

Bilateral and Unilateral Antennal Lesions Alter Orientation Abilities of the Crayfish, *Orconectes rusticus*

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Abstract

Numerous animals use chemical cues within their environments to execute various behaviors. One of these behaviors is orientation to an odor source. Crayfish, in particular, can orient to food sources under a number of different conditions. It has not been determined, however, what kind of search strategy these animals employ to successfully locate a food source. To determine the role of antennae and antennules in this behavior and to investigate different modes of orientation behavior, the orientation patterns of crayfish with complete and partial antennal lesions were examined. Detailed analysis of orientation paths confirmed that crayfish could not locate odor sources with either bilateral or unilateral lesions. This suggests that crayfish are using the spatial information obtained from these appendages to successfully orient. Animals using information from the bilaterally paired appendages in the control group exhibited increased walking speed, increased speed to source and decreased heading angles towards the source compared to these measurements taken from lesioned groups. There was no significant difference in any parameters between animals with unilateral or bilateral lesions. This strongly suggests that these animals are reliant on the spatial comparison of differences between bilaterally paired olfactory appendages for successful orientation.

Introduction

Studies focusing on chemical orientation through olfactory cues, especially in aquatic organisms, have proliferated in recent years in an attempt to understand orientation strategies (McLeese, 1973; Reeder and Ache, 1980; Moore *et al.*, 1991; Breithaupt *et al.*, 1999; Moore and Grills, 1999; Finelli *et al.*, 2000; Weissburg, 2000). Different organisms have been found to employ various orientation strategies when presented with chemical stimuli. The two main types of orientation mechanisms that have been explored in detail differ only in the degree by which spatial decisions are controlled by the chemical stimuli. These are odor-modulated optomotor anemotaxis (terrestrial) and odor-gated rheotaxis (aquatic) versus chemotaxis.

Moths have been found to use odor-modulated optomotor anemotaxis (Vickers and Baker, 1992, 1994; Mafra-Neto and Cardé, 1994, 1995). During orientation, the moth switches between a casting behavior and an upwind surge in the presence of odor. The duration of the surge is controlled by the frequency with which odor filaments contact the receptors on the antennae. Blue crabs use odor-gated rheotaxis in orientation (Weissburg and Zimmer-Faust, 1993). While orienting, the crabs use both the direction of flow and the intermittency of the chemical signal to find the odor source. Crayfish and lobsters, which orient using

chemotaxis, extract directional information directly from the odor plume, which is used in orientation (Moore *et al.*, 1991; Atema, 1996; Moore and Grills, 1999). It has been determined that the animal remains in the center of the odor plume and moves towards the source by the use of spatial and temporal comparisons. Moore *et al.* (Moore *et al.*, 1991) classified the search patterns of lobsters in odor plumes into three distinct search phases. The phases are as follows: the initial stage is marked by increased walking speeds and decreased heading angles, the intermediate stage is identified by constant walking speeds and headings and the final stage consists of decreased walking speeds and increased heading angles. Up to now the strategy used by crayfish during chemical orientation has remained unknown. Detailed descriptions of these and other orientation mechanisms can be found elsewhere (Bell and Cardé, 1984; Kennedy, 1986).

Although there has been much debate about defining specific terms related to various modes of orientation, it is widely recognized that organisms use different mechanisms to orient to a point source. These mechanisms can be classified as either a taxis or kinesis (Van Der Steen and Ter Maat, 1979). Orientation responses during a taxis, such as turn angles and heading angles, are directly guided by the spatial distribution of the stimulus (Fraenkel and Gunn,

1961; Bell and Cardé, 1984). Typically, this is performed through information obtained from spatially separated receptors. Orientation through odor-modulated optomotor anemotaxis and odor-gated rheotaxis is dependent not only on the structure of the odor plume, but also on the flow direction of the medium and the visual flow field. Therefore, both mechanoreceptors, chemoreceptors and visual receptors are necessary to successfully orient using these search strategies. During a kinesis, orientation responses, such as turning rate and walking speed, are not made with respect to the direction of the stimulus, but involve a change in the rate of behavior being performed by an organism (Fraenkel and Gunn, 1961; Bell and Cardé, 1984; Schöne, 1984). Kinesis is performed by comparing information in a temporal fashion. Spatially separated sensory appendages are not necessary to successfully perform a kinesis.

