well. A closer assessment of the functional properties of the songs of normally reared males may help to clarify these speculations. In addition, these data suggest the need to examine in more detail the developmental role of self-stimulation. It may be that the self-generated sounds of the isolate males channeled their song development toward an emphasis on the acoustic properties that contain the sexual message in cowbird song. Males reared with other males, however, whether age mates or adults, may receive acoustically more varied stimulation, may learn to modify their song components as a result of auditory and behavioral feedback from their companions (thereby leading to a dilution of the purely sexual message), or both. In any case, these data implicate self-stimulation as possibly a very important form of species-typical experience for cowbirds as has been hypothesized (*8*). Finally, although it is tempting to label the cowbird's response to isolate song as idiosyncratic or unrepresentative of other songbirds, this cannot be done, as little comparative information exists regarding the responses of other species to isolate songs. Such information would be useful in species that, like the cowbird, have courtship songs. Songs of a primarily territorial nature may be more difficult to assess.

Although these results provide one answer to the question of the mechanism by which cowbirds identify one another, they do not explain how young or juvenile cowbirds first come to recognize one another. Little is known about cowbird social behavior, yet data from our laboratory indicate that cowbirds have highly structured dominance hierarchies and complex intraspecific behaviors that may also facilitate identification or maintain social integration among acquainted cowbirds (*9*). The mechanism we have described might represent an independent system designed to ensure identification during the most important context, the breeding season, and to enhance reproductive opportunities by inducing sexually appropriate behavior in the female. Thus, although cowbirds may have multiple means to identify one another, the presence of a mechanism geared directly for reproduction may represent a critical adaptation for a parasitic species.

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4. Each chamber consisted of two concentric boxes constructed of plywood and sheetrock. Wood and acoustic tile baffles between the boxes were designed to be most effective between 2 and 16 khz. Suppression was greater than 39 db at 1000 hertz, and it increased with higher frequencies to greater than 50 db between 8 and 16 khz. The interior box was a 1.1-m cube, fabric-lined to reduce sound reflection, lighted by two 40-watt Vita Lite tubes and continuously ventilated. White noise was broadcast in the room housing the chambers.
5. The songs were recorded with a dynamic microphone (Uher 517) and tape recorders (Uher 4000). The recordings were played through a driver (JBL 2420) and horn (JBL 2340). Playback levels were determined with a sound pressure meter (General Radio 1933). The same sound pressure levels (SPL's) were used for playbacks of the normal and the abnormal songs. At 0.35 m from the speaker along the axis, the SPL was 86 ± 1.5 db slow reading and 104 ± 1.5 db impulse. Control songs of the other species were adjusted to the slow reading. The A-weighted SPL inside the chamber was 50 db slow reading.
6. Differences between categories of song were tested with *t*-tests. Reliable differences were obtained for the following comparisons: abnormal one or two versus normal song (abnormal one: $t = 11.04$, $P < .001$; abnormal two: $t = 9.95$; $P < .001$); abnormal versus control songs (abnormal one: $t = 8.85$, $P < .001$; abnormal two: $t = 19.43$, $P < .001$); normal versus control song ($t = 6.09$, $P < .001$); the "burble" phrase versus the "tsee" phrase ($t = 3.17$, $P < .05$); and either partial song versus the normal "burble" ($t = 4.22$, $P < .001$). There was no reliable difference between the two samples of abnormal song or between the "tsee" phrase and normal song.
7. Female CB's lack of response the previous year probably resulted from her being housed with a male cowbird, who repeated his song to her many times each day, thus raising her threshold for song responsiveness. Furthermore, her companion's song was undoubtedly of superior acoustic quality, a factor that might also have diminished her interest in the recorded songs.
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11. We thank G. Wilcox who designed the isolation chambers, R. Chu and D. Eastzer who helped in the field by collecting eggs and nestlings, R. E. Johnston for valuable suggestions during the initial stages of this project, and P. Cabe, W. Dilger, D. Miller, P. Ornstein, and H. Rheingold for helpful comments on the manuscript. We are especially grateful to G. Gottlieb for both technical and conceptual assistance.
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15 September 1976; revised 15 November 1976

Phenylethanolamine: A New Putative Neurotransmitter in *Aplysia*

Abstract. *Phenylethanolamine is present in the* Aplysia *nervous system in concentrations similar to that of octopamine. There are receptors that are very specific for phenylethanolamine, which on different neurons mediate sodium, chlorine, or potassium conductance increase responses. These observations indicate that phenylethanolamine may act as a neurotransmitter in* Aplysia.

