Mixtures of Octopamine and Serotonin Have Nonadditive Effects on the CNS of the Medicinal Leech

KAREN A. MESCE, KEVIN M. CRISP, AND LAURA S. GILCHRIST

Graduate Program in Neuroscience and Departments of Neuroscience and Entomology, University of Minnesota, St. Paul, Minnesota 55108

Received 26 May 2000; accepted in final form 14 February 2001

Mesce, Karen A., Kevin M. Crisp, and Laura S. Gilchrist. Mixtures of octopamine and serotonin have nonadditive effects on the CNS of the medicinal leech. J Neurophysiol 85: 2039–2046, 2001. It is well established that neural networks respond to a wide variety of modulatory substances by which they can become reconfigured, yet few studies have examined the effects of neurotransmitter mixtures on such networks. In a previous study of the medicinal leech using triple intracellular recordings, we found that stimulation of identified mechanosensory neurons activated both the serotonergic cell 21 (a swimming neuron) and the dorsal lateral octopamine (DLO) cell. Because these findings suggested that serotonin (5-HT) and octopamine (OA) may be released together, we investigated the effects of 5-HT and OA mixtures on isolated nerve cords of Hirudo medicinalis (which contained both head and tail brains). Fifty micromolar OA, 50 μM 5-HT, or a mixture of 50 μM OA and 50 μM 5-HT was bath applied to the nerve cord under constant perfusion conditions. Additional experiments were performed with combinations of either 25 or 100 μM OA and 5-HT. Neural activity was examined specifically in the segmentally repeated dorsal posterior (DP) nerve because it has been shown to contain identified swim motor units. Nonadditive effects of amine combinations were most apparent in their ability to decrease overall activity in the DP nerve and to alter patterned motor activity in the form of fictive swimming. Whereas swim burst activity has been previously shown to increase in nerve cords bathed in either 5-HT or OA solutions alone, we demonstrated that a mixture of the two amines resulted in a robust decrease in the number of swim bursts expressed and an inhibition of swim activity in preparations already swimming. Most compelling was the observation that when the amine mixture was replaced with normal saline, swim burst activity increased dramatically. We discuss that the effects of amine mixtures may be due to their interaction with descending interneurons known to trigger and inhibit swimming as the mixture-induced effects were not observed in nerve cords lacking the head and tail brains. Because the net effect of the two amines was not simply additive (i.e., 5-HT or OA is known to activate swimming, yet the mix inhibits swimming), this result reveals yet another layer of complexity inherent in “simpler” invertebrate nervous systems.

INTRODUCTION

The biogenic amines, octopamine (OA) and serotonin (5-hydroxytryptamine, 5-HT), are known to have a prominent influence over the CNS of the medicinal leech, sometimes inducing striking changes in an animal’s overall behavioral state. In invertebrates, examples of large-scale organizational effects induced by these amines include alterations in aggressive and submissive posturing (Kravitz 2000; Kravitz et al. 1985; Livingstone et al. 1980) and the expression of leech swimming and feeding-related behaviors (Lent et al. 1991; Wilson et al. 1996). In insects, OA has been repeatedly implicated in the orchestration and modulation of complex behaviors (Adamo et al. 1995; Casagrand and Ritzmann 1992; Monastirioti et al. 1996; Ramirez and Pearson 1991; Sombati and Hoyle 1984; Taylor et al. 1992). The biogenic amines are also known to have a strong influence on stomatogastric pattern-generating neural networks in crustaceans (Flamm and Harris-Warrick 1986a,b).

Over a number of years, researchers have focused on the crustacean stomatogastric nervous system to study how neuromodulators, including the biogenic amines, reconfigure neural circuits. Such studies have documented that various neuropeptides and amines are able to affect the intrinsic membrane properties and synaptic efficacies of neurons so that distinct motor patterns can ultimately emerge. The impressive number of neuromodulators found associated with the crustacean stomatogastric ganglion continues to expand. For example, the crab stomatogastric ganglion is associated with neural inputs containing nearly 20 different neuromodulatory substances, many of which act in a modulatory way (Abbott and Marder 1998). Although it is unlikely that only one modulatory substance is released at any given time, little is known about the co-activation of neuromodulatory neurons and the effects of neurotransmitter mixtures (Dickinson et al. 1997; Marder et al. 1995).

