Orienting and avoidance turning are precisely computed by the predatory sea-slug *Pleurobranchaea californica* McFarland

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Summary

Computing the direction and amplitude of orienting and avoidance turns is fundamental to prey pursuit and risk avoidance in motile foragers. We examined computation of turns in the predatory sea-slug *Pleurobranchaea californica*, observing orienting and aversive turn responses to chemotactile stimuli applied to the chemosensory oral veil. We made seven observations: (1) the relation of turn angle/stimulus site on the oral veil was linear; (2) turn amplitudes increased with stimulus strength; (3) turn responses markedly overshot the target stimulus; (4) responses to two simultaneous stimuli at different loci were averaged to an intermediate angle; (5) stimuli could induce sequential turns in which the angles of the first and third turns were similar, a form of working memory; (6) turn direction was affected by appetitive state, so that animals with higher feeding thresholds tended to avoid appetitive stimuli; and (7) avoidance turns induced by mildly noxious stimuli were computed similarly to orienting, while differing in direction. These observations appear to outline a framework of behavior that could be employed for efficient tracking of odor trails, and which is regulated by decision mechanisms that integrate sensation, internal state and experience.

Key words: trailing, orienting, odor tracking, decision.

Introduction

Actively exploring animals make precise computations in their rapid and frequent decisions to turn toward or away from the varied stimuli present in the environment. The appropriateness of their decisions for turn direction and amplitude determines success in resource acquisition and avoidance of predators and accidental injury. Such decisions, like most others, are likely to be made through integrating stimulus percepts with internal state (e.g. hunger and reproductive states) and the memories of experience. For instance, while moderately hungry animals may orient to and approach weak appetitive stimuli, satiation and learning can change these responses so food stimuli are actively avoided by satiated animals. What is more, hungry animals may avoid food items with which they have had a previous negative experience, such as induced sickness or distress from effects of strong prey defenses (Garcia and Koelling, 1966; Mpitsos and Collins, 1975; Gillette et al., 2000). Diverse studies have elucidated the computations for directional behavior in arthropods and fish (Murphey, 1971; Levi and Camhi, 2000; Zeil et al., 1985; Eaton and Emberly, 1991), but few have similarly examined elements of foraging behavior in molluscs (but see Teyke et al., 1990). Further, to our knowledge none have quantitatively studied the interactions of sensation and internal state for which these model systems are most useful.

We used the predatory sea-slug *Pleurobranchaea californica* to study the relationships between stimulus location, stimulus amplitude and modality, and internal state on orienting and avoidance turns. *Pleurobranchaea* is an opportunistic predator on numerous invertebrates, including conspecifics (Davis and Mpitsos, 1971), whose abilities to track appetitive chemotactile stimuli and to recognize and avoid potentially dangerous stimuli must be most critical to the animal's eventual reproductive success. This species generally inhabits deeper waters (20 to >1000 m) characterized by constant and changing currents. Without external eyes and imaging vision, the animals depend largely on chemotactile cues in their environmental interactions.

Foraging gastropods are well-documented for their abilities to orient to and follow odor trails of potential prey and mates (Hall, 1973; Tyndale et al., 1994; Townsend, 1974; Clifford et al., 2003; Wyeth and Willows, 2006) or to avoid those of potential predators (Wyeth and Willows, 2006). However, to date few studies have elucidated the behavioral elements of orienting or avoidance or of their neural bases. For *Pleurobranchaea*, previous work outlined a neural network of

identified neurons that mediates avoidance turning stimulated by unilateral electrical shocks to the oral veil (Jing and Gillette, 2003). This neural network, composed of bilaterally homologous pairs of neurons, was hypothesized also to mediate orienting turns if active in an inverted configuration. We have extended behavioral observations on turning to further define the constraints that must attend the neural network structure and function, through assessing the causative factors that determine the turn response in terms of direction and angle.

Escape turns induced by strongly noxious electric shocks to *Pleurobranchaea*'s oral veil have been studied (Jing and Gillette, 2003). Such turns were characterized by their vigor, large and unpredictable amplitudes, and similarity to classic fixed action patterns. However, our informal observations suggested that most turns in the actively exploring animal are of smaller amplitudes and might serve either in locating appetitive stimuli or in avoidance of non-appetitive stimuli (http://www.life.uiuc.edu/slugcity/movies.html). Thus, in the present study, we tested the effects of appetitive and only moderately aversive chemotactile stimuli. We adapted procedures for quantitatively relating stimulus site to turn response in other species (Murphey, 1971; Zeil et al., 1985; Teyke et al., 1990; Levi and Camhi, 2000) for use with *Pleurobranchaea*. Our findings indicate that the angle of the turn response to a punctate chemotactile stimulus applied to the animal's oral veil is precisely computed on the basis of stimulus site and strength, while the turn direction is a function of both stimulus modality and satiation state. These results seem to speak to chemotaxic tracking and avoidance strategies, and they suggest neural circuit hypotheses for future investigation.

