

Characterization of obstacle negotiation behaviors in the cockroach, *Blaberus discoidalis*

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Accepted 4 March 2009

SUMMARY

Within natural environments, animals must be able to respond to a wide range of obstacles in their path. Such responses require sensory information to facilitate appropriate and effective motor behaviors. The objective of this study was to characterize sensors involved in the complex control of obstacle negotiation behaviors in the cockroach *Blaberus discoidalis*. Previous studies suggest that antennae are involved in obstacle detection and negotiation behaviors. During climbing attempts, cockroaches swing their front leg that then either successfully reaches the top of the block or misses. The success of these climbing attempts was dependent on their distance from the obstacle. Cockroaches with shortened antennae were closer to the obstacle prior to climbing than controls, suggesting that distance was related to antennal length. Removing the antennal flagellum resulted in delays in obstacle detection and changes in climbing strategy from targeted limb movements to less directed attempts. A more complex scenario – a shelf that the cockroach could either climb over or tunnel under – allowed us to further examine the role of sensory involvement in path selection. Ultimately, antennae contacting the top of the shelf led to climbing whereas contact on the underside led to tunneling. However, in the light, cockroaches were biased toward tunnelling; a bias which was absent in the dark. Selective covering of visual structures suggested that this context was determined by the ocelli.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/212/10/1463/DC1>

Key words: ethogram, tactile orientation, antennae, ocelli, vision, climb, tunnel, *Blaberus discoidalis*, cockroach, dark, light, ethogram, bilateral antennectomy, short antennae, antennal lesion.

INTRODUCTION

To properly negotiate obstacles in their path, animals typically need to alter their behavior. For instance, an animal that is walking in a straight line and encounters an obstacle may respond to it by initiating any of a number of behaviors, such as climbing, tunneling, jumping, escaping or turning. However, the animal may first have to evaluate the object to determine the appropriate response. These objects may be predators (Comer et al., 2003), prey items (Catania and Kaas, 1997; Dehnhardt et al., 2001), tall obstacles (Watson et al., 2002) or walls (Camhi and Johnson, 1999; Cowan et al., 2006; Wiesel and Hubel, 1963). To respond appropriately, the animal must detect and extract specific properties of the objects it encounters. While this is often thought of as a visual process, many insects and vertebrates use mechanosensory information for navigation (Patla et al., 1999). Vertebrate examples include rats (Brecht, 2006; Mehta et al., 2007; Mitchinson et al., 2007; Towal and Hartmann, 2008), shrews (Anjum et al., 2006; Catania, 2000), harbor seals (Dehnhardt et al., 1998) and star-nosed moles (Catania, 1999; Catania and Kaas, 1997), which use their whiskers (or appendages around the nose in the case of the star-nosed mole) to sense objects in their environment. Even humans, despite our visual nature, are able to use mechanosensory information to respond to objects within our environment (Roland, 1992). While some invertebrates can use mechanosensors on their front limbs to sense obstacles (Blaesing and Cruse, 2004b; Pick and Strauss, 2005), they can also gain mechanosensory information from the antennae (Camhi and Johnson, 1999; Dürr and Krause, 2001; Horseman et al., 1997; Pelletier and McLoed, 1994; Zeil et al., 1985).

Insect antennae are covered with a variety of mechanosensors. For instance, the basal segments of the antennae possess hair plates, campaniform sensilla and chordotonal organs. The hair plates encode the position of the antenna in both the horizontal and vertical planes (Okada and Toh, 2001). This position information is an important element in obstacle localization. Without it, tactile orientation to obstacles is impaired (Okada and Toh, 2000). Campaniform sensilla are located both at the base of the antenna and along the flagellar segments (Campbell, 1972; Schafer and Sanchez, 1973). It is thought that these sensors detect where contact was made along the flagellum and are involved in wall following behavior (Camhi and Johnson, 1999). Lastly, the chordotonal organs in the base of the antenna respond to movement of the flagellum in specific directions as well as to flexion within the scape–pedicel joint (Staudacher et al., 2005; Toh, 1981). The combined information detected by each of these sensors should enable the cockroach to identify an object's location relative to itself. However, it is likely that the antennae are able to sense much more about obstacles than just their position.

Previous behavioral observations suggest that antennae are likely candidates to guide obstacle negotiation behaviors in cockroaches (Camhi and Johnson, 1999; Okada and Toh, 2000; Okada and Toh, 2006; Staudacher et al., 2005; Ye et al., 2003). Information about an obstacle is obtained prior to limb contact, encoded within the nervous system and then is used to guide the new behavior. For example, cockroaches are able to climb over an obstacle using a single front limb movement without that limb ever touching the front of the obstacle (Watson et al., 2002). If the height of the barrier is altered, the cockroach changes its

rearing height appropriately (Watson et al., 2002). Another insect that uses this strategy, the potato beetle, fails to rear when their antennae are removed, suggesting that the antennae provide critical cues for directing this behavior (Pelletier and McLoed, 1994).

While antennae may provide critical information, they are not the only sensors available to the cockroach. As with most insects, cockroaches also have two pairs of eyes: one simple (ocelli) and one compound. Ocelli have lower spatial resolution than compound eyes but have a higher transmission speed and greater sensitivity to changes in light level (Goodman, 1981; Laughlin, 1981; Mizunami, 1994). While houseflies are able to use their ocelli to orient to edges (Wehrhahn and Reichardt, 1973), ocelli are most known for their function as horizon detectors (Reichert et al., 1985; Schuppe and Hengstenberg, 1993; Stange, 1981; Taylor, 1981). Cockroach ocelli are anatomically distinct from those of other insects in that they possess a larger lens and more photoreceptors (Mizunami, 1996; Weber and Renner, 1976). While it is possible that cockroach ocelli may enable them to orient to objects, it is thought that compound eyes are responsible for most of an insect's object perception. Cockroaches have optic superposition compound eyes, which are adapted to low light conditions. This sensitivity comes at the cost of visual acuity (Land, 1981; Wolken and Gupta, 1961). Even so, nocturnal insects are able to visually navigate around stationary objects (Varju and Reichardt, 1967; Wehner, 1981), pursue moving objects (Wehner, 1981) and estimate object distance (Collett, 1978; Wallace, 1958; Wallace, 1959). There is even evidence that visual information can be used to guide antennae toward objects within the visual field (Honegger and Campan, 1981; Ye et al., 2003), suggesting that the visual and mechanosensory systems could act alone or in concert. Indeed, recent evidence has suggested that ocellar information may modulate processing of certain primary sensory inputs (Willis et al., 2008).

No matter which sensory system is employed, an appropriate response to some objects will require a more thorough evaluation of the object. Unlike simple reflex events, the process of this initial evaluation can be somewhat variable. Thus, the essential properties of the obstacle that are extracted and how they guide these behaviors are currently unknown. A quantitative description of such complex behaviors is necessary if one is to experimentally manipulate the system to gain a better understanding of underlying mechanisms. Without such a framework, alterations could be attributed to the inherent variability of the system rather than to the experimental manipulation.

Both quantitative behavioral descriptions as well as an understanding of variability within the system can be represented by an ethogram. Ethograms quantitatively describe complex behaviors by separating one complex behavioral event into a series of simpler elements. These elements form a sequence detailing the probability of transitioning from one element to the next, thereby describing the original behavior while characterizing its variability (Lehner, 1996). This type of analysis has been used previously to describe many different behaviors, such as courtship (Darrow and Harris, 2004; Pandav et al., 2007), agonistic encounters (Adamo and Hoy, 1995; Karavanich and Atema, 1998; Nilsen et al., 2004), exploratory behavior (Clark et al., 2005) and predatory behavior (MacNulty et al., 2007). Combining ethograms with other techniques has allowed researchers to determine brain structures and pathways involved in specific behaviors (Diamond et al., 2008; Ewert, 1987), establish whether a single sensory modality or a combination of multiple modalities is used for a particular behavior (Goyret et al., 2007; Raguso and Willis, 2002), characterize deficits in genetically

modified organisms (Crawley, 1999; Pick and Strauss, 2005) and create computer models for testing neurobiological hypotheses (Blaesing, 2006).

Previous behavioral studies investigated the role of mechanosensory information in gap crossing behaviors (Blaesing and Cruse, 2004a; Hutson and Masterton, 1986). In stick insects, changes in step type and velocity occur with gaps of different sizes. These alterations are influenced by whether or not the gap was detected by the antennae or by the front legs (Blaesing and Cruse, 2004a). Similarly, in rats, a gap was crossed only after the rat's whiskers contacted the other side of the gap (Hutson and Masterton, 1986).

In this paper, we investigated the role of cockroach antennae in negotiating vertical obstacles. Previous studies suggested that cockroaches use their antennae to guide movements associated with block climbing (Watson et al., 2002). Here, we examined specific behavioral elements to determine exactly which features of the obstacle are important in guiding appropriate motor responses and how these features are detected. We found that antennae played a major role in guiding the cockroach over both blocks and shelves but this occurred in a context determined by visual information.

