BEHAVIORALLY-SELECTIVE CHEMORECEPTOR LESIONS REVEAL TWO DIFFERENT CHEMICALLY MEDIATED ORIENTATION STRATEGIES IN THE RUSTY CRAYFISH, ORCONECTES RUSTICUS

Kimberly E. Kraus-Epley 1,2, Sara E. Lahman 1, and Paul A. Moore 1,*

1 J. P. Scott Center for Neuroscience, Mind and Behavior; Laboratory for Sensory Ecology; and Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403, USA
2 Cleveland Zoological Society, Cleveland, OH 44109, USA

ABSTRACT

Information from environmental stimuli is essential for animals to make behaviorally and ecologically relevant decisions. We sought to determine by studying Orconectes rusticus (Girard, 1852), the relative importance of mechanical and chemical information during orientation of crayfish to food and the type of orientation strategy crayfish employ to locate attractive odor sources. This was achieved through selectively lesioning chemoreceptors, leaving mechanoreceptors intact. Chemoreceptors were lesioned by placing the olfactory appendages in 50 ppt saltwater for two hours, then a deionized water bath for 10 minutes. Crayfish were subsequently used in orientation trials with a food source of fish gelatin. Orientation videos were digitized and orientation parameters (walking speed, heading angles and turning angles) were analyzed at a population level using a polynomial regression to determine how parameters changed as a function of distance from source and experimental treatment. Through the lesioning of chemoreceptors, we determined whether crayfish were orienting using chemotaxis or odor-gated rheotaxis. Lesioned crayfish oriented to the source with significantly decreased upstream heading angles compared to controls. Regression analysis revealed that lesioned crayfish had significantly altered orientation parameters demonstrating a change in orientation strategy. Results suggest that intact crayfish are using chemical information to guide search behavior while lesioned crayfish are performing a rheotactic-type of search.

KEY WORDS: chemotaxis, crayfish, olfaction, orientation, rheotaxis

DOI: 10.1163/1937240X-00002378

INTRODUCTION

Organisms across many taxa orient to patchily distributed resources using different sensory modalities (von Frisch and Lindauer, 1956; Stein and Magnuson, 1976; Gould, 1982; Rossel and Wehner, 1986; Wagner et al., 1987; Weisburg and Zimmer-Faust, 1993; Lehrer et al., 1995; Mafra-Neto and Cardé, 1995; Salmon and Witherington, 1995; Lohmann and Johnsen, 2000). Salmon and Witherington (1995) demonstrated that loggerhead hatchlings use visual cues to orient to the ocean. While hatchlings were known to use moonlight cues to orient toward the ocean, they were found to also use shape and elevation cues to orient away from buildings along the beach. Male moths use species-specific pheromones emitted by females to locate potential mates (Mafra-Neto and Cardé, 1995). Various physiological mechanisms have evolved in vertebrates to use magnetoreception as a cue for migration, homing and moving throughout a local habitat (Lohmann and Johnsen, 2000). Blue crabs integrate both mechanical and chemical cues to orient to food sources (Weisburg and Zimmer-Faust, 1993). The common thread through these different organisms and different sensory signals is the ability to extract relevant spatial and temporal information from the sensory landscape.

For organisms using chemical senses at larger spatial scales (>1 cm), the sensory landscape is a turbulent odor plume and can be thought of as an odor landscape (Atema, 1996). Odors are spread through an environment through the processes of molecular diffusion and turbulent dispersion (Moore and Crimaldi, 2003). These two processes create a dynamic three-dimensional distribution that can be visualized as an “odor landscape” consisting of changing peaks and valleys of stimulus concentration (Moore and Atema, 1991; Zimmer-Faust et al., 1995; Finelli et al., 1999). The structure of the odor landscape holds potential directional information for animals to use during chemical orientation (Moore and Atema, 1988; Cardé, 1996; Keller et al., 2001). To effectively navigate in this odor landscape, animals have evolved different strategies that depend upon the signal structure of the odor plume (Vickers, 2000), the hydrodynamics of the environment (Moore and Crimaldi, 2003), the types of sensory appendages used during orientation (Atema, 1996; Cardé, 1996; Mjos et al., 1999), and whether the animal moves in three dimensions (flying or swimming) (Nelson et al., 1997; Barbin, 1998; Ishida et al., 2001) or two dimensions (walking) (Wilson, 1961; Bell and Tobin, 1981; Moore et al., 1991).

