An Inducer of Molluscan Metamorphosis Transforms Activity Patterns in a Larval Nervous System

By: Esther M. Leise and Michael G. Hadfield

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Abstract:

Larvae of the nudibranch mollusc *Phestilla sibogae* metamorphose in response to a small organic com-pound released into seawater by their adult prey, the scleractinian coral *Porites compressa.* The transformations that occur during metamorphosis, including loss of the ciliated velum (swimming organ), evacuation of the shell, and bodily elongation, are thought to be controlled by a combination of neuronal and neuroendocrine activities. Activation of peripheral chemosensory neurons by the metamorphosis-inducing compound should therefore elicit changes within the central nervous system. We used extracellular recording techniques in an attempt to detect responses of neurons within the larval central ganglia to seawater conditioned by *P. compressa,* to seawater conditioned by the weakly inductive coral *Pocillopora damicornis,* and to non-inductive seawater controls. The activity patterns within the nervous systems of semi-intact larvae changed in response to both types of coral exudates. Changes took place in two size classes of action potentials, one of which is known to be associated with velar ciliary arrests.

Article:

Introduction

For a number of molluscan larvae, specific chemical compounds from the juvenile environment can act as chemosensory cues and trigger metamorphosis. For example, inductive compounds may be given off by the adult prey (Hadfield and Karlson, 1969; Hadfield, 1977, 1978; Chia and Koss, 1978, 1988; Lambert and Todd, 1994; Avila *et al.,* 1996; Lambert *et al.,* 1997), by adult conspecifics (Pechenik, 1980; McGee and Targett, 1989; Pechenik and Gee, 1993), by bacteria associated with adult conspecifics (Fitt *et al.,* 1990; Tamburri *et al.,* 1992), and by the algal food of the juveniles (Scheltema, 1961; Kriegstein *et al.,* 1974; Switzer-Dunlap and Hadfield, 1977; Morse *et al.,* 1979; Levantine and Bonar, 1986; Morse, 1990; Boettcher and Targett, 1996; Leise *et al.,* 1996). In gastropods, sensory neurons that may mediate the induction of settlement and metamorphosis occur on the head, between the ciliated velar lobes (Bonar, 1978; Chia and Koss, 1982, 1984; Wodicka and Morse, 1991; Baxter and Morse, 1992; Uthe, 1995; Marois and Carew, 1997; Kempf *et al.,* 1997), and on the foot (Chia and Koss, 1989). Our understanding of how these neurons function is still limited. Observations of Morse and colleagues (Trapido-Rosenthal and Morse, 1985; Baxter and Morse, 1987, 1992; Morse, 1990; Wodicka and Morse, 1991) strongly imply that receptors for lysine, an amino acid that modifies inducer reception, lie on chemosensory cilia in the apical sensory organ of larval abalone. If pre-competent nudibranch and abalone larvae are exposed to an inducer substance, they display habituation—that is, decreased rates of metamorphosis—when they reach competency (Hadfield, 1980; Hadfield and Scheuer, 1985; Trapido-Rosenthal and Morse, 1986; Avila *et al.,* 1996). Habituation is thus a phenomenon associated with the morphogenetic pathway that directly initiates metamorphosis.

More recent studies are beginning to elucidate further internal mechanisms that are downstream from the chemo-sensory processes. These include changes in gene expression (Degnan and Morse, 1993, 1995; Degnan *et al.,* 1997), protein synthesis, and second messenger levels (Inestrosa *et al.,* 1993). Although the cellular circuitry that actually drives metamorphosis is still unknown, recent pharmacological studies have revealed some attributes of this path-way. Serotonin, which occurs widely in larval molluscan nervous systems

(Goldberg and Kater, 1989; Marois and Carew, 1997; Kempf *et al.,* 1997), apparently acts as a neurotransmitter or neuromodulator that promotes metamorphosis in the mud snail *Ilyanassa obsoleta* (Couper and Leise, 1996). The neurotransmitter dopamine appears to be necessary for metamorphorphosis in the nudibranch *Phestilla sibogae* and the slipper limpet *Crepidula fornicata,* whereas norepinephrine may endogenously inhibit this process in *Crepidula* (Pires *et al.,* 1996, 2000). Nitric oxide appears to be yet another endogenous inhibitor of metamorphosis, as shown by studies on *Ilyanassa* (Froggett and Leise, 1999). Yet, even with these recent advances, we still have much to learn about the integrative mechanisms that follow the reception of chemosensory information to produce, ultimately, a juvenile organism.

