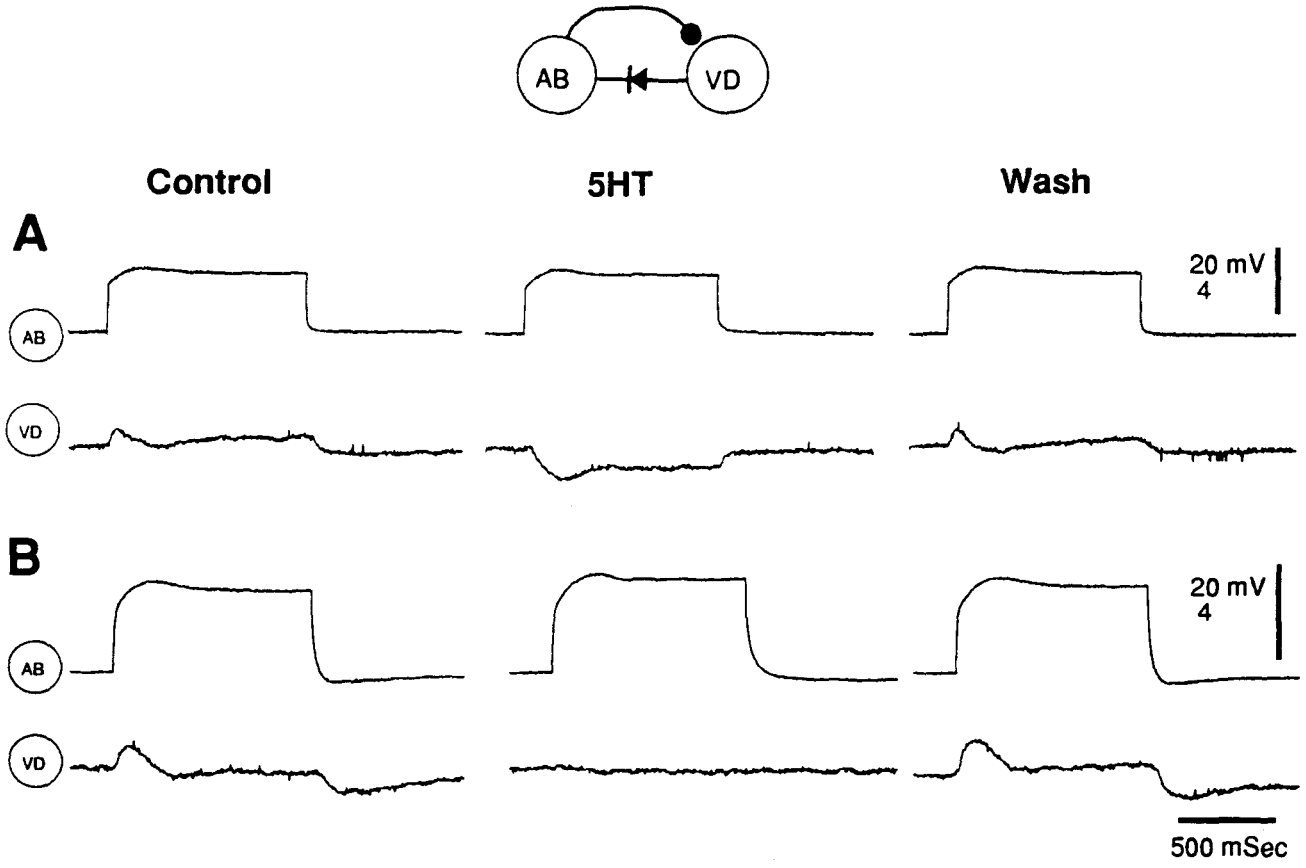

Fig. 3A,B Examples of the range of effects of 5HT (10^{-5} *M*) on the isolated AB→VD mixed synapse. **A** The first preparation shows a biphasic, net depolarizing VD response to control AB depolarization, the reversal of this net VD response by enhancement of chemical inhibition and reduction of electrical coupling during 5HT application, and recovery after a 30 min wash. **B** A different preparation showing a biphasic VD response to control AB depolarization, abolition of this response by reduction of both chemical inhibition and electrical coupling during 5HT application, and response recovery after a 30 min wash. The resting potentials of the AB and VD neurons were held at −55 mV

from LP→PY, or, alternatively, of negative current from PY→LP (Graubard and Hartline 1987; Johnson et al. 1993a) with a coupling coefficient of 0.06 in the preferred direction in the presence or absence of PTX. When the LP→PY pair was isolated from other pyloric and modulatory inputs, the chemical component of this mixed synapse was much weaker than the AB→VD interaction; consequently, biphasic potentials with clear electrical and chemical components were not usually seen. Depolarization of the LP evoked either a weak chemical inhibitory response (5 of 11 experiments, see Figs. 4A and 6B, Control), or a weak electrotonic PY depolarization with little or no chemical component (6 experiments, see Figs. 4C and 6A, Control). These different PY responses may reflect either variability in the relative strengths of the chemical and electrical components of the LP→PY synapse or the existence of a mixed population of PY subtypes (Hartline et al. 1987) with different strengths of the LP→PY synapse. However, our standard criteria of firing time in the normal pyloric rhythm and chemical and electrical synaptic connectivity were not useful for separating distinct PY subtypes in this or our previous studies (Johnson et al. 1993a). Weaker electrical coupling was observed during LP hyperpolarization, reflecting the rectifying nature of the electrotonic junction (see Fig. 4D, Control).

Similar to its effects on the AB→VD synapse, DA enhanced LP→PY inhibition (Fig. 4), which was seen as a statistically significant net inhibitory change in the mixed response (Fig. 5A). In 4 of 7 LP→PY experiments in which DA was applied, no electrical coupling was seen in the PY neuron in response to control LP depolarization: only small chemical inhibitory PY responses were detected (Fig. 4A, B, Control). Dopamine significantly enhanced these inhibitory potentials (Figs. 4, 5C). In 3 of the 4 DA-induced enhancements of the chemical component, there was no obvious change in the apparent voltage dependence of transmitter release (Fig. 4B).

