Neuromodulator-evoked synaptic metaplasticity within a central pattern generator network

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Kvarta MD, Harris-Warrick RM, Johnson BR. Neuromodulator-evoked synaptic metaplasticity within a central pattern generator network. J Neurophysiol 108: 2846–2856, 2012. First published August 29, 2012; doi:10.1152/jn.00586.2012.—Synapses show short-term activity-dependent dynamics that alter the strength of neuronal interactions. This synaptic plasticity can be tuned by neuromodulation as a form of metaplasticity. We examined neuromodulator-induced metaplasticity at a graded chemical synapse in a model central pattern generator (CPG), the pyloric network of the spiny lobster stomatogastric ganglion. Dopamine, serotonin, and octopamine each produce a unique motor pattern from the pyloric network, partially through their modulation of synaptic strength in the network. We characterized synaptic depression and its amine modulation at the graded synapse from the pyloric dilator neuron to the lateral pyloric neuron (PD,LP synapse), driving the PD neuron with both long square pulses and trains of realistic waveforms over a range of presynaptic voltages. We found that the three amines can differentially affect the amplitude of graded synaptic transmission independently of the synaptic dynamics. Low concentrations of dopamine had weak and variable effects on the strength of the graded inhibitory postsynaptic potentials (gIPSPs) but reliably accelerated the onset of synaptic depression and recovery from depression independently of gIPSP amplitude. Octopamine enhanced gIPSP amplitude but decreased the amount of synaptic depression; it slowed the onset of depression and accelerated its recovery during square pulse stimulation. Serotonin reduced gIPSP amplitude but increased the amount of synaptic depression and accelerated the onset of depression. These results suggest that amine-induced metaplasticity at graded chemical synapses can alter the parameters of synaptic dynamics in multiple and independent ways.

graded synaptic transmission; synaptic depression; lobster pyloric neurons; dopamine; serotonin; octopamine

Chemical synapses using graded transmitter release, where release is a continuous function of presynaptic membrane potential, also show short-term dynamics (Goutman and Glowatzki 2007; Jackman et al. 2009). The roles of graded synaptic dynamics have been analyzed in the pyloric network of the lobster stomatogastric ganglion (STG). In this 14-neuron central pattern generator (CPG) network, graded chemical synapses help determine the phasing of pyloric neurons to produce coordinated motor patterns (Hartline et al. 1988). The synaptic strength in this network dynamically tracks activity levels and thus alters network connectivity as a function of cycle frequency (Mouser et al. 2008; Nadim and Manor 2000). Synaptic depression during pyloric network activity is thought to control the transition to different oscillatory modes of the network (Nadim et al. 1999) and to promote phase maintenance over different cycle frequencies (Manor et al. 2003; Nadim et al. 2003).

Short-term synaptic plasticity can be regulated through neuromodulation. This is a form of metaplasticity (Philpot et al. 1999), where neuromodulators alter the characteristics of activity-dependent changes in synaptic strength (Fischer et al. 1997; Kreitzer and Regehr 2000; Parker 2001; Parker and Grillner 1999; Qian and Delaney 1997). In the pyloric network, we have previously demonstrated that monoamines such as dopamine and octopamine can alter the steady-state synaptic depression at pyloric synapses (Johnson et al. 2005, 2011). In addition, the peptide proctolin can change the sign of synaptic plasticity from depression to facilitation at a pyloric graded synapse to help stabilize the cycle period (Zhao et al. 2011).

In this article we examine amine modulation of synaptic dynamics from the pyloric dilator (PD) neuron to the lateral pyloric (LP) neuron, the PD—LP synapse. This synapse provides an important output from the pacemaker kernel, which sets the cycle frequency of its follower cells and thus helps regulate network activity. The PD—LP graded synapse shows synaptic depression when the PD neuron is driven with presynaptic square pulses (Graubard et al. 1983; Johnson and Harris-Warrick 1990) and when the PD neuron is artificially driven with realistic voltage waveforms that resemble its normal activity (Rabbah and Nadim 2007). Graded synaptic strength at nondepressed PD—LP synapses is reduced by dopamine and serotonin and enhanced by octopamine (Johnson and Harris-Warrick 1990), but the effects of amines on PD—LP synaptic strength under conditions of realistic rhythmic activity are unknown. Here we show that the three amines can have independent effects on synaptic strength, the magnitude and temporal dynamics of synaptic depression, and the recovery from depression.

MATERIALS AND METHODS

General Procedures

California spiny lobsters (Panulirus interruptus) were supplied by Don Tomlinson Commercial Fishing (San Diego, CA) and maintained in marine aquaria at 16°C. After lobsters were anesthetized in ice, the stomatogastric nervous system (STNS) was removed and pinned in a Sylgard-coated petri dish in chilled Panulirus saline of the following composition (mM): 479 NaCl, 12.8 KCl, 13.7 CaCl2, 3.9 Na2SO4, 10.0 MgSO4, 2 glucose, and 11.1 Tris base, pH 7.35 (Mullone and Selverston 1974). The STG was desheathed, enclosed in a 1-ml pool walled with Vaseline, and superfused at 5 ml/min with oxygenated Panulirus saline. Experiments were performed at 19°C to enhance the strength of graded synaptic transmission (Johnson et al. 1991). Dopamine (DA; 10−5 M), octopamine (Oct; 10−5 M), and serotonin (5-HT; 10−5 M) were dissolved in saline just before application (amine conditions). If the amine effects did not reverse after a 15- to 60-min wash, the experiment’s results were discarded. Amines were purchased from Sigma Chemical.

