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Author
Jinn, Judy

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Judy Jinn

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Committee in charge:

Dr. Lucia F. Jacobs, Chair
Dr. Frédéric Theunissen
Dr. David Foster
Dr. Robert Full

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Abstract

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The evolution of the first complex neural systems occurred in a sensory world defined by chemosensory stimuli. Despite its universality across the animal kingdom, the strategies animals use to sample for odors and orient using odors is still not clear. Odor plumes are complex structures. How animals sample their environment for odor stimuli, make decisions, and ultimately find an odorant using their sense of smell has been studied more in invertebrates and birds than mammals, yet olfaction is critical for most mammals, even humans. This dissertation will analyze the orientation and sampling strategies in two species of mammals that differ in their olfactory capacity yet are known to use odors to orient in space. It will examine olfactory navigation in both naturalistic conditions and controlled studies to understand how environmental conditions, individual differences, and olfactory cues affect search strategies, navigation behavior, and sampling behavior.

Chapter One addresses the nature of odors and olfaction, the structure of odor plumes, and a summary of our current knowledge of animal strategies for olfactory navigation through odor plumes. It also discusses how individual differences, such as sex differences and spatial cognitive abilities, may affect olfactory discrimination and subsequently, orientation.

Chapter Two investigates how a species known to be expert in olfactory navigation, the domestic dog, oriented to the outdoor scent trail of a hidden person. Search and rescue dogs were adept at following odor trails to a target person despite a number of meteorological factors affecting odor dispersion along the trail. Meteorological data collected along the odor trail revealed that hot temperatures, low humidity, and high wind speeds caused odors to disperse quickly and widely. Under these conditions, dogs searched farther from a person's original trail. Studying the dogs' behavior as they followed the odor trail also revealed that dogs were more likely to sample the air for odors when moving at high speeds. Some results suggested that they slow their speed and searched the ground for odors when they lost contact with the odor plume. This study demonstrated the ease in which animals can track odors over great distances to their source.

Chapter Three describes an experiment with humans that examines sniffing behavior modulation during olfactory navigation. In this experiment, humans in an indoor setting varied their sampling behaviors in response to spatial distance from an odorant as well as to stereo olfactory cues. People sniffed less frequently and longer while initially searching for the odor plume. After detecting the odor, people casted, a behavior characterized by zig zagging trajectories, when they were attempting to definitively locate the odor plume. Once the plume was found and they were following it to the source, they sniffed more frequently and had shorter sniffs. Overall, as people approached the source of the odor, sniffing intensity increased. Sniffs were always slower, shorter, and less intense when using stereo olfaction than when one nostril was blocked. Women also detected odor plumes earlier than men. This is the first study to show people modulate their sniffing patterns while navigating an odor plume.

The final experiment in Chapter Four asked whether people have a mental prototype of an odor plume's structure. People's movement patterns and sampling behavior were recorded as they interacted with a virtual odor plume in a virtual environment. This study revealed that people treated the virtual plume as if it were a real odor plume and exhibited similar behaviors as in the real world experiment in Chapter Three. People sampled more frequently while they searched for the stimuli, less frequently when they were following the virtual odor, and then increased their sampling frequency again if they lost contact with the stimuli. People were sensitive to as little as a 1% drop in stimulus intensity, to which they reacted by correcting their trajectory to pursue an increasing stimulus gradient instead. They were also able to successfully modulate their sampling behavior when using stereo or non-stereo cues. Overall, men performed better at this task, meaning they were able to estimate the origin of the virtual odor with higher accuracy than women.

This body of work is one of the first to investigate mammalian odor navigation. It shows that mammals have many strategies to modulate their sampling and orientation behavior to account for meteorological factors, loss of stereo cues, and individual differences. The dissertation concludes with a discussion of these three complementary experiment and suggested future studies.

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Chapter 1
Introduction

Olfaction

All animals must find resources to survive. Vision is one sensory modality that could be used. However, it is suboptimal when searching for hidden resources or resources located far away. Instead, across the animal kingdoms, olfaction is one of the primary modalities for detection and orientation, whether it is foraging for food (Lancaster, 1964; Wolf & Wehner, 2000), avoiding predators (Amo, Galván, et al., 2008; Conover, 2007; Kelley & Magurran, 2003), finding shelter (Bonadonna & Bretagnolle, 2002; Døving, Stabell, et al., 2006) or locating mates (Bakker, 2003; Rafferty & Boughman, 2006; Ruther, Reinecke, et al., 2000). Chemosensation can be found in bacteria, invertebrates, vertebrates, and even plants (Blackburn, Fenchel, et al., 1998; Boller & He, 2009; Nei, Niimura, et al., 2008; Stocker, 1994). Nematodes (*Meloidogyne incognita*) avoid toxic environments (Zuckerman, 1984); mantis shrimp (*Hemisquilla ensiguera californica*) find prey (Mead, Wiley, et al., 2003); birds such as homing pigeons (*Columba livia*), previously thought to be mostly ansomic, return to roosts or migrate using olfaction (Gagliardo, 2013a, 2013b; Papi, Fiaschi et al., 1972).

How do animals integrate statistics from odor plumes, sampling behavior, and odor perception to navigate to the source? As an animal samples the environment then detects an odor, it compares samples to identify the concentration gradient and determine the correct direction to travel. They also integrate olfactory stimuli with mechanical sensations that assist in identifying the direction of the fluid (air or water), and visual cues, which then helps the animal to maintain its bearing in the fluid, e.g., upwind (Bell & Wilson, 2016; Bell & Kramer, 1979; Frye, 2010; Miller & Roelofs, 1978).

Jacobs (2012) proposed the olfactory spatial hypothesis of vertebrate olfaction, hypothesizing that navigation is a primary function of the main olfactory system across vertebrates. Further, olfaction was the key sensory modality for early marine vertebrates to orient towards and locate remote resources underwater by creating directional maps that are similar to those seen in flying insects orienting to odor plumes. Two major components of the limbic system are the main olfactory system and the hippocampal formation. The relative size of the hippocampus in birds and mammals varies with space use under natural conditions (Jacobs, 2009; Sherry, 2006). However, cetaceans, in particular toothed whales, which have home ranges up to several hundred square kilometers in size, do not rely on olfaction and have a highly reduced olfactory system and hippocampus (Patzke, Spocter, et al., 2013; Sprogis, Raudino, et al., 2016). Jacobs' hypothesis could explain the extreme diversity in the relative size of limbic system components in vertebrates, in particular the olfactory bulb in the main olfactory system. This link between spatial orientation and olfactory function has recently been demonstrated in humans. A functional imaging study by Dahmani, Patel, et al. (2018) demonstrated that olfactory discrimination was positively correlated with ability to navigate in a visual virtual maze and that ability also was predicted by the relative volume of the right hippocampus and an olfactory structure, the left orbitofrontal cortex.

If this is so, then olfactory navigation relies on integrating both olfactory and spatial cues. Animals therefore face two key challenges during olfactory navigation, detecting the odor and properly orienting in the gradient of the odor plume to navigate to the source. This dissertation will discuss the strategies mammals use to initially search for an odor, sample the environment for odor stimuli, and then integrate odor cues with their spatial movement to navigate to an odorant. In addition, it will also demonstrate that these strategies are flexible depending on environmental conditions, cue type, and individual differences.

Olfactory Terminology

What is an odor? An odorant is any substance, living or inorganic, from which odor molecules emanate. Odor molecules are chemical compounds which are detectable by olfactory receptor neurons and elicit a scent that can be perceived. The greatest influence on odorant movement is not diffusion but transport in a fluid, such as air or water (Murlis, Elkinton, et al., 1992). Turbulent diffusion in the form of advection is mainly responsible for bulk odor transportation and dispersion. Within the larger turbulent structure, dispersion as a result of random molecule movement, intermingling of different fluids, and shearing from the velocity field causes filaments of odor packets to break off the main mass and creates the structure of an odor plume (Moore & Crimaldi, 2004). Notably, the structure of an odor plume is highly complex. It is not continuous in space, but is highly intermittent with areas and filaments containing high concentrations of odor adjacent to regions completely devoid of odors (Connor, McHugh, et al., 2018).

A plume can be represented by its probability density or mean concentration (Bossert & Wilson, 1963; Weissburg, 2000). However, studies of invertebrates such as gypsy moths (*Lymantria dispar*) and lobsters (*Homarus americanus*), show that they are not in contact long enough with a plume to map its overall structure. Instead, they sample the instantaneous concentration of the plume using a sniff or the wave of an antennae to gather information before deciding on an action (Atema, 1996; Elkinton, Cardé, et al., 1984). For this reason, the human analysis of odor plume structures is generally a measurement of short-scale or instantaneous structure using highly detailed measurements (Finelli, Pentcheff, et al., 1999). These techniques include using photoionization devices (PIDs), which detect and measure air borne organic compounds. PIDs use a single sensor, or an array of sensors placed within the flow field of a plume. They can detect odor molecules in parts per billion with rapid frequency responses (Justus, Murlis, et al., 2002). Other methods include obtaining highly detailed images of odor plume structures imagined using planar laser-induced fluorescence (PLIF; Moore & Crimaldi, 2004). This method uses mixtures of air and compounds that can be excited to reflect light such that a camera can take images with high temporal resolution.

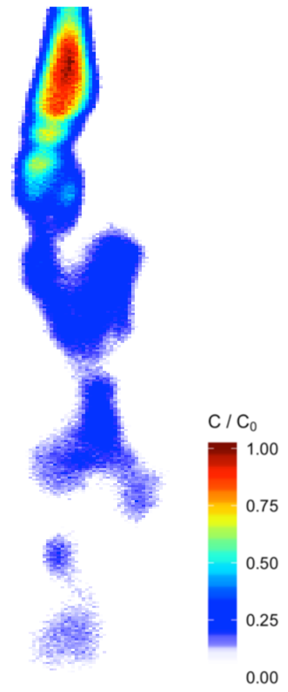

Figure 1.1. An acetone and air mixture odor plume flowing at 10 cm/s measured using PLIF. Odor plumes are characterized by empty spaces adjacent to high concentrations of odors. Colors represent normalized concentration distribution.

The landscape in which an odor plume is found affects the way it disperses. Odorants that are close to another object or substrate are subject to a viscous sublayer or boundary layer. Here, the flow of fluids is slowed and relatively laminar if the substrate is relatively flat (White, 2006). In these conditions, odor plumes tend to spread laterally across the substrate and diffusion plays a larger role in the spread of odors, resulting in plumes which show less empty regions within the main structure and fewer filaments (Connor, McHugh, et al., 2018; Jackson, Webster, et al., 2007; Paul A. Moore, Weissburg, et al., 1994). In contrast, when an odorant is located above the boundary layer, the odor plume is thinner, stretching longitudinally and sheds filaments of odors. This plume has greater patterns of intermittent of high and low odor concentrations (Fackrell & Robins, 1982). In both cases as the odor disperses laterally and longitudinally the concentration of the odors slowly diffuses into the surrounding fluid until it is no longer detectable by an organism (Markides & Mastorakos, 2006; Weissburg, 2000).

Other physical factors should affect dispersion as well, perhaps most obvious is that wind will affect a plume's structure. Higher wind speed predominately is responsible for increasing shear forces, causing increased shedding of odor filaments and a thin sinusoidal plume shape (Farrell, Murlis, et al., 2002). The effect of rapid dispersion of odor molecules also prevents the plume from rising as high and it will move laterally with the wind instead (Bursik, 2001). Temperature of the fluid carrying the odor molecules also will affect how an odor disperses. As temperatures rise, molecules are excited and move faster, causing molecules to move apart faster (Drescher, Lobascio, et al., 1995). This effect will carry odors upwards and away from the source more quickly (Baughman, Gadgil, et al., 1994). However, it should be noted that these are assumptions made from knowledge of fluid dynamics and have not been studied explicitly from measuring odor plumes.

Due to the complex nature of odor plumes, it is difficult to model odor concentrations within a plume given environmental variables. Imaging plumes while an organism moves through it is also a complex task because of the typically small scale of PLIF setups, high power of the lasers used, and restrictions on the types of odors that can imaged using PLIF. Therefore how environmental variables affect odor plume dispersion and quantifying the concentration of odors sampled by organisms that inform navigational choices during olfactory search are both areas of active research. One of the goals of this dissertation is to shed light on how organisms behave during olfactory navigation in response to olfactory plumes in variable environmental conditions.

Sampling Odors and Perception

Animals can detect odor molecules in the environment either passively or actively. Passive detection is used primarily by arthropods which have olfactory neurons concentrated on their antennae and appendages such as legs (Boeckh, Kaissling, et al., 1964; Derby & Atema, 1982; Koehl, Koseff, et al., 2001). Similarly, fish detect odors passively using nares lined with olfactory receptors in which water flows through (Cox, 2008; Hara, 1994). As odors flow over these receptors an odor is perceived without physical effort from the animal.

In contrast, active detection of odors is an intentional sampling method. For example, flounders *Lepidopsetta bilineata* and *Platichthys stellatus* will “cough”, flexing muscles that help pump water and sniff the surrounding water (Nevitt, 1991). Arthropods such as spiny lobsters (*Panulirus argus*), blue crabs (*Callinectes sapidus*), and mantis shrimp, flick their antennae to increase the probability of odor detection (Goldman & Koehl, 2001; Mead, Koehl, et al., 2002; Reidenbach & Koehl, 2011). Gypsy moths will flutter their wings to increase the transport of odors over the sensors (Carde & Hagaman, 1983).

The present dissertation focuses on active sampling in the form of sniffing in air by mammals. In mammals, sniffing is a specific rhythmic motor pattern powered by respiratory muscles to move odor molecules to the olfactory receptors in the olfactory epithelium in the nasal passages (Kepecs, Uchida, et al., 2006). The mechanical feedback of active sniffing is necessary for odor perception in humans, which without it is either dulled or is not perceived by the brain (E. Bocca, Antonelli, et al., 1965; Ettore Bocca & Battiston, 1964)(Mainland & Sobel, 2006). The same finding is also present in other mammalian species. Odors presented to anesthetized rats result in different activation patterns in main olfactory bulb than those of awake mice (Rinberg & Gelperin, 2006). Because the pattern of sniffing leads to different patterns of brain activation, it is clear that sniffing is a critical part of not just odor detection but also odor perception and categorization (Mozell, Kent, et al., 1990).

The pattern of sniffing also affects odor perception. Sniffs can vary in length, frequency, number and intensity. More frequent, short sniffs enhances the discrimination of odorants (Verhagen, Wesson, et al., 2007). The intensity of a sniff regulates the speed of airflow through the nostrils. The odor constancy model proposed by Teghtsoonian & Teghtsoonian (1978) suggests that the human brain must disentangle the speed of airflow intake and odor perception to process that a slow sniff and a fast sniff of the same volume and odor concentration are equal in their perceived intensity. However, other studies in humans have shown that the intensity of a sniff does affect the perception of an odor, with intense sniffs leading to a perception of higher odor concentrations (Laing, 1983). Parkinson patients, who often have dulled senses of smell can also be taught to sniff more intensely which leads to an increase in their ability to detect odors (Sobel, Thomason, et al., 2001). This dissertation investigated how people's sniffing intensity is controlled while they are navigating to address whether sniffing intensity is an important aspect of olfactory discrimination and navigation.

A slow, low-intensity sniff or a fast, high-intensity sniff, may all draw the same volume of air. However, the statistics of the plume for each of these conditions may differ because of the intermittent pattern of odor plumes. Therefore, how a mammal sniffs appears to be specifically adapted to what kind of information the animal is trying to extract from an odor plume. The strategies used by mammals to optimally control their sniffing while following an odor to its source is less clear. In this dissertation, sniffing behavior was examined during olfactory navigation to discover both how two disparate species of mammalian olfactory navigators, the domestic dog and the human, modulate sniffing and how such sampling is integrated with spatial navigation strategies.