Electrophysiological studies conducted on a variety of molluscan veligers have also provided some insight into their neural activities. Rapid and coordinated velum-wide ciliary arrests are driven by action potentials in the ciliated cells of the preoral band (Mackie *et al.,* 1976; Arkett *et al.,* 1987), and ramp depolarizations can slow ciliary beating on a more localized level (Arkett *et al.,* 1987). Thus, metachronal beating appears to be controlled by the relative depolarization of the ciliated cells and is modulated by excitatory neuronal input, presumably from the brain ganglia (Carter, 1926; Mackie *et al.,* 1976; Arkett *et al.,* 1987). These mechanisms are likely to be involved in the cessation of ciliary beating that accompanies larval settlement and crawling, behaviors that often precede metamorphosis. Barlow (1990) demonstrated that the ciliated velar cells in abalone larvae change their spiking activity only as an indirect response to the presence of the inducer substance. They do not act as sensory receptor cells. Arkett *et al.* (1989) recorded depolarizing receptor potentials from sensory neurons in nudibranch larvae in response to a settlement-inducing substance, although the use of cobalt anesthetic in their experiments limits the conclusions that can be drawn from their electrophysiological traces. Larvae of several molluscan species can be induced to metamorphose by an increase in external potassium ion concentration (Baloun and Morse, 1984; Yool *et al.,* 1986; Pechenik and Heyman, 1987; Todd *et al.,* 1991; Inestrosa *et al.,* 1992; Pechenik and Gee, 1993), a classical method for depolarizing nerve cells (Nicholls *et al.,* 1992), which again suggests that the peripheral nervous system, the larval central nervous system (CNS), or both are active during the initial phases of metamorphosis. If so, changes in the activity of central neurons, as well as in peripheral sensory receptors, should be detect-able as they respond to a natural inducing substance.

The full range of metamorphic phenomena will most likely be controlled by neuroendocrine products as well as by classical synaptic interactions (Scheltema, 1974; Schacher *et al.,* 1979), but molluscan metamorphosis includes at least two relatively rapid events that may be under direct neuronal control. These are loss of the velum, a process common to all molluscan veliger larvae, and shell dehiscence, which occurs in many opisthobranchs (Bonar and Hadfield, 1974; Hadfield, 1978). These events, in addition to the chemosensory initiation of metamorphosis, could involve neuronal networks within the CNS that drive appropriate effector organs. Indeed, Hadfield (1978) summarized data in support of the hypothesis that the nervous system was the most likely and sufficient regulatory system underlying all facets of metamorphosis in molluscs.

To learn more about the role played by the nervous system during the metamorphosis of marine invertebrates, we used larvae of a nudibranch mollusc, *Phestilla sibogae,* to study the response of the CNS to a natural metamorphosis-inducing compound. The scleractinian coral *Porites compressa* is the major prey for adult *P. sibogae* in Hawaii. A small organic compound that is a natural exudate from live *P. compressa* induces metamorphosis in developmentally competent larvae (Hadfield and Karlson, 1969; Hadfield, 1977; Hadfield and Pennington, 1990). Our extracellular recordings from the exposed dorsal surface of the brain ganglia provide evidence that activity patterns in the CNS change in the presence of the coral extract. We propose that the electrical changes we observed are associated with the initiation of metamorphosis, and that some of them are specific responses to larval exposure to *P. compressa.*

Materials and Methods

Veliger larvae of the nudibranch *Phestilla sibogae* Bergh were cultured in the laboratory in 0.2-μm-filtered natural seawater (FSW) using previously described methods (Miller and Hadfield, 1986; Pires and Hadfield, 1991). During initial experiments, insufficient electrical activity was recorded from the epidermal surfaces of intact larvae, so we used an *in vitro* reduced preparation to maximize our ability to record spiking activity. To

facilitate access to the larval brain, larvae without shells were used in all electrophysiological experiments. Deshelled larvae settle and metamorphose normally, although they do not undergo shell dehiscence (Pennington and Hadfield, 1989). Larval shells were decalcified by culturing about 100 larvae in a stender dish in 30 ml of artificial seawater (ASW) (Cavanaugh, 1956) lacking the usual 2.14×10^{-3} *M* sodium bicarbonate and buffered instead with 0.01 *M* Tris to pH 7.0 (Pires and Hadfield, 1993). Nine-day-old larvae were kept in ASW overnight so that metamorphically competent, shellless, 10-day-old larvae were available as experimental subjects. About 70% of larvae cultured in this fashion had no shells 14 h after immersion. Deshelled larvae were rinsed in six changes of FSW over the following 2 h to reacclimate them to normal seawater (pH 8.3) before experimentation began.