In order to understand the underlying neural mechanism that guides searching behavior, researchers have focused on

* Corresponding author; e-mail: pmoore@bgsu.edu
the sensory sources of information that allow animals to make directional decisions. A number of moths integrate information from chemical and visual stimuli. Male moths orient to species-specific sex pheromones through a mechanism of 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 upwind surge is controlled by the frequency with which odor filaments contact the chemoreceptors on the antennae. Similar to moths, blue crabs use odor-gated rheotaxis which integrates chemical and mechanical information (Weissburg and Zimmer-Faust, 1993; Finelli et al., 2000). The crabs use both direction of flow and intermittency of the chemical signal to orient to the odor source. Weissburg and Zimmer-Faust (1994) found that increases in current velocity and substrate roughness both mediate behavioral orientation changes in the blue crab by altering turbulence structure within the boundary layer. This demonstrates that orientation in blue crabs is a function of both the hydrodynamic properties of flow and the presence or absence of odor. Lobsters orient using a chemotactic strategy and extract directional information directly from the odor plume (Moore et al., 1991; Atema, 1996). Lobsters orient toward the source by comparison of the spatial and temporal differences within the odor plume. In general, these orientation mechanisms can be classified based on the source of information that guides the direction of movement (spatial information) and the source of information that control the timing of movement (temporal information). For a review of various types of orientation mechanisms and the information used for these behaviors see Fraenkel and Gunn (1961) and Kennedy (1986).

Crayfish appear to use information in the fine-scale distribution of chemical signals which controls both the direction of movement and the timing of that movement. In particular, changes in the odor landscape due to changes in the hydrodynamics of flow can influence temporal properties of orientation to food sources (walking speed and time spent moving; Moore and Grills, 1999). Foraging success and efficiency of orientation were increased by increasing the complexity (the temporal fluctuations in signal structure) of the odor landscape (Keller et al., 2001). Furthermore, crayfish altered the temporal aspects of their orientation paths (walking speed) with differences in source arrangement of food cues, namely increases in source concentration and spatial arrangement of sources and with increases in the pulsatile nature of turbulent odor plumes (Kozłowski et al., 2003; Wolf et al., 2004). While crayfish were able to orient in pulsed odor plumes, the spatial and temporal complexity of continuous plumes increased orientation success. The results of these studies demonstrate that crayfish orientation behavior is modulated by the spatial and temporal changes in the odor plume. Kraus-Epley and Moore (2002) demonstrated that crayfish require bilateral information from spatially-separated receptors to successfully orient to a food source. Crayfish with unilateral or bilateral antennal and antennular lesions were unsuccessful at finding the odor source.

To investigate the relative importance of chemical and mechanical information in guiding orientation behavior, chemosensory input to crayfish was selectively inhibited. Because both chemical and mechanical information are normally available during orientation to an odor source, the present study focused on the types of information that are necessary during orientation and how this information can guide both the spatial and temporal aspects of orientation behavior. We sought to determine whether chemoreceptor lesions to the olfactory appendages (antennae, medial and lateral antennules) altered orientation parameters and the search strategy mechanism of the rusty crayfish, *Orconectes rusticus* (Girard, 1852).

Materials and Methods

Animals

Male and female *O. rusticus* were collected from the Portage River near Bowling Green State University in Bowling Green, OH, USA. Crayfish (2.93 ± 0.47; mean ± SEM) were stored in population tanks and fed one pellet of rabbit food three times per week until approximately one week prior to use. At that time, individuals were visually and mechanically isolated in containers with a diameter of 16 cm and a depth of 9 cm. Crayfish were kept on a 14:10 light:dark cycle, and the average water temperature of the holding tank was 22.8 ± 0.3°C. All crayfish used in this study had intact olfactory appendages (antennae and antennules), chelae, and walking legs. Crayfish were starved for one week prior to trials to ensure motivation to localize the food odor (described below).

Stimulus

The stimulus for all trials consisted of one block of fish gelatin (0.5 × 2.5 × 5.1 cm). Fish gelatin was made by homogenizing fish (perch; average 45.4 ± 0.1 g) in 235 ml of cold water. Four packets of unflavored gelatin were mixed with 471 ml of boiling water, which was then mixed with the fish homogenate. The mixture was poured into a rectangular pan, covered with aluminum foil and refrigerated until solid. The gelatin was cut into cubes, individually wrapped in plastic and refrigerated until time of use. Previous studies have shown that crayfish readily orient to this type of stimulus which simulates the effects of slowly diffusing carrion in natural habitats and will consume the gelatin as a food source (Willman et al., 1994; Moore and Grills, 1999; Keller et al., 2001; Kraus-Epley and Moore, 2002).