Stereo Olfaction

Stereo olfaction plays an important role in assisting odor localization. The concept of stereo olfaction was first introduced by von Békésy (1963). In von Békésy's experiment, a ball of odor was suspended in front of blindfolded participants and they were asked to estimate the ball's location. When a nostril was obstructed localization accuracy was reduced. In American moles (*Scalopus aquaticus*), crossing the right and left olfactory inputs by inserting tubes into the nares resulted in turning away from the odor while using only one nostril caused a bias towards direction of the unobstructed nares (Catania, 2012). Porter et al. (2006) demonstrated that humans tracking a chocolate scent trail on the ground performed worse when trail following using their sense of smell if one nostril was taped shut. A similar effect was shown in rats following a trail with one nostril sewed shut (Khan, Sarangi, et al., 2011). Stereo olfaction effects have also been seen in invertebrates such as fruit flies (*Drosophila melanogaster*), honey bees (*Apis*), and crayfish (*Orconectes rusticus*; Borst & Heisenberg, 1982; Gomez-Marin & Louis, 2012; Kraus-Epley & Moore, 2002; Martin, 1965). In these experiments, one antenna was removed or the antennae were crossed. When a single antenna was removed invertebrates had a bias towards the side with the remaining antenna. If the antennae were crossed they would run in the opposite direction of the scent. Although vertebrates and invertebrates have different sampling strategies, they use stereo olfaction in similar ways. Theoretical studies have also demonstrated the value of two sensors over one sensor in the ability to determine relative location in an odor plume (Baker, Dickinson, et al., 2018). Boie, Connor, et al. (2018) described a theoretical experiment comparing sampling with a single sensor to two sensors while in a fixed location. Sampling with two sensors has a higher rate of mutual information gain in comparison to one sensor.

As shown, having access to stereo cues increases the ability to find an odorant. However, in many of the examples listed, both vertebrates and invertebrates eventually locate or followed the odorants which were presented. This was achieved by active spatial and temporal comparisons of samples (spatio-temporal strategy) by moving the whole body or a single sensor to two or more locations. Rats and people meander more widely when following a trail when a single nostril is obstructed (Khan, Sarangi, et al., 2011; Porter, Craven, et al., 2006). Bees actively flick their remaining antenna back and forth (Martin, 1965) and fruit fly larvae move their heads laterally to sample more broadly (Gomez-Marin & Louis, 2012).

Primate nose structure is highly variable. But among the great apes, only the genus *Homo* has uniquely evolved an external pyramid. One possible function for the evolution of this structure is an enhancement of stereo olfaction (Jacobs, 2019), Human olfaction has historically been underestimated (McGann, 2017; Shepherd, 2004). Sela & Sobel (2010) referred to human perceptual abilities as a "constant state of change blindness". However, humans may be able to discriminate as many odors as mammalian species, such as mice, which have nearly three times as many unique olfactory receptors (McGann, 2017; Niimura, 2012; Shepherd, 2004; Zhang & Firestein, 2002). Humans have also been shown to use odors to orient in space, both tracking a substrate-bound odor trail (Porter, 2006) and mapping an arbitrary location in a room (Jacobs et al., 2015). Thus humans are an interesting species to study the use of odors in navigation and are the focus of two experiments in this dissertation (Chapters Three and Four). These chapters will describe how humans modulate their sampling behavior when navigating using stereo or non-stereo cues.

Individual Differences

Despite its stereotyped nature, sniffing patterns vary among individuals. When people are asked to sniff at a specific intensity and frequency, their perception of an odor is different than when they sniff at their own pace (Laing, 1983). Mice also show differential patterns of sniffing between individuals when they forage for food (Wesson, Donahou, et al., 2008). Why different individuals have different sniffing patterns is unknown. It suggests, however, that sniffing is a partially learned behavior for optimizing odor detection, and develops in response to an individual's experience. Therefore, for each individual of a species in their different environments and slightly variable olfactory thresholds, unique sniffing patterns may optimize olfactory functions (Mainland & Sobel, 2006), which could include navigation.

A well-documented pattern of individual differences in olfactory ability is differences between women and men. Women have a lower olfactory threshold, i.e., they discriminate odors at lower concentrations than men (Brand & Millot, 2001; Dalton, Doolittle, et al., 2002; Koelega & Köster, 1974). Women also remember odors more accurately than men (Larsson, Lövdén, et al., 2003). If olfaction is an important source of information for navigation, then such sex differences in olfaction may be related to sex differences in spatial cognition. For example, men rely more heavily on distal cues and directional cues for navigation (Astur, Ortiz, et al., 1998; Chai & Jacobs, 2010) while women rely more on local and visual cues (Sandstrom, Kaufman, et al., 1998; Tlauka, Brolese, et al., 2005). If one of the primary drivers of increased spatial abilities is related to olfactory abilities (Jacobs, 2012) then there may be an important link between sex differences in olfaction and sex differences in spatial orientation. Thus, this dissertation also focuses on human sex differences in their ability to use odors for navigation as well as their more abstract sense of direction, predicting that the sense of direction may predict performance in odor navigation tasks.

Olfactory Navigation Behavior

When an odor is detected, odor plume or trail *following* begins. One of the most notable behaviors in many animals during odor *following* is casting (Dusenbery, 1992). Casting is characterized by broad, switch-back turns while advancing towards an odorant. This behavior is thought to occur whenever an odor plume is lost and an animal attempts to re-contact the odor (Kennedy & Marsh, 2019). Casting is well documented in gypsy moths (Kenen & Cardé, 1994), fruit flies (Van Breugel & Dickinson, 2014), blue crabs (Keller & Weissburg, 2004), *Procellariiform* birds and albatross (*Diomedea exulans*, Hutchison & Wenzel, 1980; Nevitt, Losekoot, et al., 2008), rats (Khan, Sarangi, et al., 2011), and even vertical casting in the water column by fish (*Salmo salar*; Døving, Westerberg, et al., 1985). It is logical that when an odor is lost, the animal should reverse directions to re-locate the odor resulting in the zig zagging trajectory typical of casting. However, because odor plumes can largely be devoid of odorants, olfactory navigation cannot be based on a simple strategy to reverse direction each time the odor is lost, else an animal would waste time and energy casting continuously. Animals therefore must discriminate between sampling an empty space within the plume where no direction change is required and sampling an empty space outside of the bulk plume, where casting must be initiated to reconnect with the plume. The navigators are able to form a model of the overall plume shape. Hence, casting can be seen as a behavioral signal that signifies the approximate shape and location of the odor plume. Less is understood about what changes in odor concentration cue animals the odor plume has truly been lost, a question that will also be addressed in this dissertation with experiments in humans.

Before an animal can navigate through an odor plume to find an odorant it must be able to find the odor plume itself. An understudied aspect of olfactory navigation is how animals first *search* for an odor in a *de novo* environment. In a naturalistic setting, the proportion of possible areas to search for an odor to the odor plume itself may be immense. Patch foraging models give some clues to how animals may search for odors. At smaller scales when there are visual cues that may give hints to where a resource is located, models have been proposed that optimize time spent searching in each patch before moving on in an explore-exploit tradeoff (Bénichou, Coppey, et al., 2005; Linhares, 1998; Parker & Stuart, 1976). In a large environment with few clues of where to search, animals risk time and energy searching for resource patches. One proposed search strategy is using Lévy walks to search the environment until encountering resources (Bartumeus, Da Luz, et al., 2005; Zollner, Lima, et al., 2007). In a Lévy walk, animals are modeled as walking in a direction and then performing a concentrated search within a region. A visual representation of the model appears as clusters of looping paths which are connected by the single path an animal takes to move between possible resource patches.

Lévy walks may be similar to olfactory navigation behavior, especially casting. It is possible that when animals search for an odor they begin by walking using a correlated random walk that dissuades them from returning to a previously visited location. As the animal encounters an odor plume their navigation suddenly switches to *following* the odor plume or trail to its source, which is often characterized by casting behavior. One can imagine then, that the trajectory taken by an animal as it searches and then casts to the source is quite similar to a Lévy walk and perhaps the driving factor that makes animals produce the Lévy walk pattern is because they are contacting and searching within odor plumes (Pasternak, Bartumeus, et al., 2009; Reynolds, Cecere, et al., 2015). Chapter Four of this dissertation will showcase the various *search* strategies that people used to initially find an odor plume. All chapters will discuss behaviors mammals exhibit as they *follow* odor trails and odor plumes to their source.

Dissertation Overview

Olfaction remains one of the final sensory systems to be studied in depth. Great challenges remain in understanding both the physical properties of odor plumes themselves and the neurological processes that underlie perception of odors. Scientists are tackling these questions from both sides of the spectrum, from theoretical models of odor movement to behavioral experiments. At one end, physicists are just beginning to characterize odor plumes in simple flow fields using precise measurements. Detailed information about how odors are moving in complex landscapes in response to meteorological conditions is still unknown. On the other hand, several decades of studies has examined how invertebrates and some birds may behave as they follow odor plumes. The results of these studies have shown a great deal about movement patterns as animals track odor plumes. However, minimal research has examined how animals, specifically mammals, gather information about odors. How mammals modulate their sniffing behavior, their reliance on stereo olfaction, and how they move as they follow odors is largely unknown.

The goal of this dissertation is to address this gap with three experiments investigating how mammals sample odors and navigate using olfaction. Because there has been little research on this topic, the scope of the present work investigates olfactory navigation broadly, using both field experiments under natural conditions and highly controlled laboratory experiments to examine decision-making processes in response to measurable instantaneous concentrations of odors. Each chapter represents one point along the spectrum from the natural to the controlled, from the study of navigation to complex natural odors in the field, to the oriented to simulated odor plumes in a virtual environment.

Chapter Two describes a study of one of the most familiar of olfactory navigators: the domestic dog (*Canis lupus familiaris*), utilizing trained search and rescue (SAR) trailing dogs tracking the odor trail of a human. During ordinary work conditions, trained SAR dogs navigate through odor plumes under complex and unpredictable meteorological conditions, such as variations in wind, temperatures and humidity. The study described in this chapter examined how such environmental factors affect the navigation patterns of SAR dogs and the behavioral strategies employed by the dogs. These results document quantitatively, for the first time, the how such meteorological conditions influence a SAR dog's performance and search strategy.

The second mammalian species studied in this dissertation is also familiar but is a species with a considerably less robust olfactory system, the human. Chapter Three examines humans as they searched for an odorant indoors, where participants searched for a scent diffuser, releasing a target odor, in a large indoor gridded room. The goal of this experiment was to discover how mammals, such as humans, modify their sampling behaviors as they approach an odorant, and the importance of stereo olfaction in this process. This research revealed that humans adjusted their sampling behavior when stereo olfaction was removed and hence only one nostril could be used for sampling. This research was also illuminated questions about sex differences in human olfactory navigation as well as the role played by individual differences in the sense of direction.

Chapter Four further addresses outstanding questions about human olfactory navigation by studying human olfactory navigation and sampling strategies in a virtual environment. One of the most significant challenges when studying olfactory navigation is understanding how an organism decodes the statistics of information contained in an odor plume to make adaptive navigational choices. Animals may possess a mental prototype of an odor plume, either learned through experience or through evolution (Baker, Dickinson, et al., 2018). To test this hypothesis, an odor plume was represented by an audio proxy, where tone intensity was linearly scaled to odor concentration from a quantified odor plume. Empirical measurements of a known air-borne odor plume, measured with PLIF, were set in a virtual environment where participants were asked to estimate the origin of the odor source. The trajectories of participants along with the known quantities of stimuli experienced for each sample were recorded. Participants were presented the data in stereo, mono, and as crossed (right and left stimuli reversed) to determine how these conditions affected the search strategy of the participants. These responses included movement patterns, casting and sampling rates. Strategies were found to differ both between participants of different sexes and also among individuals with different levels of self-reported sense of direction.

Chapter 2

Effects of ambient meteorological conditions on olfactory search behavior in dogs

Introduction

The structure of odor plumes can vary with many factors, such as ambient temperatures, wind, and the geographic features of the environment (Connor, McHugh, et al., 2018; Drescher, Lobascio, et al., 1995; Finelli, 2000). All of these factors have been documented and analyzed using the principles of fluid dynamics (Chapter One). However, most studies of odor plumes have been restricted to small laboratory flumes, where the flow of the odors and the variables affecting them can be tightly controlled to ensure accurate measurements. None of these experiments have specifically measured the effects of temperature and humidity on an odor plume's structure.

There are two ways odors could be affected by meteorological conditions. First, the current (prevailing) conditions could determine the spatial location of the odors in the environment. Second, the magnitude of change in the conditions between the time the odor was deposited and when the odor is detected by the navigator could have a strong effect on how the odor has dispersed. Higher temperatures should cause volatized odors to disperse more quickly (Drescher, Lobascio, et al., 1995). Humidity may affect the concentration of odorants held by substrate (Vander Wall, 2003). Ambient wind should have a significant effect, spreading odor molecules more broadly with higher wind speed (Bursik, 2001).

Understanding how an odor behaves in a complex natural environment, however, is a challenge. As discussed in the previous chapter, measuring odor plumes with PLIF is impossible in the field. Using PIDs is a possible solution, but ideally one would measure the plume at many different locations to describe the overall geometry of an odor plume. An alternative is the bioassay: using an animal as biological odor detector, as has been done with parasitic wasps (*Microplitis croceipes C.*), hawkmoths (*Manduca sexta L.*), and honey bees (*Apis melifera L.*; Rains, Tomberlin, et al., 2008).

Most studies have focused on the behaviors of animals in a single type of plume, either turbulent or laminar (Álvarez-Salvado, Licata, et al., 2018; Reidenbach & Koehl, 2011). Few studies have compared sampling behavior and spatial movements by animals during olfactory navigation with respect to the same odor source under different conditions that affect the structure of the plume (Willis, 2005). The behavior of animals should change in response to the same odorant under different conditions. Olfactory navigation in mammals is understudied, however, and no study of mammals has compared behavioral strategies used by a navigator tracking the same odorant in the same landscape under different meteorological conditions. The goals of this experiment were to study odor dispersion due to meteorological conditions in nature by studying how mammals change their behavior to successfully navigate the same odor plumes.

The Domestic Dog

The domestic dog olfactory epithelium ranges in area between 67-200 cm^2, depending on the breed and have more than 1,000 unique olfactory receptors (Quignon, Rimbault, et al., 2012). In contrast, the area of the human olfactory epithelium area is 1-5 cm^2 with less than 400 unique receptors (Malnic, Godfrey, et al., 2004). The dog has a lower olfactory threshold than humans for most odors, on the order of parts per trillion, and can discriminate more odors than humans (Walker, Walker, et al., 2006).

Humans and domestic dogs have been co-evolving for at least 14,000 years and possibly longer (Leonard, 2013). People utilized dogs to increase hunting success and as alarms for danger (Schleidt & Shalter, 2003; Steen, Mohus, et al., 1996). Modern dogs are used worldwide for their olfactory function, including hunting, contraband detection, and locating humans, such as finding missing persons during search and rescue (SAR).

A variety of different breeds used for SAR (Fenton, 1992). Most of the dogs are medium sized, chosen predominately for their ability to navigate through thick underbrush and climb over obstacles if necessary. In addition, the breeds chosen are ones that are intelligent and willing to work independently, but still responsive to their handlers. These characteristics enable the formation of a close working partnership between handler and dog. As a result, it is perhaps no surprise that the common SAR dog breeds are classic working breeds such as German Shepherds (Willis, 1991), retrievers, and collies (Rooney & Bradshaw, 2004).

There are three primary regimes used to train SAR dogs to find people. The prototypical dogs following a trail to find a person are part of a SAR group called *trailing* dogs. These dogs are trained to find the person whose scent matches a sample presented to them. The dogs work on leash with their handlers and use scents on the ground and in the air to follow the same trail made by the target person. In contrast, *area* SAR dogs which are trained to find any living human within a designated area. These dogs work off leash, typically walking transects with the handler behind them (Greatbatch, Gosling, et al., 2015). Area dogs can often be seen during the aftermath of crises such as natural disasters where people may become buried under rubble. They typically do not follow an odor trail, as there may be none, and instead sample the air to find people and will alert to the odors of any living person found. Lastly, there are *cadaver* dogs which work on leash and are trained only to find deceased individuals or their body parts, possibly hidden in substrate or within structures (Oesterhelweg, Kröber, et al., 2008). Some SAR dogs are trained under multiple paradigms, with many dogs trained both as area and search dogs. However, the dog's search behavior is dictated by how handlers cue them to search during a mission. If on a leash and given a person's odor to sample, the dogs will trail. When off leash and not given an odor sample, the dogs will exhibit area search behavior. If not given an odor and lead in a specific search pattern, dogs will search for cadavers. This ability to seamlessly search for a variety of different human odors highlights the SAR dog's highly trained abilities to track different types of odor plumes, an ability that mimics the range of strategies seen in wild carnivores (Conover, 2007).