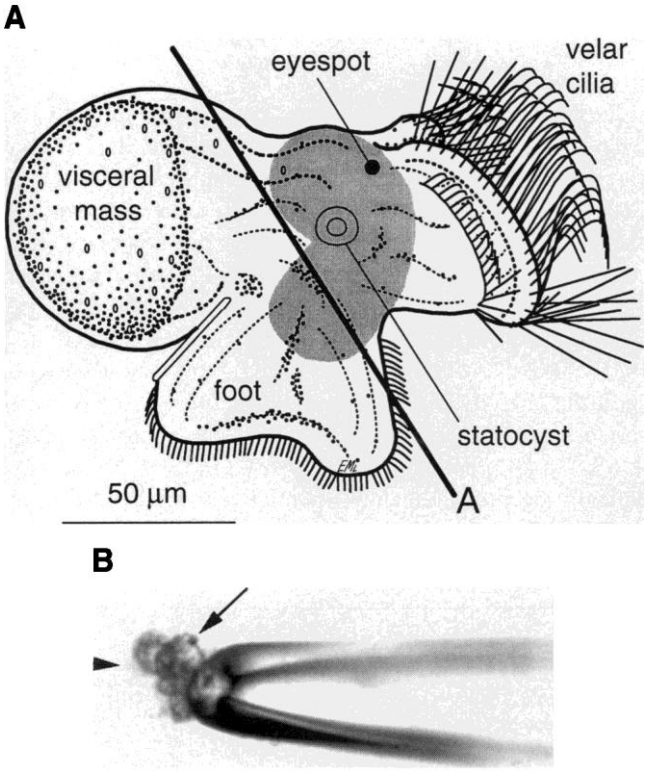

Figure 1. (A) Drawing of a deshelled larva (after Rasmussen, 1944) showing approximate location of the cut used to remove the visceral mass and foot from the head. Grey area represents approximate extent of the brain. The upper lobe containing the eyespot is likely to be a fusion product of the cerebral and pleural ganglia and may also contain elements of the parietal and buccal ganglia (Tardy, 1970). The region below the statocyst corresponds to the pedal ganglion. (B) Isolated head on the end of a suction electrode. The micropipette tip shown here is smaller than that typically used for recording purposes, to make the head more visible. The left eyespot is at arrow; right eyespot is visible through the transparent neural tissue within the open tip of the electrode. Velum is at arrowhead. For recording purposes, micropipettes were sized appropriately so that the entire cut surface could be contained by the electrode. ×178

Isolated larval heads (Fig. 1) were produced by chilling 20-25 individuals in FSW in a small petri dish in an ice water bath. Larvae became immobile as the FSW temperature approached 0°C. Small knives made from broken razor blades (Pires and Hadfield, 1993) were used to remove the visceral mass and foot from these cold, anesthetized larvae. This cut (line A in Fig. 1A) exposed the dorsal surface of the brain for extracellular recording, although it may have also eliminated part of the pedal ganglia. The eyes and statocysts remained in this isolated head preparation.

We also conducted experiments on animals from which only the visceral mass was removed (head-foot preparations). Results were similar, but we have chosen to leave those data unreported because fewer controls were conducted. Initial activity patterns in all experiments were somewhat varied (Fig. 3A, C, E, G), so data from different dissected veliger heads were not pooled.

Immediately after being cut, the chilled, isolated heads were transferred to fresh FSW at room temperature, where-upon they recovered normal metachronal beating of the velar cilia. Electrical recordings were made with a fire-polished glass micropipette suction electrode with an inner tip diameter of 40 to 50 µm. The suction electrode was appressed to the exposed dorsal surface of the brain and gentle suction was applied to maintain contact between the electrode and the larval tissue.

Larvae were exposed to one of three experimental solutions: FSW, FSW containing the natural metamorphosis-inducing compound produced by *Porites compressa* Dana (ISW), or a similar exudate from the relatively non-

inductive coral *Pocillopora damicornis* (PSW). PSW induces less than 30% metamorphosis compared to 90% induced by ISW (Hadfield, 1977). Adult *P. sibogae* do not use *Pocillopora* as prey (Hadfield, 1977). ISW and PSW were prepared by placing about 22 g of living coral into 250 ml of aerated seawater in a covered beaker. Coral tips were used to maximize the ratio of living tissue to skeleton. The coral was removed after 48 h and the resulting conditioned sea-water passed through a 1.2-μm filter. ISW and PSW were stored in the refrigerator and used within 48 h of production. Freshly made ISW normally induces more than 92% of 10-day-old intact larvae to metamorphose within 24 h. If the coral showed signs of ill health during preparation of ISW or PSW, the coral and solutions were discarded. Assays for the metamorphosis-inducing capabilities of ISW and PSW were compared to FSW controls and conducted with intact larvae as previously described (Pennington and Hadfield, 1989). Assays were examined at 24 and 48 h and scored for number of larvae, juveniles, and empty shells. We also tested 34 isolated heads for their ability to metamorphose. These heads were cultured under sterile conditions for 48 h as previously described (Pires and Hadfield, 1993), then examined for loss of ciliated velar cells.

