

RESEARCH ARTICLE

Selective prey avoidance learning in the predatory sea slug *Pleurobranchaea californica*

Vanessa Noboa^{1,*} and Rhanor Gillette^{1,2,†}

¹Department of Molecular and Integrative Physiology and ²The Neuroscience Program, 524 Burrill Hall, 407 S. Goodwin Avenue, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

*Present address: Colegio de Ciencias de la Salud, Universidad San Francisco de Quito, Quito, Ecuador

†Author for correspondence (rhanor@illinois.edu)

SUMMARY

Predator–prey interactions involving aposematic signaling, where predators learn the warning cues of well-defended prey, are clear examples of cost–benefit decisions in foraging animals. However, knowledge of the selectivity of predator learning and the natural conditions where it occurs is lacking for those foragers simpler in brain and body plan. We pursued the question in the sea slug *Pleurobranchaea californica*, a generalist forager of marked simplicity of body form, nervous system and behavior. This predator exploits many different types of prey, some of which are costly to attack. When offered *Flabellina iodinea*, an aeolid nudibranch with a stinging defense, biting attack was followed by rapid rejection and aversive turns. The predatory sea slug rapidly learned avoidance. Notable exceptions were animals with extremely high or low feeding thresholds that either ignored *F. iodinea* or completely consumed it, respectively. Experienced slugs showed strong avoidance of *F. iodinea* for days after exposure. Aposematic odor learning was selective: avoidance was not linked to change in feeding thresholds, and trained animals readily attacked and consumed a related aeolid, *Hermisenda crassicornis*. For *P. californica*, aposematic learning is a cognitive adaptation in which sensation, motivation and memory are integrated to direct cost–benefit choice, and thereby lend flexibility to the generalist’s foraging strategy.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/216/17/3231/DC1>

Key words: aposematism, decision, choice, odor learning, discrimination.

Received 3 September 2012; Accepted 1 May 2013

INTRODUCTION

Animals with noxious defenses may enhance their protection from predation by educating predators to associate prominent cues with an elevated cost of attack. Such warning signaling is known as ‘aposematism’. In predator–prey interactions this has different trade-offs in the behavioral economies of both sides. For aposematic prey, maintenance of conspicuous coloration or odor in tandem with noxious defense is energetically costly. For generalist predators exploiting a broad spectrum of prey in which aposematic species occur, there may be constant decisions related to changing prey availability and the relative costs and benefits of predation (Davies and Krebs, 1979). Thus, attack and pursuit of novel prey to learn preferences and aversions through trial-and-error under some circumstances is time consuming and inefficient, but is otherwise adaptive for exploiting changing environments (Lavery and Plowright, 1988). In particular, for an experienced predator, the costs of attacking and consuming prey known to be well defended must be weighed against its own nutritional state and the availability of safer prey. These calculations are well documented for predator species with more complex nervous systems and behavior, such as vertebrates and some higher arthropods. However, for the most part, generalist predators of species with simple nervous systems and behavioral repertoires remain to be examined.

We examined aposematic learning in the marine opisthobranch *Pleurobranchaea californica*. Our interest followed an accidental

observation of rapid attack and rejection of the colorful Spanish shawl nudibranch, *Flabellina iodinea*, and refusal to attack again by the normally voracious and opportunistic predator. Aeolid nudibranchs such as *F. iodinea* often sequester stinging nematocysts from their cnidarian prey and use them in their own defense (Greenwood and Mariscal, 1984), and their bright coloration is thought to act as a visual aposematic signal for potential predators. *Pleurobranchaea californica* is sightless with a simple nervous system, body form and behavioral repertory typical of many Nudipleura, and has been well studied in the laboratory for aspects of behavioral choice. Thus it is of appreciable interest to see whether the predator could optimize its foraging strategy with aposematic odor recognition and altered prey valuation by testing for ability to learn selective avoidance of a noxious prey species, and under what conditions it might do so.

Food avoidance learning in *P. californica* was previously shown in a classical avoidance conditioning paradigm where an appetitive squid homogenate was contingently paired with electric shock on expression of feeding behavior (Mpitsos and Collins, 1975). Moreover, animals were capable of discriminating among different odorants, including squid and sea anemone homogenates and extract of beer (Davis et al., 1980; Mpitsos and Cohan, 1986a; Mpitsos and Cohan, 1986b). Thus, *P. californica* might be expected to be able to learn positive and negative values for specific odors of actual prey. If so, the demonstrations would provide insight to the animal’s predatory lifestyle.

We found that many *P. californica* readily learned to avoid *F. iodinea*. However, notable exceptions existed in individuals that were either extremely ready to feed or were unresponsive to appetitive stimuli. Prey avoidance learning was highly specific, independent of changes in feeding thresholds, and endured through 48 h. Animals trained against *F. iodinea* still attacked and consumed the related aeolid nudibranch *Hermisenda crassicornis*. The robust and selective aposematic learning, with its notable exceptions, is consistent with a previous neural model for cost–benefit decisions that encodes stimulus valuation and risk from moment to moment in the appetitive state, and thereby determines approach–avoidance decisions (Hirayama and Gillette, 2012).

