LOCOMOTION IN *APLYSIA*: TRIGGERING BY SEROTONIN AND MODULATION BY BAG CELL EXTRACT¹

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Abstract

Locomotion in *Aplysia* is mediated by a central program. We have found that this program is triggered by the biogenic amine, serotonin (5-HT), and modulated by an endogenous source of neuropeptides, bag cell extract.

In examining the triggering of locomotion, we found that injecting 5-HT into the bloodstream of quiescent animals elicited locomotion in both intact, freely moving animals and split-foot preparations. Increasing the concentration of 5-HT from 10^{-7} to 10^{-3} M produced both a monotonic increase in the number of steps elicited over a 5-min period after injection and a monotonic decrease in the first interstep interval. The threshold for triggering locomotion was 10^{-7} M in split-foot preparations. Application of a saturated salt solution to the tail—a natural trigger for escape locomotion—facilitated 5-HT locomotion. The effects of 5-HT on locomotion were relatively specific since they were not mimicked by three other biogenic amines known to exist in *Aplysia* (dopamine, octopamine, and histamine). 5-HT could still trigger locomotion after isolating the pedal and pleural ganglia from the rest of the central nervous system, suggesting that a primary site for the action of 5-HT on locomotion lies in the pedal and pleural ganglia.

In examining the modulation of locomotion, we found that injecting bag cell extract, an endogenous source of neuropeptides in *Aplysia* which elicits egg-laying behavior, reversibly suppressed 5-HT-triggered locomotion. As in the case with serotonin, bag cell extract suppression of locomotion was still observed in animals in which the pleural and pedal ganglia were isolated from the rest of the central nervous system. The suppression of locomotion was specific to bag cell extract since no suppression was produced by extracts of other central nervous tissue (cerebral ganglia). Moreover, the suppressive effect of bag cell extract was abolished by prior treatment of the extract with pronase, suggesting that the active suppressive agent is one or more of the peptides specific to the bag cells. Thus, locomotion in *Aplysia* provides a useful model system for examining both the triggering and the modulation of a fixed action pattern.

Locomotion in *Aplysia californica* is a centrally programmed behavioral sequence which is utilized by the animal to accomplish a number of different behavioral objectives. Thus it is integrated with many other components of the animal's behavioral repertory. As a result, locomotion can be elicited and modulated by a variety of

factors including appetitive stimuli (Preston and Lee, 1973; Kupfermann, 1974; Advokat et al., 1978), noxious stimuli (Wachtel and Impelman, 1973; Walters et al., 1978), reproductive behavior (Strumwasser et al., 1969, 1980; Arch, 1976), and both nonassociative and associative learning (Walters et al., 1979, 1981). Moreover, locomotion in *Aplysia* is well suited for a cellular analysis (Jahan-Parwar and Fredman, 1978a, b, c; Henning et al., 1979). Thus, this fixed action pattern is an excellent system to explore the neural mechanisms involved in the triggering and modulation of a complex behavior. As a first step in this direction, we have examined the triggering effects of several biogenic amines and the modulating effects of bag cell extract (BCE), which contains at least four neuropeptides, including egg-laying hormone (Loh et al., 1975; Arch et al., 1976a, b; Stuart et al., 1980; Scheller et al., 1982). We found that micromolar injec-

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tions of the biogenic amine, serotonin (5-HT) into the bloodstream of non-locomoting animals triggers locomotion. This effect seems to be specific to 5-HT because it is not mimicked by other biogenic amines known to exist in *Aplysia*. We have found that 5-HT is likely to exert its primary action on neurons in the pedal and pleural ganglia since it can still elicit locomotion in preparations in which these ganglia have been surgically isolated from the rest of the central nervous system (CNS).

