One antenna, two antennae, big antennae, small: total antennae length, not bilateral symmetry, predicts odor tracking performance in the American cockroach, *Periplaneta americana* L.

Lockey, J. K., and Willis, M. A.
Department of Biology
Case Western Reserve University
Cleveland, OH 44106, USA

Corresponding author:
J. K. Lockey
jklockey@case.edu
Department of Biology
Case Western Reserve University
2080 Adelbert Road
Cleveland, OH 44106, USA

Abstract:

Determining the location of a particular stimulus is often crucial to an animal's survival. One way to determine the local distribution of odor is to make simultaneous comparisons across multiple sensors. If the sensors detect differences in the distribution of odor in space, the animal can then steer toward the source. American cockroaches, *Periplaneta americana*, have 4 cm long antennae and are thought to track odor plumes using a spatial sampling strategy, comparing the amount of odor detected between these bilateral sensors. However, it is not uncommon for cockroaches to lose parts of their antennae and still track a wind-borne odor to its source. We examined whether bilateral odor input is necessary to locate an odor source in a wind-driven environment and how the loss of increasing lengths of the antennae affects odor tracking. The tracking performances of individuals with two bilaterally-symmetrical antennae of decreasing length were compared to antennal length-matched individuals with one antenna. Cockroaches with one antenna were generally able to track an odor plume to its source. In fact, the performances of unilaterally antennectomized individuals were statistically identical to their bilaterally symmetrical counterparts when the total length of both antennae equaled the length of the single antenna of the antennectomized individuals. This suggests that the total length of available antennae influences odor tracking performance more than any specific piece of antenna, and that they may be doing something more complex than a simple bilateral comparison between their antennae. The possibility of an antenna-topic map is discussed.

Keywords:

Introduction:

An animal's survival and reproductive success often depends on its ability to localize resources. For animals using olfaction to seek food or mates, multiple strategies can be used to determine the location of an odor source (Frankel and Gunn, 1961; Kennedy, 1978; Bell and Tobin, 1982; Weissburg, 2000). In fluid environments (i.e., air and water) with zero flow, odor is dispersed by diffusion and will have a gradient in which the concentration of odor near the source is greater than the concentration far away. In environments with moving fluids, diffusion is trumped by turbulent mixing, creating pockets of clean fluid intermixed with fluid bearing odor of different concentrations (Campbell, 1977; Weissburg, 2000). These pockets of odor decrease in concentration as they are carried away from the source (Murlis and Jones, 1981; Murlis *et al*., 2000; Webster and Weissburg, 2001). In either flow condition, an animal must be able to adapt its behavior as it moves and use the information available to navigate to the source.

Orientation responses of animals to environmental information are thought to fall into two rough categories: *indirect* and *direct*. In indirect responses an animal's rate of locomotion or turning are modulated by the intensity of a specific sensory input (i.e., light, chemicals, sound, etc.). These responses have been termed kineses with modulation of locomotion rate termed ortho-kineses and turning rate termed klino-kineses (Fraenkel and Gunn, 1961). Animals might also alter their movement by steering directly toward or away from a stimulus. These orientation movements have been termed positive or negative taxes, respectively. Animals controlling their locomotion directly with respect to stimuli use two well-described methods: spatial and temporal comparisons. (Frankel and Gunn, 1961; Bell and Tobin, 1982).

A tracking strategy using spatial comparisons requires an organism to sample simultaneously from two or more sensors distributed on their body to make instantaneous comparisons of at least two points in space (Moore and Lepper, 1997; Weissburg and Dusenbery, 2002; Willis, 2008). In tropotaxis, perhaps the best understood method of making spatial comparisons, the signal from one bilaterally symmetrical sensor is compared to the signal from its contralateral sensor. For example, in the fly, *Drosophila melanogaster,* bilateral input is required for odor-guided steering in adult flies while either walking (Borst and Heisenberg, 1982), or flying towards an attractive odor (Duistermars *et al.,* 2009) or away from a repulsive odor (Wasserman *et al.,* 2012). This is in contrast to the larva, where bilateral input enhances, but is not required for odor tracking (Louis *et al.,* 2008). *Drosophila melanogaster* larvae with two intact dorsal organs (i.e., larval antennae) typically turn towards the organ detecting the higher concentration of an attractive odor. When one

dorsal organ is functionally removed, they meander more (Louis *et al.,* 2008). Many other species have likewise been reported to make use of bilateral spatial comparisons to orient toward attractive odors. The crayfish *Orconectes rusticus* requires input from both antennules to successfully orient to a food source (Kraus-Epley and Moore, 2002), and the chambered nautilus *Nautilus pompilius* requires both odor-detecting rhinophores to track a plume of food odor (Basil *et* al., 2000). Similarly, the ant *Lasius fuliginosus* steers towards the remaining antenna when one has been removed generating a predictably asymmetric and looping track depending on the antenna that has been removed (i.e., turning toward the intact antenna—a classic prediction of unilateral ablation in tropotatic animals). Their ability to track a pheromone trail is disrupted when the detected position of the trail in space is flipped by crossing their antennae (Hangartner 1967). The silkmoth *Bombyx mori* turns towards the antenna receiving delayed input or a higher odor concentration relative to the opposite side (Takasaki *et. al.,* 2012). Rats require bilateral input to accurately localize an odor (Rajan *et al.,* 2006), and bilateral input increases the accuracy of tracking an odor trail (Khan *et al.,* 2012). Even humans track an odor faster and more accurately when bilateral information is available (Porter *et al.,* 2005; Porter *et al.,* 2007). In radially symmetrical animals such as sea stars this comparison may be across multiple sensors (Moore and Lepper; 1997).

Temporal tracking requires an organism to take sequential samples as it moves, compare those samples across time, and alter its course in the direction of the larger stimulus (Bell and Tobin, 1982; Kennedy, 1978). Animals using this strategy could sum, or average readings across multiple sensors to determine whether the stimulus intensity has increased or decreased and steer accordingly (Schöne, 1984). The classic example of temporal tracking is transverse klinotaxis, whereby animals symmetrically zig-zag across their net displacement vector by changing their turning angle, sampling transverse slices of the odor plume and adjusting their net displacement vector towards the stimulus source (Kennedy, 1978), effectively constructing the spatial information over time that would otherwise be available instantaneously in tropotaxis. The side-to-side head motion of a nematode tracking a chemical (Ward, 1973), as well as the casting behavior of various moth species (Kennedy and Marsh, 1974; Vickers and Baker, 1991; Willis and Arbas, 1991), are exemplars. This is in contrast to longitudinal klinotaxis, where animals continue forward so long as stimulus intensity is increasing. When there is a decrease in intensity, a series of turns are executed and samples are compared between headings to determine the direction of increasing stimulus (Bell and Tobin, 1982; Kennedy, 1978).

Recent studies of the blue crab, *Callinectes sapidus,* show that this animal uses both temporal and

spatial strategies to guide its walking path while tracking a plume of food odor, and that these two strategies are supported by different odor sensing structures (Page et al., 2011a; Page et al., 2011b). It has been proposed that the crab's brain compares the inputs from the chemoreceptor hairs on the distal tips of the crab's walking legs to decide what direction to steer to remain in the center of the odor plume (Page et al., 2011b). The speed of walking during plume tracking is correlated with the rate at which the crab's antennules encounter filaments of odor in the plume (Page et al., 2011a). The associations between the odor signal, sensory appendage and motor performance of the crabs was made possible by video recording the fine structure of a florescent dye that had been formulated with the attractant odor and co-released. Although insects do have chemosensors on other parts of their bodies, including their feet, most of these are contact chemosensilla that are typically less centrally organized than the olfactory sensilla on the antennae, and none are known to be used in long distance orientation like plume tracking (Resh and Cardé, 2009).

American cockroaches (*Periplaneta americana* L.) are attractive models for studying odor plume tracking because they are champions of olfactory behavior and the olfactory epithelium on their 4 cm long, filamentous antennae is easily accessible. These antennae are each made up of more than 150 segments called annuli, and each annulus is covered in olfactory sensilla of multiple types. In males, about half of these sensilla are selectively sensitive to the female sex attractant pheromone, periplanone (Schaller, 1978).