Figure 2. Representative trace from an isolated head in FSW. Large spikes are truncated and correlate with spontaneous velar ciliary arrest. No stimulus was used to elicit these large ciliary arrest spikes. Bottom trace is manually controlled cue (event marker) on the PCM data recorder. Cue was depressed, yielding an upward deflection, whenever spontaneous velar cilia were observed to cease beating. Audio monitor was turned off to avoid biasing the observer. Cessation of ciliary beating coincides with the largest spikes.

Electrophysiological data were recorded for 5-10 min before and after the addition of experimental solutions. The decision to expose each head to control or experimental solutions was made before recordings were initiated. Experiments were conducted in 35 × 10 mm plastic petri dishes in about 6 ml of FSW. Changes to bath solutions were made manually; 4 ml of the bath solution were ex-changed four times over the course of 1-3 min, during which time recording continued. Solution changes some-times introduced mechanical artifacts, so results are re-ported for spiking activity occurring after solution changes were complete. Changes in spiking activity typically began 2-3 min after solutions first contacted the larval head. Data were collected from a new isolated head for each experiment, amplified through a differential AC amplifier (A-M Systems, Inc.), and recorded in digital format on videocassette tape through an Instrutech VR-100 PCM (pulse code modulation) device. This device has a manually operated event marker, or "cue" switch. When depressed, a positive 2.5-V deflection from ground is recorded on a separate channel on the videotape. Data were played back directly onto a Western Graphtek thermal chart recorder or, alternatively, collected on a 486 Insight computer and analyzed with the Enhanced Graphics Acquisition and Analysis (EGAA) software programs, ver. 3.50.02 (RC Electronics, Goleta, CA). Action potentials of different magnitudes were identified and counted using the EGAA Waveshape Recognition program, which stores start and stop times in digital data files. As necessary, files were converted to standard ASCII text format and analyzed further with Microsoft Excel 97 (Microsoft Corp.). Traces with relatively few spikes were analyzed directly from chart recorder records or the EGAA display screens. Two-sample analyses (two-tailed *t* tests) were conducted with Statgraphics Plus ver. 7.1 (Manugistics, Inc., Rockville, MD) or GB-STAT 6.0 (Dynamic Microsystems, Silver Spring, MD). Results were graphed with DeltaGraph 4.0 (SPSS, San Francisco, CA).

Results

Extracellular recordings from the dorsal surfaces of brains in isolated heads of competent veliger larvae displayed two general sizes of spiking units in FSW (Fig. 2). Continuous recordings were made while the preparations were exposed to the various experimental solutions. The largest spikes, between 200 and 500 μV, were associated with partial or velum-wide ciliary arrests that occurred spontaneously in all preparations (Figs.

2, 3; Mackie *et al.*, 1976; Arkett *et al.*, 1987). No stimulation was needed to elicit this activity. Initial patterns of activity in FSW were varied, but we recorded spontaneous ciliary arrest spikes (CASs) in all preparations (Fig. 3A, C, E, G). CAS activity typically occurred tonically, as relatively regular trains of single action potentials at 1 Hz or less. Spikes from smaller units (20-100 *μV)* also occurred spontaneously, but with less regularity (Fig. 3A, C, E, G).

