Serotonin Injections Induce Metamorphosis in Larvae of the Gastropod Mollusc *Ilyanassa obsoleta*

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Abstract:

Bath-applied serotonin (5-HT) induces competent larvae of the marine snail *Ilyanassa obsoleta* to metamorphose. Previously, the mode of action of 5-HT, whether as an external ligand or as an internal neurotransmitter, was unknown. Larvae were injected with 10^{-4} *M* 5-HT and other pharmacological agents to provide evidence that serotonergic neurons are necessary for metamorphosis in *Ilyanassa* larvae and that serotonin functions as a neurotransmitter or neuromodulator during this process. About 50% of 5-HT-injected animals metamorphose within 48 hours. Fluoxetine, a 5-HT re-uptake inhibitor, and alpha-methy1-5-hydroxytryptamine (αm5HT), a 5-HT agonist, were also effective inducers of metamorphosis. Gramine (3-[dimethyl-aminomethyl]indole), a 5-HT antagonist, inhibited the inductive activity of 5-HT, while the amino acid gamma-aminobutyric acid (GABA) resulted in rates of morphological restructuring similar to those of controls. Collectively, the results of our experiments support the idea that serotonergic neurons are active during larval metamorphosis of *Ilyanassa* and that 5-HT does not induce metamorphosis by binding to epidermal chemoreceptors.

Article:

Introduction

Most marine invertebrates spend the early portion of their lives as planktonic larvae, surviving in an environment that is drastically different from the benthic one they will inhabit as adults. Larval metamorphosis is often triggered by physical and biological features of the environment. For many animals, especially molluscs, chemical factors are the most important inducers of metamorphosis (reviewed by Crisp, 1974, 1984; Pawlik, 1992a,b). Several molluscs are known to settle and metamorphose in response to specific environmental compounds (Scheltema, 1961; Hadfield, 1977; Morse and Morse, 1984; Hadfield and Scheuer, 1985; Levantine and Bonar, 1986; Zimmer-Faust and Tamburri, 1994). The oyster *Crassostrea virginica*, whose habitat overlaps with that of the mud snail *Ilyanassa obsoleta* (Fox and Ruppert, 1985), appears to settle in response to a conspecifically derived peptide (Zimmer-Faust and Tamburri, 1994). The natural inducer for *Ilyanassa* larvae is unknown, but like the metamorphic inducers for *Crassostrea* and the nudibranch *Phestilla sibogae* (Hadfield and Sheuer, 1985), it is a small, organic, water-borne molecule (Levantine and Bonar, 1986).

Exogenous neurotransmitters can act as external ligands, mimicking the action of natural metamorphic inducers. For example, larvae of the abalone *Haliotis rufescens* are induced to settle and metamorphose by the neurotransmitter GABA and its structural analogues (Morse *et al.*, 1979; Morse and Morse, 1984; Morse *et al.*, 1984). Like the natural inducer, GABA acts at receptors on the larval epithelium (Trapido-Rosenthal and Morse, 1985; 1986a,b; Wodicka and Morse, 1991).

Exogenous 5-HT is a reliable inducer of metamorphosis in *Ilyanassa*, but its mode of action is unclear (Levantine and Bonar, 1986). Levantine and Bonar suggested that 5-HT acts either at a different site or in a manner different from that of the natural inducer. In their experiments, a low level of metamorphosis in response to mud extract (the natural inducer) was reached in 2 hours. The same metamorphic rate was reached 20 hours later for animals exposed to 5-HT. The difference in timing between these two inducers might reflect

the time required for uptake and internal release of 5-HT or its conversion to another active molecule within the larval nervous system.

The monoamine serotonin is common in both vertebrate and invertebrate nervous systems (reviewed by Kandel *et al.,* 1991; Frazer and Hensler, 1994). Its role as a neurotransmitter and neuromodulator in a wide range of behaviors in molluscs was extensively reviewed by Walker (1986). As examples, serotonin is active in controlling heartbeat rhythm (Liebeswar *et al.,* 1975) and feeding and biting behaviors (Weiss *et al.,* 1978; Kupfermann and Weiss, 1981; Gelperin, 1981; Ram *et al.,* 1981; 1991; Yeoman *et al.,* 1994), and in modulating swimming motor patterns (Parsons and Pinsker, 1989; McPherson and Blankenship, 1991; Satterlie and Norekian, 1995). It also regulates ciliary beating in larval and adult stages of several molluscan species (Gosselin, 1961; Diefenbach and Goldberg, 1990; Diefenbach *et al.,* 1991; Goldberg *et al.,* 1994). Both freshwater and marine molluscs develop serotonergic neurons as embryos and maintain populations of these neurons throughout their lives (Goldberg and Kater, 1989; Koshtoyants *et al.,* 1961; Diefenbach and Goldberg, 1990; Satterlie and Norekian, 1995), although, as discussed below, populations of serotonergic neurons are not necessarily static (Barlow and Truman, 1992).

