Abstract

Dissertation Title: Use of Multiple Cues for Navigation by the
                                          Leaf-cutter Ant *Atta cephalotes*.

                                          Kyle Alrich Vick, Doctor of Philosophy, 2005

Dissertation directed by: Associate Professor John Jeka
                                          Neuroscience and Cognitive Sciences Program

In the first chapter, there is a brief introduction to ant navigation and a review of previous literature as well as a summary chapters 2-7.

In chapter 2, I examine orientation of *Atta cephalotes* workers in the laboratory. Laden nest-bound foragers were moved from a "bridge" with or without trail pheromone present and placed on a parallel bridge with or without pheromone.

In chapter 3, I continue to examine orientation of *A. cephalotes* foragers in the laboratory. Foragers walked on a single bridge and I altered various cues and contexts and recorded which manipulations caused the ants to reverse course.

In chapter 4, I put orientation cues into direct conflict by letting the ants forage on a Y-maze. Foragers that were returning to a food source preferred visual cues to odor cues while recruited foragers consistently used odor cues.

In chapter 5, I use a vertical T-maze to investigate the role that gravity plays in *A. cephalotes* navigation. The gravitational cue was put in direct conflict with odor cues and light cues. There was an asymmetry to the ants' response to the gravity cue in that ants returning to a food source had a tendency to go up regardless of the previous position

of the food source or the position of the odor trail. Introducing a light cue changed the

angle required to make the ants respond to the gravitational cue.

In chapter 6, I investigate the anatomy of *A. cephalotes* eyes and brains. Based on

tissue sections, I measured the angles between adjacent ommatidia in the eyes, and the

volumes of sub-compartments of the brain..

In chapter 7, I use the results from the other chapters to inform my speculations

about the nature and neural basis of *A. cephalotes* navigation. I develop an hypothesis of

navigation in the wild and a simple model of its neural underpinnings.

Use of multiple cues for navigation by the leaf-cutter ant *Atta cephalotes*

Kyle Alrich Vick

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Dedicated to Alison

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Chapter 1

Introduction and Literature review

1.1: The place of ant navigation in cognitive science

Cognitive science, the study of the mind, includes the study of perception, language, attention, memory, control of movement, feelings, and consciousness (Gazzaniga, et al. 1998). Insects may lack some of these abilities, but they clearly possess some form of perception, attention, memory, and control of movement. Insects are not generally considered to have "minds" because of the cognitive abilities that they lack. Nevertheless insects make excellent subjects for studying the cognitive abilities they possess.

The primary advantage in studying cognitive abilities in insects is that insects are relatively simple (Burrows 1996). It is conceivable that in an insect, an entire neural network responsible for an activity could be identified and studied. It is also conceivable that such a network could be modeled on a neuron by neuron basis. This would allow for types of analysis that are simply not possible in vertebrate nervous systems. Furthermore, the extreme diversity of insects sometimes makes it possible to see how their cognitive abilities have evolved because different stages of development can sometimes be observed in extant species.

Navigation is a particularly interesting insect cognitive ability that incorporates perception, attention, memory, and control of movement. Navigation is not simply controlled locomotion; it is the process of choosing and maintaining a course from one

specific position to another (Gallistel 1990). The specificity of the target location differentiates navigation from taxis, i.e., following a stimulus gradient until finding some desired level or a local minimum or maximum. Taxis will get an organism to a location with specific parameters, but may be unreliable in getting an organism to a specific site such as a nest. In order to get to a specific location, an animal needs to navigate. Navigation is clearly important for central-place foragers, i.e., animals that need to return to a nest or sleeping site. As central-place foragers, ants are good subjects for navigation research.

1.2: Basics of Navigation

The central problem an organism faces in navigation is keeping track of where it is relative to where it is going. For this reason, navigational strategies are largely defined by sensory cues used to keep track of position. These strategies can be broadly categorized as either piloting or dead reckoning. When piloting, the navigator uses proximal cues to steer a course. Proximal cues include local landmarks and gradients. They generally provide the navigator with a local vector bearing that leads either to the goal or to an intermediate landmark. When dead reckoning, the navigator calculates the direction and distance to the goal and uses the distal cues to maintain a heading toward that goal. Distal cues are generally celestial objects such as the sun, moon, stars, or the polarization pattern in the sky. Landmarks on the horizon can also serve as distal cues so long as they are much further away than the distances traveled and there is relatively little change in their position on the horizon. There are also some cues used for dead

reckoning that are technically proximal because they are measured locally (e.g. the Earth's magnetic and gravitational fields). I will group these cues with distal cues because they allow the navigator to maintain a single heading for a long period of time.

1.3: Ant Navigation

Ants use a wide variety of cues to navigate. I will briefly describe the range of non-visual cues ants are known to use. Proximal cues and distal cues will be treated separately because they are used for different general strategies. I will then move the discussion closer to the focus of this study by examining how ants use visual cues for navigation. This discussion will also be divided between proximal cues and distal cues. Next, the hierarchical ordering of cue use will be discussed followed by an examination of previous investigations of *Atta* vision. Finally I will describe the neural structures likely to be involved in navigation and briefly describe their functions.

1.3.1: Non-visual navigation
Non-visual proximal cues

The most commonly used proximal cue is an odor trail laid by the navigating ant or one of her nestmates. Trail substances can originate in the hindgut or in a large variety of glands (review in Hölldobler and Wilson 1990). Another, less common proximal cue is a nestmate. In "tandem running", an ant simply follows her nestmate by keeping her antennae in contact with the lead ant's abdomen (review in Hölldobler and Wilson 1990).

Pheidologeton diversus workers also use nestmates as proximal cues, but in a slightly different fashion. When food laden, they look for other food laden ants and decide which direction to take along an odor trail according to the direction taken by the other food laden ants (Moffett 1987). The clearest demonstration of this is that laden ants traveling away from the nest are significantly more likely to reverse course on a trail with laden ants traveling in the opposite direction than on an otherwise identical trail with light traffic. This occurs whether or not the ants physically touch. *Atta cephalotes* workers are able to locate active leaf-cutting sites by orienting to the plant-borne vibrations produced by their nest mates (Roces, et al. 1993). In all of these cases, the ants are using cues previously established by nestmates. These nestmates, however, must have used different cues to navigate to the food or to the nest. In some cases, these cues are also proximal. *Serrastruma lujae* foragers are able to follow humidity gradients to find prey and return to the nest directly using path integration and an undetermined compass (Dejean and Benhamou 1993). The army ant *Neivamyrmex nigrescens* uses tactile information to orient along the edges of rocks and fallen trees (Topoff and Lawson 1979). The carpenter ant *Camponotus pennsylvanicus* also orients along the crest-lines of terrain features but stays on top of the crest by not deviating very far from a zero slope as measured by gravity detectors (Klotz et al. 1985), greatly increasing locomotory efficiency (Klotz et al. 2000).

Non visual distal cues

Ants also employ many distal cues for compass information. *Cataglyphis bicolor* workers use wind as a compass cue if other cues are unavailable (Duelli 1972). *Formica rufa* (Camlitepe and Stradling 1995), *Oecophylla smaragdina* (Jander 1998), and *Atta columbica* (Banks and Srygley 2003) use the Earth's magnetic field for compass information when other compass cues are unavailable. *Solenopsis invicta* workers had also been found to use magnetic fields for orientation (Anderson and Vander Meer 1993), but the experimental method was demonstrated to be flawed (Klotz, et al. 1997). *Solenopsis invicta* workers have been shown to respond to magnetic fields when they place brood in their nest (Slowik, et al. 1997), and to sense electric fields (MacKay, et al. 1991) so it is possible that a better experimental design will demonstrate magnetic navigation in this species as well.

Formica polyctena workers are capable of using gravity as a compass cue on an inclined surface (Markl 1964). *Myrmica ruginodis* workers can use gravity to maintain an orientation during an escape run (Vowles 1954). By placing iron filings on different parts of their bodies and subjecting them to magnetic fields, Volwes (1954) determined that the ants have gravity receptors on their antenna. These organs are not actually dedicated gravity detectors, but proprioceptors (Horn 1975). The ants, like most other insects, determine the direction of gravity by comparing information from position proprioceptors and force proprioceptors. Some other insects do appear to have specialized gravity detectors, such as occur in the cockroach genus *Arenivaga*, where specialized structures on the cerci seem to be homologous with the filiform hairs used to

detect wind in other cockroaches (Walthall and Hartman 1981). There are no known specialized gravity receptors in Hymenoptera (Horn 1975).

When insects that have a positive phototaxis (i.e., they tend to go toward light) and a negative geotaxis (i.e., they tend to go up) are presented with a light source while on an inclined plane, they tend to walk in a compromise angle (Horn 1975) indicating that a fusion exists in the processing of these cues. It is likely that more complicated navigational behaviors based on these cues are derived from these taxises and will therefore also involve such a fusion.

1.3.2: Visual navigation
Early research

Research on ant visual navigation began early in the twentieth century when Santschi (1911) claimed that ants are usually guided in their navigation by either vision or olfaction. In a famous experiment demonstrating the role of vision, Santschi (1911) used a screen to block the sun from an ant's view, and a mirror to reflect the image of the sun onto the ant from the opposite direction. A large number of ant species respond to this experiment by reversing their direction of travel so that the reflected image of the sun falls on the same part of the ant's eye as the direct image did before the manipulation. After Santschi, little attention was paid to visual orientation until the 1950's.

Proximal cues

Landmarks are the proximal cues for visual navigation. Jander (1957) found that wood ants use multiple landmarks including trees, buildings, the moon, and the polarization of light in the blue sky. As a result, wood ants are often unresponsive to Santschi's mirror experiment. Wood ants also prefer gravity to light as an indicator of direction and will use gravity on a vertical surface and light on a horizontal surface and will change what they use to orient if the incline of the surface is changed (Jander 1957). In an experiment on landmark use by *Formica rufa* workers, Vowles (1965) employed a T-maze where the walls of the maze were vertical, horizontal, or diagonal stripes. These experiments consisted of a training period when the ants explored the T-maze and a testing period when the ants' decisions were recorded. The ants were released at the base of the maze. One branch of the T led to the nest and the other dead-ended. Vowles found that *Formica rufa* workers can distinguish between vertical, horizontal, and diagonal stripes on the walls of a maze, but cannot distinguish between diagonal stripes of opposite orientation (Vowles 1965). . Vowles hypothesized that the ants do not perceive the form of the stripes, but respond to the rate of light flicker on their ommatidia. More recent studies have focused less on wood ants' visual abilities and more on how wood ants use visual cues to guide their foraging. *Formica rufa* workers show a great deal of route fidelity visiting the same feeding sites and using the same paths for multiple seasons (Rosengren 1971). They will continue to use a route as long as

there is food at the feeding site. They need visual cues to learn the route, but in time they are able to follow the route in the absence of visual cues (Cosens and Toussaint 1985). Cosens and Toussaint attributed this to the development of a spatial concept of the location of the food source, but in light of recent discoveries (Camlitepe and Stradling 1995) magnetic navigation seems a more plausible explanation. Beugnon and Fourcassie (1988) used an artificial tree to put odor cues and visual cues in conflict for *Formica nigricans* workers. The "tree" consisted of a platform on top of a pole with four arms extending off of the platform. Beugnon rotated the platform to change the direction of the odor trail with respect to visual cues. This demonstrated that *Formica nigricans* workers use visual landmarks in preference to odor cues during the day but use the odor cues preferentially at night (Beugnon and Fourcassie 1988). *Formica rufa* workers are able to remember visual cues through the winter dormancy and continue to use the same feeding sites the next spring. They are also able to use olfactory cues for this but only when the visual cues have been altered (Rosengren and Fortelius 1986). Judd and Collett (1998) found that the *Formica rufa* foragers periodically turn around and fixate objects of interest when returning to the nest from a feeding site. These turns are more frequent closer to objects of interest because the image size changes more rapidly at close range. From this, Judd and Collett (1998) proposed that *Formica rufa* foragers memorize these extended paths by creating a sequence of photograph-like images that are recalled as the ant walks along the path (Judd and Collett 1998).

If a *Cataglyphis bicolor* forager is piloting toward her nest and a larger copy of a landmark is substituted for a nest landmark, she will look for the nest farther from the landmark where the two-dimensional image matches the stored image. This fact

demonstrates that *Cataglyphis bicolor* foragers do not see the landmarks they use as distinct three-dimensional objects. Rather they attempt to match the retinal image to a stored template (Wehner and Raber 1979). *Cataglyphis bicolor* foragers only store detailed images of the landmarks near the nest. Away from the nest they still use landmarks, but not for detailed steering. They only use the landmarks for general guidance such as whether to go to the right or left of an object. This is thought to require less memory (Collett et al. 1992). The semi-random nature of *Cataglyphis bicolor* worker foraging might explain why any given homing vector disappears from their memory over 4-6 days while they remember the landmark configuration of their nests their entire lives (Ziegler and Wehner 1997). In contrast, *Formica rufa* workers tend aphids and repeatedly visit the same pasture sites. They seem to store detailed images not only of the area around the nest, but of the area around the pasture site and of landmarks in between. They may even have multiple images of the same landmark from different distances (Judd and Collett 1998).

Other species of ants also use visual landmarks. *Camponotus modoc* workers are able to reestablish a disrupted odor trail by using local landmarks to guide them past the break. If a screen prevents them from seeing prominent landmarks they become disoriented in the area where the odor trail has been disrupted (David and Wood 1980). A direct comparison demonstrated that while *Formica subsericea* workers and *Camponotus pennsylvanicus* workers use the same cues in navigating, *Formica subsericea* workers place a higher priority on visual cues while *Camponotus penssylvanicus* workers rely more heavily on the odor cues (Klotz 1987). However, when researchers shifted the substrate a trail had been laid on so that the trail continued

beyond what had been a branch point, *Camponotus penssylvanicus* workers turned off the trail at the point indicated by the visual cues, and ignored the continuing odor trail (Klotz and Reid 1992). *Camponotus aethiops* workers are able to orient on the basis of a dark stripe on the wall of a white arena, but are unable to use a similar mark on the ceiling of the arena. Although all *Camponotus aethiops* workers appeared to use the same cues, smaller nestmates were more efficient at orienting with respect to these marks than their larger sisters (Laffort, et al. 1991). Several other species of ants also learn landmarks along paths that they frequently use. Like wood ants, individuals show strong fidelity to individual paths. This general pattern of route fidelity based on a knowledge of local visual landmarks has been observed in *Pachycondyla* (formerly *Neoponera*) *apicalis* (Fresneau 1985), *Pachycondyla tesserinoda* (Jessen and Maschwitz 1986), *Temnothorax* (formerly *Leptothorax*) *unifasciatus* (Aron, et al. 1988), and *Dinoponera gigantea* (Fourcassie, et al. 1999). In the case of *Dinoponera gigantean*, the local knowledge of foragers was extensive and they were able to make novel shortcuts and account for new obstacles after a single encounter (Fourcassie, et al. 1999). *Pachycondyla tesserinoda* and *Temnothorax* (formerly *Leptothorax*) *unifasciatus* workers do lay odor trails to particularly good food finds, and they follow these when they recruit nestmates by tandem running. These odor trails are specific to individual ants and they will only follow their own trails. These trails do not seem to be used for finding the nest and removing the odor trail by disrupting the leaf litter has no effect on the ants' nestbound navigation (Jessen and Maschwitz 1986 and Aron, et al. 1988).

Distal cues

The 1950's was also the time when research into the use of distal cues resumed. In that decade it was shown that ants of the genus *Myrmica* respond to the direction of polarization of light (Vowles 1950). Carthy (1951 a, b) found that some ants use distant visual cues to maintain a straight course on a slowly rotating turntable, while others are disoriented by the rotation because rotating the turntable moves the chemical trail they were following home.

The use of distal visual cues for navigation has been most thoroughly studied in the desert ant genus *Cataglyphis*. In the late 1960's Wehner began studying the visual navigation of *Cataglyphis*. Wehner began by demonstrating that these ants use piloting when recognized landmarks are available, but use dead reckoning with a sun compass when there are no available landmarks (Wehner 1969). In the deserts these ants occupy there are quite often no suitable landmarks so they use dead reckoning quite often. It was later found that like some other ants *Cataglyphis bicolor* foragers use the polarization of the blue sky when the sun is obscured. They identify this polarization with a specialized portion of their retina that has polarization filters (Wehner and Raber 1979). This portion of the retina is arranged so that on average the filters give a maximum response when the ant's head is aligned with solar meridian (Wehner 1989). The ants are able to account for the movement of the sun during their journey by extrapolating from recently observed motion (Wehner and Lanfranconi 1981) and by drawing on extended experience with the movement of the sun over the course of a day (Wehner and Muller 1993). The degree to which the ants use polarized light or the direct position of the sun depends on the species of *Cataglyphis* and this difference appears to be driven by genetics and not the

characteristics of the sky in the different habitats these ants inhabit (Horvath and Wehner 1999). In the forest dwelling ant *Pachycondyla* (formerly *Paltothyreus*) *tarsatus*, compass information seems to be derived from the pattern of branches in the canopy (Hölldobler 1980).