Electrophysiological Recording and Cell Identification

Pyloric neuron activity was monitored by using pin electrodes to record extracellular APs from appropriate motor roots (differential AC amplifier, model 1700; A-M Systems) and intracellular electrodes (3 M KCl, 10−15 M) to record from cell bodies in the STG. We identified pyloric neuron somata during ongoing rhythmic activity by matching the extracellularly recorded APs with intracellularly recorded APs, by the characteristic shape and amplitude of membrane potential oscillations and APs of pyloric neurons, and by the known synaptic connections between pyloric neurons (Johnson et al. 2011).

Isolation of Graded PD→LP Synapse

Inhibitory glutamatergic synapses in the pyloric network were blocked by 5 × 10−8 M picrotoxin (PTX; Sigma Chemical) to pharmacologically isolate the PD→LP synapse (Bidaut 1980). The anterior burster (AB) neuron, which is electrically coupled to the PD neurons, was photoactivated by intracellular iontophoresis of 5,6-carboxyfluorescien and illumination with bright blue light (Miller and Selverston 1979). This eliminated any amine-induced AB activity spreading to the PD neuron through the AB→PD electrical synapse. We waited at least 1 h after AB photoactivation to start our experiments, to allow the preparation to recover from the acute effects of the light exposure (Flamm and Harris-Warrick 1986).

Dynamics of PD→LP Graded Synaptic Transmission

LP graded inhibitory postsynaptic potentials (gIPSPs) were recorded after 10−7 M tetrodotoxin (TTX; Tocris) was added to Panulirus saline to block APs. We studied the PD→LP graded chemical synapse using two different presynaptic stimulation protocols. The first used single-3-s square pulses as voltage-clamp commands to drive the PD neuron, whereas the second used a train of 12 linked realistic PD waveforms as voltage-clamp commands to drive PD oscillations (see Johnson et al. 2005, 2011 for realistic waveform construction). Averaged and filtered PD waveforms from pyramidal recordings had a mean amplitude of 15.5 ± 3.5 mV and a mean cycle period of 688 ± 199 ms (n = 10); we used this shape and cycle period as presynaptic stimuli. Although realistic presynaptic waveforms are more physiologically relevant than square pulse waveforms, we used both stimulation regimes for two reasons. First, some basic characteristics of synaptic depression and its modulation at the PD→LP synapse can only be measured with square pulse stimulation, for example, accurate measurements of the onset of synaptic depression (see below). Second, previous studies of graded synaptic transmission between pyloric neurons have often used square pulse stimulation (for example, Graubard et al. 1983; Johnson et al. 1995; Manor et al. 1997; Zhao et al. 2011), and we wanted to compare our results with these earlier studies and determine whether postsynaptic responses differed between the two stimulus forms. Both stimulation protocols used two-electrode voltage clamp to drive the PD from a holding potential (Vhold) of −55 mV in increasing amplitudes of 5-mV increments up to −25 mV. Stepping the PD up to −25 mV evokes near-maximum gIPSPs in the LP neuron (Johnson and Harris-Warrick 1990; Rabitto and Nadim 2007). We separated stimulation runs by a minimum of 30 s, which was sufficient to eliminate all effects of the previous stimulation. We used two-electrode current clamp to hold the postsynaptic LP membrane potential at −50 mV; postsynaptic electrodes were filled with 0.6 M K2SO4 + 0.02 M KCl and presynaptic electrodes with 3 M KCl. In these experiments we used Axoclamp-2A and 2B amplifiers (Molecular Devices).

Under control and amine conditions (at least 5 min of amine superfusion), we measured the initial peak and steady-state amplitudes of the gIPSPs to calculate input-output curves and dynamics of synaptic depression (see Fig. 1, A and B). The steady-state amplitude was measured as the gIPSP plateau level at the end of the square pulse stimulation or as the mean amplitude of the last five gIPSPs in response to the realistic PD waveform train. A synaptic depression index (DI) was calculated as the fractional gIPSP decrease from the initial peak response at steady state: DI = (initial peak amplitude − steady-state amplitude)/initial peak amplitude. Thus a larger DI value corresponds to greater synaptic depression. The time constant (τrec) of the gIPSP depression was measured during presynaptic square pulse stimulation by fitting the voltage decay from the initial peak to the steady-state amplitude with a single-exponential function (see Fig. 1A). The rate of synaptic depression was rapid enough that it could not be measured accurately from the separated peaks of the realistic waveform series.

In a separate measurement, we quantified the time constant of recovery from synaptic depression (τrec) for both realistic and square waveform stimulation under control and amine conditions. We evoked steady-state depression by stimulating the PD neuron (Vhold = −55 mV) as described above, followed by a single square pulse or realistic oscillation test stimulation at increasing times after the end of the last conditioning stimulus. The percent recovery of the test pulse back to the initial peak value over time was fitted with a single exponential in each experiment to calculate τrec (see Fig. 2, B and C).