Phenylethanolamine is a biogenic amine that differs from norepinephrine in lacking two ring hydroxyl groups. In mammals, it is synthesized from phenylalanine through the action of aromatic amino acid decarboxylase (to make phenylethylamine) and dopamine β-hydroxylase (*1*). Phenylethanolamine is present in mammalian fetal brain (*1*) but dramatically decreases in concentration with prenatal development (*2*). The presence of phenylethanolamine has usually been considered to be an accident of catecholamine synthetic pathways without a physiological function, although sympathetic nerve terminals can take up phenylethanolamine and store it to a limited extent (*1*).

The nervous system of the marine mollusk *Aplysia californica* lacks norepinephrine but contains considerable amounts of dopamine (*3*) and octopamine (*4*). Some *Aplysia* neurons have specific receptors for dopamine (*5*) and octopamine (*6*). We have detected the presence of a considerable amount of phenylethanolamine in the *Aplysia* nervous system and established the existence of specific receptors for this amine.

Phenylethanolamine was assayed as described by Saavedra and Axelrod (*1*) from buccal, pleural, pedal, cerebral, and abdominal ganglia and from various nerves (Table 1). It is present in all ganglia, its concentration being highest in the buccal (2.32 ng per milligram of protein) and lowest in the pedal (0.73 ng per milligram of protein). The identity of endogenous phenylethanolamine was confirmed by thin-layer chromatography (*1*). The amount of phenylethanolamine in these tissues does not significantly differ from that of octopamine (*4*). Phenylethanolamine is present in the ganglionic neuropil, the region of normal synaptic contact. Its concentrations in this area are lower than those in the whole ganglion in the case of the buccal, pedal, and pleural ganglia. Thus, the possibility remains that phenylethanolamine is also present in neuronal cell bodies. However, to our knowledge no phenylethanolamine has yet been found in isolated, identified neurons. Phenylethanolamine was also found in the nerves, in particularly high concentration in the pleural-abdominal connective nerves.

Electrophysiological studies were performed as previously described (*6*). Phenylethanolamine was dissolved in dis-

tilled water (2*M*, *p*H 3.5) and applied by ionophoresis through one barrel of a five-barreled ionophoretic electrode; the other barrels usually contained octopamine, dopamine, norepinephrine, and acetylcholine. In some experiments tyramine and phenylethylamine were studied, but no responses were found to these drugs. When appropriate, bath application of these putative neurotransmitters was made to confirm sensitivity or lack of sensitivity.

Figure 1 illustrates the variety of responses recorded in *Aplysia* neurons on ionophoretic application of phenylethanolamine. Only a minority of neurons showed a specific response, either on ionophoresis or bath application (14 of 71 neurons). Most responsive neurons were found in either cerebral or buccal ganglia, but occasional responsive cells were found in other ganglia. In every case the phenylethanolamine receptors were located exclusively in the neuropil. As in the case for acetylcholine (*7*), serotonin (*8*), and dopamine (*9*), three different ionic responses were found with phenylethanolamine. The neuron in Fig. 1A showed a depolarizing response, which peaked at about 2 seconds and was totally abolished by perfusion of Na^+-free ($tris^+$) seawater. These responses were rare. The cell in Fig. 1B showed a comparatively fast hyperpolarization (peak at 2 seconds), which reversed at potentials more negative than −60 mv and was abolished in Cl^--free (acetate) seawater (*10*). The neuron in Fig. 1C had a hyperpolarization with a slower time course, which did not reverse below −80 mv and was essentially unaffected by perfusion with Cl^--free seawater. All three types of response were often (but not always) associated with a measurable increase in membrane conductance. The failure to observe a conductance increase in some cases is probably a result of the fact that the receptors are located at some distance from the soma recording site (*5*). Thus we conclude that these three responses result from specific conductance increases to Na^+, Cl^-, and K^+, respectively.

Most of the phenylethanolamine responses (as in Fig. 1, A and B) were found in neurons unresponsive to other phenylethylamines, and therefore it can be assumed that the receptors were very specific for phenylethanolamine. In some cases, however, as in the neuron in Fig. 1C, there was a similar ionic response to dopamine, although it was considerably smaller in amplitude for equal charge application. Since dopamine and phenylethanolamine would cross-desensitize the receptor, they appear to be acting on a common receptor. The facts that phenylethanolamine was always more effective and that the specific dopamine receptor has a very different pattern of structure and activity requirements (*9*) indicate that functionally this is probably a phenylethanolamine receptor. It is not clear whether receptors not responsive to dopamine represent a different class of phenylethanolamine receptors. As previously reported (*4*), octopamine receptors can be activated with reduced sensitivity by both phenylethanolamine and norepinephrine. In contrast, the phenyl-

Table 1. Distribution of phenylethanolamine in *Aplysia* nervous system. Each value is the mean ± standard error of the mean for a group of six individual values. Octopamine results are from Saavedra *et al.* (*4*).