While Wehner and colleagues were developing a picture of how *Cataglyphis bicolor* foragers perceive compass information from the sky, they were also developing a picture of how this information is used. The ants use the compass information not only to maintain a straight heading on their return journey, but also to calculate the direct return course based on the twisting outward journey. The ants use an approximation to the true vector sum, which sacrifices a small amount of accuracy for a large decrease in computational complexity (Muller and Wehner 1988). The ants get the compass information for this calculation from the sun or sky polarization, and they get the distance information from the optic flow of the ground beneath them as they walk (Ronacher and Wehner 1995). *Cataglyphis bicolor* workers take torturous paths on their outward journeys while searching for food in the desert.   These journeys may take them over one hundred meters from their nest (Wehner and Lanfranconi 1981). Upon finding food the foragers' approximate calculation allows them to return to within a few meters of the nest entrance. From here they either use piloting if there are conspicuous landmarks (Wehner and Raber 1979) or engage in a systematic search if there are no landmarks (Wehner and Lanfranconi 1981). Even when the ant is piloting (Collett, et al. 1998) or searching (Muller and Wehner 1994) she continues to use her path integration system to update a home vector. This allows the ant to return periodically to the origin of the search or to

correct for a mistaken landmark. The constant updating of the home vector also allows them to deal with enforced detours on their return journey (Schmidt, et al. 1992).

Nearly all of the behavioral experiments on *Cataglyphis bicolor* workers use variations of Wehner's (1969) protocol. Ants are captured either near a feeding site or near the nest and transported to a test location where a grid has been painted on the desert floor. A researcher then follows the ant and records the path of the ant on graph paper corresponding to the grid. When the ant's sensory information is to be restricted a cart is used to restrict the information without restricting the ant's movements. The cart consists of aluminum walls with a skirt at the bottom to prevent wind from reaching the ant. The top of the cart consists of whatever light filters the researcher requires. For some experiments (Wehner and Raber 1979) visual information was further restricted by covering the eye directly with paint.

The landmark and dead reckoning navigation systems in *Cataglyphis bicolor* are not entirely independent. As noted earlier, the ants are calculating a homing vector even while they are piloting. This is probably in part a safeguard against piloting error, but it is also used to calibrate the path integration system (Collett, et al. 1999).

Sun and polarized light compass cues are also important to *Polyergus breviceps* raiders. This slave-making species has become so specialized for raiding *Formica gnava* colonies that the *Formica gnava* slaves are essential for maintaining the *Polyergus breviceps* colony. The *Formica gnava* slaves are taken as brood and are raised in the *Polyergus breviceps* colony. The raids to acquire brood are initiated by scouts that locate the target nests. These scouts use a sun and polarized light compass to navigate back to their own nest. When they return with a raiding party, all of the ants in the raiding party

lay odor trails on the outbound journey. This trail is then used to return to the home nest after the raid (Topoff, et al. 1984). Direction along the trail is still determined by a celestial compass on the return journey so that orientation is disrupted by either disrupting the odor trail, or by blocking the view of the sky (Topoff, et al. 1985). The search pattern displayed by scouts is also similar to that used by *Cataglyphis bicolor* foragers. The scouts travel in a relatively straight line away from the nest followed by a search centered at the end of this straight path (Topoff, et al. 1987). Similar results were obtained for *Polyergus rufescens* raiders. These studies more closely paralleled the displacement experiments with *Cataglyphis bicolor* workers. The scouts behaved essentially like *Cataglyphis bicolor* foragers and followed their global vector to the location of a fictive nest where they began to search. The fictive nest was the location on the experimental grounds that was in the same location relative to the release site that the nest was to the capture site. Recruited raiders did not seem to generate a global vector and began searching immediately after displacement (Grasso, et al. 1996). The reliance of the raiders on the scout for guidance seems to be absolute and when the scout ant was removed from the outbound raiding party, the raiding party generally broke up and returned to the nest (Grasso, et al. 1997).

A different kind of distal cue used by some ants for orientation is the pattern of light filtering through the canopy of a jungle. This phenomenon was first observed in *Pachycondyla* (formerly *Paltothyreus*) *tarsatus* (Hölldobler 1980). Ants were allowed to forage in an arena where the ceiling was a photograph of a jungle canopy. When the photograph was rotated by 90 degrees, the ants shifted their orientation accordingly. Canopy orientation has also been observed in *Odontomachus bauri* Emery (Oliveria and

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Hölldobler 1989). In these experiments the ants seemed to use the canopy pattern as a pure compass indicator. However, in the wild some ants that use canopy orientation may forage far enough for canopy patterns to change. In this case it would be more of a special type of landmark.

1.4: Cue Hierarchies

It is tempting and sometimes useful to assign hierarchies of cues to different species of ants. Animals in general use navigational cues in a hierarchical manner, abandoning a cue only after it has become unavailable (Able 1980). However, it must be remembered that these hierarchies can be subject to context. Prior experience has been shown to cause some ants to prefer vision to olfaction. *Leptothorax unifasciatus* workers lay individual odor trails to profitable food sources, but when visual cues are made to disagree with these odor trails the ants follow the visual cues. These ants do not follow other individuals' trails so naïve ants essentially chose their paths at random (Aron, et al. 1988). The Giant Tropical Ant *Paraponera clavata* does use odor trails for recruitment. These ants use the odor trail on their first trip to a food source but will follow visual cues in preference to the odor cues on subsequent trips (Harrison, et al. 1989). A comparative study of *Lasius niger* and *Iridomyrmex humilis* found that the former adopts visual orientation with experience while the latter continues to prefer odor cues (Aron, et al. 1993). The chief advantage of using visual cues that have been learned by following an odor trail seems to be that ants can follow visual cues more quickly than odor cues (Harrison, et al. 1989). Both the Aron and the Harrison studies used Y-mazes to force the

ants to choose between odor cues and visual cues. Aron's experiments were conducted in a lab with a light bulb as the only visual cue. Harrison's experiments were conducted in the field with natural visual cues. The conflict between visual and olfactory cues was accomplished exclusively by moving the papers covering the Y-maze branches.

1.5: Visual Navigation in *Atta*

Very little research has been done with visual orientation of *Atta*. A study of orientation in *Atta cephalotes* and *Acromyrmex octospinosus* found that *Atta cephalotes* workers use visual cues to orient along an odor trail and possibly to choose between odor trails (Vilela, et al. 1987). However, no mention was made of what these visual cues might be and visual cues and odor cues were never put in direct conflict. Visual orientation has also been shown for *Atta laevigata* (Jaffe, et al. 1990). This study demonstrated that vision facilitates trail following and that *Atta laevigata* foragers navigate better with just vision than with just odor. This study also demonstrated that in the presence of light *Atta laevigata* workers learn to run a Y-maze more quickly while there is no such improvement in the dark. Again light and odor cues were not put in direct conflict and the nature of the visual cues was not explored. Both sets of experiments employed Y-mazes formed from tubes. Visual cues were made accessible or inaccessible by using transparent or opaque tubes. The mazes were always either completely transparent or completely opaque.

1.6: Neural Anatomy

Navigation probably requires some processing in every sub-compartment of the ant's brain. These include the antennal lobes, the optic lobes, the mushroom bodies, the central body, and possibly the central bridge. The antennal and optic lobes appear to be the primary processing areas for olfactory and visual information respectively. The mushroom bodies receive input from the antennal and optic lobes and are thought to mediate complex behaviors (Gronenberg and Hölldobler 1999). The clearest evidence of the mushroom bodies mediating associative learning comes from experiments in which cooling the alpha lobe of a mushroom body of a honeybee prevented the bee from developing the proboscis extension response to a rewarded olfactory stimulus (Erber, et al. 1980). Experiments with cockroaches (Mizunami, et al. 1993) and fruit flies (de Belle and Heisenberg 1994) have shown that lesions to the mushroom bodies prevent insects from using far field visual or olfactory cues to locate themselves in space although use of landmarks is not lost. In ants in particular, a correlation has been shown between the relative volume of the mushroom bodies innervated by the optic lobes and the complexity of a species' visually mediated behavioral repertoires (Gronenberg and Hölldobler 1999). The central body receives input from all of the previously mentioned subcompartments and seems to also be associative. The central body is also the source of premotor outflow to the nerve cord. The central bridge connects the lateral parts of the central body. In navigation it could be involved in side to side comparisons of input.

1.7: Summary chapters 2-7

In chapter 2, I examine orientation of *Atta cephalotes* workers in the laboratory. Laden nest-bound foragers were moved from a "bridge" with or without trail pheromone present and placed on a parallel bridge with or without pheromone. Foragers moved from bridges with pheromone to another with pheromone and foragers moved from bridges without pheromone to another with without pheromone usually continued to orient toward the nest. Foragers moved from bridges with pheromone to bridges without pheromone did not orient preferentially towards the nest, but instead moved back and forth on the bridge. This study suggests that although *A. cephalotes* foragers do not need trail pheromone to orient, foragers moved from a pheromone trail do not orient towards their nest because they are searching for the suddenly absent trail.

In chapter 3, I continue to examine orientation of *A. cephalotes* foragers in the laboratory. Foragers walked on a single bridge and I altered various cues and contexts and recorded which manipulations caused the ants to reverse course. On a horizontal bridge there was always a significant response to a light source moving from one side of the bridge to the other. This response was significantly smaller for nest-bound ants that were non-food laden. The ants failed to reverse course for cues other than light sources or for light sources when the bridge was vertical.

In chapter 4, I put orientation cues into direct conflict by letting the ants forage on a Y-maze. Foragers that were returning to a food source preferred visual cues to odor cues while recruited foragers consistently used odor cues. There was no significant effect from kinesthetic cues or magnetic cues.

In chapter 5, I use a vertical T-maze to investigate the role that gravity plays in *A. cephalotes* navigation. The gravitational cue was put in direct conflict with odor cues and light cues. There was an asymmetry to the ants' response to the gravity cue in that ants returning to a food source had a tendency to go up regardless of the previous position of the food source or the position of the odor trail. Recruited ants consistently followed the odor trail regardless of its direction. Introducing a light cue changed the angle required to make the ants respond to the gravitational cue. This suggests that the fusion of response observed in geotaxis and phototaxis also exists in more sophisticated forms of navigation.

In chapter 6, I investigate the anatomy of *A. cephalotes* eyes and brains. Based on tissue sections, I measured the angles between adjacent ommatidia in the eyes, and the volumes of sub-compartments of the brain. These results are compared to my measurements of *Formica exsectoides* and measurements from the literature.

In chapter 7, I use the results from the other chapters to inform my speculations about the nature and neural basis of *A. cephalotes* navigation. I develop an hypothesis of navigation in the wild and a simple model of its neural underpinnings.

Chapter 2

Orientation when removed from a pheromone trail.

This chapter is based on a published paper (Vick 2004) with suitable format changes.

2.1: Introduction

A field study (Wetterer, et al. 1992) demonstrated that *Atta cephalotes* workers displaced from a natural bridge on a trail to a parallel bridge placed by a researcher became disoriented if the placed bridge lacked a pheromone trail, but oriented without difficulty if a pheromone trail had been laid on the bridge. It was unclear if the observed disorientation was the result of an inability to orient in the absence of a pheromone trail, or if the ants were searching for the suddenly absent trail.

In this study I examined the cause of the disorientation. By moving the experiment into a laboratory setting, I was able to examine the effect of displacement on ants that were not initially following a pheromone trail. This allowed me to differentiate between the hypothesis that *A. cephalotes* need a pheromone trail to orient properly and the hypothesis that they are searching for the missing trail.

2.2: Methods

This study used two colonies of *Atta cephalotes* that were collected in Trinidad in the summer of 2002, and are kept at the Smithsonian National Museum of Natural History. Each colony consists of 2-3000 workers and a fertile queen. This is much smaller than a mature wild colony, which may contain as many as 2-3 million workers

(Weber 1982). The small colony size resulted in an immature worker size distribution (Wilson 1983), and foraging strategy (Wetterer 1999). The colonies were kept in three 10 cm X 10 cm X 15 cm plastic boxes, which were connected to a 45 cm X 70 cm foraging arena. The walls of the arena were greased with mineral oil to prevent escape. The colonies were fed a diet of privet leaves and citrus rind. At any given time, 20 - 80 ants were painted using a xylene-based paint pen with a unique color pattern on the back of the head, the top of the thorax, and the top of the gaster.

The experiments were conducted on a double path apparatus (Fig. 2.1). Depending on the condition, the double path was either two copies of the single path placed side by side, or one copy of the single path and a sham path that does not have a platform or an entrance ramp. All of the experiments were conducted on the nest bound leg of an ant's foraging trip. A loose piece of path material was placed at the midpoint of the path. When a nest bound ant stepped on this piece of path, the piece was moved to the other path. For each condition, 'trail' indicates that the ants had laid a recruitment pheromone trail on the path for 30 minutes before the experiment began, 'non-trail' indicates that the tape covering that path had been replaced so that the recorded ant was the first ant on that path surface. For each trial, I recorded whether the ant "Continued" to the end of the path closest to the nest, or "Reversed" and reached the end of the path farthest from the nest. I also measured a "Search index," the number of times each ant switched its direction of movement along the path during the trial. Three of the four possible permutations (trail to trail, trail to non-trail, and non-trail to non-trail) were tested.

2.3: Results

The Non-trail to non-trail results (Table 2.1) did not differ significantly from the Trail to trail results ($X^2 = 3.16$; d.f. = 1, $P > 0.05$) but did differ significantly from the Trail to non-trail results ($X^2 = 30.93$; d.f. = 1, $P < 0.0005$). The results from the first two conditions essentially confirmed field observations by Wetterer, et al. (1992), indicating that nest-bound ants moved from a trail to a non-trail do not continue to their nest, but instead appear to search for the trail they were removed from. The general trends observed in the field were preserved in the lab, but the ants in the lab were significantly better at orienting in the home direction in the trail to trail condition ($X^2 = 9.32$; d.f. = 1, $P < 0.005$). The results in the trail to non-trail were not significantly different ($X^2 = 0.14$; d.f. = 1, $P > 0.25$). The results from the third condition indicate that ants that start on a path without a pheromone trail do not engage in search behavior when moved to a path without pheromone trail. This result rules out the possibility that the ants in the second condition are disoriented because they need the pheromone trail to navigate after a disturbance.

Table 2.1. The effect of transferring nest-bound ants on their direction of travel.

