PKC-mediated GABAergic enhancement of dopaminergic responses: implication for short-term potentiation at a dual-transmitter synapse

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Svensson E, Proekt A, Jing J, Weiss KR. PKC-mediated GABAergic enhancement of dopaminergic responses: implication for short-term potentiation at a dual-transmitter synapse. J Neurophysiol 112: 22–29, 2014. First published April 9, 2014; doi:10.1152/jn.00794.2013.—Transmitter-mediated homosynaptic potentiation is generally implemented by the same transmitter that mediates the excitatory postsynaptic potentials (EPSPs), e.g., glutamate. When a presynaptic neuron contains more than one transmitter, however, potentiation can in principle be implemented by a transmitter different from that which elicits the EPSPs. Neuron B20 in *Aplysia* contains both dopamine and GABA. Although only dopamine acts as the fast excitatory transmitter at the B20-to-B8 synapse, GABA increases the size of these dopaminergic EPSPs. We now provide evidence that repeated stimulation of B20 potentiates B20-evoked dopaminergic EPSPs in B8 apparently via a postsynaptic mechanism, and short-term potentiation of this synapse is critical for the establishment and maintenance of an egestive network state. We show that GABA can act postsynaptically to increase dopamine currents that are elicited by direct applications of dopamine to B8 and that dopamine is acting on a 5-HT receptor-like receptor. This potentiation is mediated by GABA receptors as GABA receptor agonists and antagonists, respectively, mimicked and blocked the potentiating actions of GABA. The postsynaptic actions of GABA rely on a G protein-mediated activation of PKC. Our results suggest that the postsynaptic action of cotransmitter-mediated potentiation may contribute to the maintenance of the egestive state of *Aplysia* feeding network and, in more general terms, may participate in the plasticity of networks that mediate complex behaviors.

buccal ganglia; co-transmission; classical transmitters; synaptic plasticity; neuromodulation

MULTIPLE MECHANISMS MAY CONTRIBUTE to neurotransmitter and neuromodulator-mediated synaptic potentiation. Homosynaptic potentiation can be mediated by the same transmitter that elicits excitatory postsynaptic potentials (EPSPs), e.g., glutamate can act at AMPA receptors to mediate the EPSP and at NMDA receptors to potentiate the EPSP (Collingridge et al. 1983). In heterosynaptic potentiation, one transmitter can potentiate responses to other transmitters as is the case when a transmitter released from one neuron potentiates the EPSPs elicited by another neuron that uses a different transmitter (Brunelli et al. 1976). Homosynaptic plasticity can also involve interactions of multiple transmitters as the same neuron may contain multiple cotransmitters. For instance, neuropeptides can potentiate the PSPs elicited by a classic transmitter contained in the same neuron (e.g., Fox and Lloyd 2001; Koh and Weiss 2005; Sámano et al. 2012). Also, the same neuron can contain multiple classic transmitters. Colocalization of dopamine and GABA is common in both vertebrates and invertebrates (Barreiro-Iglesias et al. 2009; Díaz-Ríos et al. 2002; Hirasawa et al. 2012; Liu et al. 2013; Maher and Westbrook 2008; Rodicio et al. 2008; Stensrud et al. 2013; Tritsch et al. 2012). Here, we have investigated the possible role of cotransmission by dopamine and GABA that are colocalized in the *Aplysia* interneuron B20 and its potential contribution to short-term synaptic plasticity (Díaz-Ríos et al. 2002; Jing and Weiss 2001).

Interneuron B20 is a component of the feeding central pattern generator in *Aplysia* (Cropper et al. 2004; Jing and Weiss 2001; Teyke et al. 1993). The B20 synapse onto motoneuron B8 exhibits activity-dependent short-term potentiation that arises as a result of generation of motor programs, and it contributes to the plasticity of the neuronal network during switching from ingestive to egestive motor programs (Proekt et al. 2004, 2007). Dopamine acts as a fast transmitter at the B20 synapse with the feeding motoneuron B8. Although GABA does not elicit any fast PSPs at this synapse, it can potentiate the dopaminergic EPSPs elicited in B8 (Díaz-Ríos and Miller 2005). However, the sites and mechanisms of GABA action at this synapse remain unknown.