We have also examined the modulating effects on 5-HT-triggered locomotion of an endogenous source of several neuropeptides in *Aplysia*, BCE, which triggers egg-laying behavior (Kupfermann, 1967). We have found that injections of BCE prior to injections of 5-HT reversibly suppress 5-HT-triggered locomotion. This suppressive effect is observed both in animals with intact nervous systems and in animals in which the pedal and pleural ganglia are isolated from the rest of the CNS but are connected to the periphery. Furthermore, the suppressive effect of BCE is abolished by prior treatment of the extract with pronase, suggesting that the active agent in the extract is a neuropeptide. Thus, locomotion in *Aplysia* provides a useful model system for examining triggering and modulation of a complex fixed action pattern on both behavioral and cellular levels.

Preliminary results of some of the experiments described in this paper have been reported previously in abstract form (Mackey et al., 1981).

Materials and Methods

In our experiments we have used two types of preparations: (1) intact, freely moving animals with a cannula chronically implanted in their anterior aorta, and (2) split-foot preparations whose CNSs are completely exposed.

Intact animals. In six animals, the anterior aorta was exposed, and a T-shaped cannula (1-mm internal diameter) was inserted into its lumen and tied at both ends (Koch and Koester, 1982). Each animal was first injected with 25% of its body weight of isotonic $MgCl_2$. A 3- to 4-cm incision was made on the side of the foot at the junction with the tegument and body wall, starting at the caudal end of the pro-segment. A 15-cm length of Silastic tubing (0.5-mm internal diameter) was attached to the long arm of the T and passed through the body wall, allowing substances to be directly injected into the artery through the Silastic tubing. The Silastic tubing was kept filled with artificial sea water (ASW) and plugged at its free end to prevent blood loss and to maintain normal blood pressure. The volume of the dead space was approximately 0.2 ml. The incision was sutured closed and the animal's initial body weight was restored with an injection of ASW into the hemocoel. The animal was allowed to recover for at least 24 hr.

Split-foot preparations. Behavioral studies were also performed using a modified split-foot preparation (Hening et al., 1979). Animals weighing between 150 and 300 gm were anesthetized with $MgCl_2$ and pinned dorsal side up in a Lucite chamber filled with isotonic $MgCl_2$ solution. The viscera and buccal mass were excised and the anterior end of the anterior aorta was tied off. A longi-

tudinal incision was made down the middle of the foot from the propodium to the tail, approximately three-fourths the length of the foot. The $MgCl_2$ solution was removed and replaced with ASW. The anterior aorta was cannulated and perfused with aerated ASW. The cannula was equipped with a diaphragm that allowed direct injections of substances into the bloodstream. The duration of injection was approximately 5 sec; transit time from injection to arrival at the CNS was approximately 15 sec. In some experiments either the pedal-cerebral, pleural-cerebral, and pleural-abdominal connectives and commissure or the nerves of the pedal and pleural ganglia were cut; otherwise the circumesophageal ganglion and the peripheral nerves were left intact. All animals were perfused with normal ASW for at least 1 hr before testing. The arterial perfusion rate was maintained at approximately 1.5 ml/min throughout the experiment.

Locomotion was recorded with three Grass FT 0.03 strain gauges attached by elastic threads to stainless steel hooks which, in turn, were hooked through silk suture loops sewn into the foot. The sutures were placed at three sites along the margin of the podium and tegument: two at bilaterally symmetrical sites on the right and left side of the pro-segment and a third on the right side at the meso- or meto-segment. This arrangement allowed us to distinguish true "steps" during locomotion from other types of contractions that were not part of the locomotor pattern, since normal steps (1) occur bilaterally at homologous sites on the foot and (2) consist of a monotaxic wave that progresses in a retrograde direction along the foot (Jahan-Parwar and Fredman, 1978a; Hening et al., 1979).