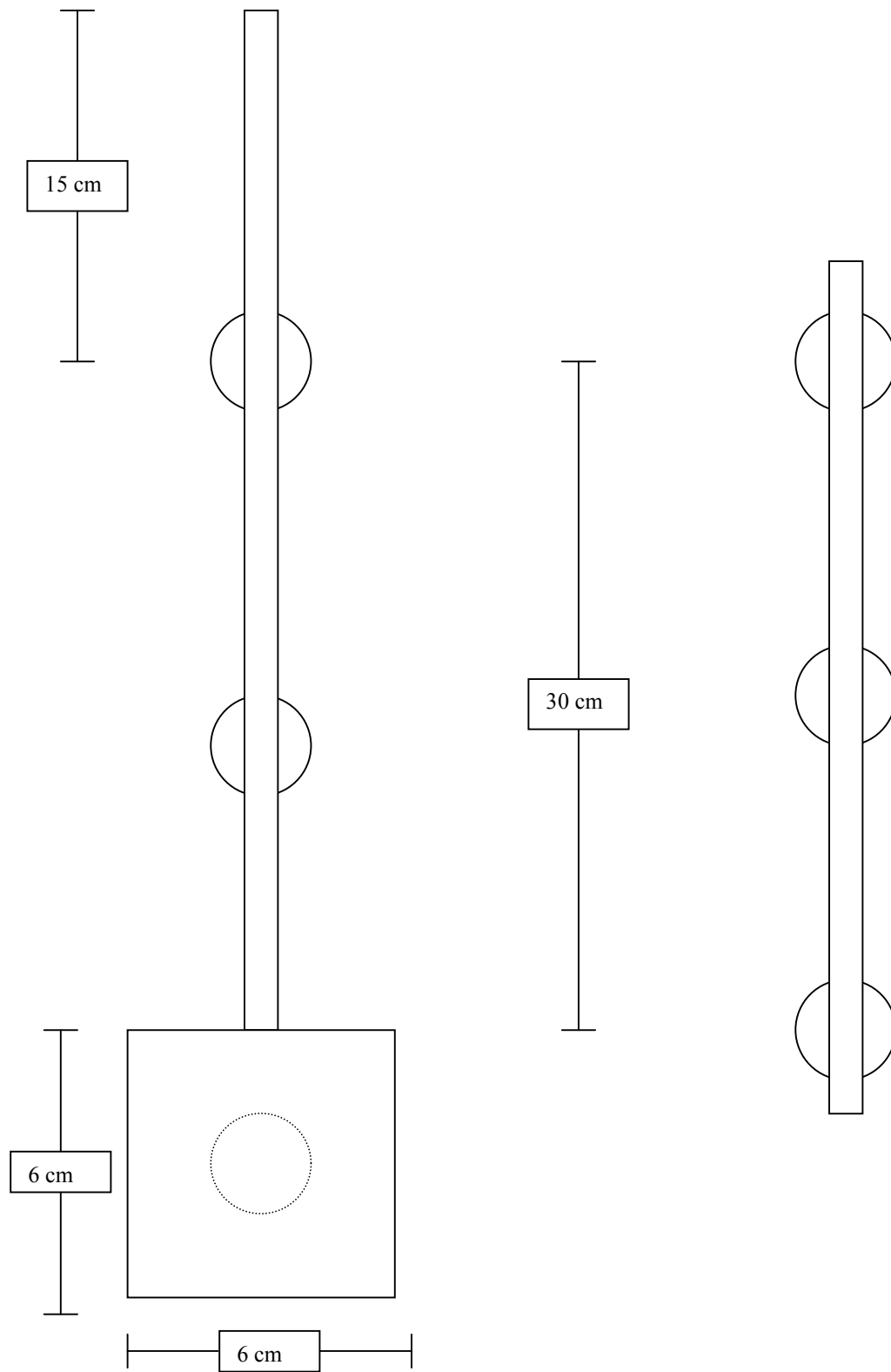|	Direction of travel		
	Continued	Reversed	Search index (+ 1 s.d.)
Trail to trail:	49	2	0. 10 $\pm$ 0. 30
Trail to non-trail:	23	27	5. 40 $\pm$ 3. 20
Non-trail to non-trail	43	7	0. 26 + 0.66

Fig. 2.1: Diagram of Path and Sham Path used in two path experiments.

2.4: Discussion

As in the Wetterer, et al. (1992) study, this study found that *A. cephalotes* do not consistently resume a nest bound course when moved from a path with trail pheromone to a path without trail pheromone. Instead the ants move back and forth along the path changing direction several times before reaching a path end at random. This study also confirmed the earlier result that ants moved from a path with trail pheromone to another path with trail pheromone orient toward the nest most of the time. In fact the laboratory ants in this study oriented slightly better than the wild ants in the earlier study probably because it was easier for the ants to identify the direction of the nest in the relatively simple lab environment.

The behavior of ants moved from a non-pheromone path to a non-pheromone path was indistinguishable from the behavior of ants moved from a pheromone path to a pheromone path in terms of final destination or number of direction reversals. This indicates that it is not the presence of the pheromone that allows the ants to orient. Rather this suggests that the reason ants moved from a pheromone path to a non-pheromone path move randomly is that they are searching for the missing pheromone trail. However, if the ants are able to orient toward the nest without the pheromone trail, why should they waste time and effort searching for it when it disappears? One possible answer is that the trails do more than help the ants orient. For well traveled trails, the ants often clear debris from the ground covered by the trail (Hölldobler and Wilson 1990). This creates a physical trail that is coexistent with the pheromone trail. This physical trail may serve as a 'structural guideline' (i.e. a relatively smooth and straight

terrain feature), which is more efficient for ants to follow (Klotz, et al. 2000). The trails

may also be a safer environment where the worker is much less likely to encounter a

spider or other predator. Finally, the ants may be able to orient for short distances (like

those in the lab) without the use of a trail, but may need the pheromone trail to navigate

larger distances making it important to locate a lost trail.

Chapter 3

The role of context in the response to a changing cue

3.1: Introduction

Santschi (1911) established that some species of ants use the position of the sun to maintain a heading so that if a researcher reverses the apparent position of the sun, the ant reverses course. Here, I investigate the role of context in this behavior for *Atta cephalotes*. The contextual factors I considered include differences in the behavioral state of the ants, such as whether they are food laden; differences in the path environment, such as whether the path is vertical or horizontal; and differences in the reference landmark, such as whether it is a light source or a dark pole.

3.2: Methods

I performed three different types of experiments. The second and third types were variations on the first. The first experiments used a single path apparatus (Fig. 3.1). The entrance ramp led to a single 30 cm long path that ended in a platform. Lamps were placed on either side of this path.

The ants were allowed to freely move on the path. Painted ants were identified either at the top of the ramp or on the feeding platform depending on the experiment. When an identified ant reached the midway point of the path, the lamp that was on was turned off and the lamp that was off was turned on, reversing the direction of lighting. The direction the ant traveled was then recorded. The ant was considered to have continued if it reached the end of the path it was heading toward. It was considered to have reversed if it made it to the end of the path it started from. Nest-bound ants were
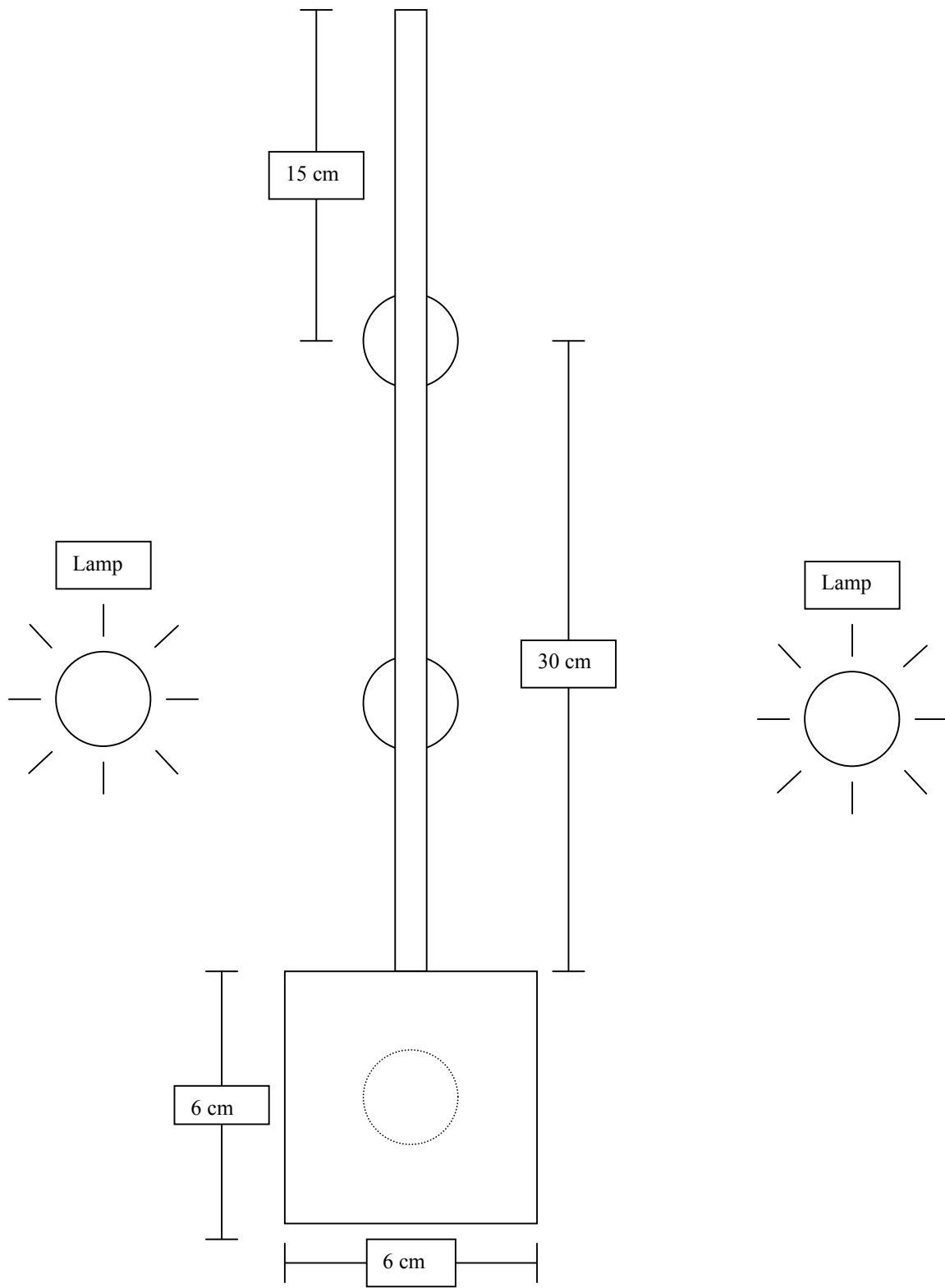
Fig. 3.1: Diagram of Single Path Apparatus

food-laden, non-food-laden, on trail, and with no trail. Outbound ants were all non-food laden and were tested "trail" and with "non-trail". In "trail" tests, the ants were allowed to lay a pheromone trail for half an hour before the experiment began and that food was present during the entire experiment. In "non-trail" tests, the tape covering the path was replaced between each recording. Food laden non-trail ants were probably laying a trail and so the path was not entirely pheromone free for the entire trial, but the ant was not following a preexisting trail. Each condition also had two control experiments where the lamp was either never on, or turned off without turning on the opposite lamp.

The second set of experiments used a vertical path apparatus. The concept of the vertical path apparatus (Fig. 3.2) was essentially the same as that used for the horizontal path experiments. This used a 50 cm tall wooden dowel with a platform on top. One side of the dowel was covered in tape smeared with mineral oil. This forced the ants to use only one side of the dowel. The lamps were positioned so that they shone on the dowel at the halfway point. The experiment was otherwise identical to the food laden, nest bound, trail following condition of the horizontal single path.

In the third experiment, the lamps were replaced with other stimuli on the horizontal single path. In the first of these a dark pole was placed where the lamps were and moved to the other side when the ant reached the midpoint of the path (Fig 3.3). In the second, a set of walls was constructed with dark and light bands such that to one side of the path the bands were horizontal and to the other side the bands were vertical. A thick band width was used to ensure the bands were viewed by different ommatidia. When the ant reached the midpoint of the path, the set of walls was rotated so that the patterns appeared to be on the other side (Fig 3.4).
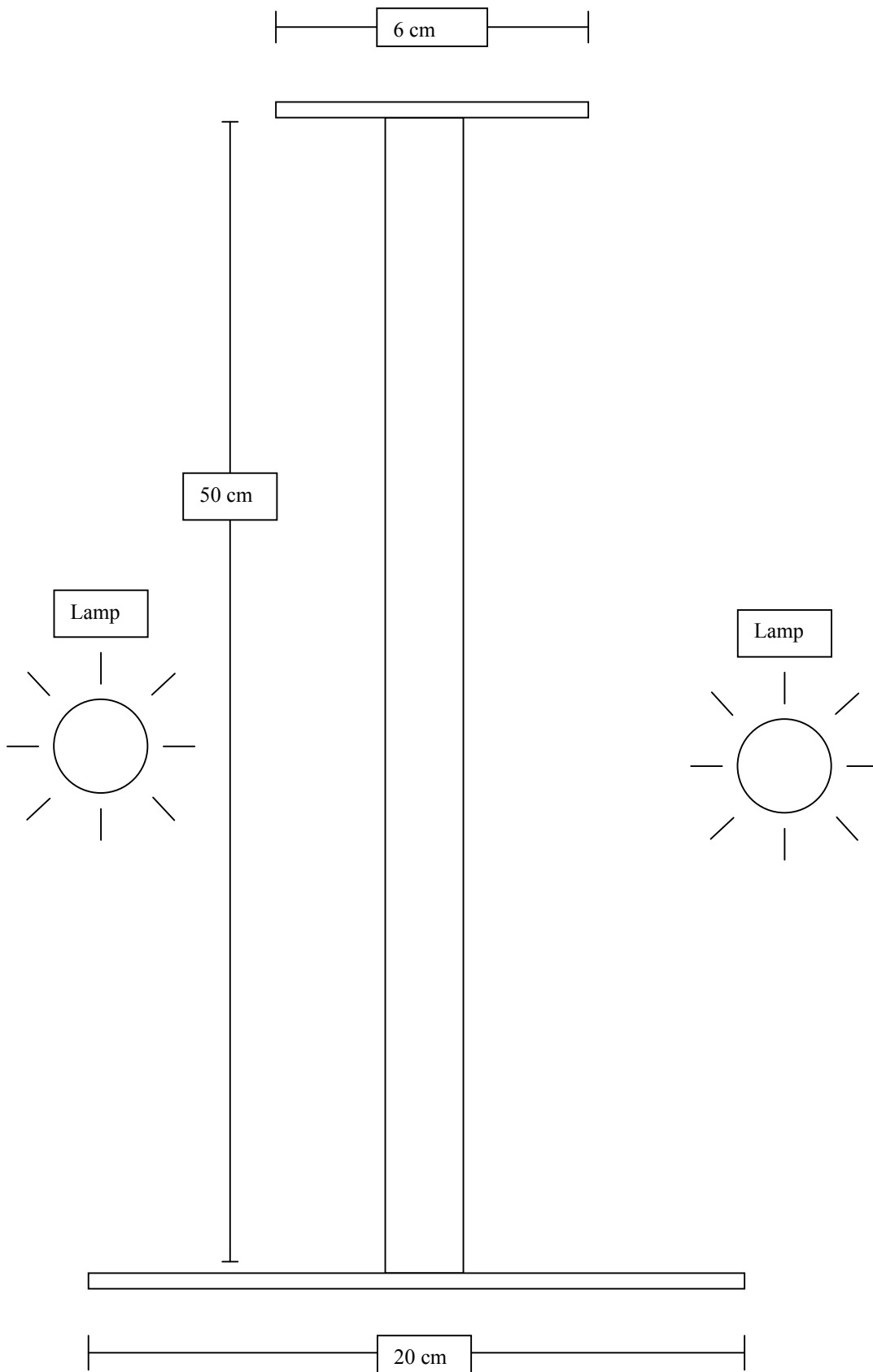
Fig. 3.2: Diagram of Vertical Single Path Apparatus. Back side of vertical dowel is covered in tape that has been smeared with mineral oil to prevent ants from
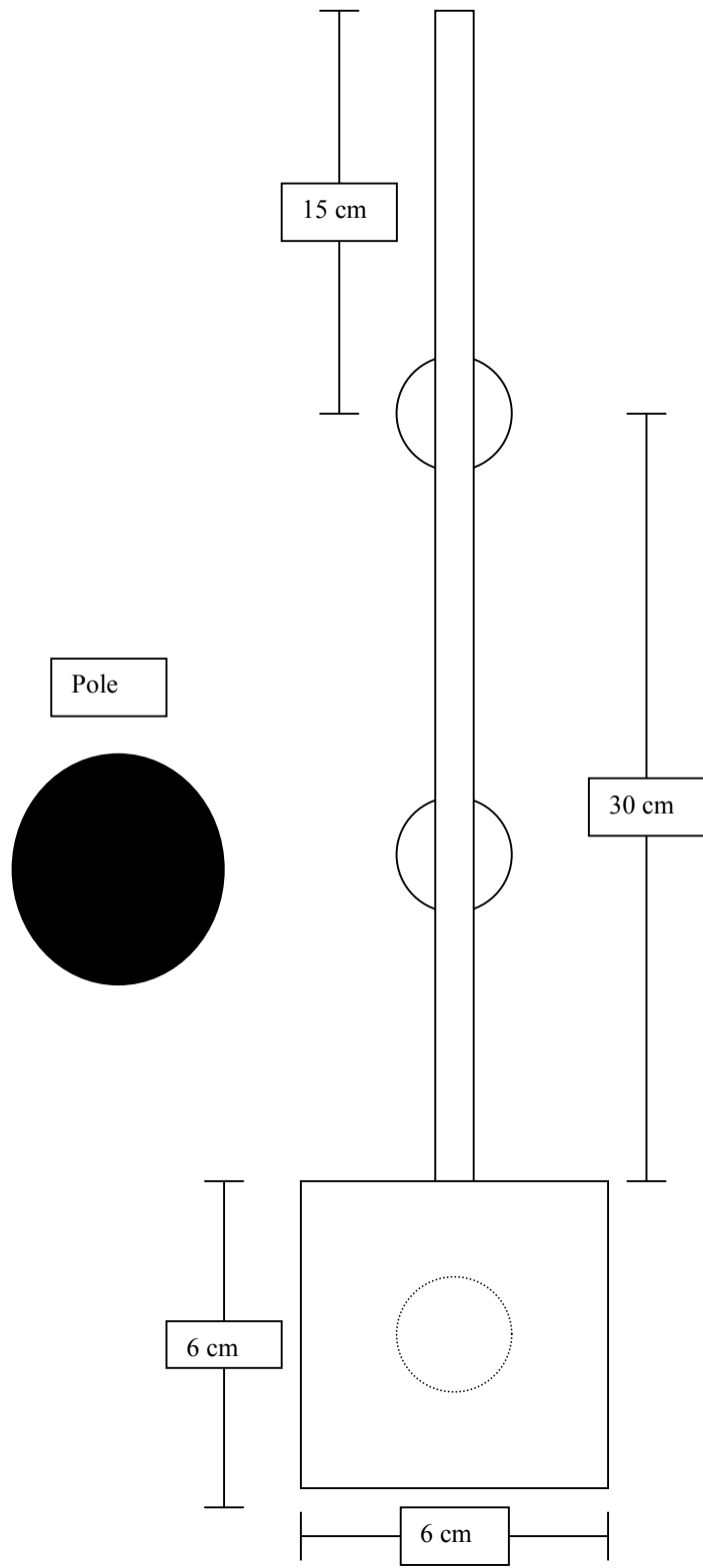
Fig. 3.3: Diagram of Single Path Apparatus with the pole substituted for the lamps. In the experiment two poles were used. When the ant reached the halfway point one pole was hidden and the other placed in sight.
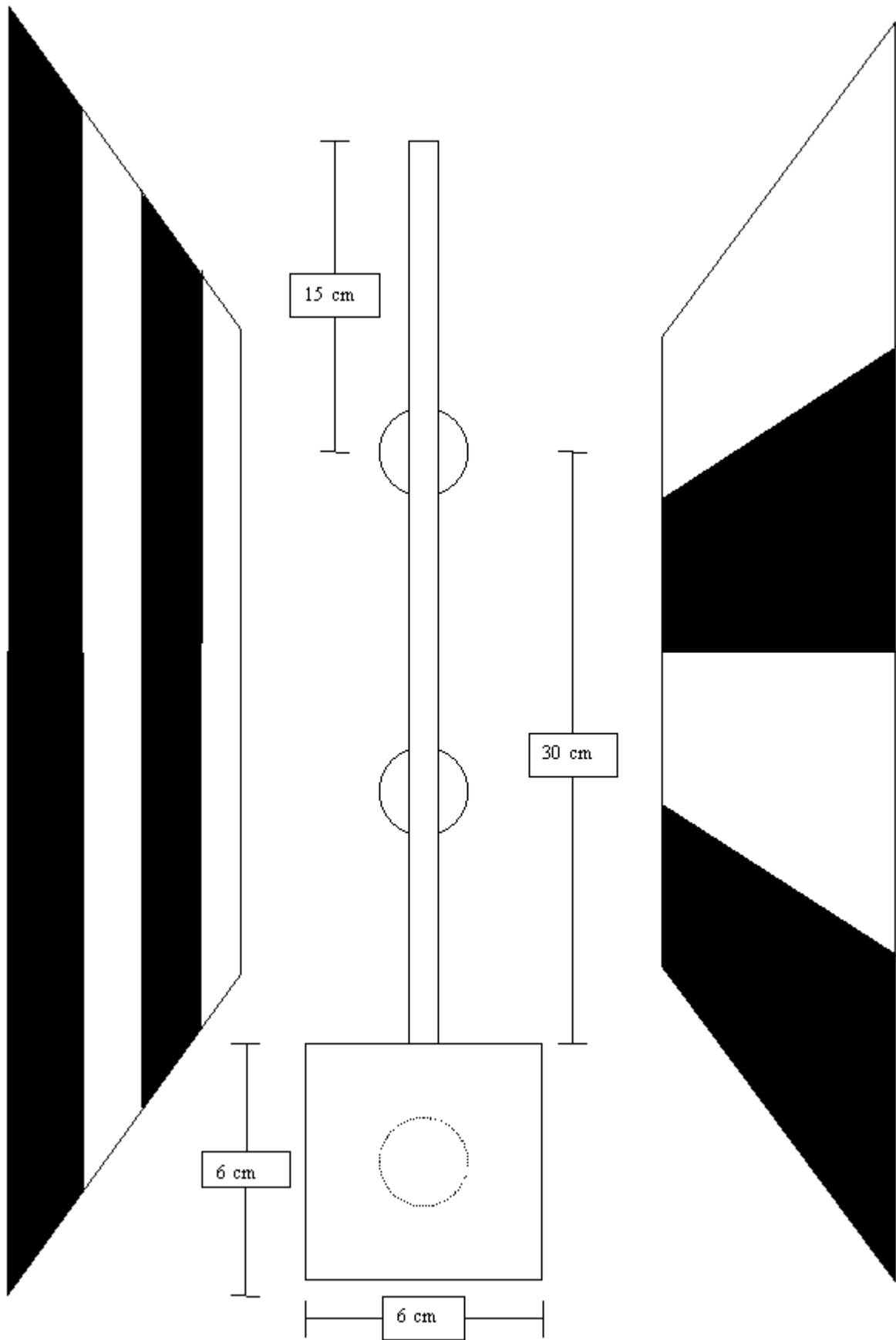
Fig. 3.4: Diagram of Single Path Apparatus with striped walls.