In the leech, either 5-HT or OA is known to promote swimming (Hashemzadeh-Gargari and Friesen 1989; Willard 1981). The dependence of swim on 5-HT has been indicated by observations that chemically induced 5-HT depletion can eliminate fictive swimming (Glover and Kramer 1982; Hashemzadeh-Gargari and Friesen 1989; O’Gara et al. 1991). The role of OA and its effects on swim burst activity have not been as extensively researched. This is likely due to the fact that the modulation of swimming activity is more sensitive to 5-HT (Hashemzadeh-Gargari and Friesen 1989) as well as the questionable identification of OA neurons, which have been confirmed relatively recently (Gilchrist et al. 1995).

Swimming in the leech results from the rhythmic output of a CNS pattern generator (Friesen 1985, 1989a,b; Friesen et al.)
Electrodes were placed in Vaseline wells containing the cut end of the petroleum jelly (Vaseline)/mineral oil mixture. Alternatively, wire electrodes were placed in Vaseline wells containing the crude oil/mineral oil mixture. The nerve and wire electrode were then surrounded with a swimming in the presence of 5-HT (Willard 1981).

As six or seven ganglia are sufficient to express fictive effects of amine mixtures were examined using only ganglia 2–14. Unless noted, all experiments were performed on isolated whole leech nerve cords, which included two compound head and tail “brains.” In one set of experiments, however, we examined the ability to evoke swimming (Hashemzadeh-Gargari and Friesen 1989). Our results demonstrate that mixtures of amines together may influence swimming activity. In the current study, the actions of mixtures of OA and 5-HT on the leech CNS are described and compared with the effects of each amine individually. Our results demonstrate that mixtures of OA and 5-HT can alter CNS neural activity in ways that are not merely additive. The knowledge that specific mixtures of neuromodulators can produce unique behavioral states adds to the ever increasing complexity of simpler neural networks and how they are regulated.

METHODS

Animals

Experiments were performed on adult Hirudo medicinalis obtained from Leeches USA (Westbury, NY) or Biopharm (Charleston, SC). Leeches were maintained in artificial pond water (0.5 g/l Hirudo-salt from Biopharm) at room temperature. More than 50 animals were used during the course of this study.

Electrophysiological recordings

All adult leeches were anesthetized by cooling on ice for 10–15 min and then dissected in cold normal leech saline (in mM) 115.0 NaCl, 1.8 CaCl$_2$, 4.0 KCl, and 10.0 Tris-maleate) (Nicholls and Baylor 1968). The leech CNS is comprised of 21 unfused ganglia, and two compound head and tail “brains.” Unless noted, all experiments were performed on isolated whole leech nerve cords, which included both the head and tail brains. In one set of experiments, however, the effects of amine mixtures were examined using only ganglia 2–14 (G$_2$–G$_{14}$), as six or seven ganglia are sufficient to express fictive swimming in the presence of 5-HT (Willard 1981).

Swim motor activity can be assayed by recording extracellularly from the segmentally repeated dorsal posterior (DP) nerve (Kristan et al. 1974). Extracellular wire electrodes were placed in Vaseline wells containing the cut end of the DP nerve. Recordings were made in two ganglia, one in a more anterior region (G$_2$–G$_6$), and one in a more posterior region of the animal (G$_{14}$–G$_{16}$). In the set of experiments that included only G$_2$–G$_{14}$, however, one recording was made in G$_2$–G$_6$ and the other in G$_{14}$–G$_{16}$.

Preparations were perfused with normal leech saline at a rate of 1.0 ml/min (Hashemzadeh-Gargari and Friesen 1989) using a Rainin Rabbit-plus perfusion pump (Woburn, MA). The volume of the bath was always maintained between 1.5 and 2.0 ml. Activity in the DP nerve was amplified using a Grass P15 preamplifier (Quincy, MA), displayed on a Tektronix 5113A storage oscilloscope (Tektronix, Beaverton, OR) and recorded with a Brush/Gold chart recorder (Cleveland, OH). In addition, some extracellular signals were displayed and recorded using the MacLab/4 s data acquisition hardware and PowerLab/MacLab Chart v3.6.3/s software (ADInstruments, NSW, Australia) installed on a Macintosh Performa 5200CD.

