Selective Prey Avoidance Learning in the Predatory Sea-Slug *Pleurobranchaea californica*

by:

Vanessa Noboa\(^1,3\) and Rhanor Gillette\(^1,2\)

Address: \(^1\)Department of Molecular & Integrative Physiology, \(^2\)The Neuroscience Program, 524 Burrill Hall, 407 S. Goodwin Ave., University of Illinois, Urbana, IL 61801, USA

\(^3\)Present Address:

Colegio de Ciencias de la Salud
Escuela de Medicina
Universidad San Francisco de Quito
Quito, Ecuador

Running title: Prey avoidance learning in a simple system

Corresponding Author:

Rhanor Gillette
Department of Molecular & Integrative Physiology
524 Burrill Hall
407 S. Goodwin Ave.
University of Illinois
Urbana, IL 61801, USA.
Email: rhanor@illinois.edu
Tel.: 217-333-0328

Manuscript information: 5 figures
Number of pages: 24 (not including figures)
Word count: 197 Summary, 546 Introduction, 1104 Discussion
Key words: aposematism, decision, choice, odor learning, discrimination
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 predator sea-slug rapidly learned avoidance. Notable exceptions were animals with extremely high or low feeding thresholds that either ignored _Flabellina_ or completely consumed it, respectively. Experienced slugs showed strong avoidance of _Flabellina_ for days after exposure. Aposematic odor learning was selective: Avoidance was not linked to changes in feeding thresholds, and trained animals readily attacked and consumed a related aeolid, _Hermisenda crassicornis_. Aposematic learning could be related within a framework for cost-benefit decision in which effects of memory, motivation and odor sum in appetitive state to direct behavioral choice.
Introduction

Animals with noxious defenses may enhance their protection from predation by educating predators to associate prominent cues with 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 cost-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 (Laverty 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 like Flabellina 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 an aposematic signal for potential predators. Pleurobranchaea is sightless with 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. It was 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 *Pleurobranchaea* 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,b). Thus, *Pleurobranchaea* 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 *Pleurobranchaea* readily learned to avoid *Flabellina*. 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 hours. Animals trained against *Flabellina* still attacked and consumed the related aeolid nudibranch *Hermissenda crassicornis*. The robust and selective aposematic learning, with its notable exceptions, is consistent with a previous neural model for cost-benefit decision that encodes stimulus valuation and risk from moment-to-moment in appetitive state, and thereby determines approach/avoidance decision (Hirayama and Gillette, 2012).

**Materials and methods**

**Pretraining procedures**

Specimens of *Pleurobranchaea californica*, 80-1000 ml volume, were obtained by trawl or trapping through Pacific Biomarine, Venice, CA, Sea Life Supply, Sand City, CA and Monterey Abalone, Inc., Monterey, CA. *Flabellina iodinea* were collected by SCUBA from pilings of the Fisherman’s Wharf in Monterey, CA, or obtained from Marinus, Inc., Santa Monica, CA, or Sea Life Supply, Sand City, CA or Monterey Abalone, Inc. This species ranges from the Galapagos Islands (Goslinger, 1991) to Vancouver Island, British Columbia, Canada (Bernard, 1970). *Flabellina* inhabits the intertidal to approximately 40 m depth, and is a specialist feeder on the colonial hydroid *Eudendrium ramosum* (McDonald, 1978). *Hermissenda crassicornis* were obtained from Sea Life Supply. Animals were maintained without feeding for 2-7 days until use. No clear loss in the aversiveness of *Flabellina* was noted over these periods.
Feeding thresholds for *Pleurobranchaea* were measured as done previously (Davis and Mpitsos, 1971; Gillette et al., 2000). Responses were observed for betaine (trimethylglycine HCl; Sigma-Aldrich) solutions in filtered artificial sea-water applied in 1.5 ml volumes over 10 seconds to the oral veil with a hand-held Pasteur pipette in a series of ascending concentrations from $10^{-6}$ to $10^{-1}$ M. Thresholds recorded were those concentrations at which animals showed proboscis extension and biting. When specimens failed to respond to the highest concentration ($10^{-1}$ M) 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 *Pleurobranchaea* 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 *Flabellina* was placed at 12-15 cm distance in front. Upon release, *Pleurobranchaea* crawled forward to physically encounter *Flabellina* 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 *Pleurobranchaea* refrained from biting the prey for 90 seconds. In multiple cases during later training a *Pleurobranchaea* made an avoidance turn before touching *Flabellina*, indicating 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 were given 5 trials with 20 minute intertrial intervals. If animals failed to reach the 90 seconds non-biting criterion after 5 trials, additional trials were given until criterion was reached. In control observations, *Flabellina* 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 hours post-training, betaine feeding thresholds were taken to check for possible changes. In tests for odor specificity of learning at 20 minutes and 24 hours post-training, animals were presented with *Hermissenda crassicornis*, an aeolid nudibranch.
relative of *Flabellina* readily consumed by *Pleurobranchaea*. At 24 hours retention tests for *Flabellina* followed 20 minutes after tests for *Hermissenda*. If an animal failed to refrain from attacking, extra training trials were carried out. Only retention tests were carried out 72 hours 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 hour 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 the threshold measures were postponed to 24 hours.