Serotonin appears to be an endogenous factor that stimulates ciliary beating in nudibranch veligers (Koshtoyants *et al.,* 1961; Coon *et al.,* 1990; Barlow, 1990). Serotonergic neurons in the CNS innervate the velum I—the swimming and feeding organ—and the foot of the nudibranch *Berghia verrucicornis* (Kempf *et al.,* 1991) and of *Ilyanassa* larvae (S. C. Kempf, Auburn Univ., pers. comm.). This discovery, along with Levantine and Bonar's (1986) result showing that metamorphosis in *Ilyanassa* occurs in response to exogenously applied 5- HT, supports the hypothesis that serotonergic neurons and internally active 5-HT are necessary for larval metamorphosis in this species. The role of 5-HT in settlement and metamorphosis in *Ilyanassa* may or may not be a cilio-excitatory one.

Although the activity of serotonergic neurons may be necessary for the induction of metamorphosis, not all of the serotonergic neurons in the larval brain are retained throughout this process. In *Haliotis,* five pairs of 5-HT-immunoreactive (IR) neurons occur in the cerebral gangion by four days after fertilization, and one of these pairs innervates the velum (Barlow and Truman, 1992). This particular neural complement remains stable until metamorphosis, when the pair of velar serotonergic neurons is rapidly lost.

As mentioned above, exogenous neurotransmitters, which are usually small molecules (Erulkar, 1994), may mimic natural compounds by acting at external chemo-receptors (Trapido-Rosenthal and Morse, 1986a,b; Wodicka and Morse, 1991). Larvae can also take up a variety of small molecules from seawater for internal us-age (Jaeckle and Manahan, 1989). *Haliotis* larvae can transport amino acids from the environment into their bodies, thereby acquiring energy (Manahan, 1983). Transported compounds can affect larval behavior and could even trigger metamorphosis. The delay in response of *Ilyanassa* to 5-HT in comparison to the natural inducer suggests that 5-HT does not act at epithelial chemoreceptors (Levantine and Bonar, 1986). Instead, exogenous 5-HT may be transported across the larval epidermis and be available to mimic the release of endogenous 5-HT and induce metamorphosis. We injected 5-HT and related pharmacological agents into the hemocoel of competent *Ilyanassa* larvae to determine if serotonergic activity in the nervous system promotes metamorphosis.

Materials and Methods

Animal culture

Adult *Ilyanassa obsoleta* (Say, 1922) were kept in the laboratory in well-aerated tanks from which egg capsules were removed twice weekly. Every day capsules were briefly rinsed in 70% ethanol to remove bacteria and were placed in fresh 0.2-μm-filtered Instant Ocean (FI0). Swimming larvae were removed from the dish and held at 7°C until enough larvae were collected to begin a new culture (Leise, 1996).

Larval *Ilyanassa obsoleta* were cultured in the laboratory from embryos (Collier, 1981) as previously de-scribed (Leise, 1996). Briefly, larval cultures were maintained in an airlift system in a 50-50 mixture of 0.2-μm-filtered

natural seawater and FIO with penicillin and streptomycin antibiotics (Miller and Hadfield, 1986). Larvae were fed algae daily: young cultures (less than 3 days old) received 40 ml of *Isochrysis galbana, Monochrysis* sp., *Dunaliella tertiolecta,* or *Skeletonema rathbun;* older cultures were also fed *Nannochloropsis* sp. Larval culture medium was changed every 6 days.

General experimental protocol

Experiments in which test solutions were applied to larvae in the bath seawater were conducted in 24-well plastic Falcon tissue culture plates. Ten larvae were added to each well along with 2 ml of the appropriate solution. Each treatment and control group was replicated three times and each experiment was repeated three times, yielding a potential total of 90 individuals. Experimental treatments and control groups for individual experiments were obtained from one culture. Different cultures were used for at least two of the repeated experiments to ensure that results were consistent and did not depend upon the culture used. Results from each experiment were pooled, and the combined results were tested for statistical significance, as described below. The graphs presented in this paper were obtained from these pooled results. Mortality was generally near zero in the bath experiments, and around 5% in the injection experiments. The results reported here include only surviving individuals. Usually, less than 400 larvae were available for experimentation in any particular culture. As a result, in many experiments the number of larvae in the control groups was reduced to maintain sufficient numbers of animals in the experimental treatments. This did not impair the statistical significance of the results.

