

RESEARCH ARTICLE

Discontinuous locomotion and prey sensing in the leech

Cynthia M. Harley*, Matthew Rossi, Javier Cienfuegos and Daniel Wagenaar

California Institute of Technology, Division of Biology, Pasadena, CA 91125, USA

*Author for correspondence at present address: University of Minnesota, Department of Entomology, St Paul, MN 55108, USA
 (cmharley@umn.edu)

SUMMARY

The medicinal leech, *Hirudo verbana*, is an aquatic predator that utilizes water waves to locate its prey. However, to reach their prey, the leeches must move within the same water that they are using to sense prey. This requires that they either move ballistically towards a pre-determined prey location or that they account for their self-movement and continually track prey. We found that leeches do not localize prey ballistically. Instead, they require continual sensory information to track their prey. Indeed, in the event that the prey moves, leeches will approach the prey's new location. While leeches need to continually sense water disturbances to update their percept of prey location, their own behavior is discontinuous – approaching prey involves switching between swimming, crawling and non-locomoting. Each of these behaviors may allow for different sensory capabilities and may require different sensory filters. Here, we examined the sensory capabilities of leeches during each of these behaviors. We found that while one could expect the non-locomoting phases to direct subsequent behaviors, crawling phases were more effective than non-locomotor phases for providing direction. During crawling bouts, leeches adjusted their heading so as to become more directed towards the stimulus. This was not observed during swimming. Furthermore, in the presence of prey-like stimuli, leeches crawled more often and for longer periods of time.

Key words: behavioral choice, crawl, leech, locomotion, sensory, swim.

Received 12 June 2012; Accepted 23 January 2013

INTRODUCTION

Finding food is of paramount necessity to all animals; predators are no exception to this rule. While predation strategies are highly diverse between species, at an abstract level, strategies can be categorized based on the use of sensory information during predation: some species continuously track their prey during their approach, while others move ballistically after an initial estimate of prey location or trajectory (Ewert, 1987; Corrette, 1990). Intuitively, continuous tracking should lead to more reliable prey capture, but it is not without problems. To be able to localize prey while moving, an animal has to distinguish self-movement from prey movement, and this adds a level of complexity to the necessary neural circuitry. For instance, hoverflies and dragonflies have separate visual pathways for processing the movement of small objects (e.g. conspecifics, prey) and for large-field movement (e.g. self-movement) (Collett, 1980; Olberg, 1981; Nordstrom and O'Carroll, 2006). As a consequence, many animals dispense with continuous tracking entirely, instead either ambushing prey or sensing prey between intermittent ballistic movements. The latter behavior, known as 'stop-and-go predation' or 'saltatory search', involves periodic stops during which animals re-orient towards their prey before initiating a new ballistic movement (Lock and Collett, 1979; Miller, 1979; O'Brien et al., 1989; Gilbert, 1997). During these movement phases the animals are effectively blind. A striking example of this is the toad *Bufo viridis*, which closes its eyes during the fast phase of pursuit and re-opens them while stationary (Lock and Collett, 1979). Stop-and-go predation allows the animal to avoid any confusion of self-movement with prey location. However, it is only effective if the animal can outrun its prey (Lock and Collett,

1979; Gilbert, 1997; Niven, 2006). Although sensing prey during locomotion comes at a price, it is not without benefit because it supplies the animal with valuable sensory information. For example, visual motion parallax generated during movements or across movement steps is utilized by insects, birds and humans alike to gain depth information from the surrounding environment (Collett, 1978; Frost, 1978; Srinivasan et al., 1990; Lehrer and Srinivasan, 1994; Poteser et al., 1998; van der Willigen et al., 2002; Medendorp et al., 2003).

Here we examine an aquatic predator, the leech *Hirudo verbana*, that determines prey location by sensing water disturbances using both visual sensors and mechanosensors (Young et al., 1981; Dickinson and Lent, 1984; Carlton and McVean, 1993; Harley et al., 2011). During prey localization, leeches readily switch between swimming, crawling and non-locomotion (for descriptions of these behaviors, see Fig. 1) (Gray et al., 1938; Sawyer, 1986; Stern-Tomlinson et al., 1986; Brodfuehrer et al., 1995a; Brodfuehrer et al., 2008), suggesting that leeches may serve as another example of stop-and-go predators. But if this is the case, then why would leeches employ two separate locomotor behaviors? While it is not uncommon for animals to have a slow and a fast mode of locomotion, these modes commonly serve different behavioral needs: the slow mode is often used for foraging when large amounts of sensory information will help the animal to localize food and negotiate obstacles (e.g. Harley et al., 2009), whereas the fast mode often directs escape behaviors, thus requiring rapidly acquired albeit less detailed sensory information (e.g. Cowan et al., 2006). Thus both sensory capabilities and sensory needs may differ between slow and fast locomotion. Our previous work described the visual and

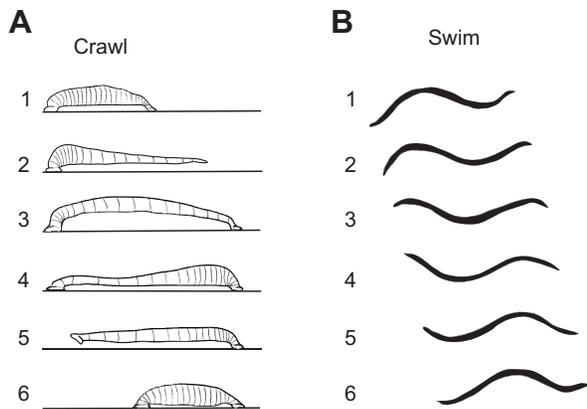


Fig. 1. Crawling and swimming are visually separable behaviors. (A) An illustration of the sequence of body postures comprising a single crawl step (adapted from Stern-Tomlinson et al., 1986). (B) An illustration of the sequence of movements comprising a single cycle of swim behavior (adapted from Chen et al., 2011; Kristan et al., 1974). In both panels the leech moves to the right.

mechanical stimuli that evoke prey localization in the medicinal leech (Harley et al., 2011). Here we consider whether swimming and crawling in the leech may serve distinct functions in prey localization, and use behavioral assays to determine the extent to which leeches are able to sense prey-like stimuli during various forms of locomotion.

MATERIALS AND METHODS

Animals and care

Adult medicinal leeches (*Hirudo verbana* Carena 1820) were obtained from Niagara Medicinal Leeches (Niagara, NY, USA) and maintained according to methods described previously (Harley et al., 2011).

Behavioral arena

Behavioral testing was performed in a plastic saucer-shaped arena (Super Saucer, Paris Co., South Paris, ME, USA) that had a diameter of 90 cm, which was filled with water ($18 \pm 0.5^\circ\text{C}$) to a depth of 20 mm. The resultant water diameter was 75 cm. An arena of this shape was chosen to minimize the reflection of water waves. Approximately 0.2 kg of white aquarium gravel was placed on the floor of the arena as it was found that gravel aided in quiescence (C.M.H., unpublished observation). The arena was placed on an air table to isolate it from external vibrations.