Video of encounters between *Pleurobranchaea* and *Flabellina* was recorded at Hopkins Marine Station (Stanford University) in Pacific Grove, CA, and can be seen and downloaded in the supplementary file.

**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, as described above. Latencies are presented as medians, and errors are presented as interquartile range (+/- IQR). Data were compared using Friedman’s non-parametric repeated measures ANOVA or Kruskal-Wallis non-parametric ANOVA where applicable, Wilcoxon’s signed rank test, and Mann Whitney test. Significance is reported for 2-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*IQR above the third quartile (Q3) and 1.5*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 *Flabellina* without rejecting it. We assayed 5 such animals with averaged proboscis extension threshold of $10^{-6.2} \pm 10^{-0.58}$ SE and bite
thresholds of $10^{-5.0} \pm 10^{-0.71}$ SE) prior to consuming Flabellina. 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 Flabellina 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 $10^{-4} - 10^{-2}$ M,
which is an intermediate range of readiness-to-feed (Gillette et al., 2000).

Latencies to bite increased with prey avoidance trials

Forty-four Pleurobranchaea were used in training trials with Flabellina. Close
approach caused mouth opening, proboscis extension and ballistic biting. These
behaviors sometimes began when the predator was 2-3 cm from Flabellina, 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 seconds from either first proboscis extension or physical
contact. Seizure of Flabellina was always followed within 2-3 seconds 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 seconds 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 degrees,
the anterior foot re-attached to the substrate, the tail lifted, 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 the supplementary file.

In control trials Flabellina 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, 4 had reached full avoidance criterion of 90 seconds
after a single trial, 21 had reached criterion by the 5th trial and 7 required a single sixth
trial. 16 control animals stimulated with Flabellina but prevented from biting did not show significant latencies changes over trials.

**Increasing avoidance with trials**

Retention was tested at 24 and 72 hours post-training. Latencies to bite remained significantly increased at 24 and 72 hours after the initial training session (Fig. 3A). Inversely, the number of trials to reach criterion 24 and 72 hours 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 1-2 more training trials to reach criterion (Fig. 3C; N=28). At 24 hours 35% actively avoided on the first trial and the remainder were all avoiding by the 3rd trial. At 72 hours 75% avoided on the first trial and all by the 2nd 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 Hermissenda as prey. 8 animals that had met avoidance criterion were presented with the aeolid nudibranch Hermissenda at 20 minutes after the final Flabellina training trial on the first day. Separately, 5 trained animals were challenged with Hermissenda at 72 hours. In each case Pleurobranchaea oriented to the Hermissenda, bit and readily devoured it. Pictures from these trials are shown in Figure 4.

Prior observations indicated that contributions of learning and handling effects could be mixed. 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 Pleurobranchaea at 24 and 72 hours from initiation of training. In this population no significant differences in thresholds for proboscis extension or biting were observed at 24 or 72 hours (Fig. 5A). However, in separate groups of 16 experimental and 16 control animals tested at 24 hours post-training, bite thresholds, but not proboscis extension thresholds, did rise significantly (not shown) by an average of
0.93 negative log units for experimentals (Wilcoxon p<0.01, W=49) and for controls an average of 0.44 unit (1-tailed Wilcoxon p=0.042, W=35). Differences between experimentals and controls were not significant in Mann-Whitney tests. The most marked difference in responses of experimental and control animals was that 100% of experimentals showed active avoidance responses to *Flabellina*, while no controls avoided. 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 further 11 instances *Pleurobranchaea* were tested with *Hermissenda* both before and 1 hour after training against *Flabellina* (Fig. 5B). The average latencies in these trials for biting *Hermissenda* were significantly higher, but avoidance behavior never occurred. However, all the trained animals did avoid *Flabellina*. Thus, handling effects, possibly including trauma from *Flabellina*’s stinging defense, may have contributed to the longer latencies for *Hermissenda* attack. It is also possible that there was some generalization of avoidance learning of *Flabellina* to the related aeolid *Hermissenda*. However, the complete absence of active avoidance of *Hermissenda* is more consistent with simple handling effects on readiness-to-feed.

