595.4

Friday Harbor Laboratories

Behavioral and neurophysiological responses to chemosensory cues associated with prey, mates, and predators in the nudibranch mollusc, *Tritonia diomedea*

Russell C. Wyeth & A.O. Dennis Willows
Dept of Biology, University of Washington, Seattle WA

595.4

Introduction

How do animals integrate different sensory cues to guide locomotion? The nudibranch *Tritonia diomedea* has several characteristics which have made it an excellent model for understanding how the nervous system controls behavior. Robust behaviors, and a small, relatively simple central nervous system (~7000 cells), with large re-identifiable cells, have given past workers the opportunity to describe how individual neurons are involved in a variety of behaviors. For example, studies in *Tritonia* have shown how small circuits of neurons can be used to produce and modulate rhythmic output (Katz et al., 1994).

Recent work has focused on two sensory modalities: mechano- and magnetosensation. *Tritonia* has a strong rheotactic (crawling upstream relative to current flow) behavior, and several neurons have been described which are sensitive to water flow (Murray et al., 1992). In addition, *Tritonia* is the only animal with identifiable magnetosensative neurons (Wang et al. 2003), which are presumed to play a role in magnetically guided behaviors. Furthermore, the recent description of a set of neurons as general locomotory 'command' neurons (Popescu & Frost, 2002) as well as a pair of neurons which control turns (R. Redondo, pers. comm.), has opened new possibilities for examining how the different sensory modalities converge to elicit locomotory behaviors.

While the laboratory focus has been on mechano- and magnetosensation, our field observations have shown that the primary cues used for navigation in this animal are odors and water flow. Information on either behavioral or neural responses to odor in *Tritonia* is sparse. Therefore, to understand sensory integration underlying navigation in this animal, we need to study chemosensation in *Tritonia*. Here, we describe behaviors guided by a combination of odors and water flow, and make the first forays into the chemosensory components of the nervous system involved in those behaviors.

Prey

Tritonia prey on sea pens (*Ptilosarcus gurneyi*), a soft coral which forms 'beds' in current-swept soft sediment habitats in the shallow subtidal of Puget Sound, WA. *Tritonia* must pounce and bite off pieces of the lower 'leaves' of the sea pen, before it retracts into the sediment. Without vision, the only senses available for the slugs to detect and locate prey are odors and water flow.

Mates

Tritonia are simultaneous hermaphrodites, and mate frequently. Our field observations show 2 or 3 matings/day is not unusual. Mating involves finding a mate sometimes more than a 1/2 hour crawl away. Both single slugs and mating pairs can be highly attractive to downstream animals. However, not all upstream animals are attractive, particularly those laying eggs.

Predators

The sunflower star, *Pycnopodia helianthoides*, is a voracious predator. When touched by *Pycnopodia*, *Tritonia* swims off the substratum, allowing it to be swept away from the predator downstream. The consequences of swimming are not known, but they could be costly, drifting the slug far from both food and mates. Thus, sea star avoidance behavior seems likely to be adaptive.

Objective I

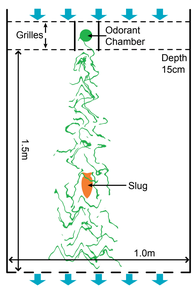
• Characterize odor guided behaviors in *Tritonia*

Method

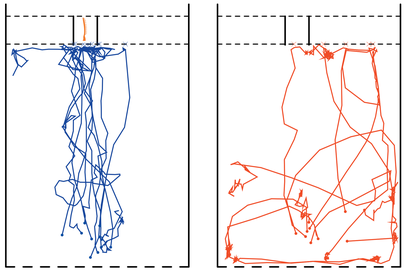
A flow tank (Fig. 1) was used to compare slug behaviors with and without experimental odor sources. Sea water flowed continuously in one end and out the other. Flow at ~1m/min was turbulent, but consistent across the tank; i.e., there were no large eddies to produce local flow reversals. The result was a flow field which approximates that found in the natural habitat of *Tritonia*. At the upstream end of the tank a pair of grilles perpendicular to flow, with movable partitions parallel to flow, created one or two flow-through odorant chambers.