MATERIALS AND METHODS

Animals

Blaberus discoidalis (Serville 1839) cockroaches were raised following methods described in Ridgel et al. (Ridgel et al. 2007). Both male (mean length 47.4 ± 2.02 mm, mean mass 2.33 ± 0.21 g, $N=38$ measured) and female (mean length 52.48 ± 2.06 mm, mean mass 4.41 ± 0.54 g, $N=35$ measured) adult insects were used. No significant behavioral differences were found in this study between males and females. There was no statistical evidence suggesting that the day on which an individual was tested influenced its behavior [generalized estimating equation (GEE) (Hanley et al., 2003; Hardon and Hilbe, 2003)].

Experimental arena and obstacles

At the beginning of each trial the cockroach was placed in a plastic release cage (measuring 50 mm wide \times 50 mm high \times 90 mm long), which was then set in the arena (measuring 50 mm wide \times 100 mm high \times 580 mm long). All trials were recorded using two digital video cameras (PhotronTM, San Diego, CA, USA), one on either side of the arena operating at 60 frames s^{-1} . This frame rate allowed us to obtain enough temporal resolution to track antennal movements, which for empty arenas and those containing a block were, respectively, on average 5.9 cycles s^{-1} (± 1.5 , $N=7$) and 4.9 cycles s^{-1} (± 1.2 , $N=7$) in the light and 3.3 cycles s^{-1} (± 1.6 , $N=7$) and 3.5 cycles s^{-1} (± 1.7 , $N=7$) in the dark. To avoid possible chemical communication, the experimental arena was cleaned with ethanol at least 30 min before the start of any trials. The obstacle within the arena was chosen at random prior to the start of the experiment through the use of a random number generator. This obstacle could either be an acrylic block (50 mm wide \times 11.7 mm high) or a glass shelf that was 1 mm-thick and measured 50 mm wide and 11.4 mm high. For tests involving 'naïve' individuals, each insect performed one trial such that no insect was influenced by experience. In tests involving surgical modification (shams, antennal shortening, antennectomy or eye coverings), each individual performed up to four trials. The release cage was opened at the beginning of the trial. If the cockroach failed to leave the release cage within 5 min or failed to complete a trial before 10 min had passed, it was removed. This removal was permanent for non-modified individuals. For modified individuals, if the individual became inactive after more than one trial, it was then removed from the arena. In the

event that modified individuals only performed one trial, they were later returned to the arena. If they became inactive again before performing a second trial, they were removed permanently.

Lighting conditions

Tests were performed under two lighting conditions: referred to simply as 'light' and 'dark'. Tests under light conditions were performed during the last three hours of the cockroach's light cycle (12 h:12 h light:dark); for the dark condition, tests were performed during the first four hours of the dark cycle. This timing was chosen because cockroaches are most active during the end of their light phase and the beginning of their dark phase (Gunn, 1940; Tobler and Neuner-Jehle, 1992). Animals were entrained to a 12 h:12 h light:dark cycle at 27°C for a minimum of 48 h prior to testing. On the day of the experiment, they were removed from the environmental chamber and placed in the experimental room for one hour prior to the start of the experiment in order to allow them to adapt to experimental room conditions. The experimental arena within this room was illuminated to 350 lx incident light (2800 lx reflected light) (Gossen Luna-Pro light meter, Nürnberg, Germany) by fluorescent lights and two infrared (IR) strobe lights, which were synchronized to the cameras (Infrared Strobe II, AOS technologies AG, Baden, Switzerland). This lighting condition approximated an overcast day. The addition of IR lights did not alter light levels over that of the fluorescent sources. At the start of the cockroach's subjective night, the room was made dark (0.17 lx, the lowest non-zero light level detected by our light meter), approximating light levels during a full moon. The cockroaches were given one hour to adapt to the dark before testing under these conditions began. Under these lower light conditions, the IR strobes added (non-visible) light to the arena causing measurable light levels to reach 7–11 lx. Under either lighting condition, the cameras were capable of recording detailed images as the insects moved. Individual insects were only tested under one of the two lighting conditions.

Measurements

The horizontal distance between the cockroach and the block was measured using the Winanalyze motion analysis software package (Mikromak, Berlin, Germany). This distance was measured as a horizontal straight line from the front of the pronotum to the plane of the block at the beginning of each climbing attempt (swing phase). For modified individuals, this measurement was only taken after the first climbing attempt to assess the onset of climbing behavior. Here, climbing attempts were defined as pronounced vertical movements, of one or both front legs, directed toward the top of the block. These movements were easily distinguishable from walking movements. In the case of elevator-type climbing movements (see below), measurements were only taken once at the beginning of the first swing because, by definition, a single elevator movement uses at least two swing movements. The Winanalyze software package was also used to calculate dwell time, the time between contact of the obstacle with the cockroach's antennae and the onset of climbing or tunneling.

This same software was used to measure antennal angles. The angle between the tip of the antenna, the pronotum and the most posterior portion of the abdomen of the cockroach was measured to approximate the position of the antennal tip relative to the body axis. As contact with an obstacle would change the antennal trajectory, we only measured prior to antennal contact with an obstacle (if one was present). The antennal angle was then organized into 5 deg. bins for each trial. The percentage of time spent in each

bin was then averaged for the treatment group. The proportion of each trial represented by a given bin was calculated. These proportions were then added together and are represented by the distance from the origin. This gives an approximation of the amount of time spent in a given region of space. For this circular data, means were calculated for raw data using Oriana 3.0 software (Kovach Computing Services, Anglesey, UK). An analysis of variance (ANOVA) was then used to compare means from individuals of different groups.

Antennal ablations

For antennal ablations, the flagellum of each antenna was cut, either to 10 mm (between half and a third of the original length) or removed entirely (leaving the scape intact) under cold anesthesia. Animals with these ablations will be referred to, respectively, as having 'short antennae' or 'bilateral antennectomies'. The tip of the cut end of the antenna was coated with VetBond (3M, St Paul, MN, USA) to prevent loss of hemolymph. After ablation surgeries, individuals were given at least 20 h to recover before behavioral testing. Sham animals for this procedure were anesthetized and handled but nothing was done to their antennae. These individuals were given at least one hour to recover prior to the start of testing.

Ethograms

Ethograms were created by separating the behavior into smaller defined elements, which do not overlap temporally (Fig. 1B). These were defined as follows:

Approach

The cockroach left the release cage thus approaching the obstacle. All sequences started with this behavioral element.

Return

The cockroach turned (more than a quarter turn) away from the obstacle, starting to return to the beginning of the arena (release cage). It then had to turn around again to re-approach the obstacle.

Antennal contact

Any part of the antenna(e) contacted the obstacle. Contact may have continued beyond this point. Contact for the shelf obstacle could be further separated into three distinct patterns: over/over (both antennae contacted the top of the shelf, under/under (both antennae contacted the underside of the shelf), over/under (one antenna contacted the top of the shelf and one contacted the underside). The over/under contact pattern also included trials where one antenna contacted the top and bottom of the shelf before the second antenna made initial contact. Both antennae always contacted the obstacle prior to climbing or tunneling. Antennae did occasionally touch the walls of the arena; however, this contact only involved the tip of the antenna and did not result in noticeable changes in behavior and, thus, was not included in the ethograms.

Body contact

The cockroach's leg, body or head contacted the obstacle. This was only noted in animals that had received bilateral antennectomies. In the other individuals, it was unusual for the body to contact the block prior to the subsequent behavior, and this contact never preceded antennal contact.

Climb

This was defined as a vertical movement, of one or both front legs, directed towards the top of the obstacle (Fig. 1Aii). This behavior

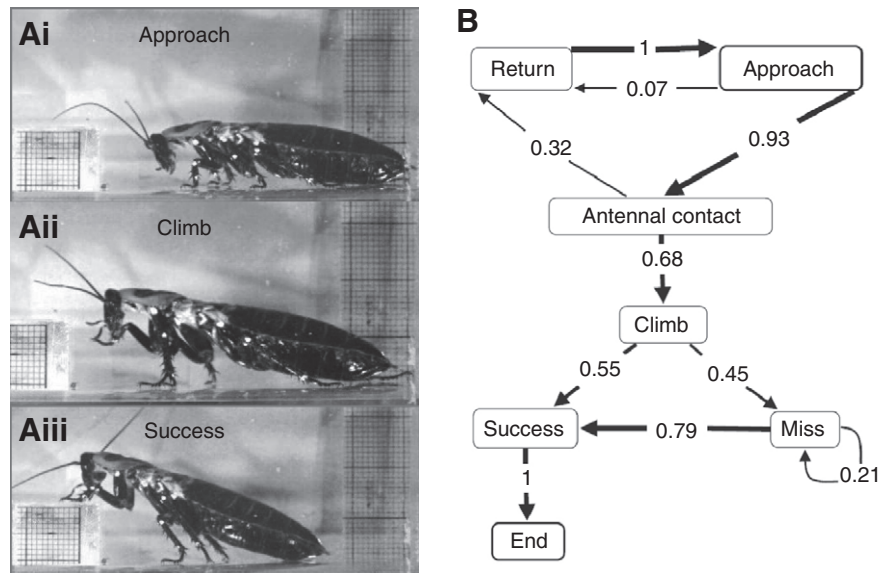


Fig. 1. (A) Block climbing behavior: approaching the block (i), swinging the leg to climb (ii) and climbing (success) (iii). (B) Ethogram of block climbing in the light. Arrows represent a direct transition from one behavior to the next. The number on the arrow and its thickness represent the frequency of that transition. This was calculated by dividing the number of times a specific transition was made by the total number of transitions exiting a specific element. All behavioral sequences begin with the cockroach approaching the block (approach). It can then turn around and walk away from the obstacle (return) before or after antennal contact (antennal contact). The cockroaches would then enter a climbing sequence (climb), which could either be successful, with their foot reaching the top of the obstacle (success), or not be successful (miss). In the event that the cockroach missed, it would then produce another climbing motion, which again could either be successful or not. The end of the behavioral sequence occurred when the cockroach climbed the block. The beginning and end of the sequence must be 'approach' and 'end', respectively, for this reason these elements are represented in bold. This sequence represents the responses of 58 individuals (one trial per individual).

often involved postural changes; however, as these were not separable elements, they were left out of the ethogram. The end point of the vertical swing movement was characterized by the location of the foot, which either may have (success) (Fig. 1Aiii) or may not have (miss) reached the top of the block. If a swing resulted in a miss, then the insect would swing again; subsequent swings could then either miss again or could be successful.