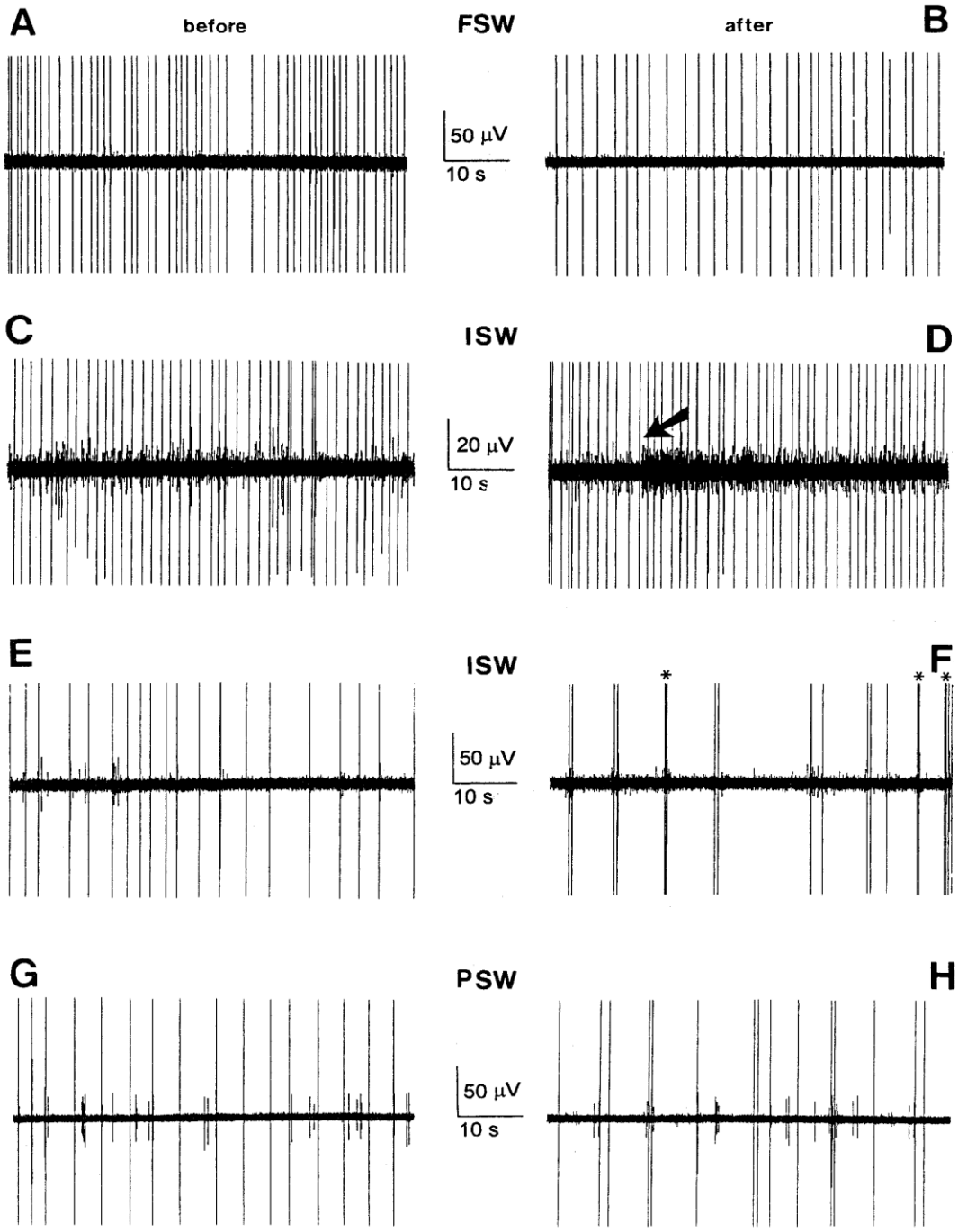

Figure 3. ***Representative 64-s traces, taken about 3 min before (A, C, E, G) and 1 min after (B, D, F, H) addition of experimental solutions, demonstrate induced changes in spiking activity. Traces A, C, E, and G, under the heading "before," all illustrate activity in filtered seawater (FSW). Trace B, a sham experiment, shows activity after the addition of FSW. Traces D and F show activity in seawater conditioned by the presence of the inductive coral Porites compressa (ISW), while trace H shows activity after the addition of seawater conditioned by the presence of Pocillopora damicornis (PSW). In all traces, most velar ciliary arrest spikes (CASs) are truncated and were maximally 200 μV in C and D and 500 μV in all other traces. Traces A and B from Expt. 90-60b, traces C and D from expt. 91-21, traces E and F from expt. 90-61, traces G and H from expt. 91-22. (A) Note relative lack of activity in small units (SU). (B) Addition of FSW did not***

significantly change the firing rates of CASs when averaged over 5 min ([|t| = 0.80] < $t_{0.005(2),8}$ = 2.31). Low activity levels in SUs were likewise unaffected (Figs. 4A, 5A). (C) Note the variable firing patterns of SUs in FSW. (D) Addition of ISW (arrow) significantly increased activity of SUs ([|t| = 3.14] > $t_{0.05(2),14}$ = 2.15, Fig. 5B), but did not affect activity of velar arrest spikes (Fig. 4B). (E) Note variable firing pattern of SUs. (F) In this experiment, addition of ISW did not significantly change activity in large or small units (Figs. 4B, 5B), but produced a qualitative change in the firing pattern of CASs. We recorded short bursts of 2-4 spikes during the 10 min after ISW addition. Longer bursts, with spike frequencies at or above 1 Hz (asterisks), coincided with a contraction of the velar lobes and cessation of ciliary beating. (G) Note variable firing patterns of SUs. (H) Addition of PSW again produced no significant changes in average number of spikes/minute in large or small units, but induced an increased variability in the firing pattern of CASs (Figs. 4C, 5C).

Ciliary arrest was often accompanied by a contraction of the entire velar lobe; during prolonged arrest periods the cilia and velar tissue were held in an upright position. At CAS frequencies below 1 Hz, velar cilia resumed beating between arrest spikes (Figs. 2; 3A, B). During spiking activity at frequencies above 1 Hz, cilia remained relatively motionless (Fig. 3F).