Materials and methods

Specimens of *Pleurobranchaea californica* McFarland (Sea Life Supply, Sand City, CA, USA) were maintained in artificial seawater at 12–13°C. A total of 18 animals were tested in this study. Feeding thresholds were measured as described previously (Davis et al., 1974; Gillette et al., 2000). Ascending tenfold dilutions of betaine (trimethylglycine) from 10^{-6} to 10^{-1} mol l^{-1} were applied to the oral veil with a pasteur pipette in 1.5 ml volumes over a period of 10 s. Those dilutions at which proboscis extension and biting responses occurred were recorded as feeding thresholds. Betaine is the single most potent feeding stimulant, and an abundant osmolyte of *Pleurobranchaea*'s invertebrate prey (Gillette et al., 2000). Animal sizes, determined by seawater displacement, ranged from 8 to 400 ml. An animal was considered quite ready-to-feed if its betaine threshold for biting was less than or equal to 10^{-3} mol l^{-1}, values at which animals tend to ignore mildly noxious stimuli to attack food stimuli (Gillette et al., 2000).

Gelatin cubes of 3–5 mm on a side were used for chemotactile stimulation. The cubes were cut from a shallow tray of unflavored gelatin powder (Knox; Kraft Foods, Northfield, IL, USA) dissolved as 1 g per 30 ml of artificial seawater with a few drops of red food color (Red #40; Supplier, Meijer Inc., Grand Rapids, MI, USA) added to improve

visibility for video data analysis. In the gelatin we also dissolved appetitive (betaine) or noxious (taurine) chemical stimuli in varying concentrations. The cubes were impaled on the tips of Pasteur pipettes, leaving no sharp glass edges exposed, for application to the chemosensory oral veil (Fig. 1).

Betaine-infused cubes were applied to elicit orienting turns. To investigate the effects of appetitive stimulus strength on orienting turns, betaine concentrations in cubes ranged 10^{-7} mol l^{-1} to 10^{-2} mol l^{-1}. Taurine-infused cubes (2-aminoethanesulfonic acid; Sigma) were applied to induce avoidance. Taurine is a noxious stimulus when applied to the skin of *Pleurobranchaea* (Gillette et al., 1991).

The seawater arenas used were large enough to permit several complete unrestrained turns and had no active water flow. Animals were positioned at one end of the arena at the beginning of trials. When an animal began to locomote straight forward, a gelatin pellet was brought in front into the water parallel with the locomotion path, taking care not to introduce ripples and currents. The stimulus was moved toward the animal, positioned within a body length in the animal's path so as to contact a desired locus on the oral veil, and held there, stationary, until contact. Only responses of animals that locomoted straight forward to touch the pellet were included in the analysis. Stimulus application to quiescent animals rarely elicited a response. In those few cases where quiescent animals actively responded, responses were not robust and those trials were not included, nor were rare occasions when an animal turned toward the stimulus before touching it. Stimulus application to actively turning animals was avoided.

When animals touched the pellet with their oral veil, it was held there for 1–2 s and then removed. The pellet was moved slowly with the animal as it locomoted forward so as not to cause appreciable distortion of the oral veil. This light, transient contact allowed clear estimation of the stimulus position on the oral veil and also prevented animals from biting the pellet. At times animals with marked readiness to feed (10^{-7} mol l^{-1}–10^{-5} mol l^{-1} betaine biting threshold) oriented to the gelatin pellet as it was brought near, thus performing an orienting turn to a stimulus not yet defined by our criteria. In other cases animals succeeded in biting the gelatin pellet and such trials were excluded from the data analysis. After stimulus removal the animal was allowed to locomote until it encountered a wall or made more than three sequential turns, and was then repositioned for the next application several minutes later. Animals on average yielded 10–12 raw data points before they ceased to respond.