**Discussion**

The principal observations and conclusions in this work were four: 1) *Pleurobranchaea* learns through experience to suppress feeding and avoid the noxious aeolid nudibranch *Flabellina*, 2) The learned avoidance is relatively long-term, lasting at least 48 hours, at which time it still manifests strongly, 3) Learned avoidance is largely selective to *Flabellina* and is independent of reduced arousal levels or non-specific changes in feeding threshold, and 4) Animals at either extreme of 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 *Flabellina*, and in retention of avoidance for 24 and 48 hour periods after training trials.

Two separate observations sustained the conclusion that learned avoidance of *Flabellina* was selective: 1) Animals trained to avoid *Flabellina* continued to attack the related aeolid nudibranch *Hermissenda*, and 2) Feeding thresholds to the general appetitive stimulus betaine did not change significantly in trained vs. 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 for attack both *Flabellina* and *Hermissenda* in experimentals. However, the experimentals’ complete avoidance of *Flabellina* and ready attack of *Hermissenda* supports a prominent role for aposematic learning.

These results extend previous observations to more closely connect *Pleurobranchaea*’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 *Pleurobranchaea*

Aposematic learning may markedly enhance the foraging strategy of a simple, generalist forager like *Pleurobranchaea*. Simplicity in CNS and behavior is characteristic of the clade Nudipleura (Gillette, 1991), whose members appear to have traded off their shells in evolution for a notable array of chemical defenses (Wägele and Klussman-Kolb, 2005; Cimino and Gheselin, 2009) and at the same time reduced need for greater neural and behavioral complexity. Their primitive statocyst equilibrium organs and soft bodies, that limit proprioceptive and motor abilities in general, appear suited to relatively simple behavioral economies often highly specialized in diet and habitat. However, for the generalist like *Pleurobranchaea*, aposematic learning is a cognitive adaptation to a variable prey population that must lend appreciable flexibility to its foraging strategy.

Animals with noxious defenses like *Flabellina* 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). Possibly some or many of the compounds identified in the skin of nudipleuran species as likely defensive chemicals (Cimino and Gheselin, 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, *Pleurobranchaea* 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, 1986c). The present results indicate that *Pleurobranchaea* 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 *Pleurobranchaea* 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 *Flabellina* odor with avoidance-inducing punishment, bringing the odor itself to finally elicit avoidance, as noted in those *Pleurobranchaea* 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. The ability for learned odor aversion has also been shown in another very simple system, the nemotode *Caenorhabditis elegans*, in which classical conditioning of aversion to a pathogenic bacterium was shown (Zhang et al., 2005).

Field observations are consistent with the likely natural occurrence of aposematic learning in *Pleurobranchaea*. Collecting trawls in 100 m depths in Monterey Bay in 1993 (examined by RG and Leonid Moroz) brought up both *Pleurobranchaea* and the aeolid *Flabellina trilineata* in the same nets, indicating cohabitation of these species at that time and place. 4 of 8 *Pleurobranchaea* tested on the boat showed marked avoidance to *F. trilineata*, while the remaining specimens showed the bite/rejection response to *F. iodinea* described here. As of August 2012, *Pleurobranchaea* 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 (personal observation, Armand Barilotti, Monterey Abalone, Inc.). It may be reasonably expected that unhappy encounters occur naturally between these two species.

These observations agree with conclusions that foraging Pleurobranchaea 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 Flabellina 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 of it. 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 editorial comments.
These studies were elaborated from preliminary observations by Dr. Rong-Chi Huang in
1985.