Each *Tritonia* was subjected to paired trials. The experimental trial had an odor source in the chamber, while the control trial had no odor source. Once flow was properly established, and the odor source added, the slug was introduced into the 1.5m x 1.0m behavior arena portion of the tank, and left undisturbed. The trials were videoed from above, and slug positions and headings were marked using custom designed Matlab software.

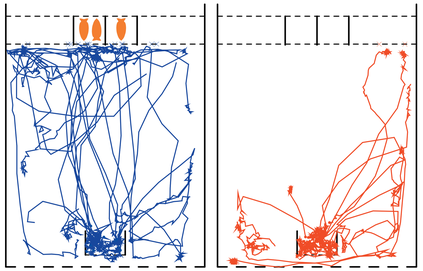
Slug paths in control and experimental trials were compared qualitatively. Both headings relative to flow and distance from the odorant chamber were compared quantitatively.



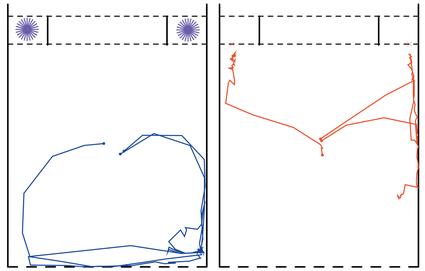
1 Flow tank schematic. Slugs were allowed to crawl freely with or without an upstream odor source.



3 *Tritonia* detect and locate prey upstream (blue), while controls (red) only have a vague tendency to head upstream.
All 10 of the experimental animals made direct contact with the upstream grille, and 9 of those found the odorant chamber (blue X's), while only 3 of 10 controls (red X's) made contact with the grille, without first touching the side walls of the tank, and only 1 of those found the (empty) odorant chamber. The experimental animals followed the direction of flow more closely (Mann-Whitney test comparing angular dispersions, P=.01) and were, on average, closer to the odorant chamber (T-test, P=.0064).



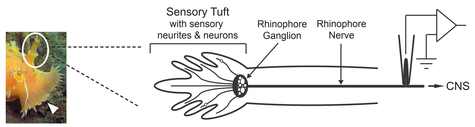
5 *Tritonia* often detect and locate conspecifics upstream (blue), while controls (red) only have a vague tendency to head upstream.
Twelve of 20 experimental animals made direct contact with the upstream grille, and 7 of those found the odorant chamber (blue X's), while only 2 of 20 controls (red X's) made contact with the grille, without first touching the side walls of the tank, and none found the (empty) odorant chamber. The experimental animals followed the direction of flow more closely (Mann-Whitney test comparing angular dispersions, .005<P=<01) and were, on average, closer to the odorant chamber (T-test, P=.0028). These results suggest *Tritonia* intermittently release odors attractive to downstream slugs.



7 *Tritonia* detect and locate predators upstream (blue), while controls (red) have a vague tendency to head upstream.
Pilot results show experimental animals (blue) turning and crawling downstream, away from predatory starfish. These results are similar to a recent series of field experiments where we placed predatory starfish upstream of the stationary slugs, and in 16 of 17 cases, the slugs turned or swam away downstream.

Objective II

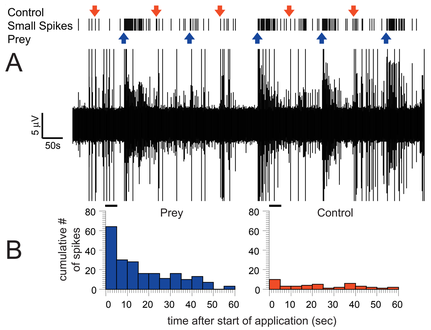
• Describe chemosensory afference from the rhinophores



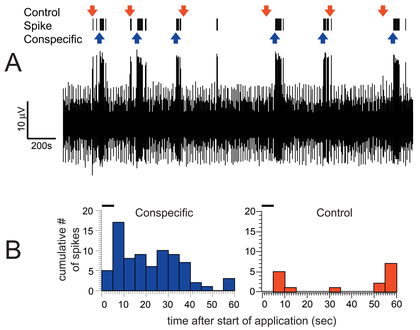
2 Rhinophores are one of the cephalic sensory structures responsible for chemosensation. Extracellular responses to various odor sources were recorded.