For stimulation and data analysis, the oral veil was divided into nine regions of roughly equal width, which denoted the position of the stimulus (Fig. 1). Thus, for the nine regions the left tentacle at the extreme edge of the oral veil corresponded to stimulus position $x=-4$, the midline $x=0$, and the right tentacle $x=+4$. Those regions could be estimated fairly well during stimulus application, and exact stimulus positions were determined during the video analysis. Responses were recorded on a Sony camcorder. Videos were digitized with a Dazzle multimedia adapter for PC and stored on computer in mpeg
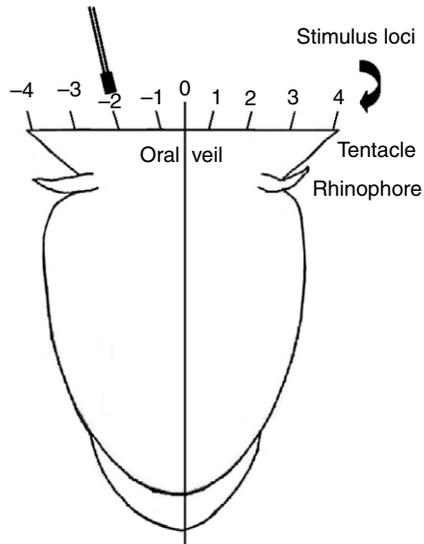
Fig. 1. Stimulus application protocol. Nine stimulus loci were distinguished on the oral veil, with 0 designating midline, and +4 and –4 designating the right and left tentacle, respectively.
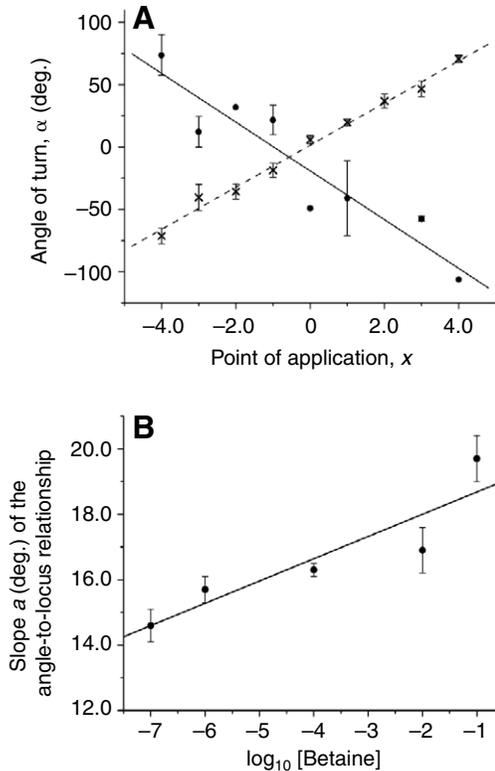

Fig. 2. Unilateral applications of chemotactile stimulus resulted in linear relationship between the turn angle and stimulus locus. (A) Responses to the appetitive stimulus betaine (crosses) and to the aversive stimulus taurine (filled circles). (B) The slope of the relationship in A for appetitive stimulus increases with the stimulus concentration. Values are means ± s.e.m.; for correlation coefficients, see text.

format (examples can be seen at http://www.life.uiuc.edu/ slugcity/movies.html). Using those mpeg files for stop-frame video analysis, we traced the locomotion paths of animals by marking the position of both tentacles approximately every 2–5 s on a transparency overlain on a computer monitor. As animals locomoted at 5–10 mm s^{-1} and turns could take more than 1 min to complete for larger animals, 12–30 data points were adequate to measure the turns, varying with animal size. Responses for oral veil regions were averaged within and across animals for the turn response plots of Fig. 2.

The locomotion traces described above were used to measure the angles of turn responses induced by stimuli. Turns were measured using a protractor with a precision of 0.5°. Completion of turns was marked either by initiation of an oppositely directed turn or by straight and forward locomotion. The angle of turn was defined as the angle between the midline of the animal at the moment of stimulus application and the midline at the end of the turn. If an animal performed only one turn in response to the stimulus, the final orientation of the midline was measured when it became clear that the animal was locomoting straight forward. If a series of turns was performed, the end of each turn was marked clearly by the change of the oral veil orientation, facilitating measurements (Fig. 4).

Multiple stimulus applications at various sites on the oral veil were performed on each animal, but the total number of applications was limited by the amount of time any given animal remained responsive. Data from all animals were combined for analysis into a single dataset for each of the following experimental conditions: single-point betaine applications, single-point taurine applications, and two-point betaine applications. Averaged responses in the entire animal

population were computed for each application site or combination of sites.

Statistical analyses employed unpaired two-tailed Student *t*-tests for statistical comparisons of slopes computed by means of linear regression. Linear regression was performed with least-squares algorithm, using an acceptable level of significance of $P<0.05$. Fisher's exact test was used to determine relationship between readiness-to-feed and prevailing nature of turn responses (orienting or avoidance).

Results

Single-point applications

Most measurements with appetitive stimulation were performed using betaine concentrations of 10^{-2} mol l^{-1}, a high, but not maximal, concentration chosen so as to cause a robust response. In trials where gelatin pellets infused with 10^{-2} mol l^{-1} betaine were presented singly to defined sites on the oral veil, a linear relationship was found to exist between the application locus *x* (between –4 to +4) and the angle of turn α (Fig. 2A; correlation coefficient $r=0.99$, $N=9$, $P<0.001$):

$$\alpha = ax + b .$$

Table 1. *Correlation analysis of the relationship between angle of turn α and concentration β of the stimulus for all conditions tested*