Location	Phenylethanolamine (ng/mg)	Octopamine (ng/mg)
Ganglion		
Buccal	2.32 ± 0.23	2.12 ± 0.52
Cerebral	1.75 ± 0.28	1.90 ± 0.71
Pleural	1.74 ± 0.28	1.55 ± 0.39
Abdominal	1.12 ± 0.11	1.19 ± 0.24
Pedal	0.73 ± 0.07	1.28 ± 0.44
Neuropil		
Buccal	1.61 ± 0.45	
Cerebral	2.91 ± 0.30	
Pleural	0.85 ± 0.22	
Pedal	0.39 ± 0.08	
Nerves		
Pleural-abdominal connectives	2.31 ± 0.48	0.72 ± 0.16
Posterior parapodial	1.41 ± 0.20	0.42 ± 0.13
Cerebral-buccal, cerebral-pleural, and cerebral-pedal connectives	1.43 ± 0.18	1.35 ± 0.22
Cerebral nerves to periphery	0.91 ± 0.10	0.67 ± 0.09

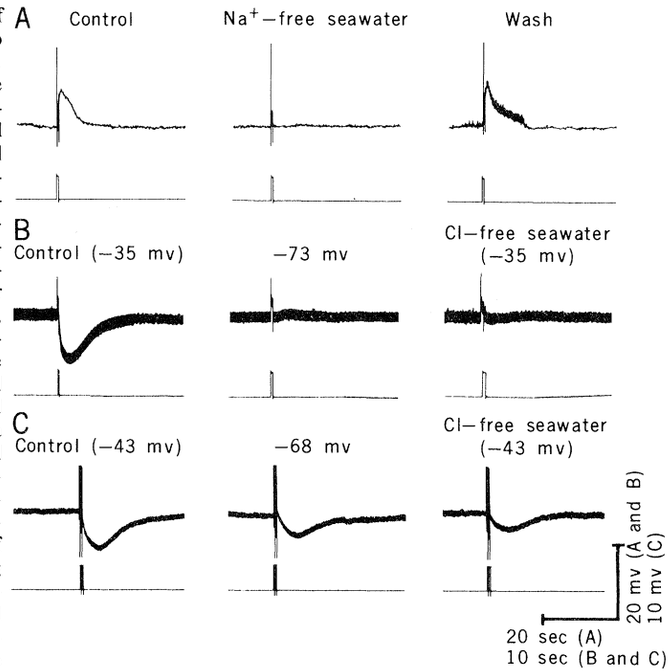

Fig. 1. Responses of *Aplysia* neurons to phenylethanolamine. The upper traces are intracellular recordings from unidentified neurons in (A) buccal and (B and C) cerebral ganglia. The ionophoretic charge application for a five-barreled electrode is indicated in the lower trace, and was 500, 500, and 1000 nanocoulombs (nc). The ionophoretic control unit was constructed so that it passed a predetermined total charge without varying the applied voltage. The technique allows better control of electroosmotic drug movement when several drugs are applied through multiple barrels. However, since time is varied rather than applied voltage, the duration of the pulse (although not the amount of applied drug) is not constant. The neuron in (A) was unresponsive to 1000 nc of dopamine and octopamine but had a depolarizing response to acetylcholine. The middle record in (A) was taken 4 minutes after beginning perfusion with seawater in which $tris^+$ replaced all Na^+; the record at the right was taken 10 minutes after washing was begun. The neuron in (B) was insensitive to dopamine, norepinephrine, octopamine, and histamine, while that in (C) had a very small hyperpolarizing (K^+) response to dopamine but was unresponsive to octopamine, norepinephrine, and acetylcholine. In (B) and (C) the neurons were penetrated with two independent intracellular electrodes for recording and current passage. The middle records show the response after membrane potential was hyperpolarized past the equilibrium potential for Cl^- (about −60 mv) and show that the response in (B) reverses while that in (C) does not. The Cl^- response in (B) is also abolished by replacement of Cl^- in the seawater with acetate [right-hand record in (B)].

ethanolamine receptors are totally insensitive to octopamine.

The observation that three types of conductance increase responses to phenylethanolamine have been found is consistent with the suggestion by Swann and Carpenter (*9*) that a single class of neurotransmitter binding site in this preparation may be coupled to at least three different ionophores. The three ionophores mediate Na^+, Cl^-, and K^+ conductance changes, respectively. This model provides an explanation for the occurrence of at least three different ionic responses to several transmitters (*7–9*) and for the similarities in several properties of the responses to different neurotransmitters, but resulting from the same ionic conductance.