3.3: Results

Reversal of primary light source

In each of the first five conditions, reversing the direction of lighting had a significant effect on the ants' direction of travel, indicating that light direction is an important source of orientation information (Table 3.1). Only in the case of a vertical path did reversal of light have no effect (Table 3.2). In this case, gravitational cues apparently superceded the light cues.

-
Table 3.1. The effect of reversing primary light source on direction of travel.

	Direction of travel		
	Continued	Reversed	X^2
Nest bound, laden, trail			
Control	75	0	
Reversed light	1	49	120.89 ***
Nest bound, laden, non-trail			
Control	67	8	
Reversed light	1	49	92.24 ***
Nest bound, non-laden, trail			
Control	62	13	
Reversed light	31	19	6.73 **
Nest bound, non-laden, non-trail			
Control	68	7	
Reversed light	36	14	7.48 **
Outbound, non-laden, trail			
Control	99	1	
Reversed light	10	65	133.89 ***
Outbound, non-laden, non-trail			
Control	70	5	
Reversed light	15	35	55.30 **
** $P < 0.01$; *** $P \ll 0.0005$			

-
Table 3.2. Response to light reversal on vertical and horizontal paths.

| | Direction of travel | | |
	Continued	Reversed	X^2
Horizontal Path	1	49	
Vertical Path	50	0	96.08 ***
*** P << 0.0005			

In the two conditions where the ants were nest bound and non-laden, they were much less likely to reverse course ($X^2 = 101.87$; d.f. = 1, P << 0.0005). These conditions are also the only ones where the ants do not have a clear destination. Outbound ants are generally movivated to get to the food source, and food laden ants are generally motivated to get to the nest..

Reversal of other stimuli

I found that reversing the position of the black pole and reversing the stripe pattern on the walls had no significant effect on the direction of travel (Table 3.3).

-
Table 3.3. The effect of reversing other stimili on direction of travel.

| | Direction of travel | | |
	Continued	Reversed	sig.
Nest bound, food laden, trail			
Control	75	0	
Reversed pole	49	1	n.s.
Reversed stripes	50	0	n.s.

-

3.4: Discussion

The horizontal single path experiments that used lamps were functionally identical to the Santschi mirror experiments. *A. cephalotes* workers proved to be very responsive to this type of light source reversal when they were on a horizontal path. Even the least responsive group (non-laden nest bound ants) reversed course significantly more than controls. The fact that non-laden nest bound ants were less responsive to the change in light source is noteworthy. This is the only group of ants in this study that does not have a presumed destination. Food laden ants are presumed to be heading for the nest and outbound ants are presumed to be heading to the food source. Many of the non-laden nest bound ants may be involved in local tasks that do not involve navigation. These ants may be more locally focused on cues such as trail edge and may ignore navigational cues such as light direction. In contrast, *A. cephalotes* workers were entirely unresponsive to reversals of light when they were on a vertical path, or to reversals of other visual cues on a horizontal path. The failure to respond on the vertical path indicates that the gravitational cue takes precedence in this case.

That the ants failed to respond to landmark cues indicates that there is something special to *A. cephalotes* foragers about light source cues. The dark poles and patterns were large enough to be seen by several ommatidia, and the black figures on white backgrounds should have provided adequate contrast for the cues to be seen, so it seems likely that the cues were seen but ignored. The reason for this may be that in the jungle there is an abundance of visual cues that would be quite taxing to track. In contrast, light source cues would form a relatively small subset of visual cues that would be limited to the position of the sun or possibly to the positions of a relatively small number of breaks

in the canopy. These cues would not only be sparser than landmark cues, they would be

more stable over relatively long distances.

Chapter 4

Responses to conflicting odor and light cues

4.1: Introduction

Two previous studies have investigated the cue hierarchy in *Atta* navigation (Vilela, et al. 1987, Jaffe, et al. 1990). These studies both examined the time it took individual ants to solve a maze in the presence or absence of various cues. These studies found that *Atta cephalotes* and *Atta laevigata* foragers navigate best in the presence of both visual cues and odor cues, next best in the presence just odor cues, next best in the presence of just visual cues and at random when neither cue is present. These results were judged both by the amount of time spent in the maze and by the arm of the maze chosen and seem to reflect a clear hierarchy of cues. However, because the cues were never placed in direct conflict, where both cues were present but indicated different destinations, the described hierarchy expresses which cue gives the ant a higher success rate and not necessarily which cue the ants prefer to use. Furthermore, these studies did not differentiate between new recruits and ants that had previously been to the food source. This creates a bias in the results against visual cues because the new recruits do not have the memory necessary to make use of visual cues.

In this chapter I examine the cue preferences of ants that have previously visited a food source with those lacking previous experience. Odor and light cues are put into direct conflict and the nature of the visual cue is examined by different manipulations of the light source.

4.2: Methods

This experiment used a Y-maze apparatus (Fig. 4.1). The entrance arm of the Y-maze was 15 cm long. Each arm was 15 cm long. The platforms were squares 6 cm on a side. The entire maze rested on PVC pipes 4 cm above the foraging arena floor. A 15 cm long ramp led from the arena floor to the maze entrance. The PVC pipes were oiled to prevent the ants from climbing onto the maze at points other than the entrance. The circles in the diagram of the maze indicate the positions where the lamps were placed roughly 15 cm above the arena floor. The paths were 1 cm wide and covered in vinyl electrical tape.

At the beginning of each Y-maze trial, the lamp was placed in the right (R), left (L), or center (C) position. Food was placed in the position of the initial trail (R or L). For the following hour or half-hour the food was watched and the identities of all painted ants visiting the food was recorded. The configuration of the maze was then changed by moving the lamp, moving (or replacing) the tape on the branches, or both. Painted ants were then identified as they entered the maze. When an ant reached the decision point at the center of the maze, the direction that ant continued in (Right, Left, or Back) was recorded. A decision was scored when the ant reached a platform or the entrance ramp. Since the lamp was on the left, right, center, or off, these positions are designated L, R, C, and O respectively. Similarly, the pheromone trail can be on the left branch, the right branch, or non-existent, designated by L, R, and O. Individual experiments can be designated by listing the before and after positions of the lamp and the before and after positions of the trail. For example, if the lamp was moved from left to right and the trail
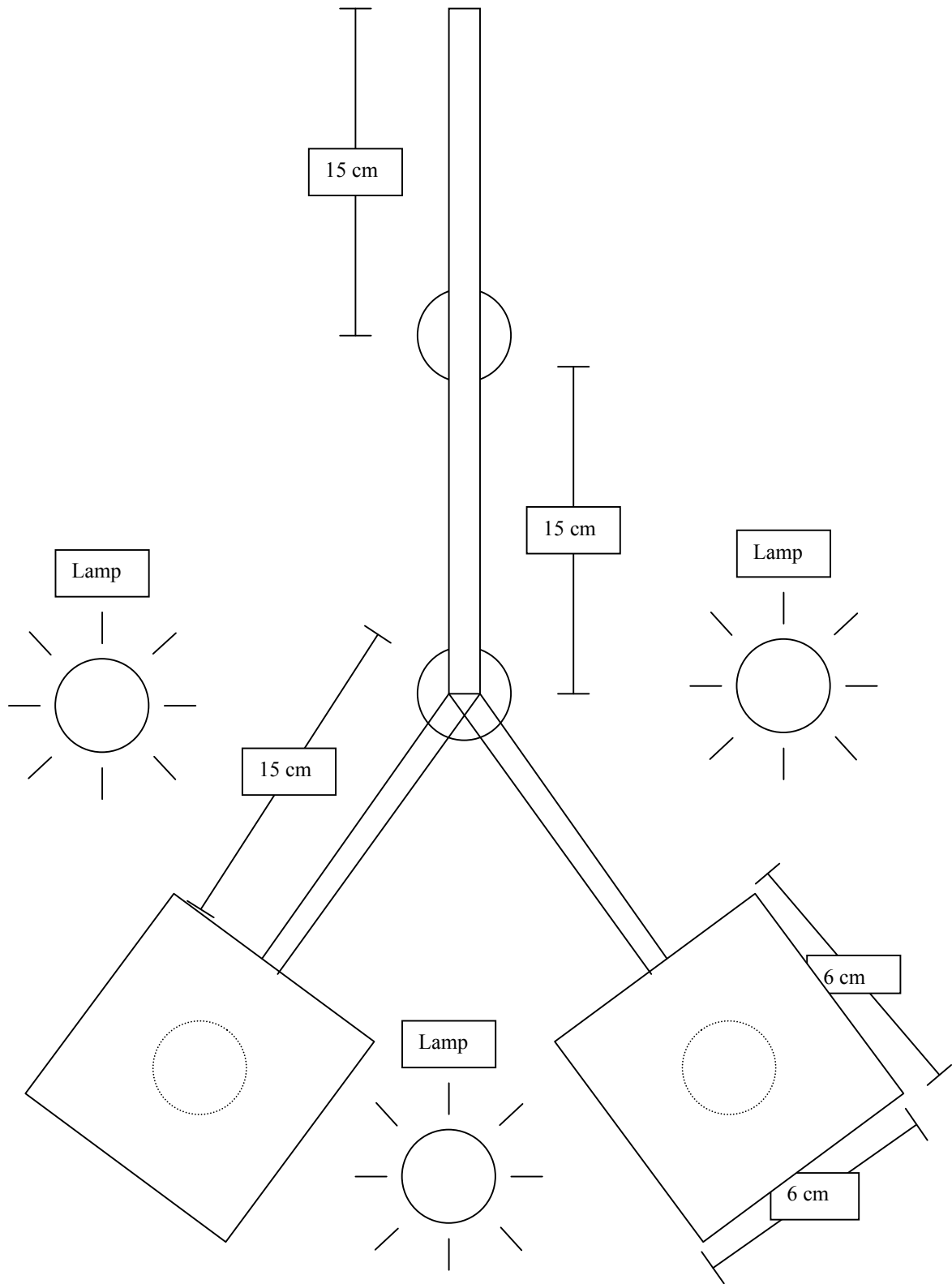
Fig. 4.1: Diagram of Y-maze Apparatus. Lamp positions indicated possible
lamp positions. At any given time, two positions were occupied and only
one lamp was on.

was moved from right to left the experiment would be designated LR RL. In the last condition, the lamp was placed in the direction of the base of the Y maze and this is indicated with a B. In all trials, the original trail was to the right, so choosing the right branch was consistent with unaltered navigation. The following configurations were tested. RR RR, RC RR, RL RR, RO RR, RR RL, RC RL, RO RL, RR RO, RC RO, RO RO, CC RR, CR RR, CL RR, LL RR, LR RR, LC RR, LB RR .

In each experiment the recruited ants were a control for the behavior of the experienced ants. Recruited ants are those ants that made their first visit to the food source during the recording portion of a trial. A significant difference in the behaviors of experienced ants and recruits indicates that the experienced ants were using cues that were unavailable to the recruits.