MATERIALS AND METHODS

Pretraining procedures

Specimens of *Pleurobranchaea californica* MacFarland 1966, 80–1000 ml volume, were obtained by trawl or trapping through Pacific Biomarine (Venice, CA, USA), Sea Life Supply (Sand City, CA, USA) and Monterey Abalone (Monterey, CA, USA). *Flabellina iodinea* (Cooper 1863) were collected by SCUBA from pilings of the Fisherman's Wharf in Monterey, CA, or obtained from Marinus Scientific (Newport Beach, CA, USA), Sea Life Supply or Monterey Abalone. This species ranges from the Galapagos Islands (Goslinger, 1991) to Vancouver Island, British Columbia, Canada (Bernard, 1970). *Flabellina iodinea* inhabits the intertidal to ~40 m depth, and is a specialist feeder on the colonial hydroid *Eudendrium ramosum* (McDonald, 1978). *Hermisenda crassicornis* Eschscholtz 1831 were obtained from Sea Life Supply. Animals were maintained without feeding for 2–7 days until use. No clear loss in the aversiveness of *F. iodinea* was noted over these periods.

Feeding thresholds for *P. californica* were measured as described previously (Davis and Mpitsos, 1971; Gillette et al., 2000). Responses were observed for betaine (trimethylglycine HCl; Sigma-Aldrich, St Louis, MO, USA) solutions in filtered artificial seawater applied in 1.5 ml volumes over 10 s to the oral veil with a hand-held Pasteur pipette in a series of ascending concentrations from 10^{-6} to 10^{-1} mol l⁻¹. Thresholds recorded were those concentrations at which animals showed proboscis extension and biting. When specimens failed to respond to the highest concentration (10^{-1} mol l⁻¹), the next highest value, 10^0 , was assigned. Tests began with a control seawater application that was assigned a value of 10^{-7} . This convention assigns conservative finite values to essentially infinitely high or low thresholds. Thus, the data are treated with non-parametric statistics using the negative logs, such that 10^{-6} is treated as 6.0 and so forth.

Training parameters

In training trials, a *P. californica* was gently removed from the holding tanks by hand, placed in the training arena and restrained until its foot attached to the substrate. An individual *F. iodinea* was placed at 12–15 cm distance in front. Upon release, *P. californica* locomoted forward to physically encounter *F. iodinea* with the oral veil. Latencies to bite were recorded either from first physical contact or when early proboscis extension was observed (sometimes up to 3 cm away), an indication of detection. Trials were halted when *P. californica* refrained from biting the prey for 90 s. In multiple cases during later training, a *P. californica* made an avoidance turn before touching *F. iodinea*, indicating a specific reaction to the water-borne odor. When that occurred the prey was moved against the predator's oral veil, a strong stimulus that tended to provoke biting in earlier phases of training. All animals participated in five trials with 20 min intertrial intervals. If animals failed to reach the 90 s non-biting

criterion after five trials, additional trials were performed until criterion was reached. In control observations, *F. iodinea* was held in front of the oral veil with padded forceps and removed before a bite could occur, eliminating the punishing ingestion phase.

At 24 h post-training, betaine feeding thresholds were taken to check for possible changes. In tests for odor specificity of learning at 20 min and 24 h post-training, animals were presented with *H. crassicornis*, an aeolid nudibranch relative of *F. iodinea* readily consumed by *P. californica*. At 24 h, retention tests for *F. iodinea* followed 20 min after tests for *H. crassicornis*. If an animal failed to refrain from attacking, extra training trials were carried out. Retention-only tests were carried out 72 h post-training on 12 animals of the total 28, of which 16 were used in separate neurophysiological experiments or spontaneously expired in the tanks. Pilot studies in which thresholds were measured at 1 h post-training indicated handling effects in some animals like those observed in food-shock training that decay with time (Davis et al., 1983); thus, to decrease handling effects, the threshold measures were postponed to 24 h.

Video of encounters between *P. californica* and *F. iodinea* was recorded at Hopkins Marine Station (Stanford University) in Pacific Grove, CA, and can be seen in supplementary material Movie 1.

Statistical analysis

Results were analyzed using non-parametric methods for the non-Gaussian distribution of the data. Feeding thresholds were treated as negative logarithms of the betaine concentration, as described above. Latencies are presented as medians, and errors are presented as interquartile range (\pm IQR). Data were compared using Friedman's non-parametric repeated-measures ANOVA or Kruskal–Wallis non-parametric ANOVA where applicable, Wilcoxon signed-rank test and Mann–Whitney *U*-test. Significance is reported for two-tailed tests, except in one instance where mentioned. Data are presented in box and whisker charts. The ends of the whisker are set at $1.5 \times$ IQR above the third quartile (Q3) and $1.5 \times$ IQR below the first quartile (Q1). When minimum or maximum values are outside this range, they are shown as outliers.