Data Acquisition and Analysis

Electrophysiological recordings were digitized at 4 kHz using a PCI-6070-E board (National Instruments) and stored on a personal computer using custom-made recording software (Scope) written in LabWindows/CVI (National Instruments). This software also controlled the injection of oscillatory and square pulse waveforms as voltage-clamp commands into the PD neuron (Johnson et al. 2005, 2011). All data were analyzed with the related custom-made software program ReadScope, also written in LabWindows/CVI; these programs were kindly provided by Dr. F. Nadim (http://www.alertnet.org/software/index.html). Data were exported to Clampfit software (Molecular Devices) to calculate τdep and τrec. For statistical comparisons, we used JMP software (SAS Institute) to run multivariate analysis, followed by post hoc tests to determine the statistical significance of differences between individual data groups. Statistical differences between mean values were accepted with P < 0.05 (2-tailed probability) for F and t values. Mean measured values and percentages are means ± SD.
RESULTS

Synaptic Dynamics of the PD→LP Graded Synapse Under Control Conditions

We first characterized the baseline synaptic dynamics of the PD→LP graded synapse under control conditions, using single 3-s square pulses \( n = 10 \) and repeated presynaptic activation with trains of 12 realistic oscillations \( n = 7 \). As described previously for square pulse stimulation of the PD neuron (Graubard et al. 1983; Johnson and Harris-Warrick 1990; Rabbah and Nadim 2007), above the threshold for graded PD transmitter release (approximately −50 mV), the LP response is a rapid initial peak hyperpolarization that rapidly depresses to a significantly smaller steady-state hyperpolarization \( (P < 0.001 \) across PD depolarizations; Fig. 1, B and D; see also Rabbah and Nadim 2007). Peak and steady-state gIPSP amplitudes increased with increasing PD voltage commands from −40 to −25 mV under both stimulation protocols (Fig. 1, C and D). The amplitude of the initial peak LP gIPSP tended to be larger when elicited with PD square pulse stimulation than with realistic waveform stimulation (compare Fig. 1, C and D, open and filled circles), and this difference was significant during PD depolarizations to −30 and −35 mV \( (P = 0.03 \) and 0.05, respectively). However, the mean steady-state gIPSP amplitudes were significantly greater when elicited with oscillations compared with square steps at strong PD depolarizations (to −30 and −25 mV; compare Fig. 1, C and D, open and filled squares; \( P = 0.05 \) and 0.03, respectively). Since square pulse stimulation evoked larger peak initial gIPSPs and smaller steady-state amplitudes than oscillation stimulation, it also evoked greater synaptic depression at all PD stimulation voltages (Fig. 1E; \( P < 0.001 \) at all PD voltages). For example, the mean DI was twice as large at PD depolarizations; Fig. 1, B and D; see also Rabbah and Nadim 2007). Peak and steady-state gIPSP amplitudes increased with increasing PD voltage commands from −40 to −25 mV under both stimulation protocols (Fig. 1, C and D). The amplitude of the initial peak LP gIPSP tended to be larger when elicited with PD square pulse stimulation than with realistic waveform stimulation (compare Fig. 1, C and D, open and filled circles), and this difference was significant during PD depolarizations to −30 and −35 mV \( (P = 0.03 \) and 0.05, respectively). However, the mean steady-state gIPSP amplitudes were significantly greater when elicited with oscillations compared with square steps at strong PD depolarizations (to −30 and −25 mV; compare Fig. 1, C and D, open and filled squares; \( P = 0.05 \) and 0.03, respectively). Since square pulse stimulation evoked larger peak initial gIPSPs and smaller steady-state amplitudes than oscillation stimulation, it also evoked greater synaptic depression at all PD stimulation voltages (Fig. 1E; \( P < 0.001 \) at all PD voltages). For example, the mean DI was twice as large at PD depolariza-

Fig. 1. Synaptic dynamics of graded synaptic transmission at the PD→LP chemical synapse. A: lateral pyloric (LP) graded inhibitory postsynaptic potentials (gIPSPs) in response to 3-s pyloric dilator (PD) neuron square pulse depolarizations. Initial peak and steady-state IPSP amplitudes and the time constant for synaptic depression \( (\tau_{\text{Dep}}) \) were measured for all PD depolarizations from a holding potential \( (V_{\text{hold}}) \) of −55 mV. B: LP gIPSPs during a train of 12 realistic PD oscillations of increasing amplitude from \( V_{\text{hold}} = -55 \) mV. The first gIPSP amplitude (initial peak) and the mean of the last 5 gIPSPs amplitudes (steady state) were measured for all PD depolarizations. C: mean input/output relationship for the amplitude of the initial peak (open circles) and steady-state (open squares) gIPSPs during square pulse PD stimulation \( (n = 11) \). D: mean input/output relationship for initial (filled circles) and steady-state (filled squares) LP gIPSPs amplitudes during PD oscillation stimulation \( (n = 7) \). E: mean depression index (DI) for square pulse (open bars) and oscillation (filled bars) stimulation across PD stimulation voltages. **Significantly greater DI for PD square pulse stimulation than for PD oscillation stimulation \( (P < 0.001 \) for all PD voltages). The DI did not show voltage dependence with either stimulation protocol \( (P > 0.20 \) for both).
tion to $-25\text{ mV}$ with the use of square waves compared with oscillations (Fig. 1E). This probably results from the greater depression during continued presynaptic stimulation than during trains of oscillations, where the synapse could partially recover between oscillations (see DISCUSSION). There was no significant effect of PD voltage on the magnitude of the depression index over the voltage range of either PD stimulation protocol (Fig. 1E; $P = 0.23$ and 0.21 for square pulse and oscillations, respectively).