The long linear array of odor sensors characteristic of *P. americana*'s antenna could allow either spatial, temporal, or both strategies to be used simultaneously for odor localization. Previous work in an environment with no predictable flow has suggested that *P. americana* with two antennae use a spatial tracking strategy, in which the insects compare the olfactory signal on one antenna with the olfactory signal on the other (Bell and Tobin, 1981; 1982). Individuals with only one antenna are thought to switch to using a temporal tracking strategy since comparisons between the antennae are no longer possible (Bell and Tobin, 1981; 1982). In this case, steering is thought to depend on changes in the olfactory input detected by the intact antenna being compared across two or more consecutive time points. Locating an odor source with only one antenna is likely to be a requirement of *P. americana* outside of laboratory experiments. It is not uncommon to find adult *P. americana* in our laboratory colony with all or part of an antenna missing, and it should not be surprising to find cockroaches in their natural, crowded, habitats suffering similar damage (Guthrie and Tindall, 1968; Roth and Willis, 1960). Until the study presented here, the impact of partial antenna loss on odor-guided navigation strategies has not been addressed systematically.

In principle, the long antennae of *P. americana* gives the animal the ability to obtain a significant amount of spatial information about its odor environment. This holds true even if one of the antennae is missing, so long as the system is able to discriminate zones of sensation along an antenna. Previous work by Hösl (1990) has shown that the firing frequency of a subset of interneurons in the processing center for female pheromone in the antennal lobe of *P. americana* males, the macroglomerular complex (MGC), is dependent on the location of pheromone stimulation along the antenna. This suggests the presence of an antenna-topic olfactory map of each antenna in the brain. The MGC is a specialized collection of glomeruli in the antennal lobe where all sex-pheromone sensitive olfactory neurons from the antenna converge (Ernst & Boeckh, 1983; Watanabe *et al.,* 2010; Watanabe *et al.,* 2011), and is the first possible place where intra-antenna comparisons or integrating can occur. If an antenna-topic map is utilized, information on the spatial distribution of odor and subsequent steering and ultimately the tracking performance should be influenced by the size and spatial resolution of the available map.

To better understand whether *P. americana* males use spatially sampled odor information from across their antennae to track a wind-borne odor, we challenged animals with both bilaterally symmetric and unilaterally ablated antenna of different lengths (Figure 1) to track a plume of female sex attractant pheromone in a wind tunnel. If *P. americana* use bilateral comparisons between their antennae to steer upwind in an odor plume then unilaterally ablated individuals should be unable to track the odor or show predictable behavioral deficits such as looping toward the intact antenna (Hangartner, 1967). Whereas individuals with bilateral input should be able to track the plume, regardless of antennae length as long as they can still detect the odor. We found that unilaterally ablated individuals were able to track odor nearly as well as their bilateral counterparts with the same total antennal length. This shows that they can track a plume when bilateral comparisons are unavailable to them. However, decreasing the length of the antennae also affected the plume tracking ability of cockroaches, with individuals with shorter antennae being less successful at locating the odor source. While the role of temporal information is still not clear, these results show that bilateral comparisons are not a requirement for odor plume tracking in *P. americana*. Further, these results show that, for male *P. americana*, the total amount of olfactory epithelium providing odor information is predictive of successful orientation to odor plumes, regardless of whether that epithelium is on one antenna or two. The fact that cockroaches with only one antenna perform as well as those with two totaling the same length suggests that they either switch from using spatial to temporal comparisons when they lose an antenna, or that spatial information continues to be available to them through a single antenna.

Results:

Successful plume tracking continues with only one antenna.

We first determined the percentage of animals in each group that were able to complete the odor tracking task and navigate to the odor source. Out of 20 individuals in each treatment group, at least one tracked the odor plume to its source except for the group with only 10 annuli remaining on the left antenna. The animals with longer antennae had higher success rates (up to 100%) than those with shorter antennae (as low as 5%) (Fig. 1). The percent of each treatment group tracking the plume to the source were significantly different according to Fisher's exact test (Fig. 1). The 10 annuli treatment length was excluded from further statistical analysis due to low success rates yielding such a small sample size. Next, we observed the behavior of the cockroaches as they navigated towards the odor source. The trajectories of our treatment groups showed considerable variability (Fig. 2), consistent with results from other plume-tracking experiments in cockroaches (Willis and Avondet, 2005; Willis *et al.,* 2008). Each group had animals with a high linearity score that walked up the plume to the source with few (if any) turns or excursions out of the plume (Fig. 2 – top row) as well as animals with low linearity scores that made many turns or excursions out of the plume (Fig. 2 – bottom row). Visual inspection of the tracks revealed no obvious trends in turning direction or exits from the plume for any group so we could not visually discriminate between the treatment groups based on their tracks. Furthermore, loss of left or right antenna did not obviously bias subsequent behavior in the direction of the intact or removed antenna (see below).

No effect of left vs right antenna loss was found, but 2 antennae vs 1 antenna of the same length showed considerable differences in behavior.

A detailed computer analysis of the tracks was performed using custom Matlab scripts to calculate 16 different track parameters and Bayesian Estimation was used to compare groups in R (see **Methods** section and Table 1 for track parameter definitions and Table 2 for grand means $\pm$ S.D.). These results were further corroborated with hierarchical Bayesian models and one-way and two-way analyses of variance (see **Methods** and the **Supplement**). Within each antenna length grouping (i.e., 4 cm, 2 cm, 1 cm), the performance of individuals with only one antenna (either left or right) was statistically identical (Fig. 3). Further, a one-way ANOVA showed 6 of the 16 track parameters to be different between all one and two antennae individuals, regardless of length (Table S6). Individuals with one antenna were pooled into groups by length and compared to animals with two antennae of that same length (e.g., one vs two 4cm long antennae) (Fig. 4). This comparison reveals that in the 2 cm and full-length (4 cm) groups the animals with only one antenna performed

differently that those with two antennae on most parameters, the main exception being average body angle and its derivatives. A one-way ANOVA & post-hoc tests likewise showed the 4 cm and 2 cm to vary on 10 of the track parameters, regardless of the number of antennae (Table S5). The 1 cm group had fewer differences in track parameters between one and two antennae individuals. This may be due to the low number of tracks and the higher variability in the behavior of animals with shorter antennae.

Animals with longer antennae performed better.

Animals with longer antennae had a higher success rate at finding the source than animals with short antennae, regardless of antennae group (left, right, or bilaterally symmetric) (Fig. 1). Groups with longer antennae found the source faster than those with shorter antennae (Figs. 5a, and 6). This is due in large part to animals with longer antennae having narrower tracks, (Fig. 5b), and making fewer turns, backtracks, or stops (Fig. 6, Table 2, Table S3). They walked more directly to the source (Fig. 5D).

Animals with the same total length of antennae were broadly similar in their behavior.

We found few differences in performance between animals with the same total amount of olfactory epithelium, regardless of where that epithelium was located (e.g., two 2 cm antennae & one 4cm antenna both have a total of 4 cm of antenna) (Fig. 7). The only difference in individuals with 4 cms of total antenna is those with bilateral input had a shorter inter-turn duration, made fewer stops and correspondingly spent less total time stopped. The same was true of individuals with 2 cm total antenna with the addition that those with bilateral input also walked faster. The only significant interaction between number of antennae and antenna length revealed by the analysis of variance was in the number of stops (see supplemental Fig. S1). This was consistent with results from the Bayesian hierarchical model. No other interactions between antennal length and bilateral symmetry were found.

Discussion:

One antenna is sufficient for odor tracking.