Bag cell and control extracts. Animals weighing between 250 and 500 gm were sacrificed, and either bag cells or cerebral ganglia were removed. The bag cell clusters or cerebral ganglia (used for control extracts) from an individual animal were placed in 0.3 ml of ASW and homogenized by hand with glass pestles in glass homogenizers, and then were kept on ice. Each homogenate was transferred to centrifuge tubes, and an additional 1.2 ml of buffer was used to rinse out the homogenizer and was transferred to the homogenate. The extract was centrifuged at 10^4 rpm for 10 min at $4^\circ C$ using a Beckman centrifuge. The supernatant fluid was pooled and frozen at $-20^\circ C$ until used. Each injection contained the equivalent of the bag cell clusters or cerebral ganglia of a single animal. Samples were used within 30 min of thawing.

Pronase treatment. Proteolytic treatment of BCE was carried out with pronase (90 units/ml) based on a modification of a technique used by Kupfermann and Weiss (1976). The pronase was incubated for 30 min to destroy any contaminating enzyme activities. BCE was prepared as described above. After centrifugation the extract was divided into control and experimental aliquots of 0.75 ml each. Pronase (0.75 ml) was added to the experimental sample, and 0.75 ml of ASW buffer was added to the control and allowed to incubate. After incubation, 75 μ l of 2 N HCl were added to each sample to eliminate enzymatic activity. The samples were then centrifuged for 10 min at low speed and the supernatant was removed. Tris buffer was added to each supernatant to return the

pH to 7.8. Both control and experimental samples contained the equivalent of bag cell from a single animal.

Statistical analysis. All statistical tests were carried out by means of a *t* test for either correlated means (within-group comparison) or independent means (between-group comparison). All comparisons were two-tailed unless otherwise stated. All errors are reported as standard errors of the mean (SEM).

Results

5-HT elicits locomotion in intact animals. Since the biogenic amine serotonin (5-HT) has a prominent role in both defensive and appetitive arousal responses (Weiss and Kupfermann, 1978; Kandel, 1979), we first examined the effect of 5-HT on locomotion in quiescent animals. We used six freely moving animals with surgical implants that allowed direct injection of 5-HT into the anterior aorta. We first recorded locomotion over a 5-min period after an injection of 1 ml of ASW to serve as a base line control for injection per se. The duration of injection was approximately 30 sec. We then injected into the anterior aorta increasing concentrations of 5-HT, from 10^{-7} to 10^{-3} M (pH of all solutions, 7.8). The duration of injection was approximately 30 sec. Each injection was separated by 10 min. The threshold concentration for triggering locomotion ranged from 10^{-6} to 10^{-4} M with 50% of the animals locomoting after a 10^{-5} M injection. These concentrations refer to the concentration of the 1-ml injection. The effective concentration was probably much less, due to dilution by the hemolymph. 5-HT elicited significantly greater locomotion (as measured by the number of steps in a 5-min period; see Walters et al., 1979) than did ASW in all animals ($p < 0.02$). In fact, ASW elicited locomotion in only one animal, and 5-HT in that animal triggered a greater number of steps. Thus, 5-HT, in effective concentrations in the micromolar range, can trigger locomotion in intact, freely moving animals.

5-HT elicits locomotion in split-foot preparations in a dose-dependent fashion. We next extended our study of the effect of 5-HT on locomotion in a modified split-foot preparation, a simplified preparation in which reliable replicas of locomotion can be observed (Hening et al., 1979; Fig. 1A). The split-foot preparation simplifies the screening of neuroactive substances and is also amenable for subsequent cellular studies. During locomotor episodes the foot exhibits rhythmic patterns of contraction, as observed in freely moving animals, with waves beginning at the anterior margin of the foot and moving posteriorly (Jahan-Parwar and Fredman, 1978a, b, c; Hening et al., 1979). We recorded steps with strain gauges attached to the foot (see "Materials and Methods"). In all experiments, we used total number of steps exhibited in a 5-min test period following injection of a substance as a measurement of the triggering effects of that substance. To distinguish between triggered and spontaneous locomotion, only steps occurring within 60 sec of injection and with less than a 60-sec interstep interval were counted as triggered locomotion.