We provide evidence that this plasticity may be postsynaptically mediated. Our results are consistent with the idea that GABA acts postsynaptically to potentiate responses to dopamine released from the same neuron and thus contributes to feeding-network plasticity. We also provide evidence that dopamine activates an ionotropic 5-HT receptor-like receptor and that GABA can potentiate the dopamine-elicited currents in B8. GABA acts through a metabotropic G protein-coupled receptor with pharmacological properties similar to the GABA receptor and activates an intracellular signal transduction pathway that involves PKC.

MATERIALS AND METHODS

*Aplysia*, obtained from Marinus Scientific (Long Beach, CA), were anesthetized by injection of isotonic MgCl2. The buccal ganglia were dissected, desheathed, and pinned in a cooled recording chamber (15–17°C) that was continuously perfused (0.5 ml/min) with artificial seawater (ASW) containing, in mM, 460 NaCl, 10 KCl, 55 MgCl2, 11 CaCl2, and 10 HEPES, adjusted to pH 7.6. Extracellular suction electrodes for nerve recordings were manufactured from polyethylene tubing. Extracellular signals were fed to a Model 1700 Differential AC Amplifier (A-M Systems, Carlsborg, WA). Sharp electrodes (4–6 MΩ) filled with 3 M potassium acetate, and 0.1 M KCl were used for intracellular recordings. Amplification and stimulations in bridge or AC Amplifier (A-M Systems, Carlsborg, WA). Sharp electrodes (4–6 MΩ) filled with 3 M potassium acetate, and 0.1 M KCl were used for intracellular recordings. Amplification and stimulations in bridge or
Dopamine was dissolved in ASW and puffed onto the B8 somata using a micropipette. Pressure pulses (50–100 ms, 30–50 psi) were given every 60–90 s to prevent desensitization. TTX (10 μM) was perfused in all puffing experiments to suppress polysynaptic actions of dopamine. GDPβS (10 mM) was iontophoretically injected into B8 using direct currents (DC; 20 nA) for ~1 h. In these experiments, neurons were impaled with a second electrode, which contained normal electrolyte, to maintain a stable membrane potential of ~70 mV to avoid secondary effects of the negative current injection. Chelerythrine, forskolin, and TTX were purchased from Tocris. Chelerythrine, forskolin, and TTX were purchased from Tocris. Dopamine was dissolved in ASW and puffed onto the B8 somata using direct currents (DC; 20 nA) for ~1 h. 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EPSPs were analyzed using the variance method of quantal analysis (Hubbard et al. 1969). The EPSPs were recorded in B8 at ~80 mV, and the membrane potential was maintained by a constant DC injection. The amplitude of the EPSPs was small (<10 mV) relative to the difference from the holding potential at ~80 mV to their reversal potential (~0 mV) and far away from the action potential threshold and therefore was not corrected for nonlinearity. The method assumes that the number of quanta of transmitter released from the presynaptic neuron follows a Poisson distribution. The average amplitude of miniature PSPs ($\bar{q}$) is estimated as the ratio of the variance of the amplitude of the PSP [var($v$)] to the mean of the amplitude of the PSP ($\bar{v}$). The number of quanta released ($m$) can then be calculated by dividing $\bar{v}$ by $\bar{q}$. Change in $m$ is indicative of a presynaptic effect. Change in $\bar{q}$ is indicative of a postsynaptic effect. Variance was calculated according to the standard equation: $\text{var}(x) = E(x - \mu)^2$ where $\mu$ is the expected value of random variable $x$ (in this case, the amplitude of the EPSP). To determine the variance, the EPSPs were grouped into 30-s bins (74–75 EPSPs in each bin as they were elicited at 2.5 Hz).

Statistical significance was tested using Student’s $t$-tests, and data are presented as means ± SE.