Our results clearly demonstrate that *P. americana* males can track a wind-borne odor with only one antenna, and thus bilateral input is not necessary for successful odor tracking when directional information is provided by the wind. By this observation alone, we can reject bilateral chemo-comparisons as the sole means of odor-guided navigation in *P. americana*. They do not need to make a comparison between their two antennae, which is consistent with the previous work by Bell

and Tobin (1981) done in a zero wind environment. Our observation that there are no appreciable differences in the behaviors performed by left and right antennectomized animals is likewise consistent with Bell and Tobin (1981). While intact animals could make bilateral comparisons, they are not a requirement to successfully track a wind-borne odor plume. Unilaterally antennectomized *P. americana* do not loop toward their intact antenna like the ant *L. fuliginosus* (Hangartner 1967). Looping toward the intact sensor is an obvious sign that an organism is comparing one senor to the other across the midline of the body (Shöne, 1984). The cockroaches also made similar numbers of leftward and rightward turns, regardless of which antenna had been removed (Table 2 and Fig. 3)— a further indication of lack of directional bias.

Total antenna length may determine spatial information for odor tracking.

We present evidence that antennal length makes a significant contribution to tracking ability in *P. americana*. The tracking behavior of animals with only a left or right antenna was remarkably similar, both to each other, and as a group to individuals with bilaterally symmetric antennae of the same total length (e.g., one 4cm vs two 2cm antenna). These results suggest that the total length of the antennal epithelium defines the size of the cockroach's window on the olfactory world. Restricting the size of the window reduces its ability to track odors rapidly and effectively. Cockroaches with less antennal surface spend significantly more time tracking the plume (Fig. 5A), stop more frequently and spend more time stopped (Fig. 7). It is particularly telling that roaches with shorter antennae generate wider overall tracks, suggesting that a smaller olfactory "window" must be moved through a larger space to inform appropriate steering maneuvers. There are at least two other ways to achieve the odor information necessary to track a plume, the first could be to move each antenna through space using their antennal muscles. While previous studies have observed intact *P. americana* males to hold their antennae in a stereotyped posture during plume tracking (Willis and Avondet, 2005), it may be that animals with missing antennae don't hold a stereotyped posture, but resolution of our video recordings precluded these measurements. Ongoing studies will address the question of antennal scanning movements in animals with different antennal lengths. A second possible way *P. americana* could be informing steering maneuvers is through a temporal tracking strategy such as transverse klinotaxis (see below).

Potential use of olfactory spatial maps.

Previous work by Hösl (1990) demonstrated that some projection neurons in the MGC of *P. americana* describe overlapping, spatially distinct receptive fields on the antenna that could form an antenna-topic map of odor space in the brain. Given our observations, if *P. americana* continue to

use instantaneous spatial information for olfactory tracking after the complete loss of one antenna, it must be through comparing between zones or integrating across zones along the length of the remaining antenna. An antenna-topic map as suggested by Hösl's (1990) study provides a possible neural mechanism to support this behavior. If the animal is able to compare olfactory inputs across such a map, it could still make spatial comparisons similar to those following the classical idea of tropotaxis using bilateral comparisons. However, such a spatial map could be far more flexible than a two-input bilateral comparison system, since much of the antenna could be lost, yet still provide enough information for chemotaxis. At a bare minimum, the animal would need enough antennae remaining for there to be two distinct receptive fields to compare across.

Alternatively, *P. americana* could be integrating across the map (Equation 1, Fig. 8), using an additive, rather than comparative olfactory tracking strategy. This integration could be over two maps, one for each antenna as described by Hösl (1990), or one continuous map stretching across both antennae made from the maps present in each MGC. Such an additive model has been shown to enhance directional olfactory performance in fruit fly larvae (Louis *et al.* 2008), rats (Khan *et al.* 2012; Rajan *et al.* 2006), and humans (Porter *et al.,* 2005; Porter *et al.,* 2007). In each of these studies, one sensor was sufficient to complete the olfactory task, and the availability of a second (bilaterally symmetric) sensor decreased the amount of wandering the animal did. If *P. americana* can integrate odor information detected across the span of one or both antennae and keep track of where on the antennal map the odor is detected, it could use the change in the pattern of activation to orient within the plume. Detecting changes in the encountered odor distribution would require a short-term memory to be generated as odor tracking progresses. For this to be successful a temporal component would be required.

Potential for a switch to temporal comparisons

An alternative hypothesis to using a spatial map could be that *P. americana* use klinotaxis (temporal tracking) upon loss of bilateral odor inputs. For *P. americana* to track an odor using a temporal tracking strategy, they would need to store information about the location of odor encounters and the displacement of their odor sensors through the environment, either by moving their antennae relative to their head or by walking or flying. Whole body displacement information could be gained through a visual (optomotor) response (Marsh *et al.* 1978) or through a proprioceptive pedometer (e.g., step counting) (Buehlmann *et al.,* 2012; Collett and Collett, 2000; Wittlinger *et al.,* 2006). Previous work by our lab has demonstrated that *P. americana* successfully track an odor plume with their eyes (both compound eyes and ocelli) covered with black paint

(Willis *et al.,* 2011), suggesting that a visually-guided temporal search strategy is not being used by these cockroaches. Antennal movement information could be gained through processes like proprioceptive feedback or efference copies. Future investigations into potential evidence for temporal tracking in *P. americana* will begin with the search for their ability to calculate displacement via a pedometer. The antennal movement hypothesis seems unlikely, as previous work has shown that intact *P. americana* hold their antennae in a stereotyped posture and move them very little (Willis and Avondet, 2005), but could be tested in the various antennae manipulation groups by tracking antennae movement through a known odor distribution. The experiments presented here were done with freely behaving animals with no control over exactly when and where the stimulus was encountered by the antennae. Future experiments will determine the contribution of any potential spatial map by experimentally applying odor to specific points on the antennae and measuring steering responses.

Proposed model

Prior to the publication of Hösl's work, Bell and Tobin (1981) suggested that *P. americana* with an antenna removed must use a temporal tracking strategy, and went on to further suggest that cockroaches normally may use a combination of both instantaneous spatial comparisons (tropotaxis) and temporal comparisons (longitudinal klinotaxis) (Bell and Tobin 1982). Briefly, they proposed a flow chart in which instantaneous left-right comparisons of the gradient were used to steer left or right (towards whichever concentration was higher), and that temporal comparisons were made along their track to determine if the heading should be maintained (i.e., concentration is increasing), or to make an abrupt sharp turn (i.e., concentration is decreasing) (Bell and Tobin, 1982). It should be noted that this flow chart assumed zero wind conditions with a detectable concentration gradient.

In light of our results and in consideration of Hösl's (1990), we propose a new flow chart featuring integration across the antenna olfactory receptor array (Fig. 8). Specifically, the animals could use changes in the pattern of excitation across the antenna to inform turning (i.e., which odor receptive fields on the antenna are receiving odor vs. which are not). One way to summarize this pattern of excitation would be to calculate a central "balance point" along the sensory array. If this balance point moves to the left along the antennae, the animal should adjust their course to the left, and likewise to the right (see inserted graphs in Fig. 8). This can be modeled mathematically by Equation 1, where c is the "center of olfaction," n_a is the number unique olfactory receptive fields on the antennae, $\rho(a)$ is the density of sensory input across the receptive fields, r is a binary (0/1)

input indicating whether a given zone detects odor.

$$c = \frac{1}{n_a} \int_{ant} \rho(a) r \; da$$ (Equation 1)

This is analogous to the center of mass for a one-dimensional system, only instead of applying weights along a line, we are interested in odor detection along the array of sensors. The turns we observe would be due to large course corrections (if c made a large shift along the antennae), or if odor was lost and searching behavior was initiated. Such a tracking strategy could be particularly effective at tracking the high-contrast edge encountered at the plume's lateral margins. The transition from odor plume to clean air at the lateral edge typically occurs within ca. 1cm (Willis et al., 2013, plume measurements in this study – please see **Methods** below). In support of this idea, it is noteworthy that the narrowest tracks generated by *P. americana* males are in response to the narrowest pheromone plumes (Willis and Avondet, 2005).