Despite the historical and economic importance of search dogs, there has been surprisingly little research their olfactory abilities and search behavior. These studies used a variety of differently trained dogs, including trailing, area, and cadaver dogs as well as dogs which search for explosives and biological material, each of which utilizes different training and search strategies to detect different characteristics of odors (Greatbatch, Gosling, et al., 2015; Lazarowski, Haney, et al., 2018; Reed, Bidlack, et al., 2011). For example, trailing dogs search for odor trails, while area and cadaver dogs search for point source odors (Dorriety, 2007; Hepper & Wells, 2005); these two kinds of odor sources represent two very different odor plume structures and hence it is difficult to compare strategies across these studies.

I used trailing dogs in this experiment because they are trained to search for a unique scent along an odor trail that could be replicated under various meteorological conditions. Trailing dogs also search mostly without influence of their handlers and exhibit more natural tracking behavior unlike other SAR dogs which are guided to search by their handlers. Furthermore, I could design a clear path for the dogs to follow could be created that avoided structures and tree canopies that may interfere with GPS signals.

Dogs were predicted to search farther from the trail when it was windy, dry, and hot. Under these conditions, the rate of odor dispersion should increase and spread odors laterally away from a human's trail, as well as upwards into the air. Dogs may be able to follow the odor to find a person just as accurately under these conditions because the odors are spread in a wider cone. However, if the odor plume becomes too dispersed, then dogs will lose the ability to follow the odor gradient effectively and become lost. Under these hot conditions, dogs should rely on sampling the ground for odors that are trapped close to substrate instead of sampling the air.

Methods

Study Animals

Six domestic dogs were used in this study, four females (three German Shepherds, one English Border Collie) and two males (German Shepherd and Labrador Retriever; mean age 6 ± 1.67; average ± standard deviation). All dogs were SAR dogs trained for trailing humans. They were mission ready certified by the California Rescue Dog Association. Research was approved by the Animal Care and Use Committee of the University of California, Berkeley.

Field Site

A field site was chosen in Briones Regional Park, Martinez, California at the Bear Creek staging area entrance. An approximately 800 m trail was recorded at 5 Hz using a custom global positioning system (GPS) logger (Ultimate GPS Module - MTK3339 chipset and Adafruit Assembled Data Logging Shield) designed and assembled by Alex Reben. The maximum altitude change of experiment site was 36 m. The trail began at the edge of a parking lot, and briefly followed the edge of a tree line before predominately crossing over open fields. After approximately 300 m into the trail a low wood fence was crossed by teams. Immediately following the fence a single switchback was put into the trail before the trail continued east. A small valley was situated between two low hills around two-thirds into the trail. The end of the

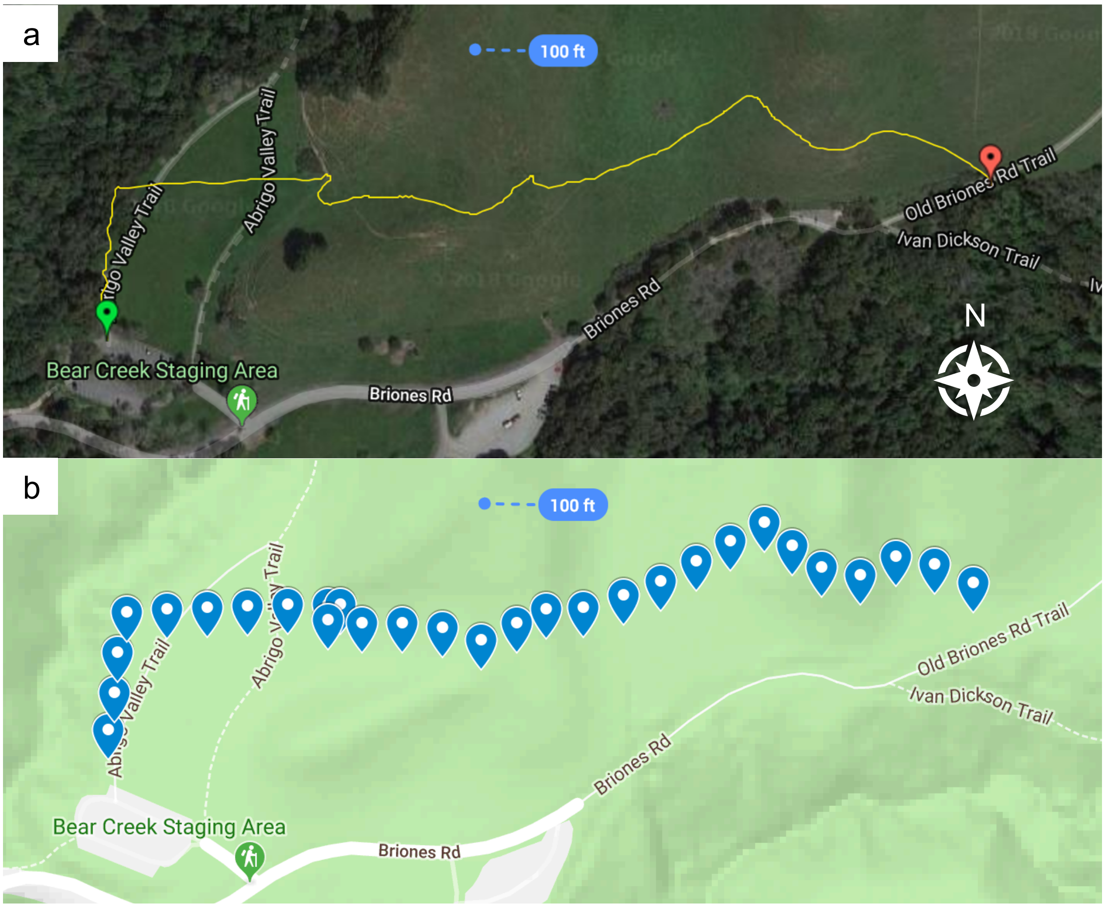

Figure 2.1. (a) Trail created by researchers acting as the “missing” person for SAR teams to follow. All researchers walked the same direction from west to east. Green marker is the start of the trail, red is the end. (b) Thirty-one waypoints were marked 25 m apart on the trail, including the start and end point of the trail. The research team collected data about ambient weather conditions at each waypoint. The geography of the landscape is also shown in this panel.

trail was hidden behind a tall shrubs and trees which occluded the target person from the handler's and dog's view (Figure 2.1a).

The field site had little to no brush or tree cover. This ensured the GPS readings were accurate and allowed researchers to carry meteorological equipment. The site was a field devoid of foxtails (*Hordeum*) which are a danger to dogs if they are inhaled. In addition, the field avoided manmade trails which could bias the dog's path choice. Furthermore, the location had relatively low elevation changes because the effects of geography on odor dispersion were not a primary focus of the study. In addition, the field site avoided geographic or vegetation "corridors" which would constrain the trail to a narrow region surrounded by hills or trees. This ensured the dogs were not navigating the trail because it was the only possible direction to travel. Finally, the site included an exit route for the target person to leave without needing to overlapping the experimental trail. The exit route purposefully gave the experimental trail a wide berth to prevent dogs from following the exit route instead of the experimental trail and prevented interference with the next dog searching the trail.

GPS and waypoints

GPS is susceptible to measurement errors up to hundreds of meters depending on the environment in which the GPS is located and the GPS equipment used (Thomson, Börger, et al., 2017). Errors can arise for many reasons including poor satellite geometry, obstruction of the satellite signal by trees, or atmospheric refraction (Johnson & Barton, 2004). Strategies used to reduce measurement errors, include averaging multiple consecutive recordings, differential corrections, and data screening (Lewis, Rachlow, et al., 2007; Moen, Pastor, et al., 1997; Oderwald & Boucher, 2003). I averaged multiple GPS points to mitigate the effects of measurement error. The GPS logged data at 5 Hz and synced to time stamped data. Every five points were averaged to find the location for every second of movement. A visual examination of the mapped experimental trail compared to the known path showed that the averaging of GPS waypoints was highly accurate.

I marked waypoints for collecting meteorological data on the map, and were measured after GPS averaging and located every 25 m along the trail. In total there were 31 waypoints including the start and endpoints of the trail (Figure 2.1b). The experimental trail and waypoints were uploaded to Google MyMaps. Researchers used their cell phone's built in GPS to follow both the trail and detect the waypoints during the study.

Equipment

A microphone collar was made from 2” (5 cm) wide elastic. An Omron Sprague Rappapport Stethoscope was dissembled such that only the diaphragm was left attached to the chest piece. The casing of a Neewer 3.5mm microphone was removed from its casing and glued to the stem of the stethoscope chest piece using hot glue. Any open holes were glued shut on the chest piece and the exposed microphone wiring was bound with electrical tape. The stethoscope microphone was then attached to the collar with two overlapping 2” (5 cm) D-rings sewed to the elastic strap. Velcro was sewn onto the collar to allow for size adjustments for each dog breed. In addition, two ¼” (6 mm) elastic button hole strips with a matching button was sewed to collar and assisted securing the microphone collar to the dog’s primary collar to prevent the microphone from slipping from its position on the dog’s trachea (Figure 2.2). Audio was recorded using a Zoom H1 Handy Digital Recorder. Spectra 360 Electrode Gel was placed on both the dog’s neck over their trachea and on the stethoscope diaphragm before securing the collar to reduce sound from friction of the dog’s fur.

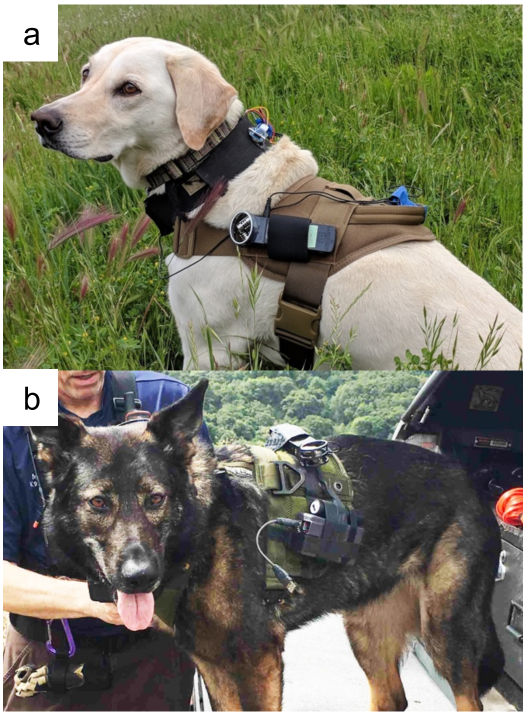

Figure 2.2. SAR dog wearing research harnesses. (a) Audio recorder, microphone collar, and tilt switch (blue chip attached to collar) are shown. (b) Data logger for GPS and tilt switch.

Custom dog harnesses were modified with elastic bands and Velcro. Each harness held the Zoom recorder and the data logger containing the GPS. In addition, an Adafruit tilt ball switch sensor was also connected to the data logger. Dogs walked with their heads aligned with their spine while searching, and were considered to be air-scenting while in this position. The tilt switch was glued to a wood shaving cut at a 20 degree angle to ensure the switch registered a “head up” when the dogs were in their ordinary search posture. When the dog’s head was lowered greater than 20 degrees downwards the data logger would record a “head down” position as the dog’s sampled the ground for odors. The tilt switch was clipped to the microphone collar (Figure 2.2a).

Ambient meteorological conditions were recorded using Davis Weather Stations (6250 Vantage Vue). The weather station was mounted on a 6’ (1.8 m) speaker tripod (PylePro) and pointed north at each waypoint to ensure accurate measurements of wind direction. A handheld console paired with the weather station showed the relative humidity, temperature (Celsius), wind (m/s), and barometric pressure (mmHg). Absolute humidity was calculated from relative humidity and temperature during analysis (Oyj, 2013).

Procedure

Searches occurred during three times of day, morning (08:00 – 10:00), afternoon (13:00 – 15:00), and evening (18:00 – 20:00). The study took place between June and December of 2017. These three times of day were chosen to capture a range of meteorological conditions. The consistent climate of this location in the Bay Area of California allowed similar weather patterns to be captured for each trial despite study days occurring weeks to months apart.

One hour prior to the arrival of the SAR teams, a researcher acting as the target odor walked the experimental trail. The researcher walked at a normal pace and did not try to conceal their scent. Upon completing the trail they sat and waited to be found for the duration of the trials. Six different researchers laid the trail for the SAR teams. No dog searched for the same person twice and when possible the same person was asked to act as the target for the corresponding time of day to maintain consistency across all searches. Walking the trail took approximately 15 minutes.

Immediately following the departure of the target, a team of researchers followed on the trail, recording ambient temperature, humidity, barometric pressure, wind velocity, and wind magnitude at each waypoint. The weather vane was stopped and pointed due North upon reaching each waypoint to ensure wind conditions were from the waypoint location and not a result of researcher movement. Researchers waited one minute per waypoint before recording data to allow the weather station to calibrate. Data collection took approximately 30-40 minutes for the entire trail of 31 waypoints.

Each SAR team searched the experimental trail three times. At most, four teams participated in the study on the same day for a given session (morning, afternoon, or evening). One team searched the trail at a time. The next team would not begin until the previous team had found the target. The order in which dogs searched the trail per session were counterbalanced to ensure dogs were not following the trails of other search teams, instead of the target's scent. Each team did not return to the experiment site for a minimum of one week between searches.

A scent article was left by the target person at the start of the trail, typically an unwashed t-shirt or a scent sample taken by wiping the neck with a cotton swab. To reduce "scent contamination" (i.e. mixing of multiple people's odors), the scent article was placed into sealed plastic bags by the target. SAR dogs were presented with the scent article by opening the plastic bag to expose the odorant. Often this was the only instance which the dog was exposed to the odorant. On rare occasions if the scent was lost by the dog, the handlers presented the scent article a second time.

Search teams were lead to the start of the experimental trail by the research team collecting meteorological data. Researchers turned on recording equipment on the dog's harness and dictated to the audio recorder the date, name of dog, and the current time. The SAR handler presented the dog with the scent article and then they began searching for the target. Data collection researchers then proceeded to record meteorological data again, staying behind SAR teams to prevent revealing information about the experimental trail.

During each of the three searches, the dog was given the scent of a new target, thus the dog would follow a new scent and presumably to the dog, a new trail, as they were trained to do. Often, the researcher who had previously acted as the target during a prior search was part of the meteorological data collection team following the SAR team. Thus, if a dog was attempting to follow the target odor from a prior search, the dog should locate the target person standing behind them. This never occurred, therefore it was unlikely the dog was biased towards following a previous trail.

Each handler was told not to guide their dogs and allow them to search naturally. The handler was not shown the trail map and could only see the initial departure of teams before them. Therefore, although the handler remembered where the target was hiding, it was unclear to the handler if the path they had previously walked was correct. A handler biased towards their prior search paths would lead them to repeat the exact path taken in prior searches which would be evident in post-analyses.

Analyses

To understand how average environmental conditions during each search affected odor dispersion and therefore the search behavior of dogs, metrics to quantify dog behavior were carefully chosen. Three possible metrics were considered: average distance from the trail, tortuosity, and the area of the region captured between the experimental trail and the dog's trail. Each GPS point of a dog's trail was matched to the nearest GPS point of the experimental trail `using a K-D decision tree (Python *scikit-learn*) to find the distance between the dog and the trail. However, taking an average of these distances proved to be a poor measure for overall performance during a search because some dogs occasionally would stray far from the path before returning and walking close to the original trail. The average distance would become skewed and this metric was not reported. Tortuosity, the ratio of the length of the dogs' path to the length of the true path, was also considered. However, tortuosity gave no insight into how far the dogs were from the experimental trail and was also not used. A "between trails area" was chosen to assess how closely dogs followed the human's trail. The area of a polygon with a perimeter created by the dog's path and the experimental trail was calculated. A larger area meant a dog was far from the experimental trail, while a small area meant the dog stayed close (Figure 2.3a). In addition, the average frequency of ground sampling was found for the duration of the search.