RESULTS

Short-term potentiation at the B20-to-B8 synapse. Previous work demonstrated that the B20-to-B8 synapse exhibits activity-dependent synaptic plasticity (Proekt et al. 2004). In the following set of experiments, we sought to characterize a possible contribution of postsynaptic mechanisms to the potentiation of the B20-to-B8 synapse.

We previously found that a 5-min esophageal nerve stimulation, which elicits ~10 motor programs and is sufficient to establish an egestive network state, results in the potentiation of the B20-to-B8 synapse, and activity of B20 in these esophageal nerve-elicited motor programs is both necessary and sufficient for the facilitation of the B20-to-B8 synapse (Proekt et al. 2004). To characterize the site at which potentiation occurs in this paradigm, we analyzed EPSPs using the variance method (see MATERIALS AND METHODS), which is an indirect way to measure this. After esophageal nerve-elicited motor programs, the amplitude of the EPSP increased to 289 ± 54% of control (Fig. 1A; $n = 6$; $P < 0.001$) and with time returned to baseline. There was a significant increase in the calculated quantal size ($q$) to 507 ± 175% of control ($P < 0.05$) without any significant change of the calculated $m$ (Fig. 1A2).

To characterize the locus of this potentiation, we stimulated B20 and analyzed the EPSPs using the variance method. Intracellular stimulation of B20 (10 Hz, 30 s) potentiated the B20-to-B8 EPSPs. The amplitude increased to 231 ± 26% of control (Fig. 1B; $n = 5$; $P < 0.001$). The amplitude of the potentiated EPSPs recovered to control values within ~10 min.
Dopamine activates a 5-HT$_3$-like receptor in B8. Given the limitation of the variance analysis, we sought to determine directly whether GABA is able to increase the amplitude of the dopaminergic responses in B8.

To evaluate the action of dopamine on B8, the neuron was single-electrode voltage-clamped, and dopamine was puff-applied onto B8 neurons through a sharp electrode. Dopamine-evoked fast inward currents in B8 reversed at −90 mV, indicating that nonselective cation channels are involved (Fig. 2A; n = 3) as previously suggested (Díaz-Ríos and Miller 2005).

Previous work showed that fast dopamine responses in Aplysia can be blocked by sulpiride (Díaz-Ríos and Miller 2005). This effect is unexpected as sulpiride is a recording showing the effect of tropisetron on the dopamine responses. The effect of dopamine recovers after washout of tropisetron. C: an histogram that summarizes the effects of the 5-HT$_3$ receptor antagonists tropisetron (25 μM, n = 4; 50 μM, n = 5) and MDL-72222 (50 μM, n = 4; 100 μM, n = 3) on the dopamine-evoked responses in B8 neurons. ***P < 0.001.

GABA acts postsynaptically to potentiate dopamine-elicited currents in B8. Previous experiments (e.g., Díaz-Ríos and Miller 2005) demonstrated that the dopaminergic EPSPs elicited by B20 in B8 were potentiated when GABA was exogenously applied, but the mechanisms of this action remained uncharacterized. Here, we sought to determine whether GABA can elicit the potentiation in the presence of TTX to minimize the possibility of polysynaptic effects.

A 5-min perfusion of GABA (100 μM) induced a significant potentiation of the amplitude of dopamine currents to 143 ± 8.8% of control (Fig. 3A; n = 5; P < 0.001). These currents returned to control values after washout of GABA. Our experiments thus far demonstrated that GABA can directly potentiate dopamine-evoked currents in B8.

GABA acts through a GABA$_B$-like receptor. We investigated the pharmacological profile of the receptor through which GABA acts on B8 to facilitate dopamine-evoked currents. We examined the effects of the GABA$_B$-receptor agonist baclofen, which increases the size of B20-to-B8 EPSPs (Díaz-Ríos and Miller 2005). Baclofen (100 μM) mimicked the action of GABA, as it potentiated dopamine currents to 168.4 ± 29.2% of control (Fig. 3B; n = 5; P < 0.05). This potentiation reversed to control after washout of baclofen. The GABA$_B$-receptor antagonist phaclofen (100 μM) blocked the action of both GABA (Fig. 3, A and C; n = 3; P > 0.05) and baclofen (Fig. 3, B and C; n = 3; P > 0.05) on dopamine responses. Thus GABA acts on a receptor with pharmacological profile similar to that of the GABA$_B$ receptor.