4.3: Results

Table 4.1. Results of Y-maze experiments

Light and Trail unchanged (Baselines)

Lt. Tr.		Left	Right	Back	X^2 (L & R)
RR RR					
	Recruited	2	18	0	
	Experienced	6	16	1	2.92 * (2.03) *
CC RR					
	Recruited	1	13	0	
	Experienced	3	17	1	1.18 * (0.49) *
LL RR					
	Recruited	4	13	1	
	Experienced	1	31	0	6.77 ** (5.04) **

Light and Odor cues altered but indicated same trail

		Left	Right	Back	X^2
RC RL					

	Left	Right	Back	X^2
Recruited	12	2	2	
Experienced	12	0	1	2.04 * (1.86) *

Light and Odor cues indicate different trails

	Left	Right	Back	X^2
RC RR				
Recruited	4	15	0	
Experienced	21	7	0	(13.23) ***
CL RR				
Recruited	0	8	2	
Experienced	18	10	4	10.15 ** (10.29) **
RR RL				
Recruited	9	2	0	
Experienced	8	11	0	(4.47) **
LB RR				
Recruited	0	11	0	
Experienced	10	13	3	8.48 ** (6.78) **

No Light Cue

	Left	Right	Back	X^2
RO RR				
Recruited	7	11	4	
Experienced	5	19	2	2.82 * (1.64) *
RO RL				
Recruited	14	3	1	
Experienced	14	3	2	0.31 * (0.00) *

No Odor Cue

	Left	Right	Back	X^2
RR RO				
Recruited	3	8	2	
Experienced	2	18	0	4.78 * (1.57) *
RC RO				
Recruited	7	8	1	
Experienced	15	3	4	6.19 ** (4.95) **

No Cues

	Left	Right	Back	X^2
RO RO				
Recruited	15	17	1	
Experienced	10	10	4	3.28 * (0.05) *

Ambiguous Light Cues

	Left	Right	Back	X^2
RL RR				
Recruited	5	6	2	

Experienced	8	8	8	1.43 * (0.05) *
CR RR				
Recruited	0	7	0	
Experienced	0	24	1	0. 11 *
LR RR				
Recruited	0	5	1	
Experienced	2	18	5	0. 59 * (0.54) *
LC RR				
Recruited	1	5	0	
Experienced	0	13	2	3.31 * (2.29) *

* P > 0.05; ** P < 0.05; *** P < 0.0005

Figures 4.2-4.7 display the total results for the cue categories. These figures clearly indicate that recruited ants follow the odor trail when it is available and navigate randomly when there is not an odor trail. Experienced ants reliably follow the light cue when it is available but will use the odor cue if the light cue is absent or ambiguous and will only navigate randomly when no cues are present.

4.4: Discussion

The results of these experiments indicated a strong tendency for experienced ants to use visual cues when they are available. The specific visual cue they use seems to be the position of a light source. The results of the trials with ambiguous light positions demonstrate that the ants are using the position of the light source rather than brightness of illumination on the path. If they were using the latter cue then the RL RR experiment would have led to better orientation than the RC RR experiment, but this did not happen.

Despite the clear preference for light source cues *A. cephalotes* foragers displayed in these experiments, the results may not reflect a rigid hierarchy of cue use. It may be that the light cues in this experiment were particularly salient or that the odor cues were particularly weak. The results certainly demonstrate that both were detectable, but the

results are consistent with the ants choosing a direction based on a weighted comparison of cues where the light cues generally won in this context.

It may be the case that *A. cephalotes* foragers have a rigid hierarchy of cues with vision over olfaction, or they may use whichever cue is more salient. These experiments indicate that they do not have a rigid hierarchy of cues where olfaction dominates over vision.
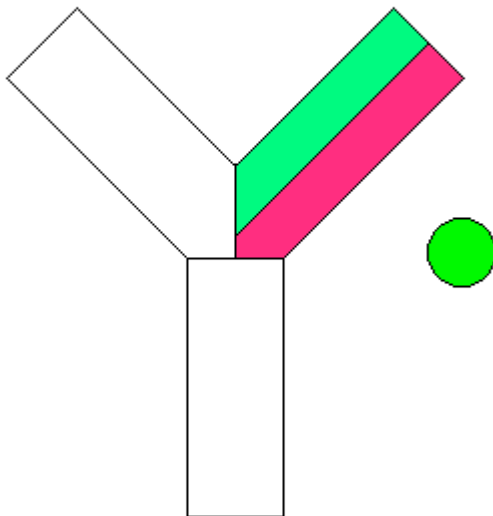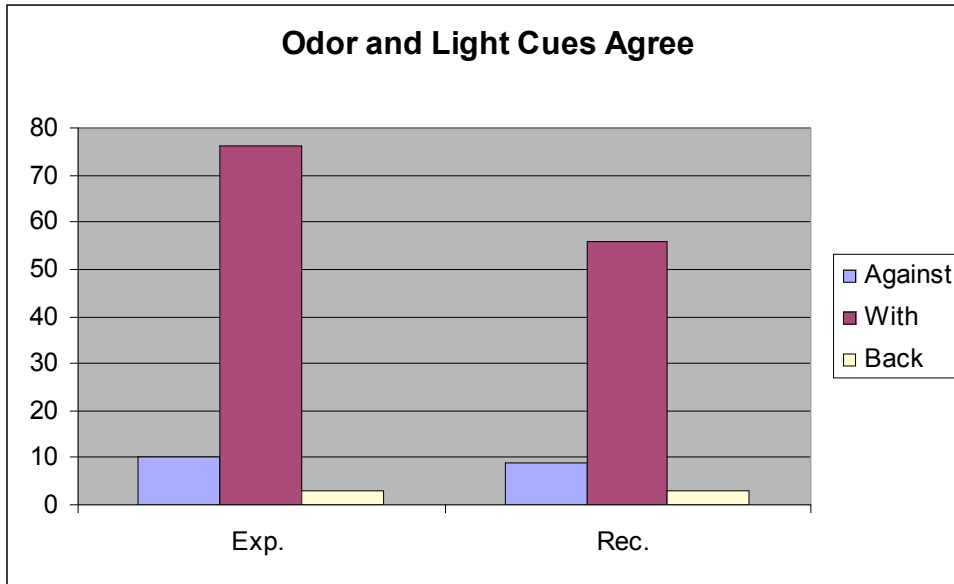
Odor and Light Cues Agree

Fig. 4.2: Response of experienced and recruited ants when the odor and light cues indicate the same arm of the maze. The diagram illustrates an example of this situation.
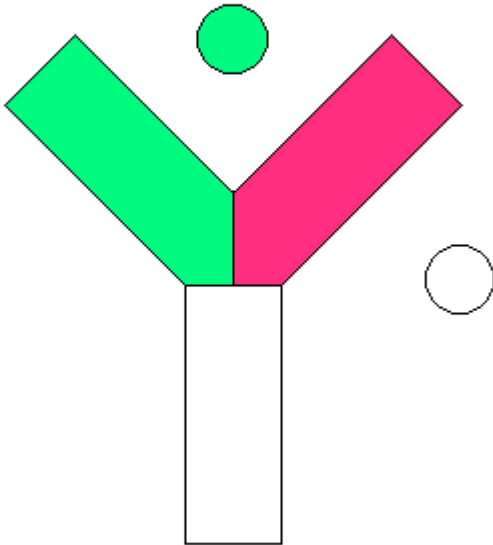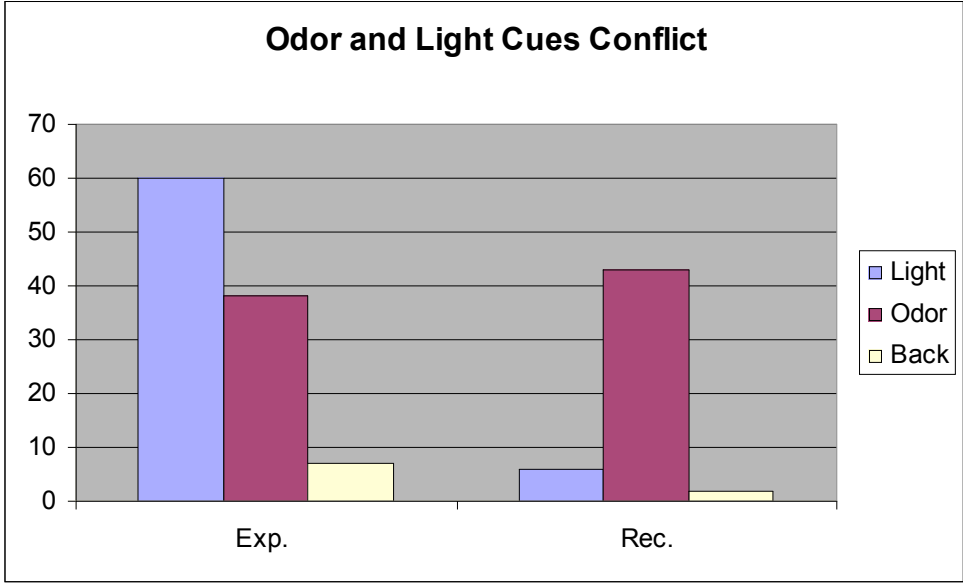
Fig. 4.3: Response of experienced and recruited ants when the odor cues and light cues indicated different arms of the maze as indicated in the diagram.
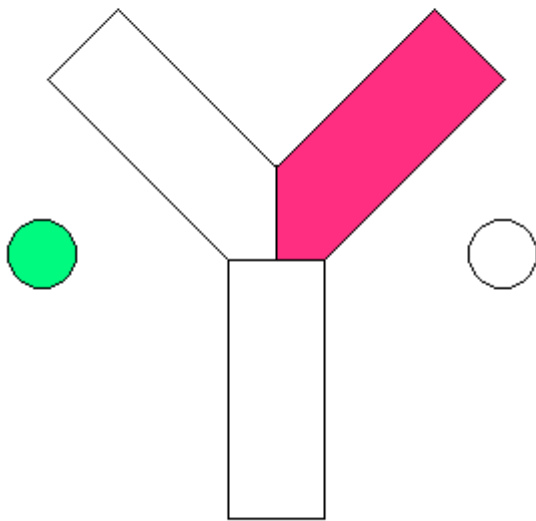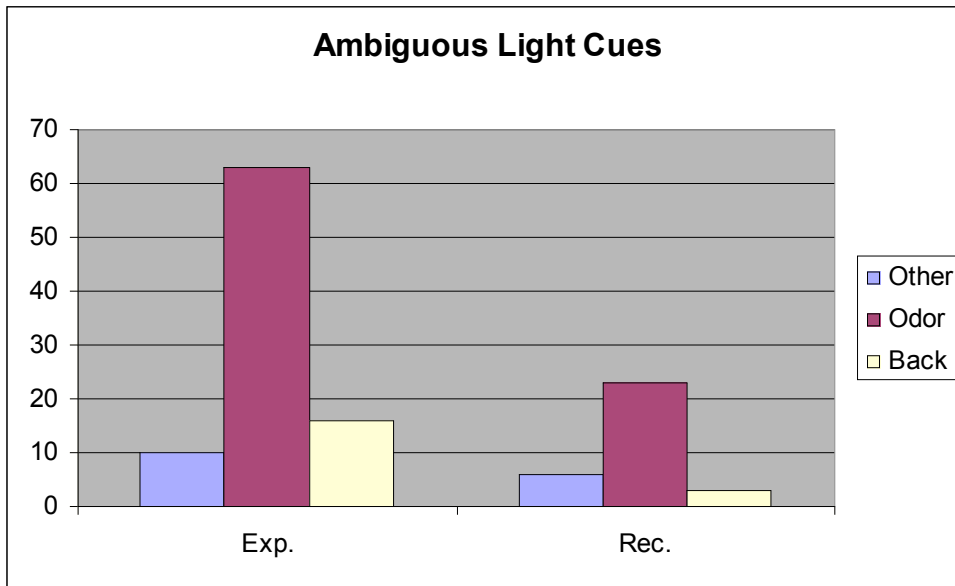
Ambiguous Light Cues

Fig. 4.4: Response of experienced and recruited ants when the light cues were present, but did not specify an arm of the maze. The diagram depicts and example of this situation.
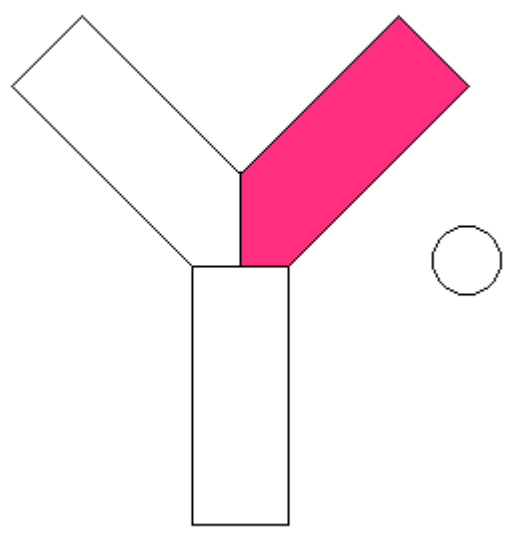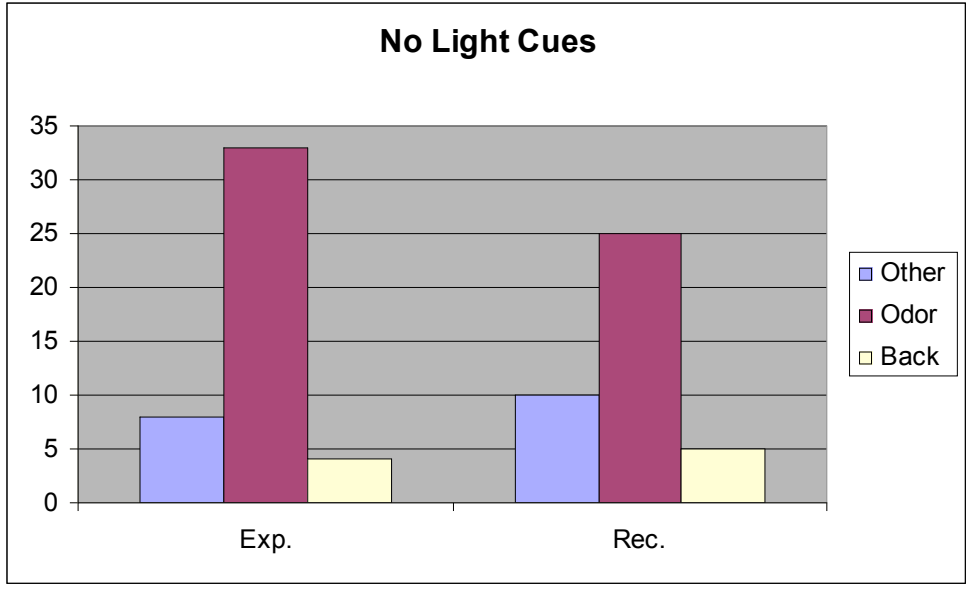
No Light Cues

Fig. 4.5: Response of experienced and recruited ants when there is an odor trail, but no light cue. The diagram depicts an example of this type of trial.

No Odor Cues

Fig. 4.6: Responses of experienced and recruited ants when a light cue was provided but the odor cue was removed. The diagram depicts an example of such a trial.