In 3 of 7 DA experiments, only electrotonic coupling was evident in control conditions, with no detectable chemical component (Fig. 4C,D, Control). As we have previously reported (Johnson et al. 1993b), the DA enhancement of LP→PY inhibition in these experiments was so powerful that DA reversed the synaptic interaction from net depolarization to net hyperpolarization during LP depolarization (Fig. 4C,D). Thus, DA converted these synapses from domination by electrotonic coupling

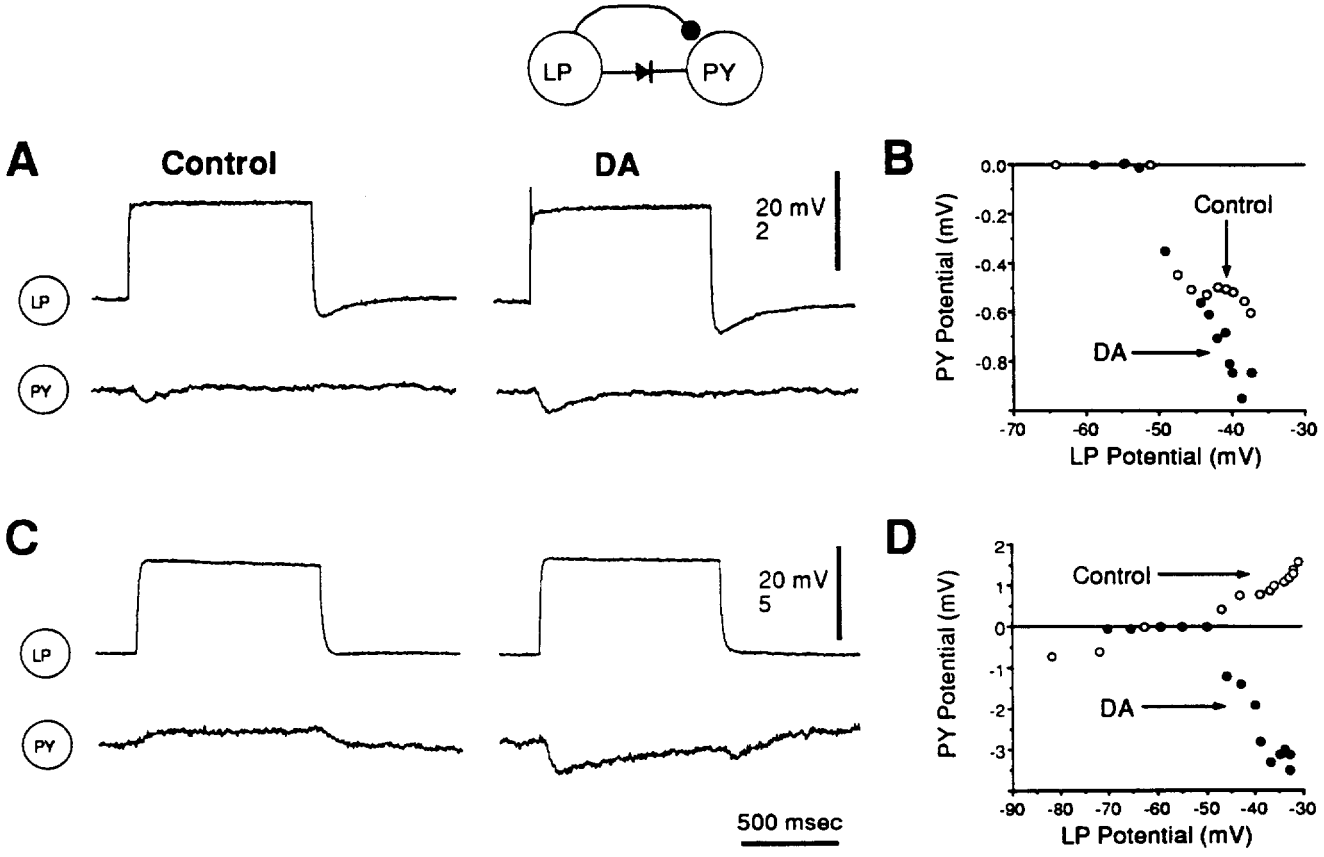

Fig. 4A–D Dopamine enhancement of inhibition at isolated LP→PY mixed synapses. The digitized traces show the LP response to 1 s depolarizing current injections and the resulting PY synaptic responses from two different preparations in control conditions (10^{-7} *M* TTX-saline) and during 10^{-4} *M* DA superfusion. **A** A preparation showing a PY chemical inhibitory response during control LP depolarization and the enhancement of this response by DA. **B** I/O curve from the experiment in **A** showing the PY chemical inhibitory response during LP depolarizations enhanced by DA. **C** A preparation showing a PY electrotonic response during LP control depolarization and the reversal of this PY response by DA. **D** I/O curve from the experiment in **C** showing the electrotonic PY responses during control LP polarizations converting to chemical inhibition of PY during DA application. The resting potentials of the AB and VD neurons were held at −55 mV. The time calibration bar applies to the traces in **A** and **C**

to domination by chemical inhibition. When the electrotonic component is isolated by blocking the chemical component with PTX, DA greatly reduces or eliminates LP→PY electrotonic coupling (Johnson et al. 1993a,b). Similar results are obtained when the chemical component of the synapse is intact and electrical coupling is measured during LP hyperpolarization (Figs. 4D, 5B). DA-induced enhancement of pre-existing chemical inhibition (Fig. 4A,B), the activation of "silent" chemical inhibition (Fig. 4C,D) and the reduction of electrical coupling (Fig. 4C,D) all suggest that DA-induced reduction of electrical coupling and enhancement of chemical inhibition act together to increase LP→PY inhibition.

Serotonin caused a very small but statistically significant change in the net LP→PY mixed interaction which slightly increased PY inhibition during LP depolarization ($n = 9$; Fig. 5A). In 6 of 9 5HT experiments, only PY electrotonic depolarization was seen during LP depolarization in control conditions. Serotonin caused a weak reduction in this electrical coupling whether or not the chemical synapse was blocked with PTX (Fig. 5B). In 3 5HT experiments, only PY chemical inhibition was observed during control LP depolarizations; 5HT had no significant effect on the amplitude of these chemical inhibitory potentials (Fig. 5C). Thus, the slight enhancement of LP→PY inhibition appears to result mainly from 5HT's weak reduction of electrical coupling, with no effect on chemical transmission.