Method

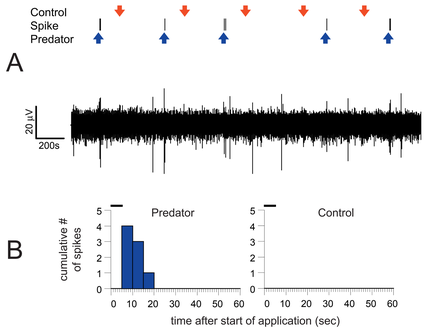
Tritonia has two chemosensory organs: the paired rhinophores (one circled, Fig. 2) and the oral veil (white arrowhead, Fig 2). We focused on the rhinophores because these structures have been shown in other sea slugs to be important for distance chemoreception (Audesirk, 1975), and because rhinophore lesions eliminated the odor guided behaviors described here. Rhinophores were isolated with a long portion of the rhinophore nerve exposed to allow *en passant* recording with a suction electrode. No chemosensitive responses were recordable from the cut end. The rhinophore was pinned in a sylgaard lined dish with continuous sea water perfusion. Odor and regular sea water (to control for mechanical effects) were applied to the sensory tufts, and the resulting activity was amplified (A-M Systems Model 1700) and digitized (CED, Micro 1401 MkII and Spike2 software). Once an odor elicited response was found, a series of 5 paired applications of control and odor sea water was made to characterize afferent activity produced by the odors. Odors were isolated from prey, mates, and predators by either sampling the effluent from a tank containing the odor source or by manually acquiring the sea water near the odor source. In either case, control sea water was acquired in a similar manner from a tank without an odor source.



4 Rhinophore afferents respond to prey odors. This example shows a group of small, high frequency units which respond more to application of prey sea water (blue arrows) than to control sea water (red arrows). Spike shapes in the extracellular record (A) were sorted to isolate the group of small responsive units (Small Spikes). The cumulative histograms for all 5 trials (B) show a short latency and long lasting response to the ~5 second application (horizontal black bar) of sea pen sea water but not controls. The perfusion flow flushes the recording dish in ~60 seconds. These characteristics suggest the small spikes belong to primary chemosensory neurons which respond selectively to prey odors.



6 Rhinophore afferents respond to conspecific odors. This example shows a large, medium frequency unit which responds almost exclusively to application of conspecific (blue arrows) sea water. The spike shapes in the extracellular record (A) were sorted to isolate the single responsive unit (Spike). The cumulative histograms for all 5 trials (B) show a longer latency and long lasting response to the ~5 second application (horizontal black bar) of conspecific sea water but not controls. These characteristics suggest this unit is afference from peripheral chemosensory processing in the rhinophore ganglion.

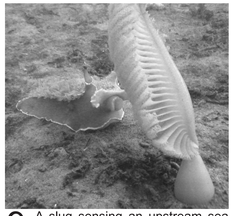


8 Rhinophore afferents respond to predator odors. This example shows a large, low frequency unit which responds exclusively to application of predator (blue arrows) sea water. The spike shapes in the extracellular record (A) were sorted to isolate the large responsive unit (Spike). The cumulative histograms for all 5 trials (B) show a long latency and acutely phasic response to the ~5 second application (horizontal black bar) of predator sea water, but not controls. These characteristics suggest this unit is afference from peripheral chemosensory processing in the rhinophore ganglion.

Conclusion I

• *Tritonia* uses odors to guide three major navigational behaviors, using chemosensation to find upstream prey and mates, and to avoid upstream predators

The ability to locate upstream odor sources probably relies upon a combination of odor and current cues. Strict chemotaxis is not possible in the turbulent flows found in the slug's natural habitat. Rather, the most likely mechanism is odor gated rheotaxis, as found in many other aquatic animals (Weissburg & Webster, 2001). Similarly, the predator avoidance behavior probably involves crawling downstream once the predator's odor is detected. Because *Tritonia* lacks vision, the only distant navigational cues useful for interaction with other organisms in its habitat are water flow and odors. Thus, navigation in *Tritonia* likely involves sampling the current flow for different odors and then choosing to crawl in different directions relative to flow, based on the different odors detected.