Stimuli

A function generator (Pasco Scientific, Roseville, CA, USA) was used to drive a speaker (Pasco Scientific). A thin aluminum rod with a clear plastic circular foot (4.7 cm in diameter) at its tip was connected to the speaker. This foot was placed such that it lay flat on the surface of the water. This stimulator created waves in the testing arena containing the leech. Throughout this study, we used waves with a frequency of 8 Hz, because those were found to be most readily localized by adult leeches under our experimental conditions (Harley et al., 2011). The arena was illuminated using a 750 W halogen flood lamp (McMaster-Carr, Santa Fe, CA, USA).

Using a piece of cardboard measuring 7×7 cm we projected a shadow of approximately that same size into the arena. This encouraged the leeches to reach a quiescent state (C.M.H.,

unpublished observations; Gee, 1913; Herter, 1936; Mann, 1962). A trial was started only after the leech remained within the shaded region for 1 min. Once this time had passed, the shadow was removed and the stimulus was started. The stimulus duration was determined by the behavioral assay (see below). Individuals were given 20 min to reach a quiescent state and complete a trial. If a trial was not completed, due to a failure of the leech to quiesce within 20 min of introducing the individual to the arena or if the individual left the arena three times, it was removed from a given day's testing.

Data acquisition

Videos were acquired using a Logitech pro 9000 webcam (Fremont, CA, USA) suspended above the arena. Videos were acquired at 25 frames s^{-1} at a resolution of 1600×1200 pixels (2 Mpixels).

Stimulus paradigms

To determine whether leeches update their internal representation of stimulus location during locomotion, we used two different stimulus paradigms: constant *versus* interrupted stimulation, and dual speaker stimulation. A third paradigm called conditional stimulation was used to determine whether ongoing locomotor activity disturbed the leech's ability to sense stimuli.

Constant *versus* interrupted stimulation

'Constant stimulation' consisted of a speaker that delivered wave stimuli constantly throughout the 5 min trial. 'Interrupted stimulation' consisted of wave stimuli delivered only until the leech moved from its initial position; once the leech either swam or crawled from its initial position, stimulation was stopped.

Dual speaker

Stimulation was provided through one of two speakers (A or B). The speakers were placed 120° apart in the arena (see Fig. 2, inset). The two speakers were calibrated according to methods described previously (Harley et al., 2011), such that both speakers created waves of the same amplitude. A simple switch inserted between the waveform generator and the speakers was used to change which speaker was active at a given time. At the beginning of a trial, one speaker was active. Then, once the leech moved away from its initial position, the switch was toggled and (only) the other speaker became active.

Conditional stimulation

Stimuli were only presented while the leech was in a particular locomotor state: crawl, swim or non-locomotion (see below). The arena was equipped with an LED indicating when the stimulator was active. This information was used to calculate the fraction of each trial during which stimulation occurred.

Identification of locomotor state

Locomotor states were easily separable by visual inspection and were defined as follows (Fig. 1).

Crawl

Vermiform crawling occurs as a series of steps (Stern-Tomlinson et al., 1986) (Fig. 1A). Initially, the body is shortened and both suckers are attached to the substrate. The front sucker then leaves the substrate and the front half of the body is extended. Once this extension is complete, the front sucker re-attaches to the substrate. At this point the animal's rostral annuli contract while the caudal annuli extend. Once the caudal section is fully extended, the rear sucker releases, propelling the front of the leech forward as the body contracts. The

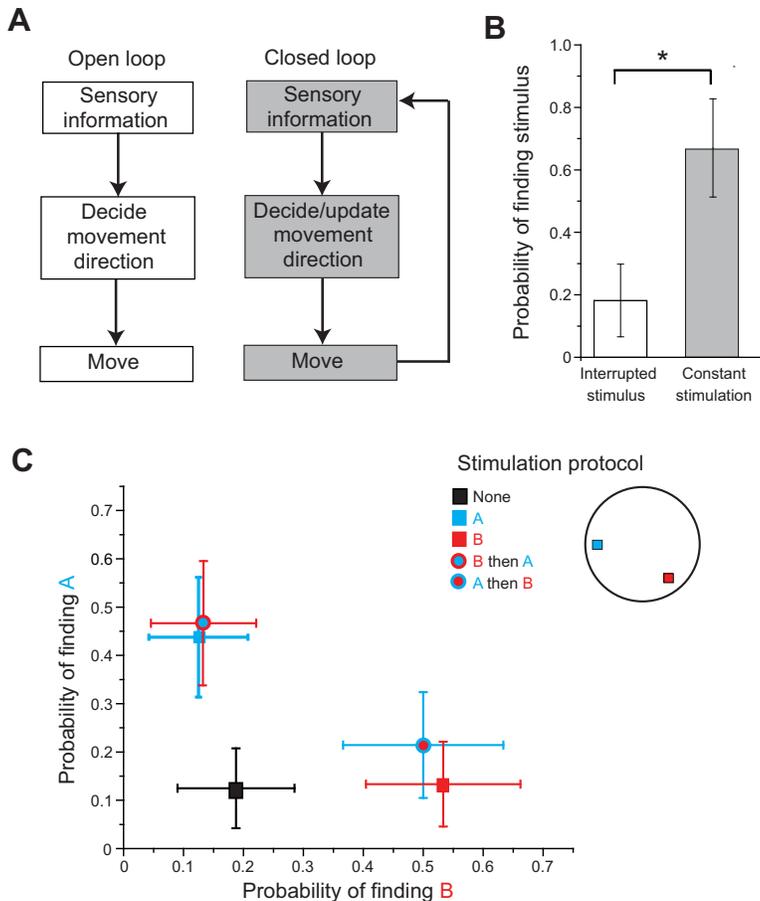


Fig. 2. Leeches require closed loop sensory feedback to localize stimuli. (A) A schematic illustrating open-loop and closed-loop control of behavior. (B) The probability of finding the stimulus location under interrupted (white, $N=11$) and constant (gray, $N=9$) conditions. * $P<0.05$ based on a χ^2 test. (C) The probability of localizing either of the two stimulus sources, A (vertical axis) and B (horizontal axis). Individuals were tested under the following conditions: no stimulus (black, $N=16$), stimulus A (blue, $N=15$), stimulus B (red, $N=16$), stimulus A then B (red circle with blue outline, $N=15$), and stimulus B then A (blue circle with red outline, $N=14$). In the latter two cases, the switch from one stimulus source to the other occurred after the first locomotor movement. Stimulus locations were as indicated in the inset.

crawl step is complete once the body is shortened and both suckers are again attached to the substrate. Often, there is a delay of a few seconds before the next step initiates. For the purpose of conditional stimulation experiments, a delay that lasted more than 5 s was considered the start of a non-locomotor period. If the head began to move in a crawling motion, it was understood that the leech was then continuing to crawl.