We also characterized the time constants of synaptic depression and recovery from depression at the PD→LP graded synapse using the square pulse series. The decay of the LP response to PD stimulation (Fig. 1A) was well fit by a single exponential (fit correlation $r = 0.99 \pm 0.001$, $n = 11$). The mean time constant of synaptic depression, $\tau_{\text{Dep}}$, was $400 \pm 200\text{ ms}$ during PD square pulse steps to $-25\text{ mV}$. There was no voltage dependence of $\tau_{\text{Dep}}$ during square step stimulation ($P = 0.95$; Fig. 2A). Because of the comparatively slow cycle frequency of the oscillation stimulation (cycle period 688 ms), we were unable to calculate the faster $\tau_{\text{Rec}}$ from the oscillation data.

In a separate measurement, we characterized the time constant of recovery from depression, $\tau_{\text{Rec}}$, by using both stimulation protocols (PD steps to $-25\text{ mV}$ only) and fitting the gIPSPs at increasing poststimulation intervals with a single exponential (Fig. 2, B and C; square pulse: exponential fit correlation $r = 0.95 \pm 0.03$; oscillation: fit correlation $r = 0.98 \pm 0.01$). Recovery from synaptic depression was over twice as fast with square pulse PD stimulation ($n = 13$) as with oscillation stimulation ($n = 12$; Fig. 2D; $P = 0.003$). This may result from increased mobilization of the recovery process during maintained presynaptic depolarization (see DISCUSSION).

![Fig. 2. Synaptic depression and recovery at the PD→LP graded synapse. A: mean $\tau_{\text{Dep}}$ across PD square pulse stimulation voltages ($n = 11$). $\tau_{\text{Dep}}$ did not show any voltage dependence ($P = 0.95$). B: measurement of the time constant of LP gIPSP recovery from depression ($\tau_{\text{Rec}}$) in response to PD square pulse depolarization to $-25\text{ mV}$. C: measurement of $\tau_{\text{Rec}}$ in response to PD oscillation depolarization to $-25\text{ mV}$. D: mean $\tau_{\text{Rec}}$ for LP gIPSPs elicited by PD square pulse ($n = 13$) and oscillation stimulation ($n = 10$). *Significantly longer $\tau_{\text{Rec}}$ with PD oscillation stimulation ($P = 0.003$).](http://jn.physiology.org/doi/10.1152/jn.00586.2012)

![Fig. 3. Dopamine (DA; 10$^{-5}$ M) modulation of synaptic strength at the PD→LP synapse. A–C: examples showing variable effects of DA to reversibly enhance (A), reduce (B), or have no effect (C) on the initial LP gIPSP amplitude with PD square pulse stimulation. D: mean effects of DA on amplitudes of initial peak and steady-state LP gIPSPs elicited by PD square pulse (open bars, initial peak; light gray bars, steady state) and oscillation stimulation (solid bars, initial peak; dark gray bars, steady state) at $-25\text{ mV}$ PD depolarization. E: example showing DA enhancement of the initial peak gIPSP during PD oscillation stimulation. Ctrl, control.](http://jn.physiology.org/doi/10.1152/jn.00586.2012)
Amines Change the Synaptic Dynamics of the PD→LP Graded Synapse

DA, 5-HT, and Oct affect synapses in the pyloric network by a complex set of pre- and postsynaptic actions (Harris-Warrick and Johnson 2010; Johnson and Harris-Warrick 1997). In addition, they can alter synaptic strength indirectly by modifying the waveforms of the pre- or postsynaptic neuronal oscillations (Johnson et al. 2005). To focus on synaptic metaplasticity, we attempted to limit the other consequences of amine modulation. For square pulse stimulations, we held the presynaptic neuron at −50 mV by voltage clamp both before and during amine application. For oscillation stimulation, we used the realistic waveforms obtained from PD oscillations during the normal pyloric rhythm for both control and amine conditions. Thus the effects of amine-evoked changes in PD oscillations were eliminated. The postsynaptic LP neuron was held at −55 mV under all conditions to prevent indirect effects of neuronal membrane potential changes evoked by the amines (Flamm and Harris-Warrick 1986).