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

References
educated predators strategically trade off the costs and benefits of consuming aposematic
prey. Behav. Ecol. 18, 645-651.
Columbia: based on faunistic surveys since 1950. Syesis 3, 75-94.
Cimino, G., and Ghiselin, M. T. 2009. Chemical defense and evolution of
Behavioral and electromyographic study of ingestion and egestion in intact specimens. J.
Comp. Physiol. 145, 277-287.
Organization of synaptic inputs to paracerebral feeding command interneurons of


**Figure Legends:**

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

Figure 2. Comparison between experimental and control latencies to bite during training sessions. 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 2nd trial (Wilcoxon Signed Rank Test: W= -181, *p=0.02) and grew over 3rd (W= -293, **p<0.0001), 4th (W= -389, **p<0.0001), and 5th trials (W= -406, **p<0.0001). The 28 experimentals differed significantly from 16 controls on the 3rd trial (Mann Whitney test, U=128.5, ●p<0.02), 4th trial (U=80.5, ●●p<0.001) and 5th trial (U=8.0, ** p<0.0005). Control animals were exposed to a Flabellina that was removed before it could be bitten.

Fig 3. Prey avoidance at 24 and 72 hours post-training. A. Median latencies of 28 animals on the first trial of sessions remained high at 24 hours and were still higher for 12 animals at 72 hours. Kruskal-Wallis non-parametric ANOVA: H= 32.846, p<0.0001. Wilcoxon Signed Ranks test: 0 vs. 24 hr ***p<0.0001, 0 vs. 72 hr Mann Whitney test: U=347. B. The median number of trials needed to reach criterion decreased, non-parametric ANOVA p<0.0001, H= 52.368, 0-24 hr ***p<0.0001; 0-72 hr, ***p<0.0001. C. The percentage of animals actively avoiding Flabellina increased with trial number and over days of training.

Figure 4. Species selectivity of prey avoidance training. In a post-training test, an animal is shown actively avoiding Flabellina (A-C). The same animal 20 minutes later quickly attacked and ingested the related nudibranch Hermissenda crassicornis (arrows; D-F). The animal’s foot appears raised as it touches the glass wall of the aquarium.

Figure 5. Selectivity of prey avoidance training. A. Median feeding thresholds for proboscis extension and bite to the appetent betaine were not significantly different after training at 24 and 72 hours (N=12; Wilcoxon Signed Rank tests). B. Latencies to bite Hermissenda were also elevated 1 hour after training (Wilcoxon signed-rank test, W= 66, p<0.001), but to a lesser extent than for Flabellina, compared to pre-training measures for
11 Pleurobranchaea, perhaps due to handling effects (see text). All 28 trained animals showed active avoidance of Flabellina, which was not seen in any controls.

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

Figure 2. Comparison between experimental and control latencies to bite during training sessions. 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 2nd trial (Wilcoxon Signed Rank Test: $W=-181$, *$p=0.02$) and grew over 3rd ($W=-293$, **$p<0.0001$), 4th ($W=-389$, **$p<0.0001$), and 5th trials ($W=-406$, **$p<0.0001$). The 28 experimentals differed significantly from 16 controls on the 3rd trial (Mann Whitney test, $U=128.5$, ●$p<0.02$), 4th trial ($U=80.5$, **$p<0.001$) and 5th trial ($U=8.0$, **$p<0.0005$). Control animals were exposed to a *Flabellina* that was removed when the control attempted to bite it.
Fig 3. Prey avoidance at 24 and 72 hours post-training.  A. Median latencies of 28 animals on the first trial of sessions remained high at 24 hours and were still higher for 12 animals at 72 hours. Kruskal-Wallis non-parametric ANOVA: H= 32.846, p<0.0001. Wilcoxon Signed Ranks test: 0 vs. 24 hr ***p<0.0001, 0 vs. 72 hr Mann Whitney test: U=347.  B. The median number of trials needed to reach criterion decreased, non-parametric ANOVA p<0.0001, H= 52.368, 0-24 hr ***p<0.0001; 0-72 hr, ***p<0.0001. C. The percentage of animals actively avoiding Flabellina increased with trial number and over days of training.
Figure 4. Species selectivity of prey avoidance training. In a post-training test, an animal is shown actively avoiding *Flabellina* (A-C). The same animal 20 minutes later quickly attacked and ingested the related nudibranch *Hermissenda crassicornis* (arrows; D-F). The animal’s foot appears raised as it touches the glass wall of the aquarium.
Figure 5. Selectivity of prey avoidance training. A. Median feeding thresholds for proboscis extension and bite to the appetent betaine were not significantly different after training at 24 and 72 hours (N=12; Wilcoxon Signed Rank tests). B. Latencies to bite Hermissenda were also elevated 1 hour after training (Wilcoxon signed-rank test, W= 66, p<0.001), but to a lesser extent than for Flabellina, compared to pre-training measures for 11 Pleurobranchaea, perhaps due to handling effects (see text). All 28 trained animals showed active avoidance of Flabellina, which was not seen in any controls.