Our integration model is similar to the proposed behavioral tracking strategy in the crab *C. sapidus* (Page *et al*., 2011b), but with key differences. Page *et al*. (2011b), describe the crabs as detecting the distribution of odor concentrations, calculating a center-of-mass (COM), and steering towards that center of the distribution. Odor concentration is not directly measured by *P. americana* olfactory sensilla, but rather threshold changes in concentration (Tichy et al., 2005). This is the rational for treating r as binary and merely measuring the distribution of odor in space, invariant of concentration (either it is above the necessary threshold or not). Moths flying and crabs walking upcurrent while tracking odor plumes both reveal that the temporal structure of the plume (i.e., odor onset, offset and interval duration) and changes in that structure have a greater effect on the tracker's behavior than the odor concentration in the plume (Mafra-Neto and Cardé, 1995; Page et al. 2001a). Odor concentration is trumped by encounter rate in predicting *C. sapidus* odor tracking velocity (Page *et al.* 2011a). Further, the balance point c is only a summary statistic of the pattern of activation along the antennae. There are other statistics a nervous system could encode to yield a similar result, but we cannot say without proper neurophysiological recordings what is encoded by the nervous system. By focusing on changes in the pattern of activation, the integration model would expect animals to maneuver through the plume in no particular position. They could follow the edge of the plume or walk up the center—all of which have been observed in plume tracking *P. americana* (Willis and Avondet, 2005), including the present study (Fig. 2). Whereas in the COM tracking strategy described by Page *et al.,* the animals' steering tends towards the center of the

plume. If integration happens continuously across both antennae, it would have further explanatory power in that it accounts for the observed similarity in behavior of animals with antennae of the same total length (Fig. 5 A & B, Fig. 7). Loss of receptive fields would decrease the accuracy of c in describing the plume, and presumably decrease the effectiveness of the animal's tracking behavior. This is consistent with our observation that cockroaches with shortened antennae generate wider walking tracks, perhaps increasing their lateral movements to move their remaining antennal receptive fields across more of the environment.

This model is consistent with our observations and the observations of others and supports the idea that it does not matter if you have one big antenna or two small antennae: it is the total amount of available olfactory epithelium that is important in the odor tracking performance of *P. americana*.

Methods:

Animals were taken from the institutional colony.

Adult, male *Periplaneta americana* L. were removed from our lab colony and held for one week to increase their sensitivity to female sex attractant pheromone. They were then placed in an environmental chamber on a 12:12 light:dark cycle for a minimum of two days before experimentation.

Animals were divided into twelve treatment groups and challenged to track an odor plume.

For each experimental day, 24 animals were divided into three groups one hour before the onset of the dark phase (scotophase): *left antenna* (right antenna removed)*, right antenna* (left antenna removed), and *bilateral* (two bilaterally symmetric antennae). Each group was further subdivided into four groups by antenna length: *4 cm* (full length)*, 2 cm, 1 cm*, and *10 annuli* (ca. 0.5 cm). These twelve treatments had two individuals included on each experimental day. Complete antennectomies were performed by cutting the antenna between the pedicel and the first annulus. This removes all olfactory sensilla while preserving the mechanoreceptive Johnston's organ. The full length group had intact antennae with no apparent missing segments. The remaining groups were cut to the appropriate length with micro scissors. 10 annuli was chosen as the shortest length because previous work suggested that a minimum of 8-14 segments are needed for *P. americana* to track the female sex pheromone (J. K. Lockey, unpublished). Cutting just above the tenth annuli should result in a more consistent number of olfactory sensilla being included than using a total length of 0.5 cm. Counting annuli in the treatments with longer antennae was not possible within a reasonable time.

At the scheduled onset of scotophase, the animals were placed under red and infrared lighting (i.e., wavelengths thought to be undetectable by the eyes of *P. americana*). Behavioral recordings were conducted from two to four hours post-sunset, centering the experiments on male *P. americana's* peak behavioral response time to female sex attractant pheromone (Zhukovskaya, 1995). The testing arena consisted of a raised aluminum platform (91 x 152 cm) in a laminar flow wind tunnel (25 cm/s). 0.1 ng of periplanone B (Kitahara *et al*., 1987; Kuwahara and Mori, 1990) was placed on a filter paper disk (diameter 1 cm) held 2 cm above the platform and centered at the upwind end. The filter paper was oriented perpendicular to the direction of flow, yielding a plume 14.2 ± 2.3 cm wide (mean ± S.D.) at the downwind end of the platform. Experiments began and ended with intact control (full-length antennae) trials to verify that the odor source was viable and the cockroaches were still responding. All other trials were done in random order and the treatment was not known to the experimenter. Trials began by placing the animal in its release cage centered at the downwind end of the platform. After 30 seconds of acclimation time, the individual was released from its cage to track the plume.

Pheromone plume tracking behavior was recorded by infrared camera and digitized.
Behavior was recorded on an infrared camera at 30 Hz. The video was sub-sampled at 15 Hz because the animals moved slowly enough to yield sufficiently high resolution of the behavior for our analysis. The video was digitized in Matlab (Mathworks, Natick MA) with the DTLdv5 application (Hedrick, 2008). Track parameters were calculated using a custom Matlab script (modified from Rutkowski *et. al.,* 2009). All trials where the animal tracked the odor plume to the source were digitized, save for one full-length, left antenna individual because the video file was corrupted. The track parameters we measured are described in Table 1.

Electroantennograms establish time averaged plume area.
The time-averaged plume boundary in the track figures (Fig. 2) was determined using an eletroantennogram preparation, similar to previous work in our lab (Willis *et al.,* 2013, Talley, 2010). Because *P. americana* antennae are too slender to accept a fine silver wire into their lumen, we placed them between saline-filled capillaries, connected to silver-wire electrodes. The preparation was then moved across the wind tunnel in 1 cm steps and a 30sec sample of the response was recorded. The boundary coordinates of five separate antennae were averaged to give the mean width of 14.2 ± 2.3 cm (mean ± S.D.), and the plume envelope displayed in the figures (Fig. 2).

Bayesian estimation reveals treatment effects in behavioral patterns.

Statistics were performed in R (R Core Team, 2014) using the BEST package (Kruschke and Meredith, 2014). The BESTmcmc function was used to compare groups. This function uses Bayesian estimation to compare two groups (analogous to Student's T-test (Gosset, 1908)). The results were corroborated with a hierarchical Bayesian model with two nominal predictors (antenna length and ablation) and a metric predicted variable (a track parameter), this is analogous to an analysis of variance with a post-hoc test (Kruschke, 2014). Bayesian approaches are used because they are less sensitive to outliers, allow us to accept the null hypothesis (that two groups are Practically Equivalent), are robust in estimating effect size, and are generally more conservative than traditional Frequentest methods (Kruschke, 2013; 2014). The reported statistic in Figures 3, 4, 6, and 7 (the columns of dots next to track parameters) are a graphical representation of the 95% credible interval (Highest Density Intervals) on the difference between means with a Bonferroni adjustment for 8 groups (Gelman *et al.*, 2012). If this HDI excluded zero, it is analogous to significant at the 0.05 level (Kruschke, 2014), this is represented by a dot to the left or right of the zero line. The placement of the dot is determined by the difference between means: a dot to the right indicates that the first group has a greater estimated value for the given track parameter than the second, and vice versa to the left. The groups are indicated at the top of each column. Conversely, if the credible intervals contain zero (the dot straddles the zero line), the groups are Practically Equivalent (i.e., we can accept the null hypothesis (Kruschke, 2013; 2014)). The Highest Density Intervals used to generate the tables are supplied in the supplemental tables (Tables S1-S4), as well as the corroborating statistics from the hierarchical model and the ANOVA (Table S5 and S6).