RESULTS

Pre-selection of experimental subjects

In selecting subjects for training, two groups were found to be essentially incapable of participating. In one, animals with markedly lower feeding thresholds were found to bite and completely ingest *F. iodinea* without rejecting it. We assayed five such animals with a mean (\pm s.e.m.) proboscis extension threshold of $10^{-6.2 \pm 10^{-0.58}}$ and bite thresholds of $10^{-5.0 \pm 10^{-0.71}}$ prior to consuming *F. iodinea*. More commonly we found many animals in the second group, with quite high thresholds at or above $10^{-1}/10^0$ for proboscis extension/bite. These animals tended not to attack *F. iodinea* and in many cases completely ignored it. Thus, in selection of experimental subjects, both those animals extremely ready to feed and those unresponsive to betaine were eliminated in threshold measures, and subjects were favored with bite thresholds of 10^{-4} – 10^{-2} mol l⁻¹, which is an intermediate range of readiness-to-feed (Gillette et al., 2000).

Latencies to bite increased with prey avoidance trials

Forty-four *P. californica* were used in training trials with *F. iodinea*. Close approach caused mouth opening, proboscis extension and ballistic biting. These behaviors sometimes began when the predator was 2–3 cm from *F. iodinea*, and at latest upon oral veil contact. A typical encounter is shown in Fig. 1. All subjects bit prey in the first presentation within 25 s from either first proboscis extension or physical contact. Seizure of *F. iodinea* was always followed within

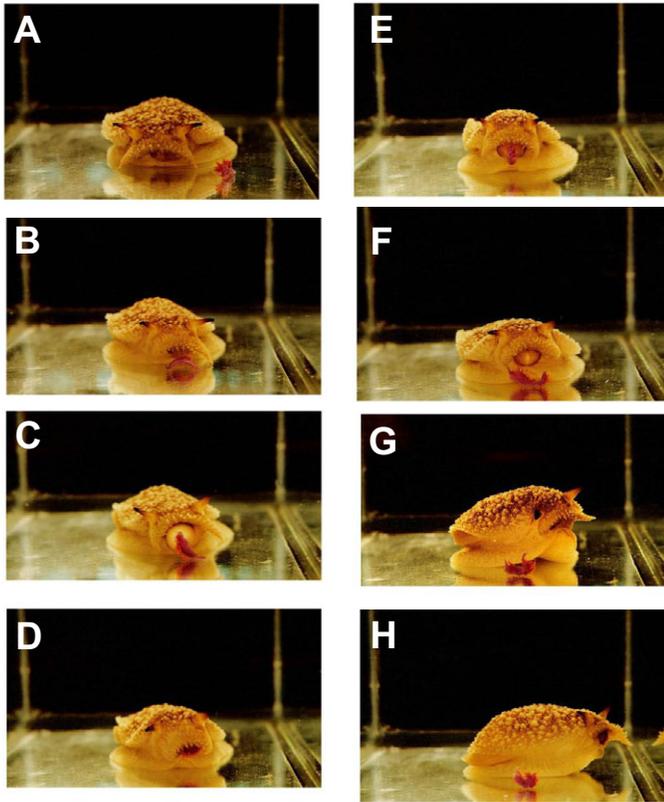


Fig. 1. Prey avoidance experience in *Pleurobranchaea californica*. Approach and attack of a *Flabellina iodinea* by a naïve animal (A–D). Seconds after partial ingestion into the buccal cavity, the prey is egested (E–F). A stereotypic avoidance turn (G–H) follows shortly with the proboscis extended and continued cyclic rejection movements of the radula.

2–3 s by expulsion and active rejection movements of the radula (Croll, 1981), serving in some cases to visibly expel a few orange cerata lost by the nudibranch in the attack. Within 5 s predators initiated a stereotypic avoidance turn (Gillette et al., 1991; Jing and Gillette, 2003), where the anterior part of the foot was lifted slightly off the substrate as the animal flexed right or left away from the stimulus, pivoting on its broadened posterior foot/tail region, which remained attached to the substrate. After a lateral flexion ranging from 30 to 250 deg, the anterior foot re-attached to the substrate, the tail lifted and rotated, and forward locomotion began. During the aversive turn and subsequent locomotion, some animals continued cyclic rejection movements with the buccal apparatus. Video of pre- and post-training encounter is available in supplementary material Movie 1.

In control trials, *F. iodinea* were removed just following detection/orienting but prior to physical contact. Latencies of experimentals increased monotonically with trials. Statistically significant differences were seen by trial 2 and continued to increase (Fig. 2). Of the 28 experimental animals, four had reached full avoidance criterion of 90 s after a single trial, 21 had reached criterion by the fifth trial and seven required a single sixth trial. Sixteen control animals stimulated with *F. iodinea* but prevented from biting did not show significant changes in latency over trials.