Swim

During swim bouts the whole body is flattened and moves in a series of quasi-sinusoidal dorsoventral contractions that progress from the front of the leech towards the back (Fig. 1B) (Kristan et al., 1974; Chen et al., 2011). During swimming, unlike crawling, neither of the leech's suckers is attached to the substrate.

Non-locomotion

Periods during which the leech did not progress forward, for a period of 20 s or more, were defined as non-locomotion. In 'conditional stimulation' experiments, individuals stimulated during non-locomotor states were stimulated after cessation of swimming or crawling behavior.

Analysis

The following are the definitions of specific terms used to describe the results.

Find

When an individual remained within a radius of 7 cm from the center of the stimulus for a minimum of 30 s during its 5 min trial we defined

the trial as a 'find'. This timing was chosen to separate steps in forward movement occurring because the individual was investigating the stimulus from those that occur naturally as part of crawling behavior (Harley et al., 2011). No more than one find was counted per trial. To calculate the probability of finding the stimulus, we divided the number of finds for a given treatment by the number of trials.

Time spent non-locomoting in a region

The arena was divided into a grid of 32 squares. Whenever an individual spent time in a non-locomotor state, the duration of this state was recorded along with the location where it occurred. Across individuals, we calculated the total amount of time spent non-locomoting in each square, and normalized this by the total amount of time spent non-locomoting anywhere. To test for significance, we calculated the standard deviation of the results among the 32 squares and multiplied the result by either 2.99 to obtain a $P<0.05$ confidence bound or by 3.45 to obtain a $P<0.01$ confidence bound. (These values incorporate the Bonferroni correction.) A square was deemed to be significantly overvisited if the time spent there exceeded the confidence bound.

Heading

The leech's heading was determined by measuring the angle between the animal's body axis and the stimulus using the program Tracker (Brown, 2012). Heading was measured at the start and end of each locomotor bout. If an animal actively senses the stimulus location during a locomotor behavior, it should change its heading over the course of the bout such that it ends up more directly

traveling toward the stimulus. Rayleigh tests were used to determine whether distributions of angles significantly differed from random, and *V*-tests were used to test whether heading angles were significantly directed toward the stimulus.

Representative tracks were analyzed using our Wormfinder program (Wagenaar and Kristan, 2010). The behavior during a given bout was determined visually.

RESULTS

Prior to this study it was known that leeches are able to utilize information from water disturbances to localize their prey (Friesen, 1981; Young et al., 1981; Dickinson and Lent, 1984; Harley et al., 2011). However, it was unknown whether they did so ballistically (under open-loop control) or *via* the guidance of constant sensory input (closed-loop control). In our first attempt to address this question, we employed the use of two stimulus protocols: one in which the stimulus was turned off after the leech initiated locomotion ('interrupted stimulation') and another in which the stimulus remained on for the duration of the trial ('constant stimulation'). An animal that does not update its sensory picture of the stimulus location would respond equally well to constant and interrupted stimulation. In contrast, an animal that requires additional sensory input during locomotion to update its direction of travel will better localize a constant stimulus. Our results show that individuals presented with constant stimulation located the source significantly more often than those given an interrupted stimulus ($z=2.276$, $d.f.=1$, $\chi^2=5.179$, $P<0.05$; Fig. 2B).

Alternatively, these results could be interpreted to indicate that leeches specifically detect the cessation of the stimulus and use that as a cue to stop searching; after all, searching when no prey is present would be a waste of valuable energy. To distinguish between this possibility and the possibility that leeches need constant information to update their heading, we designed a second paradigm in which we did not terminate the stimulus but rather 'moved' it to a new location. Two stimulators were placed in the arena, ~120deg apart, such that at any time either location could be cued. Control trials established that animals 'found' cued locations significantly more readily than non-cued locations ($z=1.981$, $d.f.=1$, $\chi^2=3.923$, $P<0.05$; Fig. 2C), which were 'found' at chance levels. Next, we created a more complex scenario in which we cued one location initially and, once the animal started moving, switched such that we were cueing the other location. A leech that, after initiating movement, moves ballistically towards a stimulus would approach the first stimulus cued, whereas a leech that constantly senses stimuli within its environment would move towards the most recently sensed stimulus location, and thus localize the second location that was cued. In fact, leeches did not find the original stimulus location more often than would be expected by chance ($z=0.126$, $d.f.=1$, $\chi^2=0.016$, $P>0.4$). Instead, they approached the new location ($z=2.518$, $d.f.=1$, $\chi^2=6.34$, $P<0.05$). Even though leeches were not given any extra time to find the new location (trials were limited to 5 min under all conditions), no significant difference was found between the find rates of the new location in these trials and the find rates in the control trials in which the source was not moved. The movement toward the second stimulus coupled with a find rate comparable to when only one stimulus is present suggests that leeches use sensory information to update their sensory picture of the environment and do not localize prey through ballistic movements.

Both of these results indicate that leeches obtain and process sensory information while locomoting towards prey but they do not unambiguously say whether the older information is retained and whether that information still influences the behavior. In trials in