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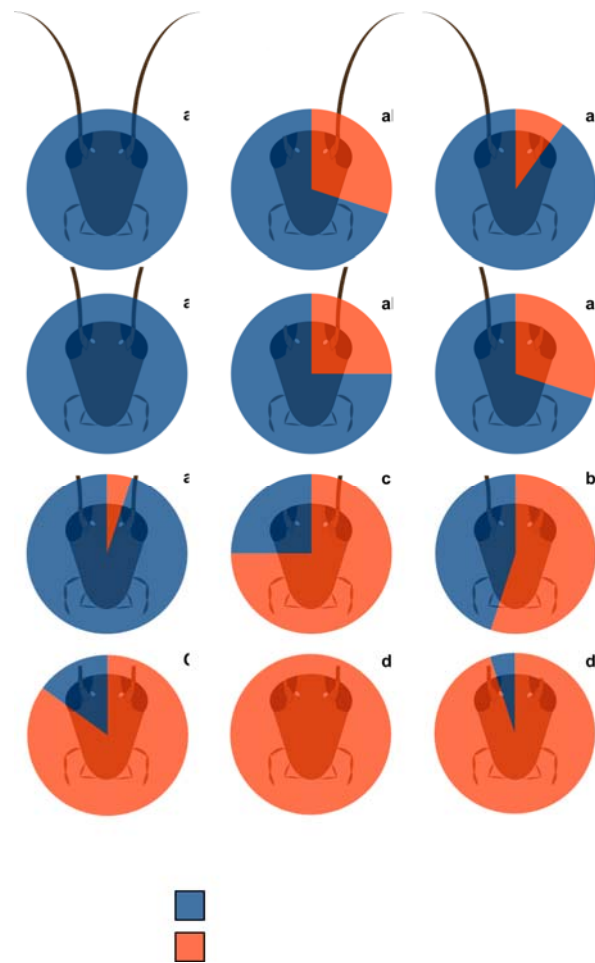
Figures:

Figure 1
Rates of success at locating the pheromone source of the different experimental treatment groups. The left column shows a diagram of *P. americana* with both antennae (bilateral input), the middle column with its right antenna removed (left antenna remaining), and the right column with its left antenna removed (right antenna remaining). The first row represents animals with intact 4 cm antennae and antennae length decreases from top to bottom (2 cm, 1 cm, 10 annuli). Success-rates are presented in the superimposed pie charts; blue indicates success, red indicates failure. A Fisher's exact test for all groups was highly significant ($p=1.0\cdot10^{-4}$). Sub-sampling the contingency table to compare each group shows that in general, animals with the longest antennae have higher success rates than those with the shortest antennae. Groups with the same letter are similar after a Bonferroni correction for 12 groups ($p<0.00417$).
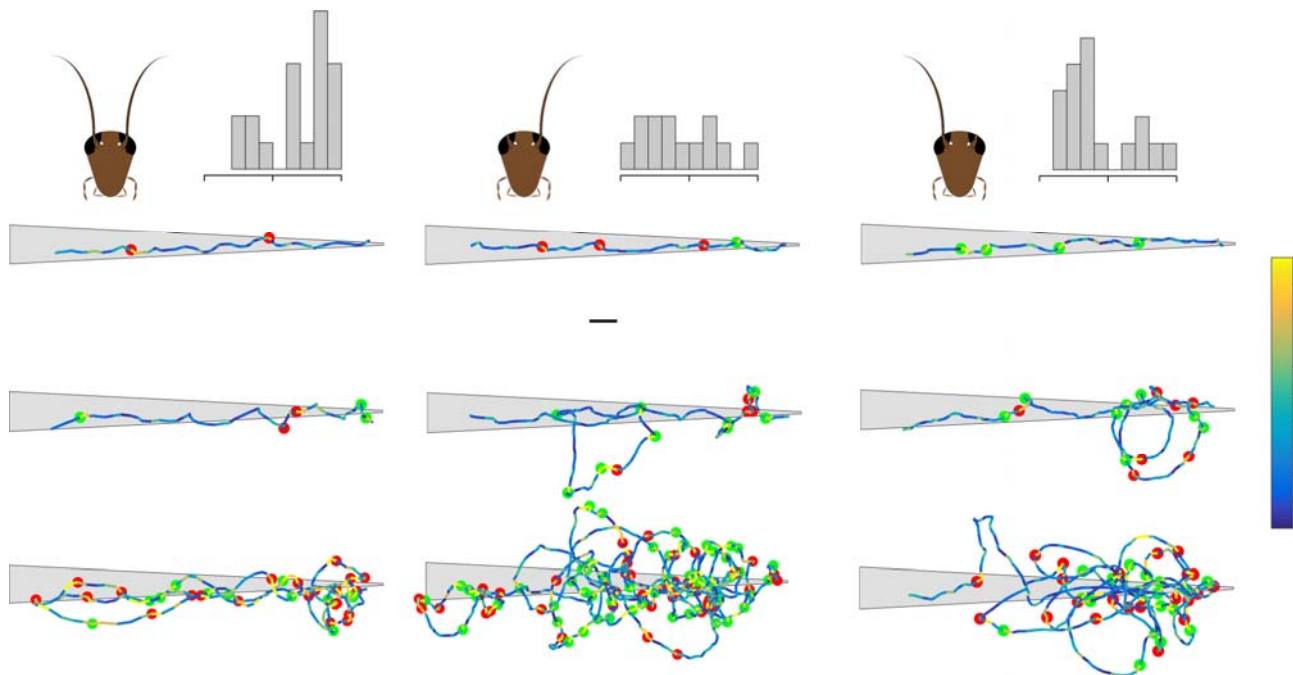
Figure 2
Cockroaches with full-length antennae displayed a range of tracking behaviors. Tracks for animals with two antennae (left column), a left antenna (middle column) and a right antenna (right column) are shown. In each column, the top trace represents the track with the highest linearity score (the straightest track), in the middle is a track with the median linearity score for that treatment, and on the bottom is the track with the lowest linearity score. The distribution of linearity scores for the treatment groups are given in the inset histograms. The time averaged plume boundaries (as measured by electroantennograms) are represented by a light gray triangle. The trajectory follows the cockroach's head position and the color is scaled to the animal's angular velocity. Turns were defined as points where the angular velocity was either >360 °s^{-2} or <-360 °s^{-2}. Left and right turns are denoted by red and green circles, respectfully. Scale bar represents 10 cm. Wind blows from right to left at 25 cm s^{-1}.
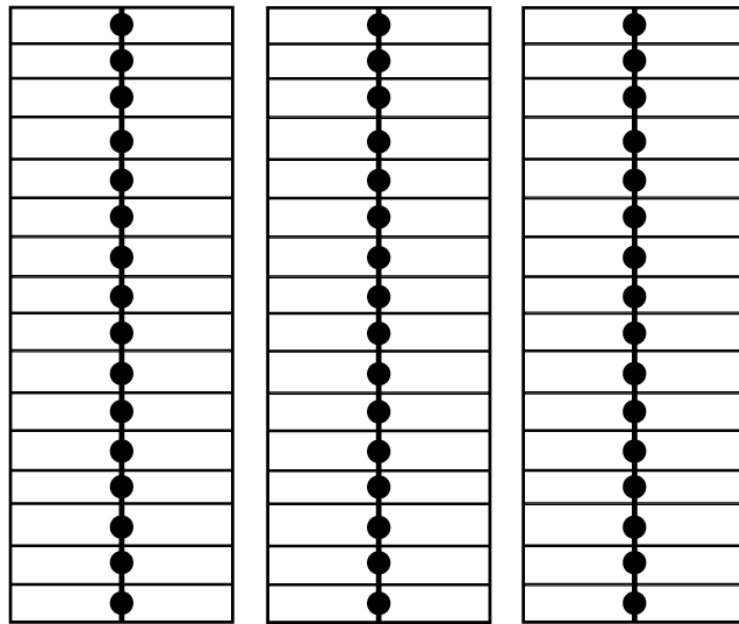
Figure 3
Left and right antennectomized animals behaved the same. This figure presents credibility intervals on the difference in means between animals with only a left or right antenna. A dot to the left of the 0 line indicates that the credibility interval is to the left of zero (negative), a dot on the 0 line indicates that the credibility interval includes zero (the groups are Practically Equivalent), and a dot to the right of the 0 line indicates that the credibility interval is to the right of zero (positive). A negative credibility interval indicates that first group in the comparison is smaller than the second group whereas a positive credibility interval indicates the first group is larger than the second. In this case, we compared left to right, and all groups are equal for all track parameters. (See Table S1 for the HDIs used to compile this figure.)
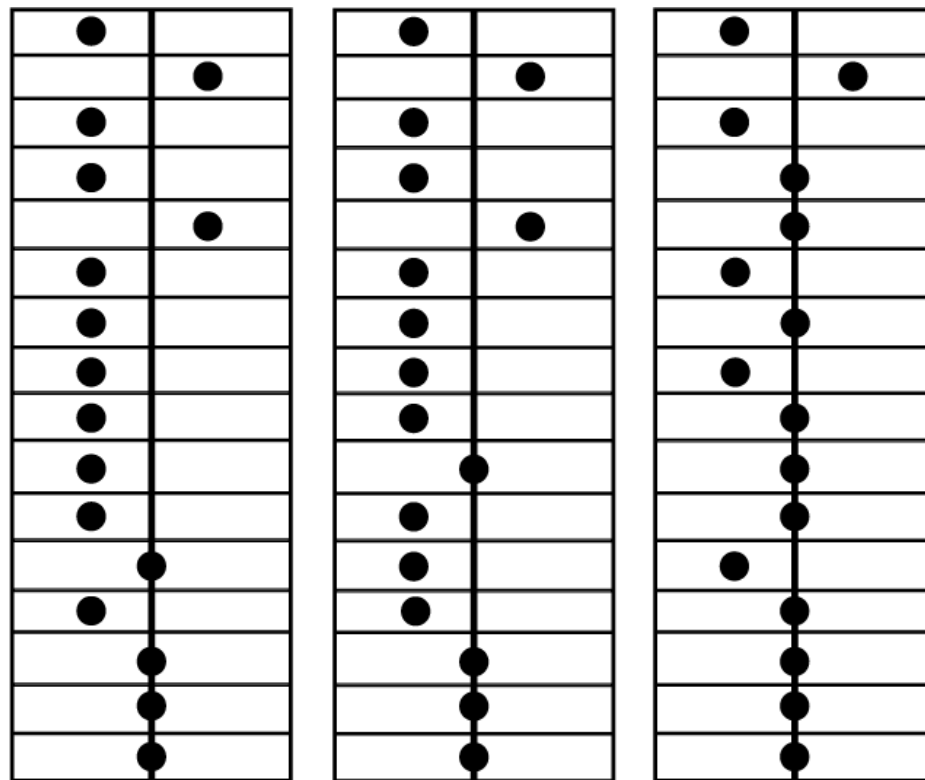
Figure 4