Increasing avoidance with trials

Retention was tested at 24 and 72 h post-training. Latency to bite remained significantly increased at 24 and 72 h after the initial

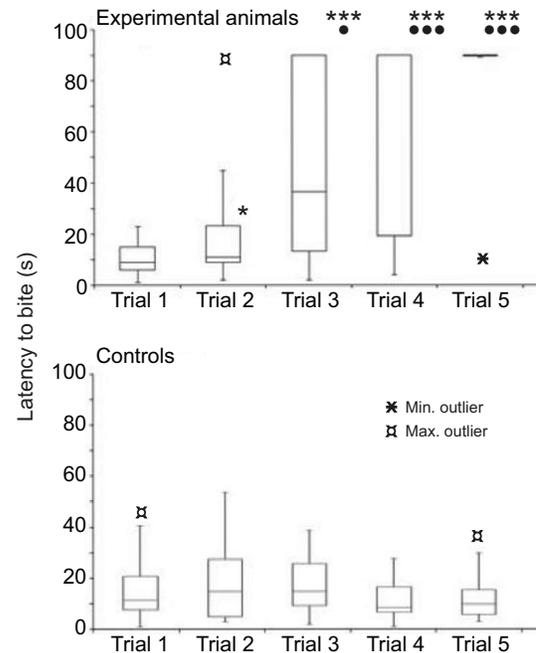


Fig. 2. Comparison between experimental and control latencies to bite during training sessions with *Pleurobranchaea californica*. Experimental latencies ($N=28$) differed significantly over the trials (Friedman's non-parametric repeated-measures ANOVA, $\chi^2=60.5$, $P<0.0001$). Marked differences from the first trial appeared by the second trial (Wilcoxon signed-rank test, $W=-181$, $*P=0.02$) and grew over the third ($W=-293$, $***P<0.0001$), fourth ($W=-389$, $***P<0.0001$) and fifth trials ($W=-406$, $***P<0.0001$). The 28 experimental animals differed significantly from 16 controls on the third trial (Mann–Whitney U -test, $U=128.5$, $*P<0.02$), fourth trial ($U=80.5$, $***P<0.001$) and fifth trial ($U=8.0$, $***P<0.0005$). Control animals were exposed to a *Flabellina iodinea* that was removed before it could be bitten.

training session (Fig. 3A). Inversely, the number of trials to reach criterion 24 and 72 h after training decreased significantly (Fig. 3B).

Orienting turns were usually observed in the early stages of training, but as training advanced, these were replaced by active avoidance (Fig. 3C). On the first day of training, 71% of animals actively avoided the prey by trial 5, with the remaining animals requiring one to two more training trials to reach criterion ($N=28$; Fig. 3C). At 24 h, 35% actively avoided on the first trial and the remainder were all avoiding by the third trial. At 72 h, 75% avoided on the first trial and all by the second trial ($N=12$).

Selectivity of prey avoidance learning

Learning selectivity was assayed in experiments comparing the readiness of trained animals to accept the aeolid nudibranch *H. crassicornis* as prey. Eight animals that had met avoidance criterion were presented with the aeolid nudibranch *H. crassicornis* 20 min after the final *F. iodinea* training trial on the first day. Separately, five trained animals were challenged with *H. crassicornis* at 72 h. In each case *P. californica* oriented to the *H. crassicornis*, bit the prey and readily devoured it. Pictures from these trials are shown in Fig. 4.

Prior observations indicated that contributions of learning and handling effects could be mixed. Davis and co-workers (Davis et al., 1983) reported transient effects of handling in raising feeding thresholds during food-avoidance conditioning. Thus, we followed feeding thresholds for a set of 12 *P. californica* at 24 and 72 h from