Dopamine. The physiological DA concentrations normally achieved in vivo will depend on the spike frequency of the dopaminergic neurons and are not known. We demonstrated previously that 10⁻⁴ M DA greatly reduces or abolishes the LP gIPSP in response to square pulse PD stimulation (Johnson and Harris-Warrick 1990). This also occurs using realistic waveform PD stimulation (data not shown), so we decided instead to use a lower concentration of 10⁻⁵ M DA, which does not eliminate PD→LP transmission (Fig. 3), to examine DA’s effects on PD→LP graded synaptic dynamics. At this lower concentration, DA had weak and highly variable effects on the LP gIPSPs in different preparations. With the use of square pulse PD stimulation, DA reversibly increased initial gIPSP amplitudes in about one-half of the preparations (Fig. 3A) and reduced or had little effect on gIPSP amplitudes in the other half (Fig. 3, B and C); all of these effects reversed upon washout of DA. We suggest that this variability is caused by the known opposing effects of DA to decrease presynaptic PD transmitter release and increase postsynaptic LP input resistance (Harris-Warrick and Johnson 2010), but to differing amounts in different preparations. On average, 10⁻⁵ M DA did not significantly change the mean amplitude of the peak and steady-state components of the LP gIPSP during PD square pulse stimulation (Fig. 3D, n = 5, P > 0.3 for both). In contrast, during oscillation stimulation, DA significantly increased the mean first gIPSP amplitude when compared across all PD depolarizations (example shown in Fig. 3E; P = 0.02, n = 16). However, this effect was weak and only produced a trend to increase initial peak gIPSP amplitude at single PD voltage step amplitudes (Fig. 3D; P = 0.08, n = 4 each). DA did not significantly change the steady-state gIPSP amplitude across PD oscillation steps (Fig. 3D; P = 0.26).

With regard to synaptic depression, there was no significant effect of DA on the amplitude of synaptic depression, as measured by the DI, for either square pulses (control DI: 0.68 ± 0.07, DA DI: 0.70 ± 0.10 at −25 mV) or oscillations (control DI: 0.32 ± 0.06, DA DI: 0.37 ± 0.17 at −25 mV) at any PD voltage (P > 0.10 for both).

Despite these weak and variable effects on the amplitudes of LP gIPSPs, DA consistently accelerated both the onset of, and the recovery from, synaptic depression. Figure 3C shows a typical square wave measurement, where τ<sub>Dep</sub> was accelerated by DA compared with the control gIPSP response, with little change in gIPSP amplitude. The mean τ<sub>Dep</sub> was significantly decreased over the entire PD voltage range (Fig. 4A; n = 5, P < 0.005), but the DA effect showed no significant voltage dependence (P = 0.78). Recovery from depression was also faster with both square pulse and realistic waveform stimulation (Fig. 4A). This can be seen in the example using oscillation stimulation in Fig. 4B, which shows more complete recovery of...
the depressed gIPSP 1 s after the stimulus train during DA (89% recovery) than under control conditions (72% recovery). The mean $\tau_{\text{Rec}}$ at PD depolarization to $-25$ mV was significantly faster in DA for both square pulse PD stimulation (Fig. 4A, 39 ± 23% $\tau_{\text{Rec}}$ decrease; $P < 0.001$, $n = 5$) and realistic waveform PD stimulation (Fig. 4A, 43 ± 19% $\tau_{\text{Rec}}$ decrease; $P = 0.03$, $n = 4$). This acceleration of onset and recovery from depression did not depend on the sign of the rather variable DA effect on gIPSP amplitudes described above. As summarized in Fig. 4C, in all but one experiment the $\tau_{\text{Dep}}$ and $\tau_{\text{Rec}}$ values were smaller while DA either increased or decreased gIPSP amplitude. Thus, although DA ($10^{-5}$ M) had highly variable effects on gIPSP amplitude, it had reliable, significant, and independent effects to accelerate the time course of synaptic depression onset and recovery from synaptic depression.

**Octopamine.** We previously demonstrated that $10^{-5}$ M Oct strengthens the PD→LP synapse when tested with square wave PD stimulation (Johnson and Harris-Warrick 1990). In our current experiments, Oct consistently increased the amplitude of gIPSPs over the full range of presynaptic voltages using both square pulse ($n = 5$) and realistic ($n = 4$) PD waveforms (Fig. 5, A and B). The peak and steady-state responses to square pulse stimulation were significantly larger during Oct application at all PD depolarizations ($P < 0.0001$ and $P = 0.004$, respectively): during a PD step to $-25$ mV, the mean peak gIPSP increased by 55 ± 29% whereas the steady-state gIPSP increased by 157 ± 32% (Fig. 5C). As shown in Fig. 5C, Oct increased the steady-state gIPSP amplitude significantly more than the initial peak amplitude across the voltage steps ($P = 0.04$). With the oscillation protocol, Oct also increased the first peak and steady-state gIPSP amplitudes across all PD stimulation voltages ($P < 0.0001$ for both); for example, at $-25$ mV, the peak amplitudes increased by 57 ± 33% whereas the steady-state amplitudes increased by 48 ± 37% (Fig. 5C). Unlike the results using square pulse stimulation, during oscillation stimulation there was no significant difference between Oct’s enhancement of the peak and the steady-state gIPSP amplitudes at any PD voltage ($P = 0.15$; Fig. 5C). There was no voltage dependence of the Oct effect on gIPSP amplitude.