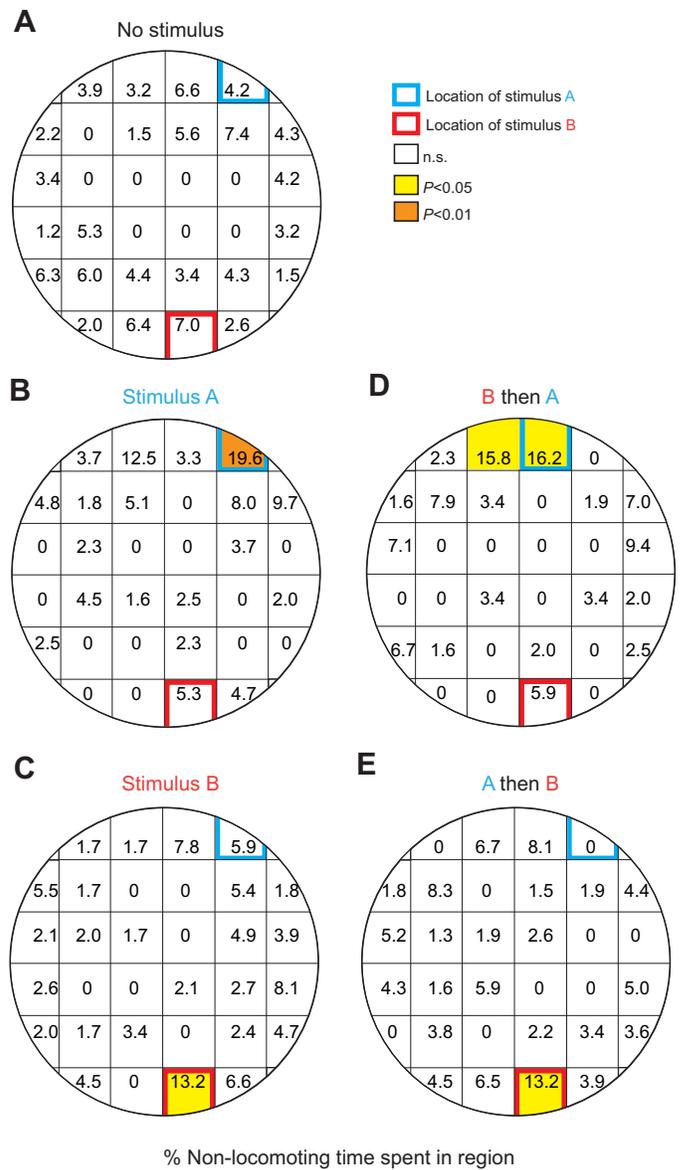


Fig. 3. Leeches use only the most current information to determine stimulus location. Large circles represent the behavioral arena, and numbers in each sector represent the time that leeches spent non-locomoting in that region as a percentage of the total time spent non-locomoting. The locations of the two stimuli are marked in blue and red. (A) No-stimulus control. (B) Location A cued. (C) Location B cued. (D) Location B cued until the leech started moving, then location A cued. (E) Location A cued until the leech started moving, then location B cued. Sectors in which the time spent was significantly above the average were color-coded as follows: yellow for $P<0.05$, orange for $P<0.01$ according to statistical methods described in the Materials and methods. Sample sizes are as in Fig. 2C.

which the stimulus was moved, rather than heading directly to one stimulus or the other, a leech could actually proceed to stop between the two stimuli. To investigate this possibility, we examined all instances in which a leech stopped locomoting for at least 20s and noted the location where this happened as well as the amount of time the leech spent there. These data were combined among trials and animals and used to compute, for each region of the arena, the fraction of time spent non-locomoting in that region (see Materials and methods). Significant increases in non-locomotor behavior were

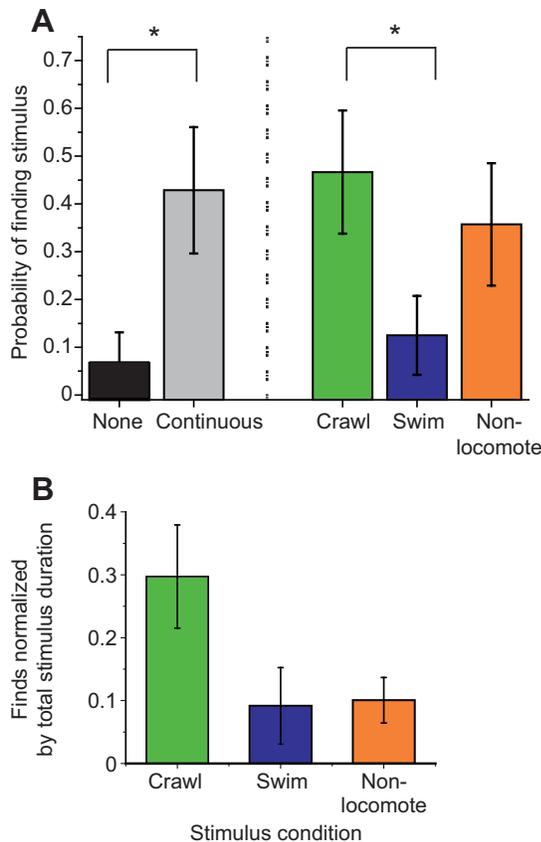


Fig. 4. Locomotor state affects the leech's ability to localize stimuli. (A) Probability of finding a stimulus when it was presented continuously (gray) or only during specific locomotor behaviors: crawling (green), swimming (blue) or non-locomotion (orange). Black: no-stimulus control. * $P < 0.05$ based on a χ^2 test. (B) Find rate normalized to the amount of time the stimulator was active.

only found in regions near the stimulus (Fig. 3). This was true whether the stimulus was moved (Fig. 3D,E) or not (Fig. 3B,C). In no case was non-locomotion significantly concentrated at non-cued locations, and, in particular, it was not significantly concentrated at an intermediate point between the two cued locations. Furthermore, in the case where the cued stimulus was switched during the trial we found that there was no significant increase in non-locomotor bouts at the original cued stimulus (Fig. 3D,E). Thus, older information did not substantially influence the prey-finding behavior.

Our data suggest that leeches obtain and process sensory information as they locomote towards prey. However, the very locomotor state the leech uses to approach its prey may affect its ability to sense water waves. Thus we compared the leech's ability to orient towards stimuli while in its different locomotor states: swimming, crawling and non-locomoting. Under this paradigm, stimulation was provided only during one of the locomotor behaviors. Leeches presented with stimulation only while crawling showed no difference in their ability to find the stimulus from those stimulated constantly (Fig. 4A), whereas individuals presented with stimulation only during swimming showed a significant decrease in their ability to find the stimulus source ($\chi^2 = 2.094$, d.f. = 1, $P < 0.05$), reducing them to a find rate not significantly different than if there was no stimulus present. Leeches presented with stimulation only during non-locomotion exhibited intermediate find rates,

which did not differ significantly from either that of constant stimulation or when no stimulus was present. It is worth noting that the three locomotor-related behaviors, swimming, crawling and non-locomoting, were not equally prevalent (occurring during 22.7, 26.2 and 59.2% of the trial, respectively). As a result, stimulating only during crawling or only during swimming resulted in significantly less total stimulation than stimulating during non-locomotion (ANOVA Tukey means comparison, $q = 9.15$ and 8.27 for crawling and swimming, respectively, when compared with non-locomoting, $P < 0.05$). Normalizing the find rate by the total amount of time an individual was in a specific locomotor state (and thus received stimulation) revealed that of the locomotor behaviors, crawling yielded the highest number of finds per unit stimulation time (Fig. 4B). In contrast, swimming and non-locomoting resulted in find rates similar to when no stimulus was present. It is possible that the inability to localize stimuli during non-locomotor bouts may be due to a decrease in the willingness to locomote.