Dunham *et al.* (Dunham *et al.*, 1997) found, through ablation, that antennules are not necessary for detection of sucrose in the crayfish *Cambarus bartonii*. However, the antennules were found to be necessary for substrate searching during orientation and also for leg-to-mouth feeding. Also, Giri and Dunham (Giri and Dunham, 2000) collected data supporting the hypothesis that antennules are necessary for orientation to an odor source. They found that the total ablate groups in the study initiated search behavior at a much closer distance to the source than the partial ablates and intact animals. Several studies focusing on the lesioning of olfactory organs have shown that unilateral ablation of lobster lateral filaments resulted in the loss of directional choice ability while unilateral medial lesioning had no effect (Devine and Atema, 1982). Belgane *et al.* (Belgane *et al.*, 1997) found that lobsters must have both antennules to orient in the far field. Crayfish were the ideal organisms for the present study due to the fact that they respond to chemical stimuli present in their environment (Hazlett, 1985a,b) and forage on patchily distributed food sources.

The present study attempted to interpret whether or not full or partial lesioning of crayfish antennae and medial and lateral filaments affected chemical orientation to a food source. It has not been determined if lesioned crayfish, both bilaterally and unilaterally lesioned, are capable of locating a food source. With this in mind, if crayfish were using taxis, it would be expected that individuals with both antennae lesioned would not be receiving chemical or mechanical stimulation at either antenna and, therefore, would not exhibit a search behavior. Animals with a unilateral lesion will receive mechanical and chemical stimulation on one side only. It would be expected that these animals would be unable to successfully locate the odor source due to the fact that there is no spatial separation between unlesioned receptors, a condition necessary for taxis. This study sought to determine what mechanism is used to successfully orient, namely to differentiate between the use of kinesis or taxis.

Materials and methods

Animals

The crayfish (*Orconectes rusticus*) used in this study were from the Portage River near Bowling Green State University in Bowling Green, Ohio. The animal carapace lengths were measured and only intermolt males (3.3–32.0 g) with intact appendages were used (i.e. antennae, lateral and medial filaments, chelae and walking legs). Animals were considered to be intermolt if they did not molt for 2–3 weeks after experimentation. All animals were stored in individual containers with a diameter of 16 cm and a depth of 9 cm. Animals were visually and mechanically isolated for a minimum of 72 h in a recirculating housing tank on a light cycle of 14 h light:10 h dark before use. The animals were fed one pellet of dried rabbit food three times per week.

Stimulus

The stimulus for all trials consisted of a block of fish gelatin (average 9.0 ± 0.78 g, average $2.5 \pm 0.20 \times 2.5 \pm 0.13$ cm). Fish gelatin was made by homogenizing cod (average 47 ± 1.6 g) and 235 ml of cold water. Four packets of Knox unflavored gelatin were mixed with 471 ml of hot water, which was then mixed with the fish homogenate. The mixture was poured into a rectangular pan, covered with plastic and refrigerated until solid. The gelatin was cut and each cube was individually wrapped in plastic and refrigerated until time of use.

Flume

All trials were conducted in a 760 l recirculating flume (complete dimensions, $200 \times 62 \times 52$ cm; working section, $104 \times 53 \times 27$ cm). The flume was constructed using clear plexiglas sheets for the sides of the chamber and the frame was constructed of wood beams. Two sheets of fluorescent light grating (egg crates, 169 mm^2 holes) wrapped with plastic screen (1 mm^2 holes) were placed upstream and one placed downstream to serve as collimators. Average flow velocity (3.63 ± 0.16 cm/s) was measured in the middle of the flume with a Marsh-McBirney Model 2000 Portable Flow Meter. The air-powered motor (Eclipse Systems, model no. 9-4300-14A) was allowed to run for at least 1 h before the first acclimation period. A maximum of three trials were run each time the flume was filled.