Flume

All trials were conducted in a 2001 l recirculating flume (complete dimensions 568 × 57 cm; working section 244 × 57 × 61 cm) with a water temperature of 20.1 ± 0.1°C. The frame for the working section and end tanks of the flume were constructed of stainless steel and the sides and bottom of the working channel were glass. Upstream collimators were constructed from polycarbonate core honeycomb (2.54 cm thick; 2.54 cm diameter) and sheets of fluorescent light grating (egg crates, 169 mm2 holes) wrapped with plastic screen (1 mm2 holes). The three-dimensional flow profile was quantified using an Acoustic Doppler Velocimeter (Nortek® AS P107/01). During orientation trials, average flow velocity was kept at 5.0 ± 0.1 cm/s as measured in the middle of the flume with a Marsh-McBirney® Model 2000 Portable Flow Meter. A centrifugal pump (WEG, Model No. 005180P3E184JM) powered by an adjustable speed drive (Baldor, Model No. ID15H205-E) was allowed to run for at least one hour before the first acclimation period for the crayfish. A D.E. Jacobs filter system (Emerson motor, model No. S55CXPED-7500 CAT; Landslide filter, model No. LS40-SILGT9-3-HK6-LH) was used for 10 minutes between trials to eliminate background accumulation of organic residue and odors. The flume was emptied, cleaned, and refilled after one hundred trials. The bottom of the flume was lined with a thin layer of gravel (0.6 ± 0.04 cm) which was glued to a Plexiglas sheet in order to simulate the substrate of crayfish natural habitat.

Experimental Protocol

The treatment groups consisted of the following lesions for behavioral response: 1) control (no lesions/handling control), n = 20; 2) bilateral antennae and antennular chemoreceptors lesions, n = 13; 3) right antenna and antennule chemoreceptors lesions, n = 16; 4) left antenna and antennule chemoreceptors lesions, n = 16. A total of 114 crayfish were used in the control group to achieve 20 successful trials. A total of 118 trials were run for the 16 successful trials...
for the left and right lesioned groups while a total of 121 trials were run to achieve a total of 13 successful trials in the bilaterally lesioned group.

Lesion Protocols
Crayfish were restrained upside-down with their appendages to be lesioned placed in a micro-pipette tip (Universal tip, 1000 μl). The pipette tip was filled with 50 ppt saltwater for 2 hours. A 2-hour saltwater bath was followed placing the appendages in deionized water for 10 minutes to complete the lesion. All crayfish underwent lesioning on the day they were used in trials.

The effectiveness and selectivity of the lesion treatment was tested using a behavioral assay. A lesioned animal was placed in a small holding tank containing aged tank water. A stimulus bolus of fish homogenate was directed at the antennules of the lesioned animal through a 3 ml syringe with a 25 gauge needle. Preliminary studies showed that this method of introduction provided primarily chemical and mechanical stimuli. When presented with this stimulus, control crayfish would move forward and grasp the syringe with their major chelae, demonstrate flicking, and a general level of increased activity. A crayfish was considered to be “lesioned” if the behavioral response was evident when stimulated by water, but not present when stimulated with fish homogenate injections. Unilaterally lesioned crayfish responded with chela grabbing at the syringe or feeding movements. To test for sensitivity to mechanical stimuli, crayfish antennules were stimulated with a jet of aged tap water from a 3 ml syringe without a needle. When presented with a similar injection of just control water, crayfish responded less vigorously with their chelae and with little to no increase in flicking. In these studies, the crayfish would react quite negatively as a result of the stimulus, as all control and lesioned crayfish responded with a tailflip (an escape response) to this mechanical stimulus. Control animals were handled in an identical fashion and were put through the lesion protocol. The only difference was that aged tap water was used in place of the saltwater and deionized water.

A similar lesion technique has been used in lobsters (Gleeson et al., 1993; Belgane et al., 1997) and has been speculated that reduced osmotic pressure lyses the dendrites of the chemoreceptors while leaving the mechanoreceptors intact. Although we cannot confirm whether the chemosensory dendritic membranes are destroyed using this technique, we can confirm with behavioral tests that these animals are lesioned and, henceforth, we will refer to these animals as lesioned for a behavioral response. Preliminary evidence from post-trial behavioral assays suggests that lesions of this type can be effective for several days.

Orientation Testing Methods
Crayfish were placed at the downstream end of the flume in a shelter made of fluorescent light grating (dimensions 12.7 × 12.7 × 12.7 cm) for a 20-minute acclimation period to flow and temperature. Fish gelatin was at a 2007). Orientation paths of all groups resembled those of crayfish in previous studies (Fig. 2). Overall, animals followed a direct path toward the source in the general direction of upstream. Lesioned animals appeared to orient more directly towards the source, almost straight upstream, than controls under these specific hydrodynamic conditions. Paths of lesioned individuals were much straighter and less convoluted than the paths of controls (Fig. 2).