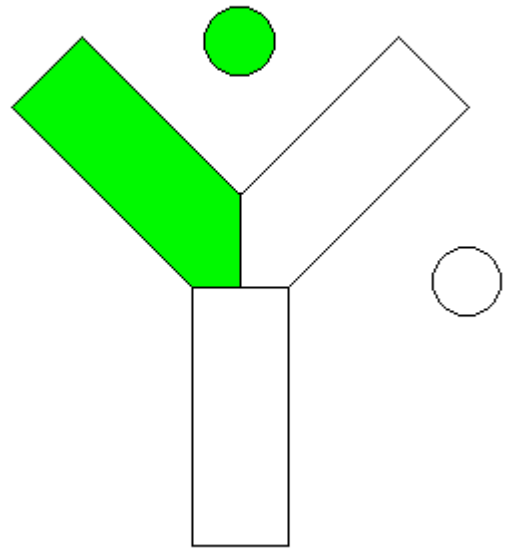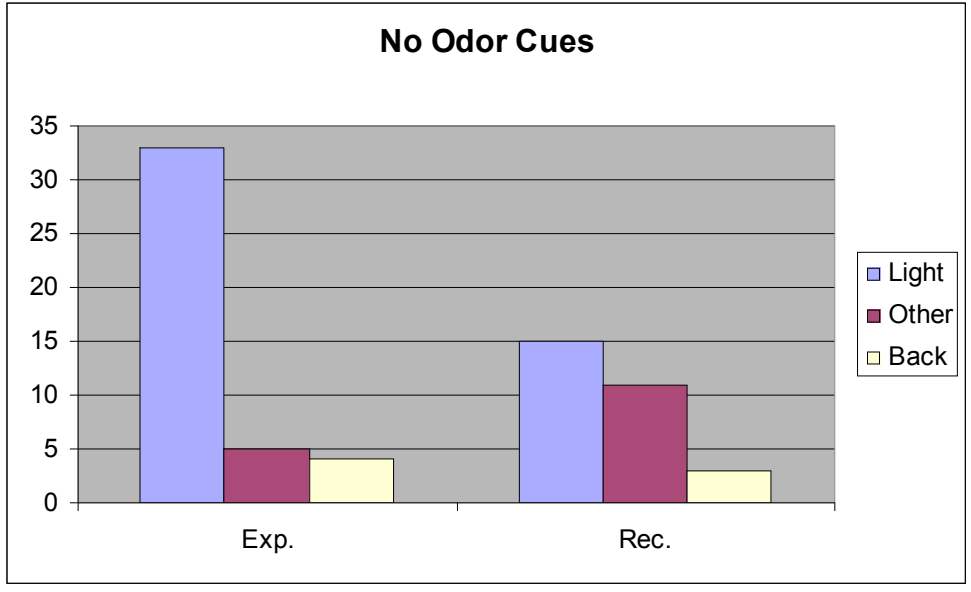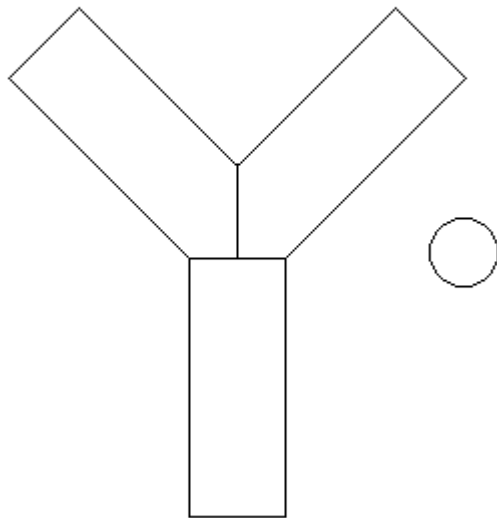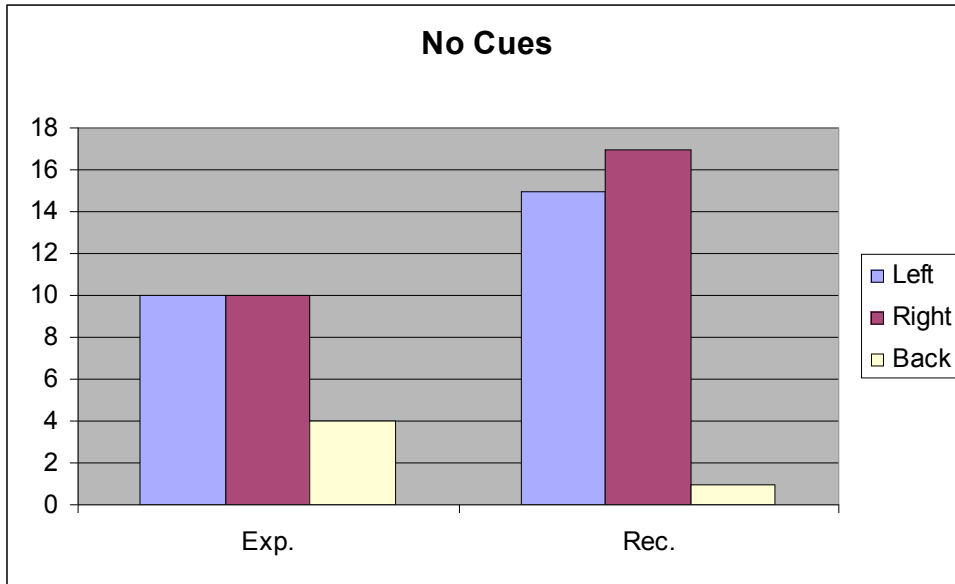Fig. 4.7: Responses of experienced and recruited ants when no cues were provided during the testing period. The blank Y-maze figure depicts this configuration, and the blank circle represents the position of the lamp during the initial period.

Chapter 5

Responses to conflicting odor and gravity cues

5.1: Introduction

      This set of experiments examined the role that gravity plays in *Atta cephalotes* navigation by putting gravity cues in direct conflict with odor and visual cues. I varied the strength of the gravity cue by changing the angle of the path. This allowed me to investigate the threshold for using gravity as a cue and to see if this threshold changed when conflicting cues changed. I also examined the form of the transition from not using gravity cues to using gravity cues.

5.2: Methods

      I conducted these experiments on a vertical T-maze with a rotating crosspiece (Fig. 5.1). Unlike with my earlier Y-maze experiments, I made no attempt to directly manipulate the odor trail on this apparatus. Instead, the food was always on the same arm, which presumably had the stronger odor trail. Since the odor trail was always on the same arm I refer to this arm as the odor arm and the other as the gravity arm even in the experiments where it does not carry a gravity signal.

      As in the Y-maze experiments, during an initial observation period I recorded the identities of ants collecting food. Then, in the trial period, I recorded how many ants chose each of the two possible paths for the next half hour. In most cases, I recorded the behavior of experienced ants for each experiment. I used x^2 analyses to compare the results of these trials with the behavior of the recruited ants in all of the trials combined.
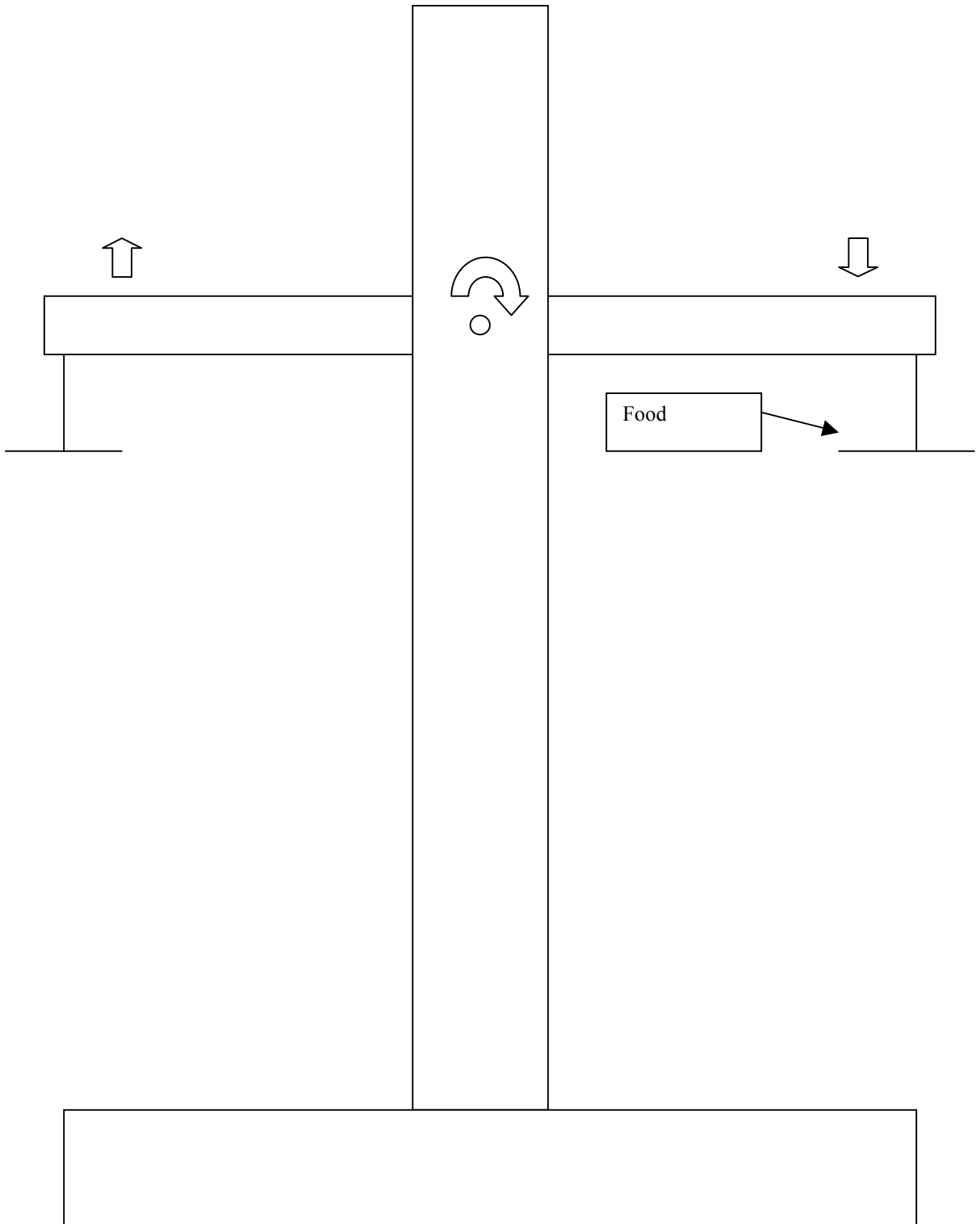
Fig. 5.1: Diagram of the T-maze apparatus.

In the control trials, where I specifically examined the behavior of recruits, I recorded recruit behavior and compared it to the pool of recruit behavior. I have designated these trials with a "C", and I did not include these results in the pool of recruit data that I used for comparisons.

In control trials, I examined the effect of the odor trail in the absence of any change in gravitational cues. In level ("0/0") control trials, the crosspiece was horizontal during both the initial observation and during the trial period. (Note that in these trials, the "gravity arm" does not carry any gravity signal.) In the "0/0 C" trials, I used an initial observation period of ten minutes so that most of the ants in the trial period would be new recruits. In the "0/0" trials, I used an initial period of one hour.

In gravity control trials, I examined if there was a bias to go up or down an incline when seeking food in recruited ants. The two configurations tested were odor arm up 60° (+/+ C, and odor arm down 60° (-/- C). In these trials, I used an initial period of ten minutes so that most of the ants in the trial period would be new recruits.

In my experimental trials, during an initial one-hour observation period, I recorded the identities of ants collecting food from the apparatus in an initial configuration. Then, I changed the apparatus and recorded the choices of the ants to the new configuration for half an hour.

In "+/-" experimental trials, the odor arm was raised 5° for the initial observation and lowered by 5° during the trial period. On successive trials the angle of inclination was raised by 5° each trial until the maximum inclination of 60° was reached. Then the angle of inclination was decreased until the transition from using gravity to not using

gravity was crossed. This was to check for any hysteresis in the curve of responsiveness versus angle of inclination that would indicate a priming effect.

The "-/+" experimental trials were similar, but I lowered the odor arm in the initial period and raised it in the trial period. This allowed me to investigate whether the ants have an inherent bias to go up an incline when seeking food and down it when nest bound.

Finally, the "+/- L" experimental trials returned to raising the odor arm for a one-hour initial period and lowering it in the trial period, but with a lamp added as a non-diffuse light source. This put the gravitational cue in conflict with both an odor cue and a light cue. The trials started at 60˚ and then the angle of inclination was decreased until the ants no longer used the gravitational cue.

5.3: Results

In the absence of any change in the apparatus, the vast majority of ants followed the odor arm, confirming the importance of the pheromone odor trail in directing ants to a food source.

With small angle changes, the odor cue continued to dominate. At higher angle changes, however, the gravity cue dominated (Table 5.1 and Fig. 5.2). In the +/- experiments, the threshold for using the gravitational cue, defined as the point at which the behavior of returning ants differs significantly from the behavior of 0/0 trial ants, was between 40˚ and 45˚. There was no hysteresis in the results so they were combined. In the -/+ trials the ants never used the gravitational cue, and in the +/- L experiments the threshold shifts to between 50˚ and 55˚.
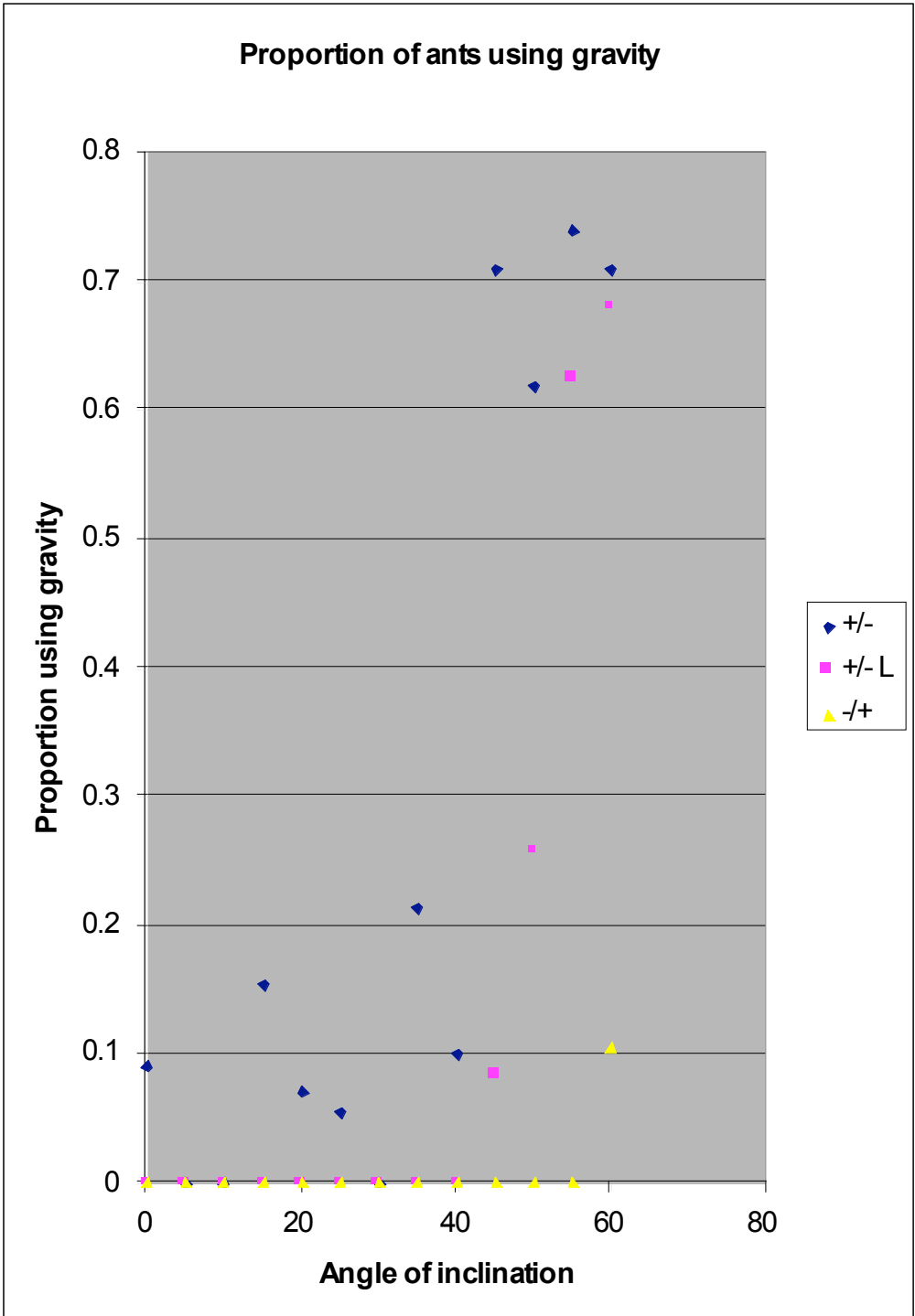
Fig. 5.2: Graph of experienced ants behavior on the vertical T-maze.

Table 5.1. The results of gravitational experiments.

		Arm chosen		
		Odor	Gravity	X^2
Recruits Pool		77	7	-
0/0 C	0°	7	0	0.63
0/0	0°	10	1	0.01
+/+ C	60°	14	0	1.26
-/- C	60°	16	3	0.98
+/-	60°	9	22	47.11**
	55°	6	17	44.63**
	50°	8	13	31.27**
	45°	9	22	47.11**
	40°	27	3	0.08
	35°	11	3	2.25
	30°	10	0	0.90
	25°	17	1	0.16
	20°	13	1	0.02
	15°	11	2	0.66
	10°	7	0	0.63
	5°	9	0	0.81
-/+	60°	17	2	0.09
	55°	7	0	0.63
	50°	12	0	1.08
+/- L	60°	8	17	39.95**
	55°	12	20	38.07**
	50°	23	8	6.10
	45°	11	1	0.00

** $p \ll 0.0005$; all other n.s.

5.4: Discussion

The results of the +/- experiments provide strong evidence that *A. cephalotes* foragers use gravitational cues for navigation when the angle of the incline is 45° or greater. Every other cue the ants could be using indicated the odor arm and yet most of the ants chose the gravity arm. The transition from not using gravitational cues to using gravitational cues was extremely sharp indicating that the threshold is consistent, to within 5°, across individuals. The -/+ results indicate that there is an asymmetry to the ants' use of the gravitational cue. Specifically the ants will choose a path going up, in preference to a path with a pheromone trail going down, if they have previously visited a feeding site above the decision point. When faced with the inverse situation, the pheromone trail going up when the remembered food source was down, the ants chose to go up along the pheromone trail. There are several possible explanations for this result.