The bottom of the flume was lined with a thin layer of gravel (~5–8 mm). A shelter (6×12 cm) was constructed from a PVC pipe cut in half lengthwise. This shelter was used to acclimate the animal to the tank and was placed at the downstream end of the working section on the middle of the back wall. The bottom of the shelter was enclosed in a wire screen and the downstream end was enclosed in plastic grating. A weighted, removable plastic grate was placed at the upstream end to completely enclose the shelter.

The food source was placed directly on the substrate

upstream of the animal. The exact position of the gelatin was 71.36 cm upstream from the downstream collimator of the flume.

The exterior walls of the flume were covered with black material so as not to disturb the animals with outside activity. A video camera (Panasonic Color CCTV camera, model no. WV-CI350) was mounted above the flume. Trials were recorded on a Panasonic VHS recorder (model no. AG-1970). All orientation trials were performed between 08:00 and 17:00 hours.

Lesion protocols

The treatment groups consisted of the following: (i) no treatment or control (herein called control); (ii) both antennae and antennules lesioned (herein called both); (iii) right antenna and antennule lesioned (herein called right); (iv) left antenna and antennule lesioned (herein called left). Twelve individuals were used in each of the four experimental groups. Experimental animals were placed on ice for 10 min before being placed in restraints and glued with Kwik-Fix brand superglue. Animals were put on ice to further slow their movements while in the restraints. The glue was applied using the applicator tip of the glue tube. Glue was applied to the antennae and antennules of experimental animals beginning at the base and extending to the tip and covering the aesthetasc hairs. Controls were put on ice for 10 min and a similar amount of glue was placed at the base of the carapace to ensure that behavior was not affected by the smell of the glue. Antennae and antennules of controls were washed with flume water using a syringe. This was done to simulate physical stimulation of the antennae and antennules of experimental individuals during gluing. Extreme care was taken to avoid getting glue on any anatomical parts other than the antennae and antennules. The glue was completely dry before the individual was placed in the holding container for temperature acclimation.

Testing methods

Before being placed in the flume, crayfish were acclimated to the temperature change from the holding tank (average $24.5 \pm 0.29^\circ\text{C}$) to the flume (average $21.3 \pm 0.41^\circ\text{C}$) for a minimum of 20 min in a holding container with flume water.

After gluing and temperature acclimation, the individual was placed at the downstream end of the flume in the shelter. Animals were allowed to acclimate to the flow for 19 min before the start of the trial. The gelatin was removed from the refrigerator at the beginning of each acclimation so it would be at room temperature for the trial. Each animal was exposed to the odor source for 1 min before video taping began and the start gate of the shelter removed. A trial was terminated when the animal found the food source or if the animal did not locate the odor source within 10 min. A successful trial was one in which the animal physically contacted the food source. A new piece of gelatin was used for each trial.

Analysis

A Peak Motus Motion Analysis System was used to digitize the taped trials. For each trial the tip of the rostrum was digitized at 1 point/s. The rostrum was digitized because these animals can walk sideways and backwards. We were not concerned with body orientation but with the overall movement of the body. These parameters were chosen based on Moore and Grills (Moore and Grills, 1999). Many lesioned animals spent the entire 10 min walking around the tank. For all animals that located the source the whole orientation path was included in the analysis. The trials of animals that did not successfully locate the odor source were cropped to the average time of control trials. Since some orientation parameters, namely walking speed and speed to source, are sensitive to total time spent orienting, we only analyzed the first 70 s of experimental trials. This time period was chosen because it was the average orientation time of control trials. We analyzed the following orientation parameters: percent success of finding the source, distance from the source (cm), speed to the source (cm/s), walking speed (cm/s), heading angle towards the source, net to gross ratio or straightness of path, heading angle upstream and turning angle towards the source [Figure 1, taken from (Moore *et al.*, 1991)]. A MANOVA was performed to detect differences between groups for each parameter, followed by a *post hoc* comparison of LSD with a *P* value of <0.05 . The success rate of each group in locating the source was analyzed using a χ^2 statistic. We then used the Dunn-Šidák