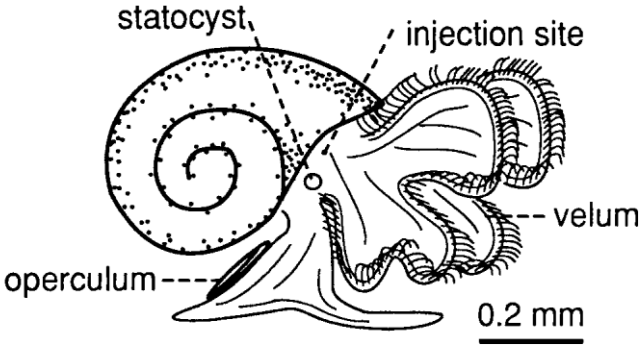

Figure 1. Diagram of a competent larva (redrawn from Fretter and Graham, 1962). Injection site is approximately 50 μm dorsal to the statocyst and probably adjacent to the cerebral ganglia (Lin, 1995).

Injection experiments

Animals that attained a shell length of about 630 μm or longer were described as competent by Scheltema (1962). In our cultures, 12-40-day-old animals measuring 600-625 μm were usually competent and were used for all injection experiments. Larvae that were smaller than 600 μm showed a lower percentage of metamorphosis in response to 5-HT or the natural inducer. Older, larger larvae (>700 μm) were not useful for injection experiments as a relatively high percentage of these animals metamorphosed spontaneously and in response to FIO (control) injections. This obscured the effects of 5-HT and other experimental compounds.

To facilitate access to the larval hemocoel, larval shells were decalcified by culturing the animals in a low pH and calcium-free artificial seawater bath overnight (Pires and Hadfield, 1993). Larvae were reacclimated to normal seawater (pH 7.9) using several changes of FIO. Prior to injection, larvae were embedded in a 1% wgt/vol solution of low-melting-point agarose (Type VII, Sigma Chemical Co.). Animals were then teased from the gel and pipetted into the experimental chamber. This procedure temporarily impeded ciliary beating and swimming activity so that the larvae lay on the bottom of the dish or swam very slowly and were easily injected. Larvae recovered from this treatment within several hours and swam slowly in the test chamber. Injected animals were less active than uninjected animals, but showed no increase in mortality over the period of most experiments.

Decalcified larvae were injected with a small volume (about 6 nl or about 10% of larval volume) of the test solution using a Picospritzer II (General Valve Corporation) (McCaman *et al.,* 1977). The injection site was approximately 50 μm dorsal to the statocyst, just under the epidermis. This site was just lateral to the brain and

avoided direct injection into ganglionic neuropils (Fig. 1) (Lin, 1995). Glass micropipettes were pulled on a Flaming/Brown Model P-87 automatic puller (Sutter Instruments).

Following injection, larvae were observed for signs of metamorphosis. Metamorphic induction was considered to have begun when ciliated cells were dropped from the larval swimming organ, or when the velum was dropped in pieces (Fig. 2). Results were scored at 48 hours; a positive result (metamorphosis) was scored only if metamorphosis was complete. Partial metamorphosis (animals retaining mounds of velar supportive cells or ciliated cells from the pre- or post-oral bands) (Pires and Hadfield, 1993) was not considered to be a positive result. Control and experimental animals were taken from the same culture. Positive (5-HT bath) (Levantine and Bonar, 1986) and negative (FIO bath) controls were included in each experiment to determine the capability of that age group for developmental change.

Test solutions

Fluoxetine was a generous gift from the Eli Lilly Re-search Laboratory; αm5HT and 5-HT were purchased from Research Biochemicals International; GABA, gramine, and L-ascorbic acid were purchased from Sigma Chemical Co. Chemical solutions used for injections were 10^{-4} *M* (unless otherwise noted) because larval response was usually optimal at this concentration. The optimal response for fluoxetine was seen at 10^{-6} *M*. Chemicals were introduced into solution in 1 ml of distilled, deionized water, and then diluted in 99 ml of FIO to achieve a 10^{-4} *M* solution. Solutions of 10^{-6} and 10^{-8} *M* were made by serially diluting aliquots of the 10^{-4} *M* solution. The one exception to this procedure involved gramine, which was first dissolved in 1% ethyl alcohol. Injections of 1% ethanol into competent larvae resulted in no significant induction of metamorphosis.

Data were analyzed with 2-way or 3-way chi-square tests (Sprinthall, 1994). For cells with low expected frequencies, a Yates correction was performed to ensure that the chi-square was not inflated (Sprinthall, 1994). Percentage data were normalized using an arc-sin transformation before analysis (Rohlf and Sokal, 1981), but were transformed back to percentages for graphing. Error bars are standard deviations of the mean. Graphs were produced with the Statgraphics software program (Manugistics, Inc.).