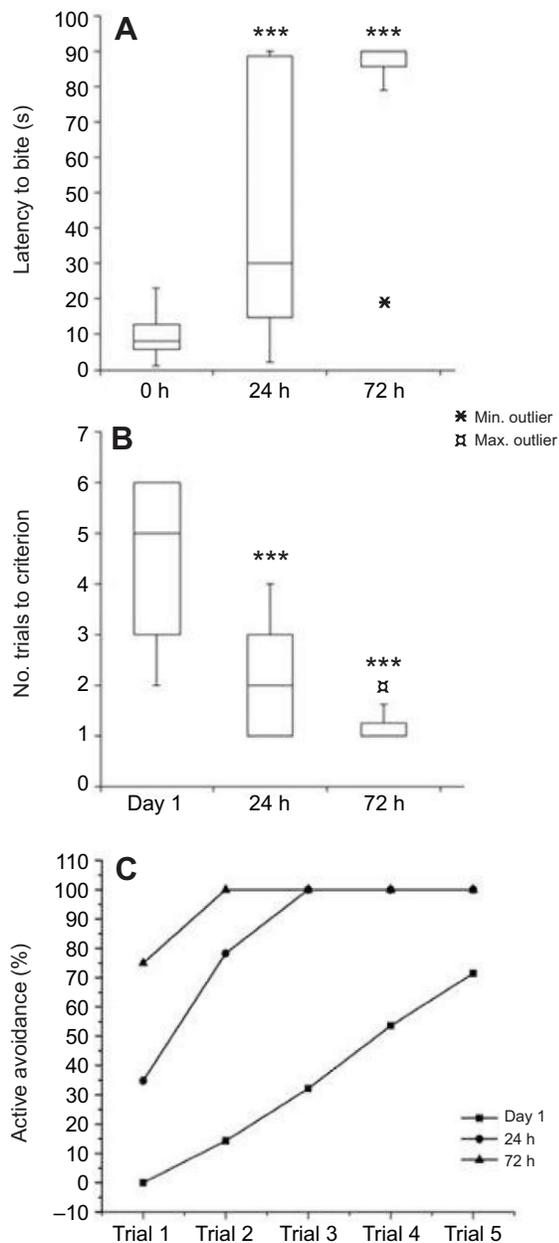


Fig. 3. Prey avoidance at 24 and 72 h post-training. (A) Median latencies of 28 *Pleurobranchaea californica* in the first trial remained high at 24 h and were still higher for 12 animals at 72 h (Kruskal–Wallis non-parametric ANOVA, $H=32.846$, $P<0.0001$; Wilcoxon signed-rank test, 0 versus 24 h, $***P<0.0001$; Mann–Whitney test, 0 versus 72 h, $U=347$). (B) The median number of trials needed to reach criterion decreased (non-parametric ANOVA, $P<0.0001$, $H=52.368$; 0–24 h, $***P<0.0001$; 0–72 h, $***P<0.0001$). (C) The percentage of animals actively avoiding *Flabellina iodinea* increased with trial number and over days of training.

initiation of training. In this population, no significant differences in thresholds for proboscis extension or biting were observed at 24 or 72 h (Fig. 5A). However, in separate groups of 16 experimental and 16 control animals tested at 24 h post-training, bite thresholds, but not proboscis extension thresholds, did rise significantly (not shown) by an average of 0.93 negative log units for experimental animals (Wilcoxon, $P<0.01$, $W=49$) and 0.44 units for controls (one-tailed Wilcoxon, $P=0.042$, $W=35$). Differences between

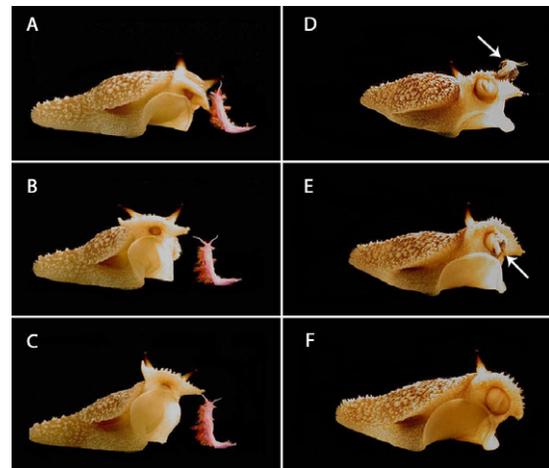


Fig. 4. Species selectivity of prey avoidance training. In a post-training test, a *Pleurobranchaea californica* is shown actively avoiding *Flabellina iodinea* (A–C). The same animal 20 min later quickly attacked and ingested the related nudibranch *Hermisenda crassicornis* (arrows; D–F). The animal's foot appears raised as it touches the glass wall of the aquarium.

experimental and control animals were not significant in Mann–Whitney U -tests. The most marked difference in responses of experimental and control animals was that 100% of experimental animals showed active avoidance responses to *F. iodinea*, while no controls avoided this prey. Thus, while there appeared to be an effect of handling on feeding thresholds and general arousal, similar to those noted in food avoidance training experiments that decay with time (Davis et al., 1983), that did not by itself account for the robust effects on prey avoidance.

In 11 further instances, *P. californica* were tested with *H. crassicornis* both before and 1 h after training against *F. iodinea* (Fig. 5B). The average latencies in these trials for biting *H. crassicornis* were significantly higher, but avoidance behavior never occurred. However, all the trained animals did avoid *F. iodinea*. Thus, handling effects, possibly including trauma from *F. iodinea*'s stinging defense, may have contributed to the longer latencies for *H. crassicornis* attack. It is also possible that there was some generalization of avoidance learning of *F. iodinea* to the related aeolid *H. crassicornis*. However, the complete absence of active avoidance of *H. crassicornis* is more consistent with simple handling effects on readiness-to-feed.

DISCUSSION

There were four principal observations and conclusions from the present study: (1) *P. californica* learns through experience to suppress feeding and avoid the noxious aeolid nudibranch *F. iodinea*, (2) the learned avoidance is relatively long-term, lasting at least 48 h, at which time it still manifests strongly, (3) learned avoidance is largely selective to *F. iodinea* and is independent of reduced arousal levels or non-specific changes in feeding threshold, and (4) animals at either extreme of the appetitive state fail to engage in the attack–rejection sequence of aposematic learning experience.