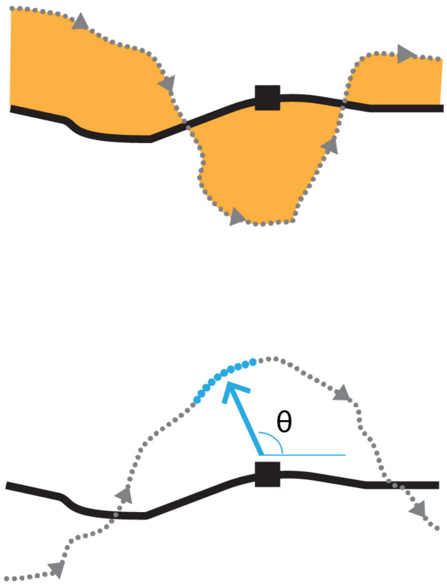

Figure 2.3. A schematic representing the two metrics used to assess how closely to the person's trail a dog was during the trials. Example dog trail shown in dotted gray line, example target trail in black. A waypoint is represented by a black square. (a) The between trails area was calculated by finding the area of the region (orange) between the dog's trail and the person's trail. (b) the distance and angle from weather station waypoint to the dog was found by averaging the ten closest points on the dog's trail to the weather station.

The trail's waypoints were diverse geographic areas and vegetation. It was feasible then that odors may disperse at different rates along the trail. The nearest ten GPS locations of a dog to a waypoint were found using a K-D tree (Figure 2.3). Ten GPS points were used in case one location biased the dog's location to be farther or closer than the true distance to the waypoint. The average distance from these ten GPS points was then found. In addition, an average vector was found between the waypoint and the dog's location using the same ten GPS points to determine the angle from the waypoint to dog. The average frequency of ground sampling behavior was also calculated near each weather station from the ten data points (Figure 2.3).

Meteorological data was categorized into two groups, prevailing conditions and the temporal condition changes. Prevailing conditions represented the meteorological data collected when the dog searched the trail. Temporal changes were measured by the magnitude of change in each meteorological reading during the time period between when the trail was laid and when each SAR team began their search. This was found by subtracting the meteorological readings at each waypoint during the time of the dog's search from the respective readings taken at the waypoint when the trail was laid.

Data were retrieved and cleaned using Python 3.6.1. Statistical analyses were performed with R 3.5.1. Linear mixed models (LMMs) and mixed effects logistic regressions were used to determine the prevailing and temporal conditions that influenced dog search behavior the most (R *lme4*). Dog IDs were included in each model as a random effect. Meteorological conditions were stepped backwards removing the least significant variable or until only significant variables remained. Conditional r^2 values are reported using the *MuMIn* R package for linear mixed effects models. Alpha was set to 0.05. Averages and standard deviations are reported throughout text.

Results

Four of the six dogs completed all three time of day searches. The remaining teams completed one trial each, an evening and an afternoon trial. In total 15 searches were performed. Only one search was unsuccessful and results were not analyzed for this experiment because the team was unable to recover the experimental trail. Although recordings were made for each search, the sound of the dogs' fur interfered with respiratory recordings and hence audio data could not be analyzed.

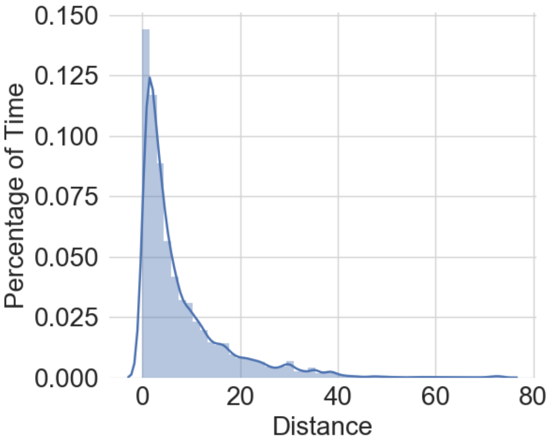

Figure 2.4. Distribution of distances from the person's trail dogs for all searches. The majority of the time dogs are within 10 m of the person's trail.

Dogs predominately stayed within 10 m of the experimental trail (Figure 2.4). This suggested that the bulk of the odor was concentrated within 10 m of the target person's trail. Search teams averaged 14.23 ± 4.34 minutes to locate the target. Dogs searched at an average speed of 0.89 ± 0.82 m/s, with some dogs averaging higher speeds, up to 1.6 m/s. Overall, dogs traversed 0.61 ± 0.1 miles in comparison to the 800 m long trail laid by the person. The average area of the between trails region was 5373.0 ± 2378.32 m^2.

Search trajectories for dogs can be seen in Figure 2.5. Dogs stayed relatively close to the experimental trail. During two searches, the same dog strayed far from the trail before recovering the odor and returning to the correct trajectory (Figure 2.5b). The beginning of the trail purposefully hugged a tree line. However, it was also located adjacent to a sidewalk. Some dogs followed the trail correctly along the trees while other dogs followed the sidewalk and then were able to successfully cut east to continue following the trail. At the switchback approximately a third of the distance into the experimental trail (waypoints 11 and 12), the dogs almost never followed the experimental trail and instead shortcut directly the trail. A small hill was present in the last quarter of the trail. The dogs often searched below the main trail, perhaps signaling that odors were more likely to disperse down the slope. When farther from the experimental trail, dogs were observed making loops before returning to the main trail.

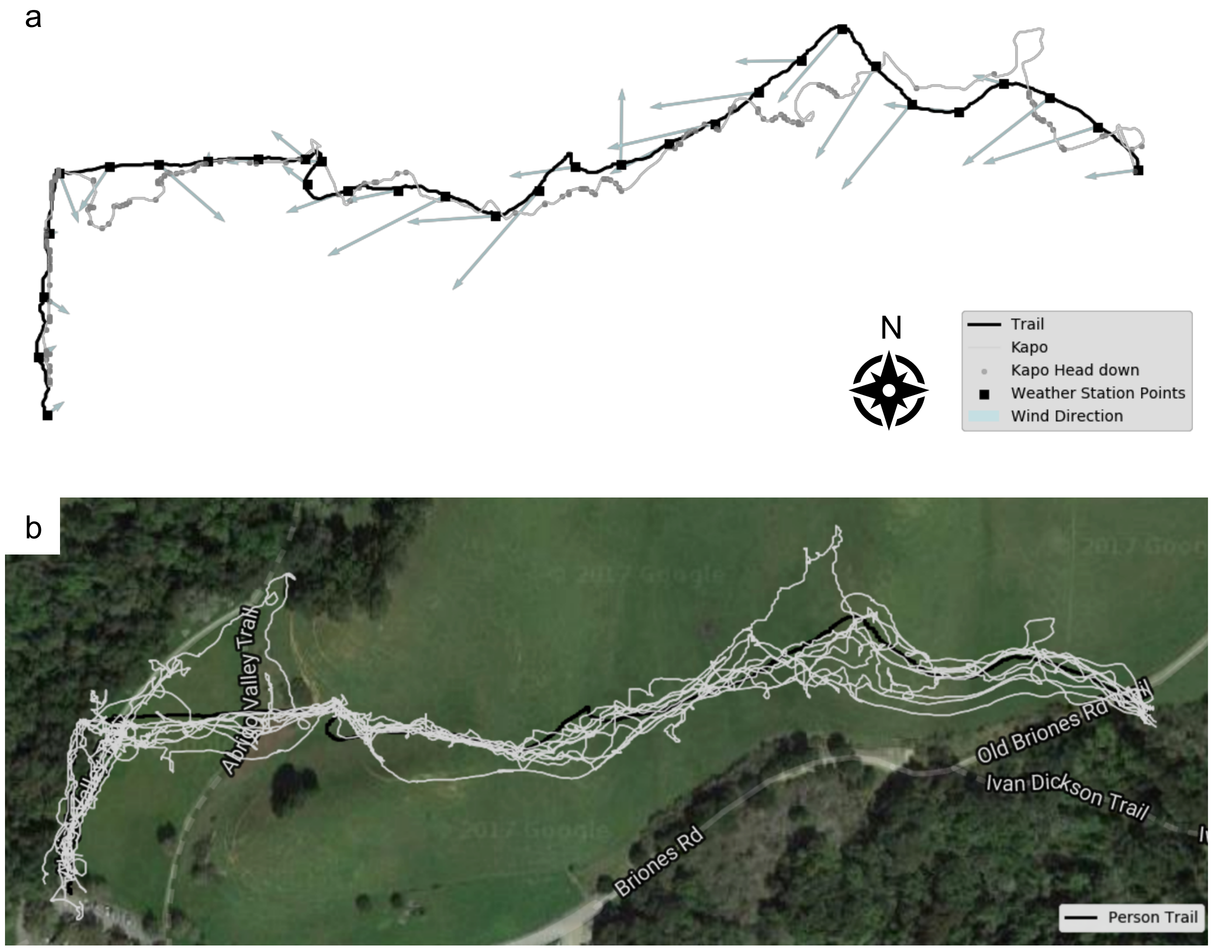

Figure 2.5. Two different overhead views of the target person's trail and dogs' trails. (a) An example of a search performed by a SAR trailing team. Trails ran from west to east. Black line shows the experimental trail walked by a person. Black squares show the location of each waypoint where meteorological data was recorded both before and during the dogs' searches. Light gray line is the dog's path. Gray circles on the line show whenever a dog sampled odors from the ground. Blue vectors show relative wind speed and direction. (b) All 15 search paths taken by dogs during the experiment.

Meteorological Conditions

In total, 434 meteorological data points were collected. Average prevailing meteorological conditions from the different time periods can be seen in Table 2.1. Morning conditions and evening conditions are similar. However, mornings were drier and windier than evenings. Afternoon conditions were especially hot and dry. A correlation matrix was created that included temperature, humidity, absolute humidity, barometric pressure, and wind speed. A strong correlation was found between temperature and relative humidity (r = -0.86) as well as temperature and absolute humidity (r = 0.82). Temperature was excluded from linear mixed effects models to avoid multicollinearity.

Table 2.1 also shows the temporal change in conditions between when the trail was laid and when the dogs searched for trail. There was a decrease in humidity in the morning trials between when the target person laid the trail and when a dog began its search. An inverse pattern occurred with temperature decreasing and humidity increasing during the evening trials.

Table 2.1. Average meteorological conditions for each period of the day when searches took place. Wind direction is direction from which it originates with 0 degrees at North and continuing clockwise.

Prevailing Conditions during Search

	Temperature (C)	Relative Humidity (%)	Absolute Humidity (%)	Barometric Pressure (mmHg)	Wind Speed (m/s)	Wind Direction (degrees)
Morning	16.77 ± 0.86	61.11 ± 10.18	8.70 ± 1.31	742.55 ± 2.36	2.51 ± 1.55	184.81 ± 79.29
Afternoon	30.96 ± 5.82	41.79 ± 11.70	12.89 ± 1.16	756.39 ± 6.70	1.81 ± 0.96	153.13 ± 107.2
Evening	16.60 ± 6.85	74.92 ± 8.62	10.89 ± 3.05	751.46 ± 5.75	0.97 ± 1.12	189.07 ± 90.55

Temporal Changes between Trail Laying and Search

	Temperature (C)	Relative Humidity (%)	Absolute Humidity (%)	Barometric Pressure (mmHg)	Wind Speed (m/s)	Wind Direction (degrees)
Morning	2.01 ± 0.83	-5.48 ± 4.20	0.33 ± 0.48	0.44 ± 0.92	0.73 ± 1.25	-18.19 ± 104.16
Afternoon	1.14 ± 1.08	-2.12 ± 2.54	0.02 ± 0.55	-0.57 ± 0.38	0.38 ± 1.11	38.63 ± 108.25
Evening	-4.35 ± 1.48	13.53 ± 4.48	-0.51 ± 0.35	3.32 ± 6.11	-0.63 ± 1.04	-57.75 ± 104.17

Design Method Effects

Each trial was separated by an average of 9.63 ± 3.96 days, yet it was unclear how long dogs may have remembered a previously searched area and could have attempted to search the same trail. Yet, a LMM showed that the number of searches each dog had previously performed had no effect on the time it took to complete a trial (14.23 ± 4.34 min; $\beta = -48.06$, $t_{8.4} = -0.79$, $p = 0.45$; cond. $r^2 = 0.21$). Nor did the number of previous searches affect the between trails area calculated (6919.10 ± 2378.33 m^2; $\beta = -826.02$, $t_{8.9} = -1.12$, $p = 0.29$; cond. $r^2 = 0.22$). Repeating searches on the same trail did not improve performance.

A second design concern was the effect of having multiple search teams search the same trail one after another on a given day. Dogs could have followed the scent of the previous search team. Hence, the starting order of dogs for days with multiple search teams was randomized so no search team was the first to search more than once. LMM results revealed that starting order had no effect on time ($\beta = -35.06$, $t_{8.8} = -0.61$, $p = 0.56$; cond. $r^2 = 0.53$) nor between trail area ($\beta = 6.36$, $t_{10.5} = 0.01$, $p = 0.99$; cond. $r^2 = 0.11$). Start order had no effect on search performance.

Environmental Conditions Affect Odor Dispersion from Trails

A LMM was created that predicted the between-trails area using overall prevailing weather conditions and the overall temporal change in conditions. In total, ten metrics were included in the model (prevailing and temporal changes in humidity, wind speed, barometric pressure, relative humidity, and absolute humidity. Variables were sequentially removed by least significant result until only significant factors remained. Prevailing relative humidity remained as the factor which had the most effect on how closely dogs searched to the original trail (Appendix A, Figure 2.6). The higher the relative humidity, the closer dogs searched to the experimental trail. Each percent increase in relative humidity, on average, led to a reduction of approximately 90 m^2 in between-trails area.

I also investigated the small-scale variations in the conditions along the trail and their effects on odor dispersion. This was determined by modeling a LMM with all 434 readings from the weather stations and regressing them against a dog's average distance to each respective waypoint. The ten meteorological measurements of prevailing conditions and temporal condition changes were added to the model and stepped backwards until only significant variables remained. Within the microclimates surrounding each waypoint, prevailing higher relative humidity resulted in dogs searching closer to the person's trail. In addition, higher wind speed resulted in dogs searching farther away (Appendix B). Temporal changes in weather conditions had no effect on a dog' search distance from each weather station.

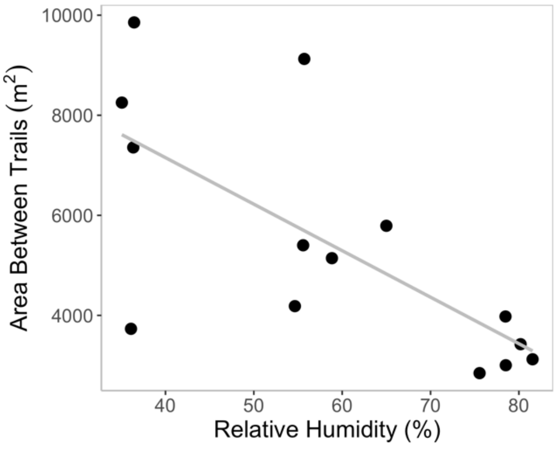

Figure 2.6. Relationship between relative humidity and the area between trails. Odor disperses less in high humidity and dogs are able to follow closer to a person's scent trail.

Wind direction should also push dogs to search downstream in the same direction as the wind. A LMM with wind direction regressed against the angle from the waypoint to the dog's location showed that wind direction, however, had no impact on where the dog was located ($\beta = 0.06534$, $t_{352.8} = 1.26$, $p = 0.21$).

Sampling Behavior

Dogs spent approximately 16.7 ± 13.1% of search time sampling the ground. A mixed effects logistic regression model revealed that the faster a dog was moving the lower the odds it was sampling the ground (Appendix C). For every increase of one m/s in speed, the odds of dogs sampling the ground dropped by 0.22.

A second mixed-effects logistic regression showed a marginal relationship between distance from the trail and the odds of ground sampling. This effect was low, with only a decrease of 0.01 in odds for air-sampling for each meter away from the person's trail that the dog traveled ($\beta = 0.004$, $z = -1.73$, $p = 0.08$). dogs were slightly more likely to sample the ground as they moved farther from the experimental trail.

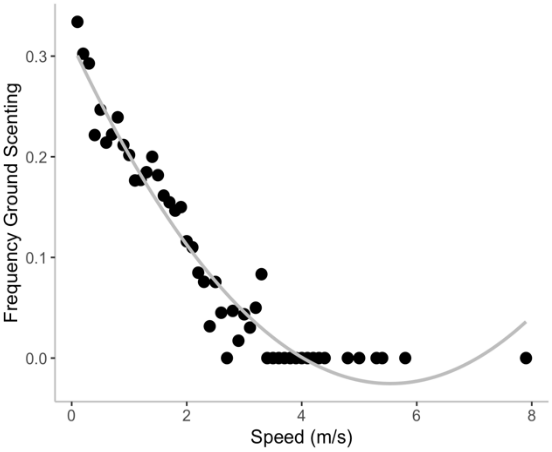

Figure 2.7. Frequency of time spent sampling the ground when graphed against dogs' speed. On average, the faster a dog moved, the less likely it was to sample for odors on the ground.