The effects of Oct on the LP→PY synaptic interaction were also examined ($n = 11$). In 7 Oct experiments, control LP depolarizations evoked only PY electrotonic depolarization. In 5 of these experiments, the rise to the peak PY electrotonic response was retarded during Oct superfusion compared to the control condition, with little or no change in the ultimate peak electrotonic response itself (Fig. 6A). This suggested that Oct enhanced the chemical inhibitory component of the mixed synapse, which rises to a peak and then decays during the LP voltage step. However, this chemical inhibition was not large enough to outweigh the opposing electrical coupling in these experiments. Indeed, in 4 experiments where PY chemical inhibition predominated during control LP depolarization, Oct increased the amplitude of the chemical

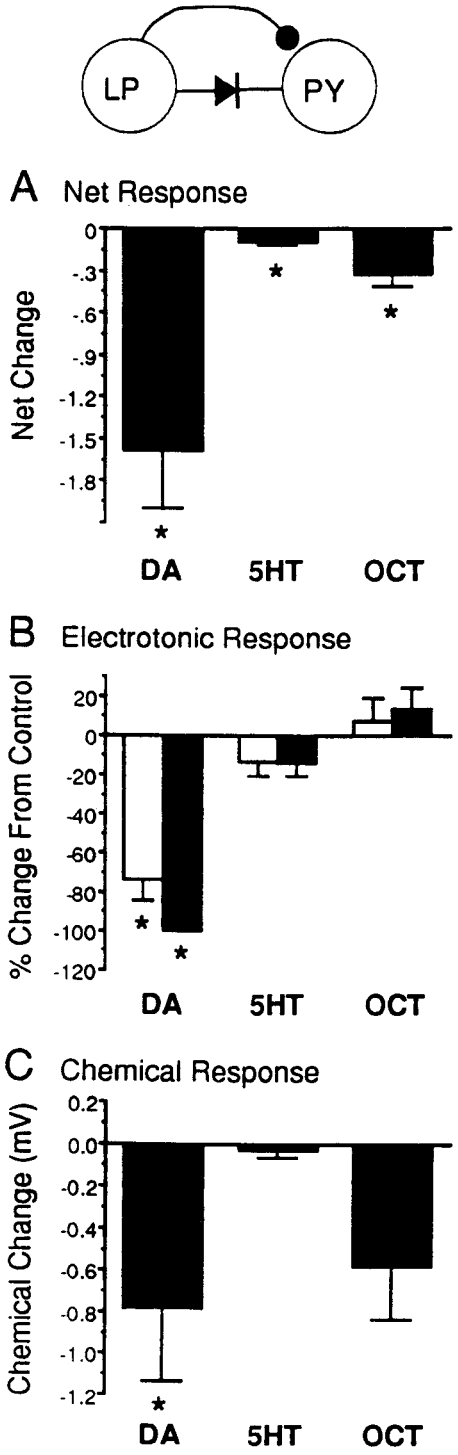

Fig. 5A–C Summary of amine effects on the LP→PY mixed synapse. **A** Mean changes from control values evoked by amines in the PY net response to LP depolarization. Means are from all LP→PY experiments. Net change units are mV·s. **B** Mean% change from the control electrical coupling coefficient evoked by amines in the preferred direction (i.e. during LP depolarization). The *open bars* indicate mean changes when the chemical component is blocked with PTX (Johnson et al. 1993a) and the *closed bars* indicate mean changes when the chemical component is intact. Means are only from experiments which showed PY electrotonic depolarization in response to LP depolarization. **C** Mean changes from control values evoked by amines in the chemical component of the mixed synapse. Means are only from experiments which showed PY inhibitory responses to control LP depolarization. * indicates a statistically significant change from the mean control value

inhibitory potential (Fig. 6B). Although consistent in all 4 experiments, this effect was not large enough with this small sample size to be statistically significant (Fig. 5C, $P = 0.10$). Three of the 4 Oct-induced increases in the LP→PY chemical inhibition showed hyperpolarizing shifts (from 8–16 mV) in the apparent voltage dependence of transmitter release. Again, some of the variability in these results may be due to the existence of different subtypes of PY neurons. Octopamine did not affect the mean amplitude of the PY electrotonic response, measured either in the presence or absence of PTX to block the chemical component (Fig. 5B). One experiment, shown in the I/O curve example of Fig. 6C, indicated that Oct, like DA, could reverse the sign of the LP-PY synaptic interaction. In this experiment, the Oct enhancement of chemical inhibition changed the synaptic interaction from domination by electrical coupling to domination by chemical inhibition without any effect on the electrical coupling (see responses during LP hyperpolarization). These results suggest that Oct evokes a statistically significant change in the net LP→PY interaction (Fig. 5A), specifically by enhancing the strength of chemical transmission, with no effect on electrical coupling.

Amine effects on the PY→LP mixed synapse

The PY→LP mixed synapse shows rectifying electrical coupling, with preferential passage of hyperpolarizing current from PY→LP (Graubard and Hartline 1987; Johnson et al. 1993a); in addition, some PY cells inhibit LP via a glutamatergic chemical synapse (Marder 1987). PY depolarization evoked no response in 5 LP neurons (example in Fig. 7A,B, control), caused small LP inhibitory responses in 5 other LP neurons (example in Fig. 7C,D, control), and elicited a small electrotonic depolarization in 2 LP neurons. Again, this variability may reflect heterogeneity in the PY cell subtypes.

We have previously provided preliminary evidence that DA can enhance PY→LP inhibition by both strengthening chemical inhibitory transmission and weakening electrical coupling (Johnson et al. 1993b). In 5 of 8 DA experiments reported here, no chemical component of the PY→LP synapse was detectable under control conditions. Dopamine activated these "silent" chemical synapses so that PY depolarization now evoked an LP hyperpolarizing response (Fig. 7A, I/O curve example in Fig. 7B). In 3 of the DA experiments, weak chemical inhibitory transmission was detectable in control conditions and DA enhanced the amplitudes of these chemical potentials (Fig. 7C, I/O curve example in Fig. 7D). In each of these experiments, there was a hyperpolarizing shift in the LP chemical inhibitory response to