A further indication that stimuli are more readily localized during crawling than during either of the other behaviors is the animals' heading relative to the stimulus before and after bouts of swimming and crawling. Before either swim or crawl bouts, headings were randomly distributed (a Rayleigh test yielded non-significance, $Z = 0.46$ for crawls and 0.908 for swims; Fig. 5A,B, gray boxes, mean directions were 295 and 301 deg with vector lengths of 0.037 and 0.168 , respectively); a fact that additionally indicates that non-locomotor bouts did not orient the leech towards the stimulus. At the end of swim bouts, these headings were still randomly distributed (Rayleigh test, $Z = 0.106$; Fig. 5B, magenta circles mean direction and vector length were 333 deg and 0.057 , respectively). In contrast, at the end of crawl bouts leeches were heading in the direction of the stimulus (V -test, $u = 2.302$, $P < 0.01$, Rayleigh test, $Z = 2.931$, $P < 0.05$; Fig. 5A, magenta circles, mean direction and vector length were 342 deg and 0.294 , respectively). To illustrate the differential effects of swimming and crawling on heading, Fig. 5C,D displays tracks of six representative crawling bouts and seven swimming bouts from the same trial (in which, incidentally, the stimulus was not found).

Because locomotor state affects the ability to sense prey location, is the probability of a given locomotor behavior itself changed by the presence of a stimulus? To examine this possibility, we measured the duration of crawling bouts in 24 individuals in the presence and absence of stimuli and found a significant increase in the number of long bouts (lasting more than 30, 60 or 90 s; χ^2 , each $P < 0.05$) when a stimulus was present compared with when no stimulus was present (Fig. 6A). We also noted a stimulus-induced increase in the fraction of time spent crawling and a corresponding decrease in the fraction of time spent non-locomoting (Fig. 6D). In addition, the probabilities of an animal transitioning from either non-locomoting or swimming to crawling were significantly higher when a stimulus was present ($\chi^2 = 1.989$, $P < 0.05$, and $\chi^2 = 9.350$, $P < 0.005$, respectively; Fig. 6E). No significant stimulus-induced change was seen in the number of long swim bouts, swim bout duration or the fraction of time spent swimming (Fig. 6B,D).

DISCUSSION

Prior to this study it was unknown whether leeches found their prey using a ballistic strategy, whereby the movement heading was chosen before its onset, or whether they regularly adjusted their trajectory toward their prey while locomoting. Adding complexity to this issue, leeches utilize two distinct modes of locomotion, swimming and crawling, during prey localization (Fig. 1). Either or both of these behaviors could be ballistically aimed or leeches could use sensory information to adjust their heading. We found, however, that

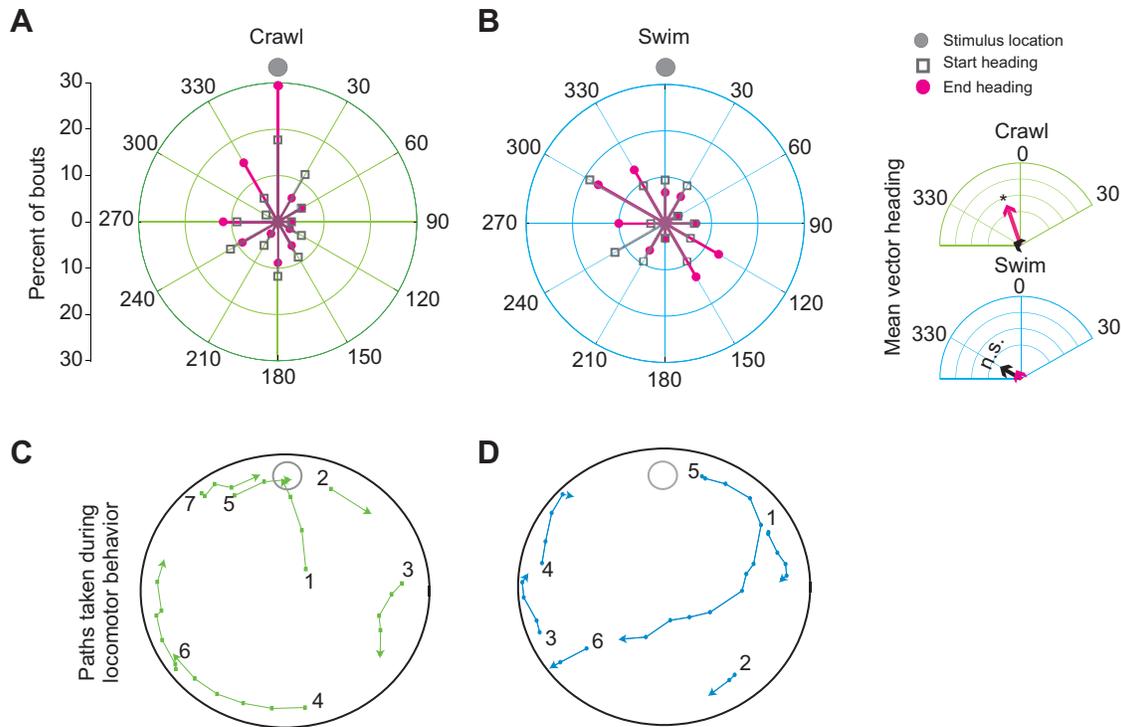


Fig. 5. Crawling is directed towards the stimulus. (A,B) With the stimulus presented constantly, the directional heading of nine leeches was measured at the start (gray) and end (magenta) of bouts of crawling (A) or swimming (B). Data represented are from 35 crawl bouts and 32 swim bouts. The dot at 0 deg represents the direction towards the stimulus. Mean vector headings for the start and end of the bouts are represented by black and pink arrows, respectively, to the right of panel B. * $P < 0.01$ based on a V -test; n.s., not significant. (C,D) Traces of paths recorded during consecutive bouts of crawling (C) and swimming (D) in a single representative video. The gray circle represents the stimulus location. Individuals were tracked every 6.4 s during crawling or every 0.66 s during swimming, which was the average amount of time it took for the leech to travel a single body length (C.M.H., personal observation).

leeches do not move towards prey ballistically, but rather that their movement requires continual sensory feedback. Without a constant source of sensory input, leeches were unable to localize the source of a stimulus (Fig. 2B). In addition, changing the location of the perceived stimulus mid-trial resulted in the leech retargeting its search to the new stimulus location (Fig. 2C) without regard for the old location (Fig. 3). We conclude that prey-capture behavior is not ballistically aimed, but rather is one in which the leech uses sensory information to update its direction of movement.