Using split-foot preparations, we first recorded locomotion over a 5-min period in previously non-locomoting animals after an injection of ASW to serve as a control for the effects of injection per se. We then injected

increasing concentrations of 5-HT, from 10^{-7} to 10^{-3} M, into the anterior aorta. The effective concentration was probably much less, due to dilution in the cannula and anterior aorta. Injections were separated by 10 min or until the animal stopped locomoting for 10 min after a prior injection. An example of 5-HT-triggered locomotion is shown in Figure 1B, and the relationship between 5-HT concentration and number of steps is shown in Figure 2A. After an injection of 10^{-7} M 5-HT, 50% of the animals ($N = 4$) locomoted within 60 sec of injection. The mean number of steps was 1.5 ± 0.87 as compared to control injections of ASW, which produced no locomotion in any of the animals tested. Increasing the concentration of 5-HT injected from 10^{-7} to 10^{-3} M caused a monotonic increase in number of steps, reaching a mean of 25.0 ± 5.67 steps after an injection of 10^{-3} M 5-HT. Moreover, increasing the concentration of 5-HT produced a monotonic decrease in the first interstep interval, ranging from a mean of 24.5 ± 13.5 sec after 10^{-7} M 5-HT to a mean of 8.8 ± 1.46 sec after 10^{-3} M 5-HT (Fig. 2B). Thus, with increasing 5-HT concentration, animals not only showed more locomotion but also a faster initial rate of locomotion.

We next examined how locomotion triggered by 5-HT interacts with locomotion triggered by salt, a natural stimulus of escape locomotion. There were three parts to the experiment. (1) We first injected 10^{-4} M 5-HT and measured locomotion over a 5-min period to establish a base line. (2) We then administered a second injection of 5-HT 20 min later, but immediately prior to this injection, 5 ml of a saturated salt solution was applied to the tail. (3) Finally, after a 20-min interval, we applied 5 ml of saturated salt solution to the tail in the absence of 5-HT, and locomotion was recorded over a 5-min period. The locomotor effects of salt and 5-HT were additive (Fig. 3). The mean amount of 5-HT-triggered locomotion was 14.2 ± 4.2 steps ($N = 6$). Salt solution alone elicited 7.0 ± 2.9 steps. Salt followed by 5-HT elicited 21.7 ± 3.3 steps. The locomotion elicited by salt alone was qualitatively similar to that triggered by 5-HT at a concentration of 10^{-5} to 10^{-4} M.

Other biogenic amines do not trigger locomotion. To examine the specificity of 5-HT, we tested the triggering effects of three other biogenic amines known to exist in *Aplysia*: dopamine, octopamine, and histamine. Injections of each amine were tested in non-locomoting split-foot animals ($N = 3$ for each amine tested) and compared with the response to an equivalent concentration of 5-HT in the same animal. We found that injections of from 10^{-4} to 10^{-3} M of dopamine and octopamine failed to elicit any locomotion in any of the animals tested, while 10^{-3} M histamine elicited only a slight degree of locomotion (2.3 ± 2.3) compared to 10^{-3} M 5-HT (34.7 ± 4.3). These results indicate that of four biogenic amines tested, triggering of locomotion appears to be relatively specific to serotonin (Fig. 4).

Previous work has shown that *Aplysia* contains at least five different receptors to 5-HT (Gerschenfeld and Paupardin-Tritsch, 1974). At least one class of receptors is coupled to adenylate cyclase (Cedar and Schwartz, 1972; Brunelli et al., 1976; Weiss et al., 1978). We were interested to see whether 5-HT-triggered locomotion

Figure 1. Locomotion is triggered by 5-HT in a split-foot preparation. **A**, Diagrammatic view of the split-foot preparation. The animal is split down the middle leaving the CNS attached to the periphery (see Hening et al., 1979). The anterior aorta is cannulated and perfused continuously with ASW. This preparation exhibits reliable replicas of locomotion. **G.**, ganglion. **B**, A strain gauge record of locomotion triggered by 1 ml of 10^{-3} M 5-HT injected into the anterior aorta. Upward deflections reflect forward extension of the foot.