Tunnel

This behavior only occurred with the shelf and was defined by the cockroach's tarsus passing under the shelf (Fig. 5Aii). To be counted as a tunnel the entire tarsus had to break the front plane of the shelf.

End

Sequences ended when the individual's second foot reached the top of the obstacle or in the case of shelf tunneling when the individual's thorax passed under the shelf.

These elements are either physical movement of the insect or the actions of the antennae. These items were combined in the attempt to understand how antennal contact with an obstacle is involved in these behaviors. The timing of transitions between elements in the behavioral sequence was determined by examining the video records with the Photron Fastcam ViewerTM (San Diego, CA, USA) software. We combined data from all individuals to create a first-order transitional probability matrix. This matrix recorded the number of times one behavioral element followed another. As this was a first-order matrix, we only considered the immediate transition from one behavior to the next. The matrix was then used to create a sequence of elements that represents the entire behavior. While additional transitions were possible, they were not exhibited in any trials by any individuals and thus

were not included in the ethograms. While many of the elements within these ethograms could be further divided into even smaller elements, we felt that additional detail was not essential for this study. Further work is necessary to understand the active tactile sampling strategy of the antennae. In the Results section, we use insets of these ethograms to highlight the behavioral element discussed in each figure.

Climbing strategies

Climbing swings or attempts could be separated into multiple strategies, which were defined as follows:

Controlled rear

During or before the climbing swing, the cockroach raised the front of its body, changing the body-substrate angle (Cruse, 1976; Staudacher, 2005; Pelletier and McLoed, 1994; Watson et al., 2002; Yamauchi et al., 1993). It then swung its leg toward the top of the block.

Elevator

The front leg swung and either failed to contact the block or contacted the face of the block; it then swung higher toward the top of the block (Cruse, 1980; Pearson and Franklin, 1984; Watson et al., 2002).

Brute force

The cockroach pushed its head and body into the obstacle until that force resulted in its body pushing up and over the obstacle.

T1 on top

The cockroach used a high limb trajectory such that its foot contacted the block while its body remained horizontal. It then used

this front leg (also known as a T1 leg because it is located on the first thoracic segment) to pull its body up and over the block (provided that the leg reached the block) (Watson et al., 2002).

All six

The cockroach simultaneously extended all six legs, elevating the whole body during or prior to the climbing swing [described previously as 'elevate' in Watson et al. (Watson et al., 2002)].

Jump

The cockroach extended both hind legs in a jumping movement that propelled the body both upward and forward in order to climb the block (Watson et al., 2002). This often involved simultaneous climbing trajectories from both front legs.

It should be noted that some of these strategies such as 'controlled rear' and 'all six' require coordination of multiple limbs to move the whole body whereas others such as 'T1 on top' and 'elevator' only require the coordinated movement of a single limb. Thus, these strategies present distinct motor control issues, which should be further analyzed in the future. In some trials, cockroaches made multiple attempts to climb prior to successfully reaching the top of the block. The climbing strategy was recorded for each attempt regardless of success. Each attempt began when the leg started to move, whether it was picked up from the substrate or resumed movement while in the air. The leg would then swing, either landing on top of the block or missing the block and continuing to extend toward the substrate. The cockroach's leg would then stop moving forward, defining the end of the attempt.

Shelf height

To examine the effect of changing shelf height on climbing and tunneling behavior, we set shelves at each of the following heights (mm): 8.9, 10.3, 10.8, 11, 11.7, 12.3, 12.9, 14. These heights were chosen to test four that were higher and four that were lower than the 11.4 mm height used in the original shelf experiments. Before the experiment began, a random sequence of shelves was determined and assigned to each individual. Unlike the other shelf and block experiments, here animals performed more than one trial with each encountering at least three (up to all eight) shelf heights. No individuals faced the same shelf height more than once. Prior to the start of each trial, the appropriate shelf was placed in the arena. The cockroach was then placed in a release cage, which was then set in the arena (measuring 50 mm wide × 580 mm long). Statistics for this experiment were performed using the GEE protocol in SAS (Cary, NC, USA). This statistical method allowed us to account for multiple uses of the same individual (Hanley et al., 2003).

Eye coverings

For eye coverings, dental wax was melted and mixed with carbon powder to create an opaque black wax (Roberts, 1965). This was used to cover the ocelli, compound eyes or both. Shams were cold anesthetized and both their simple and compound eyes were covered with wax that had not been mixed with carbon and thus was still translucent. The melted wax was poured into thin flexible sheets that were attached to the eyes using moderate heat. These individuals were cold anesthetized and were given one day to recover prior to behavioral experiments. To prevent these individuals from removing the wax, they were placed on corks and restrained with pins placed through the pronotum and wings. Special care was taken to make sure that the pins did not damage the legs. Before the beginning of their trials, these subjects were freed and allowed to walk within a container for a few minutes. Upon completion of the trials, they were

examined post-mortem to ensure that their eyes were still covered. Due to the proximity of the eyes and antennal joints, all individuals were examined before the start of behavioral trials to ensure full mobility of antennal joints.

RESULTS

Block obstacles

To diagnose changes in block climbing behavior associated with experimental treatments, we first had to determine the inherent variability of the behavior in naïve individuals. To accomplish this task, we broke the entire behavioral sequence into simpler elements by creating an ethogram (Fig. 1B). Each number on the ethogram along with its arrow represents the frequency of transitions from one state to another. The weight of the arrow is also indicative of this transition frequency. All sequences started with the cockroach approaching the block (approach). The insect could then return to the beginning of the arena (return) without having contacted the block with their antennae or after they contacted the block with their antennae (antennal contact) (Fig. 1Ai). They then proceeded into climbing behavior (climb), which often involved changes in body-substrate angle called rearing (Fig. 1Aii) (see Watson et al., 2002). Rearing either occurred before or during the leg movements associated with the actual climb. The climb began with the cockroach swinging one or both of its front legs to either reach the top of the block (success) (Fig. 1Aiii) or failure to reach the top (miss). If a swing resulted in a miss, then the insect would swing again; subsequent swings could either miss again or be successful. Here, under light conditions, our 58 individuals made 88 climbing attempts (58 successes, 30 misses). Once their tarsi had successfully reached the top of the block, the middle and hind legs would extend to push the insect's body upward, thereby surmounting the block and ending the sequence.

Naïve cockroaches missed the top of the block 45% of the time (Fig. 1B). Because success of climbing attempts depended on the cockroach using information about the obstacle's height and distance to target its limb trajectory, it was possible that there was an optimal distance from the block where climbing attempts were most successful. To examine this possibility, we measured the distance between the cockroach and the block during climbing attempts. The majority of climbing trajectories (58 out of 88 attempts) occurred at distances less than 11 mm and most of these (50) were successful. Conversely, 22 of the 30 misses occurred at distances greater than 11 mm (Fig. 2A). Even when the distance from the block is normalized to account for insects of varying body length, we notice the same separation between successes and misses (Fig. S1 in supplementary material). Together these observations suggested that distance from the block is important to climbing success and thus would be an important parameter for the cockroach to sense.

Effect of altered antenna length

If antennae do indeed detect the distance from the block, then shortening the antennae should influence the distance at which climbing commences. To investigate this possibility, we measured how far cockroaches with sham lesions, short antennae and bilateral antennectomies were from the block when they made their first climbing attempt (Fig. 2B). Shams were, on average, the farthest from the block when they attempted to climb (7.5 mm). Cockroaches with short antennae were significantly closer at 5.9 mm ($P < 0.001$, GEE). Individuals with no antennal flagellum were the closest to the block at 3.2 mm ($P < 0.001$, GEE).