During square pulse stimulation, the greater Oct enhancement of the steady state over the peak gIPSP amplitude resulted in a significant decrease in the DI across PD depolarizations ($16 ± 6%$ decrease at $-25$ mV; $P = 0.0001$; Fig. 5D). However, there was no equivalent DI decrease during oscillation stimulation, because Oct did not differentially increase the first peak and steady-state amplitudes of the gIPSP (Fig. 5, C and D; $P = 0.15$).

Octopamine significantly altered the onset and recovery of synaptic depression in response to square pulse PD stimulation in a way that is quite different from DA. Octopamine significantly slowed $\tau_{\text{Dep}}$ across PD steps (increase by $80 ± 41%$ at $-25$ mV; $n = 5$, $P < 0.0001$; Fig. 6A). This slowing of synaptic depression was not caused simply by the larger gIPSPs evoked during Oct. When gIPSP amplitudes were matched in the presence and absence of Oct with the use of different amplitude presynaptic PD steps in the same preparation, $\tau_{\text{Dep}}$ remained slower during Oct (Fig. 6B). In contrast, Oct significantly accelerated the recovery from depression during square pulse PD stimulation (Fig. 6C) by an average of $47 ± 24%$ using $-25$-mV steps ($n = 4$, $P = 0.026$; Fig. 6A). However, when measured with oscillation stimulation, Oct had highly variable effects on $\tau_{\text{Rec}}$ that could either accelerate or slow down and were not significant overall (control: 1,120 ± 610 ms, Oct: 1,510 ± 1,510 ms; $P = 0.63$). Thus the main effect of Oct was to increase PD→LP synaptic strength during both PD stimulation protocols. With the use of square pulse PD stimulation, Oct reduced the depression amplitude and slowed the onset of synaptic depression while speeding up the rate of recovery from depression.

**Serotonin.** We previously showed that $10^{-5}$ M 5-HT weakens the PD→LP synapse when tested with square wave PD
Serotonin significantly accelerated the time course of synaptic depression measured with square pulse PD stimulation and tended to accelerate the time course of recovery from depression (Fig. 8A), although this effect did not reach statistical significance. The acceleration in \( \tau_{\text{Dep}} \) was not caused by a smaller gIPSP during 5-HT. For example, the trace in Fig. 8B shows that when sized matched (using different presynaptic voltage steps), the gIPSP recorded during 5-HT depressed more quickly than in control conditions. Serotonin significantly accelerated \( \tau_{\text{Dep}} \) with square pulses across PD voltages \( (P = 0.02) \); at \(-25\) mV PD depolarization, \( \tau_{\text{Dep}} \) was reduced by 25 \pm 34\% (Fig. 8A; \( n = 4 \)). In 3 of 3 experiments, 5-HT decreased \( \tau_{\text{Rec}} \) by 28 \pm 14\%, but this did not reach statistical significance with the small sample size and variability \( (P = 0.10 \); Fig. 8A). Serotonin also decreased \( \tau_{\text{Rec}} \) in 5 of 5 experiments by 26 \pm 27\%, measured by oscillation stimulation to \(-25\) mV (see example in Fig. 8C), but this also did not reach our criterion for statistical significance \( (P = 0.07 \); Fig. 8A). Thus 5-HT reduces PD→LP synaptic strength and increases the rate of onset of synaptic depression and the amount of synaptic depression while tending to weakly accelerate the recovery from depression.

### DISCUSSION

**Amine-Induced Metaplasticity at the PD→LP Graded Synapse**

Graded chemical synapses of the pyloric and gastric CPG networks of the lobster STG show marked synaptic depression with prolonged voltage steps or repeated trains of presynaptic activation; all pyloric synapses are tonically depressed at normal pyloric cycle frequencies (reviewed by Hartline and Graubard 1992; see also Johnson et al. 2005, 2011; Mamiya and Nadim 2004; Manor et al. 1997). Synaptic depression is commonly seen at graded chemical synapses, such as those between retinal neurons and from hair cells to afferent fibers (Edmonds et al. 2004; Wan and Heidelberger 2011), although not all graded chemical synapses show short-term activity-dependent changes in synaptic strength (Narayan et al. 2011; Simmons 1981). Aside from studies with pyloric network synapses, little previous work has examined neuromodulation of synaptic dynamics at graded chemical synapses.

We have previously described amine effects on the strength of the PD→LP graded synapse (Johnson and Harris-Warrick 1990), and here we demonstrate that the magnitude and kinetics of short-term synaptic dynamics are also modulated by amines at this synapse. The most surprising conclusion from our results is that the synaptic strength and the magnitude of synaptic depression, as well as the kinetics of its onset and recovery, can be independently modulated by each amine. We suggest that this may occur through multiple pre- and postsynaptic mechanisms of neuromodulatory actions that shape synaptic strength in a functioning network.