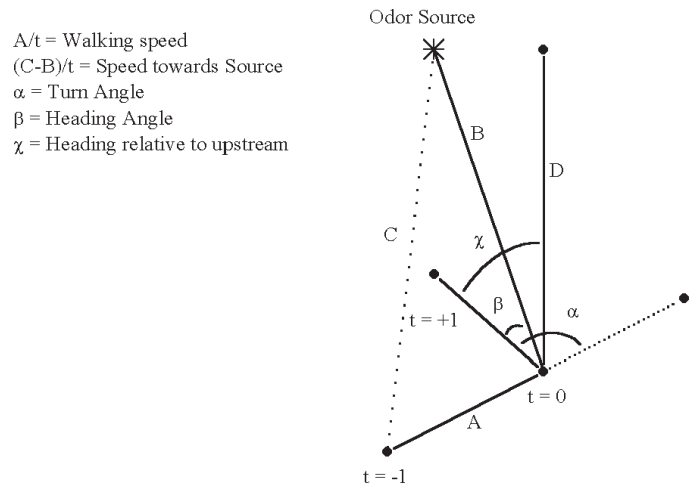


Figure 1 Three successive positions ($t = -1, 0$ and $+1$; ●) on a hypothetical orientation path used to define behavioral parameters. The dashed line represents the crayfish's projected path (if it had continued in a straight line), from which a turn angle value (α) at point $t = 0$ is calculated. Line B represents the straight line to the odor source (asterisk) from which a heading angle relative to the odor source (β) is calculated. Line D represents a path that is straight upstream, from which a heading angle relative to upstream (γ) is calculated. A indicates distance moved from time point $t = -1$ to $t = 0$, the walking speed. The difference between distance to the odor source at time $t = -1$ (dashed line C) and distance to the odor source at time $t = 0$ (line B) is the walking speed towards the source.

method to correct for multiple comparisons. The new P value used to determine significance was $P < 0.013$.

Results

Qualitative description of orientation

Orientation paths were qualitatively different between control and experimental groups (Figure 2). Control individuals (no treatment) were clearly attracted to the fish gelatin, whereas experimental individuals showed no stereotyped paths. Orientation paths were visibly different between the four treatment groups.

The success rate of finding the source was markedly different between groups. All lesions had significant effects. Significance was found between the control group and each of the experimental groups (Figure 3A). Numbers of animals finding the source were as follows: 9 of 12 controls located the source, 1 of 12 individuals with bilateral lesions found the source, 1 of 12 individuals with the right lesion located the source and 0 of 12 individuals with the left lesion located the source. Statistical differences were as follows: control group and both ($\chi^2 = 69.8$, $n = 12$, $P < 0.001$); control group and right ($\chi^2 = 68.8$, $n = 12$, $P < 0.001$); control group and left ($\chi^2 = 6.75$, $n = 12$, $P < 0.01$).

Effects of impairment on walking

Antennal impairment was also found to affect walking speed. Significant differences were noted between control individuals and all treatment groups with respect to the average speed to the source (Figure 3B). Lesioned animals had slower walking speeds towards the source. Significant differences were found between the control group and each of the experimental groups. Statistical differences were as follows: control group and both ($n = 12$, $P < 0.0001$); control group and right ($n = 12$, $P < 0.002$); control group and left ($n = 12$, $P < 0.0003$). Lesioned animals were also found to walk more slowly in the presence of odor (Figure 3C). The control group was found to be significantly different from both ($n = 12$, $P < 0.003$) and right ($n = 12$, $P < 0.02$). Significance was also found between both and left ($n = 12$, $P < 0.05$).