As distance from the block was related to climbing success, and antennal length affected this distance, we would expect changes in

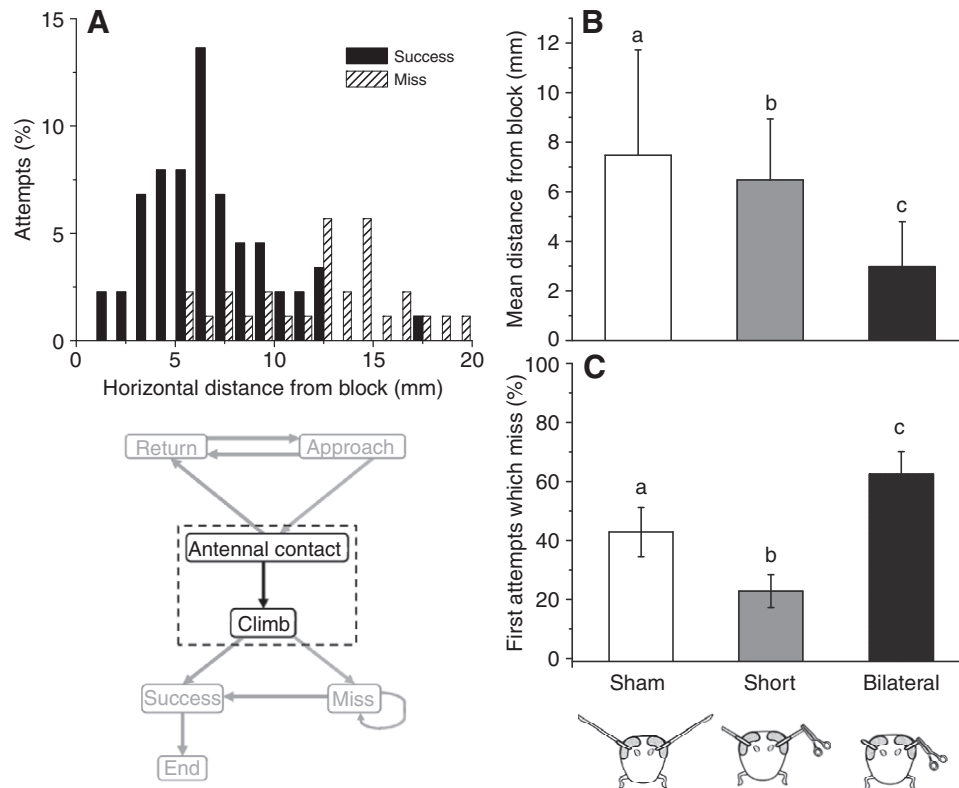


Fig. 2. Distance from obstacle, and climbing success. (A) Success is dependent on the distance from the block. The horizontal straight line distance was measured between the cockroach and the obstacle at the beginning of each climbing attempt. Climbing attempts were categorized as success or miss. The data from 88 steps by 58 individuals are represented as a histogram. The distance from the obstacle for successful attempts is significantly less than that for unsuccessful attempts [$P < 0.0001$, generalized estimating equation (GEE)]. Statistics were performed on raw data. (B) Shortening antennae results in cockroaches getting closer to the block before climbing. Mean distance from the block on first attempts in sham (white), short (gray) and bilateral (black). Each individual performed up to four trials. The horizontal distance was measured in the same manner as in A. The number of trials (n) and number of individuals (N) is as follows: sham ($n=36$, $N=14$), short ($n=42$, $N=15$) and bilateral ($n=31$, $N=14$). Individuals with bilateral antennectomies were significantly closer than individuals with short antennae and shams ($P < 0.001$, GEE). Individuals with short antennae were significantly closer to the block than shams ($P < 0.001$, GEE). (C) Shortening antennae changes success for first attempts. Black bars indicate bilateral antennectomies ($n=40$, $N=16$), gray bars indicate shortened antennae ($n=57$, $N=15$) and white bars indicate shams ($n=35$, $N=14$). The first climbing attempt made in each trial of each individual is counted as either a success or a miss, calculated as the percentage of first attempts that are misses for a given individual. No significant differences existed between light and dark. Individuals with shortened antennae miss less than shams ($P < 0.05$ Tukey means comparison) and individuals with bilateral antennectomies miss more often than shams ($P < 0.01$, Tukey means comparison). Letters in B and C indicate the independence of statistical comparisons whereby if two conditions were not significantly different they would share the same letter.

the rate of climbing success in individuals with modified antennae. Definite changes in the frequency of misses were present (Fig. 2C) but the relationship of miss frequency compared with shams was different between the two antennal treatments. Insects with bilateral antennectomies missed significantly more than shams on their first attempt ($P < 0.01$, Tukey means comparison). However, animals with short antennae missed significantly less often than shams ($P < 0.05$). Why might success increase with shorter antennae? Successful climbing was related to the cockroach's distance from the block, with most successful attempts occurring within 6 mm of the block (Fig. 2A). With their mean distance of 5.9 mm from the block, cockroaches with shortened antennae attempted to climb in the optimal range more often than sham animals. Interestingly, for bilateral antennectomies, the mean distance for successful first attempts was 2.4 mm whereas it was 3.8 mm for misses, suggesting that an additional factor was altered in these trials.

The increase in misses in cockroaches with bilateral antennectomies could be attributed to a total change in climbing strategy. There are multiple strategies a cockroach can employ in order to climb over a block. However, rearing was the most

commonly used by intact cockroaches (57%) (Fig. 3) (Watson et al., 2002). In this strategy, the cockroach raised the front of its body so that a typical front leg swing would place the front foot on top of the block (Ritzmann et al., 2004). This targeted limb motion and compensatory change in body posture appeared to be guided by the height and position of the obstacle. If the necessary information was acquired by the antennae, cockroaches without antennae might be compelled to switch to a completely different climbing strategy. Cockroaches with short antennae still predominantly used rearing to surmount the block (57%) (Fig. 3). However, cockroaches with bilateral antennectomies changed to elevator leg movements (47%) with only 22% attempting controlled rearing. In the elevator strategy, the cockroach would swing its leg toward the top of the block; the leg would miss (often touching the block face) and was then swung higher searching for the top of the block. Clearly a strategy that relies on trial and error would result in increased misses. Bilateral antennectomies also showed an increase in brute force climbing behavior (25%), which occurs when the cockroach pushes its body into the block such that it slides up the object's face to the top. Both of these strategies occurred close to the block (Fig. 2B) and relied

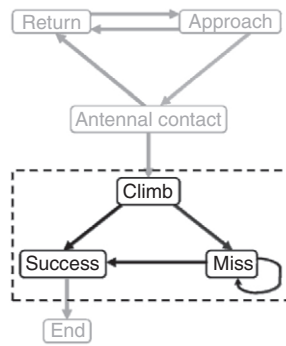
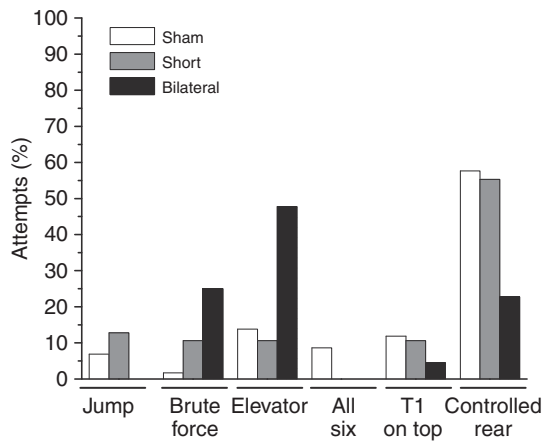


Fig. 3. Strategies used for block climbing in sham (striped), short (gray) and bilateral antennectomies (black). There is no significant difference between the distribution of climb strategies in individuals with short antennae and shams but there is a significant difference between both of these groups and bilateral antennectomies ($P < 0.001$, Pearson's χ^2 test). Each animal performed up to four trials, which may have contained more than one climbing attempt. The number of trials (n), number of measured climbing attempts and number of individuals (N) are as follows: sham ($n=36$, attempts=56, $N=14$), short ($n=42$, attempts=46, $N=15$) and bilateral ($n=31$, attempts=48, $N=14$). Despite the fact that by definition they contain more than one climbing swing, elevator strategies were only counted once.

on trial and error, suggesting that these individuals used the strategy available to them with their decreased sensory information.

If spatial information provided by the antennae was critical to normal barrier responses, shortening or removing the antennae could also alter other aspects of those behaviors. These explorative behaviors are variable by nature. However, by comparing the ethograms associated with the different treatments, we were still able to assess differences (Fig. 4). For bilateral antennectomies, we had to modify the antennal contact portion of the sequence to body, head or limb contact. Body contact (body contact) was not observed prior to antennal contact (antennal contact) in shams or insects with short antennae.

In addition to the aforementioned changes in success and climbing strategy, the ethograms also revealed altered frequency in returns after antennal contact (Fig. 4, broken circles). There was a general trend whereby animals with short antennae and bilateral antennectomies returned after antennal contact at a much lower frequency than their sham counterparts ($P < 0.001$, $P < 0.01$, respectively, χ^2). This decrease in variability suggested that by modifying the antennae we decreased the availability of stimuli to which the animals reacted.