Application	Slope *a*	Intercept *b*	*r*	*N*
Single-point applications				
10^{-1} mol l^{-1} betaine	19.7±0.7	0.8±1.9	0.99	9
10^{-2} mol l^{-1} betaine	16.9±0.7	1.5±1.7	0.99	9
10^{-4} mol l^{-1} betaine	16.3±0.2	−1.1±0.6	0.99	9
10^{-6} mol l^{-1} betaine	15.7±0.4	0.2±0.9	0.99	9
10^{-7} mol l^{-1} betaine	14.6±0.5	−3.2±1.2	0.99	9
10^{-2} mol l^{-1} taurine	−19.5±2.9	-19.1±7.9	−0.93	8
Relationship between slope and [β]	0.7±0.2	19.4±0.8	0.91	5
Relationship between intercept and [β]	0.6±0.3	1.9±1.2	0.77	5
Unilateral two-point applications				
10^{-2} mol l^{-1} betaine; orienting	6.0±1.5	14.5±7.1	0.83	10
10^{-2} mol l^{-1} betaine; avoidance	−8.4±1.4	15.5±6.9	−0.87	12
Bilateral two-point applications				
10^{-2} mol l^{-1} betaine	9.3±1.6	7.3±4.2	0.91	9
Unilateral two-point applications angle correlations				
Third turn *vs* first turn	1.0±0.2	−3.7±8.2	0.84	17
Second turn *vs* first turn	0.0±0.2	22.9±8.1	0.08	17
Bilateral two-point applications angle correlations				
Third turn *vs* first turn	0.6±0.3	34.5±17.4	0.53	12
Second turn *vs* first turn	−0.4±0.4	−40.4±21.7	−0.32	12

$\alpha = a\beta + b$, where α is the angle of turn and β is the stimulus concentration. Values of slope *a* and intercept *b* are means ± s.e.m. *N*, sample size; *r*, correlation coefficient.

The table also shows correlation analysis for first and second angles of turn for sequences of turns elicited by 1- and 2-point applications.

To determine the possible effect of chemical stimulus strength, we used a range of betaine concentrations, from 10^{-7} mol l^{-1} to 10^{-1} mol l^{-1}. A linear relationship between the angle of turn and the site of stimulus application existed at all concentrations (Table 1). In addition, there was a linear correlation between the slope *a* and the concentration of the feeding stimulant (correlation coefficient $r=0.91$, $N=5$, $P=0.0326$). As concentration of the stimulus increased, the slope *a* also increased (Fig. 2B), reflecting that animals performed turns of greater amplitude at higher stimulus strengths. The average value of the relation's intercept *b* was very close to zero ($b=-0.36±0.82$, significance level 95%), indicating that animals tended to continue locomoting straight when the stimulus was applied to the midline.

Pleurobranchaea overshoot during orienting turn

In these experiments chemotactile stimuli were applied to the oral veil to simulate direct contact with prey. We initially expected *Pleurobranchaea* to perform an orienting turn that would approximately center the stimulus on the oral veil, such that the connection between stimulus locus and subsequent turn angle would follow a simple relationship where angle=arctan $(xk/4)$, where *k* is the ratio of the oral veil width to the body length *L* as shown in Fig. 3A. However, the results unexpectedly revealed that animals overshot by approximately fourfold (Fig. 3B).

Two-point applications

In normal sensory stimulation, sensory stimuli can impinge on multiple sites or wide areas on the oral veil. To investigate how *Pleurobranchaea* respond to simultaneous stimuli at separate loci, we applied betaine-infused gelatin pellets at two sites on the oral veil simultaneously. The stimuli were applied both unilaterally and bilaterally and all possible combinations of the loci were tested. Under the assumption that the two loci are equivalent, we fitted the data to a simple linear regression formula for two independent variables weighted with the same coefficient:

$$\alpha = a_2(x_L + x_R) + b_2 ,$$

where index L corresponds to the leftmost of the two stimuli, and index R corresponds to the rightmost one. The subscript 2 indicates that these values of slope and intercept refer to the 2-point applications. Linear regression analysis showed that a linear relationship did indeed exist between the turn angle and the sum of the two stimulus application sites (Table 1). This result stood for both unilateral (correlation coefficient $r=0.83$, $N=10$, $P=0.0032$) and bilateral (correlation coefficient $r=0.91$, $N=9$, $P=0.0006$) applications. In addition, the slopes for bilateral and unilateral applications were not significantly different ($P=0.1505$, $N=17$, unpaired two-tailed *t*-test).