We compared firing rates of CASs and the smaller units (SUs) before and after addition of experimental and control solutions to 13 isolated heads. In one experiment, addition of FSW elicited statistically significant changes in firing frequencies of both CASs and small spikes (Figs. 4A, 5A). In the remaining two experiments, as expected, no statistically significant differences were seen in spiking activity after the addition of FSW (Figs. 3A, B; 4A; 5A).

In contrast to larval heads that were exposed to FSW, those exposed to ISW exhibited some type of statistically significant change in firing pattern, in either CASs, SUs, or both, in 6 of 7 experiments (Figs. 4B, 5B). In only one experiment, #90-61 (Fig. 3E, F), did we fail to observe any statistically significant differences in spiking activity in response to ISW. However, in this experiment, after the addition of ISW, CASs tended to occur in short bursts of 2-4 spikes (Fig. 3F). Short bursts of spikes elicited longer periods of ciliary arrest than did single CASs, and were often accompanied by contractions of the velar lobes. We observed similar results from preparations with an intact foot on several occasions (data not shown). In 4 of the 7 experiments, addition of ISW elicited a significant decrease in the frequency of CASs (Fig. 4B) and a change in the spiking activity of SUs (Fig. 5B).

The addition of PSW to isolated heads elicited no statistically significant changes in firing rates (Figs. 4C, 5C), but in all cases, PSW elicited a qualitative change in CAS activity. With PSW, the firing pattern of the CASs became irregular (Fig. 3H), which accounted for the significant increase in variance that occurred in all experiments (Fig. 4C). No such increase in variance was detected for the firing rates of small spikes.

Finally, we tested 34 isolated heads for their ability to metamorphose. The results were equivocal: four (12%) lost velar cilia, suggesting that isolated heads may be able to detect and respond to ISW, depending, perhaps, upon the amount of intact central nervous tissue. Because a large proportion (56%) died within 48 h, we cannot make a definitive conclusion about the metamorphic capabilities of isolated heads.

Discussion

Metamorphosis in the nudibranch *Phestilla sibogae* is triggered by a chemosensory event, namely, the perception by a competent larva of a small organic compound given off by its adult prey, the coral *Porites compressa* (Hadfield and Scheuer, 1985; Hadfield and Pennington, 1990). In 6 of 7 experiments, we recorded statistically significant changes in electrical activity from *in vitro* heads of larval *P. sibogae* shortly after the addition of a metamorphic inducer. In 3 of the 4 experiments in which spiking activity in small units changed, activity increased. In 4 of the 7 experiments with ISW, firing rates of velar ciliary arrest spikes decreased. Although we did not record consistent responses from all preparations, it is clear that long-lasting changes in electrical activity are initiated within minutes of initial exposure to the coral inducer.

Competent larvae of *P. sibogae* display a rapid behavioral response to ISW that can be reliably observed under laboratory conditions (Koehl and Hadfield, unpubl. obs.). These larvae, which are negatively buoyant, stop swimming and rapidly sink when encountering ISW (Hadfield, unpubl. data). In the field, such a response would increase the chances of a larva contacting its adult food source. External signs of metamorphosis occur only 18-20 h after larvae have been exposed to an inducer substance for at least 4-6 h (Hadfield, 1977; Hadfield and Pennington, 1990). During this delay period, crucial physiological transformations and biochemical pathways must be activated as a prelude to the more obvious morphological transformations of metamorphosis.