Potentiating actions of GABA require G protein and PKC activation but do not involve cAMP/PKA. Since GABA$_B$ receptors are G protein-coupled receptors, we interfered with G protein functions by loading B8 with nonspecific G protein inhibitor GDP$eta$S from the microelectrode (see MATERIALS AND METHODS). Following this treatment, GABA failed to enhance dopamine-elicited currents (Fig. 4A; n = 4; P > 0.05). In control experiments, which excluded GDP$eta$S from the microelectrode, GABA potentiated the dopaminergic responses (data not shown; n = 2). The GDP$eta$S experiment also provides addi-
Previous work on short-term heterosynaptic potentiation in *Aplysia* implicated two signal transduction pathways, PKA and PKC (Braha et al. 1990; Brunelli et al. 1976). Thus we characterized the possible role of PKA and PKC in the GABAergic facilitation of dopamine-elicited currents. We treated the preparation with PKC and PKA blockers and activators known to be effective in *Aplysia* (Braha et al. 1993; Hochner and Kandel 1992; Klein 1993; Manseau et al. 1998; Villareal et al. 2009). In preparations preincubated with the PKC antagonist chelerythrine (20 µM), GABA failed to potentiate dopamine currents (Fig. 4; *n* = 4; *P* > 0.05). The PKC activator PDBu (10 µM) mimicked the effect of GABA and induced a significant potentiation of the amplitude of dopamine currents to 132.3 ± 6.5% of control (Fig. 4C; *n* = 4; *P* < 0.01). Preincubation with the PKC antagonist chelerythrine before PDBu was applied completely blocked the potentiation actions of PDBu on dopamine currents (Fig. 4C; *n* = 4; *P* > 0.05).

To test the involvement of PKA, buccal ganglia were incubated with the PKA antagonist Rp-cAMP (100 µM). Rp-

Fig. 3. GABA and baclofen potentiate dopamine currents in neuron B8. *A*: potentiation of dopamine currents by perfusion of GABA (100 µM) and block of this effect by the GABAB-receptor antagonist phaclofen (Phaclo; 100 µM). *Insets* illustrate dopamine currents under various conditions. *B*: perfusion of the GABAB-agonist baclofen (100 µM) potentiates the amplitude of dopamine currents in B8. The effect of baclofen was blocked by phaclofen (100 µM). Traces at the top illustrate dopamine currents under various conditions. *C*: summary data of the effects of GABA (*n* = 5), GABA + phaclofen (*n* = 3) and baclofen (*n* = 5), and baclofen + phaclofen (*n* = 3) on dopamine currents in B8. ***P < 0.001; *P < 0.05.

Fig. 4. GABA-induced potentiation of dopamine currents involves G protein and PKC. *A*: inhibition of GABA-induced effects on dopamine responses in B8 neurons loaded with GDPβS (10 mM). *Insets* illustrate dopamine currents under various conditions. *B*: chelerythrine (20 µM) blocks the potentiating action of GABA. Traces at the top show that in the presence of chelerythrine, GABA fails to potentiate dopamine currents. *C*: phorbol 12,13-dibutyrate (PDBu; 10 µM) potentiates the amplitude of dopamine currents. The effect was blocked in the presence of chelerythrine (20 µM). Traces at the top illustrate the effect of PDBu on dopamine currents, and the 2nd *inset* shows the lack of effect of PDBu in the presence of chelerythrine. *D*: summary of the blocking effects of GDPβS and chelerythrine on GABA (*n* = 3) and PDBu (*n* = 4) induced potentiation of dopamine responses in B8 neurons. The lack of effects of the PKA agonists (forskolin, *n* = 3; Sp-cAMP, *n* = 4) and the antagonist Rp-cAMP (*n* = 3) are also shown. ***P < 0.001; *P < 0.05.
cAMP did not block GABA-elicited potentiation of dopamine currents as in the presence of GABA the currents increased to 137.0 ± 15% of control (Fig. 4D; n = 3; P < 0.05). Furthermore, forskolin, which stimulates the synthesis of cAMP (10 μM) and the PKA agonist Sp-cAMP (100 μM), neither increased nor blocked the potentiating actions of GABA on dopamine-elicited currents (Fig. 4D; forskolin: n = 3, P > 0.05; Sp-cAMP: n = 4, P > 0.05). Altogether, our results indicate that in the neuron B8, GABA activates an intracellular pathway that involves G protein-mediated activation of PKC, but not PKA, and that this action contributes to a postsynaptic potentiation of dopamine-elicited responses.