Selective odor avoidance learning

Rapid and long-term odor learning was shown in increasing latencies to bite and replacement of feeding attack with avoidance on repeated exposure to *F. iodinea*, and in retention of avoidance for 24 and 48 h periods after training trials.

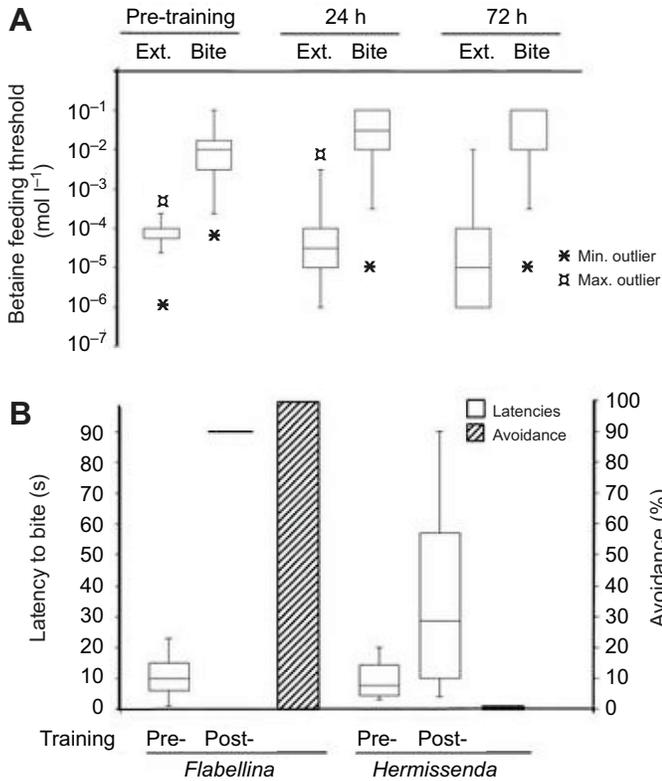


Fig. 5. Selectivity of prey avoidance training in *Pleurobranchaea californica*. (A) Median feeding thresholds for proboscis extension and bite to the appetent betaine were not significantly different after training at 24 and 72 h ($N=12$; Wilcoxon signed-rank tests). (B) Latencies to bite *Hermisenda crassicornis* were also elevated 1 h after training (Wilcoxon signed-rank test, $W=66$, $P<0.001$), but to a lesser extent than for *Flabellina iodinea*, compared with pre-training measures for 11 *P. californica*, perhaps due to handling effects (see Results). All 28 trained animals showed active avoidance of *F. iodinea*, which was not seen in any controls.

Two separate observations sustained the conclusion that learned avoidance of *F. iodinea* was selective: (1) animals trained to avoid *F. iodinea* continued to attack the related aeolid nudibranch *H. crassicornis*, and (2) feeding thresholds to the general appetitive stimulus betaine did not change significantly in trained versus control animals, an internal control for possible changes in arousal level due to handling or passage of time. Thresholds did rise for some control animals during training, which may have contributed somewhat to increases in latencies of attack on both *F. iodinea* and *H. crassicornis* in experimental animals. However, the experimental animals' complete avoidance of *F. iodinea* and ready attack of *H. crassicornis* supports a prominent role for aposematic learning.

These results extend previous observations to more closely connect *P. californica*'s learning abilities to their likely benefit in the natural environment. In so doing, they also indicate how learning is integrated with sensation and internal state in foraging decisions.

Aposematic learning in *P. californica*

Aposematic learning may markedly enhance the foraging strategy of a simple, generalist forager such as *P. californica*. Simplicity in the central nervous system and behavior is characteristic of the clade Nudipleura (Gillette, 1991), whose members appear to have evolutionarily traded off their shells for a notable array of chemical

defenses (Wägele and Klussman-Kolb, 2005; Cimino and Ghiselin, 2009) and at the same time reduced the need for greater neural and behavioral complexity. Their primitive statocysts (equilibrium organs) and soft bodies, which limit proprioceptive and motor abilities in general, appear suited to relatively simple behavioral economies often highly specialized in diet and habitat. However, for generalists such as *P. californica*, aposematic learning is a cognitive adaptation to a variable prey population that must lend appreciable flexibility to this species' foraging strategy.

Animals with noxious defenses, such as *F. iodinea*, enhance predation protection with their aposematic coloration. The present results indicate that they also possess aposematic odor. Aposematism in odor signaling parallels visual warning coloration and patterns to enhance message distribution (Camazine, 1985). Some or many of the compounds identified in the skin of nudipleuran species as likely defensive chemicals (Cimino and Ghiselin, 2009) may act otherwise or also as aposematic cue odors. The aposematic strategy is dependent on some animals suffering initial attempts at predation, the results of which are learned directly by the predator and deter further attack. Thus, for the predator it is beneficial to recognize potentially dangerous prey. Previously, *P. californica* was shown to be capable of odor discrimination learning in trials pairing electric shocks with extracts of squid, shrimp, sea anemone or beer (Davis et al., 1980; Mpitsos and Cohan, 1986a). The present results indicate that *P. californica* can evaluate potential prey in terms of past experience to assess risk and reward in its foraging strategies. Thus, here a natural role for odor learning is shown for differentiating prey species of differing values.