Two appetitive stimuli applied simultaneously at different loci constitute a distributed stimulus, which from the animal's perspective is a minimum estimate of the size of the object
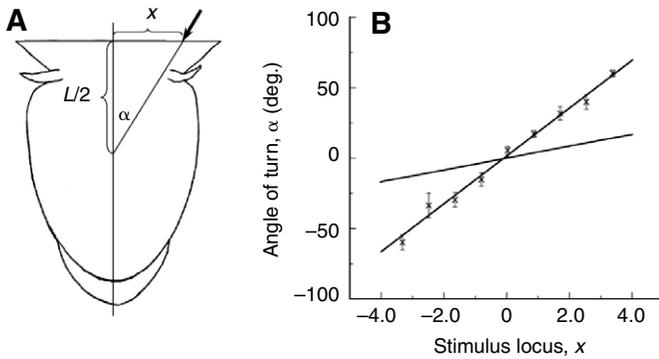
Fig. 3. Animals overshoot in orienting turns by approximately a factor of 4. (A) If animals were turning to position the stimulus in the center of their oral veil, then the angle of turn would relate to the stimulus locus according to the relationship: angle=arctan(xk/4), where k is the ratio of the oral veil width to the body length L (solid line without datapoints in B). (B) The relationship determined from data (solid line with data points, replotted from Fig. 2A) gives a slope 4 times steeper than the predicted relationship for k=0.3.
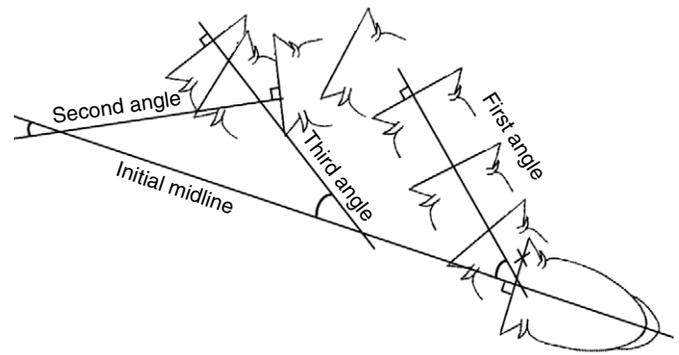

Fig. 4. Sequential turns stimulated by a single stimulus application. A significant fraction of animals performed multiple turns in response to unilateral oral veil stimulation. Three turns were most common, of which the first and third turn angles had the animals going in a closely similar direction. The second turn angle, to the opposite direction, was not correlated with the first or third. See text for further details.

causing the stimulus. This posed a question: do *Pleurobranchaea* average their response to the two sites of stimulation, as if they were turning toward the center of the object? If so, then the slope of the relationship for 2-point applications should be one-half of the value for single point applications, $a_2=a/2$. Indeed, we found the difference between a_2 and $a/2$ was not significant (Table 3) for both unilateral (P=0.1723, N=17, unpaired two-tailed t-test) and bilateral applications (P=0.6056, N=16, unpaired two-tailed t-test).

Sequential turn angles are correlated

In unilateral 2-point applications, we observed that 8 of 18 animals performed 2–5 sequential turns before their paths straightened or they ceased locomoting (Fig. 4). A sequence of three turns was most frequently observed, on average in 2 of 10 stimulus applications for each animal that performed multiple turns. Fig. 5 summarizes our analysis of those cases, in which data from all animals are combined into a single dataset. Correlation analysis of 17 cases showed that the angle of the third turn was highly correlated with and was not significantly different from the angle of the first turn (correlation coefficient r=0.84), whereas the second angle and first angle were not correlated (correlation coefficient r=0.08).

Application of 2-point stimuli bilaterally did not result in such sequential turns (Table 1).

Appetitive state affects turn direction

We found that a fraction of animals avoided the feeding stimulant betaine, instead of orienting to it. This suggested that the animals' readiness to feed was affecting the direction of the turn. We combined data from multiple trials on all tested animals and related feeding thresholds to turn direction. We found that animals with biting thresholds for betaine lower than 10^{-3} mol l^{-1} were indeed significantly more likely to orient to
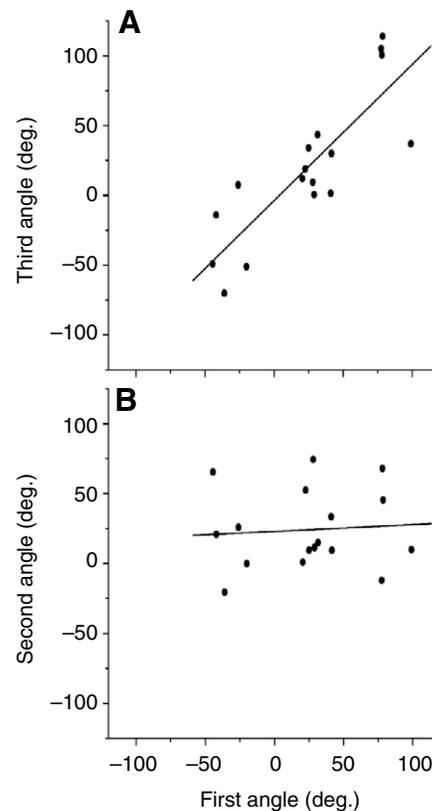

Fig. 5. (A) Correlation analysis of 17 cases of sequential turns showed that the angle of the third turn was highly correlated with and was not significantly different from the angle of the first turn (r=0.84). (B) The second angle and first angle were not correlated (r=0.08).

betaine than the animals with higher thresholds (Table 2). The frequencies of orienting and avoidance turns strongly correlated with readiness to feed (Fisher's exact test; P<0.001).