could also be mimicked by a cAMP analogue—benzylthio-cAMP. Previous work in *Aplysia* has shown that millimolar concentrations of benzylthio-cAMP can simulate cAMP-mediated effects produced by serotonin receptors that are coupled to adenylyl cyclase (Weiss et al., 1979). Injections of up to 10^{-3} M benzylthio-cAMP failed to elicit locomotion in any animals tested ($N = 5$). Moreover, preliminary experiments suggest that cinnanserin—a pharmacological blocker of 5-HT receptors coupled to adenylyl cyclase (Brunelli et al., 1976)—has no effect on 5-HT-triggered locomotion. Thus our results suggest that 5-HT-triggered locomotion is not mediated by cAMP-coupled receptors.

Site of action of 5-HT. The central program for locomotion is located in the circumesophageal ring ganglia which consist of paired cerebral, buccal, pleural, and pedal ganglia. The majority of foot and body wall motor

neurons are located in the pedal and pleural ganglia (Hening et al., 1979; Fredman and Jahan-Parwar, 1980). We wanted to determine the site of action of 5-HT. As a first step, we examined whether 5-HT-triggered locomotion could be elicited in a single pedal and an adjacent pleural ganglion isolated from the rest of the circumesophageal ganglia. To accomplish this we cut the pedal commissures and the connectives that run between the cerebrobuccal and the pleural-pedal ganglia (see Fig. 5, inset). Before lesioning, spontaneous locomotion was often observed, but after lesioning, only weak pedal waves were observed. However, injections of 10^{-4} M 5-HT into the aorta still elicited locomotion qualitatively similar to (and not significantly different from) locomotion elicited before the lesions. In the four animals tested, 5-HT elicited a mean of 17.5 ± 2.4 steps after isolating the pedal-pleural ganglia compared to 22.8 ± 4.91 steps be-

Figure 2. 5-HT-triggered locomotion is concentration dependent. *A*, Increasing concentrations of 5-HT (serotonin) produce a monotonic increase in the number of steps recorded during a 5-min period following injection ($N = 5$ for ASW and all concentrations of 5-HT except 10^{-7} M, where $N = 4$). Threshold concentration is 10^{-7} M. *B*, Increasing concentrations of 5-HT also produce a monotonic decrease in the interstep interval of the first two steps.

Figure 3. The locomotor effects of a natural trigger for locomotion (salt) and 5-HT are additive. Three episodes of triggered locomotion are shown ($N = 3$). *A*, Salt-triggered locomotion alone (0.5 ml of salt solution). *B*, Locomotion triggered after injection of 10^{-4} M 5-HT. *C*, Locomotion triggered by a 0.5-ml salt solution applied to the tail, followed by injection of 10^{-4} M 5-HT. As shown on the right (*A + B*), the locomotor effects of salt and 5-HT are additive.

fore the lesion (Fig. 5). Cutting the pedal nerves from the right pedal ganglion abolished 5-HT-elicited locomotion on that side. These results suggest that a locus for 5-HT-elicited locomotion lies within the pedal and pleural ganglia. 5-HT does not trigger locomotion in peripheral neurons or muscles that have been isolated from the CNS, nor does it require the presence of the cerebral or other central ganglia. More recently, we have obtained

Figure 4. 5-HT-triggered locomotion is specific to 5-HT. Locomotion was recorded after a 1-ml injection of 10^{-3} M dopamine ($N = 3$; left solid bar), octopamine ($N = 3$; middle solid bar), or histamine ($N = 3$; diagonal line bar). Dopamine and octopamine failed to produce any locomotion, while histamine produced only a slight effect. In each case, 5-HT (open bars) produced significantly more locomotion than did the other biogenic amine being tested.

preliminary evidence that locomotion can also be triggered in an isolated pedal ganglion connected only to the foot.