One possible explanation is that the ants simply prefer to go up when looking for food. The results of the +/+ and -/- trials indicate that the bias to go up does not overwhelm other cues. Recruited ants follow the odor trail whether it goes up or down, so a memory of a food source above the decision point seems to be necessary. It is also possible that the observed bias is not inherent to the ants, but is due to an asymmetry in the experimental situations. The asymmetry lies in the fact that the decision point was above the arena floor so the ants had to travel up to get to the decision point. This means the up paths were a continuation of moving in the same direction while the down paths were a change in direction. The transition from the base of the maze to the crossbar was sufficiently difficult for the ants that there was not a direct orientation bias caused by the

ants simply continuing to walk along a line, but there may have been an analogous cognitive bias.

The results of the "+/- L" trials indicate that there is a comparison of cues in the ants' navigational decision process. The presence of a light cue distinctly shifted the threshold for using the gravitational cue and may have reduced the overall responsiveness of the ants to the gravitational cue but not significantly. The process of combining cues may be a fusion where the result is a hybrid of the inputs, or it may be a winner take all mechanism where the final behavior is dictated by the winning cue as if it were the only cue. These two alternatives cannot be distinguished with the current experiment because of the forced choice nature of the maze, but the fusion hypothesis is more consistant with the results with geotaxis and phototaxis experiments in other insects (Horn 1975).

Chapter 6
Anatomy of eyes and brains

6.1: Introduction

In this chapter I will discuss my anatomical investigations of *A. cephalotes*.

These include a rough estimate of the angle between adjacent ommatidia in the eyes, and

a measurement of the relative volumes of sub-compartments of the brain. There are also

results for *Fomica exsectoides* that provide some context for the *A. cephalotes*

measurements. Reference to the literature will provide further context.

The primary reason for these anatomical investigations is to get an idea of what

neurological resources *A. cephalotes* has at its disposal for navigation. This in turn may

help interpret the behavioral results.

In describing the eyes I use a couple of technical terms. Ommatidia are the units

of the compound eye. Each ommatidia consists of a lens at the surface, a crystalline cone

to focus the light, and a bundle of photoreceptors. Each photoreceptor is called a

rhabdomere, but in ants, the eight rhabdomeres are fused into a single structure called a

rhabdom.

6.2: Methods

The *A. cephalotes* workers in this study were taken from the behavioral colonies.

The *F. exsectoides* workers were collected at the Patuxent National Wildlife Refuge,

Beltsville MD.

I sacrificed each ant by decapitation and removed the portion of the head anterior

to the antennae. This portion consisted mostly of the mandibles and other parts of the

mouth. For eye investigations I cut the head sagitally, for brain investigations it was left

intact. I immediately fixed the dissected head in alcoholic Bouins solution for 24 hrs. I

then dehydrated the fixed head in mixtures of ethanol and butanol. Next I infiltrated the

dehydrated head with a catalyzed monomer JB-4 A for two days and then set it in a

mixture of this monomer with another monomer JB-4 B. After the polymer had

hardened, I trimmed, mounted, and sliced the block into 5-micron sections with a

microtome. The resulting sections were mounted on slides, bleached to remove the

natural pigment in the eyes (this step was skipped for brain investigations), rinsed,

stained with toludine blue, rinsed again, dried, and coverslipped. I then photographed the

slides at 100x (50x for brain investigations) magnification with a digital camera mounted

on a Zeiss axiolab compound microscope. I measured these photographs using the Java

version of NIH Image. For the eye measurements I individually measured the orientation

of each ommatidium by drawing a line though the rhabdom and comparing the angle of

the line to a common reference. When the rhabdom was visible in adjacent ommatidia I

recorded the angle between these ommatidia. I averaged these measurements for each

species. For the brain measurements, I measured the area of each sub-compartment in

each section and calculated the volumes by adding the slice areas together and

multiplying by the slice thickness. Missing slices and slices where sub-compartments

were not discernable were accounted for by taking the average of the adjacent slices.

6.3: Results

The average angle between ommatidia for *A. cephalotes* was 9.0˚ +/- 2.0˚ s.d.

while for *F. exsectoides* it was 6.8˚ +/- 1.1˚ s.d. This suggests that *F. exsectoides* has

better resolution in its vision. A sample slice of each type of eye is provided in figures 6.1 and 6.2.

Table 6.1: Relative volumes of important brain subcompartments

Species Name	Optic/Brain (%)	Olfactory/Brain (%)	Mushroom/Brain (%)	Brain (μm^3)
Atta cephalotes	2.2	13.4	49.9	2.5×10^7
Formica exsectoides	5.6	14.1	40.9	6.8×10^7

I have followed Gronenberg and Hölldobler's (1999) convention of displaying the volumes of sub-compartments as percentages of the brain volume because this seems to be the behaviorally relevant metric. It can be seen in table 6.1 that the two species have nearly the same proportion of their brains devoted to olfactory lobes and mushroom bodies, but *F. exsectoides* has nearly triple the percentage devoted to optic lobes. A sample slice is shown in Fig. 6.3.

6.4: Discussion

Based on these results, *A. cephalotes* does not have exceptional visual tools. The method I used for determining the angle between ommatidia was crude, but would seem to be reasonably accurate given that *Cataglyphis bicolor* have an equivalent angle of 4˚ (Zollikofer, et al. 1995) measured by a much more accurate method. By comparison, the functionally equivalent angle in humans is on the order of a minute of arc (Woodhouse and Barlow 1982). It is possible that *A. cephalotes* has flatter regions of its eyes that act

Atta cephalotes eye

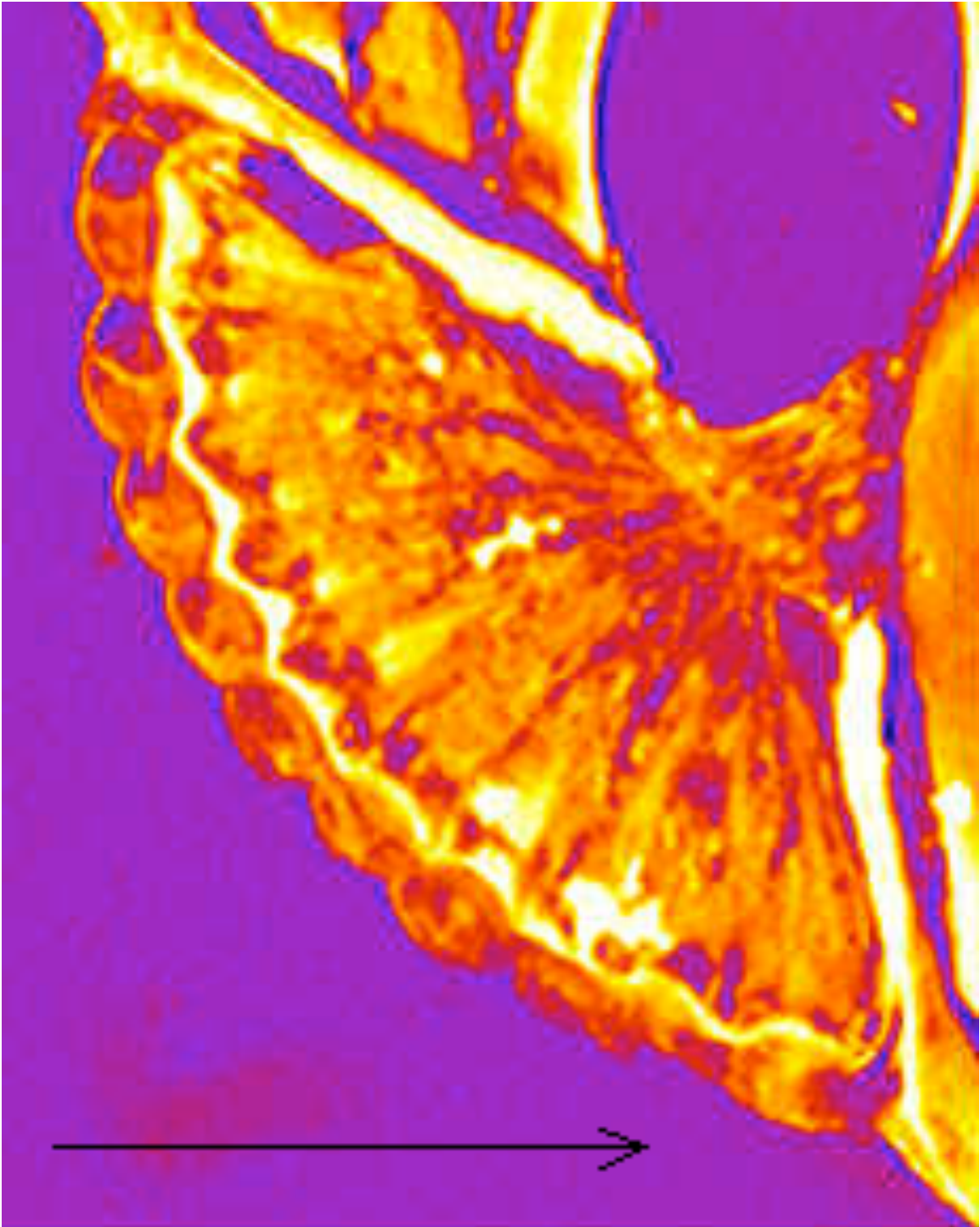Fig. 6.1: Sample slice of _Atta cephalotes_ eye. Scale bar is 100 microns.
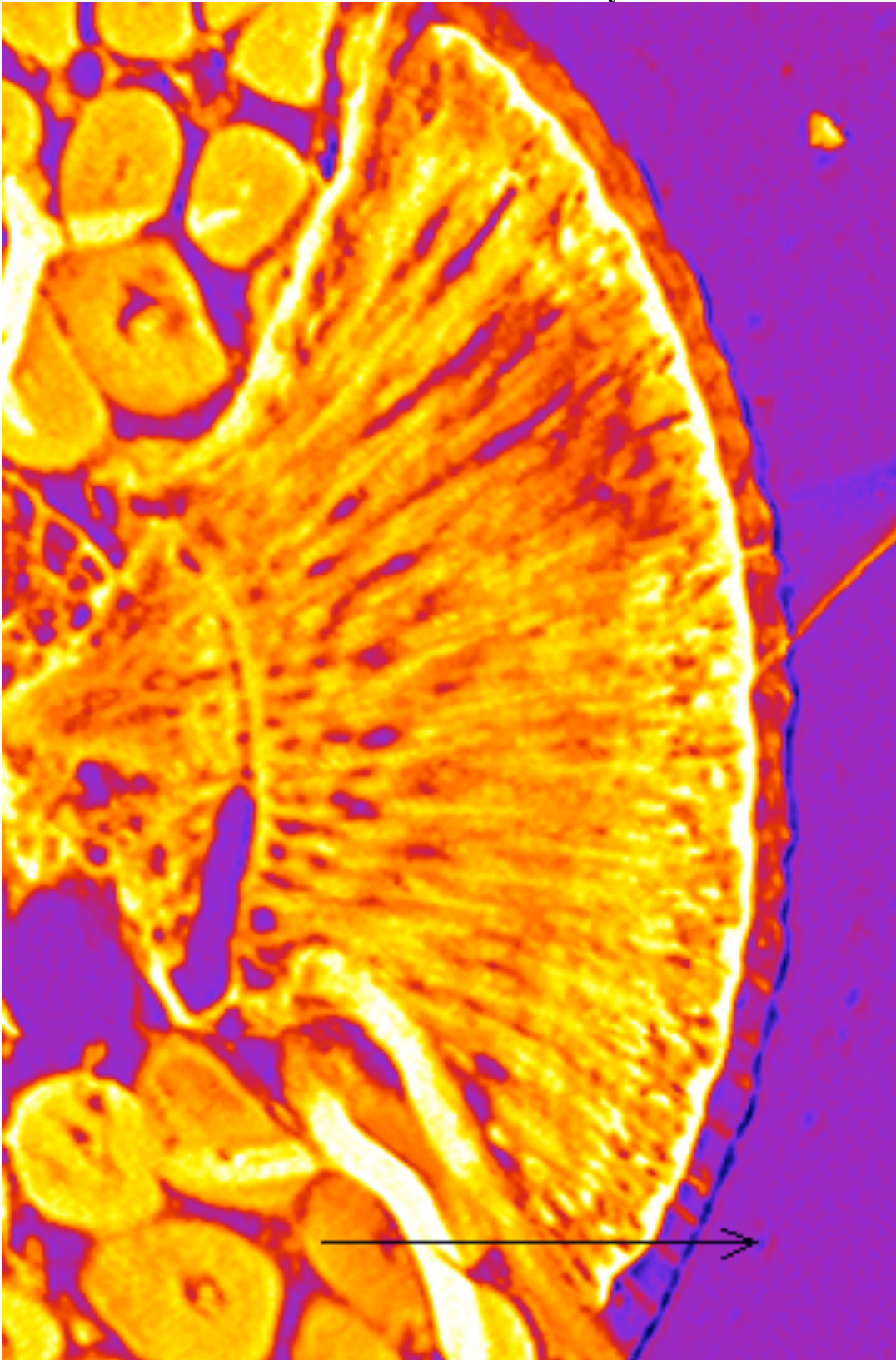
Fig. 6.2: Sample slice of *Formica exsectoides* eye. Scale bar is 100 microns.
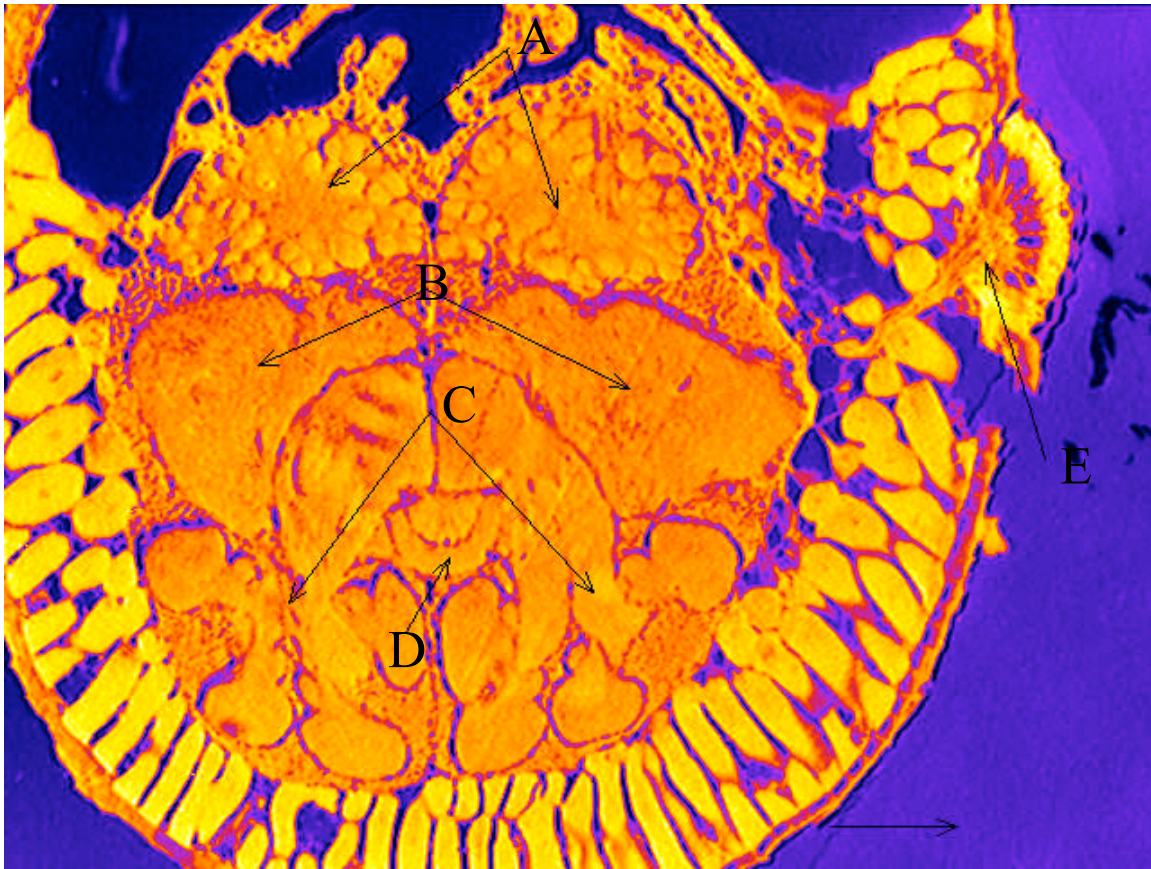
Atta cephalotes brain

Fig. 6.3: Sample slice from a brain of an _Atta cephalotes_. A: Antennal lobes. B: Central Bodies. C: Mushroom Bodies. D: Central Bridge. E: Eye with attached optic nerve. The scale bar in the lower right is 100 microns.

as fovea, but since there is no evidence for this from either behavior or a casual inspection of the eye shape in the sections, it is unlikely that there is a fovea that is distinctly different from the rest of the eye. This means that *A. cephalotes'* visual resolution is about 600 times coarser than human visual resolution.