As dogs moved far away from the experimental trail, their speed increased and decreased following a quadratic pattern within the first 40 m (Appendix E; Figure 2.8). Beyond 40 m, dog speed linearly increased with distance (Appendix F; Figure 2.8). A closer examination of sampling behavior when dogs are within 40 m gives insight to their air vs ground sampling behavior. Dogs sampled the ground more when they were close to the odor trail (less than 10 m) and also when they were between 20-30 m away. Finally, at the farthest distances (more than 40 m), dogs sampled the ground more frequently.

A LMM model with the ten weather metrics was stepped back to find the most important variables that predicted frequency of ground sampling. The prevailing wind speed had an effect on how often dogs sampled the ground (Appendix G). As wind speeds increased, dogs sampled the ground more. However, this effect was small, an increase of wind speed by one m/s resulted in an increase of ground sampling by 2%.

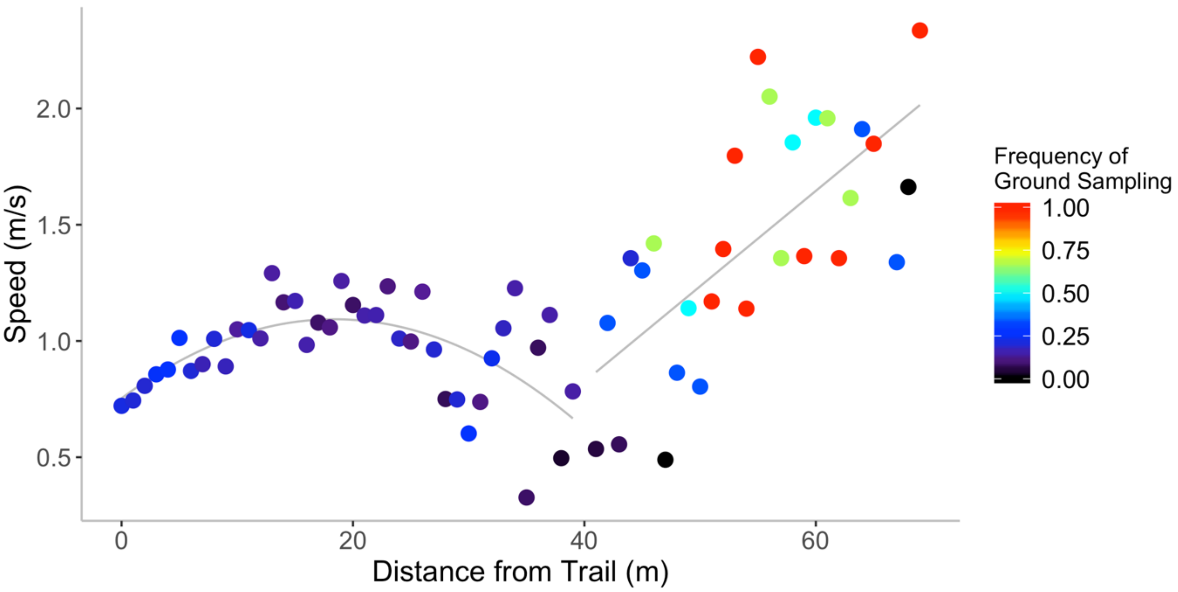

Figure 2.8 Relationship between movement speed, distance from the trail, and ground sampling frequency. Dogs appeared to display two distinct behaviors depending on their distance from the trail. Within 40 m, dogs display a quadratic relationship between distance, travel speed, and frequency of ground sampling. Beyond 40 m dogs appear to have lost the odor trail and begin to increase their speed linearly as well their frequency of ground sampling.

Discussion

This is the first study to examine how environmental conditions influence SAR *trailing* dog search and sampling behavior. The dogs were very accurate, there was a 93% success rate for finding the target at the end of trail. This is congruent with other studies which show success rates between 65-82% (Greatbatch, Gosling, et al., 2015; Woidtke, Dreßler, et al., 2018). Past studies have investigated the success rate of searches and found little correlation between the success and failure of a search with ambient weather conditions (Greatbatch, Gosling, et al., 2015). However, meteorological conditions do affect search behavior and SAR dogs use different strategies to compensate for these changes to successfully complete a search.

Impacts of Meteorological Conditions on Odor Trail Following

Dogs strayed farther from the original trail laid by the target during hot, dry conditions. The increased mixing of odors in turbulent, hot air may create a wider odor plume that contain more pockets of clean air and odor-rich air (Drescher, Lobascio, et al., 1995; Gandhi, 2004). Dogs may become confused when navigating within these highly intermittent plumes. Instead, dogs wandered away from the experimental trail to find the edge of the odor plume before tracking it to the source.

Finelli, et al. (1999, 2000) argued for the importance of odor plume edge detection by animals during olfactory navigation. He described a strategy where animals find the gradient of an odor plume by identifying the edges of the plume where there is a cleaner delineation between the bulk of the odor and clean air. This plume edge detection behavior is qualitatively seen in many studies of olfactory navigation, but rarely described as a navigation strategy (Willis, 2005; Zimmer-Faust, Finelli, et al., 1995). Instead, most studies only describe casting behavior broadly, failing to acknowledge where casting occurred in relation to the plume structure, which should be at edges of the plume (Kenen & Cardé, 1994). Simple observations of animals navigating through an odor plume therefore gives valuable insight to the basic structure of a plume without requiring precise measurements with equipment, such as a PID.

Relative humidity during the time of the search was the most important variable that determined where dogs searched and hence how far odors may have dispersed from the experimental trail. On cooler, humid, calm days, the odors must not have spread as widely as the dogs appeared to find the edges of the odor plume within a short distance from the experimental trail. Charlton, Kanno, et al. (1993) also observed that gypsy moths behaved as if they were tracking a narrower plume at cooler temperatures, flying with tighter turns and a more direct flight to the odor source. A studies on perceived odor from feces near toilets and animal facilities also supports the idea of odors spreading wider rather than more narrowly when temperatures are high (Afful, Oduro-kwarteng, et al., 2016; Dalton, Caraway, et al., 2011; Guo, Dehod, et al., 2005).

A physiological explanation may also explain why dogs were able to follow the trail more closely on humid days. A study in humans showed that an increase in humidity results in a lowering of olfactory thresholds (Kuehn, Welsch, et al., 2007). This same effect may occur in dogs and allowed them to detect the odor trail more easily.

Soil moisture in combination with meteorological factors may also play an important role in how easily odors can be detected. Rodents (*Peromyscus maniculatus, Perognathus parvus,* and *Tamias amoenus*)have difficulty locating buried seeds when there is little soil moisture (Vander Wall, 2003). The chemical explanation for this effect is that active competition for binding sites on substrates between water and odor molecules is responsible for odor dispersion. When the ground or some substrate is damp, odor molecules cling to substrates along with water particles. As humidity increases though, saturating the ground, water competes to bind to the substrate and wins, forcing odor molecules which were originally attached to the substrate into the air (Spencer & Cliath, 1970).

When temperatures and wind were low when it was humid, the target trail may have been trapped in substrate on or near the experimental trail. Even in the presence of light wind, odor molecules may have remained near the substrate level or continued to be pushed out of the soil slowly for the dogs to detect. During the afternoon when it was hot and dry, however, fewer odor molecules would have been able to sink into the substrate. Instead the odor would have remained suspended in the air and then, given the heightened turbulence in hot air, the odors would have lifted higher. Left to be spread by wind, odor molecules then dispersed far from the experimental trail which could have resulted in dogs searching farther from the trail. Air dispersion models have shown that this is a possible mechanism for relatively low odor perception in these meteorological conditions (Xing, Guo, et al., 2007). Studies on pesticide vaporization have shown that wind is capable of picking up volatized substances, such as odor vapors, from substrates and mixing them into the air (Kimball & Lemon, 1971). Indeed, as observed in the study, higher wind speeds resulted in dogs searching farther from the trail.

Prevailing ambient conditions during search for the target affected a dog’s search trajectory more so than the magnitude of change that occurred between when the target trail was laid and when the dogs searched. This is important for two reasons. First, it showed that the concentration of odor from the original trail remained high enough that it could be affected by current ambient conditions hours after the trail is laid without disappearing. Secondly, it has a practical implication for SAR, that search accuracy will peak during cool, humid conditions.

Sampling Behavior

Dogs moved quickly and confidently when a high concentration of odors was found in the air near the trail, i.e. between 10-20 m from the trail. At farther distances of 20-40 m though, dogs increased ground sampling and reduced their speed. This distance may be within the limits of where dogs can still detect odors, at least when dogs are searching for tortoises (Cablk, Sagebiel, et al., 2008). However, odor concentrations may have been lower at these distances. Ground sampling may be a strategy dogs use when they lost contact with an odor and attempt to locate odors trapped within the boundary layer (Paul A. Moore, Weissburg, et al., 1994). Odors in the boundary layer are close to substrate and flow at slower speeds, holding them lower to the ground (Paul A. Moore, Weissburg, et al., 1994).

A dog's speed was also low when less than 10 m from the trail and at this distance the dogs were more likely to sample the ground. A high concentration of odors may be deposited in the footsteps of the target person or on the grasses by the trail. Therefore at close distances to the trail, a dog may have kept its head low to the ground to follow high concentrations of odor trapped on the substrate.

At distances beyond 40 m, a dog's speed increased but ground sampling also increased. A handler is highly attuned to their dog's behaviors and can identify when a dog has lost an odor. Dogs at this distance were observed circling when disoriented, which can be seen in trails from Figure 2.5. A dog will gradually expand the diameter of the loops or search back and forth until they re-locate the odor and continue on. This behavior is reminiscent of casting behavior. Here, a dog often sniffed the ground while performing the behavior which further supports the theory that dogs may sample the ground more when they lose contact with odors. This is the first study that has demonstrated strategic tradeoffs between sampling behaviors in SAR dogs during active search.

Although sampling patterns from audio recordings of respiration could not be used for this experiment, researchers could observe sniffing behaviors. During hot days and when dogs were moving quickly, dogs panted to cool themselves (Crawford, 2012). The act of panting interferes with active sniffing behavior because dogs cannot pant and sniff simultaneously (Gazit & Terkel, 2003; Settles, Kester, et al., 2003). Dogs would close their mouths to sniff, but this tradeoff between panting and sniffing reduces the number of odor samples a dog can make. Therefore on hot days, it may have been more difficult for the dogs to sniff for odors and contributed to the increased distance from the experimental trail if they were more likely to lose the trail.

In addition, as wind speeds increased, dogs were marginally more likely to sample the ground. Odor molecules in the air at high wind speeds may have become too diluted to reliably follow the odor gradient, thus sniffing the ground for trapped odor molecules was a better strategy.

Individual Differences

Each dog had different frequencies of ground sampling. The English Border Collie preferred to sniff the ground for odors much more than the German Shepherds and Labrador Retriever in this study. The collie also maintained a slower pace than all the other dogs, but was as accurate as the others. Therefore, these two prevailing strategies, to search slowly and on the ground, or fast and in the air, worked equally well and are likely adapted to how far away from the target trail a dog is searching and the meteorological conditions.

Implications

In conclusion, two main important results emerged from this study. First, SAR dogs in this study gave valuable insight into how odor plumes disperse in nature by acting as biological odor detectors, their behavior demonstrated that odors disperse quickly and widely in hot, dry, windy conditions. Second, few studies have been published on the effectiveness of SAR teams searching for missing individuals and none have investigated how SAR dogs' search patterns are affected by meteorological conditions. This study suggests that searches which take place during cool, humid, calm times of day may lead to higher success rates.

Finally, this experiment revealed how a large cursorial mammal, the dog, actively modifies its sampling behavior in response to different meteorological conditions. Dogs are able to move slowly and sample for odors on the ground or move quickly while following air-borne odors. They switch between the two strategies depending on both distance to the odor trail and meteorological conditions.

Chapter 3

Modulation of sniffing behavior during olfactory navigation in humans

Introduction

Chapter two revealed how environmental variables affected odor dispersion and the different search strategies SAR dogs used to follow odor trails. Unfortunately, the experiment was not able to capture sniffing behavior. It was unclear if the dogs modified their sniffing behavior in response to distance from the odor trail or ambient weather conditions. This chapter will investigate how sampling is modulated during mammalian olfactory navigation, specifically in humans. No study has previously described how humans both detect and follow an odor to its source during an odor navigation task.

Jacobs (2019) proposed that the unique shape of the human nose may have evolved for olfactory navigation. People may modulate sampling frequency, length, intensity, and overall number of samples as they navigate through an odor plume. Olfactory navigation can be split into two phases: *searching* for the odor and *following* the odor plume. If humans are like dogs and rats, when they are actively *searching* for the odor, sniffing frequency should be high and are expected to slow their sampling frequency as they *follow* an odor plume to its source (Khan, Sarangi, et al., 2011; Thesen, Steen, et al., 1993),. Sniffing length should be inversely related to sniffing frequency—the faster the sniffs, the shorter the sniffs. Studies of sniff intensity have both claimed that it has no effect on odor perception or that it heightens perception (David G. Laing, 1983; Teghtsoonian & Teghtsoonian, 1978). Therefore, it remains to be seen in this experiment if sniffing intensity will not change as people both *search* and *follow* the odor or if people sniff less intensely as odor concentration increases.

In traditional hunter-gatherer roles, men and women had separate spatial skills. Human cognition evolved specializations in each sex to fulfill these roles (Hawkes, 1996; Lovejoy, 1981). Men outperform women when using distance or distributed cues for navigation such as distal mountains or the geometric cues, whereas women excel using discrete landmarks (Chai & Jacobs, 2010; Silverman, Choi, et al., 2000). An olfactory plume is a directional cue so men may outperform women. However, women should have a lower threshold for odors (Koelega & Köster, 1974) and may detect an odor earlier than men.

Mammals, including humans, smell in stereo and their ability to localize an odor are negatively affected if they are restricted to sampling with only one nostril (Catania, 2012; von Békésy, 1963). People in this experiment completed the task using stereo olfaction or with one nostril blocked during a portion of trials. The first chapter presented two hypotheses of how mammals gather information from odor plumes during navigation. They may compare odor concentrations from both nostrils during each sample to decide which way to travel, or they may rely on physically moving to new locations and comparing the odor concentration between the two locations (spatio-temporal strategy). If humans used a spatio-temporal strategy to navigate odor plumes, blocking a single nostril should have no effect on the success of finding the odorant.

However, navigation behavior should be affected during plume *following* with a bias towards the side with the unobstructed nostril before correcting their trajectory. In addition, when using one nostril, people should need to sniff more frequently or more intensely in order to draw in the same amount of odors as they would have when using both nostrils. This is to ensure that the same amount of information can be gained per sample as when they are using stereo olfaction.

Methods

Subjects

Participants were 54 University of California, Berkeley students (female n = 36, male n = 18) between 18-30 years of age (21.1 ± 2.51). They were recruited through the Department of Psychology Research Participation Pool and given class credit for completing the experiment. This research was approved by the Committee for the Protection of Human Subjects, the Institutional Review Board for the University of California, Berkeley. Participants signed consent forms prior to the start of the experiment. All participants were of good health with no illness or allergies that prevented them from using their noses normally.

Experiment Setup

The experiment took place within the empty Education-Psychology library in Tolman Hall on the University of California, Berkeley campus. The room was approximately 15 m wide and 12 m long. The left side of the room was occupied with book shelves and two large tables. Windows were to the right of the experiment arena which were kept shut with the blinds closed for all trials. Room doors were also kept closed. An area outside of the doors was used as a waiting arena between each trial.

A 6 m x 10.5 m gridded area was taped onto the ground with masking tape such that there were 7 rows, called “zones”, and 12 columns, which will be referred to as “lanes”. At either ends of the grid, lanes were ID’d using large numbers from 1-12 taped to the ground (Figure 3.1).

Twelve scent diffusers (Greenair Scent Pod Oil Diffuser) were placed approximately one meter above the ground at the end of the experiment site and aligned with the center of each lane. The diffusers were 83 cm away from the last zone. The tear-dropped shaped diffusers were battery operated and a small fan within the diffuser pushed scents out from the elongated end. Due to the spin direction of the fan, the air from the exited the diffuser with a slight trajectory to the left as verified by sprinkling sawdust in front of each diffuser. Consequently, diffusers were angled approximately 45 degrees to the right such that when sawdust was sprinkled in front of each diffuser the air current flowed longitudinally down the experiment arena. Each diffuser had a micro fiber pad. However, only one scent diffuser was marked as the target diffuser which was loaded with anise seed oil (*Pimpinella anisum*) purchased from Lhasa Karnak, Berkeley, CA.