While the leech's sensory picture of the environment needs to be continually updated for it to localize its prey, actually reaching the prey requires that the leech moves within the same water that it is using to sense its prey. This motion inevitably disturbs the water and thus the sensory evidence for prey location. How might water disturbances generated by the leech's self-movement be separated from those generated by its prey? One possibility would be that non-locomotor phases direct subsequent ballistic locomotor phases. However, our data reveal that the reality is more complex. Leeches stimulated during crawling were able to localize stimuli just as well as individuals receiving a continuous stimulus despite the lack of stimulation during non-locomotor bouts. This ability to localize stimuli while crawling could be taken to suggest that self-induced sensory flow assists with prey localization, as is the case in many animals. However, if sensory flow information is helpful, then why is stimulation received during crawling effective for prey localization while that received during swimming is so ineffective (Fig. 4A,B)? One reason may be that swimming occurs at a faster speed than crawling and that the leech's swimming stimulates its water

movement sensors in a way that masks information from our prey-like stimulus. Alternatively, perhaps it is not that swimming is ineffective for prey localization but rather that crawling is effective. What may contribute to an enhanced ability of leeches to localize prey while crawling? Crawling exists as a series of steps and pauses. It is possible that these discrete steps result in the integration of additional information, which may provide a more accurate picture of prey location. This notion would liken crawling behavior to saccades present in the visual behavior of mammals as well as some insects (Rossel et al., 1992; Hollands et al., 1995; Lappe et al., 1999; van Hateren and Schilstra, 1999; Drew et al., 2008). Furthermore, during crawling, it is normal for at least one of the leech's suckers to remain in contact with the substrate. This attachment may provide a reference point between the leech's movements and the ground – a point of reference not available during swimming behavior. This static reference point may make it easier for the leech to separate self-induced and external sources of water disturbance. Supporting this notion is the fact that individuals stimulated during non-locomotor bouts where at least one sucker would be attached to the substrate, similar to those stimulated during crawling bouts, exhibit a find rate comparable to that of individuals that receive constant stimulation (Fig. 4A). However, these individuals receive significantly more stimulation than their locomoting counterparts and exhibit a much lower find rate per minute of stimulation (Fig. 4B). This may be due to their decreased likelihood of movement, a decreased receptivity to sensory stimuli, or, alternatively could be because moving in and of itself gives the animal information as to the location of its prey. Certainly, crawling

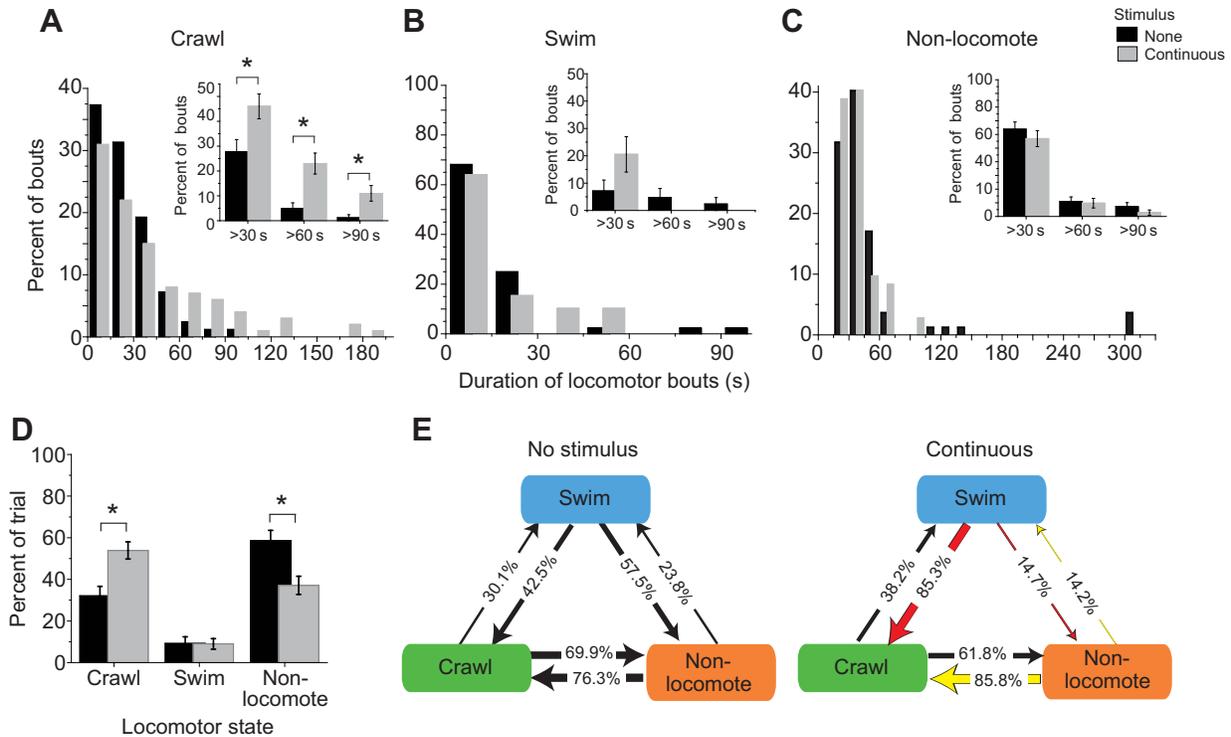


Fig. 6. Longer crawling bouts occur when the stimulus is present. (A–C) Histograms of the duration of bouts of crawling (A), swimming (B) or non-locomotion (C) in the presence (gray) or absence (black) of stimulation. Bin size was 15 s. Insets: cumulative percentage of bouts longer than 30, 60 and 90 s. (D) Overall time spent in each of the locomotor states as a percentage of trial duration. * $P < 0.05$, ANOVA Tukey means comparison. (E) Transitions between locomotor states. Numbers by arrows indicate the percentage of exits from a given state that led to a certain other state. Colored arrows indicate transitions that occurred at a significantly different frequency in the presence of stimuli (χ^2 ; yellow: $P < 0.05$, red: $P < 0.01$). Data are from 24 leeches observed during constant stimulation and 23 non-stimulated controls.

directs the leech's heading towards the stimulus (Fig. 5), making crawling an effective behavior for prey localization.

The very presence of prey-like stimuli biases leeches towards crawling: crawl bouts were longer and transitions to crawling from other locomotor states were more common when a sensory stimulus was present (Fig. 6). The underlying mechanisms associated with this bias may involve neurons in the segmental and cephalic ganglia, which have been shown to promote crawling while inhibiting swimming (Brodfuehrer et al., 1995b; Esch et al., 2002; Briggman et al., 2005). Because dopamine plays a prominent role in promoting crawling while also suppressing swimming, prey detection most likely involves this amine (Crisp and Mesce, 2004; Crisp and Mesce, 2006; Puhl and Mesce, 2008). Hungry leeches, however, have been shown to have elevated levels of serotonin (Willard, 1981; Lent and Dickinson, 1984), which stimulates swimming (Brodfuehrer and Friesen, 1984; Lent and Dickinson, 1984; Puhl and Mesce, 2008). Although serotonin-activated swimming in hungry leeches may enable a previously sedentary leech to cover a larger area in which to encounter its prey, our study suggests that prey-specific sensory stimuli induce crawling, shown by others to be regulated by dopamine, not serotonin. Because mechanosensory interneurons have been shown to be electrically coupled to dopamine-secreting neurons in the leech (Crisp and Mesce, 2004), a rapid positive feedback system is in place – one that biases the system towards crawling and may be able to guide the leech towards its prey.