Data analysis

We analyzed activity in the DP nerve during various treatment situations. Data were collected so that we could decipher two different characteristics of DP nerve activity: overall activity, as measured by the number of action potentials recorded, and changes in patterned activity. To determine a value for overall activity in the DP nerve, the
number of action potentials recorded extracellularly was counted during three 5-s intervals within every 1 min of recording. In terms of overall activity in the DP nerve, the total number of action potentials recorded was counted, and no attempt was made to differentiate between particular units. For example, swim-related units were not omitted. Within a 1-min period of recording, spike counts were started at 0, 20, and 40 s. The value for each minute interval was the average of the three readings. The dependent variable was the log base 10 of these mean values. The choice to analyze unpatterned DP nerve activity on a log scale reflects our assumption that a slight decrease in activity on a log scale reflects our assumption that a slight decrease in the three readings. The value for each minute interval was the average of these mean values. The choice to analyze unpatterned DP nerve activity on a log scale reflects our assumption that a slight decrease in spike number in a very active preparation is not as physiologically significant as a decrease of the same magnitude in a less active preparation. Data were analyzed using a two-way ANOVA for repeated measures (JMP 4 Statistical Discovery Software, SAS Institute, Cary, NC). P values of < 0.05 were deemed significant. Post hoc analyses were conducted using a Tukey’s studentized range (HSD) test for least-squared means comparisons with a significance level of 0.05.

For changes in patterned activity, we specifically looked for swim motor activity in the DP nerve. This was defined as three or more consecutive bursts with a cycle period of 0.4–2.0 s (Kristan et al. 1974). The bursting activity of the central swim oscillatory network (Friesen 1985; Friesen et al. 1978) is imposed on motoneuron 3 and other motor neurons. Activity of cell 3, an excitatory motoneuron of the dorsal longitudinal muscles (Kristan et al. 1974), is easily identified as a large spike recorded from the DP nerve. The number of bursts in the DP nerve defines the number of cycles in the swim episode and is also used for determining the length of the swim episode. Use of the words swim, fictive swimming, and swim burst activity are used synonymously in this paper. To determine whether the form of the swim motor pattern varied among the different amine treatments, swim cycle period (onset to onset) and burst duration were measured. Contingency tables were used to examine the number of animals swimming in two treatment conditions: 50 μM 5-HT or 50 μM OA alone and 50 μM 5-HT/OA mix. Numbers of animals swimming after 30 min of amine treatment and after 30 min of washout were analyzed separately using the Fisher exact test for independence (Rees 1995). P values of < 0.05 were deemed significant.

RESULTS

Overall DP nerve activity

Application of 50 μM 5-HT alone to the isolated nerve cord (delivered at t = 30 min) resulted in a slight and variable decrease in DP nerve activity as compared with baseline activity (n = 7). A representative example of such a response and its time course is depicted in Fig. 1A. As shown in Fig. 1A, activity tended to decline within the first few minutes of 5-HT perfusion and recovered to baseline or above baseline levels within the first 15 min of washout. This depression, however,
was found not to be statistically significant from baseline (see following text). In contrast, perfusion of a 50 μM OA solution did not show a similar trend, and no measurable alteration in the number of action potentials was observed (Fig. 1B; n = 5).

Perfusion of nerve cords with the 50 μM 5-HT/OA mix (n = 11), however, resulted in a significant decrease in DP nerve activity at 15 and 30 min after mixture application (Fig. 1C, see t = 45 and 60 min). The statistical significance of this depression was ascertained using an ANOVA for repeated measures (see METHODS). Specifically, this analysis revealed a significant treatment versus time interaction (F15,90 = 3.39; P < 0.001; Fig. 2). Post hoc analysis for significant comparisons (P < 0.05) showed that the 50 μM 5-HT/OA mix was significantly depressed at t = 45 min and t = 60 min relative to its saline baseline (t = 30 min) and washout (t = 90 min; P < 0.05). At t = 45 min, the 50 μM 5-HT/OA mix was also significantly different from OA at all time points and from control saline at t = 60 min and t = 90 min (P < 0.05). Additionally, at t = 45 min, the 100 μM 5-HT/OA mix (n = 5) was statistically different from OA at all time points and from control saline at t = 60 min and t = 90 min (P < 0.05). The saline control (n = 5), OA treatment (n = 5), and 25 μM 5-HT/OA mix (n = 5) resulted in no statistically significant depression in DP nerve activity.