Effects of BCE on 5-HT-triggered locomotion. Egg laying in *Aplysia* is triggered by BCE, which contains a family of neuropeptides including egg-laying hormone, a peptide of approximately 4400 M_r , produced by two clusters of bag cells—approximately 400 cells/cluster—located in the abdominal ganglion (Kupfermann, 1967; Strumwasser et al., 1969; Arch, 1972a, b). Injections of BCE into intact, sexually mature animals induces egg laying within 1 hr after injection (Kupfermann, 1967; Strumwasser et al., 1969; Arch, 1976). Egg laying is pre-

Figure 5. The pedal and pleural ganglia are sites of 5-HT action. After cutting the pedal commissures and the connectives from the pedal-cerebral, pleural-cerebral, and pleural-abdominal ganglia (G.) (see *inset*), thus isolating the pleural and pedal ganglia from the rest of the CNS, 5-HT still triggers significant locomotion (*shaded bar*).

ceded by a stereotypic behavioral sequence including buccal protraction, head waving, and a decrease in spontaneous locomotion (Strumwasser et al., 1969; Arch, 1976). We were therefore interested to see whether BCE could suppress locomotion triggered by 5-HT. In control animals ($N = 7$), we injected 10^{-4} M 5-HT four times at 20-min intervals to assess the effects of repeated 5-HT administration. Over the four test periods there was a slight but progressive tendency for repeated 5-HT applications to produce fewer steps (Fig. 6, *control*). Experimental animals ($N = 5$) received the same treatment as controls except that they received a 1-ml injection of BCE prior to the second 5-HT injection. These animals showed a mean decrease of $52 \pm 19.2\%$ in total number of steps after the second injection of 5-HT, which was a significantly greater reduction ($p < 0.003$) than that exhibited by the control group (mean decrease = $6 \pm 8.8\%$). Moreover, unlike the control group, the group receiving BCE tended to increase locomotion after the third and fourth injections of 5-HT (returning toward base line levels), suggesting that the effect of BCE was gradually wearing off.

We next determined whether the suppressive effect of BCE could be demonstrated in pedal and pleural ganglia isolated from the rest of the CNS. After isolating the pedal and pleural ganglia, we compared repeated injections of 5-HT in control animals to repeated injections of 5-HT with an injection of BCE preceding the second injection of 5-HT. Application of BCE still caused a significant reduction in 5-HT-triggered locomotion ($p < 0.05$, one-tailed test; Fig. 7). Thus, these results suggest that a site of BCE modulation of 5-HT-triggered locomotion is located in the pleural and pedal ganglia.

To show that the suppression of 5-HT-triggered loco-

motion was specific to the BCE, we also examined the effect of extracts of other nervous tissue (cerebral ganglia) in 10 animals using the same experimental procedure as above. Cerebral extracts had no suppressive effects on locomotion; they produced results that were comparable to control injections of ASW.

Previous studies have shown that BCE contains at least four neuropeptides including ELH (egg-laying hormone) (Loh et al., 1975; Arch et al., 1976a, b; Stuart and Strumwasser, 1980; Scheller et al., 1982). Moreover, Toevs and Brackenbury (1969) have shown that the egg-laying efficacy of BCE is sensitive to pronase treatment. To examine whether the suppression of 5-HT-elicited locomotion is produced by a peptide in the BCE, we subjected aliquots of BCE to pronase digestion (see "Materials and Methods"). The results of our experi-

Figure 6. BCE (containing at least four neuropeptides) suppresses 5-HT-triggered locomotion. *Left*, Four control injections of 5-HT separated by 20 min produce a slight but progressive decrement in locomotion ($N = 7$). *Right*, In five experimental preparations, injection of 1 ml of BCE prior to the injection of 5-HT significantly suppresses 5-HT-triggered locomotion ($N = 5$, $p < 0.003$). The suppression is specific to bag cells: injection of extracts of other CNS regions (e.g., cerebral ganglia) do not produce suppression.