Shelf obstacles

A shelf obstacle created a more complex paradigm whereby the cockroach had two different paths available to it. Now they could either climb over or tunnel under the object (Fig. 5A). To understand what factors were involved in path selection we constructed another set of ethograms. The shelf ethogram was similar to that which was developed for block climbing in that it starts with an approach (approach), which can lead to the cockroach turning around (return) before or after it contacted the shelf with its antennae (antennal contact). Initial contact was always with the antennae and could be classified in one of the following three ways: (1) both antennae contacting the top of the shelf (over/over), (2) both contacting the underside (under/under), or (3) one contacting the top of the shelf while the other contacts the underside (over/under). Subsequent contacts involved transitions from one of these contact states to another. These could occur multiple times in one sequence before the cockroach proceeded to the final behavior; either climbing over (climb) or tunneling under (tunnel) the shelf, which marked the end (end) of the sequence (Fig. 5B). Both antennae always contacted the shelf prior to climbing or tunneling behavior.

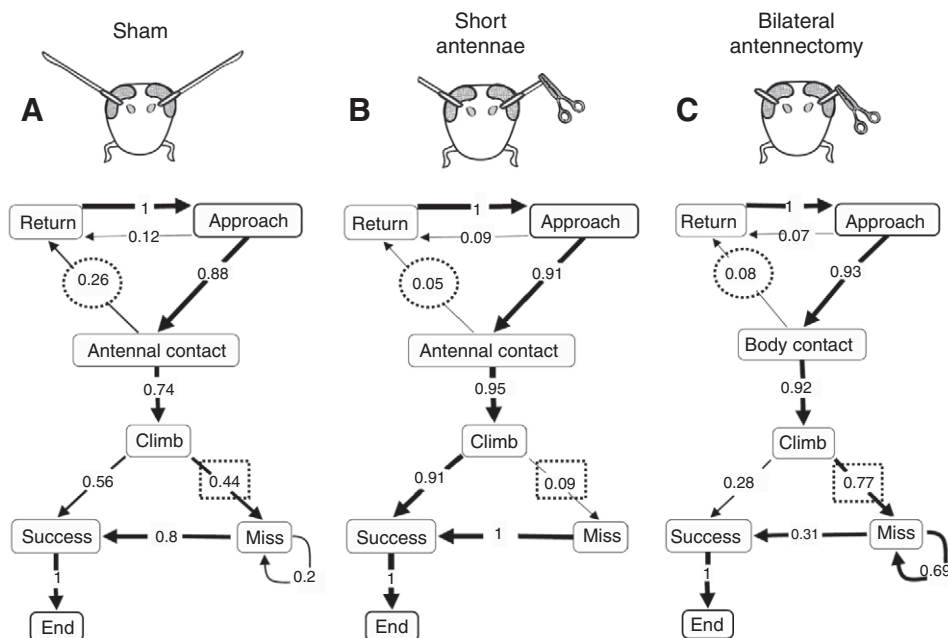
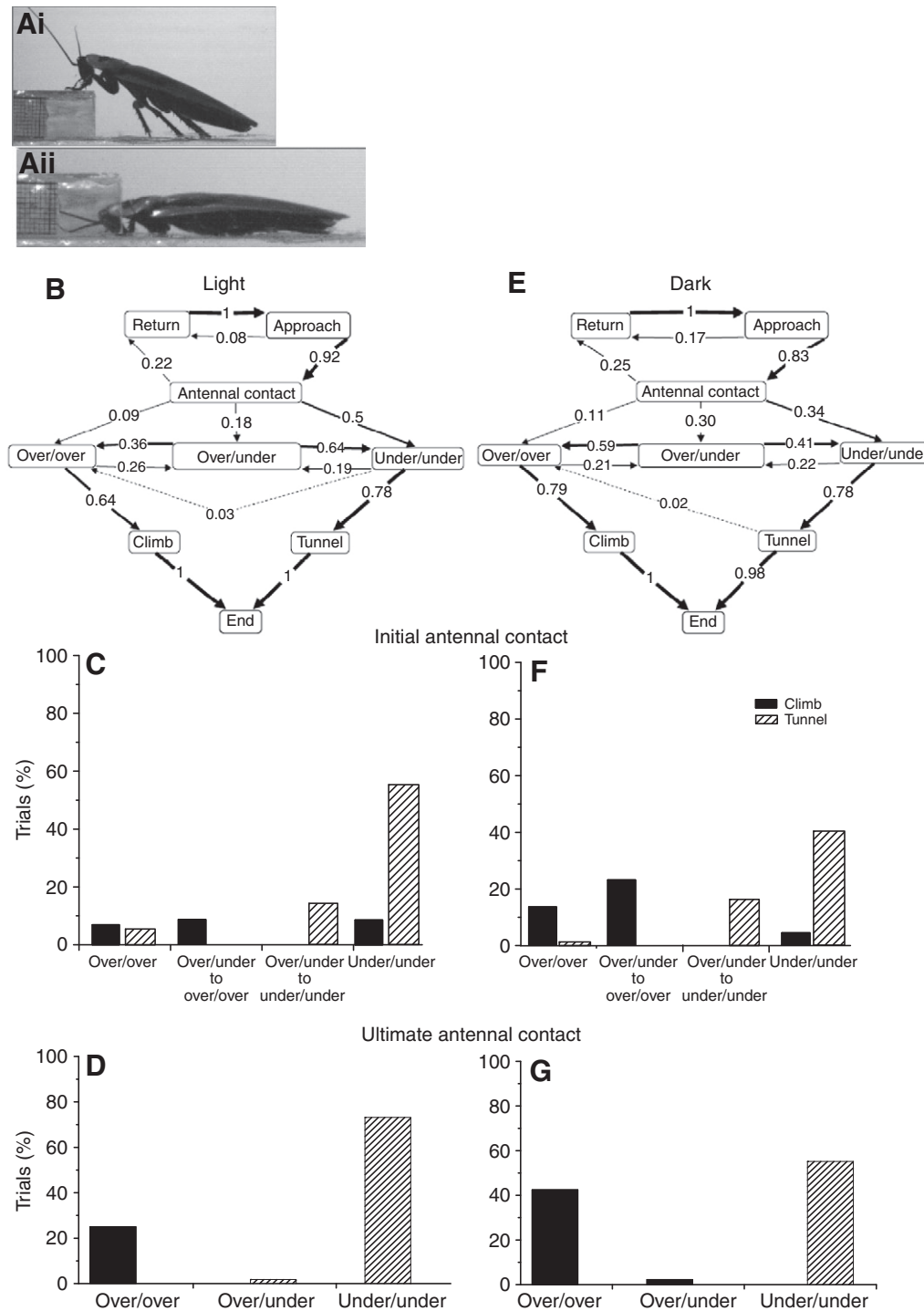


Fig. 4. Ethograms of block climbing in sham (A), shortened antennae (B) and bilateral antennectomy (C) cockroaches under light conditions. Arrows represent a direct transition from one behavior to the next (behaviors described in Fig. 1). The number on the arrow and its thickness represent the frequency of that transition. Broken squares and circles represent regions, which will be focused on within the paper. Each individual performed up to four trials; number of trials (n) and number of individuals (N) is as follows: sham ($n=36$, $N=14$), short ($n=42$, $N=15$) and bilateral ($n=31$, $N=15$).



The frequency of approach and return for shelf climbing was similar to block climbing (Fig. 5B). Differences occurred after antennal contact when the insect proceeded along one of the two paths: climbing or tunneling.

Two critical instances were examined; initial antennal contact and ultimate antennal contact just prior to climbing or tunneling. 'Initial contact' refers to the state of contact when the antennae first touched the shelf. 'Ultimate contact' refers to the situation just prior to climbing or tunneling actions. Whether the cockroach climbed over or tunneled under the shelf was highly correlated with the manner in which its antennae contacted the block at both of these time points. The

Fig. 5. Shelf climbing and tunneling is related to antennal contact.