In summary, our results only indirectly suggest that the short-term potentiation of B8 EPSPs induced by a persistent egestive state or by tetanic B20 stimulation may be mediated by postsynaptic effects of GABA. Unfortunately, attempts to block B20-induced potentiation with the GABA antagonist phaclofen were unsuccessful. Bath-applied phaclofen induced a large amount of spontaneous network activity and had multiple effects on B20 with the result that B8 recordings were unpredictable and unstable. The use of baclofen has similar undesirable effects (Dacks and Weiss 2013). Despite these experimental limitations, our results are consistent with a postsynaptic role for the B20 cotransmitter GABA in the short-term potentiation of dopamine-mediated EPSPs in B8.

DISCUSSION

We examined a dual-transmitter synapse in the feeding network of Aplysia. The results presented here, combined with those from previous work (Díaz-Ríos and Miller 2005), indicate that whereas one transmitter contained in the presynaptic neuron, dopamine, acts to mediate the EPSPs, the other transmitter, GABA, acts to potentiate these EPSPs. Our data suggest that dopamine is mediating these EPSPs by acting on a postsynaptic 5-HT3-like receptor and that GABA potentiates these EPSPs by activating GABAB-like receptors in the postsynaptic neuron (Fig. 5). Consistent with the involvement of GABA in mediating synaptic plasticity under physiological conditions, we show that synaptic potentiation elicited by both direct stimulation of the presynaptic neuron and by incorporation of its activity in the feeding motor programs appears to operate postsynaptically.

Fast dopaminergic transmission. D1-like and D2-like dopamine receptors occur in Aplysia (Barbas et al. 2006). Thus the finding that dopamine is mediating its fast synaptic transmission by acting on a 5-HT3-like receptor is surprising, but it is well-known that dopamine is a partial agonist on this receptor in mammalian model systems (Dubin et al. 1999). However, in mammals, serotonin is the endogenous ligand that mediates fast synaptic transmission through this receptor (Férézou et al. 2002; Sugita et al. 1992). The lack of effect of loading B8 with GDPβS on the dopamine responses further strengthens the conclusion that dopamine acts directly on an ionotropic receptor and not on a metabotropic G protein-coupled receptor.

Cellular basis of GABA actions. Our studies with GABA_A agonists and antagonists indicate that GABA-mediated potentiation of dopaminergic currents in B8 is mediated by GABA_A-like receptors. GABA, acting at presynaptic sites through GABA_A receptors, can enhance or reduce synaptic transmission in both vertebrates (Brenowitz et al. 1998) and invertebrates (Gutovitz et al. 2001). In our experiments, postsynaptic potentiating actions of GABA were effectively antagonized by the PKC antagonist cherythrine and mimicked by the PKC agonist PDBu. The ability of GABA_A receptors to activate PKC has previously been shown in vertebrates (Bray and Mynlieff 2011; Shen and Slaughter 1999; Tremblay et al. 1995), and there are a number of mechanisms that in principle could implement them (Chitwood et al. 2001; Connolly 2008; Henley et al. 2011; Malenka 2003; Triller and Choquet 2005). The potentiation of dopamine responses evoked by exogenous applied GABA was in general smaller than endogenous evoked EPSP potentiation induced by B20 stimulation and may be due to discrepancies in the effective concentration of GABA under different conditions. GABA also slightly increased the input resistance in B8, which could contribute to the potentiating effect of GABA on the dopaminergic EPSPs but not on dopamine response in B8 recorded during voltage-clamp (Díaz-Ríos and Miller 2005). Similarly, GABA has been recently shown to increase the intrinsic excitability of B8 (Dacks and Weiss 2013). Regardless of which specific mechanism(s) actually operate(s) at the B20-to-B8 synapse, our results indicate that GABA can potentiate fast synaptic transmission of dopamine via a postsynaptic pathway involving PKC activation in B8 (Fig. 5).