Figure 7. The pedal and pleural ganglia are sites of BCE suppression of 5-HT-triggered locomotion. The pedal commissures and the pedal and pleural connectives were cut isolating the pleural and pedal ganglia (as in Fig. 5). Using the same protocol as in the intact CNS (see Fig. 6), BCE still reversibly suppresses 5-HT-triggered locomotion.

Figure 8. BCE suppression of 5-HT-triggered locomotion is blocked by pronase treatment. *Left*, BCE (control, $N = 2$) suppresses 5-HT-triggered locomotion. *Right*, BCE treated with pronase ($N = 3$) no longer produces suppression, suggesting that the active agent in the BCE is one of the peptides synthesized by bag cells.

ments are shown in Figure 8. The suppressive effect of BCE on 5-HT-triggered locomotion is completely blocked by prior treatment of the extract with pronase. These results thus suggest that the active agent in the BCE is likely to be one of the neuropeptides released by bag cells.

Discussion

Studies in a variety of vertebrate and invertebrate systems have shown that many cyclic behaviors (locomotion, feeding, scratching) are centrally programmed (for review see Grillner, 1975; Stein, 1978). Several important questions have arisen concerning (1) the identity of the neuroactive substances (neurotransmitters or neurohormones) involved in eliciting and modulating these motor programs, (2) the site and mechanism of these transmitters' actions, and (3) the way in which cyclic behaviors are integrated into more complex behavioral sequences (such as avoidance behavior, appetitive behavior, and reproduction). In attempting to answer these questions, considerable attention has recently focused on two classes of neuroactive substances: biogenic amines and hormones—in particular, neuropeptides.

Triggering of central programs by biogenic amines. There is good evidence that several central programs for locomotion can be triggered or facilitated by biogenic amines (Grillner, 1975; Shik and Orlovsky, 1976; Willard, 1980). M. L. Shik and G. N. Orlovsky have examined locomotion in cats with brainstem transections in which the mesencephalon remains intact. Electrical stimulation of a circumscribed area, nucleus cuneiformis, also known as the mesencephalic locomotor region (MLR), evokes locomotion (Shik, 1971). In chronic preparations, maintained stimulation produces continuous locomotion (Sirota et al., 1973). Intravenous injection of phenoxybenzamine (an α -adrenergic blocker) blocks MLR-evoked lo-

comotion (Grillner, 1973). This suggests that noradrenergic neurons originating in the lower brainstem give rise to one or more descending pathways which mediate MLR-evoked locomotion. Moreover, in acute and chronic spinally injected cats, intravenous injection of 3,4-dihydroxyphenylalanine (DOPA), a precursor of norepinephrine, elicits locomotion similar to that triggered by MLR stimulation. Intravenous injections of clonidine (an α -agonist) also elicit locomotion. Finally, intravenous injection of 5-HT mimics the locomotor effects of DOPA or clonidine and also increases the excitability of motor neurons (Ahlman et al., 1971). The triggering of locomotion by biogenic amines is not limited to vertebrates. Willard (1980) has examined the effect of 5-HT on the program for swimming in the leech. Using an isolated nerve cord preparation, Willard (1980) has shown that swimming programs can be elicited after exposure to 5×10^{-6} to 10^{-5} M serotonin in the bathing medium. Using a very small volume chamber, he has also found that stimulation of the Retzius cell, a serotonergic neuron, can trigger locomotion. Finally, injection of 100 μ g of 5-HT into intact animals increases the occurrence of swimming sequences. Thus, biogenic amines are implicated as possible triggers of locomotion in both vertebrates and invertebrates.