Directed behaviors are often the output of a population code that translates sensory information into a guided movement

(Georgopoulos et al., 1988; Churchland and Sejnowski, 1992; Katz, 1996; Lewis and Kristan, 1998a; Lewis and Kristan, 1998b; Sanger, 2003; Sakura et al., 2008). However, when that guided movement is directed towards another animal and not a stationary object, the system must have a way of updating the encoded direction of movement. There are multiple strategies for this updating. First, the animal could simply head (ballistically) to the location of the original stimulus. Second, the animal could sense two stimulus locations and would conservatively proceed to a location between the two. Third, the animal could simply ignore the first stimulus and update its heading toward the more recent input. In our experiments, the leech did not head toward the first stimulus or a location between the two, but rather only to the location of the last cued stimulus (Fig. 2C, Fig. 3). Furthermore, the leech's response to the second stimulus was no different from single source responses, suggesting that older information is not considered in the new percept of the stimulus location (Fig. 3D,E). This suggests that the new sensory information rewrites the old, directing the leech towards the most up-to-date prey percept. This could be an adaptive response to prey movement during the course of the leech's rather slow orientation response. By only sensing the most up-to-date stimulus location, the system does not need memory of the previous stimulus location. This suggests a simple processing scheme for the sensory information. Such a method would be faster and could be expected to require fewer neurons, something that complements the numerical simplicity of the leech's nervous system.

A previous study suggested that, in addition to crawl bouts, swim bouts are also guided by water disturbances (Young et al., 1981).

Several differences in experimental conditions may explain this discrepancy. Firstly, Young et al. used a square arena measuring 42 cm in width whereas our saucer-shaped arena measures 90 cm in diameter. Secondly, as stimulus frequency influences the leech's behavior (Harley et al., 2011), it is important to note that Young et al. utilized a 1 Hz stimulus whereas we used an 8 Hz stimulus. Lastly, Young et al. had some evidence that a leech's level of satiety plays a role in its orientation response. It is possible that our leeches were simply at a different level of satiety than those used by Young et al. Regardless of the reason behind this discrepancy, it is a further indication that crawling and swimming have different roles in prey localization and that they may be advantageous under different environmental and internal conditions and contexts. And it points to the enticing possibility that water disturbances bias the leech not only in movement direction but also in the form of locomotion it uses to get there.

ACKNOWLEDGEMENTS

The authors thank Karen Mesce for her valuable input on the discussion of this manuscript, as well as two anonymous reviewers, whose comments aided in improving the manuscript.

AUTHOR CONTRIBUTIONS

C.M.H. and D.A.W. designed the experiments. C.M.H., J.C. and M.R. performed data analysis and experiment execution. C.M.H. wrote the manuscript. D.A.W. and C.M.H. edited the manuscript.

COMPETING INTERESTS

No competing interests declared.

FUNDING

Funding for this study was provided by the Burroughs Wellcome Fund and the Broad Foundations (to D.A.W.).