Corelease of dopamine and GABA. The presynaptic regulation and differentiation of the corelease of dopamine and GABA at the B20-to-B8 synapse is likely to be frequency- and duration-dependent since the GABA-mediated potentiation only appears after a repeated stimulation of B20.

Our results combined with those of Díaz-Ríos and Miller (2005) suggest that dopamine is the fast transmitter at this synapse. GABA, which acts here as a slow modulator, may be costored and coreleased with dopamine from the same vesicles as in vertebrate striatum (Stensrud et al. 2013), or GABA might be stored in separate vesicles and potentially released extrasynaptically only during high-frequency stimulation resulting in a slow, diffuse volume transmission (Descaries et al. 2008). The spatial and temporal action of dopamine and GABA could also be regulated by their inactivation systems, e.g., the reuptake.

Cotransmission in homosynaptic potentiation and its behavioral consequences. Potentiation of synaptic transmission can assume a multitude of forms that can differ in terms of locus of induction and expression as well as in cellular mechanisms (Antonov et al. 2010; Fisher et al. 1997; Jin and Hawkins 2003; Roberts and Glanzman 2003; Zucker and Regehr 2002). It is likely that different forms of synaptic potentiation evolved...
within neuronal networks to meet various behavioral demands. Unfortunately, the understanding of the role of synaptic potentiation is limited by a paucity of well-defined networks in which specific behavioral functions can be attributed to synaptic plasticity.

Recent studies provide such a behavioral framework for understanding the role of potentiation of the B20-to-B8 synapse in the establishment of behavioral states. When the feeding network is first activated by esophageal nerve stimulation, it fails to produce egestive motor programs. With repeated stimulation, however, the network begins to generate egestive responses. When the input is then switched to an ingestive pathway, the network disregards the switch and continues to generate egestive motor programs. These and related observations suggest that the repeated stimulation of the esophageal nerve establishes a persistent egestive network state (Proekt et al. 2004). Potentiation of the B20-to-B8 synapse is established during repeated esophageal nerve stimulation and is critical for the evolution toward and maintenance of the egestive network state. The importance of this potentiation provides physiological relevance for the cotransmission of GABA and dopamine from B20 to B8 (Proekt et al. 2004, 2007).

Potentiation of B20-to-B8 EPSPs during the evolution of the network toward an egestive state can involve mechanisms similar to those involved in potentiation of this synapse elicited by direct stimulation of B20. Repeated activation of the esophageal nerve recruits B20 firing into the motor programs to a degree similar to that used to elicit synaptic potentiation by direct B20 stimulation. Furthermore, activity of B20 during these motor programs is both necessary and sufficient for the potentiation of the B20-to-B8 synapse (Proekt et al. 2004). Finally, both posttetanic potentiation (PTP) and firing of B20 during motor programs appear to potentiate the B20-to-B8 synapse by postsynaptic mechanisms as indicated by the indirect quantal analysis and by the direct effect of GABA on the dopamine-induced responses. It has been shown that GABA can increase the input resistance in B8 by 10% (Díaz-Ríos and Miller 2005), and although it is possible that this change in resistance affects the EPSP amplitude, the 10% change in the input resistance is unlikely to account for the severalfold increase in \( q \), and the variance of the amplitudes stays approximately constant as evidenced by the relative constant size of the error bars in Fig. 1. Postsynaptic mechanisms participate in homosynaptic plasticity of a simple reflex (Roberts and Glanzman 2003). However, in networks involved in the generation of complex multiphasic behaviors, multiple synapses have to be coherently regulated during behavioral plasticity. The particular mechanism of synaptic plasticity employed by B20 may provide a clue as to how B20 may coordinate plasticity at multiple synapses. The functional role of coreleasing GABA and dopamine from B20 could also be to potentiate selectively the synapses of other dopaminergic neurons that converge onto the same targets. Furthermore, some synapses can be preferentially affected depending on the expression of GABA receptors and the presence of appropriate signal transduction machinery.