(A) Pictures of climbing (i) and tunneling (ii) behavior. Ethograms of shelf behavior in the light (B) and dark (E). Arrows represent a direct transition from one behavior to the next. The number on the arrow and its thickness represent the frequency of that transition (behaviors are described in Fig. 1). Dotted lines were used when two or fewer individuals preformed a specific transition. Antennal position relative to the shelf was determined as being both over the shelf (over/over), both under the shelf (under/under) or if one antenna contacted the top of the shelf and the other contacted the underside the pattern was recorded as (over/under). Either the first [(C,F) initial antennal contact] or last antennal contact [(D,G) ultimate antennal contact] with the shelf prior to climbing or tunneling was recorded. For over/under initial antennal contacts, the following contact pattern was also recorded. This situation always resolved such that both antennae were on one side. Usually the two antennae contacted the shelf around the same time. In cases when one antenna contacted the shelf first on one side then the opposite before the second antenna could contact the shelf, that contact was scored the same as if one antenna contacted the top of the shelf and the other contacted the underside. Data for light (B–D) represents 58 sequences from 56 individuals (14 climbs and 42 tunnels). Data for dark (E–G) represents 86 sequences from 86 individuals.

cockroach initially contacted the underside of the shelf with both antennae in 36 trials. Of these instances, 31 resulted in tunneling behavior (Fig. 5C). Trials where initial contact had both antennae over the shelf were evenly split between climbing and tunneling (4 of 7). Cockroaches with one antenna initially on either side of the shelf moved both antennae to one side and proceeded accordingly (Fig. 5B,C). The ultimate antennal contact pattern perfectly predicted whether the cockroach climbed or tunneled (Fig. 5D). That is, climbing always occurred immediately after both antennae were placed above the shelf (14/14) whereas tunneling always occurred after both antennae were placed underneath the shelf (41/41). Thus,

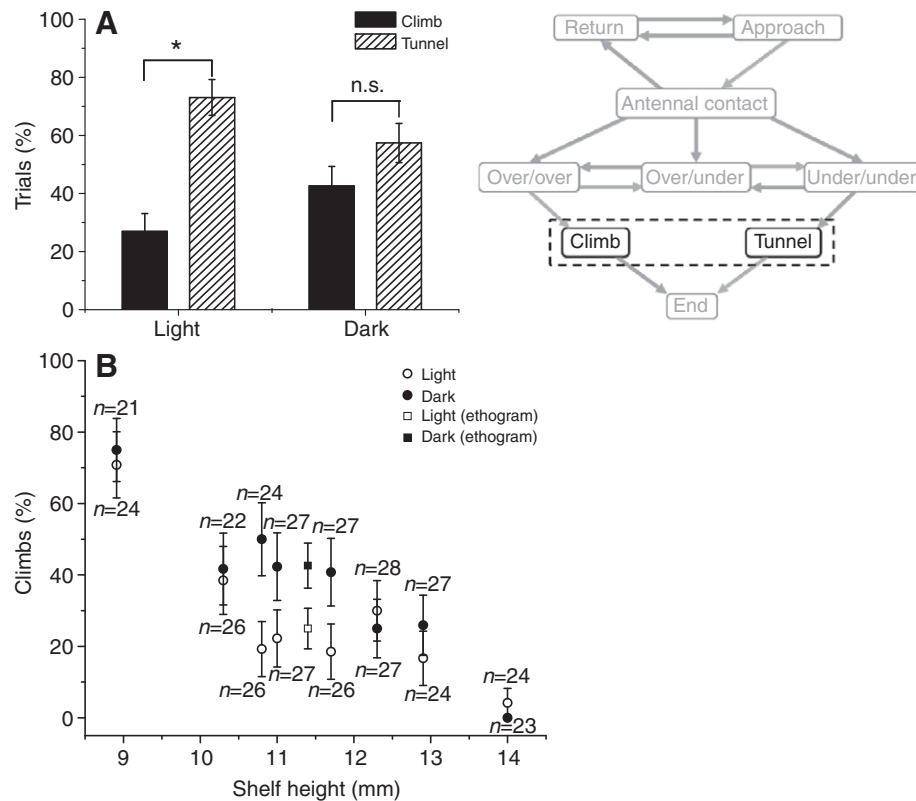


Fig. 6. The influence of shelf height and ambient lighting on climbing behavior. (A) Climbing and tunneling in insects presented with a shelf under different ambient lighting conditions. Naïve cockroaches were placed in the experimental arena with an obstacle they could climb over or tunnel under. The light condition represents 56 trials (14 climbs and 42 tunnels). The dark condition represents 61 trials (26 climbs, 35 tunnels). The error bars in this and subsequent figures represent the \pm standard deviation (\pm s.d.) (calculated using methods for binomial data). In the light, the climbing and tunneling percentages are significantly different ($P < 0.01$, χ^2 test). In the dark, this difference is not significant ($P > 0.5$, χ^2 test). (B) Prevalence of climbing with different shelf heights. The open circles represent the light condition whereas the closed circles represent the same behavior under dark conditions. Individuals faced multiple shelf heights but were not exposed to a single height more than once. All responses from all individuals were averaged to create a percentage response. When examined as a whole distribution, the light and dark distributions are significantly different at $P < 0.05$ [generalized estimating equation (GEE)]. The effect of shelf height on climbing proportion is significant ($P < 0.001$, GEE). When examined individually, points at 10.8, 11 and 11.7 mm differed significantly from their counterparts in the opposite lighting condition ($P < 0.001$, GEE). Square symbols represent data from the shelf height used to create the ethogram (Fig. 5A,D) and represents 58 naïve individuals for the light and 86 naïve individuals for the dark.

the animal appeared to resolve the initial over/under antennal pattern but then acted according to the ultimate pattern.

Effects of ambient lighting

In our light condition, we noted that nearly three-quarters of trials resulted in tunneling (Fig. 5C,D; Fig. 6A) ($P < 0.01$, χ^2 test). Because cockroaches are nocturnal animals, we suspected that the bright ambient light conditions may have affected the relative probability of climbing or tunneling by causing the subjects to seek out shelter (Kelly and Mote, 1990) thereby biasing them toward tunneling (Halloy et al., 2007; Jeanson and Deneubourg, 2007). We, therefore, repeated the observations under dark conditions. Although the data in the dark still appeared to have a slight bias toward tunneling (Fig. 6A), the difference between climbing and tunneling was, in fact, no longer significant ($P > 0.5$, χ^2 test).

Similar to the light, in the dark, initial antennal contact revealed that 35/39 trials in which the cockroach contacted the underside of the shelf with both antennae resulted in tunnelling, and 12/13 trials in which the cockroach had both antennae on top of the shelf resulted in climbing (Fig. 5E,F). In the dark, the pattern seen in the behavioral outcome of the ultimate antennal contact also reflects whether the cockroach climbed or tunneled. That is, all 37 individuals that had

both antennae over the shelf right before responding chose to climb whereas all 48 individual with both antennae under the shelf tunneled (Fig. 5G). Under both lighting conditions, there were a few individuals that responded to the shelf before both antennae were on one side. In this situation, the cockroach in the light tunneled and both individuals in the dark climbed (Fig. 5D,G).

Biases related to lighting conditions may result in changes in the delay between antennal contact and subsequent behaviors. For this reason, we determined the amount of time the individuals dwell within the antennal contact states before moving on to climbing or tunneling (Table 1). Overall this dwell time was significantly longer in the dark than it was in the light ($P < 0.05$ Tukey means comparison). However other trends were noted. The shortest dwell time in the light occurred when both antennae were under the shelf, leading to tunneling (Table 1); this was significantly shorter than the same situation in the dark ($P < 0.05$ Tukey means comparison). Interestingly, in the dark, the shortest dwell time occurred in the opposite situation when both antennae were above the shelf.

Role of vision in light/dark response

Cockroaches have two pairs of eyes that are both capable of sensing light. To assess the involvement of compound eyes vs ocelli in the

Table 1. Dwell time after specific antennal contact patterns

	Over/over		Over/under		Under/under		All	
	Mean (s)	n	Mean (s)	n	Mean (s)	n	Mean (s)	n
Light	2.96±1.8	6	2.56±1.89	15	1.80±1.98 ^a	33	2.14±1.95 ^b	54
Dark	1.79±1.61	8	4.07±3.8	27	3.37±3.67 ^a	25	3.48±3.55 ^b	60

This is a measure of the time between initial antennal contact and the initiation of climbing or tunneling behavior. Note that the mean dwell time (s) of all trials in the dark is significantly longer than that of individuals in light ($P<0.05$ Tukey means comparison). Furthermore, the dwell time for individuals whom contacted the shelf with both antennae under is significantly shorter for individuals in the light than it is in the dark ($P<0.05$ Tukey means comparison). It should also be noted that the shortest dwell times occur in the dark when both antennae are on top of the shelf and in the light when both are underneath, which may suggest preference for a specific side of the shelf in these conditions.

light vs dark behavioral bias, we covered the eyes with carbonized wax or, in the case of shams, non-carbonized wax (Fig. 7). Shams and individuals with covered compound eyes still showed a significant bias toward tunneling in the light ($P<0.05$, ANOVA, Tukey means comparison). This bias was absent in individuals in which the ocelli or both compound eyes and ocelli were covered ($P<0.77$ and $P<0.9$, respectively, ANOVA, Tukey means comparison). Indeed, individuals with both ocelli covered showed no difference in the light than shams or normal animals in the dark. Climbing prevalence in the light for shams was significantly different than that of ocellar coverings and combination compound eye and ocellar coverings ($P<0.01$ and $P<0.001$, respectively, GEE).

The effects of changing shelf height

Shelf height could affect the probability of climbing or tunneling. At low shelf heights, the behavioral outcome was biased toward climbing whereas at high shelf heights, tunneling was more prevalent (Fig. 6B). At a shelf height of 8.9 mm, 17/24 insects climbed in the light and 17/22 climbed in the dark. By contrast, at a shelf height of 12.9 mm, 4/24 insects climbed in the light and 7/27 climbed in the dark. It was important to note that even at these extreme test heights, both climbing and tunneling behaviors did occur. Therefore, the shelves were never placed at a height where only one outcome was physically possible. Despite their similarity at high and low shelf heights, between 10.8 and 11.8 mm heights, the climbing and tunneling behavior revealed significant differences between the two lighting conditions ($P<0.001$, GEE). These differences are consistent with the data reported in Fig. 6A for a shelf height of 11.4 mm.