Patterned activity: fictive swimming

Swimming activity is known to occur when OA or 5-HT is applied to the nerve cord (Hashemzadeh-Gargari and Friesen 1989; Willard 1981). In the majority of nerve cords (head and tail brains attached) in which we recorded overall levels of DP nerve activity, changes in patterned activity were also examined. A given nerve cord was deemed to express fictive swimming when the DP nerve produced three or more bursts that were present in multiple ganglia (see METHODS). In response to 5-HT or OA, Fig. 3, A and B, shows representative extracellular DP nerve recordings obtained during fictive swimming.

Unexpectedly, we observed that swimming was not reliably activated in response to the 50 μM 5-HT/OA mix but rather in response to its removal. The form of this washout-induced swimming after 30 min of washout of each amine (50 μM 5-HT and OA alone) was 70% of preparations displayed swim burst activity during the 50 μM 5-HT/OA washout, but none displayed swimming after 30 min of mix application. Also shown are the data on brainless preparations (G2-G14) in which the 50 μM 5-HT/OA mix was applied and washed out.

To examine further the influence of the 5-HT/OA mixture on swim activation, we assessed if a given preparation was swimming 30 min after initial amine application (t = 60 min) and 30 min beyond washout (t = 90 min). Above a concentration of 25 μM 5-HT/OA, we observed that most preparations expressed swimming in response to the washout of the amine mixture, not during its application (Fig. 4). For example, after washout of an amine mixture (pooled data for the 50 and 100 μM 5-HT/OA mix), 93% of animals were swimming as compared with only 17% of animals swimming after washout of either 50 μM 5-HT or 50 μM OA alone. Presented in Table 1 is the contingency table depicting the numbers of animals swimming after 30 min of washout of each amine (50 μM 5-HT or OA) versus the 50 μM 5-HT/OA mix. Using a Fisher exact test for independence, washout of the mix was correlated with a greater number of animals swimming and fewer non-swimming animals as compared with either of the amines alone (n = 22, P < 0.05). In contrast, at the end of amine application (t = 30 min), there was no significant difference in the number of animals swimming between the two treatment conditions (n = 22; Table 2). Because application of either of the two amines induced swimming in only one-third of our preparations at this time point, this probably can account for the lack of significance between the two groups even though 100% of

![FIG. 3](image-url)  Representative DP nerve recordings of swimming episodes induced by different conditions. A: swim episode recorded during perfusion of 50 μM 5-HT; B: swim episode recorded during 50 μM OA perfusion; C: swim episode recorded during the washout period following a 30-min perfusion of the nerve cord with the 50 μM 5-HT/OA mix.

![FIG. 4](image-url)  Percentage of preparations displaying spontaneous (i.e., no electrical stimulation) swim burst activity in the DP nerve at the end of a 30-min perfusion of amines and at the end of a 30-min amine washout. Note that while 5-HT and OA alone resulted in an increase in swim burst activity in a number of preparations, the 5-HT/OA mixture, at a concentration >25 μM, yielded little swimming at the end of amine application (1 of 15 preparations). Also note that whereas preparations treated with OA continued swimming after 30 min of washout, preparations treated with 5-HT no longer displayed swim burst activity at this time. It is noteworthy that 70% of preparations displayed swim burst activity during the 50 μM 5-HT/OA washout, but none displayed swimming after 30 min of mix application.
animals in the mixture group were not swimming. From this data set alone, however, we were unable to determine whether the mix was ineffective in eliciting swimming or whether it was acting to inhibit it.