**Functional role of cotransmission on homosynaptic potentiation and intrinsic/extrinsic neuromodulation.** The modulatory effect of GABA released from B20 in the *Aplysia* feeding network is considered as an intrinsic modulation by a cotransmitter since it modulates the circuit it is a part of (Katz and Frost 1996). This is quite unusual since in most cases homosynaptic potentiation is mediated by a neuromodulatory cotransmitter in projection neurons and is thus an extrinsic modulation of the network (Morgan et al. 2000). The command neuron CBI-2 colocalizes cerebral peptide 2 (CP2) and the feeding circuit-activating peptide (FCAP), and their action converges and contributes to PTP of its fast cholinergic EPSP in B61/62 (Koh et al. 2003; Koh and Weiss 2005). Another example on intrinsic modulation by cotransmitters is the midline neurons in the lamprey spinal cord that colocalize 5-HT, dopamine, and a tachykinin peptide. These neurons are an integral component of the spinal locomotor network, and the cotransmitters modulate and interact to tune the spinal locomotor network activity (Svensson et al. 2001).

Extrinsic neuromodulation of neuronal networks by colocalized neurotransmitters/modulators in sensory or projection neurons is more common than intrinsic neuromodulation. In the mollusk *Tritonia*, acetylcholine is colocalized with small cardioactive peptide (SCP) in the neuron B11, and the cotransmitters/modulators have both central effects on modulating the feeding network as well as peripheral modulatory actions on the gut motility (Lloyd and Willows 1988; Willows et al. 1988).

The role of cotransmission in extrinsic neuromodulation has been extensively investigated in the crustacean stomatogastric ganglion (STG), which displays both colocalization of small classic transmitters and neuropeptides (Nusbaum et al. 2001). The gastroplyoric receptor cells are neurons that colocalize 5-HT and acetylcholine. Acetylcholine is the fast excitatory transmitter, and 5-HT is mediating a slow depolarization of the membrane potential in the postsynaptic STG neurons (Katz and Harris-Warrick 1989, 1990; Kiehn and Harris-Warrick 1992). There are also projection neurons called inferior ventricular neurons that corelease histamine and the neuropeptide FLRF-amide, which have complementary effects in modifying the STG neuronal network activity (Christie et al. 2004; Kwiatkowski et al. 2013). The modulatory commissural neuron 1 colonizes GABA and the neuropeptides proctolin and the tachykinin-related peptide CabTRP Ia, which have divergent actions on STG neurons to modify their activity (Stein et al. 2007).

The action of cotransmitters in tuning neuronal network activity has to some extent been understood in simpler invertebrate and lower vertebrate model systems. However, the functional role in more complex neuronal networks remains to be elucidated.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS
E.S., A.P., J.J., and K.R.W. conception and design of research; E.S., A.P., and J.J. performed experiments; E.S., A.P., and J.J. analyzed data; E.S., A.P., J.J., and K.R.W. interpreted results of experiments; E.S. and A.P. prepared figures; E.S., A.P., and K.R.W. drafted manuscript; E.S., A.P., J.J., and K.R.W. edited and revised manuscript; E.S., A.P., J.J., and K.R.W. approved final version of manuscript.

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