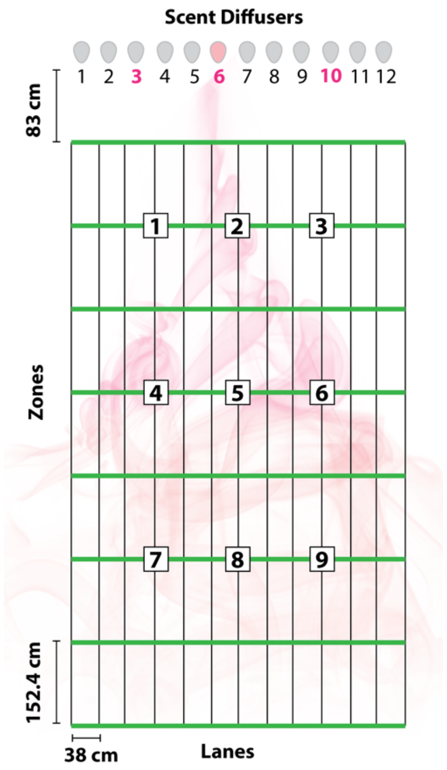

Figure 3.1. A schematic showing an overhead view of the experiment arena. Green lines mark each “zone” and black lines designate each of the 12 “lanes”. Twelve diffusers were set at the end of the experiment area, one which was the target diffuser filled with anise seed essential oil. Diffuser numbers in red represent the three lanes the target diffuser was placed for the trials. Boxed numbers represent the nine locations where PID measurements were taken. An image of an example odor plume is superimposed over the grid.

Respiratory patterns, breathing and sampling (i.e. sniffing), were recorded. Custom microphone neckbands were made for participants to wear which were identical to the ones used in Chapter Two. Trials were recorded using cameras placed at the front (Canon FS300) of the room and back of the room (Canon Rebel T6 with 58mm wide angle lens) at the height of the scent diffusers between lanes 6 and 7.

The experiment consisted of three experimental trials in a specific order: stereo (both nostrils open), mono left and mono right (one nostril open). In the text when left or right nostril is stated it will refer to the unobstructed nostril. The first 28 participants experiment were only given three trials in the same order: stereo, left nostril only, right nostril only. However, due to concerns about a learning order effect, the remaining participants were given a training trial at the start of the experiment where the diffuser was located in lane 7 and participants were allowed to use their noses normally. Following this training trial, all participants were given the same order of experimental conditions, left nostril only, right nostril only, and then a final stereo condition trial. The locations of the target diffuser were counterbalanced between the experimental trials such that the diffusers alternated between three correct locations: lane 3, lane 6, and lane 10 for each participant with combinations of the stereo, left nostril only and right nostril only trials. In total there were six permutations of experimental condition and diffuser location (Appendix H). The experiment lasted approximately 60-90 minutes.

Trial Procedure

Participants were shown the experiment arena and told they would be identifying which diffuser contained the anise seed oil scent. The three different experiment conditions were also explained. Participants were given a sample of anise seed oil to smell in the waiting area. Their height was recorded using a ruler fixed to the wall as well. Lastly, they were fitted with the microphone neckband and asked to keep the neckband on for the duration of the experiment and asked to wait outside of the experiment room until called. Before each trial began, the ambient air temperature was recorded using a Kestrel 5000 Pocket Weather Meter.

Disposable nitrile gloves were worn when handling the scent diffusers, the essential oils, and the micro fiber pads. While wearing gloves the target air diffuser was placed in the correct lane for the trial. The anise seed oil was then brought to the diffuser and the microfiber pad was loaded with three drops of anise seed oil at the front end of the diffuser. The diffuser was then turned on for one minute. All other diffusers were also turned on and cameras began recording at this time. The participant was invited into the room and the audio recorder was turned on. If in the mono condition for the trial, the participant was given a disposable earplug to block a single nostril.

Participants were instructed to advance through the arena one zone at a time, preventing them from walking directly to the diffusers and smelling each in turn to immediately locate the target diffuser. Participants were asked to raise a hand for the duration they perceived the odor as they searched through all the zones. If the odor was lost, they lowered their hand. Researchers counted down from five before participants could enter the first zone. Participants were given thirty seconds to walk in the zone with a five second warning before the time ended. At the conclusion of the thirty seconds, they vocally identified or gave their best estimate of which lane (1-12) led to the target diffuser and their answer was recorded. The researcher would then count down from five again after which the participant could advance forward to the next zone. This procedure was repeated until all seven zones were completed. Each trial lasted approximately 5-6 minutes.

At the conclusion of each trial, the participant was told the correct lane, the audio recorder and cameras were switched off, participants exited the room, and waited outside while the next trial was prepared. While wearing gloves, the microfiber pad from the target diffuser was removed directly from the diffuser as it still sat on the shelf. Both the used microfiber pad and the gloves were double bagged after each trial. The diffuser was then moved to its new location. An industrial air filter (Hurricane 12” Inline Duct Fan, Sunlight Supply) was brought to the center of the experiment area and a standing, rotating fan was turned on in front of the air diffusers. The room was filtered for a minimum of four minutes, but averaged between 5-10 minutes. The air filter and fans were then turned off and stored as set up for the next trial then began. After all trials in the experiment were completed, a minimum of an hour and a half passed before another participant was tested in the room with the air filter running.

Between the second and third trial, participants were given a survey to complete. This survey gathered demographic information (age and gender) as well as self-reported odor perception abilities (below average, average, above average), and their sense of direction (Hegarty, Richardson, et al., 2002). Participants reported answers to questions regarding their sense of direction (SOD) using a seven-point Likert scale. This 15 item psychometric survey measures the ability to spatially orient within an environment and more accurately assesses individuals' abilities to move themselves through space when compared to measures that rely on map reading or spatial rotation tasks (Hegarty & Waller, 2004). The results of the survey were calculated following the instructions included with the survey and averaged into a single score between 1-7 with 7 being the highest SOD score possible. SOD scores were then binned into low (scores 2-3), average (scores 4-5), and high (scores 6-7) to ensure each bin of SOD scores contained both women and men.

Coding Behaviors

Researchers clapped before the start of each trial when the cameras and audio recorder were turned on, and the participant was waiting to begin. Adobe Premiere CC was used to sync the video and audio together using the clap as a marker. The Observer XT (Noldus, Leesburg, VA) was used to code the behavior of participants as they moved through each trial. Zone and lane location were recorded as well as the duration of time spent in each location. Time spent with hand raised as well as the locations in which the hand was raised were also recorded.

Audio from the synced video file was coded using 2.1.1Audacity®. Noise reduction was used to filter ambient sounds from each recording. Files were independently coded for sniffs by labeling segments of audio. Respiration was quiet and often hard to hear on recordings, whereas sniffs had 2-3 times the frequency and amplitude of regular breathing. Duration of the sniff and audio intensity of each sniff were recorded. Audio intensity acted as a proxy for sniffing intensity. From 54 participants, 38 had high quality audio with sniffing behaviors that could be quantified.

Odor Plume Measurements

Full-field spatiotemporal measurements of scalar quantities like an odor plume in fluids (e.g. air or water) can give detailed quantitative information about plume dynamics and visualize their structure. However, images of this quality are only possible in controlled laboratory environments in water or air flumes using techniques such as planar laser-induced fluorescence (PLIF). Furthermore, when measuring the dynamics of scalar quantities, recordings are usually confined to small scales ranging from less than 5 cm^2 to 1 m^2 (Connor, McHugh, et al., 2018; Töger, Bidhult, et al., 2016; Webster, Rahman, et al., 2003). A previous model for simulating the flow of an odor in a room has been conducted to elucidate how bomb dogs search for odors indoors (Foat, Parker, et al., 2018). Similarly, knowing how the anise seed scent dispersed in the experiment room was necessary to understand when and how sniffing behavior was modulated.

A photoionization detector (PID; ppbRAE 3000) was used to measure the odor plume in the experimental room. PIDs are able to take continuous single-point readings of volatile organic compounds. They are sensitive to < 1 ppm and capable of detecting temporal changes in plume structure (Justus, Murlis, et al., 2002). Anethole is the principle component in anise seed oil perceived by humans. However, the PID did not include anethole as a detectable gas in its library. Thus, the PID was calibrated to detect isopropanol instead. The diffusivity of isopropanol (diffusion coefficient 0.0959) and anise seed essential oil were different (diffusion coefficient 0.039; ("Anethole - PubChem," 2019; "Isopropyl alcohol - GSI Environmental," n.d.). However, in a complex environment, diffuser of odor molecules in fluids is dependent on temperature, the fluid transporting them, and the dynamics of the fluid more so than their diffusivity coefficient (Drescher, Lobascio, et al., 1995; Gandhi, 2004; P. Moore & Crimaldi, 2004; Vickers, 2001). In the dynamic environment of the experiment room, volatilized isopropanol and the volatilized anise seed essential oil plume dynamics should behave similarly.

Unlike PLIF, PIDs measure scalar quantities in one location. Measurements in multiple locations must be taken with a PID to capture detailed dynamics of a plume's structure. One option is to form an array of PIDs simultaneously measuring a plume. The disadvantage of using multiple PIDs is that they are physically introduced to the fluid medium in which they are recording, altering the dynamics of the plume. In this study, a single PID was used to measure concentrations of isopropanol in the room to leave the plume as undisturbed as possible. Measurements from nine sites were taken in the grid for each of the three possible lanes the diffuser was located during trials for a total of 27 combinations of diffuser lane and site location (Figure 3.1). The PID was set at the average height of a participant's nostrils (160 ± 12.2 cm).

A microfiber cloth was soaked with isopropanol and placed into the target diffuser in one of the three lanes. All dummy diffusers were turned on to mimic experimental conditions. The PID logged a measurement every second for a minimum of 250 seconds, the length of time for a participant to advance through all seven zones within a single trial. Following the 250 seconds of recording, the microfiber pad was bagged and the air filter and fan were turned on in the room. The room was allowed to ventilate until the PID showed isopropanol concentrations lower than 50 ppb which took between 5-20 minutes. Up to four measurement were taken at each site and ambient temperature were recorded for each trial (23.6 ± 2.3 C). Prior to each measurement, an initial background measurement was made. Any traces of remaining isopropanol in the room prior to the following measurement were subsequently subtracted from the final analyses.

Analyses

An estimate error was used to determine how accurately participants identified the location of the target diffuser. The estimate error was calculated by finding the difference between a participant's estimate of which lane they thought held the target diffuser and the true location of the diffuser. The estimate error had a negative value if the diffuser was located to the right of where the participant estimated, or it had a positive estimate if the diffuser was located to the left of the participant's estimate. An absolute estimate error was also calculated to measure overall error.

Data from video coding and audio coding were compiled and cleaned using Python 3.6.1. Statistical analyses were performed in R 3.5.1. Linear mixed effects models (LMM) and mixed effects logistic regressions (R *lme4* package) were used to analyze data where each participant crossed by trial was included as a random effect in each model. Conditional r^2 values were reported for all LMMs using the *MuMIn* package. Comparisons between categorical variables in the LMMs were performed using Wald chi-squared tests from the *Anova* function in the *car* package because random effects were included in the models. In addition, type II ANOVAs were also calculated using the *Anova* function from the *car* package. Any further pairwise comparisons were conducted with Tukey HSD adjustments from the *emmeans* package. The package *chisq.test* in R was used to conduct goodness of fit chi-squared tests. Average values and standard deviations are reported throughout the text. Alpha level was set to 0.05.

Results

Measuring the Odor Plume

PID measurements of isopropanol were made from nine unique sites in the experiment area to estimate the odor concentrations in the room during each trial (Figure 3.2a). Sites 1, 2, and 3 were almost directly in front of lanes 3, 6, and 10 respectively. As expected, the PID measurements for each of those sites were highest when the target diffuser was also located in the corresponding lane (Figure 3.2a). The concentration of the odor began to decrease between 4-5 meters away from the target diffuser in sites 4, 5, 6. The odor plume appeared to disperse evenly throughout the room gradually widening laterally with increasing distance from the target diffuser. This is evident by looking at the odor concentrations when lane 6 (green line Figure 3.2a) held the isopropanol. The concentration odor was the highest in site 2 and was lower in concentration but present in site 5 for the full 250 seconds. Initially, isopropanol concentrations were not detected or low in sites 4 and 6, but after approximately 60 seconds the odor diffused to the two sites. Isopropanol was also detected in very low quantities

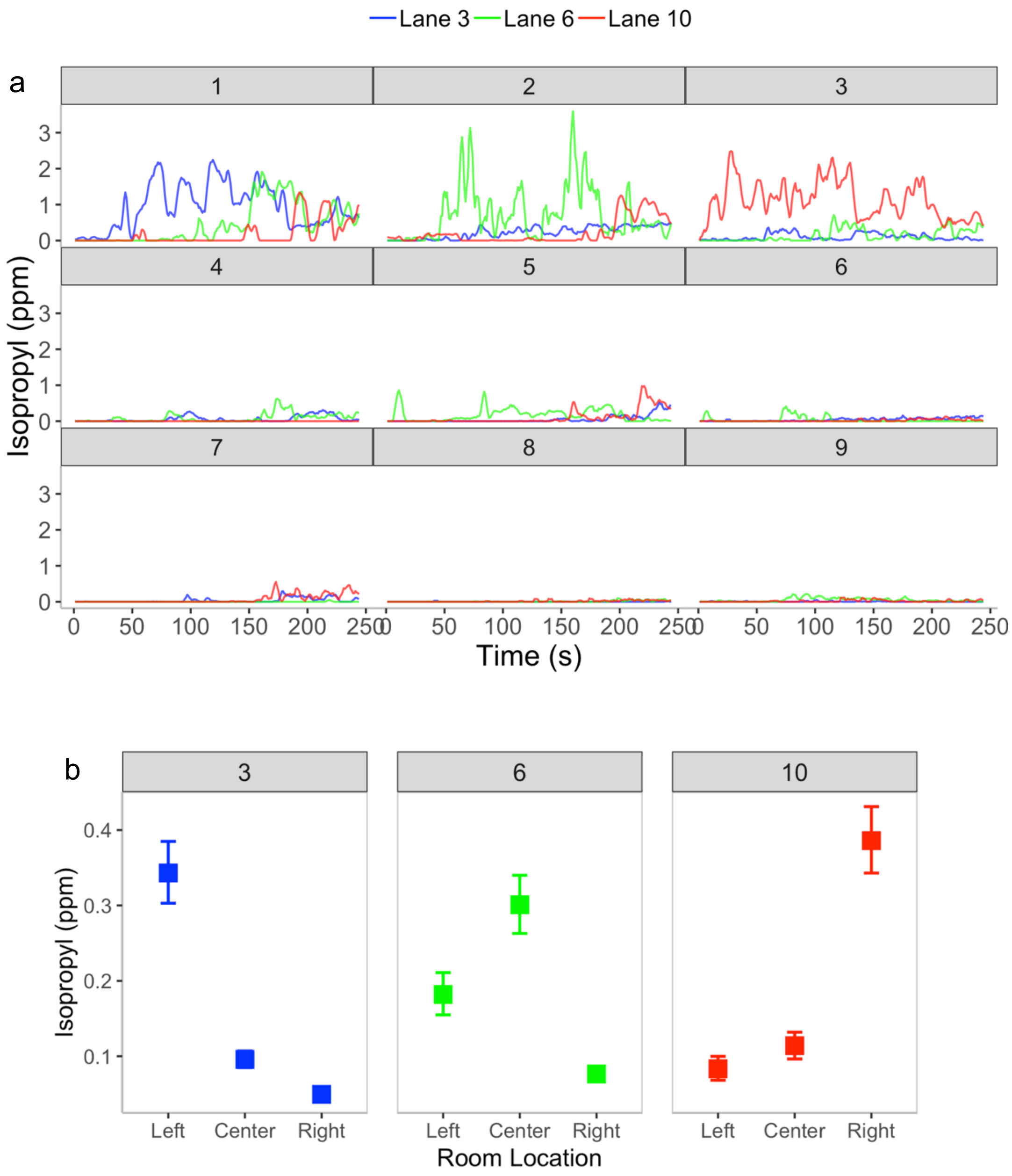

Figure 3.2. (a) PID measurements from nine sites in the experimental room (refer to Figure 3.1). The measurement sites are in a 3 x 3 grid – diffusers are located in front of sites 1-3 and the back of the experiment site extends beyond site 7-9. Different colored lines represent which lane the diffuser loaded with isopropanol was located in for the duration of the measurements. Higher concentrations of isopropanol from one lane are located in the sites which are in line to the lane holding the diffuser. For example, lanes 2, 5, and 8 all show the highest concentrations of isopropanol when the diffuser was in lane 6. As time goes on isopropanol is eventually detected from diffusers in other lanes. (b) Average concentrations of the odor plume during PID measurements when isopropanol is located in lane 3, 6, and 10 (labeled in gray). Room location are the nine PID measurement sites divided into the left side of the room (sites 1, 4, 7), center (2, 5, 8) and right (3, 6, 9). Statistically significant differences between 6-center and 10-right. 6-left also statistically higher than 3-center and 10-center.

throughout sites 7-9 after some time. A similar pattern of dispersion can be seen when the target diffuser was located in sites 3 and 10.