**Mixture-induced suppression of swimming activity**

Based on our observation that swimming rarely occurred during application of the 50 or 100 μM 5-HT/OA mix, we wanted to establish whether the mixture indeed had an inhibitory effect on swim activity. Thus to examine this possibility, an additional set of experiments was conducted (n = 5). After an initial 30-min baseline period (in saline), the 50 μM mixture was perfused through the bath for 20 min and then washed out for 20 min; this was repeated a second time. We observed that fictive swimming, induced by washout of the first mixture, was dramatically inhibited by application of the second mixture (Fig. 5). Upon washout of the second application of mix, nerve cords again resumed spontaneous swimming (Fig. 5). This inhibition is particularly compelling because application of either 5-HT or OA alone is known to promote swimming, as opposed to inhibiting it.

Next, we examined the robustness of this mix-induced inhibition by examining whether we could electrically induce swimming during periods of suppression. We used electrical stimulation of the DP nerve as a means to activate swimming (see Methods), as this method has been shown routinely to be a reliable way to induce swimming. Whereas either 5-HT or OA alone is known to promote swimming and could in fact inhibit swimming in preparations that were already swimming (Fig. 5). This mix-induced suppression was quite robust because DP nerve stimulation (a local swim bout, bursts in a single ganglion) by mix application. In four preparations, local swim bursts were initiated within 10 min of washout. In two preparations, local swim bursts were observed in multiple ganglia in anterior ganglia (G2–G4), but these occurred in phase without the usual phase lag observed during normal fictive swimming.

**Correlation between amine treatment and swimming activity**

Table 1. Contingency table for amine treatment and swimming activity after 30 min of washout

<table>
<thead>
<tr>
<th></th>
<th>Not Swimming</th>
<th>Swimming</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single amines†</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Mixture‡</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>

Correlation between amine treatment and swimming activity is significant (Fisher exact test, P < 0.05). † 50 μM serotonin (5-HT) or 50 μM octopamine (OA) alone. § 50 μM 5-HT/OA mix.

**DISCUSSION**

As compared with control nerve cords perfused with normal saline, we observed that application of a 50 μM 5-HT/OA mix resulted in a statistically significant decrease in DP nerve activity. This mixture-induced depression of DP nerve activity was reversible (Fig. 2). The effects of the 5-HT/OA mixture, which were nonadditive, were also apparent with respect to their actions on fictive swimming. Whereas either 5-HT or OA alone was shown to increase the percentage of preparations swimming during application (Fig. 4), a combination of the two amines in the perfusate resulted in few bouts of spontaneous swimming and could in fact inhibit swimming in preparations that were already swimming (Fig. 5). This mix-induced suppression was quite robust because DP nerve stimulation (a reliable way to induce swimming) was ineffective at initiating swim activity after 30 min of mix application. After mix washout, however, DP nerve stimulation could induce swimming in 80% of preparations previously tested (n = 5).

Another compelling observation was that after washout of the amine mixture, an increase in swim activity was seen in the majority of preparations examined. In preparations that showed no fictive swimming prior to removal of the mixture, the onset of swim bursts always occurred within the first 10 min after washout was initiated. Of 10 preparations tested with the 50 μM mixture, none were swimming after 30 min of mix perfusion, but all were swimming after 30 min of washout.

**TABLE 2. Contingency table for amine treatment and swimming activity after 30 min of application**

<table>
<thead>
<tr>
<th></th>
<th>Not Swimming</th>
<th>Swimming</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single amines†</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Mixture‡</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

Correlation between amine treatment and swimming activity is not significant (Fisher exact test). † 50 μM 5-HT or 50 μM OA alone. § 50 μM 5-HT/OA mix.
Possible mechanisms underlying mixture effects on leech swimming

Swimming in the leech results from the rhythmic output of a CNS pattern generator (Friesen 1985, 1989a,b; Friesen et al. 1978) and neuronal elements comprising the swim pattern-generating circuitry are, for the most part, segmentally distributed. A number of swim oscillatory neurons have been identified (Friesen 1989b). In addition, the head and tail brains are a primary source for higher-order swim control that factors into the decision to swim or not (Brodfeurer and Burns 1995). Activation of brain trigger neurons results in a prolonged activation of the gating neurons: cells 204, 21, and 61 (Brodfeurer et al. 1995). Although much is known about the identity of swim neurons and their connections, the entire swim network has yet to be described. How exactly 5-HT or OA influences the swim network remains unclear.