Treatment effects on orientation

Lesioned animals were found to have significantly greater heading angles (Figure 3D). This indicates that a less direct path was taken to the odor source by lesioned animals. Significance was found between the control group and each of the experimental groups: control group and both ($n = 12$,

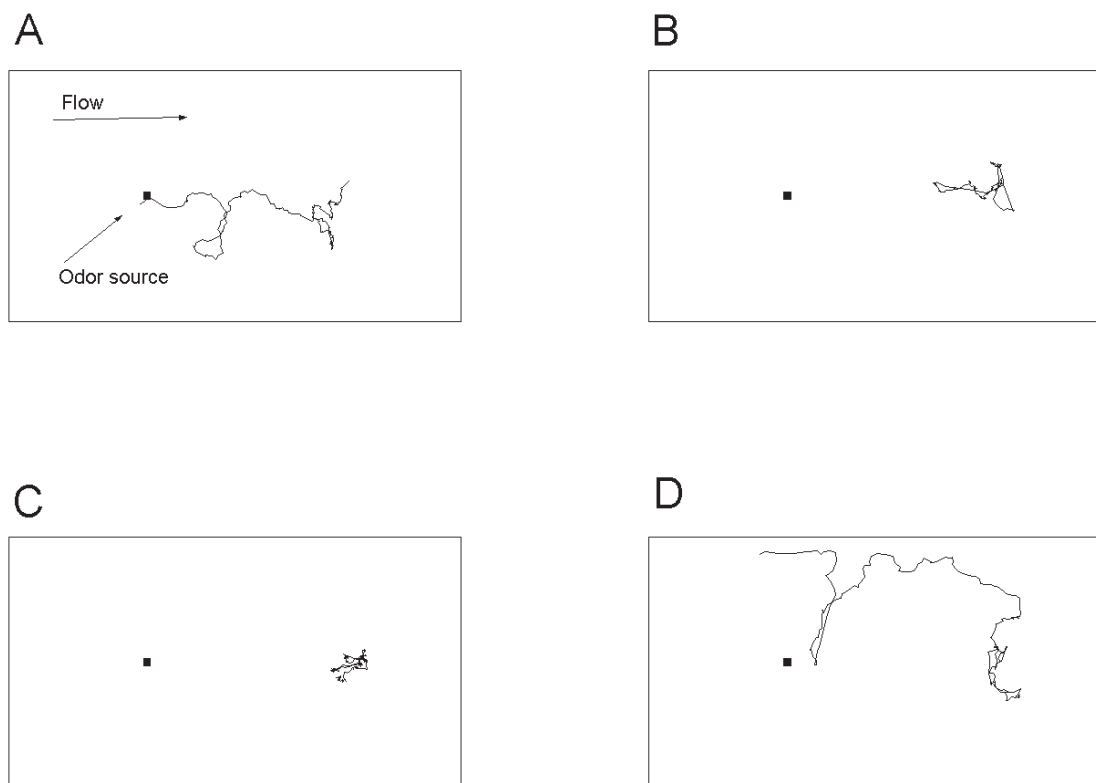


Figure 2 (A) Orientation path of a control animal: walking speed 6.07 cm/s; total duration of trial 160 s. (B) Orientation path of an animal with bilateral lesions: walking speed 4.98 cm/s; total duration of trial 70 s. (C) Orientation path of an animal with unilateral antennal lesions on the right side: walking speed 2.36 cm/s; total duration of trial 70 s. (D) Orientation path of an animal with unilateral antennal lesions on the left side: walking speed 8.48 cm/s; total duration of trial 70 s. Lines are derived from paths digitized at one point per second. Flow is in the direction indicated by the arrow. The square indicates placement of the stimulus.