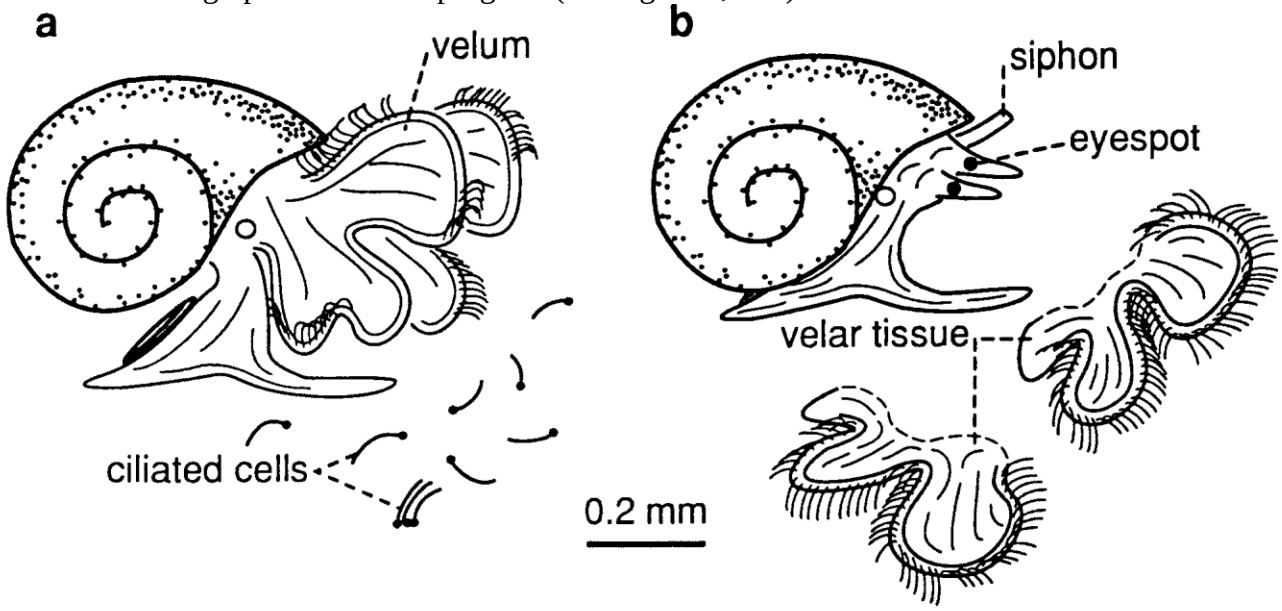

Figure 2. (a) Diagram of a larva at the beginning of metamorphosis. Ciliated cells are being individually dropped from the velum. (b) Drawing of a newly metamorphosed juvenile. The velum has been lost in large pieces (modified from Fretter and Graham, 1962).

Results

Injection and bath-application of 5-HT induced significantly more metamorphosis than did FIO controls (Fig. 3). The results of 5-HT injections were also statistically different from those produced by bath-application of 5-HT. Fluoxetine, a 5-HT reuptake inhibitor (Kandel, 1991; Frazer and Hensler, 1994; Ramamoorthy *et al.*, 1993), and am5-HT, a 5-HT agonist (Walker, 1986; Chaouche-Teyara *et al.*, 1993), also induced significant levels of metamorphosis compared to controls (Figs. 4, 5). The results of 10^{-6} *M* fluoxetine injections did not

differ statistically from those seen in the 5-HT bath condition. Injections of 10^{-4} *M* fluoxetine induced less metamorphosis than injections of 10^{-6} *M* fluoxetine *(cf.* Figs. 4, 6). When 10^{-4} *M* fluoxetine and 5-HT are injected together there is an additive effect (Fig. 6). Gramine, a 5-HT antagonist (Zhang and Harris-Warrick, 1994), did not promote metamorphosis when injected alone, and when injected along with 5-HT it resulted in decreased rates of metamorphosis (Fig. 7). To determine if these results were indeed specific to serotonergic neurons and their influence on larval physiology, GABA was injected as a negative control. Injections of this neurotransmitter did not induce significant levels of metamorphosis (Fig. 8).