Success Rate
The rate of successfully localizing the odor source for crayfish in the control group was 17.54% (20/114), 13.56% (16/118) for groups of crayfish with left side lesions and crayfish with right side lesions, and 10.74% (13/121) for the group of crayfish with bilateral lesions. Overall, there was no significant difference between the success rates of locating the odor source for each group of crayfish (Tukey multiple comparisons for proportions test, Zar, 2007).

Impact of Lesions on Spatial Parameters
Lesioned crayfish showed several significant differences in their orientation parameters when compared to control crayfish (Table 1, F(3, 76) = 107). Heading angles relative to the upstream direction of crayfish with left unilateral lesions and bilateral lesions were significantly higher than the angles calculated for control crayfish (Fisher LSD, p < 0.05). Similar results were seen for heading angles relative to the odor source as bilaterally and left unilaterally lesioned crayfish had significantly lower mean angles than the control group (Fisher LSD, p < 0.001). Mean turning angles of left unilaterally and bilaterally lesioned crayfish were found to be significantly lower than that of controls (Fisher LSD, p < 0.005). There was no significant difference between the mean values of heading angles relative to upstream, heading angles toward the odor source, and turning angles relative to the upstream direction, and net-to-gross ratio (NGR) (Moore et al., 1991). The absolute value of all angle measures was used in subsequent data analysis to allow the use of linear statistics to analyze angle distributions, which ranged in values from 0 to 180°. For all of the parameters, a single average value was calculated for each individual using the digitized paths. Population average values, which were used for statistical analyses, were calculated from the individual average values. Statistical differences for each orientation parameter between different lesion treatments were compared using a one-way MANOVA with Fisher LSD post-hoc tests with each lesion as a factor. To investigate how orientation paths changed as a function of distance, each parameter value (walking speed, etc.) was averaged over five centimeter bins along the length of the orientation path for each animal. A second order polynomial regression (parameter versus distance) was performed for each parameter for each animal. The coefficients for each regression were used in a subsequent one-way MANOVA (Fisher LSD post-hoc test) to test for significant differences in population-wide regression values across treatments. Each polynomial regression was considered significant if p < 0.01 and significance for regression coefficients was set at p < 0.01 as dictated by a Bonferroni correction. The number of right-hand turns versus left-hand turns was compared between unilateral behaviorally-lesioned treatments using a chi-square statistic (p < 0.05). Differences in the rates of successfully locating the sources between experimental groups of crayfish were statistically analyzed using a Tukey multiple comparisons for proportions test (Zar, 2007).
Walking Speed = A/t
Speed Towards Source = (C-B)/t
Turn Angle = α
Heading Angle = β
Heading Relative to Upstream = χ

Fig. 1. A hypothetical orientation path taken by a crayfish localizing an odor source (represented by a star) that displays three successive positions \((t = -1, 0, \text{ and } +1)\) used to define walking parameters. The dashed line symbolizes the projected path if the crayfish were to continue on in a straight line. This projected line is used to calculate the value of a turn angle \((α)\) at the \(t = 0\) position. Line B depicts a straight path to the odor source from which a heading angle relative to the position of the odor source \((β)\) is calculated. Line D represents the path straight upstream and is used to calculate a heading angle relative to upstream \((χ)\). The distance traveled by the crayfish from time \(t = -1\) to \(t = 0\) is indicated by Line A. This line also represents walking speed. The walking speed toward the odor source is determined by the difference between the distance to the odor source at time \(t = -1\) (represented by dotted Line C) and the distance to the odor source at time \(t = 0\) (Line B). This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1937240x.

Table 1. Mean ± SEM for orientation parameters, analyzed with a one-way MANOVA with Fisher LSD post-hoc analysis \((F_{(3,76,0.05)} = 107, p < 0.05)\).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Left lesion</th>
<th>Right lesion</th>
<th>Bilateral lesion</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heading angle upstream (°)</td>
<td>82.5 (3.0)*</td>
<td>76.3 (5.1)</td>
<td>84.8 (2.5)*</td>
<td>69.2 (2.8)</td>
</tr>
<tr>
<td>Heading angle to source (°)</td>
<td>19.9 (1.9)*</td>
<td>29.4 (3.8)</td>
<td>19.2 (2.5)*</td>
<td>34.5 (2.5)</td>
</tr>
<tr>
<td>Turning angle to source (°)</td>
<td>15.3 (2.2)*</td>
<td>26.0 (4.9)</td>
<td>13.7 (2.4)*</td>
<td>32.6 (2.9)</td>
</tr>
<tr>
<td>Walking speed to source (cm/s)</td>
<td>3.6 (0.2)*</td>
<td>2.8 (0.2)</td>
<td>3.2 (0.2)*</td>
<td>2.2 (0.2)</td>
</tr>
<tr>
<td>Walking speed (cm/s)</td>
<td>3.9 (0.2)*</td>
<td>3.2 (0.2)</td>
<td>3.5 (0.2)*</td>
<td>2.7 (0.2)</td>
</tr>
</tbody>
</table>

*Significance from control.
for crayfish with right unilateral lesions and the control group.