Table 2. *Animals with low feeding thresholds perform significantly more orienting turns to betaine than animals with high thresholds*

	Frequency of turns (%)	
	Low feeding threshold (*N*=7)	High feeding threshold (*N*=8)
Orienting	86	59
Avoidance	14	41

Fisher's exact test; *P*<0.001.

Table 3. *Statistical comparisons of the linear relationship between the turn angle and application locus for the conditions tested*

Compared quantities	*P*	d.f.
Single-point: orienting *a* vs avoidance *a*	0.2729	15
Orienting: unilateral *a* vs single-point *a*/2	0.1723	17
Avoidance: unilateral *a* vs single-point *a*/2	0.6600	18
Bilateral *a* vs single-point orienting *a*/2	0.6056	16
Bilateral *a* vs unilateral orienting *a*	0.1505	17
Unilateral: orienting *a* vs avoidance *a*	0.2569	20

d.f., degrees of freedom.

Avoidance turns

Avoidance turns were reliably elicited by applying gelatin pellets infused with taurine, a stimulus noxious to *Pleurobranchaea* (Gillette et al., 1991). The transient and focal nature of the stimuli used here, unlike for previous observations where taurine solutions were pipetted onto the oral veil, did not result in full escape turns where animals stopped locomoting to make a large turn, but simply caused a smooth change in movement similar, but oppositely directed, to orienting. In addition, we analyzed those incidents where avoidance, instead of orienting, turns were caused by betaine pellet applications.

Avoidance turns showed a relationship to the stimulus locus similar to orienting turns. The slope was linear (Fig. 2A, Table 1), and the differences between the slopes for orienting and avoidance in case of single- and double-point applications were not statistically significant in either treatment, except for sign relationship. From the data in Table 3, it may be seen that the slopes of the relations for 10^{-2} mol l^{-1} taurine avoidance were not significantly different from those for avoidance of 10^{-2} mol l^{-1} betaine (*P*=0.3716, two-tailed *t*-test), nor was the difference between slopes for orienting and avoidance of 2-point unilateral betaine application (*P*=0.2569; two-tailed *t*-test). Moreover, for unilateral 2-point applications, animals averaged their avoidance responses to 10^{-2} mol l^{-1} taurine much as they did in orienting to 10^{-2} mol l^{-1} betaine. The difference between a_2 and a/2 was not significant (*P*=0.6600, two-tailed *t*-test).

A consequence of the similar, but oppositely signed, turn angle/stimulus locus relation for both orienting and avoidance was that the transient, mildly noxious stimulus to the very midline of the oral veil caused no turn. Adaptive significance of this non-response in the absence of directional stimulus information is suggested from observations where such stimuli were maintained: either the animal stopped and initiated an escape avoidance turn (Gillette et al., 1991) or the animal initiated a normal closed-loop turn.

Discussion

In prey detection and location, foragers in the fluids of air and water like *Pleurobranchaea* variously use chemical and tactile information from odor plumes carried by turbulent eddies in prevailing current, from odor trails embedded in the substrate, and from direct contact with prey or other predators. The transient punctate stimuli that we applied to oral veil loci define computations within the sensory map that confer the direction and amplitude of the turn. The emerging observations have yielded relationships that cast light on details of the animal's foraging and avoidance behavior, and have suggested simple, heuristic neural models of turn computation and behavioral choice.

Search strategies

The four initial findings of this study seem to illuminate the foraging animal's search strategy and provide clues to the possible structure of the underlying computational neural circuitry. First, *Pleurobranchaea* calculated the angles of its orienting as linear functions of the site of application of transient appetitive stimuli across the oral veil. However, we next found that orienting turns tended to markedly overshoot the target stimulus site, and that overshoot increased with stimulus strength. Finally, the angle of the turn was averaged when two stimuli at different loci were presented to the oral veil simultaneously.

These first three findings resemble those of an earlier study on *Aplysia* (Teyke et al., 1990). The commonalities emerge despite the marked differences in the foraging strategies of the two animals, in the anatomy of the head chemosensory region, and in the ways in which the experiments were conducted. *Pleurobranchaea* is a predatory opportunist whose oral veil presents a fairly horizontal shelf overlying the mouth. Stimuli were applied to active animals locomoting on the substrate; non-locomoting animals did not perform well in turns. In contrast, *Aplysia* is an herbivore whose mouth lies between the frond-like tentacles, the oral veil homo/analogs, and which commonly feeds on leafy red and green algae. Stimuli were presented to *Aplysia* while non-locomoting, attached by the posterior foot to a vertical aquarium glass with the ventral surface of the anterior head region at the water surface. The strong similarities suggest that orienting turn mechanisms could be conserved broadly across molluscan species, and that in both cases the similar computations optimize search strategies of animals distinctly differing in form and habit.