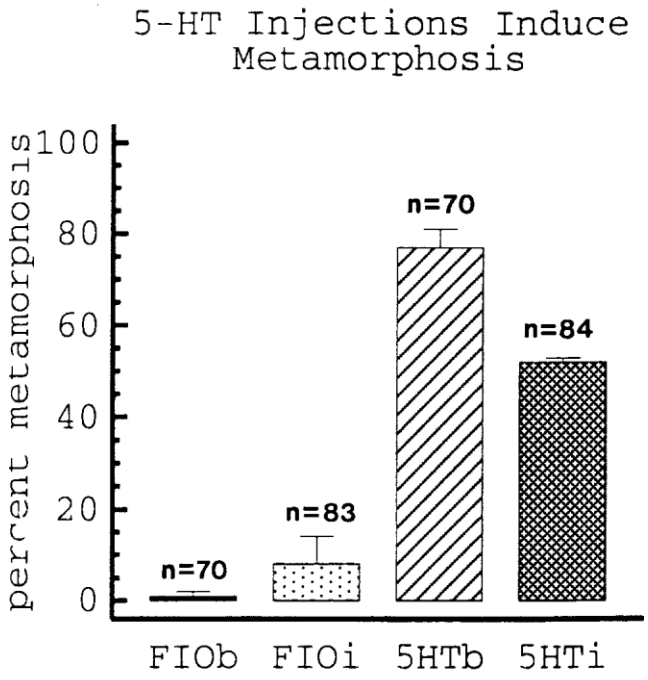

Figure 3. Injections of 10^{-4} *M* 5-HT (5HTi) induced metamorphosis in about 50% of larvae in comparison to FIO injections (FIOi) which induced an average of 8% metamorphosis ($\chi^2_{.01(1)} = 31.69$). Larvae were scored after 48 hours in all experiments. Comparison of 5-HT bath (5HTb) and 5HTi showed a statistically significant difference between these two treatments ($\chi^2_{0.1(1)} = 11.02$). Control conditions using bath-applied 5-HT (5HTb) and bath FIO (FIOb) induced 77% and 1% metamorphosis, respectively.

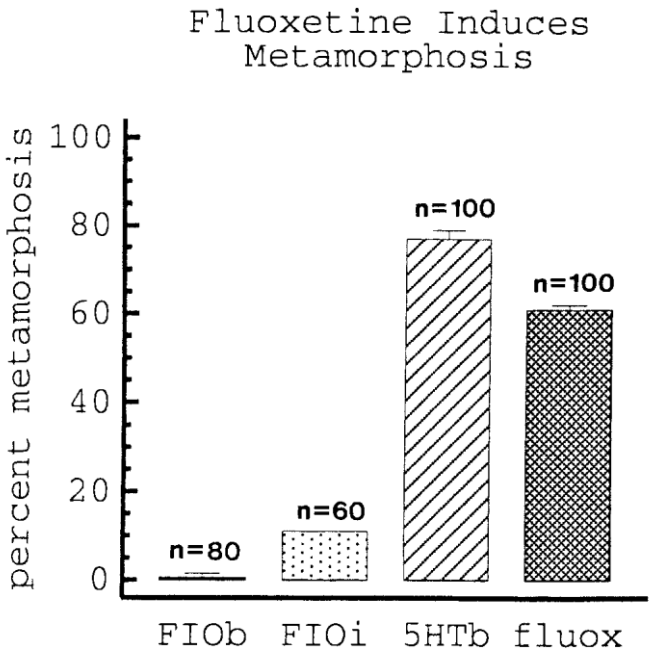

Figure 4. 10^{-6} *M* fluoxetine injections (fluox) induced 61% metamorphosis. Statistical analysis comparing 10^{-6} *M* fluoxetine injections with FIO injections showed a highly significant effect ($\chi^2_{.01(1)} = 34.67$). The fluoxetine treatment was not significantly different from the 5HTb condition ($\chi^2 = 2.42$; accept H_0).

Discussion

To partially test the hypothesis that serotonin and serotonergic neurons are directly involved in metamorphosis, behaviorally relevant amounts of 5-HT were injected into competent *Ilyanassa* larvae. Injections induced a high level of metamorphosis, suggesting that serotonin may be normally released as a neurotransmitter during metamorphosis. Because metamorphosis was considered to have occurred only if the loss of the velum and development to the juvenile state were completed within the experimental period, percentages of metamorphosis reported in this study may be somewhat less than those of other authors. Even with these more rigorous criteria, the results of our injection experiments were nonetheless significant.

Many compounds can be oxidized by seawater, losing or changing potency and thus acting in unexpected ways within larvae (Pires and Hadfield, 1991). Metamorphosis in response to bath-applied 5-HT could reflect the activity of this neurotransmitter within the larval nervous system or the activity of some unidentified oxidized product of this compound. Direct injection of 5-HT into the hemolymph eliminates the confounding factor inherent in bath-application studies, although it does not eliminate the possibility that serotonin is metabolized within larvae and that it is some metabolite, rather than serotonin, that was active during our experiments. Still, these experiments allow us to further define the locus of 5-HT activity in the larval nervous system.