The nine sites were partitioned by their location in the experiment room into the left side (sites 1, 4, 7), center (sites 2, 5, 8), and right (sites 3, 6, 9) to assess if there was a bias for the odor to pool in one side of the experimental arena. Assuming the odor dispersed conically like a typical free flowing plume, partitions should contain the highest concentration of odors when the target diffuser was located within the partition during measurements. For example, if the diffuser was located in lane 3 (blue line), then the left side of the room should contain the majority of the odor concentration (Figure 3.2b). A two-way ANOVA with room partition and diffuser lane was performed (diffuser lane: $F_{2,\ 6579} = 4.24$, $p = 0.01$, partition: $F_{2,\ 6579} = 5.64$, $p < 0.01$, interaction: $F_{4,\ 6579} = 170.92$, $p < 0.001$). Pairwise comparisons of the significant interaction terms were assessed. The sites closest to the isopropanol-loaded diffuser (3-Left, 6-Center, 10-Right) had the highest concentration of odors, as expected. 3-Left (0.34 ± 0.55 ppm) and 6-Center (0.30 ± 0.53 ppm) were not statistically different. 3-Left and 10-Right were also the same (0.39 ± 0.60 ppm). However 6-Center had a lower average concentration then 10-Right. This difference is approximately 0.1 ppm, which is below the threshold of what an average human would be able to distinguish as two different concentrations of odors (Nagata, 2003). The average concentration of the odor should diminish laterally in distance from the diffuser and the diminished concentration should be equal regardless of whether the diffuser was located in lane 3, 6 or 10. A pairwise comparison between combinations of diffuser lane and room partition (e.g. 6-Left vs. 6-Right, 6-Left vs. 3-Center, etc.) showed no significant differences except 6-Left was higher when compared to both 3-Center and 10-Center. Although significant, this difference was negligible because 6-Left was less than 0.1 ppm greater than the other locations which is not a perceptible difference to humans. Overall, odor appeared to be dispersing equally in the experimental arena.

Order Effect

No learning order effect was found. Two separate LMM models were created for the set of participants who only had three trials and those who had four trials. Trial order had no effect on the absolute estimate error in the three-trial experiment ($\beta = -0.40$, $t_{29.96} = -1.27$, $p = 0.22$) or the four-trial experiments ($\beta = 0.01$, $t_{30.0} = -0.08$, $p = 0.98$). No learning effect was present. Estimate error was the same regardless of trial number. Therefore, the three experimental trials from the four-trial experiments were combined with the three-trial experiments for a combined total of 54 participants. A LMM of the effect of trial order on absolute estimate error with all 54 participants also showed no effect of trial order ($\beta = 0.30$, $t_{53.7} = -1.09$, $p = 0.28$).

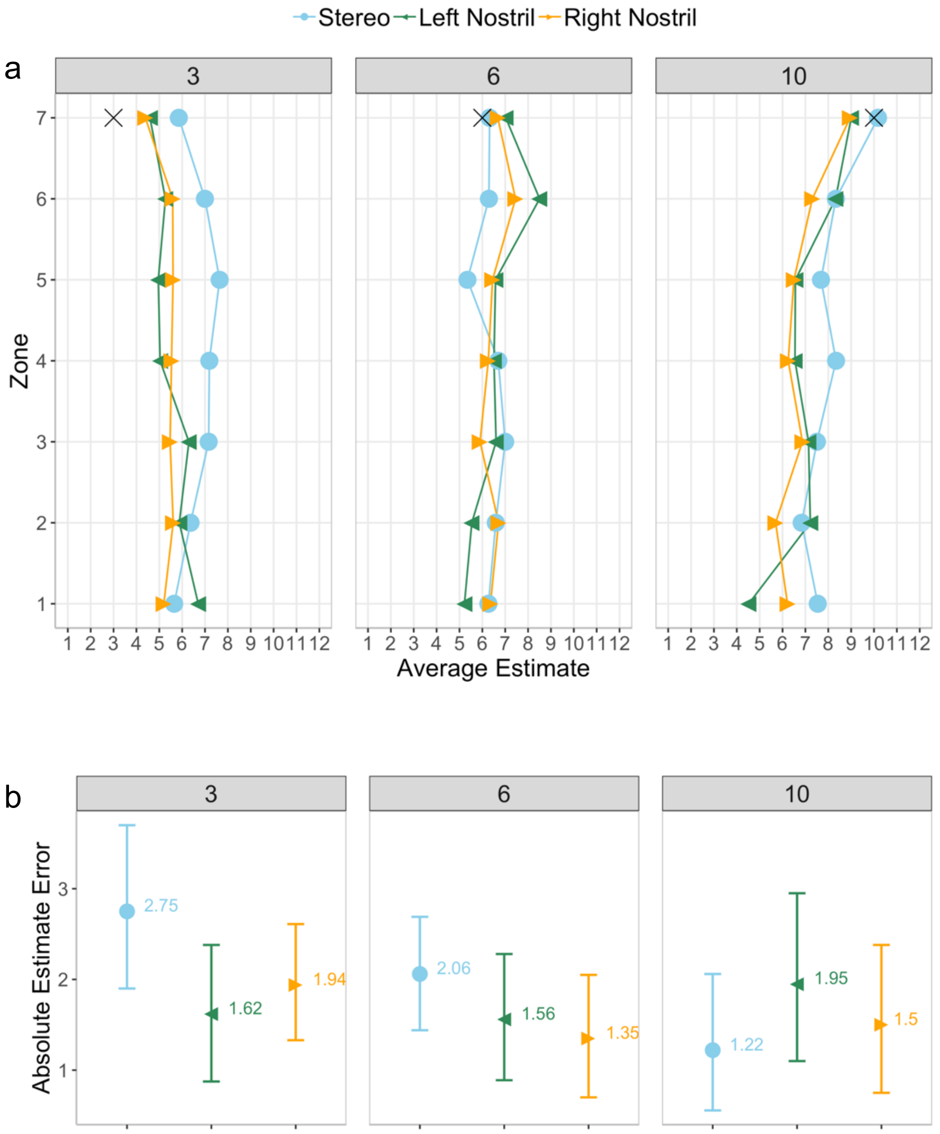

Figure 3.3. (a) Average estimate by each zone for all three experimental conditions and correct diffuser lane. X represents the lane where the correct diffuser was located. Colors represent the trial condition. Left and right nostril designate which nostril was unobstructed. (b) Plot of participants' absolute estimate error in the final zone of the experiment with 95% confidence intervals. Mean is labeled. Absolute error is the absolute difference between a participants' estimate of which lane held the target diffuser and the true location of the diffuser. No significant differences exist between any of the conditions or lanes.

Locating the Odor

Three diffuser lanes were used to ensure one location was not easier or more challenging than the others. A LMM with the three trial conditions (stereo, left nostril, right nostril) and three possible diffuser lanes (3, 6, 10) were regressed against the final estimate of the diffuser's location by participants in zone 7. No differences in absolute estimate errors were found amongst the three conditions nor the amongst the three possible diffuser locations (Appendix I, Figure 3.3b). From this point forward, the effect of correct diffuser lane will no longer be included in LMM models because it has been proven to have no impact on estimate error. No diffuser location was easier or more challenging to estimate. Furthermore, the location of the diffuser was not an effect of interest for this study.

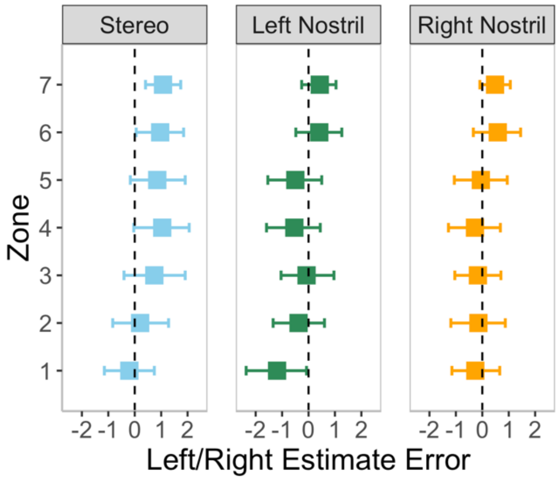

Figure 3.4. Estimate error by zone for different trial conditions. During the stereo trials, participants were statistically significantly biased towards estimating to the right of the diffuser. However, having either only the right or left nostril unobstructed did not bias the overall estimate.

Although trial condition had no effect on the final estimate error, blocking one nostril could lead to a bias for estimating to the right or left of the diffuser's true location. The average estimate error for all seven zones for each of the three trial conditions was found. Three t-tests showed that during the stereo condition, participants were more likely to guess too far to the right of the diffuser's location (0.67 ± 3.72; $t_{377} = 3.48$, $p < 0.001$). No bias existed for the left nostril (-0.26 ± 3.64; $t_{375} = -1.39$, $p = 0.17$) or right nostril conditions (0.02 ± 3.41; $t_{375} = 0.12$, $p = 0.90$; Figure 3.4). Blocking one nostril did not bias participants to search more for the odor on one side of the experimental arena.

A LMM with SOD and sex as variables showed no sex differences in absolute estimate errors (men: 1.72 ± 1.72; women: 1.81 ± 1.77; Wald $\chi^2(1, 162) = 0.01$, $p = 0.92$). No difference in absolute error was also found for those with low (1.96 ± 1.89), average (1.77 ± 1.76), or high SOD (1.5 ± 1.31; Wald χ^2 (2, 162) = 0.12, $p = 0.94$). Men, however, had a higher SOD (4.7 ± 0.65) than women (4.01 ± 0.93; $t_{52} = 2.80$, $p < 0.01$).

Temperature was recorded for each trial (23.51 ± 0.89 C). A LMM examining the effect of temperature on the absolute estimate error in zone 7 showed an almost significant effect ($\beta = 0.13$, $t_{146.9} = 1.783$, $p = 0.08$; $p < 0.001$, cond. $r^2 = 0.12$). Participants may have been less accurate when estimating the location of the diffuser when temperatures were high.

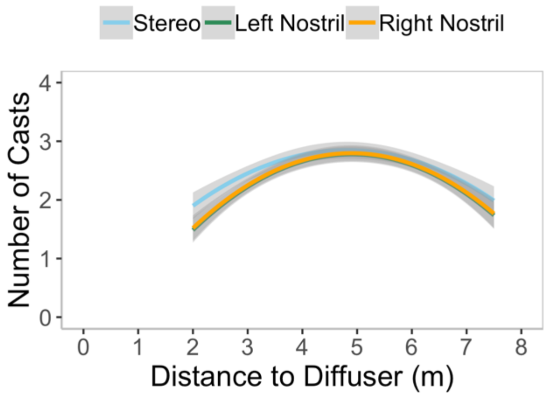

Figure 3.5. The average number of casts by distance to the correct diffuser not including random effects of participant and trial. Casting initially increases and then decreases again as participants approach the odor source. There is no effect of experimental condition (stereo, left nostril, right nostril) on the number of average casts.

Casting

Each time a participant switched directions while walking was considered a cast. A quadratic LMM showed that participants initially increased casting as they approached the odorant source before decreasing casting (Appendix J, Figure 3.5). No difference in the rate cast number increased or decreased with respect to distance to the target diffuser was found between the trial conditions. However, the total number of casts was significantly higher in the stereo trials (27.7 ± 8.92) in comparison to the right only (24.9 ± 7.36) and left only trials (24.0 ± 7.53; $\chi^2(2, 193) = 14.33$, $p < 0.001$).

A Wald chi-squared test performed on a LMM with sex and SOD showed women made more casts (27 ± 8.4) than men (25.9 ± 7.55; $\chi^2(1, 193) = 4.31$, $p = 0.04$). There were no differences in total cast number depending on SOD (low 23.6 ± 7.55, average 25.56 ± 8.7, high 28.69 ± 10.36; $\chi^2(1, 193) = 1.24$, $p = 0.54$).

Whole body movements like casting are effective for detecting changes in odor concentration when sampling for an odor. However, participants derived no benefit from casting in this task. An increase in the number of casts did not affect the absolute estimate error ($\beta = -0.007$, $t_{96.3} = -0.45$, $p = 0.66$).

Sampling Behavior

Other strategies must have been used to locate the target diffuser. Modulating sniffing length, frequency, and sniffing intensity without moving the whole body could also give the individual information about the odor plume. Participants in the study exhibited a range of different sampling patterns (Figure 3.6). Sniffing patterns were consistent throughout an entire experiment and were reflective of individual differences.

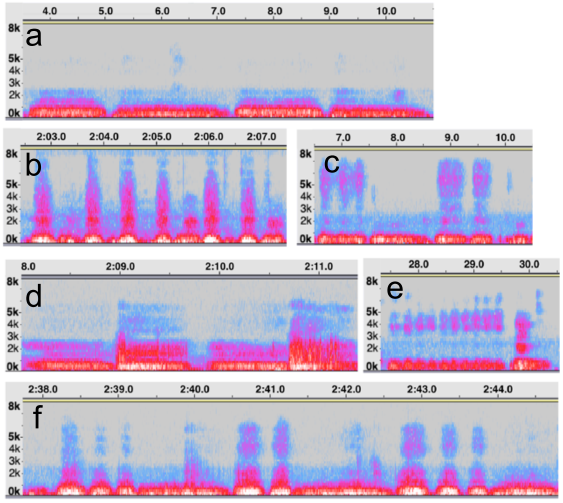

Figure 3.6. Spectrograms showing examples of sniffing patterns exhibited by participants. X-axis is time in minutes and seconds. The y-axis is frequency (Hz). (a) A pattern starting with a sniff followed by exhale (repeated twice). This was the most common sniffing pattern -- characterized by having a slightly more intense inspiration followed by a single exhale of approximately the same length or slightly longer. (b) Sniff, small exhale, sniff x3, small exhale, sniff x2, exhale. This pattern exhibits slight pauses between short sniffs. (c) Sniff x3, exhale, repeated twice. No pause between sniffs. (d) Inverse of pattern a. A gentle exhale followed by intense sniff and a light pause before exhalation. (e) Series of rapid sniffs followed by sharp exhale. (f) Repeated pattern of 2-3 sniffs followed by long

The number of sniffs per meter from the target diffuser followed a quadratic pattern. Number initially increased with a peak approximately 4 m from the target diffuser before decreasing. The quadratic relationship was consistent across all trial conditions and there were no differences among conditions for the rate at which the number of samples increased or decreased as participants approached the diffuser (Figure 3.7a; Appendix K).

During the stereo trials participants sampled on average 118.13 ± 52.34 times, the left nostril trials 107.67 ± 61.57, and sampled 106.95 ± 47.91 times with their right nostril. A Wald chi-squared test following a LMM predicting total number of samples taken during different trials showed that individuals sampled more during the stereo trials in comparison to the left nostril trial. They also sampled more when using the left nostril than the right ($\chi^2(2, 5827) = 11.19$, $p < 0.01$).

A Wald chi-square test from a LMM with sex and SOD as variables showed that sex had no effect on total number of sniffs ($\chi^2(1, 162) = 0.22$, $p = 0.64$), but SOD almost had an effect ($\chi^2(2, 162) = 6.43$, $p = 0.04$). It is clear that as SOD increased, the number of total casts increased (Table 3.1). However, a Tukey HSD adjusted pairwise comparison showed close, but not significant differences between the three groups. Both the average and high SOD participants had almost statistically significant higher total casting numbers than participants with low SOD.