Linear relationships for directional turn angles *versus*

stimulus location have resulted from investigations in a variety of arthropods, including the water strider *Gerris remegis* (Murphey, 1971), water boatman *Notonecta* sp. (Murphey, 1973), cockroach *Periplaneta americana* (Levi and Camhi, 2000) and crayfish *Cherax destructor* (Zeil et al., 1985). For terrestrial arthropods in particular, turns are mediated by combinations of leg movements, the joint angles of which determine the direction and amplitude of the turn movement (Levi and Camhi, 2000; Mu and Ritzmann, 2005). A similar linear relation is also found for the earliest movement component of the fish escape response (Eaton and Emberley, 1991). In each of these cases the anatomy underlying the turns is very much more complex than for the soft-bodied sea-slug *Pleurobranchaea*, in which turns are simply mediated by musculature that shortens one side of the body relative to the other (Jing and Gillette, 2003). However, Murphey pointed out (Murphey, 1973) that a quite simple neural computation based on lateral inhibition among the sensory pathways could produce the linear relation; the simplicity would lend it useful for the core computations observed in both simple and complex animals. The commonality across species of the linearity of the turn/stimulus relation is notable. It suggests that the relation is particularly advantageous to both the goals of directional orienting and avoidance, and/or that its simplicity lends itself to use in more complex computations; for instance in the integration of other sensory information like vision into the movement (Ye et al., 2003), or adding a third movement dimension, or incorporating temporal modifications to directional movement.

The further finding, that *Pleurobranchaea* precisely computes the average of two stimuli presented to separate loci and then swings to the intermediate angle, could supply the animal with the ability to strike at the center of an appetitive stimulus. However, the tendency to overshoot the appetitive turn past the stimulus site might appear to reduce the utility of the computational precision. Is this really the case?

In view of the precision of the turn response, the inaccuracy introduced by the overshoot, itself also precise, must confer some advantage. The nature of that advantage may be sought in consideration of the conditions when the open-loop search most often occurs. Our informal observations in our marine system indicate that *Pleurobranchaea* resembles many other aquatic molluscan foragers in its abilities to accurately navigate upstream to the source of appetitive odor plumes in currents as well as to follow slime trails of potential prey (often smaller *Pleurobranchaea* specimens) (cf. Hall, 1973; Townsend, 1974). *Pleurobranchaea* resembles *Tritonia diomedia* in general body form, habitat choice and odor tracking abilities, for which odor tracking has been documented most elegantly in the wild (Wyeth and Willows, 2006). The oral veil, sited closer to the less-stirred aqueous layer next the substrate, is used during locomotion to repeatedly sample the substrate (an example for *Pleurobranchaea* may be seen at www.life.uiuc.edu/slugcity/movies.html). In slime trail following, the overshooting turn can bring larger portions of the oral veil into the trail stimulus area and thereby enhance the
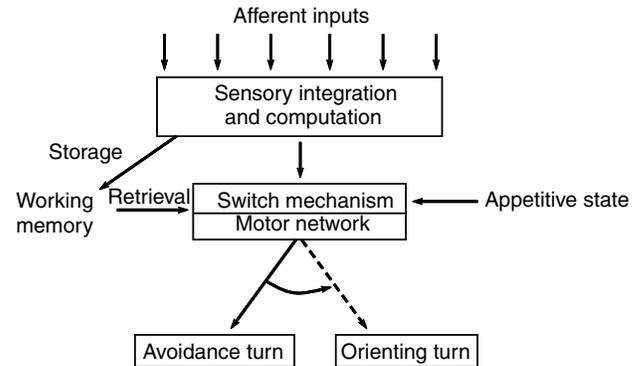

Fig. 6. A summary model of function in turn behavior. Chemotactile afferents of oral veil are conceived as being integrated in an interneuronal layer that averages amplitude and laterality of chemotactile input. The output of the interneuronal layer is translated to the turn motor network through a switch mechanism regulated by the animal's appetitive state and memory of experience. The switch acts to invert the laterality of the output to the turn motor network to result in orienting or avoidance turns.

computation of a subsequent turn toward the source. The overshoot caused by lateral stimulation of the oral veil should bring in corrective turn action by involving more medial or opposite side stimulation. Moreover, if the trail should abruptly turn laterally then the overshooting turn to the same side would enhance tracking.