Crayfish with unilateral lesions were found to turn in the direction of the side on which the intact, unlesioned antennules were located. Animals with right behavioral lesions were found to turn left significantly more often than right ($\chi^2 = 53.8, n = 6, p < 0.001$). Conversely, animals with left behavioral lesions turned right significantly more often than left ($\chi^2 = 53.8, n = 6, p < 0.001$). Bilaterally lesioned crayfish and control crayfish did not display skewed turning ratios toward either the right or left side.

Spatial Regression Values

Heading angle upstream shows considerable variation as a function of distance from source (Fig. 3A). Control crayfish have heading angles relative to upstream that are the maximum value ($72^\circ$) at distances farther away from odor source and decreased to a minimum value close to the source ($64.2^\circ$). The regression analysis for all groups of lesioned crayfish showed a significant difference in all three coefficients of regression when compared to controls (Fisher LSD post-hoc tests, $p < 0.05$), but showed no significant difference when compared to each other (Table 1). Heading angles calculated for unilaterally (either right or left) lesioned crayfish were at the highest values farthest from the source, while bilaterally lesioned crayfish had higher angles close to the source. Toward the middle section of the orientation paths of lesioned crayfish, heading angles decreased significantly to the lowest values and then increased as crayfish approached the odor source. Within the lesioned groups of crayfish, there was an inverse relationship between heading angle upstream and distance to the source, as compared to the control group.

The regression analysis showed that heading angle relative to the source varied considerably as a function of distance from source (Fig. 3B). In orientation pathways of control crayfish, heading angles calculated farther from the source were intermediate in value ($34.5^\circ$) as compared to heading angles of lesioned crayfish. Heading angles toward the source decreased as control crayfish approach the source where the lowest value ($30^\circ$) was calculated. The regression analysis for groups of lesioned crayfish showed no significant differences in two coefficients of regression when compared to controls (Fisher LSD, $p < 0.05$), and also showed no significant difference when compared to each other (Table 1). Heading angles within the pathways of lesioned crayfish had the highest values at distances farthest away from the odor source, decreased significantly within the middle section of orientation pathways and finally increased as crayfish approached the odor source. A significant difference between the control and lesioned groups was seen in the inverse relationship between heading angle toward the source and distance from the source of lesioned crayfish ($p < 0.05$).

Similar regression patterns were shown in all groups of crayfish in regards to turning angle toward the source (Fig. 3C). The turning angles calculated for orientation paths of control crayfish had higher average values at distances farthest and closest to the odor source, reaching the lowest value in the middle of the pathway. The turning angles calculated for pathways of control crayfish at distances farthest from the source ($51.5^\circ$) and closest to the source ($45.2^\circ$) were significantly higher than the turning angles calculated in the middle of the pathway ($26.4^\circ$). The regression analysis for lesioned crayfish showed a significant difference in one coefficient of regression when compared to controls (Fisher LSD, $p < 0.05$), but showed no significant difference when compared among lesioned groups of crayfish (Table 1). Turning angles toward the source for lesioned crayfish were highest in value farthest away and close to the odor source. Toward the middle section of the orientation paths, heading angles decreased significantly to the lowest values and then increased as crayfish approached the odor source.

Impact of Lesions on Temporal Parameters

Crayfish from the control group had significantly faster mean walking speeds toward the odor source compared to crayfish that underwent left and bilateral lesion treatments (Fisher LSD, $F_{(3,76,0.05)} = 107, p < 0.005$; Table 1). The mean overall walking speeds of crayfish in left and bilaterally lesioned groups were significantly faster compared to speeds of crayfish from the control group (Fisher LSD, $p < 0.005$; Table 1). There was not a significant difference between the mean walking speed toward the source or over-
Fig. 3. Spatial orientation parameters of behavioral lesion treatments analyzed with a 2nd-order polynomial regression analysis. A, heading angle upstream: left behavioral lesions (dashed line), right behavioral lesions (dotted line), bilateral behavioral lesions (dash-dot line), controls (solid line); B, heading angle toward the source: left behavioral lesions (dashed line), right behavioral lesions (dotted line), bilateral behavioral lesions (dash-dot line), controls (solid line); C, turning angle toward the source: left behavioral lesions (dashed line), right behavioral lesions (dotted line), bilateral behavioral lesions (dash-dot line), controls (solid line).

all walking speed of crayfish with right unilateral lesions and the control group.