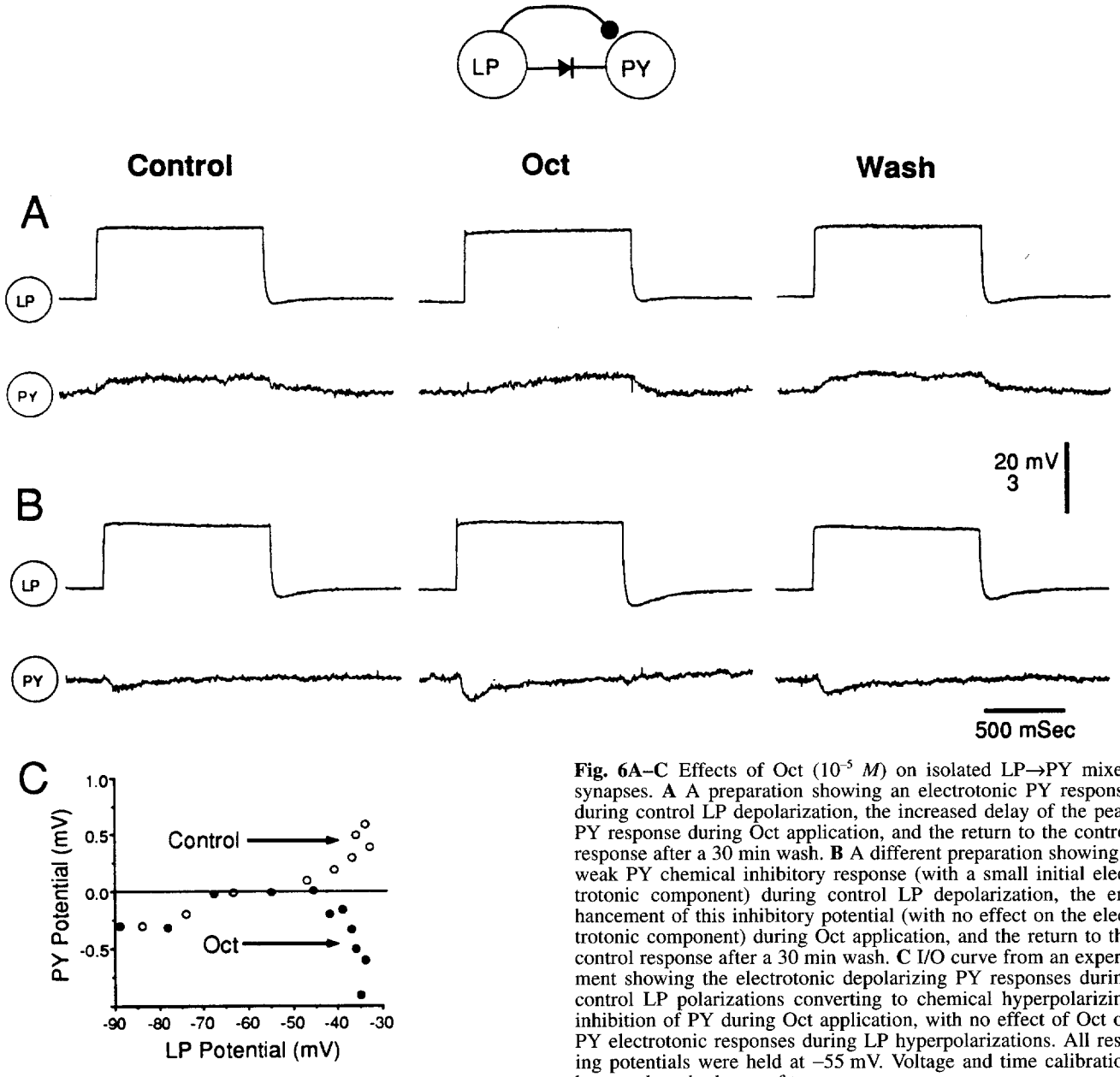

Fig. 6A–C Effects of Oct (10^{-5} *M*) on isolated LP→PY mixed synapses. **A** A preparation showing an electrotonic PY response during control LP depolarization, the increased delay of the peak PY response during Oct application, and the return to the control response after a 30 min wash. **B** A different preparation showing a weak PY chemical inhibitory response (with a small initial electrotonic component) during control LP depolarization, the enhancement of this inhibitory potential (with no effect on the electrotonic component) during Oct application, and the return to the control response after a 30 min wash. **C** I/O curve from an experiment showing the electrotonic depolarizing PY responses during control LP polarizations converting to chemical hyperpolarizing inhibition of PY during Oct application, with no effect of Oct on PY electrotonic responses during LP hyperpolarizations. All resting potentials were held at –55 mV. Voltage and time calibration bars apply to both sets of traces

PY depolarization (mean, 9 ± 5 mV). However, with this small sample size, this change was not statistically significant.

In previous experiments where PTX was added to abolish the chemical component of the PY→LP interaction, we reported that DA enhanced electrical coupling (Johnson et al. 1993a, see Fig. 8B). However, the opposite occurred in the present experiments, when the chemical synapses were intact: DA reduced the functional electrical coupling during PY hyperpolarizations, as seen in the example of Fig. 7E. We are not sure why this occurred, but it may be related to the PTX-dependent effects of DA on the LP input resistance. Dopamine caused a 10 ± 5% increase in LP input resistance when PTX was present (data from Johnson et al. 1993a) but an 11 ± 10% decrease in input resistance when PTX was absent (mean from the experiments reported here). These different effects of DA on PTX- and non-PTX-treated LP input resistance are nearly significant ($P = 0.06$), and suggest trends that may explain DA's opposite effects on PY→LP electrical coupling (see Discussion). Dopamine's enhancement of PY→LP chemical inhibition and reduction of PY→LP electrical coupling were both statistically significant (Fig. 8B,C) and caused a significant increase of the net PY→LP inhibition (Fig. 8A). Thus, DA acts to enhance chemical synaptic strength and reduce functional electrical coupling at all the pyloric mixed synapses.

Serotonin had no effect on those synapses which did not show LP chemical inhibition during control PY de-