**Dopamine.** We have previously demonstrated that DA \( (10^{-4} \text{ M}) \) increases graded synaptic strength at the LP→PY and LP→PD synapses, but only changes (increases) the amount of synaptic depression at the LP→PY synapse (Johnson et al. 2005, 2011). Thus DA can have differential effects on synaptic depression and synaptic strength depending on the postsynaptic target. We used \( 10^{-5} \text{M} \) DA in the present study because

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**FIG. 6.** Octopamine modulation of synaptic depression and recovery at the PD→LP synapse. A: Oct increases \( \tau_{\text{Dep}} \) (left, \( n = 5 \)) and decreases \( \tau_{\text{Rec}} \) (right, \( n = 4 \)) at \(-25\) mV PD square pulse depolarization. *Significant effects \((P < 0.03\) for both). B: example using PD square pulse depolarizations of differing amplitudes to adjust the LP gIPSP to the same amplitude under control and Oct conditions, showing the Oct-induced increase in \( \tau_{\text{Dep}} \) in size-matched LP gIPSPs (black traces, control; gray trace, Oct). C: example of normalized initial peak gIPSPs in control (black traces) and Oct (gray traces), with more rapid gIPSP recovery in Oct during PD square pulse depolarizations to \(-25\) mV.

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**FIG. 7.** Amine-induced metaplasticity at a graded synapse. Discrete amplitude steps are shown for each amine and voltage level. **A:** Oct increases \( \tau_{\text{Dep}} \) and decreases \( \tau_{\text{Rec}} \). B: example using PD square pulse depolarizations of differing amplitudes to adjust the LP gIPSP to the same amplitude under control and Oct conditions, showing the Oct-induced increase in \( \tau_{\text{Dep}} \) in size-matched LP gIPSPs (black traces, control; gray trace, Oct). C: example of normalized initial peak gIPSPs in control (black traces) and Oct (gray traces), with more rapid gIPSP recovery in Oct during PD square pulse depolarizations to \(-25\) mV.
10^{-4}M DA abolishes functional synaptic transmission at the PD→LP graded synapse (Johnson and Harris-Warrick 1990). At the lower concentration, DA consistently accelerated $\tau_{\text{Dep}}$ and $\tau_{\text{Rec}}$ despite small and variable effects on PD→LP synaptic strength and on the magnitude of synaptic depression. This demonstrates that the kinetics of synaptic depression can be modulated independently of synaptic strength and the magnitude of synaptic depression. At present, we cannot correlate $10^{-5}$ M DA’s variable actions on synaptic strength with its effects on other cellular properties. None of the known effects of DA on pre- or postsynaptic ionic currents described previously (Harris-Warrick and Johnson 2010) can account for the DA-induced changes in depression kinetics. DA may be acting postsynaptically to accelerate the kinetics of transmitter receptor desensitization and recovery from desensitization (Papke et al. 2011). Motor patterns produced by the lobster pyloric network are qualitatively different when treated with $10^{-4}$ and $10^{-5}$ M DA (Flamm and Harris-Warrick 1986). Concentration-dependent effects on the onset and recovery of synaptic depression may contribute to shaping distinct DA-induced network activity.

Octopamine. Octopamine’s enhancement of synaptic strength was accompanied by reduced synaptic depression, slowed $\tau_{\text{Dep}}$ and accelerated $\tau_{\text{Rec}}$ for PD square pulse stimulation but not for PD oscillation stimulation. Part of the synaptic strength increase by Oct may be due to its increase in postsynaptic calcium currents (Johnson et al. 1993), as described above for DA. Octopamine’s effects could reflect presynaptic mechanisms such as a reduction in voltage-dependent calcium currents, which would reduce release and slow the rate of vesicle mobilization. Octopamine may have additional postsynaptic effects besides decreasing LP input resistance (Johnson et al. 1993), as described above for DA. Octopamine showed a trend to accelerate both the onset and recovery from depression, which would allow greater flexibility of the synapse as the cycle frequency changes; the accelerated recovery may limit the reduction in synaptic strength during the normal oscillating activity of the neurons in the pyloric rhythm. More work is needed to clarify the mechanisms for the metaplastic effects of amines, but it is clear that amines have complex and sometimes opposing effects on short-term dynamics of graded synaptic transmission in the pyloric network.

Characteristics of Synaptic Dynamics at the PD→LP Graded Synapse

The use of both square pulse and oscillation stimulation protocols allowed us to probe characteristics of synaptic depression and its modulation at the PD→LP graded synapse of the pyloric that might not be evident with either stimulation protocol alone. First, we found that the magnitude of the synaptic depression was greater with square pulse stimulation of PD than with trains of PD oscillations, emphasizing that...
realistic oscillation stimulation allows a more physiological characterization of pyloric synapses (Manor et al. 1997). Weaker synaptic depression with realistic oscillation stimulation may be explained simply by the partial recovery from depression that occurs between the PD oscillations. Second, we could more accurately determine the time course of onset of depression with long square pulse PD stimulation. The onset of depression, measured with square pulse PD stimulation, had a time constant of \( \tau_{\text{Dep}} \approx 400 \text{ ms} \) and could not be accurately measured with oscillations occurring every 688 ms. Values for \( \tau_{\text{Dep}} \) vary from milliseconds to tens of seconds at other AP-evoked and graded chemical synapses (Cho et al. 2011; Wang and Manis 2008; Zucker and Regehr 2002). The recovery from depression was over twice as fast with square pulse PD stimulation (\( \tau_{\text{Rec}} \approx 600 \text{ ms} \)) as with PD oscillations (\( \tau_{\text{Rec}} \approx 1,600 \text{ ms} \)). Our \( \tau_{\text{Rec}} \) times are longer than those previously reported by Rabbah and Nadim (2007) at the PD→LP graded synapse, but the stimulation protocols differed between the two studies. Time constants for recovery from depression range from tens of milliseconds at hair cell synapses to seconds at other graded synapses, again depending on experimental conditions (Rabl et al. 2006; Wan and Heidelberger 2011). Finally, amines sometimes affected synaptic depression and its onset and recovery kinetics differentially (see DISCUSSION above), suggesting more complex actions on synaptic dynamics than would be seen with PD oscillation stimulation alone.