Animals with one antenna behaved differently from animals with two antennae. This figure presents the credibility intervals on the difference in means between bilaterally symmetric animals (B) and antennectomized animals (A). In this case, we compared bilateral to antennectomized animals. The first row of the first column should be interpreted as "individuals with bilateral input took less time to walk to the source than antennectomized individuals." (See legend on figure 3 for a more complete explanation of interpretation, and Table S2 for the HDIs used to compile this figure.). Means (±S.D.) of these values can be found in Table 2.
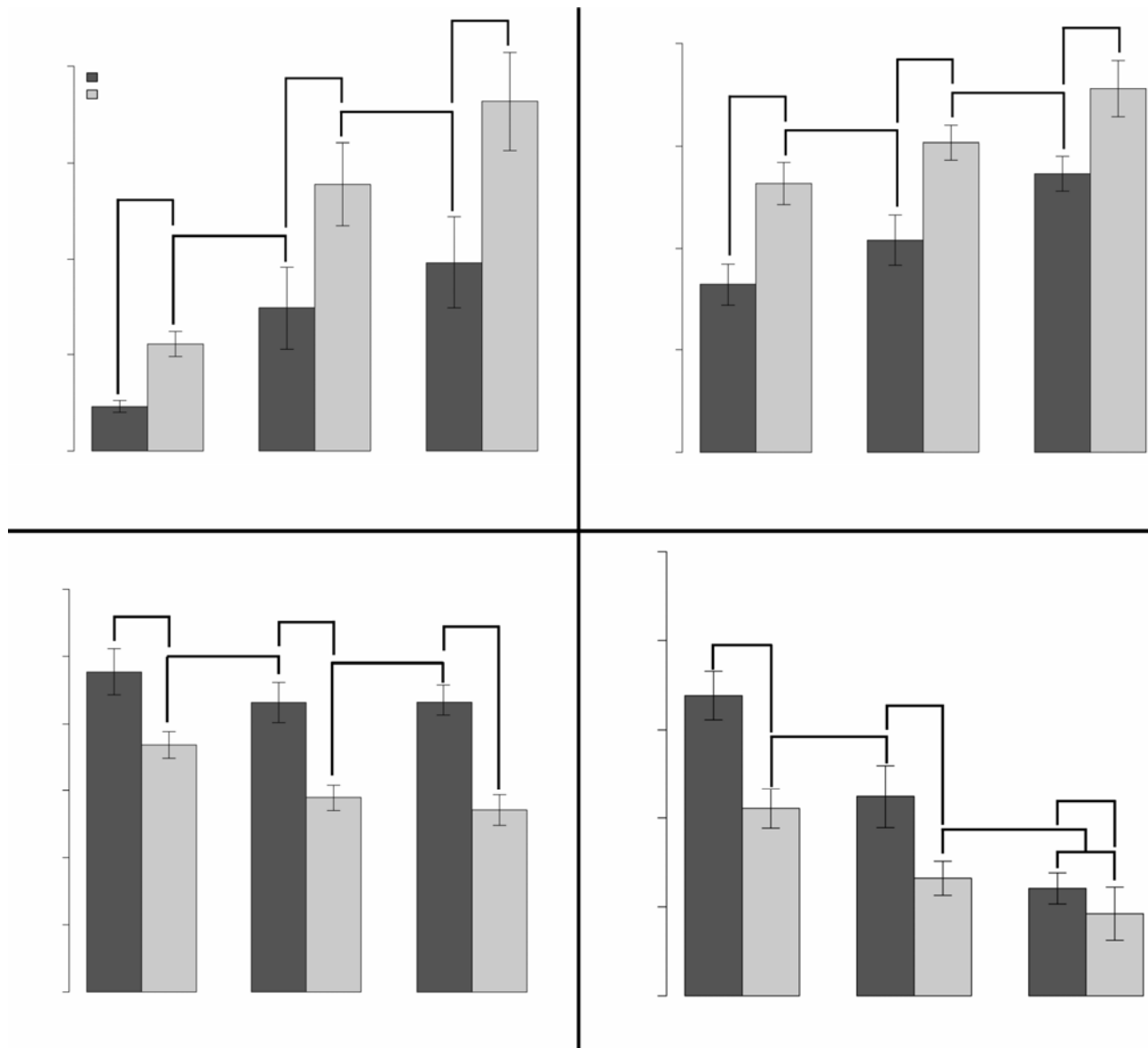
Figure 5
Most track parameters vary with antennal length, yet animals with equal amounts of total antenna are indistinguishable in their behavior. For example, tracking time was shorter (A), track width was narrower (B), walking speed was faster (C), and tracks were straighter (D) in all bilateral individuals with two antennae than those with one antenna of the same length (paired bars)—except for 1 cm individuals on linearity (D). Animals with the same total antenna (4 cm antennectomy and 2 cm bilateral, or 2 cm antennectomy and 1 cm bilateral) had statistically identical tracking times (A), track widths (B), Speeds (C), and linearity (D)—except for total 2 cm individuals on speed (C). Bayesian estimation for 2 groups (BEST package in R) was used to measure effect size and differences between means, * denotes groups with no overlap on the 95% credibility interval on the means, indicating that the groups are significantly different. Likewise, N.S. denotes groups that have overlapping credibility intervals, indicating that the groups are Practically Equivalent. For comparisons on all track parameters, see Figures 4, 6, & 7.
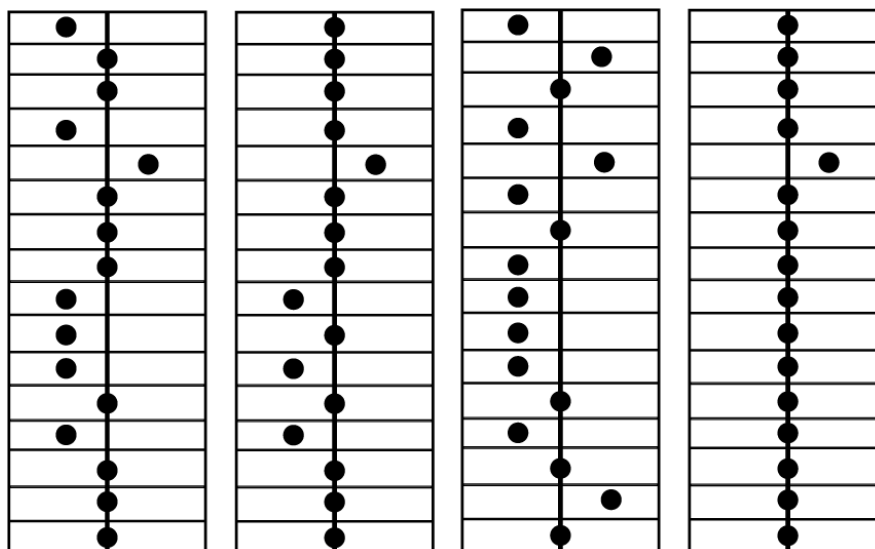
Figure 6
Bilateral or antennectomized animals with different length antennae behaved differently. This figure presents credibility intervals on the difference in means between animals with different length antenna (4 cm vs 2 cm, 2 cm vs 1 cm) within treatment groups (bilateral & antennectomy). (See legend on figure 3 for a more complete explanation of interpretation, .and Table S3 for the HDIs used to compile this figure.)