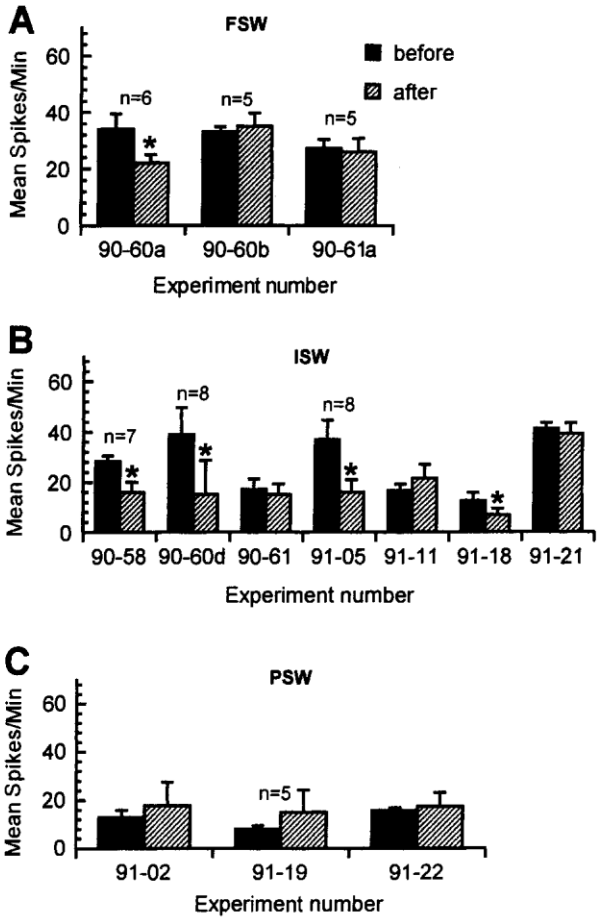

Figure 4. Mean number of velar ciliary arrest spikes recorded per minute before and after addition of experimental solutions, ± one standard deviation. Asterisks (*) indicate mean firing rate is significantly different from initial conditions ($P < 0.05$) after addition of control or experimental solution. Experiments 90-60 (a, b) incorporated different isolated heads. Means were averaged from 10 min of continuous recordings whenever possible. Exceptions are noted on graphs as $n = x$ number of minutes. (A) In one experiment, addition of FSW elicited a significantly slower rate of firing of CASs ($[|t| = 4.85] > t_{0.05(2),12} = 2.18$). (B) Addition of ISW elicited a significant decrease in the firing rate of CASs by 40% or more in 4 of the 7 experiments (*e.g.*, expt 91-18, $[|t| = 4.16] > t_{0.05(2),18} = 2.10$). (C) No change in mean number of arrest spikes per minute was recorded from isolated heads after addition of PSW (*e.g.*, expt 91-19, $[|t| = 1.96] < t_{0.05(2),8} = 2.30$). However, addition of PSW elicited a significant increase in the variance in all experiments (*e.g.*, expt 91-22, $[F = 11.4] > F_{0.05(2),9,9} = 4.03$).

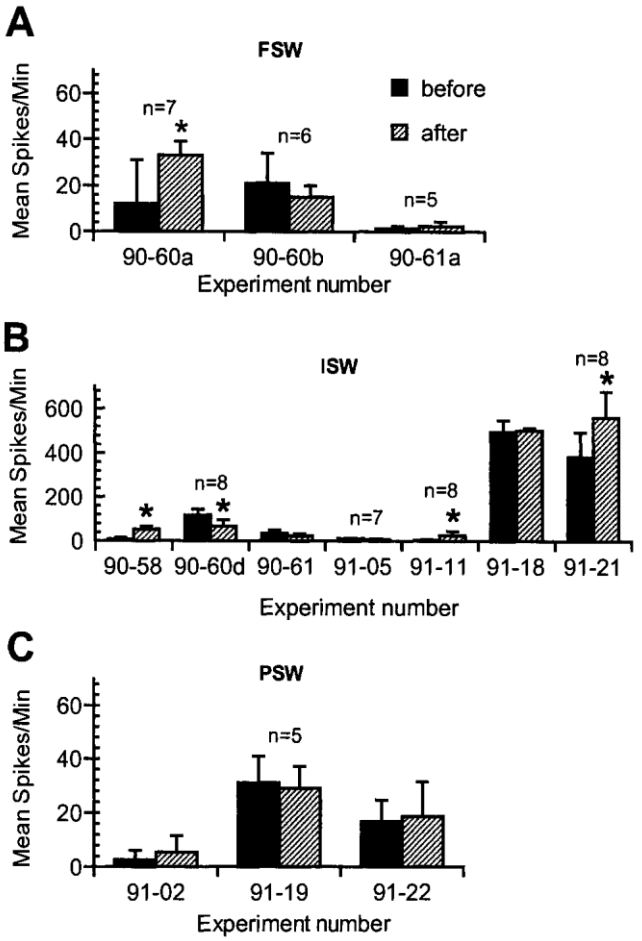

Figure 5. Mean number of spikes per min recorded from smaller units before and after addition of experimental solutions, ± standard deviation. Means calculated from 10 min before and after addition of experimental solutions, except as indicated on graph ($n = x$ number of minutes). Asterisks (*) indicate that mean firing rates before and after addition of experimental solution were significantly different. (A) Addition of FSW in one experiment elicited a significant increase in the number of SUs ($[|t| = 4.16] > t_{0.05(2),18} = 2.10$). (B) Activity levels of SUs were highly variable both before and after addition of ISW. Firing rate of SUs increased significantly after addition of ISW in three experiments (*e.g.*, expt 91-21, $[|t| = 3.13] > t_{0.05(2),14} = 2.15$), but decreased in one experiment. (C) Addition of PSW elicited no change in firing rates of SUs. Variances were similar in all of these experiments, both before and after PSW addition (*cf.* Fig. 4C).