Aposematic odor learning in *P. californica* shows elements of both classical conditioning and operant learning, as may be characteristic of most natural learning (Colomb and Brembs, 2010). Learned odor aversion emerges from pairing of *F. iodinea* odor with avoidance-inducing punishment, bringing the odor itself to finally elicit avoidance, as noted in those *P. californica* that began avoidance several centimeters from contact. However, it is the operant consequences of the predator's actions that lead to learned modification of the behavior. An ability for learned odor aversion has also been shown in another very simple system, *Caenorhabditis elegans*, for classical conditioning of aversion to a pathogenic bacterium (Zhang et al., 2005).

Field observations are consistent with the likely natural occurrence of aposematic learning in *P. californica*. Collecting trawls at 100 m depths in Monterey Bay in 1993 (examined by R.G. and L. Moroz) brought up both *P. californica* and the aeolid *Flabellina trilineata* in the same nets, indicating cohabitation of these species at that time and place. Four of eight *P. californica* tested on the boat showed marked avoidance to *F. lineata*, while the remaining specimens showed the bite-rejection response described here for *F. iodinea*. As of August 2012, *P. californica* has been collected by divers at 6–7 m depth in the Monterey harbor (the first since 1982), occurring within 100 m of *F. iodinea* on the wharf pilings (A. Bariolotti, Monterey Abalone, personal observation). It may be reasonably expected that unhappy encounters between these two species occur naturally.

These observations agree with conclusions that foraging *P. californica* can assign values and make cost-benefit decisions (Gillette et al., 2000; Hirayama and Gillette, 2012). It was notable that in pre-selection of individuals for this study we found two unsuitable classes of animals: those with markedly low feeding thresholds that were extremely ready to feed and quickly consumed *F. iodinea* without rejection, and those with very high feeding thresholds that never showed appetitive behavior on the initial trial. These animals acted according to classic observations that there exist

optimal arousal levels for performance and learning, on either side of which individuals may be either too highly aroused to perform the task or too lethargic to engage (Yerkes and Dodson, 1908; Hebb, 1949; Berlyne, 1969).

In terms of behavioral economics, animals in extreme hunger can benefit by overruling negative memories to acquire a badly needed, though well-defended, resource, and those that do not need the resource may not sufficiently value it or the risky opportunity to learn about the resource. Other data are consistent with this interpretation; for instance, European starlings increase their attack rates on chemically defended insect larvae when their body masses and fat stores are experimentally reduced (Barnett et al., 2007). A simple model (Hirayama et al., 2012) captures the cost–benefit computation of approach–avoidance and risk assessment as seen in these experiments. In it, effects of sensation, internal state and memory of experience sum in the homeostatic neuronal network for feeding, where effects of hunger, taste, pain, and negative and positive associations interact to toggle the approach–avoidance decision. The excitation state of the feeding network thus represents appetitive state, and its corollary outputs determine the switch between attack and retreat behavior.

ACKNOWLEDGEMENTS

Marine laboratory facilities were graciously lent for part of this work by Dr W. F. Gilly of Hopkins Marine Station, Stanford University. We thank Dr Rimmon Fay, Michael and Gay Morris, Lee Bradford and Trevor Fay for their unstinting support in animal supply. We especially thank Mike Morris for introducing us to the Spanish shawl. We thank Avery Ketcher for behavioral observations and Jeff Brown for comments on an earlier version of the manuscript. These studies were elaborated from preliminary observations by Dr Rong-Chi Huang in 1985.

AUTHOR CONTRIBUTIONS

This work was conceived from initial observations by R.G. Evolving conception and design, and interpretation of results were products of continuing interactions between V.N. and R.G., who were also both closely involved in drafting and revising the article. V.N. largely executed the behavioral studies and the still photography. Video recording was done by R.G.

COMPETING INTERESTS

No competing interests declared.

FUNDING

This work was supported by a grant from the National Science Foundation [IOB 04-47358].

REFERENCES

Barnett, C. A., Bateson, M. and Rowe, C. (2007). State-dependent decision making: educated predators strategically trade off the costs and benefits of consuming aposematic prey. *Behav. Ecol.* **18**, 645–651.