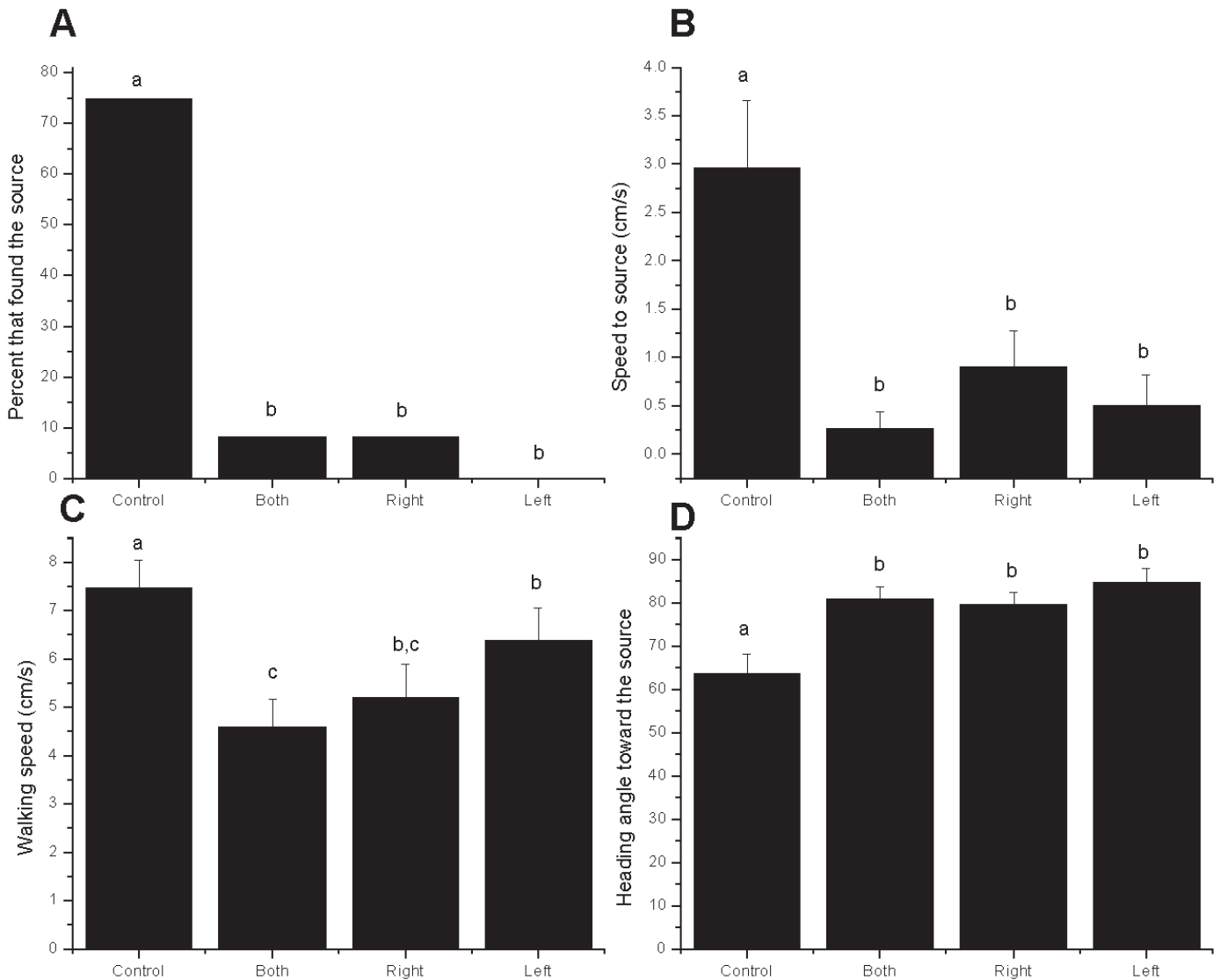


Figure 3 Percent success rate for finding the source (A), mean \pm SE speed to source (B), mean \pm SE walking speed (C) and mean \pm SE heading angle toward the source (D). Bars represent values for $n = 12$ animals. Values for groups with the same letter (a, b or c) are not significantly different, whereas values for groups with different letters are significantly different [χ^2 by Dunn-Sidak method ($P < 0.013$) or MANOVA ($P < 0.05$)]. For percent success rate for finding the source the statistics were run using the raw numbers and not percentages.

$P < 0.02$); control group and right ($n = 12$, $P < 0.03$); control group and left ($n = 12$, $P < 0.004$).

Discussion

While antennae and antennule sensory input are necessary for successful orientation to a food source, it is the bilateral information received through these appendages that is a crucial element of this behavior in crayfish. Lesioned crayfish rarely located the odor source, had much slower walking speeds and greater heading angles. This suggests that impairment of bilateral information from chemoreceptors and mechanoreceptors significantly affects the ability of crayfish to orient to odor sources. Since there is no significant difference between unilaterally and bilaterally

lesioned groups, this indicates that spatial information is critical to orientation behavior.