Just as the eyes of *A. cephalotes* do not provide a very high-resolution signal, the brain does not devote many resources to vision. The results for the optic lobe and olfactory lobe agree well with measurements made on *A. sexdens* (2% and 12% respectively; Gronenberg 1999). The mushroom body measurements do not agree, but this seems to be because my measurements include the peduncle while theirs appears to include only the calyx. In any case, *A. cephalotes* devotes a relatively small amount of its brain to visual processing. This is especially true when compared to visual insects like the ant *Gigantiops destructor* where the optic lobes are 33% of the brain, or the honeybee Apis mellifera where the optic lobes are 20% of the brain (Gronenberg and Hölldobler 1999). *G. destructor* is an extreme example, but even the optic lobes of *F. exsectoides* take up nearly three times as much of its brain as those of *A. cephalotes*. Clearly the visual processing performed by the optic lobe is not a high priority for *A. cephalotes*. I will explore why this might be the case in chapter 7.

Chapter 7

Conclusions and ideas

7.1: Introduction

In previous chapters I have reported the results of several experiments and discussed my interpretations of those individual results. In this chapter I will address the broader issues of how these results relate to natural *Atta cephalotes* navigation and how their nervous system performs this navigation. These discussions will be speculative in nature, but they will add perspective to the studies reported and will suggest possible courses for future research.

7.2: Navigation in the wild

The lab environment is useful because it is easy to manipulate, but it is also very artificial. Wild *A. cephalotes* probably rarely encounter desk lamps or rubberized plastic ramps covered in electrical tape. For this reason, lab experiments tell a great deal about what ants can do, but may tell little about what they do in nature. It is nonetheless useful to think about and predict how behaviors in the lab relate to natural behaviors. In these experiments I have examined the use of visual, olfactory, and gravitational cues. In the following discussion I will look at how each of these might translate to nature.

Visual cues

In the single path and y-maze experiments *A. cephalotes* used the lamps as visual cues but did not seem to respond to other objects. In the single-path experiments this was explicitly tested while in the y-maze experiments it is implicit in the fact that the ants

ignored some potential cues such as the researcher. In fact, the ants did not so much use the lamps themselves as the light emitted from the lamps since when the lamps were off they were as ignored as a source of other potential cues. This indicates that the source of light is a special cue to *A. cephalotes*.

In nature the most obvious source of light is the sun and when they can see it *A. cephalotes* probably use the sun as a navigational aid. To do so they probably have an acquired ephemeris function similar to the one demonstrated in *Cataglyphis* (Whener and Muller 1993). The sun may not be the only visual cue *A. cephalotes* use. Living in rainforests, the sun is probably only visible to *A. cephalotes* when they are foraging high in the canopy. On the forest floor the effective source of light is more likely to be breaks in the canopy. In this way, *A. cephalotes* might employ a slightly simplified version of canopy orientation (Hölldobler 1980) where the exact image of the canopy is unimportant, but the positions of breaks in the canopy are extremely important.

Olfactory cues

The only olfactory cue I manipulated in these experiments was the pheromone recruitment trail. Since the ants produced these trails themselves I assume that they are similar to natural trails with the following caveats. First, the physiological condition of the ants in the lab may be slightly different from that of wild ants due to difference in the lab environment such as diet. Second, the surfaces the trails were laid on were very unnatural and may have changed the characteristics of the trails. For example the rate of evaporation of the pheromone may have been different in the lab than it would be in nature. Finally, it is unclear what type of natural trail to compare the lab trails to. In nature there are well established trails near the nest and more temporary trails near the

food source (Weber 1982). It is possible that the ants treat these differently for navigational purposes (Wilson 1983). In these experiments the trails were on branching structures, near food, and were generally on surfaces that did not contain any colony odor before the experiment. These are all characteristics of trails at the edges of the trail system. However the trails were also less than half a meter from the nest entrance. To the extent that trails near the nest are treated differently from trails in the periphery this may have caused some distortion in the results. However, I think the results make the most sense for trails in the periphery. A future experiment might investigate whether extending the tubing between the nest and the foraging arena makes any difference in the results.

Gravity

Gravity may be the most natural cue investigated since it is relatively constant across the surface of the planet. The decision point on the T-maze is roughly analogous to a branch point on a tree where two branches are at different inclinations, or to a place were two branches cross each other.

An hypothesis of ant navigation

Considering the results of these experiments as well as some earlier experiments it is possible to hypothesize about ant navigation. This account is consistent with the available information and seems likely, but it may not be the only account that is consistent with the available information and hence may not be the actual story.

There are three general ways an ant can get to a food source. She may discover it on her own, she may be recruited to it, or she may return to it after a previous visit. The navigational task in each of these conditions is different. In the case of the exploring ant

there is not a specific destination and so the ant is not, strictly speaking, navigating. This does not mean that the exploring ant's movements will be entirely random. The movement may be guided by biases such as the bias to go up or the bias to go away from the nest. Whatever guides the ant in her explorations, she must acquire the information that will allow her to get back to the nest. In the case of *A. cephalotes*, this information probably takes the form of learning light source cues and gravity cues. There is probably very little dead reckoning not only because the canopy obscures the sky, but because the tree branches greatly constrain where the ant can go. This makes the navigational problem faced by *A. cephalotes* analogous to navigating through a large city while the problem faced by *Cataglyphis bicolor* on open terrain is more like navigating on the open ocean. It is possible that *A. cephalotes* foragers simply learn the appropriate cues at each branch point.

When recruited to a food source, an *A. cephalotes* forager simply needs to follow the odor trail laid down by her nest mates. At each branch point there will be odor differences between the branches and the ant simply needs to pick the branch with the more enticing odor. The level of enticement may be simply because the odor is stronger, or there could theoretically be chemical messages about the food source in the pheromone that make one pheromone trail preferable to another. While the presence of chemical messages is a theoretical possibility, it is highly unlikely given that extensive studies on trail following behavior in ants have never found any evidence of such messages (Hölldobler and Wilson 1990). Near the end of the trail the vibrations produced by stridulating while cutting may lead the ant to one of the leaves where cutting is occurring (Roces, et al. 1993).

Whether she discovered the food source, or a nestmate recruited her there, if the food source is a good one the ant is going to want to get back to it. The experimental results presented here suggest that she does so by following her individual memory of visual and upward gravitational cues. The bias for upward cues may indicate that higher food sources are somehow preferable and so given the choice between a known food source down and a recruitment trail up the ants choose to go up. In all other cases studied, when the ants were given a choice between a path indicated by individual memory and a path indicated by an odor trail, the ants chose the path indicated by individual memory.

There are many possible reasons for this reliance on individual memory and they may all play a role in selecting for this reliance. One reason is that it may simply be faster. I did not take direct measurements of this effect, but returning ants did seem to move faster than recruits. Another reason could be that trails are more subject to disruption. A trail may evaporate before it is well established, or something might disrupt the surface the trail was laid upon. Visual and gravitational cues may be less subject to this type of disruption. Finally, visual and gravitational cues would allow the ant to return to a specific food source even when a different food source is equally or more strongly indicated by the odor trail. This could be especially important for *A. cephalotes* since not all food sources are equally attractive to all nestmates. Tough leaves that cannot be cut by small ants are preferentially harvested by large ants (Wetterer 1994). While it may be possible to indicate such differences with chemical signals in the odor trail, there is no evidence for such a system (Hölldobler and Wilson 1990). Reliance

on individual memory would properly bias the distributions of ants at feeding sites without the need for chemical signals.

A final curiosity in the story of *A. cephalotes* navigation is that the ants seem to be aware of the odor trail even when they may not be using it for navigation. Some of the ants in the two path experiment were probably returning ants yet they were all disturbed by being removed from an odor trail. More interesting yet is that the passive removal from the odor trail in the two path experiments initiated a search for the missing trail while there was no search when they actively left the trail in the y-maze experiments. This seems to indicate some level of feedback that differentiates between being moved from a trail and moving from a trail. This feedback seems to be active even in returning ants that seem not to be using the odor trail.

7.3: Navigation in the nervous system

It seems likely that a different neural pathway controls each of the three methods of reaching a food source. The behavior of non-laden nest bound ants in the single path experiments suggests that *A. cephalotes* may have other navigational systems for other tasks, but I do not know enough about these tasks to speculate on what these systems might be like. Similarly, I do not know enough about what cues if any *A. cephalotes* employs during exploration to speculate on how they orient during this task. Clearly they develop memories during exploration that they can retrieve during return trips, but I do not know how they orient during those explorations.

Trail following

The mechanism for following an odor trail is probably fairly simple. The most complex part is probably the identification of the trail pheromone. This probably takes place in the olfactory lobe. The signal from the olfactory lobe probably indicates the strength of the trail odor and may also include information about the food source if the trail pheromone carries such information. Keeping to the trail is probably as simple as comparing the signal from each antenna and turning toward the antenna that is receiving the stronger signal (Hangartner 1967). Such a comparison could be by the motor system similar to the way the motor system of cockroaches compares the input from giant fibers during an escape response (Ritzmann and Pollack 1986). As with exploration, the ants also develop memories while following an odor trail that they can make use of during return trips.

Navigating from memory

Navigation based on individual memory is probably more complex. To begin with, both visual and gravitational cues seem to play a role in this type of navigation. It seems likely to me that systems based on each of these cues calculate course corrections based on the difference between stored values and the current sensory input. The correction signals could be fed to the motor system independently, but I suspect that the fusion takes place a step before the motor system in the mushroom bodies. I also suspect that when this system is generating a course correction signal, this system also inhibits the path following systems input into the motor system.

Visual memory

The specific pathways taken by the visual information are probably something like the following. The optic lobes are probably dominated by object recognition processing (Gronenberg and Hölldobler 1999). This is supported by the fact that ants that hunt prey visually like *Gigantiops destructor* have huge optic lobes (33% of the brain), ants that use visual landmarks like *Cataglyphis bicolor* have medium sized optic lobes (13% of the brain), and ants that only respond to light sources like *Atta sexdens* have small optic lobes (2% of the brain) (Gronenberg and Hölldobler 1999). In contrast, the part of the mushroom bodies that processes visual information (called the collar) seems to be involved in visual memory (Gronenberg and Hölldobler 1999). When considered as a ratio between the size of the collar and the size of the optic lobes *A. sexdens* has the largest collar for the size of its optic lobes of the ants considered. This is due in part of course to the extremely small size of the optic lobes, but it does suggest that *A. sexdens* foragers do a large amount of memory processing of the small amount of visual information they get. I suggest that it is here in the mushroom bodies where the current sensory input is compared to memory and a correction signal is generated.

Gravitational memory

The gravitational pathway is difficult to predict without further study. Ants detect gravity based on comparing the information from position proprioceptors and force proprioceptors on a large number of joints including the leg joints, the joints between body segments, and the joint in the antennae between the scape and the pedicellus (Horn 1975). There has been speculation that gravity perception in different joints mediates different behaviors (Horn and Föller 1998) and if this is the case, the antennal gravity

perception seems to be the dominant source of information for navigation (Horn and Föller 1998). If this is assumed, a reasonable hypothesis for the gravitational pathway emerges.

Since the gravitational signal originates in the antenna, the signal must pass through the olfactory lobe. There may be some processing in the olfactory lobe, or the axons may pass directly to the mushroom bodies without synapsing. In either case, the memory processing probably occurs in the mushroom bodies. It would be interesting to know how much of the portion of the mushroom bodies thought to be dedicated to olfactory processing are actually devoted to gravitational processing. As with the visual signal, the mushroom bodies probably generate the course correction signal. There is a portion of mushroom bodies where input from the optic lobe and input from the olfactory lobe mix (Gronenberg and Hölldobler 1999). If the visual and gravitational course corrections are fused before they reach the motor system it is likely to occur here.

Relationships between systems

I have claimed that the trail following system and the individual memory system are separate systems, but there must be some interaction. To begin with, the ants must acquire memories while the ant is exploring or following trails in order for the individual memory system to work. I have already described how the individual memory system seems to inhibit the output of the trail following system. The two path experiments indicate that even when the trail following system is being inhibited by the individual memory system, the trail following system is generating signal. When a passive displacement disrupts this signal it induces search behavior. If a signal from the memory system disrupts the trail following signal, then search behavior is not initiated. This

could be accomplished by having the search routine inhibited by both the trail following system and the memory based system. I have diagramed these relationships in Fig. 7.1.

7.4: Final thoughts

The model described above and pictured in Fig. 7.1, as simple as it is, is highly speculative and undoubtedly wrong in many ways. Yet it is still useful in two very important ways. It provides a framework for thinking about the problem of how ant navigation works, and it serves as guide for future experimentation. Aside from the model, the experiments themselves provide insights into the navigational tasks preformed by the simple nervous system of an ant.
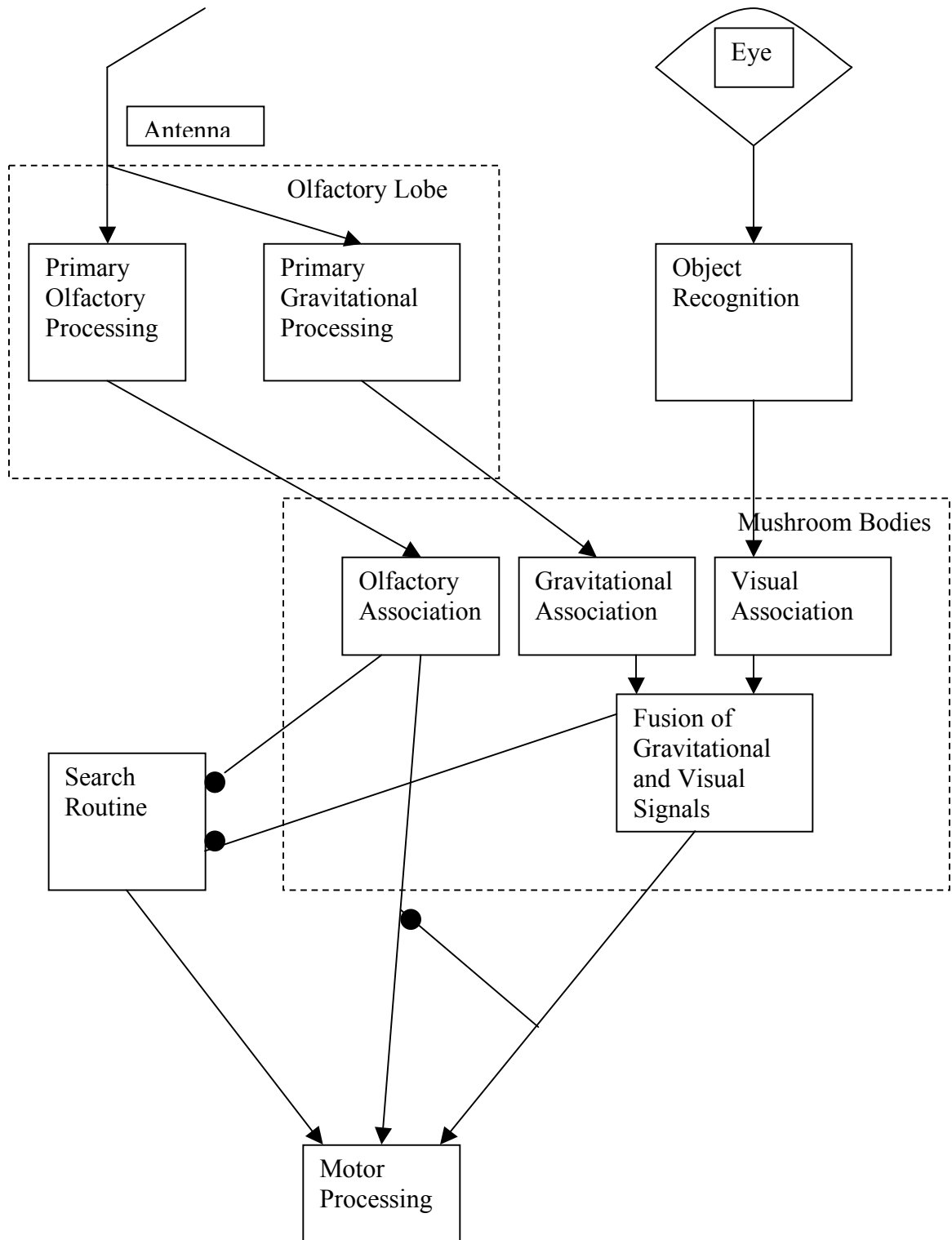
Fig. 7.1. Diagram of neural model.

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