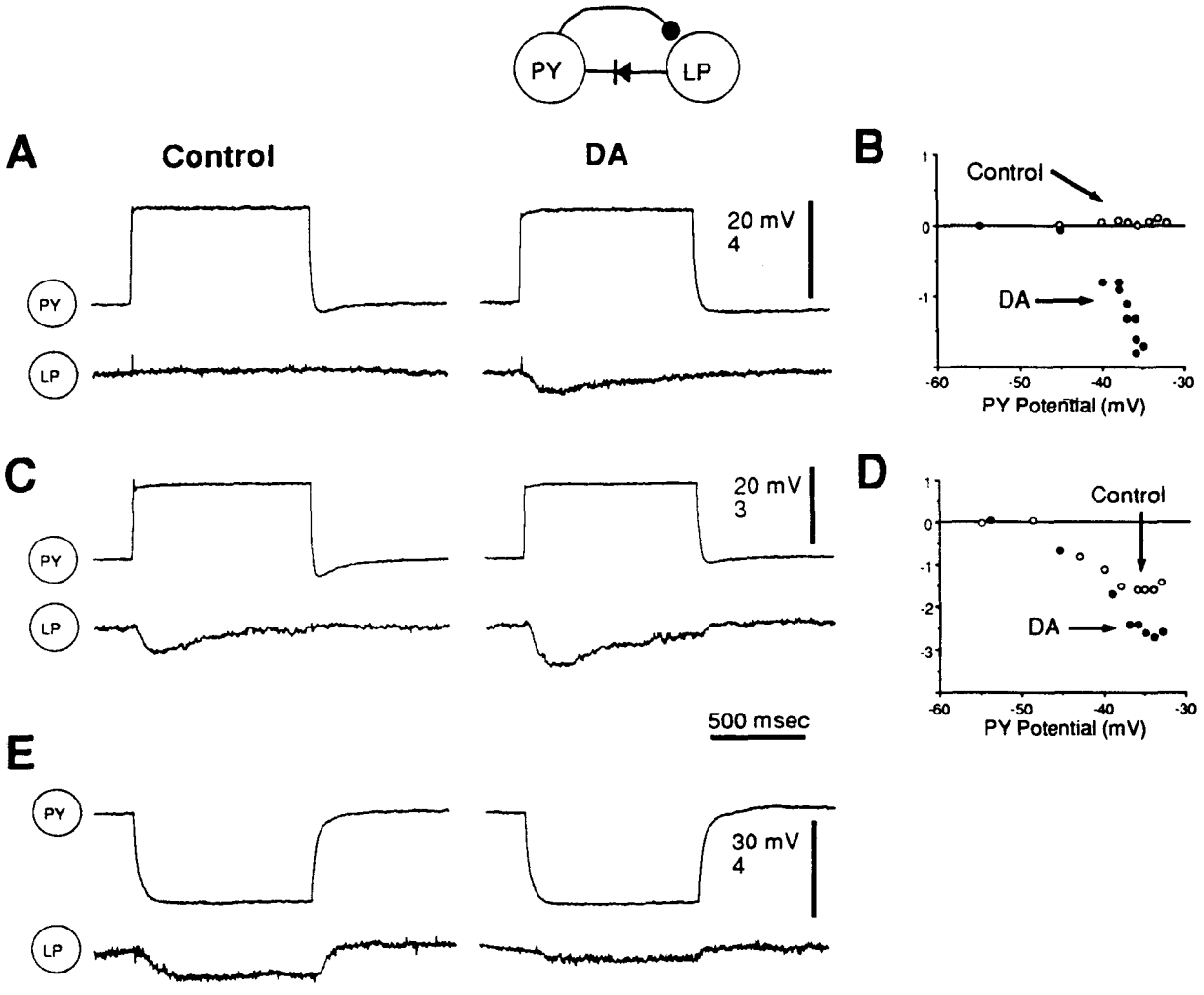

Fig. 7A–E Dopamine activation of a silent chemical synapse, enhancement of chemical inhibition and reduction of electrical coupling at isolated PY→LP mixed synapses. **A** This preparation showed no LP responses during control PY depolarization, changing to LP inhibition during DA application. **B** I/O curve from the experiment in **A**, showing the creation of a functional chemical inhibitory synapse by DA. **C** This preparation showed an LP chemical inhibitory response during PY depolarization that was enhanced by DA. **D** I/O curve from the experiment in **C**, showing the enhancement of LP chemical inhibitory responses during PY depolarizations. **E** Traces showing the electrotonic LP response during PY hyperpolarization and its reduction by DA. All resting potentials were held at −55 mV. Time calibration bar applies to **A**, **C** and **E**

polarizations. Thus, its effects were quantitatively examined in 5 preparations which showed at least small LP inhibitory responses during PY depolarization. Serotonin caused a small, but not significant, decrease in the mean net inhibitory PY→LP interaction (net positive change, Fig. 8A). There was no significant effect of 5HT on PY→LP electrotonic coupling in these or in earlier experiments where PTX blocked the chemical component (Fig. 8B). In all five experiments, 5HT did, however, cause a consistent reduction in PY→LP chemical inhibition (positive chemical change, Fig. 8C, see example in Fig. 9A). This effect approached statistical significance with this small sample size ($P = 0.06$), and probably contributed to the small decrease in PY→LP net inhibition. In 3 of these experiments, 5HT completely abolished chemical inhibitory transmission (Fig. 9A); in the other two experiments, the reduction of chemical inhibition was accompanied by a 4 ± 2 mV depolarizing shift in the threshold LP response to PY depolarization. The I/O curve example of Fig. 9B typifies 5HT's reduction of chemical synaptic strength while not changing electrical coupling.

Like 5HT, Oct had no apparent effect when the LP did not respond to PY depolarization. The effects of Oct on the PY→LP mixed synapse were thus quantitatively examined in 8 preparations which showed at least small LP inhibitory responses during control PY depolarizations and in one preparation which showed a small LP depolarizing electrotonic response to PY depolarization. Octopamine caused a small, but statistically significant, enhancement of the mean net inhibitory PY→LP interaction (Fig. 8A) without any significant effect on the peak electrotonic response (Fig. 8B). This amine weakly but consistently enhanced LP chemical inhibitory potentials in all 8 experiments (see example in Fig. 9C), though

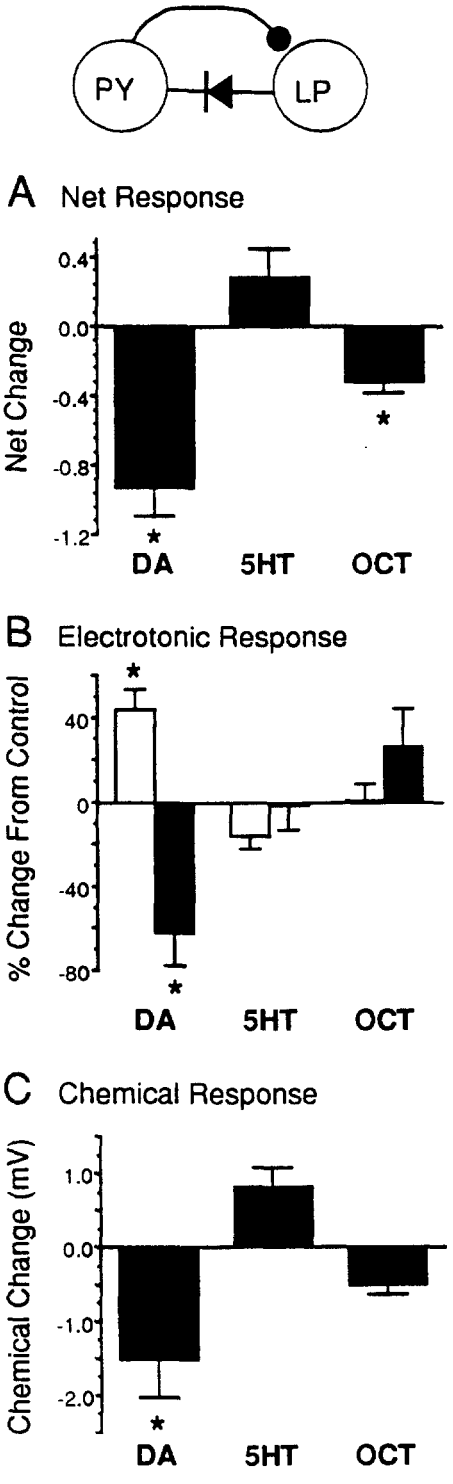

Fig. 8A–C Summary of amine effects on the PY→LP mixed synapse. **A** Mean changes from control values evoked by amines in the LP net response to PY depolarization. Net change units are mV·s. **B** Mean% change from the control coupling coefficient evoked by amines in the preferred direction (i.e. during PY hyperpolarization). The *open bars* indicate mean changes when the chemical component is blocked with PTX (Johnson et al. 1993a) and the *closed bars* indicate mean changes when the chemical component is intact. **C** Mean changes from control values evoked by amines in the chemical component of the mixed synapse. * indicates a statistically significant change from the mean control value

again not quite enough to be statistically significant with this small sample size ($P = 0.06$; Fig. 8C). The Oct enhancement of PY→LP chemical inhibition was not accompanied by a shift in the apparent voltage dependence of transmitter release. In one experiment, Oct reversed the sign of the net PY→LP interaction by inducing a small LP chemical inhibitory potential when only a small LP electrotonic potential was seen during control PY depolarizations. The I/O curve example of Fig. 9D shows the enhancement of chemical inhibition by Oct, again without any change in the electrical coupling. This suggests that the Oct enhancement of chemical inhibition alone caused the enhancement of the net PY→LP inhibition.