Figure 7
Animals with the same total antennae length behaved broadly similar. This figure presents
credibility intervals on the difference in means between animals with the same total length antenna
(2 cm bilateral vs 4 cm antennectomy, 1 cm bilateral vs 2 cm antennectomy). (See legend on figure
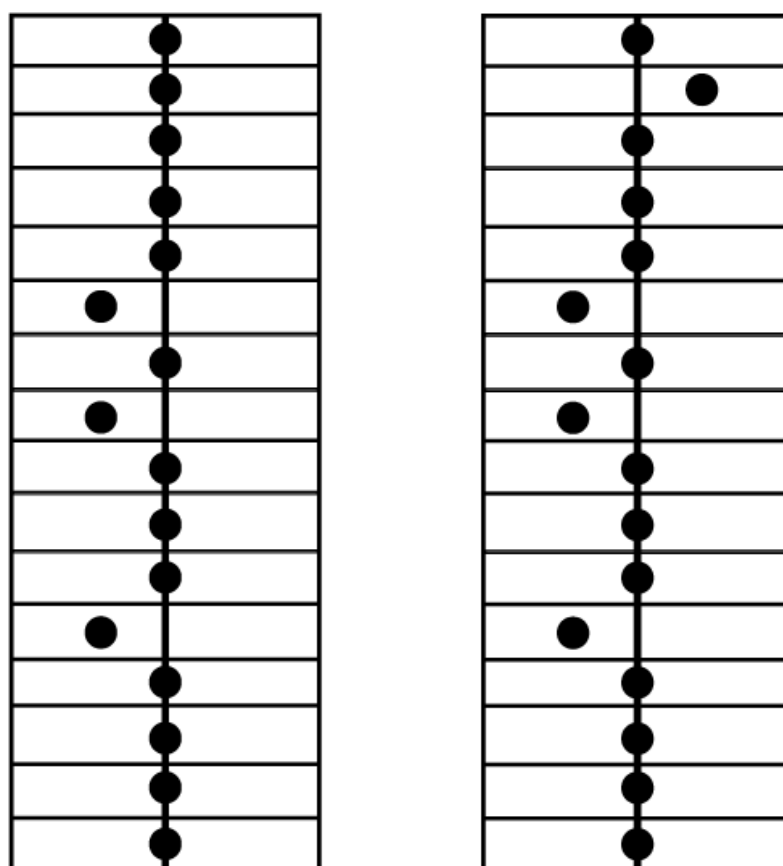3 for a more complete explanation of interpretation, and Table S4 for the HDIs used to compile this
figure.)

Figure 8
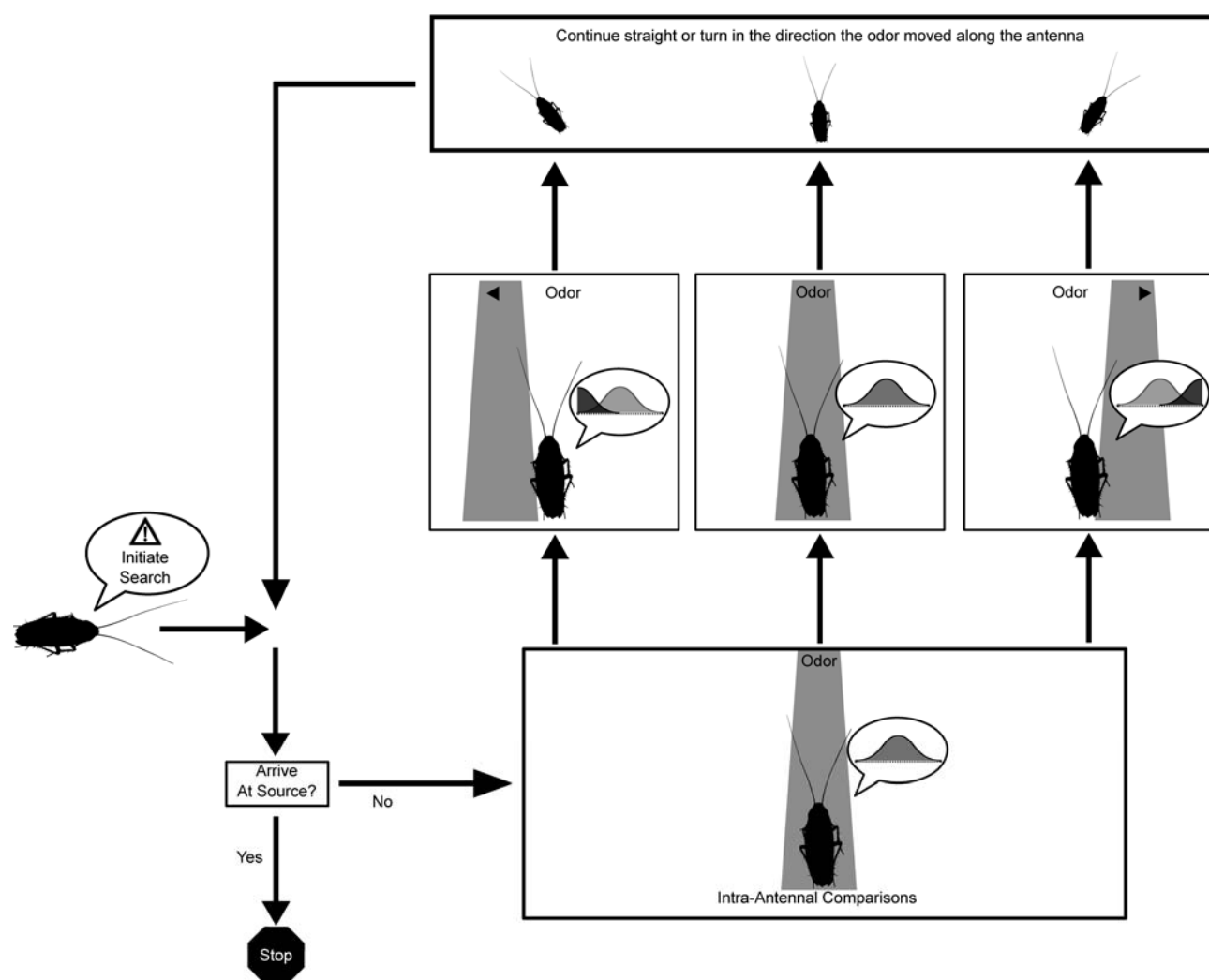**Proposed flow chart of odor tracking behavior in *P. americana*.** When the animal is tracking an odor, turning is dependent on changes in the center of stimulus. If the odor moves to the right along the antenna, the animal should turn to the right, whereas if the odor moves to the left, the animal should turn to the left. The inset distributions show possible activation patterns across the antenno-topic map.