Body posture under differing light conditions

The differences in shelf directed behavior between the two lighting conditions could possibly have been explained by changes in posture. If, in the light condition, cockroaches maintained a lower posture as they walked (i.e. held their body closer to the ground), they would be predisposed to contact both antennae under mid-range shelves, leading to a greater incidence of tunneling behaviors. Conversely, a higher posture in the dark would result in a greater incidence of antennal conditions, leading to climbing. If this were the case, the pattern of antennal contact with the shelf could be a consequence of altered posture rather than a causal step in the choice of climbing or tunneling. To assess this possibility, we measured the height of cockroach over the floor in an empty arena under both lighting conditions as the cockroach walked from the beginning of the arena to where the obstacle would be. We then calculated the mean of these values. In the light, pronotum height was 9.35 mm [nine trials from nine individuals (three males, six females)] whereas in the dark the value was 9.0 mm [eight trials from eight individuals (three males, five females)]. These were not significantly different ($P=0.39$,

two sample *t*-test). Furthermore, despite size differences, we found no significant difference between the pronotum height or climbing probability of males and females.

Differences in antennal position under differing light conditions

Differences in antennal movements between the two lighting conditions could also have resulted in changes in antennal contact leading to behavioral differences. For this reason we digitized the angle between the antennal tip, antennal base and most posterior portion of the abdomen for the antenna closest to the camera. Because it was possible that objects in the visual field could change antennal trajectory (e.g. Ye et al., 2003), we examined antennal movements when there was an obstacle (Fig. 8C,D) in the arena as well as when the arena was empty (Fig. 8A,B). We found no differences in mean antennal direction between the two lighting conditions (Fig. 8). Nor did we find any differences in antenna direction with or without an object in the arena. As the distribution of the data is skewed, it seems as though the mean is not located near the most prominent antennal angles. This skewing is better illustrated in Fig. S2 in supplementary material.

DISCUSSION

When an animal encounters an obstacle, it often must modify its behavior in order to negotiate the obstacle. This often requires the animal to redirect its leg movements so that it can climb, tunnel, turn or step over the obstacle. These tasks are dependent upon sensory information for the animal to appropriately adjust its course. For instance, cats walking in cluttered environments are able to avoid stepping on objects to a high degree of accuracy; a feat that is dependent upon visual information to plan targeted limb trajectories (Sherk and Fowler, 2001). Such targeted limb trajectories are not limited to mammals; cockroaches are able to swing their foot from the ground to the top of an obstacle in preparation for a climb (Watson et al., 2002). In naïve insects these swings were successful at reaching the top of the block the majority of the time (Fig. 1B). Furthermore, the majority of attempts occurred at a distance of less than 11 mm from the block where climbing swings were successful, suggesting that distance from the block was a factor in the initiation of climbing behavior (Fig. 2A).

Here, our data suggest that while other sensors are available to the cockroach, they rely heavily upon their antennae to guide climbing and tunneling behaviors. We confirmed this notion by altering the antennae and demonstrating a series of predictable alterations in the behavior of our experimental groups. Previous studies suggested antennal involvement in navigation through wall following (Camhi and Johnson, 1999; Cowan et al., 2006), anemotaxis (Linsenmair, 1973; Rust and Bell, 1976) and escape (Comer et al., 1994). In other studies, antennae were shown to take

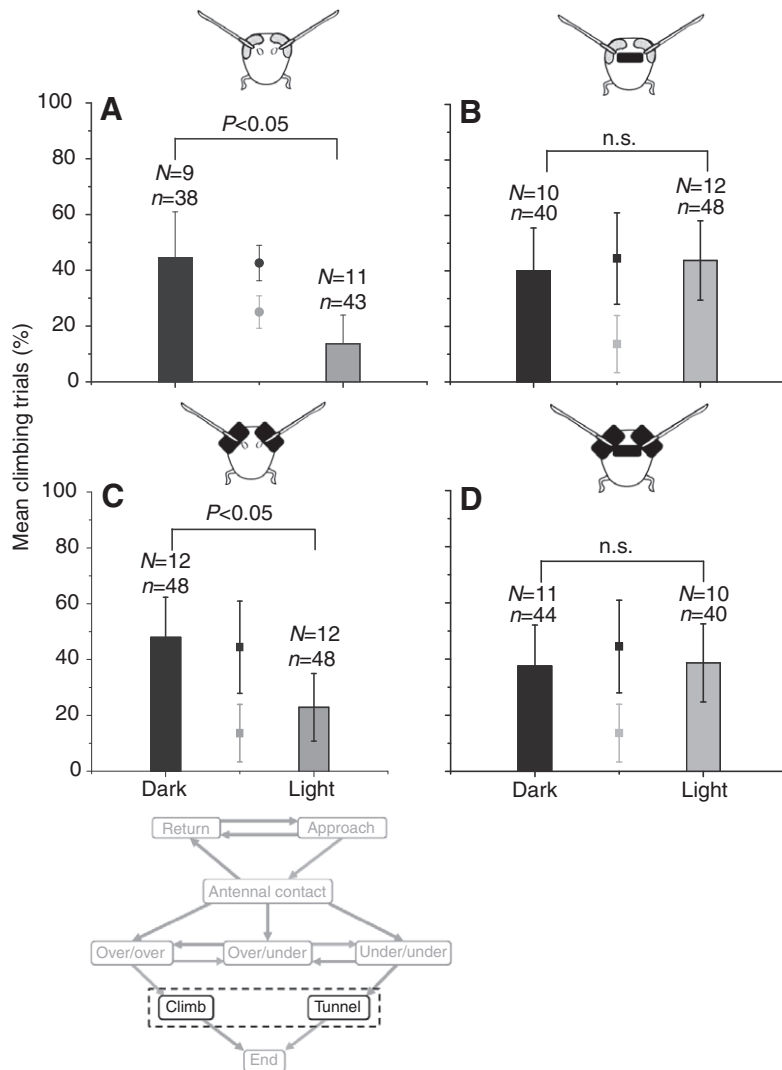


Fig. 7. Ocelli determine if it is light or dark. Each individual performed at least two and up to four trials. Climbs were scored as 1 and tunnels were scored as 0. The trials for each individual were then averaged to get the individual's response. The mean response of all individuals was then averaged creating the mean percentage of climbs (y axis). All experiments were performed in both the dark (black) and light (gray) conditions. One day before the start of any trials, the eyes were covered with translucent wax (A) sham eye covering or carbonized wax, (B) ocelli covering, (C) compound eye covering and (D) compound eye and ocelli covering. Squares on B, C and D represent the sham light (gray) and dark (black) responses. Closed circles in A represent our ethogram data for naïve individuals in light (gray, $n=56$) and dark (black, $n=86$). Both shams (A) and compound eye covering (C) showed a significant difference in climbing proportion between the light and dark conditions ($P < 0.05$, ANOVA, Tukey means comparison). This was not present in ocelli (B) or coverings of both the compound eyes and ocelli (D). Light values for the ocelli (B) and combination compound eye and ocelli (D) coverings were significantly different than that of the shams (A) [$P < 0.01$, generalized estimating equation (GEE) and $P < 0.001$, GEE, respectively]. Compound eye coverings and shams were not found to be significantly different ($P < 0.24$, GEE). The error bars represent \pm standard deviation (\pm s.d.).

part in active searching (Okada and Toh, 2000) where movement of antennae can be guided by visual stimuli (Honegger and Campan, 1981; Ye et al., 2003), leading to object tracking (Honegger and Campan, 1981). These antennal related mechanisms can lead to orientation toward obstacles (Blaesing and Cruse, 2004b; Dürr, 2000; Dürr and Krause, 2001; Okada and Toh, 2000; Staudacher et al., 2005; Zeil et al., 1985) or postural adjustments associated with obstacle contact (Dürr and Brenninkmeyer, 2001; Pelletier and McLoed, 1994; Watson et al., 2002). Here, we were able to show that antennae clearly played a role in directing obstacle climbing and tunneling behaviors. We were also able to identify some of the properties that the cockroach extracts from antennal information in navigating these barriers.

Distance from a block affects climbing success rate

What factors were involved in whether or not a swing was successful? For a swing to be accurately targeted toward the top of a block, the cockroach must be able to establish both the height of the obstacle and its own distance from the obstacle (Fig. 1B). Previously it was shown that cockroaches adjust their body position accordingly for obstacles of different heights (Watson et al., 2002). The active use of sensory information to alter body height and leg trajectory suggests that an active sensing mechanism was involved. This evaluation could be a simple closed-loop

scenario whereby the cockroach raises its body until a specific antennal angle is obtained, at which point it begins a climbing swing. Alternatively, the cockroach could calculate the height of the block from antennal information prior to climbing and move accordingly. Of course, in either case, control could reside in other sensory modalities, such as vision. However, if that were the case we would not have expected to see a change in climb strategy in individuals without antennae (Fig. 3). Instead, the increase in elevator and brute force strategies after antennectomy suggested that cockroaches without antennae were unable to obtain the sensory information necessary to employ their usual controlled rearing strategy. Interestingly, the elevator strategy has been shown to be a prominent climbing strategy in intact locusts and stick insects (Cruse, 1980; Pearson and Franklin, 1984), suggesting differences in obstacle sensing behavior between cockroaches and these insects.