Our studies provide further support for the findings, based both on vertebrates and on the leech, that biogenic amines—in particular, 5-HT—can have a triggering effect on central locomotor programs. In *Aplysia*, cutting the cerebral-pleural connectives abolishes locomotor activity (Jahan-Parwar and Fredman, 1979). This suggests that cells which trigger locomotion are located in the cerebral ganglia. More recently, Fredman and Jahan-Parwar (1981) have identified neurons in the cerebral ganglia which may constitute a "command system" for locomotion. It will be interesting to determine whether these cerebral neurons might be serotonergic or whether they might stimulate serotonergic followers.

Modulatory effects of hormones and peptides. In the last decade it has become clear that hormone receptors are widely distributed in nervous systems and that in addition to their effects on target organs, hormones have important triggering and modulatory roles in a variety of behaviors. A major principle which has emerged is that the behavioral effects of hormones are often linked to their other physiological actions. For example, Pfaff (1978) has demonstrated in the rat that the sex steroid, estrogen, which has receptors in the reproductive organs, also has receptors in the rat hypothalamus which appear to trigger copulatory behavior (lordosis). Similarly, Arnold et al. (1976) have shown that testosterone, which determines the development of male sexual characteristics, also has a critical role in the development of birdsong in the male finch—an important courting behavior. Finally, Fitzsimmons (1978) and Weindl (1973) have shown that the octapeptide, angiotensin II, which maintains body volume through several physiological mechanisms including increased resorption of Na^+ and water (via stimulation of the release of aldosterone and vasopressin) and direct vasoconstriction of arteries, also has binding sites in the subfornical organ of the rat brain which induce drinking behavior.

Modulation of neural circuitry by a given class of

hormones may occur in many regions of the nervous system. Perhaps the best examples are the bag cell neuropeptides, including ELH. Mayeri et al. (1979a, b) have shown that the neurosecretory products of bag cells exert a broad range of changes in the activity of identified neurons which are part of previously defined neuronal circuits in the abdominal ganglia of *Aplysia*. These include ink motor neurons, the command cell of the cardiovascular system (L10), and motor neurons of the gill and siphon withdrawal reflex. Stuart and Strumwasser (1980) have extended these findings to the buccal ganglia and feeding circuits and have shown that direct application of ELH to the buccal ganglia activates an identified neuron (bn 3) which causes retraction of the buccal mass, thus perhaps participating in terminating feeding. This observation is particularly significant in that inhibition of feeding is important prior to the deposition of the egg mass, and it highlights the coordination between the behavioral effects of ELH and its other physiological effects.

As described above, egg-laying behavior has many components that are coordinated into a complex, integrated sequence. This is especially interesting in light of the fact that bag cells, like other neuroendocrine tissue, release not only ELH but at least four peptides (Arch, 1972a, b; Strumwasser et al., 1980; Scheller et al., 1982). Like pro-opiomelanocortin, at least some of these peptides are derived from a common precursor polypeptide that is post-translationally cleaved to yield active molecules. However, unlike pro-opiomelanocortin, which is encoded in a single gene, Scheller et al. (1982) have shown that there are at least five genes in the haploid genome containing ELH sequences. Furthermore, the multigene family yields at least five discrete mRNA transcripts which in turn are processed and transcribed to yield polypeptides. It will be interesting to determine whether some of these distinct peptides are responsible for mediating the different aspects of egg-laying behavior, including the suppression of locomotion.

Our results suggest that BCE, probably through the action of one or more neuropeptides, suppresses locomotion triggered by serotonin. At least one site of this action lies in the pedal-pleural ganglia. In order to deposit its eggs, an animal must cease locomoting. Thus, suppression of locomotion by BCE provides yet another example of the coordination of motivationally consistent behavior by a neuropeptide hormone. Moreover, our results provide a system that is advantageous for studying the interaction of a biogenic amine and neuropeptide(s) on a central motor program. The next step will be to extend our behavioral studies to a cellular level by recording from identified motor neurons involved in locomotion in order to analyze the mechanisms and sites of action of these two classes of neuroactive substances on triggering and modulating of a complex fixed action pattern.

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