Table 1 – Definitions of measured tracking parameters.

Term	Definition
Time to source	The time (sec) from leaving the release cage to finding the source.
Walking speed	The mean ground speed (distance/time).
Walking distance	The total distance traveled by the animal.
Linearity	The walking distance ÷ the straight line distance from the release point to the source.
No. Stops	No. of times the animal moved less than 0.2 cm in > 0.133s
Stop duration	The mean time spent during each stop.
Total stop time	Sum of all time spent not moving.
No. backtracks	No. of down excursions > 8 cm (two body-lengths).
No. left turns	No. of spikes in the angular acceleration $< 360\ °/s^2$.
No. right turns	No. of spikes in the angular acceleration $> 360\ °/s^2$.
Inter-turn duration	The mean time between turns.
Magnitude body angle	The mean absolute value of the body angle.
Body angle	The mean angle between the head-thorax axis of the animal and the central axis of the plume.
Angular velocity	The mean change in body angle.
Angular acceleration	The mean change in angular velocity.

Table 2 – Grand means & standard deviations of track parameters (with number of individuals in each group).

	Bilateral			
	Full length (20)	2 cm (20)	1 cm (19)	10 annuli (3)
Time to source (s)	9.24 ± 5.26	29.7 ± 38.6	39.3 ± 41.7	69.9 ± 14.9
Walking speed (cm/s)	23.9 ± 7.59	21.6 ± 6.68	21.6 ± 4.87	22.9 ± 10.5
Track width (cm)	3.28 ± 1.84	4.17 ± 2.19	5.46 ± 1.48	6.63 ± 2.87
Walking distance (cm)	205 ± 123	737 ± 1140	862 ± 963	1500. ± 380.
Linearity	0.68 ± 0.24	0.45 ± 0.32	0.24 ± 0.15	0.08 ± 0.023
No. stops	4.65 ± 3.82	5.45 ± 4.70	10.6 ± 14.5	15.7 ± 11.9
Stop duration	0.11 ± 0.15	0.11 ± 0.084	0.13 ± 0.095	0.10 ± 0.060
Total stop time	0.57 ± 0.59	0.87 ± 1.45	2.14 ± 3.83	1.98 ± 1.68
No. backtracks	1.15 ± 1.69	9.25 ± 16.4	12.4 ± 12.7	23.3 ± 4.51
No. left turns	4.65 ± 5.43	24.2 ± 38.8	26.2 ± 27.9	39.7 ± 2.52
No. right turns	4.40 ± 3.80	21.5 ± 34.0	28.6 ± 34.0	43.7 ± 21.2
Inter-turn duration	1.43 ± 1.23	0.85 ± 0.53	0.78 ± 0.23	0.87 ± 0.35
Magnitude body angle	31.4 ± 20.7	49.4 ± 27.7	66.0 ± 15.3	78.4 ± 5.07
Body angle	3.10 ± 11.6	-0.54 ± 8.11	-2.77 ± 9.12	-0.20 ± 7.88
Angular velocity	6.54 ± 22.8	-0.42 ± 17.6	-0.62 ± 23.2	6.57 ± 42.9
Angular acceleration	-4.24 ± 9.91	-0.52 ± 7.00	0.60 ± 5.51	-1.00 ± 3.03

	Left			
	Full length (13)	2 cm (15)	1 cm (4)	10 annuli (0)[†]
Time to source (s)	23.0 ± 17.0	53.5 ± 38.0	73.1 ± 73.4	NA
Walking speed (cm/s)	18.6 ± 6.30	14.5 ± 4.85	17.4 ± 4.69	NA
Track width (cm)	5.33 ± 2.36	6.04 ± 1.82	7.50 ± 1.91	NA
Walking distance (cm)	419 ± 355	691 ± 413	1190 ± 396	NA
Linearity	0.42 ± 0.26	0.23 ± 0.16	0.10 ± 0.034	NA
No. stops	9.46 ± 7.64	22.7 ± 21.5	32.5 ± 28.4	NA
Stop duration	0.14 ± 0.14	0.15 ± 0.084	0.17 ± 0.052	NA
Total stop time	2.13 ± 3.16	4.53 ± 5.06	5.85 ± 6.22	NA
No. backtracks	4.92 ± 6.44	11.5 ± 7.74	20.8 ± 5.50	NA
No. left turns	10.6 ± 11.4	17.9 ± 13.0	30.3 ± 7.09	NA
No. right turns	12.6 ± 15.6	20.2 ± 12.3	30.5 ± 4.12	NA
Inter-turn duration	1.32 ± 1.01	1.43 ± 0.74	1.22 ± 0.72	NA
Magnitude body angle	51.1 ± 20.6	66.0 ± 16.4	76.3 ± 6.13	NA
Body angle	1.29 ± 11.7	3.91 ± 10.4	4.52 ± 7.06	NA
Angular velocity	0.68 ± 21.4	-11.6 ± 14.1	1.71 ± 15.86	NA
Angular acceleration	1.32 ± 7.85	0.38 ± 3.68	1.84 ± 2.93	NA

Right

	Full length (18)	2 cm (14)	1 cm (9)	10 annuli (1)[‡]
Time to source (s)	21.6 ± 12.9	57.8 ± 55.1	72.6 ± 38.4	33.9 ± NA
Walking speed (cm/s)	18.3 ± 5.06	14.4 ± 5.86	11.8 ± 2.56	3.5 ± NA
Track width (cm)	5.23 ± 2.31	6.10 ± 1.87	6.95 ± 2.10	19.0 ± NA
Walking distance (cm)	370. ± 219	681 ± 504	903 ± 584	120. ± NA
Linearity	0.42 ± 0.25	0.30 ± 0.25	0.22 ± 0.25	0.94 ± NA
No. stops	10.2 ± 9.58	30.6 ± 24.4	31.6 ± 36.4	9.00± NA
Stop duration	0.16 ± 0.10	0.18 ± 0.091	0.19 ± 0.090	0.11 ± NA
Total stop time	2.10 ± 2.39	7.03 ± 6.88	6.35 ± 8.43	1.00 ± NA
No. backtracks	4.50 ± 4.22	11.1 ± 10.0	18.4 ± 15.3	1.00 ± NA
No. left turns	8.78 ± 6.34	20.9 ± 18.4	22.3 ± 17.1	1.00 ± NA
No. right turns	8.78 ± 5.94	21.9 ± 19.0	21.1 ± 18.3	2.00 ± NA
Inter-turn duration	1.42 ± 0.92	1.71 ± 1.07	3.01 ± 3.37	11.1 ± NA
Magnitude body angle	54.1 ± 26.19	62.7 ± 23.2	69.0 ± 23.5	81.2 ± NA
Body angle	0.16 ± 11.6	1.33 ± 11.5	1.40 ± 3.68	60.3 ± NA
Angular velocity	7.43 ± 16.7	1.37 ± 19.3	0.23 ± 10.8	-4.06 ± NA
Angular acceleration	1.36 ± 5.06	-1.05 ± 4.40	-0.068 ± 2.59	-0.66 ± NA

[†]No Animals in the 10 annuli, left antenna group tracked (See figure 1)
[‡]Only one animal in the 10 annuli right antenna group tracked (See figure 1)