Discussion

Mixed chemical inhibitory and electrical synapses have been physiologically demonstrated in other invertebrate preparations besides the lobster pyloric network, including the retinular network of *Limulus* (Dennis 1967), interneurons from the buccal (Gardner 1977) and abdominal (Hawkins et al. 1981) ganglia of *Aplysia*, the leech swim circuit (Friesen 1985), the lobster gastric network (Selverston 1987) and stomatogastric networks from other crustaceans (Hermann 1979; Meyrand and Moulins 1988; Tazaki 1993). Mixed chemical inhibitory and electrical transmission between neurons in these preparations allows for great synaptic plasticity if the strengths of the opposing synaptic components can be differentially modulated. To determine the range of possible plasticity at mixed synapses, we examined the effects of DA, 5HT and Oct on the mixed synapses of the pyloric network. The net communication at these synapses was modulated by amines to produce not only quantitative changes in net synaptic strength, but also qualitative changes, such as net sign reversal and the activation of silent chemical synapses. All 3 amines could reverse the net sign of at least some of the individual synapses examined. Dopamine enhanced the net synaptic inhibition at all the pyloric mixed synapses; 5HT weakly enhanced LP→PY inhibition; Oct had no effect on the net AB→VD interaction but enhanced LP→PY and PY→LP net inhibition. Thus, when these amines had a statistically significant effect on the mixed synapses between pyloric neurons, they shifted the balance of the electrical and chemical inhibitory components towards greater net inhibition.

Differential modulation of mixed chemical and electrical components

The amine-induced increase in net inhibition at the pyloric mixed synapses could occur by reducing the electrical component and/or enhancing the chemical inhibitory component. Dopamine, 5HT and Oct each enhance inhibition by different means (summarized in Table 1). Dop-

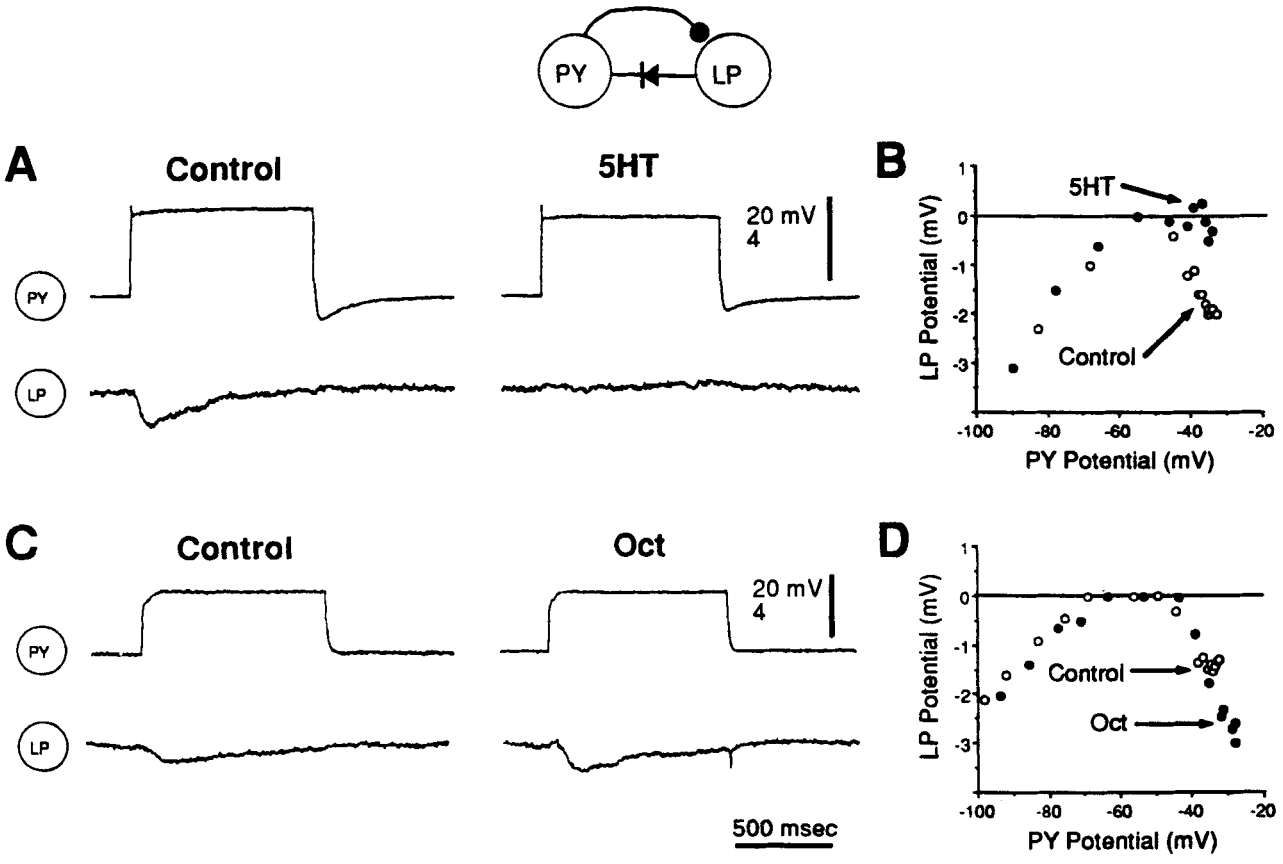

Fig. 9A–D Effects of 5HT and Oct on isolated PY→LP mixed synapses. **A** A preparation showing a LP inhibitory chemical response during PY depolarization abolished by 5HT. **B** I/O curve from the experiment in **A**, showing 5HT reduction of LP chemical inhibitory responses during PY depolarizations, with no effect on LP electrotonic responses to PY hyperpolarizations. **C** A different preparation showing a weak LP chemical inhibitory response during PY depolarization enhanced by Oct. **D** I/O curve from the experiment in **C**, showing Oct enhancement of LP chemical inhibitory responses during PY depolarizations, with no effect on LP electrotonic responses during PY hyperpolarizations. All resting potentials were held at −55 mV. Time calibration bar applies to **A** and **C**