Temporal Regression Values
Regression analysis revealed significant differences in temporal parameters between control crayfish and groups of lesioned crayfish (Fig. 4A). Control crayfish exhibited slowest walking speeds toward the source farthest from the source and reached faster speeds closest to the odor source. Walking speed toward the odor source was significantly slower for groups of lesioned crayfish as compared to controls at distances farthest downstream (Fisher LSD, \( p < 0.05 \), but showed no significant difference when compared among right unilateral, left unilateral, and bilateral lesions (Table 2). Within groups of lesioned crayfish, walking speeds toward the source were significantly faster in the middle of the orientation pathways as compared to the walking speed of control crayfish (Fisher LSD, \( p < 0.05 \), but showed no significant difference when among the different lesion treatments (Table 2). Walking speeds of crayfish from lesioned groups were slowest farthest from the source and increased to the maximum speeds in the middle of the orientation pathway before decreasing as the crayfish moved toward the source.

DISCUSSION
The results from this work clearly demonstrate two findings. First, selectively lesioning chemoreceptors on the antennules and antennae do not significantly impair the ability of crayfish to successfully orient to chemical sources. Second, lesioning these same chemoreceptors does alter the spatial and temporal dynamics of the behavioral pathways taken during orientation behavior. These findings support that crayfish may be receiving chemical information from other chemoreceptors located at various locations on the body and/or relying on odor-gated rheotaxis as an alternative search strategy when unable to perform a chemotaxis.

The technique used to lesion chemoreceptors leaves mechanoreceptors intact, allowing crayfish consistent mechanical stimulation and the ability to make small course corrections in regards to information from flow (Gleeson et al., 1993; Belgane et al., 1997). Although, crayfish with bilateral or unilateral antennular lesions successfully found odor sources, both the spatial and temporal aspects of their
Orientation pathways were significantly altered (Figs. 3 and 4). Most notably, the heading angles relative to the odor source increased as a function of distance, which is indicative that crayfish in the lesioned groups are using an odor-gated rheotaxis (Moore et al., 1991: Fig. 7). Additionally, in an odor-gated rheotactic strategy, the heading angles relative to upstream flow remain close to zero during the surge part of the strategy, a result displayed by the lesioned crayfish in this study. Consequently, the spatial and temporal parameters of orientation displayed by crayfish in lesioned groups are guided by mechanical information obtained by flow (Weissburg and Zimmer-Faust, 1993, 1994; Finelli et al., 1999, 2000). Furthermore, in an odor-gated rheotactic strategy, walking speeds and speed toward the odor source would be significantly higher than those of animals that are orienting using the fine-scale chemical information in odor plumes since crayfish would be receiving constant mechanical stimulation during orientation. Our data are consistent with this conclusion (Fig. 4).

Interestingly, there are some areas where the “left” lesioned animals showed significant differences when compared to other animals (Table 1). It is unclear on why this difference existed and was consistent across all of the behavioral measures. One possibility is that there were sections of the odor plume (due to subtle differences in hydrodynamics) that produced different turbulent structures that created pockets of information to the left side of the flume as opposed to the right side. This flume and odor parameters have been used in previous studies, but have not shown this difference before (Kozlowski et al., 2003; Kraus-Epley and Moore, 2002). Crayfish, unlike the American lobster, Homarus americanus Milne Edwards, 1837 does not show handedness in the claws and thus any possibility of a dominant sensory side of the animal seems unlikely. Still, these differences exist within this data set and present a interesting possibility of future studies.

Comparison of Different Orientation Strategies

In this study, the use of flow information from the surrounding environment, which was obtained from known mechanoreceptors (Tautz et al., 1981; Tautz, 1987), resulted in successful orientation in the absence of available chemical information. Interestingly, animals in the treatment groups of this study, presumed to be impaired with chemoreceptor lesions, appear to orient more efficiently than controls as evidenced through increased walking speeds and decreased heading angles producing a faster, more direct path toward the odor source. This result may be due to the unnatural flow conditions in our flume which produce reliable and consistent information about the upstream direction as well as a chemical gradient from a single attractive odor source. In natural stream settings, turbulent and chaotic flow in streams, local flow direction does not necessarily signal upstream direction due to large scale back flows and eddies (Hart et al., 1996; Moore et al., 2000). Furthermore, multiple odor cues are present in a natural environment and animals must differentiate between these various signals. Consequently, in a natural environment, a rheotactic-type mechanism may not be advantageous due to increased turbulence and complex chemical signals (Atema, 1995; Keller et al., 2001).