REFERENCES

- Briggman, K. L., Abarbanel, H. D. and Kristan, W. B., Jr (2005). Optical imaging of neuronal populations during decision-making. *Science* **307**, 896-901.
- Brodfuehrer, P. D. and Friesen, W. O. (1984). A sensory system initiating swimming activity in the medicinal leech. *J. Exp. Biol.* **108**, 341-355.
- Brodfuehrer, P. D., Debski, E. A., O'Gara, B. A. and Friesen, W. O. (1995a). Neuronal control of leech swimming. *J. Neurobiol.* **27**, 403-418.
- Brodfuehrer, P. D., Parker, H. J., Burns, A. and Berg, M. (1995b). Regulation of the segmental swim-generating system by a pair of identified interneurons in the leech head ganglion. *J. Neurophysiol.* **73**, 983-992.
- Brodfuehrer, P. D., McCormick, K., Tapyrik, L., Albano, A. M. and Graybeal, C. (2008). Activation of two forms of locomotion by a previously identified trigger interneuron for swimming in the medicinal leech. *Invert. Neurosci.* **8**, 31-39.
- Brown, D. (2012). Tracker video analysis and modeling tool. Available at <http://www.cabrillo.edu/~dbrown/tracker/>.
- Carlton, T. and McVean, A. (1993). A comparison of the performance of two sensory systems in host detection and location in the medicinal leech *Hirudo medicinalis*. *Comp. Biochem. Physiol.* **104**, 273-277.
- Chen, J., Friesen, W. O. and Iwasaki, T. (2011). Mechanisms underlying rhythmic locomotion: body-fluid interaction in undulatory swimming. *J. Exp. Biol.* **214**, 561-574.
- Churchland, M. M. and Sejnowski, T. J. (1992). *The Computational Brain*. Cambridge, MA: MIT Press.
- Collett, T. S. (1978). Peering-a locust behaviour pattern for obtaining motion parallax information. *J. Exp. Biol.* **76**, 237-241.
- Collett, T. S. (1980). Angular tracking and the optomotor response: an analysis of visual reflex interaction in a hoverfly. *J. Comp. Physiol. A* **140**, 145-158.
- Corrette, B. J. (1990). Prey capture in the praying mantis *Tenodera aridifolia sinensis*: coordination of the capture sequence and strike movements. *J. Exp. Biol.* **148**, 147-180.
- Cowan, N. J., Lee, J. and Full, R. J. (2006). Task-level control of rapid wall following in the American cockroach. *J. Exp. Biol.* **209**, 1617-1629.
- Crisp, K. M. and Mesce, K. A. (2004). A cephalic projection neuron involved in locomotion is dye coupled to the dopaminergic neural network in the medicinal leech. *J. Exp. Biol.* **207**, 4535-4542.
- Crisp, K. M. and Mesce, K. A. (2006). Beyond the central pattern generator: amine modulation of decision-making neural pathways descending from the brain of the medicinal leech. *J. Exp. Biol.* **209**, 1746-1756.
- Dickinson, M. H. and Lent, C. M. (1984). Feeding behavior of the medicinal leech, *Hirudo medicinalis* L. *J. Comp. Physiol. A* **154**, 449-455.
- Drew, T., Andujar, J. E., Lajoie, K. and Yakovenko, S. (2008). Cortical mechanisms involved in visuomotor coordination during precision walking. *Brain Res.* **57**, 199-211.
- Esch, T., Mesce, K. A. and Kristan, W. B. (2002). Evidence for sequential decision making in the medicinal leech. *J. Neurosci.* **22**, 11045-11054.
- Ewert, J. P. (1987). Neuroethology of releasing mechanisms: prey-catching in toads. *Behav. Brain Sci.* **10**, 337-368.
- Friesen, W. O. (1981). Physiology of water motion detection in the medicinal leech. *J. Exp. Biol.* **92**, 255-275.
- Frost, B. J. (1978). The optokinetic basis of head bobbing in the pigeon. *J. Exp. Biol.* **74**, 187-195.
- Gee, W. (1913). The behavior of leeches with special reference to its modifiability. *Univ. Calif. Publ. Zool.* **11**, 197-305.
- Georgopoulos, A. P., Kettner, R. E. and Schwartz, A. B. (1988). Primate motor cortex and free arm movements to visual targets in three-dimensional space. II. Coding of the direction of movement by a neuronal population. *J. Neurosci.* **8**, 2928-2937.
- Gilbert, C. (1997). Visual control of cursorial prey pursuit by tiger beetles (Cicindelidae). *J. Comp. Physiol. A* **181**, 217-230.
- Gray, J., Lissman, H. W. and Pumphry, R. J. (1938). The mechanism of locomotion in the leech (*Hirudo medicinalis* Ray). *J. Exp. Biol.* **15**, 408-430.
- Harley, C. M., English, B. A. and Ritzmann, R. E. (2009). Characterization of obstacle negotiation behaviors in the cockroach, *Blaberus discoidalis*. *J. Exp. Biol.* **212**, 1463-1476.
- Harley, C. M., Cienfuegos, J. and Wagenaar, D. A. (2011). Developmentally regulated multisensory integration for prey localization in the medicinal leech. *J. Exp. Biol.* **214**, 3801-3807.
- Herter, K. (1936). *Die Physiologie Der Hirudineen*. Leipzig: Akademische Verlagsgesellschaft.
- Hollands, M. A., Marple-Horvat, D. E., Henkes, S. and Rowan, A. K. (1995). Human eye movements during visually guided stepping. *J. Mot. Behav.* **27**, 155-163.
- Katz, P. S. (1996). Neurons, networks, and motor behavior. *Neuron* **16**, 245-253.
- Kristan, W. B., Stent, G. S. and Ort, C. A. (1974). Neuronal control of swimming in the medicinal leech. I. Dynamics of the swimming rhythm. *J. Comp. Physiol.* **94**, 97-119.
- Lappe, M., Bremner, F. and van den Berg, A. V. (1999). Perception of self-motion from visual flow. *Trends Cogn. Sci.* **3**, 329-336.
- Lehrer, M. and Srinivasan, M. V. (1994). Active vision in honeybees: task-oriented suppression of an innate behaviour. *Vision Res.* **34**, 511-516.
- Lent, C. M. and Dickinson, M. H. (1984). Serotonin integrates the feeding behavior of the medicinal leech. *J. Comp. Physiol. A* **154**, 457-471.
- Lewis, J. E. and Kristan, W. B., Jr (1998a). Quantitative analysis of a directed behavior in the medicinal leech: implications for organizing motor output. *J. Neurosci.* **18**, 1571-1582.
- Lewis, J. E. and Kristan, W. B., Jr (1998b). A neuronal network for computing population vectors in the leech. *Nature* **391**, 76-79.
- Lock, A. and Collett, T. (1979). A toad's devious approach to its prey: a study of some complex uses of depth vision. *J. Comp. Physiol. A* **131**, 179-189.
- Mann, K. M. (1962). *Leeches (Hirudinea). Their Structure, Physiology, Ecology and Embryology*, pp. 79-100. London: Pergamon Press.
- Medendorp, W. P., Tweed, D. B. and Crawford, J. D. (2003). Motion parallax is computed in the updating of human spatial memory. *J. Neurosci.* **23**, 8135-8142.
- Miller, P. (1979). A possible sensory function for the stop-go patterns of running in phorid flies. *Physiol. Entomol.* **4**, 361-370.
- Niven, J. E. (2006). Visual motion: homing in on small target detectors. *Curr. Biol.* **16**, R292-R294.
- Nordström, K. and O'Carroll, D. C. (2006). Small object detection neurons in female hoverflies. *Proc. Biol. Sci.* **273**, 1211-1216.
- O'Brien, J. W., Evans, B. I. and Browman, H. I. (1989). Flexible search tactics and efficient foraging in saltatory searching animals. *Oecologia* **80**, 100-110.
- Olberg, R. (1981). Object- and self-movement detectors in the ventral nerve cord of the dragonfly. *J. Comp. Physiol. A* **141**, 327-334.
- Poteser, M., Pabst, A. and Kral, K. (1998). Proprioceptive contribution to distance estimation by motion parallax in a praying mantid. *J. Exp. Biol.* **201**, 1483-1491.
- Puhl, J. G. and Mesce, K. A. (2008). Dopamine activates the motor pattern for crawling in the medicinal leech. *J. Neurosci.* **28**, 4192-4200.
- Rossel, S., Mathis, U. and Collett, T. (1992). Vertical disparity and binocular vision in the praying mantis. *Vis. Neurosci.* **8**, 165-170.
- Sakura, M., Lambrinos, D. and Labhart, T. (2008). Polarized skylight navigation in insects: model and electrophysiology of e-vector coding by neurons in the central complex. *J. Neurophysiol.* **99**, 667-682.
- Sanger, T. (2003). Neural population codes. *Curr. Opin. Neurobiol.* **13**, 238-249.
- Sawyer, R. T. (1986). *Leech Biology and Behaviour I: Anatomy, Physiology, and Behavior*. Oxford: Clarendon Press.
- Srinivasan, M. V., Lehrer, M. and Horridge, G. A. (1990). Visual figure-ground discrimination in the honeybee: the role of motion parallax at boundaries. *Proc. R. Soc. B* **238**, 331-350.
- Stern-Tomlinson, W., Nusbaum, M. P., Perez, L. E. and Kristan, W. B. (1986). A kinematic study of crawling behavior in the leech, *Hirudo medicinalis*. *J. Comp. Physiol. A* **158**, 593-603.
- van der Willigen, R. F., Frost, B. J. and Wagner, H. (2002). Depth generalization from stereo to motion parallax in the owl. *J. Comp. Physiol. A* **187**, 997-1007.
- van Hateren, J. H. and Schilstra, C. (1999). Blowfly flight and optic flow. II. Head movements during flight. *J. Exp. Biol.* **202**, 1491-1500.
- Wagenaar, D. A. and Kristan, W. B., Jr (2010). Automated video analysis of animal movements using Gabor orientation filters. *Neuroinformatics* **8**, 33-42.
- Willard, A. L. (1981). Effects of serotonin on the generation of the motor program for swimming by the medicinal leech. *J. Neurosci.* **1**, 936-944.
- Young, S. R., Dedwylder, R. D. and Friesen, W. O. (1981). Responses of the medicinal leech to water waves. *J. Comp. Physiol. A* **144**, 111-116.