Table 1 Summary of amine effects on the electrical (*E*) and chemical (*C*) components of the pyloric mixed synapses

Synapse	DA		5HT		OCT	
	E	C	E	C	E	C
AB→VD	↓	↑	↓	O	↓	O
LP→PY	↓	↑	O↓	O	O	O↑
PY→LP	↓	↑	O	O↓	O	O↑

↓ Reduction of synaptic strength
↑ Enhancement of synaptic strength
O No change in synaptic strength
O↓ No statistically significant change, but a strong trend to reduction of synaptic strength (see text)
O↑ No statistically significant change, but a strong trend to enhancement of synaptic strength (see text)

amine's enhancement of net inhibition arises from its ability to both reduce the electrical components and enhance the chemical inhibitory components of the AB→VD, LP→PY and PY→LP pyloric mixed synapses (Johnson et al. 1993b, Table 1). In preparations where the electrotonic coupling dominated the control net interaction at AB→VD and LP→PY synapses, DA reversed the sign of the net interaction. In preparations where no LP chemical response was seen during PY depolarization in control conditions, DA activated the silent chemical synapse. Dopamine can also reverse the sign of the net interaction at other pyloric mixed synapses by weakening graded chemical synapses. For example, the PD neuron sometimes shows a mixed synapse with the IC neuron (Johnson and Harris-Warrick 1990). Dopamine eliminates the chemical component of this PD→IC mixed synapse, so a hyperpolarizing IC response during control PD depolarization is converted to a weak depolarizing response. Dopamine also modulates a vertebrate mixed synapse through its effects on both the individual synaptic components (Pereda et al. 1992): it increases the gain of mixed excitatory transmission from the VIII nerve afferents to the Mauthner neuron by enhancing both the electrical and chemical excitatory components. At pure electrotonic or chemical synapses from different preparations, DA can both enhance and reduce synaptic strength. For example, DA blocks electrical coupling between snail neurons (Berry and Cottrell 1979) and between neurons in the vertebrate retina (Dowling 1991;

Hampson et al. 1992), and enhances chemical synaptic strength at a variety of invertebrate synapses (Watson and Groome 1989; Miller et al. 1985; Lingle 1981; Govind and Lingle 1987; Casagrand and Ritzmann 1992). However, DA also eliminates the graded synaptic transmission from the pyloric PD neuron onto its follower cells (Johnson and Harris-Warrick 1990).

Dopamine's reduction of the electrical component at the PY→LP mixed synapse, contrasts with our previous report that DA enhances PY→LP electrical coupling when PTX is used to eliminate the chemical component (Johnson et al. 1993a). To understand this apparent contradiction, it is important to remember that there are actually 8 PY neurons in our preparation, only one of which is being held at −55 mV. Some of the remaining PY neurons will depolarize above threshold for graded transmitter release in the presence of DA (Flamm and Harris-Warrick 1986b; Johnson et al. 1991). Thus, with chemical synapses intact, the LP neuron is receiving tonic inhibition from these uncontrolled PY neurons. When an uncontrolled PY neuron is hyperpolarized in the presence of DA, the LP neuron depolarizes due to relief from this tonic inhibition (Johnson, Peck and Harris-Warrick, unpublished observations). The PY inhibitory transmitter, glutamate, opens ion channels and reduces the input resistance of the LP cell. This reduction in input resistance in turn causes the apparent reduction in electrical coupling at the mixed PY→LP synapse. When chemical transmission is blocked with PTX, DA instead evokes an increase in LP input resistance and PY→LP electrical coupling is correspondingly enhanced (Johnson et al. 1993a). We believe that this is the direct effect of DA on PY→LP electrical coupling. However, the reduction in net coupling observed when the chemical inhibition is intact is likely to be more physiologically relevant in understanding how the pyloric network is modified by DA.

Serotonin had only weak effects at the pyloric mixed synapses. The significant but weak reduction that 5HT caused in the LP→PY net synaptic interaction appears to be due to 5HT's reduction of LP→PY electrical coupling; this amine had no consistent effect on the chemical inhibition (Table 1). Serotonin did significantly reduce the electrical component of the AB→VD mixed synapse, at least in part through reducing VD input resistance (Johnson et al. 1993a). Serotonin also reduces electrical coupling between leech Retzius neurons (Colombaioni and Brunelli 1988) and between identified peptidergic neurons in the snail *Lymnaea* (Wildering and Janse 1992). Despite the significant reduction in AB→VD electrical coupling, 5HT had no mean effect on the mixed net AB→VD synaptic interaction. There was great variability in 5HT's effect on the chemical component of the AB→VD interaction, ranging from large decreases to large increases in AB→VD chemical synaptic strength, which resulted in the non-significant mean effect on the AB→VD net interaction. We do not understand the source of this variability, but we have previously noted that 5HT can have quite variable effects on induction of rhythmic membrane potential oscillations in synaptically isolated, TTX-treated AB neurons (Johnson et al. 1992). It appears that the cellular mechanisms mediating 5HT reduction in AB→VD electrical coupling are more consistently effective than those responsible for enhancing AB→VD chemical synaptic strength or inducing AB rhythmic membrane potential oscillations.

Octopamine consistently enhanced the reciprocal net inhibition between the LP and PY neurons. This appears to be due to an Oct enhancement of chemical inhibition alone; this amine had no effect on the electrical component of these reciprocal mixed synapses (Table 1). This enhancement of inhibition, however, was weak compared to that caused by DA. We observed Oct's effects on the LP→PY chemical synapse mainly as a delay in the rapid rise of the electrical synaptic component, but electrical coupling continued to dominate most of the LP→PY mixed interactions. Oct did cause a weak, but consistent, increase in the amplitudes of those LP→PY and PY→LP chemical inhibitory potentials that were detectable under control conditions. Since Oct had no significant effect on the electrical component of these mixed synapses, we suggest it acts by specifically increasing the strength of the chemical component. Octopamine also increases the strength of graded chemical transmission from the pyloric PD neurons onto their follower cells (Johnson and Harris-Warrick 1990) and of action potential-evoked chemical transmission at crustacean (Breen and Atwood 1983; Fischer and Florey 1983; Harris-Warrick and Kravitz, 1984; Govind and Lingle 1987; Bustamante and Krasne 1991) and other arthropod synapses (Watson and Groome 1989; Calabrese 1989; Casagrand and Ritzmann 1992). Our experiments were not designed to study the detailed mechanisms of amine action on the graded chemical and electrical components of mixed transmission. These could be modulated by direct effects on synaptic transmission or by more general effects on cellular input resistance (Johnson and Harris-Warrick 1990; Johnson et al. 1993a).