Classical orientation definitions provide that crayfish with unilateral lesions and a loss of bilateral and spatial information might be expected to display a more circuitous path in the direction of the intact side (Fraenkel and Gunn, 1961). The results of the present crayfish study support previous findings in the lobster (McLeese, 1973), suggesting that integration of chemical information from other parts of the body, namely mouthparts, chelae and walking legs, may assist in spatial and temporal chemical comparisons of an odor plume allowing for a straighter path. Devine and Atema (1982) found through ablating various appendages and lesioning aesthetasc hairs that intact lobsters orient through a tropotaxis mediated primarily through aesthetasc receptor input. However, non-aesthetasc receptors on the lateral antennules and the first two pairs of walking legs were found to contribute to spatial input. Since crayfish have various types of chemoreceptors located at numerous points across their bodies (Belanger and Moore, 2006, 2009), the possibility of orientation decisions based on bimodal chemosensory input cannot be completely discarded. However, results from this study do not allow determination as to which chemoreceptors on different parts of the body are used during the rheotactic-type of orientation.

The results of the current study demonstrate that with mechanosensory input from the antennae and antennules, i.e., bilateral behavioral lesions receiving only mechanosen-
Turning angle

Blue crabs use their walking legs (Derby and Atema, 1982a, b; Johnson et al., 1993, 1994). While loss of sensory input from these appendages, successful orientation is possible due to an apparent shift in reliance upon stimulus type and thus the sensory modality used for orientation. Similar findings suggesting an alternative orientation mechanism when the primary modality is impaired were found by Steullet et al. (2002) through ablating specific sets of chemoreceptors on the antennules of the spiny lobster, *Panulirus argus* (Latreille, 1804). Behavioral responses to food odors were altered in the spiny lobster; however, while both aesthetasc and non-aesthetasc chemoreceptors contribute to activating odor-mediated behavior, neither type of receptor was necessary for successful orientation. Kraus-Epley and Moore (2002) showed that by completely lesioning the primary olfactory appendages of crayfish, eliminating both chemo- and mechanoreceptor input, successful localization of an odor was significantly decreased suggesting that neither mechanism of orientation was possible.

Beyond the antennules, there are other chemosensory appendages on the decapod crustaceans, most notably the walking legs (Derby and Atema, 1982a, b; Johnson et al., 1984). Although considered taste organs for crustaceans, these appendages could be used as secondary sources of information for distance orientation. Blue crabs use their walking legs to obtain information from odor plumes to orientation to potential prey (Weissburg and Zimmer-Faust, 1993, 1994). Given the dynamics of odor dispersal, walking legs and the odors being detected are often trapped within the benthic boundary layer and may contain different types of distance and directional information about odor sources (Moore et al., 1994).