The most common mechanism of synaptic depression at both AP-evoked and graded chemical synapses is transmitter depletion during prolonged or repeated synaptic activity (von Gersdorff and Matthews 1997; Zucker and Regher 2002); other reported presynaptic mechanisms include calcium channel inactivation (Xu et al. 2007), autoreceptor activation (Davies et al. 1990), and accumulating refractoriness of the transmitter release mechanism (Waldeck et al. 2000). Our experiments were not designed to determine the site of depression, but they suggest a mixed mechanism for depression at the PD→LP graded synapse. Changing the length of the presynaptic square pulse or the duration of the PD oscillations altered the level of depression (Rabbah and Nadim 2007). In addition, the faster \( \tau_{\text{Rec}} \) using square pulse PD stimulation, which would accumulate more intracellular calcium, is consistent with a calcium-dependent replenishment of the readily releasable transmitter pool, seen at AP-evoked (Neher and Sakaba 2008) and graded synapses (Babai et al. 2010; Gomis et al. 1999). We also found that the magnitude of PD→LP depression and its onset kinetics were not sensitive to presynaptic voltage stimulation, suggesting that depression was independent of the amount of transmitter release. The presynaptic voltage independence of both the depression magnitude and \( \tau_{\text{Dep}} \) is inconsistent with a simple presynaptic transmitter depletion model of synaptic depression. The lack of correlation between the amount of synaptic depression and gIPSP amplitude at the PD→LP graded synapse is also seen at AP-evoked synapses from other preparations where synaptic depression is clearly due to a presynaptic mechanism but its magnitude is independent of PSP amplitude (Hefft et al. 2002; Wu et al. 2005). There may also be a postsynaptic contribution at our synapse, such as receptor desensitization (Jonas and Westbrook 1995; Papke et al. 2011; Trussell et al. 1993).

Possible Functional Importance of Metaplasticity in the Pyloric Network

Short-term synaptic depression plays an important role in organizing a flexible pyloric motor pattern. The magnitude of depression and its onset and recovery kinetics dynamically determine the synaptic strength at different pyloric cycle periods and when pyloric rhythm periods are perturbed by interactions with linked networks such as those from the cardiac sac and gastric mill motor networks (Ayali and Harris-Warrick 1998; Russell and Hartline 1981; Thurma and Hooper 2002, 2003). Synaptic depression promotes phase maintenance as the pyloric network changes its cycle frequency (Greenburg and
Manor 2005; Manor et al. 2003; Nadim et al. 2003) and contributes to stabilization of the rhythm period (Mamiya and Nadim 2004). It can also switch between different forms of network bistability (Manor and Nadim 2001; Nadim et al. 1999) and can even determine the sign of a synaptic interaction (Johnson et al. 2005; Mamiya et al. 2003).

Neuromodulator-induced metaplasticity could help maintain or reset synaptic strengths between network neurons to adjust the postsynaptic neurons’ firing phase. For example, the 10^{-5} M DA effects to accelerate both τ_{Dep} and τ_{Rec} with no change in gIPSP amplitude or depression could stabilize PD→LP synaptic strength as the cycle frequency changes in DA (Johnson et al. 2011). Octopamine enhances LP excitability but does not change the LP firing onset phase (Johnson et al. 2011). The greater PD→LP synaptic strength in Oct, with reduced depression and faster onset and recovery kinetics, could balance enhanced LP excitability to maintain LP phasing at its pre-Oct values (Johnson et al. 2011). There is also no change in LP onset firing phase during 5-HT despite a reduction in LP excitability (Johnson et al. 2011). A faster τ_{Dep} might contribute to weakening the PD→LP gIPSP and to enhanced depression, and this may be mitigated by the accelerated τ_{Rec} to maintain the LP firing onset phase. At the crab LP→PY graded synapse, the peptide proctolin changes the sign of synaptic plasticity from depression to facilitation at certain presynaptic voltage levels and functions to help stabilize the cycle period (Zhao et al. 2011).

Metaplasticity through the actions of many different neuromodulators is a common feature of many neural networks (Barriere et al. 2008; Carey et al. 2011; Kreitzer and Regehr 2000; Parker 2001; Sakurai and Katz 2009) and should be considered one of the building blocks of network operation. Although well studied at AP-evoked synapses, there is relatively little known about metamodulation at graded chemical synapses. The pyloric network of crustaceans provides an excellent model system to explore the ranges of expression of metaplasticity at graded synapses and its functional role in organizing network operation and plasticity (Johnson et al. 2005, 2011; Zhao et al. 2011).

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AUTHOR CONTRIBUTIONS

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