5-HT plays numerous roles in molluscan nervous systems (Walker, 1986) and major groups of serotonergic neurons have been identified in several gastropod taxa (Koshtoyants *et al.*, 1961; Walker 1986; Jahan-Parwar *et*

al., 1987; Goldberg and Kater, 1989; Diefenbach and Goldberg, 1990; McPherson and Blankenship, 1991; Fitzgerald and Carew, 1991). For example, 5-HT regulates neurite extension and growth-cone motility in the freshwater snail *Helisoma trivolvis* (Price and Goldberg, 1993); stimulates motor neurons in *Aplysia californica* (Ram *et al.,* 1991); plays an important role in molluscan peripheral tissues, especially the heart (Walker, 1986); and heightens motor activity in embryonic gastropods in a developmentally sensitive manner (Koshtoyants *et al.,* 1961; Diefenbach *et al.,* 1991). In these young animals exogenous serotonin increases ciliary activity during early embryonic stages, then sensitivity to 5-HT decreases to an intermediate level. Eventually, ciliary beating alternates with long pauses and contraction of the velar lobes (Koshtoyants *et al.,* 1961). Results from these studies suggest that 5-HT is an endogenous factor partially responsible for controlling the characteristic ciliary beating that occurs during embryonic development in many gastropods.

Full metamorphosis in the prosobranch *Ilyanassa* is similar to the partial metamorphosis of *Phestilla sibogae* seen in response to hydrogen peroxide and catecholes (Pires and Hadfield, 1991). The first sign of metamorphosis is the loss of velar metachronal rhythm (Bonar, 1973). This loss may be due to the uncoupling of gap junctions at the bases of the preoral ciliated cells. If the velar ciliated cells are separated from the central nervous system (CNS), coordinated ciliary arrests are abolished and the cilia show continuous metachronal beating (Mackie *et al.,* 1976). Studies by Koshtoyants *et al.* (1961) revealed the cilio-excitatory role of 5-HT, but electrophysiological studies have shown that spikes generated by electrical stimulation of the velar cells elicit ciliary arrests (Mackie *et al.,* 1976; Arkett, 1988), indicating that 5-HT is not responsible for cessation of ciliary beating. Serotonin is thus unlikely to be the neurotransmitter at neurociliary junctions but may act at extrasynaptic receptors (Mackie *et al.,* 1976). In *Ilyanassa,* 5-HT injections induce a high percentage of metamorphosis within 48 hours, but we do not know if the larval response to this injection involves a cilio-excitatory action of 5-HT, a modulatory effect on extra-synaptic receptors near the ciliated epithelium (Mackie *et al.,* 1976), activity on a separate set of cells—perhaps within the CNS, or a combination of these effects.

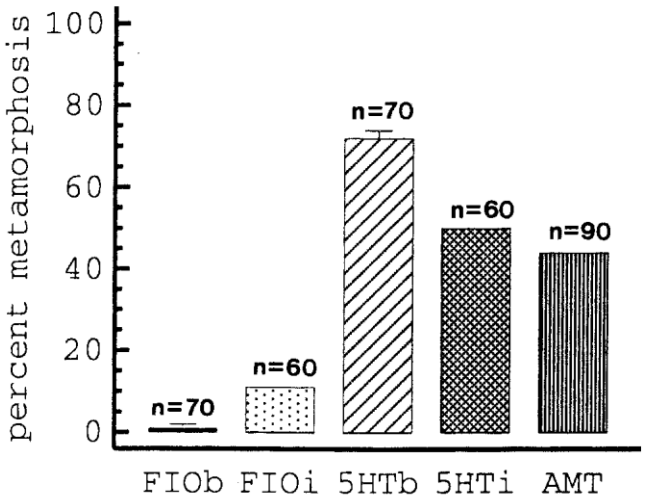

Figure 5. Injections of alpha-methyl-5-HT (AMT) induced 44% metamorphosis. This 5-HT agonist was as effective as 5-HT. FIOi induced 11% metamorphosis in this set of experiments. Metamorphic induction was significantly higher for AMT than for FIOi animals ($\chi^2_{.01(1)} = 17.97$).