Serotonin has been shown to act on motoneurons to facilitate swimming activity and to depolarize the swim-gating neuron 204 (Friesen 1989a,b; Mangan et al. 1994a,b; Nusbaum and Kristan 1986). Cellular mechanisms involved in 5-HT activation of swimming include changes in postinhibitory rebound, afterhyperpolarization potentials, and delayed rectification (Angstadt and Friesen 1993a,b; Mangan et al. 1994a,b). Although it is not yet understood how OA might alter swim activity, some evidence points to alterations in the membrane properties of mechanosensory neurons (Belardetti et al. 1984; Catarsi et al. 1995).

Because either 5-HT or OA is known to activate swimming, it remains a puzzle how a combination of the two, acting at the level of the segmental swim oscillators, could cause an inhibition of the swim rhythm. That removal of the inhibitory mixture can induce swimming is somewhat less puzzling. Perhaps postinhibitory rebound of the swim triggering or gating neurons, and/or postinhibitory hyperexcitability of the sensory neurons, may contribute to the washout-induced effects we observed.

For swimming to occur, not only must the swim activating system be on, but a parallel swim-inactivating system must be turned off. Swim inhibitory interneurons (the SIN1 cells) in the head ganglion, for example, have been found to be part of this descending parallel pathway. Perhaps the inhibitory influence of the mix can be best explained by a potential inhibition of the activating pathway and an excitation of the inhibitory neurons. This idea is supported by our results that in the absence of the compound ganglia (n = 6), fictive swimming could not be elicited in response to mix washout. Additionally, segmentally uncoordinated “local” swim bursts were observed during mixture application, suggesting the absence of an inhibitory influence. In whole nerve cords with brains attached, we also saw this inhibitory influence. This was reflected by the observation that application of 5-HT alone resulted in fewer preparations swimming spontaneously (Fig. 4) as compared with others’ studies whose preparations typically lacked the head and tail brains (Friesen 1985; Hashemzadeh-Gargari and Friesen 1989; Kristan and Calabrese 1976).

Swimming behavior and amine concentrations

The concentrations of biogenic amines we used to induce fictive swimming were similar to those reported in numerous studies. For example, both Willard (1981) and Hashemzadeh-Gargari and Friesen (1989) have used concentrations in the 10 to 100 μM range or above. Because we did not desheath the ganglia, CNS exposure to bath-applied amines was probably less than that in the perfusate. Although bath application of amines has been the standard way to deliver neuromodulators to the CNS, and both amines have been found in the blood of leeches (ca. 2 μM for OA, Webb and Orchard 1980; ca. 15–80 nM for 5-HT, Willard 1981), Willard (1981) proposed that the initiation of swimming is likely not normally initiated by increases in circulating amine levels. Furthermore treatment of leeches with the toxic 5-HT analogue 5,7-DHT eliminates swimming in intact leeches (Glover and Kramer 1982) despite the fact that blood concentrations of 5-HT increase up to 58-fold (Lent 1984).

Assuming that swim induction is associated with the local or synaptic release of amines within the CNS, might the amine concentrations we delivered to the CNS be physiologically relevant? A recent study by Bruns et al. (2000) supports this possibility. An average intravesicular concentration of 270 mM 5-HT was determined for the leech Retzius neurons. In a complimentary study, intracellular stimulation of the Retzius cell, for as little as 10 min (3–6 Hz), was sufficient to generate a concentration of 5-HT equaling 37 nM in a 50 μl volume of saline (Willard 1981). Thus a prolonged activation of these serotoninergic neurons could easily result in relatively high levels of 5-HT distributed locally within the CNS.

Last, we argue against the idea that an amine concentration above 100 μM is the factor underlying the mixture effects we observed. Although the combined (mix) amine concentrations we used were in the 100 to 200 μM range, when a 100 or 200 μM solution of 5-HT or OA alone was applied to nerve cords, swim suppression and washout-induced activation of swimming were not observed (K. A. Mesce, personal observation). In addition, Hashemzadeh-Gargari and Friesen (1989) used a concentration of OA as high as 2,000 μM and 5-HT at a level of 100 μM; they reported that higher amine concentrations readily activated swimming rather than inhibit it.