This finding complements that for lobsters found by Belgane *et al.* (Belgane *et al.*, 1997), that orientation by lobsters was impaired by the loss of an entire antennule but was not affected by the loss of only the chemoreceptors on one antennule. The lesioning technique used by Belgane *et al.* consisted of dipping the antennule in deionized water for 5 min, resulting in the lysing of chemoreceptor dendrites while the mechanoreceptors remained intact. By using glue to lesion the animals in the current study, not only the chemoreceptors but also the mechanoreceptors were blocked. Our findings also support the conclusions of Webster *et al.* (Webster *et al.*, 2000) that spatial information is important to and, in fact, is used during orientation.

Upon analysis of the results, the data suggest that crayfish are using taxis and not kinesis in orientation. Because we lesioned both chemo- and mechanoreceptors on the antennae and antennules, it is difficult to say how much of a role perception of flow direction plays in orientation. Our results clearly show that with a deficit of spatial information it is difficult for these animals to orient towards an odor source. Weissburg (Weissburg, 1997) states that although the term 'chemotaxis' has been used to describe orientation in turbulent plumes, it is unlikely that this behavior is a strict taxis (i.e. a behavior guided by a concentration gradient). This is because smooth concentration gradients cannot persist in turbulent waters. Blue crabs use odor-gated rheotaxis, or the directionality of flow patterns and the intermittency of the chemical signal, to orient to odor sources (Weissburg and Zimmer-Faust, 1993). It is thought that the animal measures the angle at which it exited the odor plume and re-enters the plume at the exact same angle. Therefore, blue crabs find the odor source by following the edges of the odor plume. In odor-modulated optomotor anemotaxis, as seen in moths, the animal switches from a casting behavior to an upwind surge in the presence of an odor signal (Vickers and Baker, 1992, 1994; Mafra-Neto and Cardé, 1994, 1995). The duration of this behavior is controlled by the frequency with which odor filaments contact the antennae. Finally, it appears that lobsters extract detailed information from the odor plume which provides directional information to the animal during orientation (Moore *et al.*, 1991; Atema, 1996). Spatial and temporal comparisons of information within the plume allow the lobster to find the center of the plume and move towards the odor source.

If crayfish used a kinesis type of orientation, we would expect to find significant differences between the treatments with both appendages lesioned and those with a unilateral lesion and no differences between controls and those with unilateral lesions. In kinesis, even with unilateral lesions, the animals would still have the capability to extract temporal information from the odor plume and successfully orient. We suggest that the animals with one intact antenna are attempting to orient using the unilateral signal they are receiving. Because the animal cannot interpret the spatial characteristics of the odor plume with only one antenna, the individual will follow the signal it is receiving until it has exited the odor plume and no longer receives the signal. The individual then engages in non-directed search behavior.

The results of our study also suggest that other sensory appendages, such as walking legs and mouthparts, do not play a primary role in orientation. We can conclude that the antennules and/or antennae are necessary for orientation and that orientation cannot occur with information from the walking legs and mouthparts only. From an ablation study using the crayfish *Procambarus clarkii*, Giri and Dunham (Giri and Dunham, 1999) suggested that information being received through the antennules is necessary for

long-distance orientation but not for orientation over short distances and that the lateral filaments are most likely the primary olfactory organ. Although these findings appear to relate to the findings of the present study, it is difficult to determine the true relevance of the study by Giri and Dunham due to a lack of experimental information. This is contrary to how intact lobsters have been found to behave during a local food search (Moore *et al.*, 1991). It has been determined that the walking legs of lobsters play a role in extracting spatial information during chemical orientation (Devine and Atema, 1982). Animals in this study showed preferential turning towards the intact side only when chemoreceptors on the walking legs of the lobsters were also lesioned using glue. This suggests that in lobsters the receptors on the walking legs play a secondary role in spatial orientation. It was also determined that leg receptor input was not necessary for successful orientation if the antennules were left intact.

These results give us insights into the behavioral orientation of an impaired animal in its natural environment. Animals with full or even partial impairment will not be able to successfully orient from long distances to food sources or any other chemical sources of interest, such as mates or predators.

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