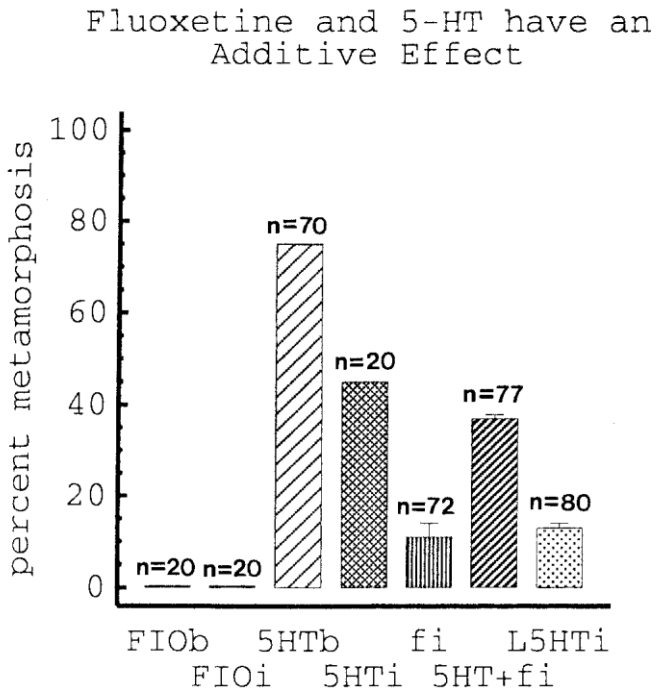

Figure 6. When 10^{-8} *M* 5-HT (L5HTi) and 10^{-4} *M* fluoxetine (fi) are injected in combination (5HT + fi) there is an additive effect in comparison to injections of these compounds alone ($\chi^2_{.01(2)} = 23.16$). 10^{-8} *M* 5-HT injections resulted in significantly less metamorphosis than injections of 10^{-4} *M* 5-HT ($\chi^2_{.01(2)} = 9.76$).

Both *Ilyanassa* and *Phestilla* lose their vela early in metamorphosis. *Phestilla* ingests its velar ciliated cells and incorporates the remaining supportive cells into the cephalic epidermis (Bonar and Hadfield, 1974). *Ilyanassa* may lose the velum *in toto* (Scheltema, 1962), or ciliated cells may drop off individually or in clumps (Couper

and Leise, 1994). Like *Phestilla, Ilyanassa* metamorphoses a number of hours after initial exposure to an inducer (Bonar and Hadfield, 1974; Levantine and Bonar, 1986). The reason for this long delay is unknown.

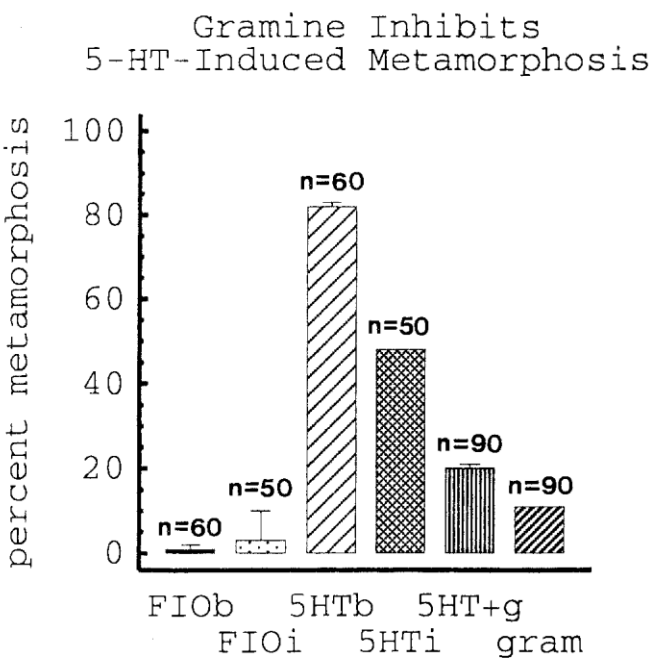

Figure 7. The 5-HT antagonist gramine reduces 5-HT-induced metamorphosis from 48% to 20%. A 3-way chi-square analysis comparing levels of metamorphosis induced by injections of gramine (gram), gramine + 5-HT (5HT+g), and 5-HT alone, showed that this reduction is significant ($\chi^2_{.01(2)} = 26.27$).

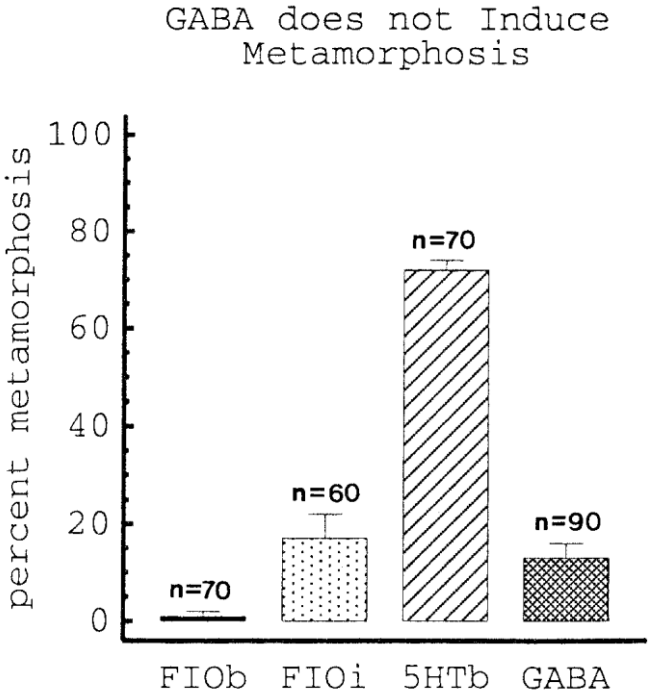

Figure 8. The amino acid GABA was used as a negative control. Results of GABA injections showed no increase in metamorphosis in comparison to FIOi controls, suggesting that metamorphosis is specific to 5-HT or 5-HT analog ($\chi^2 = 0.24$; accept H_0).