Our observations on the oral veil speak less strongly to *Pleurobranchaea*'s capacity to follow odor plumes composed of uneven turbulent eddies of widely varying stimulus concentration. The animal's rhinophores, located high on its head, appear better adapted to following current-born odors. However, the oral veil might well contribute to plume following and certainly to final prey capture. Thus, the built-in overshooting computation appears well adapted to enhance the tracking abilities of a motile forager largely dependent on odor cues either fluid-borne or embedded in the substrate.

The positive effect of stimulus intensity, where stronger appetitive stimuli cause larger overshooting turns, may be interpreted in a similar fashion by taking into account the animal's ability to average stimuli along the oral veil. Of multiple simultaneous stimuli, such as might be provided by substrate trail or odor plume, those sites with stronger stimulation would bias the turn amplitude to bring a larger surface of the oral veil into contact with stimuli for the next turn computation.

It remains to deal with one of the most interesting observations of this study: apparent working memory in tracking. Following single-point appetitive stimulation, animals often made sequential turns. The first turn, when unrewarded with a new stimulus, was succeeded by a second turn in the opposite direction whose own angle was random with respect to the first. However, the third turn was back to the original direction and repeated the angle of the first. This

observation leads to inference of a persistent memory of the first turn angle calculation, its retrieval, and the associated problem of how this could be encoded in the neural circuitry.

The significance of the sequential turn computations seems most immediately accessible in terms of efficient search strategy. In the process of following an odor trail, if a lateral stimulus suggests a lateral movement of the trail but further stimulation is not encountered upon a turn in that direction, it would most reasonably imply that the stimulus source itself moved again to either right or left. A choice to continue the original turn path would diminish the opportunity to sample the other direction. The second brief turn in the other direction would accomplish a quick check and, if negative, the original direction could be pursued with the increased likelihood that it was appropriate. These maneuvers resemble the 'casting flight' of moths following a pheromone odor plume, in which, after losing an odor during flight, they may fly in broad swings back and forth across the wind (Willis and Arbas, 1991). Thus, this form of working memory in odor tracking could markedly enhance tracking efficiency.

Behavioral choice of orienting and avoidance

Pleurobranchaea switches behaviorally from orienting to avoidance responses to appetitive stimuli when trained in a food-avoidance paradigm using paired food and shock (Mpitsos and Collins, 1975), or when satiated (Gillette et al., 2000). We did not distinguish between learning and satiated state here, but simply observed that orienting and avoidance were associated with readiness-to-feed, as assessed by measures of the animals' feeding thresholds. The findings that the choice to express orienting or avoidance turns correlated with low or high feeding thresholds, respectively, resembles previous observations on hungry and satiated animals (Gillette et al., 2000). However, in the present context the results seem to speak directly to the nature and control of a neural switch that may regulate the asymmetry of activity in the premotor turning network.

Avoidance turns induced either by normally appetitive or by noxious stimuli applied to the oral veil followed the same computational rule for turn angle *vs* stimulus site as did orienting turns, save that the direction was opposite. These observations are consistent with a marked economy of neural circuitry in an animal with a small nervous system, where degree of turning for opposite actions is probably calculated in the same neural network, and the direction of the turn is then based on an estimation of the stimulus value – positive or negative, as decided through the animal's integration of stimulus nature, its internal state and learning experience. The savings in network maintenance by use of a simple circuit for both orienting and avoidance might be justified by a low average cost of potential behavioral errors.

A framework for chemotactile sensory integration and decision

The observations presented above suggest the outline of an integrated neural model for odor tracking/avoidance in *Pleurobranchaea*. The schematic of Fig. 6 portrays the initial integration of afferent inputs from the oral veil in an interneuronal layer, whose function would be to encode and average amplitude and laterality of chemotactile input. The turn motor network in *Pleurobranchaea* is a bilaterally symmetrical circuit whose lateralized activity determines turn direction (Jing and Gillette, 2003). Thus, translation of the output of the interneuronal layer into an orienting or avoidance turn requires an intervening switch mechanism regulated by the animal's appetitive state. The switch would act to invert the laterality of the output to the turn motor network.

Conclusion

We documented seven simple parameters of turning behavior in *Pleurobranchaea*: (1) linearity of the relation of turn angle to stimulus site on the oral veil; (2) increase of turn amplitude with stimulus strength; (3) overshoot of turn angle past target; (4) averaging of stimulus loci in the response; (5) memory and retrieval in sequential turns; (6) influence of appetitive state on turn direction; and (7) similarity between avoidance and orienting turn computations, differing only in direction. Collectively these seem to compose a behavioral strategy for efficiently tracking an odor trail. Tracking is regulated by a decision-making process capable of integrating sensation, internal state and experience in toggling between orienting and avoidance turns. Future work should be aimed at computational testing of a general model incorporating the above seven parameters, and at elucidating their bases in biological neural networks.

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