The reduced preparation that we used may have produced neural activity different from that which occurs in an intact organism. The isolated heads retained most of the brain ganglia as well as intact velar lobes, eyespots, and statocysts. However, central circuits may have been damaged by a loss of ganglionic tissue, resulting in decreased connectivity and insufficient afferent information. This in turn may have led to unusual patterns of activity. Because we are reporting results from a relatively small number of experiments with a limited number of controls, we cannot fully explain the variability in endogenous activity, nor the variability in our results. The

responses to *Porites compressa* that we recorded in four experiments would lead to an increase in larval sinking, but not to a complete cessation of ciliary beating, as seen in the behavioral responses mentioned above. This suggests that the isolated heads are not responding in a completely normal fashion.

Larval *Phestilla* can apparently differentiate between their adult prey and at least one other coral species in their reef habitat. In addition to positive metamorphic responses, negative responses to unfavorable or even potentially lethal juvenile environments have been reported for other invertebrates, including several polychaete species (Woodin, 1986, 1991; Woodin *et al.,* 1993; Walters *et al.,* 1996), bryozoan larvae (Walters *et al.,* 1996), and veligers of the gastropod *Ilyanassa obsoleta* (Leise *et al.,* 1996). The ability of *Phestilla* larvae to respond differentially to species of *Porites* and *Pocillopora* is thus not without precedent. How many coral species these small larvae can distinguish remains to be investigated.

Beat frequency of the velar cilia is modulated by excitatory neural input in veliger larvae of the snails *Mangelia nebula* (Mackie *et al.,* 1976) and *Calliostoma ligatum* (Arkett *et al.,* 1987) and the abalone *Haliotis rufescens* (Barlow, 1990). Velum-wide ciliary arrests are caused by an action potential that propagates throughout the velar ciliated cells. The large action potentials we recorded were always associated with ciliary arrests and were smaller than, but similar to, the signals recorded from the velum of *Mangelia* and *Calliostoma* (Mackie *et al.,* 1976; Arkett *et al.,* 1987). The exact origin of the large spikes in *Phestilla* is unclear; they may be the propagated action potentials of the ciliated cells, or a combination of these spikes plus the summed output of central activity that drives ciliary arrests. In her work with larval abalone, Barlow (1990) found that expo-sure to an inducer substance increased the likelihood and duration of ciliary arrests. In our experiments, we mostly observed a decrease in firing frequency of the CASs, which would lead to fewer, not more, ciliary arrests. Only the qualitative change to short bursts of CASs, as seen in some experiments *(e.g.,* #90-61) would lead to longer ciliary arrests.

The behavioral relevance of the spiking activity in the smaller-sized units is unknown. We do not know if their activity arises from circuits that detect environmental odor-ants or drive motor activities, such as crawling or changes in swimming speed or direction. As elicited by ISW, the bursts of smaller action potentials are irregular, unlike bursts from any of the well-known molluscan motor systems *(e.g.,* Getting and Dekin, 1985) or recently described olfactory circuits (Gelperin and Tank, 1990; Gelperin *et al.,* 1993, 1996; Laurent and Davidowitz, 1994; Laurent *et al.,* 1996; Delaney *et al.,* 1994). Activity in the smaller larval units was also quite variable, with firing rates ranging from a few spikes per minute to hundreds per minute. We have no explanation for such variability, beyond suggesting that the amount of SU activity may reflect the amount of tissue lost during dissection. We also have no explanation for the increase in SU activity seen in one control experiment (Fig. 5A). Extracellular recordings from distal stumps of either the rhinophoral or oral-tentacle nerves of adult *P. sibogae* display changes in firing activity of small units in response to *Porites compressa* that are similar to the changes we record from SUs in response to ISW (Boudko and Hadfield, unpubl. data). We can only speculate that the SUs recorded from larval *P. sibogae* might indicate olfactory activity.

The high mortality rate that occurred in experiments on the metamorphic capabilities of isolated heads does not allow us to make a definitive statement about their ability to metamorphose. Isolated velar lobes do not metamorphose-that is, they retain their ciliated velar cells in the presence of ISW- but such lobes lack the neural apparatus that can respond to a metamorphic inducer (Pires and Hadfield, 1993). Although our results support the idea that larval perception of an inducer substance depends upon peripheral chemosensory neurons and central processing circuitry, an additional caveat is warranted. Suction electrodes do not provide a tight seal against passage of fluid between the bathing medium and the core of the electrode. Thus, in our experiments, ISW in the bath seawater could have been interacting directly with neurons of the CNS as well as with epidermal sensory neurons. Thus, the neural activity we recorded in response to ISW may or may not duplicate neural activity occurring within intact larvae at the initiation of metamorphosis. Still, the responses we recorded suggest that the beginning of this process in *Phestilla sibogae* is accompanied by lasting changes in central neural activity.

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