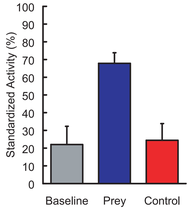
9 A slug sensing an upstream sea pen (bending towards the slug in the current) exemplifies the odor guided behaviors described here.

Conclusion II

• *Tritonia* rhinophores are chemosensory, sending a variety of odor stimulated afference to the CNS.

Rhinophore afference can be stimulated by both mechanical and chemical stimuli. However, extracellular activity can be recorded with increased or exclusive responses to odor sea water application. For five rhinophores tested with prey sea water, odor sensitive units were significantly higher than baseline after odor application, but, on average, did not respond to application of sea water to control for mechanical effects (ANOVA, P=.0101; Dunnett's Test for Difference from Baseline: Prey P<.05, Control P>.05).

Odor sensitive rhinophore afference varies greatly from preparation to preparation. Both tonic and phasic responses can be observed, with a variety of spike sizes, shapes, and latencies. Furthermore, they can respond exclusively to odors or alter their firing pattern in ways specific to odor application. Thus, it seems likely rhinophore afference includes a combination of direct chemosensory units and activity from chemosensory processing in the rhinophore ganglion.



10 Rhinophore afferent activity is consistently stimulated by prey odors.
For each rhinophore, odor sensitive units were counted during 5 x 60 second baseline, and following prey sea water, and control sea water applications. Counts were standardized to the maximal response for each rhinophore (always to prey sea water), and then averaged to derive activity levels for each rhinophore. 5 rhinophores were tested, and shown are the activity level means and standard errors for the 3 treatments.

Future Experiments

• Examine chemosensory input on locomotory control neurons in the CNS. How do odor stimuli affect the activity of 'command' and 'turn' neurons?

• Explore crawling in variable current flows to understand how *Tritonia* might use a combination of odors, water flow, and magnetic fields to find upstream targets.

References

Popescu,I.R. and Frost,T.M. 2002. Highly dissimilar behaviors mediated by a multifunctional network in the marine mollusk *Tritonia diomedea*. J. Neuroscience **22:** 1985-1993.

Katz,P.S., Getting,P.A., and Frost,W.N. 1994. Dynamic neuromodulation of synaptic strength intrinsic to a central pattern generator circuit. Nature **367:** 729-731.

Wang,J.H., Cain,S.D., and Lohmann,K.J. 2003. Identification of magnetically responsive neurons in the marine mollusc *Tritonia diomedea*. J. exp. Biol. **206:** 381-388.

Murray,J.A., Hewes,R.S., and Willows,A.D. 1992. Water-flow sensitive pedal neurons in tritonia - role in rheotaxis. J. Comp. Phys. A **171:** 373-385.

Weissburg,M.J. and Webster,D.R. 2001. Chemosensory guidance cues in a turbulent chemical odor plume. Limnol. and Oceanogr. **46:** 1034-1047.

Audesirk,T.E. 1975. Chemoreception in *Aplysia californica*. I. Behavioral localization of distance chemoreceptors used in food-finding. Behav. Biol. **15:** 45-55.

Acknowledgements

We thank M. Tam, S. Hung & S. Hardy for help with flow tank experiments, O.M. Woodward for hours of SCUBA work, O.M. Woodward, S.D. Cain, and M. Baltzley for experimental advice and editorial comments, and the staff of Friday Harbor Labs, in particular C. Staude and D. Duggins for logistical support.
RCW thanks C.P. Holmes for her support.
This work was supported by a grant to AODW from Packard Foundation, by a fellowship to RCW from the National Sciences and Engineering Research Council (Canada), and a grant to RCW from the Conchologists of America.