Functional importance of mixed synapse modulation

Dopamine, 5HT and Oct each generate a distinct motor pattern when bath applied to a quiescent pyloric network (Flamm and Harris-Warrick 1986a). Dopamine, for example, induces a motor pattern characterized by rhythmic activity in the AB, LP, PY and IC neurons; the PD and VD neurons are inactive. We do not yet have enough information on how amines affect all the neuronal "building blocks" that produce a network's output (Getting 1988) to fully explain the activity of all the pyloric neurons during amine-induced activity. It is clear already, however, that amine effects on different cellular properties summate to influence the activity of network neurons. For example, the silence of the VD neuron during DA application can be explained by DA's actions to hyperpolarize the VD both directly (Flamm and Harris-Warrick 1986b), and by increased AB→VD chemical inhibition. Also, the DA depolarization of the LP and PY

neurons (Flamm and Harris-Warrick 1986b), would increase the driving force for graded chemical inhibition (Graubard et al. 1983; Johnson and Harris-Warrick 1990) and further increase the DA-induced enhancement of chemical synaptic strength that we measured when the resting potentials are held constant at −55 mV. The relative degree of enhancement of the chemical component of the mixed synapse by DA should thus vary with the rhythmic voltage oscillations of the post-synaptic neuron during pyloric rhythmic activity.

If shifts in the relative strengths of the electrical and chemical components of mixed synapses occur during patterned pyloric motor activity, they would be expected to alter the firing phases of the synaptically interacting neurons. Theoretical studies suggest that these synaptic strength changes can have profound effects on patterned activity. For example, Mulloney et al. (1981) modeled a pyloric-like network with pairs of reciprocally inhibitory neurons connected by mixed non-rectifying electrical and chemical inhibitory synapses. As the chemical component of the mixed synapse was strengthened, the firing times of the neuron pairs progressed towards stable, alternating bursts. When the electrical component was strengthened, the pairs progressed towards synchronous firing. Another, more recent theoretical study used mixed artificial electrical coupling and an artificial chemical synapse between two pyloric neurons to show that gradual increases in the strength of the electrical component cause the post-synaptic neuron to progressively synchronize its firing with the pre-synaptic neuron (Sharp et al. 1992). There is also more direct experimental evidence that changing the relative strengths of the mixed synaptic components can alter the pyloric motor pattern. When PTX is used to weaken the AB→VD chemical synapse during pyloric motor cycling, the VD shifts to fire synchronously with the AB (Bidaut 1980). In addition, firing of the anterior pyloric modulator neuron changes the VD firing phase from alternating with the AB/PD pacemaker group to firing synchronously with it, apparently by weakening AB→VD chemical inhibition (Nagy and Dickinson 1983).

In highly distributed neural networks, small changes in the efficacy of synaptic transmission spread across many synapses can influence the network output as much as large changes at only a few synapses. For example, Lockery and Sejnowski (1993) recently modeled nonassociative learning in the distributed network that controls the local bending reflex in the leech. They demonstrated that small changes (3–5%) in the amplitude of graded synaptic potentials, when spread across all the network synapses, could account for a 50% change in motor output required for the conditioned behavior. This leads us to propose that the relatively small 5HT and Oct effects on the mixed synaptic interactions, relative to DA, might still be important for the 5HT and Oct-induced pyloric motor patterns. These small changes could work in concert with the 5HT and Oct effects (both large and small) on graded synaptic strength at other pyloric synapses (Johnson and Harris-Warrick, 1990; Johnson et al. 1993a; Johnson, Peck and Harris-Warrick, unpublished observations) and on the intrinsic firing properties of the component neurons (Flamm and Harris-Warrick 1986b) to create the distinct pyloric motor patterns produced by each amine (Flamm and Harris-Warrick 1986a).

Functional expression of anatomical synapses

All the synaptic interactions between neurons of the pyloric network have been described in previous studies (reviewed by Mulloney 1987). We were thus surprised to find the chemical components of the synaptically isolated mixed synapses to be sometimes weak and even nonexistent. Application of DA, and sometimes Oct, was required to reactivate this silent chemical component. One major difference between our and earlier studies is that we examined isolated pairs of neurons bathed in TTX-saline. This removed the *in vivo* modulatory input that normally maintains the functional integrity of the pyloric chemical synapses. In the intact spiny lobster, the STG receives modulatory input from neurons in other ganglia via approximately 120 axons in the stomatogastric nerve (Harris-Warrick et al. 1992b). There are many different modulatory compounds present in these inputs, including DA and Oct, that act to modulate synapses (reviewed in Harris-Warrick et al. 1992a; Marder and Weimann 1992). Other neuromodulators, including 5HT, affect the STG as circulating hormones (Beltz et al. 1984). It is likely that these modulatory inputs are active in varying mixtures to enhance, diminish or even eliminate the pyloric chemical synapses (Dickinson et al. 1990; Johnson and Harris-Warrick 1990). Thus, not only the strength but the very existence of synaptic interactions within the pyloric network may depend on the appropriate modulatory milieu.

The modulatory environment may also be important to achieve the full expression of the chemical component at other mixed synapses. There is clear morphological evidence for chemical transmission at mixed synapses where only the electrotonic component appears functional (Bennett 1977; Oesterle and Barth 1981; Bosch 1990; Lee and Krasne 1993). For example, morphological studies show both chemical and electrical anatomical specializations for dual transmission at one of the best known vertebrate mixed synapses, from the VIIIth nerve afferent to the Mauthner neuron in fish (Korn et al. 1990). Physiological transmission at this morphologically mixed synapse, however, is mostly electrical; silent chemical synapses can be activated using 4-aminopyridine (Lin and Faber 1988a,b). As mentioned above, DA may be one of the modulatory inputs that restores function to the chemical component of this mixed synapse (Pereda et al. 1992). Vertebrate spinal cord preparations, both in vivo and in culture, also contain anatomically defined chemical synapses that do not appear functional (Jack et al. 1981; Redman and Walmsley 1983; Henneman et al. 1984; Pun et al. 1986). Thus, the silence of an-

atomically defined chemical synapses in vertebrate preparations may be an artifact of removing modulatory inputs that are active in vivo.

The flexible and variable motor systems of both vertebrates and invertebrates are governed by highly adaptable neural networks (Pearson 1993). The pyloric motor network, for example, changes its output depending on the animal's satiation state (Turrigiano and Heinzel 1992). In addition to functioning as a flexible but discrete motor network, the pyloric neurons can be induced to join other ongoing stomatogastric networks and even participate in the creation of novel networks (Dickinson and Moulins 1992). The kinds of synaptic plasticity, especially synaptic sign reversal, that the amines create at mixed synapses could obviously contribute to the many different outputs produced by anatomically defined networks (Selverston 1988; Harris-Warrick and Marder 1991).

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