These experiments provide strong evidence that phenylethanolamine may have a direct role in synaptic transmission in *Aplysia*. The presence of specific receptors for a substance present in nervous tissue does not necessarily prove a neurotransmitter role. However, the facts that the receptors are localized to the natural synaptic region, are found on only a small minority of neurons, and are usually highly specific for phenylethanolamine suggest that these responses do not result from some nonspecific membrane interaction.

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10. It is somewhat surprising that the Cl^- response was only abolished and not reversed in Cl^--free seawater. This was not a function of duration of exposure to the Cl^--free solution and also was not due to any effect on membrane resistance. Whereas we have always been able to reverse responses to acetylcholine and γ-aminobutyric acid, where receptors are located on the cell body, the apparent Cl^- responses to several other putative neurotransmitters with receptors located only in the neuropil could not be reversed (D. O. Carpenter, J. W. Swann, P. J. Yarowsky, *J. Neurobiol.*, in press).

30 July 1976; revised 18 October 1976

Ecdysis: Neural Orchestration of a Complex Behavioral Performance

Abstract. *Cricket ecdysis (molting) requires continuously changing output in hundreds of motoneurons over a period of several hours, and exhibits considerable plasticity. Despite this complexity, analysis of identified motor units reveals a highly organized three-layered infrastructure, and indicates that the "small systems" paradigm currently applied to simple invertebrate motor programs can be extended to much more sophisticated behavioral performances.*

The goal of neurobiology is to explain behavior in terms of the operation of the nervous system. Success in fairly complete analysis has so far been confined primarily to invertebrate behaviors. Here, the "small systems" approach, investigation of identified nerve cells, has been very fruitful at the level of synaptic function, diversity, and plasticity and, more recently, at the level of motor program generation, sensory modulation of motor output, executive control of motor output, and decision-making (*1, 2*). However, the behaviors examined have been conspicuously restricted both spatially, to a portion of the body, and temporally, to acts that are episodic (crayfish tail flips; *Aplysia* gill withdrawal), rhythmic (leech swimming; locust flight), or tonic (postural) and have a duration or repetition time on the order of milliseconds (*1, 2*). Behavior treated by ethologists, on the other hand, tends to involve extensive sequences of motor patterns (waterfowl or fish courtship; ant or bee foraging; caddis-fly nest building) and to occur on a time scale of minutes, hours, or days. A question of considerable import has been whether or not such "real" behaviors involve a quantum jump in sophistication that renders them inaccessible to the concepts and techniques of small systems neurobiology.

In this report we analyze a complex invertebrate behavior, cricket ecdysis (molting), in terms of the output of identified motoneurons. The behavior lasts over 4 hours and demands the carefully controlled coordination of practically every muscle in the animal in a sophisticated series of operations; it is normally stereotyped but can evince substantial flexibility under unusual conditions. Analysis reveals that the complete performance is brought about by the hierarchically controlled integration of numerous subelements, each of which appears comparable to the simpler motor programs previously analyzed. The main features are (i) sequential activation of about 48 relatively discrete motor programs, (ii) temporal and spatial coordination of concurrently active motor programs by a bout rhythm generator, and (iii) modulation of the bout rhythm and clustering of the programs into four major phases. This multilayered arrangement is effected by the interaction of central neural elements, sensory feedback, and very likely endocrine triggering. Therefore, to the degree that this behavior is typical, complex performances are brought about by the incorporation of many simpler motor programs, of the sort already studied, with the addition of overall coordinating and controlling elements.

Crickets, *Teleogryllus oceanicus*, were raised in the laboratory and isolated before the final molt. Ecdyses were videotaped (Sony V-32; AVC 3210 videocamera; 1:1.8 zoom lens with three-stage close-up lens) and analyzed on a frame-by-frame basis (20 msec per frame). Participation of single, identified motor units was determined by intramuscular recording from 25-μm insulated silver electrodes and stored on tape (TEAC TCA 40). In some cases, the behavior and an oscilloscope display of motor unit discharge were simultaneously videotaped. Recordings from 22 muscles were made during 77 ecdyses, 11 complete ecdyses were videotaped, and additional manipulations or observations were performed on another 66 animals, for a total of 154 ecdyses analyzed.

The ecdysial process is a mechanically difficult task effected by the following four operations. (i) A preparatory phase serves to loosen and split the old exuvia and to anchor it to the substrate. It involves restless locomotor and grooming activity; rhythmic leg movements, which fix the tarsal claws into the substrate; abdominal contractions and air-swallowing to exert pressure on the ecdysial line where splitting will occur; and assumption of a characteristic posture that facilitates emergence. (ii) The ecdysial phase extracts the animal from the old cuticle. Peristaltic abdominal waves propel the body forward and increase hemolymph pressure to widen the ecdysial split as appendages are extricated by a complex sequence of muscle contractions pulling them up and forward. (iii) The expansional phase inflates the new cuticle, protects