### Table 2.
Mean ± SEM of coefficient values for the polynomial regression: $y = AX^2 + BX + C$. *Significant differences compared to controls (one-way MANOVA with Fisher LSD post-hoc test, $p < 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heading angle relative to upstream</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$-0.2 \times 10^{-3} (0.4 \times 10^{-3})$</td>
<td>0.08 (0.07)</td>
<td>64 (2.9)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>$0.9 \times 10^{-3} (0.1 \times 10^{-3})^*$</td>
<td>$-0.39 (0.07)^*$</td>
<td>20 (2.5)*</td>
</tr>
<tr>
<td>Left</td>
<td>$0.2 \times 10^{-3} (0.3 \times 10^{-3})^*$</td>
<td>$-0.09 (0.08)^*$</td>
<td>18 (2.8)*</td>
</tr>
<tr>
<td>Right</td>
<td>$0.2 \times 10^{-3} (0.3 \times 10^{-3})^*$</td>
<td>$-0.22 (0.06)^*$</td>
<td>6 (3.2)*</td>
</tr>
<tr>
<td>Heading angle relative to source</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$-0.3 \times 10^{-3} (0.3 \times 10^{-3})^*$</td>
<td>0.08 (0.06)</td>
<td>30 (2.3)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>$2.6 \times 10^{-3} (0.2 \times 10^{-3})^*$</td>
<td>$-0.44 (0.04)^*$</td>
<td>33 (1.4)</td>
</tr>
<tr>
<td>Left</td>
<td>$3.5 \times 10^{-3} (0.3 \times 10^{-3})^*$</td>
<td>$-0.57 (0.06)^*$</td>
<td>33 (2.2)</td>
</tr>
<tr>
<td>Right</td>
<td>$2.3 \times 10^{-3} (0.3 \times 10^{-3})^*$</td>
<td>$-0.43 (0.06)^*$</td>
<td>40 (2.3)</td>
</tr>
<tr>
<td>Turning angle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$2.2 \times 10^{-3} (0.4 \times 10^{-3})$</td>
<td>$-0.40 (0.07)$</td>
<td>45 (2.9)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>$2.0 \times 10^{-3} (0.3 \times 10^{-3})$</td>
<td>$-0.29 (0.05)^*$</td>
<td>18 (2.2)*</td>
</tr>
<tr>
<td>Left</td>
<td>$2.4 \times 10^{-3} (0.3 \times 10^{-3})$</td>
<td>$-0.37 (0.05)$</td>
<td>23 (1.9)*</td>
</tr>
<tr>
<td>Right</td>
<td>$2.5 \times 10^{-3} (0.3 \times 10^{-3})$</td>
<td>$-0.31 (0.06)$</td>
<td>21 (2.3)*</td>
</tr>
<tr>
<td>Walking speed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$-0.7 \times 10^{-4} (0.1 \times 10^{-4})$</td>
<td>$9 \times 10^{-3} (2 \times 10^{-3})$</td>
<td>2.5 (0.09)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>$-2.4 \times 10^{-4} (0.2 \times 10^{-4})^*$</td>
<td>$40 \times 10^{-3} (3 \times 10^{-3})^*$</td>
<td>2.5 (0.13)</td>
</tr>
<tr>
<td>Left</td>
<td>$-2.6 \times 10^{-4} (0.2 \times 10^{-4})^*$</td>
<td>$50 \times 10^{-3} (3 \times 10^{-3})^*$</td>
<td>2.1 (0.12)</td>
</tr>
<tr>
<td>Right</td>
<td>$-2.3 \times 10^{-4} (0.2 \times 10^{-4})^*$</td>
<td>$37 \times 10^{-3} (3 \times 10^{-3})^*$</td>
<td>2.3 (0.13)</td>
</tr>
<tr>
<td>Speed to source</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$-0.4 \times 10^{-4} (0.1 \times 10^{-4})$</td>
<td>$3.7 \times 10^{-3} (2 \times 10^{-3})$</td>
<td>2.2 (0.09)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>$-2.7 \times 10^{-4} (0.2 \times 10^{-4})^*$</td>
<td>$50 \times 10^{-3} (3 \times 10^{-3})^*$</td>
<td>1.8 (0.14)*</td>
</tr>
<tr>
<td>Left</td>
<td>$-2.9 \times 10^{-4} (0.2 \times 10^{-4})^*$</td>
<td>$54 \times 10^{-3} (4 \times 10^{-3})^*$</td>
<td>1.7 (0.14)*</td>
</tr>
<tr>
<td>Right</td>
<td>$-3.1 \times 10^{-4} (0.2 \times 10^{-4})^*$</td>
<td>$48 \times 10^{-3} (4 \times 10^{-3})^*$</td>
<td>1.7 (0.14)*</td>
</tr>
</tbody>
</table>

Overall Conclusions

While loss of chemical input from various organs may alter food-related behaviors in decapod crustaceans, loss of information from the olfactory appendages did not affect overall orientation success under the hydrodynamic conditions in the present study. Crayfish have been thus far classified as an animal that relies on a chemotactic search strategy for efficient localization of important ecological odors. This study demonstrated that rusty crayfish can compensate for impairment to one sensory modality and switch orientation mechanisms, between both chemotaxis and odor-gated rheotaxis, and successfully localize a distant odor source. Furthermore, the present findings show that both chemical and mechanical information are important in crayfish orientation. The relative importance of these chemical stimuli is dependent upon both flow regime and sensory appendage availability for information. Having multiple receptors located across the body and the ability to utilize various types of information efficiently carries obvious advantages as these animals are often found in natural habitats with missing various appendages (Derby and Steulet, 2001). Crayfish appear to exhibit some plasticity of behavior in that these animals can change their orientation strategy based on available information.
ACKNOWLEDGEMENTS

We would like to thank the Laboratory for Sensory Ecology at Bowling Green State University for assisting with early revisions of this manuscript. This project would not have been possible without Steve Queen of the Biology Department at Bowling Green State University and Fletcher Machine, Inc. of Wood County, Ohio for help with the construction of the flume. This work was funded by NSF grant IBN-0131320 to PAM and a Sigma-Xi Grant-in-Aid of Research to KEE. Experiments comply with the “Principles of Animal Care.” Publication No. 80-23, revised 1985 of the National Institute of Health and also with the current laws of the United States of America.

REFERENCES


_____ and _____. 2009. The role of the major chelae in the localization and sampling of female odours by male crayfish, Orconectes rusticus (Girard, 1852). Crustaceana 82: 653-668.