Endogenous 5-HT may activate targets postsynaptic to the chemosensory neurons that mediate a larva's response to the natural inducer. Injections of 5-HT may mediate either the settlement response, which includes the cessation of ciliary beating, or the metamorphic response (loss of the velum) or both. Irrespective of its causative role in metamorphosis, if 5-HT is active as a neurotransmitter or neuromodulator, then 5-HT and related pharmacological agents should affect the metamorphic process. We tested several relevant compounds in bath-application and injection experiments in an attempt to support the idea that 5-HT has an internal mode of action. The results support the hypothesis that serotonergic neurons need to be activated for metamorphosis to proceed.

The compound am5-HT is a 5-HT_2 agonist in vertebrate nervous systems (Chaouche-Teyara *et al.*, 1993) and a 5-HT agonist in invertebrate nervous systems (Walker, 1986). Although receptor classification is not identical for molluscs and vertebrates, structure-activity studies using 5-HT and chemical analogs indicate that the preferred form of the ligand for activation of any particular 5-HT receptor is probably similar for different species (Walker, 1986). As was expected, αm5-HT induced rates of metamorphosis similar to those seen with 5-HT injections. These results support the idea that 5- HT, and not a metabolite, is active internally as a neurotransmitter in *Ilyanassa.*

Gramine selectively antagonizes activity generated by 5-HT application in the crab stomatogastric ganglion (Zhang and Harris-Warrick, 1994). When 10^{-4} *M* gramine was injected into *Ilyanassa* along with 10^{-4} *M* 5-HT, induction of metamorphosis was half of that seen with injections of 5-HT alone. This decreased level of metamorphosis indicates that gramine inhibited the action of 5-HT in *Ilyanassa* larvae. Whether gramine and 5-HT compete for the same binding site is unknown.

GABA is a common molluscan neurotransmitter (Osborne, 1971) and can have mixed excitatory and inhibitory effects (Kerkut and Walker, 1961). It induces metamorphosis in *Haliotis* by mimicking the actions of the natural inducer at external chemoreceptors (Trapido-Rosenthal and Morse, 1985, 1986a,b; Barlow, 1990; Wodicka and Morse, 1991). Injection of GABA provided a negative control for our study.

In summary, 5-HT was dearly the most powerful metamorphic inducer of the compounds tested.

The synaptic effects of 5-HT are terminated by the binding of its molecules to specific transporter proteins (re-uptake) in serotonergic terminals; this mechanism is prevented by selective 5-HT reuptake inhibitors like fluoxetine, which are used as effective antidepressants for humans (Kandel, 1991; Ramamoorthy *et al.*, 1993; Frazer and Hensler, 1994; Barondes, 1994). Fluoxetine acts to increase the availability of 5-HT at the synapse in a number of mammalian nervous systems (Sprouse *et al.*, 1993; Biegon *et al.*, 1993), although Sloley *et al.* (1993) provided evidence that this effect may not occur in all vertebrates. Uptake mechanisms for 5-HT occur in brains of the snail *Helix aspersa* and the squid *Loligo pealeii* (Osborne *et al.*, 1975; Feldman and Dowdall, 1973) and are probably widespread in molluscs. As anticipated, fluoxetine injections induced levels of metamorphosis similar to those of 5-HT injections. Fluoxetine injections also induced levels of metamorphosis that were not statistically different from those seen in 5-HT bath conditions. However, 5-HT injections produced rates of metamorphosis that were statistically different from 5-HT bath-application rates. Injected 5-HT may not be available for an extended time in the nervous system of *Ilyanassa.* Injecting a 5-HT reuptake inhibitor could allow 5-HT to persist in the larval nervous system for a longer time, enabling it to exert its metamorphic effects to a greater extent.

The additive effect seen with injections combining 10^{-8} *M* 5-HT (which by itself induces low levels of metamorphosis) and 10^{-4} *M* fluoxetine corroborates that serotonin and serotonergic neurons are involved in metamorphosis. It is unclear whether 5-HT acts in the nervous system of *Ilyanassa obsoleta* larvae as a CNS neurotransmitter or as a neuromodulator, but the results of this study provide evidence that it is an endogenous compound that plays an important role in metamorphosis.

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