The following scenario may provide behavioral significance for the mixture effects we observed. T and P mechanosensory activation of the DLOs (Gilchrist and Mesce 1997) causes OA to be released locally into the CNS, possibly as the leech approaches its host. Because hungry leeches possess higher 5-HT levels than sated leeches (Lent et al. 1991), both amines might reach a peak at a time when the animal needs to stop swimming and initiate feeding-related behaviors. After 20–40 min of feeding (Dickinson and Lent 1984) with no additional release of 5-HT or OA, the levels of amines may become lowered to a point where the inhibition of swimming is relieved and the animal is free to locomote to a new location. In support of this idea, at least for 5-HT, is the finding that the large serotoninergic Retzius neurons become quiescent at the onset of the consummatory phase of feeding (Wilson et al. 1996), thus no longer contributing to a source of 5-HT within the CNS.

Mixture effects in other model systems

Evidence is accumulating to show that the effects of a neuromodulator are indeed dependent on the physiological state of the animal (Chrachri et al. 1994; Dickinson and Nagy
1983; Dickinson et al. 1997; Hooper and Marder 1987; Marder and Nusbaum 1989; Prier et al. 1994). Dickinson et al. (1997) have clearly documented for the crustacean cardiac sac motor pattern that one neurotransmitter can alter a network’s response to a second neurotransmitter. The mechanisms underlying the interactions of these two peptidergic modulators (proctolin and RPCH), however, are not yet understood. The behavioral implications of neurotransmitter combinations have also been studied in the blue crab, Callinectes sapidus (Wood 1995). Data from this study support the idea that shared neural elements for swimming and courtship behavior can be influenced by the relative amounts of dopamine, OA, and the neuropeptide proctolin.

Because amines often influence second-messenger systems (Evans 1980, 1984a,b; Nathanson 1977), the actions of two amines on a single cell might result in membrane characteristics that differ from a single modulator. Because second-messenger systems do not always act independently of each other (Berridge 1987), such interactions could contribute to a mixture phenomenon. In addition, as enzymes and ion channels can be modified directly and via second-messenger systems, a vast array of alterations in the functioning of a single cell can be achieved. In the leech, for example, the same chloride ion channels in the P mechanosensory neuron appear to be dually regulated: by 5-HT, acting directly on the channel, and by dopamine, acting via the cAMP pathway (Ali et al. 1998). Importantly, the actions of a mixture of dopamine and 5-HT were found not to be simply additive with respect to changes in the probability of ion channel opening (Ali et al. 1998).

Even if no one cell in a network has receptors for more than one neuromodulator, the output of a circuit could easily be altered by modulator-induced alterations in the membrane properties and/or synaptic efficacies of just a few cells (Harris-Warrick and Johnson 1989; Harris-Warrick and Marder 1991; Marder and Calabrese 1996). For example, LTP potentiation in the hippocampus can be strengthened by prior opioid-induced suppression of inhibitory neurons (Bramham 1992), enabling glutamameric excitatory pathways to activate the N-methyl-D-aspartate receptors more strongly.

That the effects of 5-HT and OA on the leech CNS are not simply additive, once again attests to the complexity of “simple” nervous systems (Harris-Warrick et al. 1992). A cellular analysis of the effects of OA and 5-HT on identified neurons in the swim network may provide insights into how nonadditive phenomena arise and ultimately how behaviors are organized.

We are especially grateful to Prof. Sanford Weisberg, School of Statistics, and Prof. Roger Moon, Dept. of Entomology, for expert assistance with the statistical analyses. We also thank K. Schreiber, C. Wottus, A. Sandoval, and K. Klukas for technical contributions throughout the course of this project.

This research was supported by National Science Foundation Grants IBN-9419216 and IBN-9813995 to K. A. Mesce, a Louise T. Dosdall Fellowship to L. S. Gilchrist, and a Graduate School Fellowship to K. M. Crisp.

Present address of L. S. Gilchrist: Masters Program in Physical Therapy, College of St. Catherine, 601 25th Ave. S., Minneapolis, MN 55454.

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