In contrast to obstacle height, sensing distance appears to employ a more passive antennal function. While it was possible that this sensory task could be the result of active sensory discrimination, it was more likely related to the mechanical properties of the antenna. Decreasing antennal length resulted in a decrease in the distance at which the cockroach first attempted to climb (Fig. 2B). Thus, the cockroach appears to simply rely upon contact with an appropriate length antenna to establish proximity to the obstacle leading to a

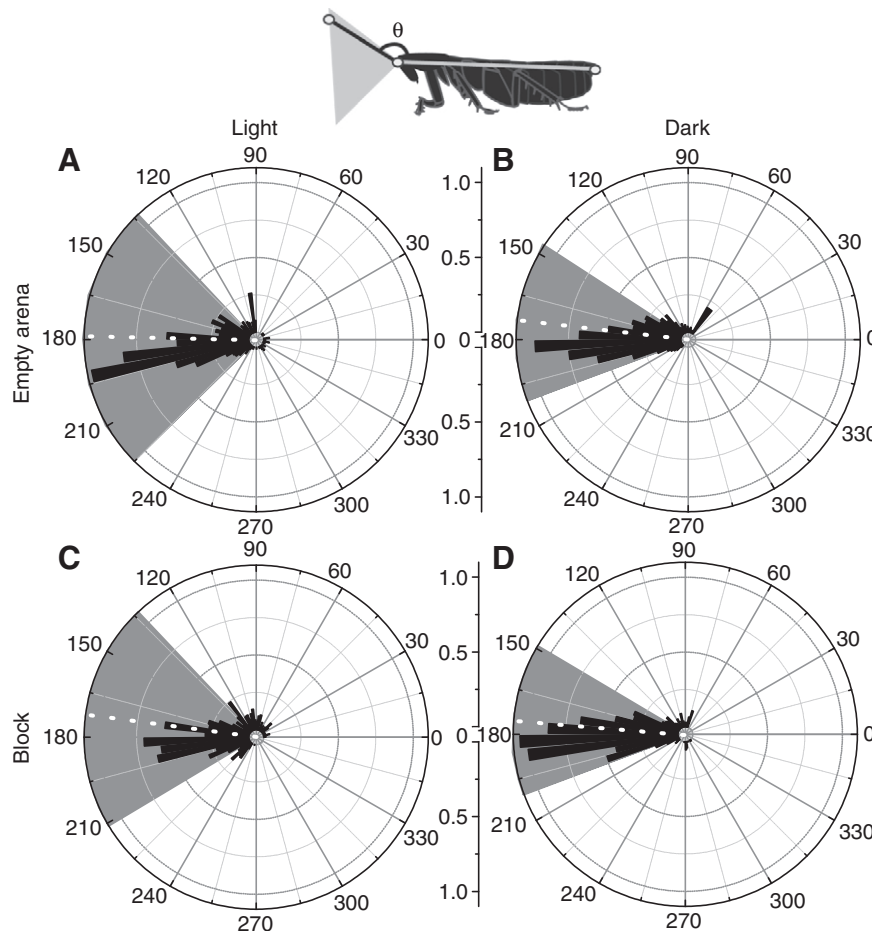


Fig. 8. Antennae sample the same space regardless of lighting. Naïve cockroaches were placed in the empty arena (A,B) or in the arena when a block obstacle was present (C,D). Vertical movements of the antenna ipsilateral to the camera (occurring prior to antennal contact with the block when it was present in the arena) were digitized. This is presented above in black where the angle is the angle between the antennal tip, antennal base, and the most posterior point on the abdomen (to approximate the body axis). To calculate distance from the origin, the antennal movements were divided into 5 deg. bins. The proportion of each trial represented by a given bin was calculated. These proportions were then added together for the individuals with a given treatment to approximate the amount of time spent in a given region of space. The circular means are represented by the dotted white line and standard deviations by the gray triangle. No significant differences were found between the means or variances of treatment groups (ANOVA). Means and standard deviations (\pm s.d.) are as follows:
empty arena light, mean=179.6 deg., s.d.=46.3 deg.;
empty arena dark, mean=173.1 deg., s.d.=26.8 deg.;
block light, mean=172.7 deg., s.d.=38.9 deg.;
block dark, mean=175.3 deg., s.d.=26.1 deg.

climb. Previous studies suggested that this is the case when insects are maintaining distance from a wall (Camhi and Johnson, 1999; Cowan et al., 2006; Dürr et al., 2003; Dürr and Matheson, 2003).

Detecting alternate routes

To create a more complex situation, we presented the cockroach with a shelf that allowed it to take one of two paths; it could climb over or tunnel under the shelf (Fig. 5). As with block climbing, several sensory modalities were available to the cockroach to establish an appropriate path over or under the shelf. However, again antennal contact appeared to play a dominant role. This was made evident by the strong relationship between the form of antennal contact immediately preceding climbing or tunneling and the pathway that was actually taken (Fig. 5). A similar critical point is seen in stick insects as they cross gaps. Once the far side of the gap is either touched by a front leg or the antennae leg movements are re-directed from walking patterns so that the stick insect can successfully span the gap and reach the other side (Blaesing and Cruse, 2004b).

Ambient lighting influences whether a cockroach climbs or tunnels

While antennal contact clearly affected the path that the cockroach took over or under a shelf, it was not the only factor involved (Fig. 5). Cockroaches were biased toward tunneling in the light and in the dark this bias was absent (Fig. 6A), suggesting that the light created a context around this behavior. Previous work demonstrated that cockroaches tend to seek out shelter from light when placed in an arena (Halloy et al., 2007; Jeanson and Deneubourg, 2007; Kelly

and Mote, 1990; Meyer et al., 1981; Okada and Toh, 1998). Other insects have also been found to change their behavior under different lighting conditions. For instance, tropical katydids change mate-attracting strategies under certain lighting conditions. During the new moon they call to attract mates whereas during the full moon they use tremulations more often than calling; a method which does not transmit the signal as far but reduces the predation risk present under relatively bright lighting conditions (Belwood and Morris, 1987; Lang et al., 2006). Similarly, here, the cockroaches may be predisposed to find shelter from predators in the light while exhibiting normal foraging behavior in the dark. All of these studies point to the context-dependent nature of complex behaviors, which should be considered in neuroethological studies.

The bias toward tunneling in the light was only present for moderate shelf heights

Similar to antennal effects, the effect of ambient lighting was not absolute. A bias toward tunneling in the light was not found at all shelf heights (Fig. 6A). At low shelf heights climbing was the predominant behavior regardless of lighting conditions whereas at higher elevations tunneling prevailed. A window existed between 10.8 and 11.8 mm within which a consistent difference in the proportion of the behaviors between the two lighting conditions was revealed.

A similar contextual bias is present in leeches, which are biased to crawl in waters less than 10 mm in depth and swim at greater depths (Esch et al., 2002). In the leech, each of these behaviors has a unique pattern of cellular activity. While a large population of cells is responsible for the decision to swim or crawl, manipulation

of a single cell can bias the system to perform one behavior or the other (Briggman et al., 2005). It is possible that a similar population of cells exists in the cockroach brain, whose activity controls whether the cockroach climbs or tunnels and that ambient lighting and antennal inputs change their activity. Indeed, populations of antennal sensitive cells that were also sensitive to ambient light have recently been described in the cockroach central complex (Ritzmann et al., 2008). Similar light-related changes in crayfish behavior have been found to be associated with changes in neural activity (Liden and Herberholz, 2008).

Ocelli detect light levels and influence the light-based bias on shelf behavior

For these behaviors to differ in light and dark, light levels must somehow be detected. While the compound eyes have been implicated in controlling the shade response (Okada and Toh, 1998), our data showed that the ocelli were solely responsible for light-related biases in shelf behavior (Fig. 7). Cockroach ocelli are large in comparison with those of other insects and have a unique anatomy (Mizunami, 1995a). Neurons related to the ocellar system have been shown to project to a number of brain regions, including the central complex (Goodman and Williams, 1976; Mizunami, 1995b). While ocelli in other insects have been implicated in flight control (Reichert et al., 1985; Stange, 1981; Taylor, 1981; Schuppe and Hengstenberg, 1993), no behavioral function in cockroaches had previously been demonstrated.

We would like to thank Greg Sutton, Amy Casselman, Jennifer Avondet, Nick Kathman, Jason Funt and John Bender for their input regarding this manuscript, Dan Kinnamon for his assistance with GEE statistics, Sheryl Petersen for assistance with SAS, Mark Willis and Mike Branicky for their input regarding the experimental methods, Mike Branicky for instruction regarding Markov chains and transitional probability matrices and Al Pollack for his technical assistance. Lastly, we would like to thank two anonymous reviewers whom made suggestions that greatly improved this manuscript. This material is based upon work supported by the National Science Foundation under Grant No. IOB 0516587 and the AFOSR under grant FA9550-07-1-0149 both to R.E.R. and NSF IGERT training grant DGE 9972747 that provided graduate support to C.M.H.

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