- Berlyne, D. E. (1969). Arousal, reward and learning. *Ann. New York Acad. Sci.* **159**, 1059–1070.
- Bernard, F. R. (1970). A distributional checklist of the marine molluscs of British Columbia: based on faunistic surveys since 1950. *Syesis* **3**, 75–94.
- Camazine, S. (1985). Olfactory aposematism: association of food toxicity with naturally occurring odor. *J. Chem. Ecol.* **11**, 1289–1295.
- Cimino, G. and Ghiselin, M. T. (2009). Chemical defense and evolution of opisthobranch gastropods. *Proc. Calif. Acad. Sci.* **60**, 175–422.
- Colomb, J. and Brembs, B. (2010). The biology of psychology: 'simple' conditioning? *Commun. Integr. Biol.* **3**, 142–145.
- Croll, R. P. W. J. D. (1981). Motor program switching in *Pleurobranchaea*. I. Behavioral and electromyographic study of ingestion and egestion in intact specimens. *J. Comp. Physiol.* **145**, 277–287.
- Davies, N. B. and Krebs, J. R. (1979). *Behavioral Ecology: An Evolutionary Approach*. Oxford: Blackwell Scientific Publications.
- Davis, W. J. and Mpitsos, G. J. (1971). Behavioral choice and habituation in the marine mollusk *Pleurobranchaea californica* MacFarland (Gastropoda, Opisthobranchia). *Z. Vgl. Physiol.* **75**, 207–232.
- Davis, W. J., Villet, J., Lee, D., Rigler, M., Gillette, R. and Prince, E. (1980). Selective and differential avoidance learning in the feeding and withdrawal behavior of *Pleurobranchaea californica*. *J. Comp. Physiol.* **138**, 157–165.
- Davis, W. J., Gillette, R., Kovac, M. P., Croll, R. P. and Matera, E. M. (1983). Organization of synaptic inputs to paracerebral feeding command interneurons of *Pleurobranchaea californica*. III. Modifications induced by experience. *J. Neurophysiol.* **49**, 1557–1572.
- Gillette, R. (1991). The molluscan nervous system. In *Comparative Animal Physiology: Neural and Integrative Animal Physiology*, 4th edn (ed. C. L. Prosser), pp. 574–611. New York: Wiley-Liss.
- Gillette, R., Saeki, M. and Huang, R.-C. (1991). Defense mechanisms in notaspid snails: acid humor and evasiveness. *J. Exp. Biol.* **156**, 335–347.
- Gillette, R., Huang, R. C., Hatcher, N. and Moroz, L. L. (2000). Cost-benefit analysis potential in feeding behavior of a predatory snail by integration of hunger, taste, and pain. *Proc. Natl. Acad. Sci. USA* **97**, 3585–3590.
- Goslinger, T. M. (1991). *Galapagos Marine Invertebrates: Taxonomy, Biogeography, and Evolution in Darwin's Islands*. New York, NY: Plenum Press.
- Greenwood, P. G. and Mariscal, R. N. (1984). The utilization of cnidarian nematocysts by aeolid nudibranchs: nematocyst maintenance and release in *Spurilla*. *Tissue Cell* **16**, 719–730.
- Hebb, D. O. (1949). *The Organization of Behavior; a Neuropsychological Theory*. New York, NY: Wiley.
- Hirayama, K. and Gillette, R. (2012). A neuronal network switch for approach/avoidance toggled by appetitive state. *Curr. Biol.* **22**, 118–123.
- Hirayama, K., Catanho, M., Brown, J. W. and Gillette, R. (2012). A core circuit module for cost/benefit decision. *Front. Neurosci.* **6**, 123.
- Jing, J. and Gillette, R. (2003). Directional avoidance turns encoded by single interneurons and sustained by multifunctional serotonergic cells. *J. Neurosci.* **23**, 3039–3051.
- Lavery, T. M. and Plowright, R. C. (1988). Flower handling by bumblebees: a comparison of specialists and generalists. *Anim. Behav.* **36**, 733–740.
- McDonald, G. R. N. (1978). Additional notes on the food of some California nudibranchs with a summary of known food habits of California species. *The Veliger* **21**, 110–118.
- Mpitsos, G. J. and Cohan, C. S. (1986a). Differential Pavlovian conditioning in the mollusc *Pleurobranchaea*. *J. Neurobiol.* **17**, 487–497.
- Mpitsos, G. J. and Cohan, C. S. (1986b). Discriminative behavior and Pavlovian conditioning in the mollusc *Pleurobranchaea*. *J. Neurobiol.* **17**, 469–486.
- Mpitsos, G. J. and Collins, S. D. (1975). Learning: rapid aversive conditioning in the gastropod mollusk *Pleurobranchaea*. *Science* **188**, 954–957.
- Wägele, H. and Klussmann-Kolb, A. (2005). Opisthobranchia (Mollusca, Gastropoda) – more than just slimy slugs. Shell reduction and its implications on defence and foraging. *Front. Zool.* **2**, 3.
- Yerkes, R. M. and Dodson, J. D. (1908). The relation of strength of stimulus to rapidity of habit-formation. *J. Comp. Neurol.* **18**, 459–482.
- Zhang, Y., Lu, H. and Bargmann, C. I. (2005). Pathogenic bacteria induce aversive olfactory learning in *Caenorhabditis elegans*. *Nature* **438**, 179–184.