A LMM showed a quadratic relationship between distance from the diffuser and the length of sniffs during all three experimental trial conditions (Appendix L). Participants increased sampling length as they approached the correct diffuser at distances greater than 4 m to the diffuser and then began decreasing their sniffing length when closer than 4 m (Figure 3.7b). Sniffing length was shortest in the stereo trials (718 .5± 444.05 ms) in comparison to the left nostril (826.92 ± 606.83 ms) and right nostril trials (834.29 ± 569.76 ms; Wald $\chi^2(2, 41874) = 28.04$, $p < 0.001$). Neither sex ($\chi^2(1, 41874) = 0.26$, $p = 0.61$) nor SOD ($\chi^2(2, 41874) = 1.09$, $p = 0.58$) had an effect on sampling length (Table 3.1).

Sampling frequency was found by averaging the number of sniffs per second. Sniffing frequency decreased as participants approached the diffuser before increasing again within 4 m of the diffuser (Appendix M; Figure 3.7c). Sampling frequency was significantly different between all three conditions with stereo having the slowest frequency (1.26 ± 0.58 Hz) on average, followed by the right nostril (1.28 ± 0.58 Hz), and lastly the left nostril (1.32 ± 0.64 Hz). No difference in sampling frequency was found between participants with different SOD (χ^2(2, 19805) = 3.30, p = 0.19) or between sex (χ^2(1, 19805) = 0.94, p = 0.33).

A LMM predicting sniffing intensity in decibels revealed that participants were increasing their sniffing intensity linearly as they approached the target diffuser (Appendix N; Figure 3.7d). In the stereo trials, the average acoustic intensity was -50.11 ± 9.21 dB, -48.79 ± 8.68 dB for the left nostril, and -48.52 ± 9.90 dB for the right nostril. Sniffing during the stereo trial was almost significantly quieter than when using the left or right nostril. During the stereo trials and left nostril unobstructed trials, participants intensified their sniffs as they approached the diffuser more rapidly than during the right nostril trials. Men also sniffed more forcefully (-45.31 ± 8.39 Hz) than women (-51.55 ± 9.02 dB, χ^2(1, 41874) = 3.79, p = 0.05). No differences in sniffing intensity were seen between participants with different SOD (χ^2(2, 41874) = 0.15, p = 0.93).

Table 3.1. Average and standard deviations of sampling behaviors (mean ± sd).

	Number of Samples	Sampling Length (ms)	Sampling Frequency (Hz)	Sampling Intensity (dB)
Stereo	118.13 ± 52.34^{a}	718 .5± 444.05^{a,b}	1.26 ± 0.58^{a,b}	-50.11 ± 9.21$^{a., b}$
Left Nostril	107.67 ± 61.57^{c}	826.92 ± 606.83	1.28 ± 0. .58^{c}	-48.79 ± 8.68^{c}
Right Nostril	106.95 ± 47.91	834.29 ± 569.76	1.32 ± 0.64	-48.52 ± 9.90
Low SOD	33.07 ± 48.94$^{e.}$	989.86 ± 465.13	1.04 ± 0.20	-50.41 ± 9.46
Average SOD	82.01 ± 70.17$^{f.}$	792.74 ± 555.03	1.230 ± 0.60	-48.74 ± 9.16
High SOD	110.92 ± 39.80	620.71 ± 443.50	1.37 ± 0.70	-52.86 ± 9.22
Female	27 ± 8.38	824.35 ± 572.64	1.30 ± 0.64	-51.55 ± 9.02^{h}
Male	23.63 ± 7.55	708.25 ± 472.72	1.26 ± 0.52	-45.31 ± 8.39

	p < 0.05 codes:	^{a}stereo vs left	^{e}low vs average	^{h}female vs male
		^{b}stereo vs right	^{f}low vs high	. almost significant
		^{c}left vs right	^{g}average vs high	

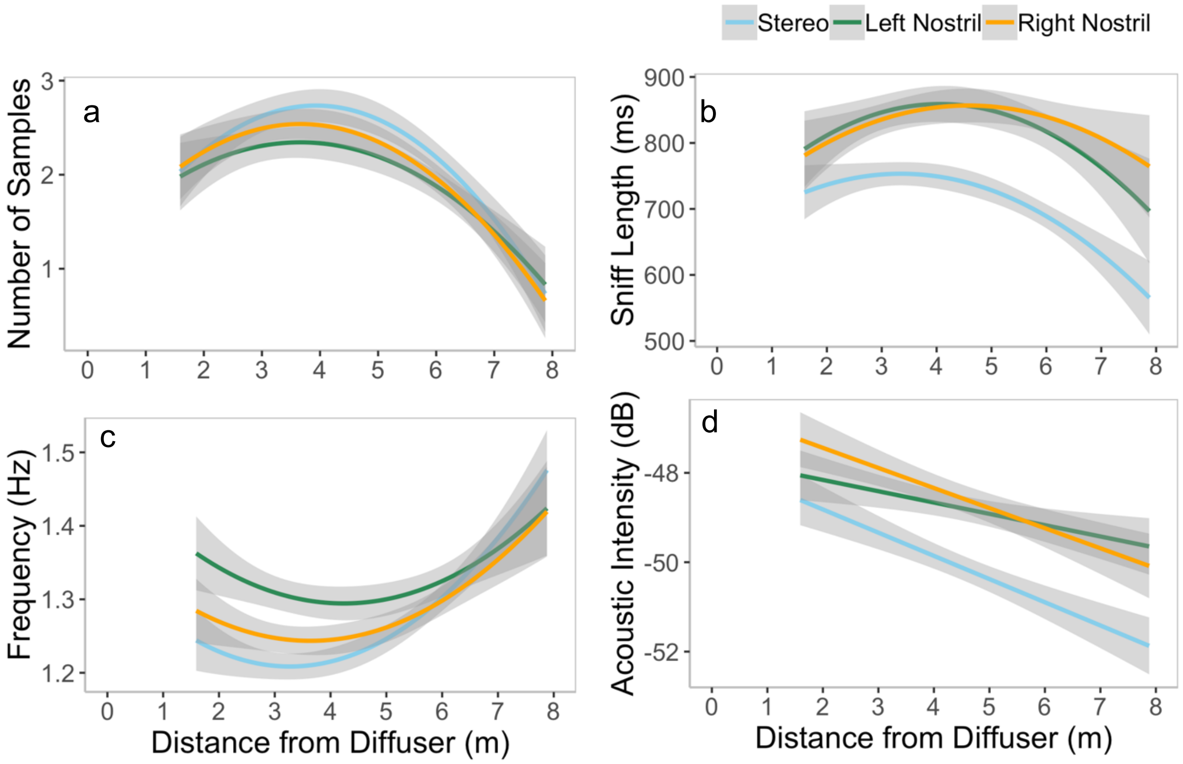

Figure 3.7. Plots of fitted lines to sampling behaviors. (a) Number of samples taken on average by distance from the target diffuser. Number of samples increased and then decreased slightly for all trial conditions. Participants sniffed significantly more in the stereo trials, followed by the left nostril only trials, and lastly the right nostril trials. (b) The change in sniff lengths as predicted by distance from the diffuser. Sniff lengths increased in length as participants approached the correct diffuser then decreased again. During the stereo trial participants had significantly shorter sniffs on average. (c) Change in sampling frequency as participants approach the diffuser with anise seed oil. Frequency initially drops, but the rises again. All three trial conditions had significantly different sampling frequencies. (d) Change in sniffing intensity measured by decibels by distance from the correct diffusers. Sniffing intensified as participants approached the diffuser across all experimental trial conditions. During the stereo trails, participants sniffed significantly less intensely.

Odor Perception

Participants rated their olfactory abilities by answering “below average”, “average”, or “above average” in the survey. Overall, there were no differences in absolute error estimate of the diffuser’s location based on olfactory ability (Wald $\chi^2(2, 162) = 0.20$, $p = 0.91$). Wald chi-squared tests were also performed on LMMs that included self-reported odor perceptual ability and trial condition when examining sampling behaviors. Perceived odor ability had no effect on the number of samples a participant made ($\chi^2(2, 162) = 4.05$, $p = 0.13$), sampling length ($\chi^2(2, 12161) = 0.25$, $p = 0.88$), sampling frequency ($\chi^2(2, 12161) = 2.74$, $p = 0.25$), or the intensity of their sniffing behavior ($\chi^2(2, 12161) = 0.35$, $p = 0.84$). Total casting number was similarly unaffected by self-reported olfaction ratings ($\chi^2(2, 193) = 0.185$, $p = 0.91$). A goodness of fit chi-squared test shows no relationship between sex and reported olfactory ability ($\chi^2(2, 54) = 0.13$, $p = 0.94$). A one-way ANOVA also showed no differences in average SOD for those with self-reported below average, average, or above average sense of smell ($F_{2,190} = 1.02$, $p = 0.36$). In conclusion, self-reported olfactory abilities were uncorrelated with any measure of sampling behavior or overall performance on the task.

Participants were asked to raise a hand when they could perceive the anise seed oil. Women raised their hands at a farther distance from the diffuser than men, meaning they perceived the odor faster. This was especially prominent in the stereo trials. The peak in Figure

3.8 shows that women on average raised their hands, signaling they had smelled the odor, when they were 6.7 meters from the correct diffuser. Men in the stereo trial signaled they initially detected the plume around a distance of 5.2 m. These differences disappeared once one nostril was obstructed.

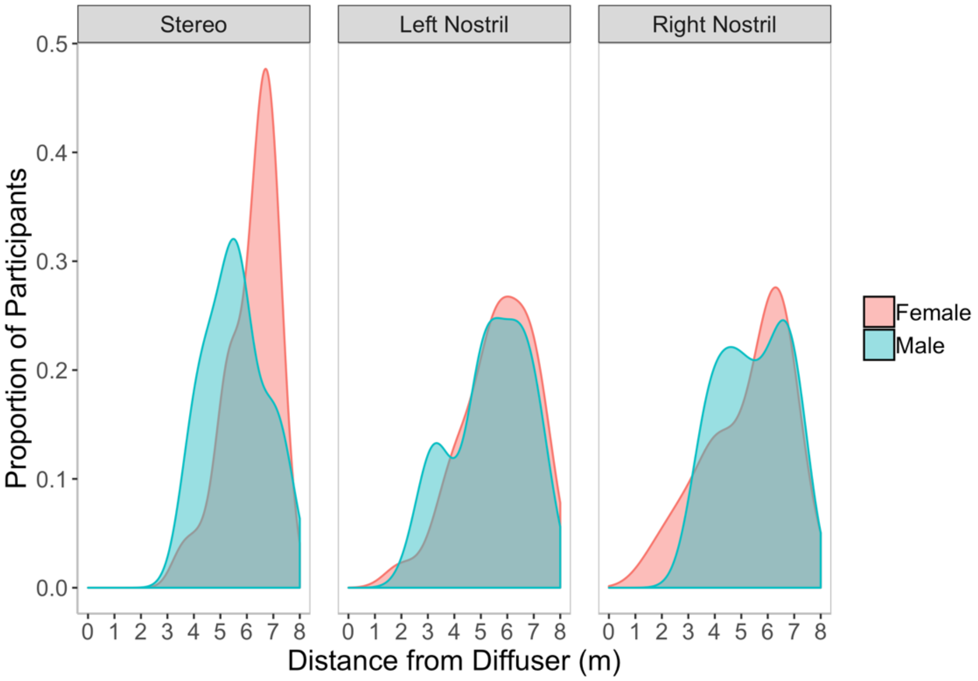

Figure 3.9. Density plots showing when men and women first raise a hand, signaling they had detected the anise seed oil odor in the air. Women raised their hands earlier than men.

As participants approached the diffuser, the time they spent signaling they had perceived the odor increased (Appendix O) and remained fairly consistent when they were less than 4 m from the correct diffuser. Thus, participants were able to smell the odor throughout the task. Habituation to the odor did not occur. Total time spent with their hand raised did not differ between the three trial conditions (stereo 83.24 ± 50.78 s, left nostril 83.63 ± 63.24 s, right nostril 80.67 ± 61.79 s ; Wald $\chi^2(2, 153) = 0.21$, p = 0.90). Figure 3.9 shows the average

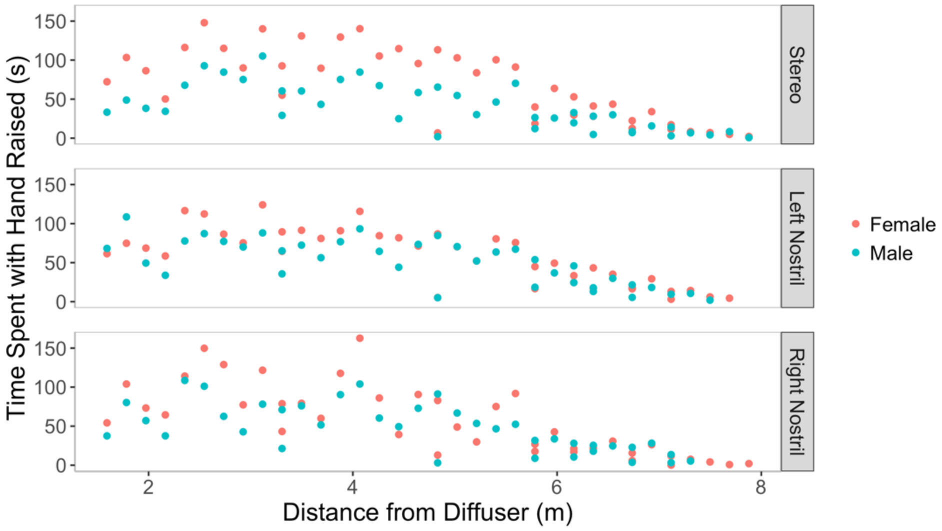

Figure 3.8. Time spent with hand raised by distance for men and women during each experimental trial. Women signaled they smelled the odor more than men. During the stereo trial, participants also signaled for longer. Signaling that the odor was detected begins to increase at distances less than 6 m from the loaded diffuser.

time spent with a hand raised in both men in women for distance from the loaded diffuser. Variation is low at distances greater than 6 m, however, at closer distances, time with the hand spent raised increases signifying people are detecting the odor.

A mixed effects logistic model was created which included distance from the diffuser, sex, and trial conditions as variables (Appendix P). The odds of a hand being raised decreased by 0.36 for each meter away from the diffuser. The model also predicted that in comparison to stereo trials, when the right or left nostril were used, the odds of raising the hand in the stereo condition was between 0.37 and 0.38 higher. Lastly, the odds of raising a hand was predicted to be three times higher in women than in men.

Discussion

This is the first study to describe how humans modulate sampling behavior during a plume following task. Participants successfully followed an odor plume to its source using both stereo and non-stereo odor cues, suggesting that humans use a spatio-temporal strategy during olfactory navigation. Sampling behaviors were modulated to both gather information about the odor plume's structure and successfully find an odorant with or without stereo olfactory cues.

Individual Differences in Sampling and Perception

A variety of sniffing patterns were seen amongst participants during this study. Some participants preferred to steadily alternate long sniffs with long exhales. Others preferred to take multiple short sniffs before releasing a large exhale and then repeating the pattern. Still other participants alternated quick sniffs with rapid exhales between each. Unique, individual sniffing patterns are not exclusive to humans and has been observed in rats as well (Wesson, Verhagen, et al., 2015). These individual sniffing patterns appear to serve a purpose. Laing (1983) demonstrated that humans perform optimally when sniffing at their natural pace suggesting that individuals may learn to optimize their sniffing patterns during their lifetime. In both rats and humans, sniffing patterns tend to be stable even with a change in difficulty of an olfactory tasks (Wesson, Verhagen, et al., 2015)—in this case, blocking a nostril did not change a person's sniffing pattern.

Sniffing is an important part of olfactory percept. Without the sniff, perception of the odor will not occur even with direct injections of odor molecules to the olfactory epithelium (Bocca & Battiston, 1964). Paired mechanical stimulation and stimulation of the olfactory epithelium by odor molecules is required for perception. Sniffing, therefore is not wholly characterized by short bursts of inspiration, but also by individualized behavior to optimize odor perception.

In the stereo olfaction trials, initial onset of hand raising by women occurred when they were farther from the target diffuser when compared to men. An experiment by Dalton, Doolittle, et al. (2002) showed that women were better than men at discriminating odors and other studies have shown that women also more sensitive to odors as well (Brand & Millot, 2001). In the current study, men showed a tendency to sniff harder than women which may assist in odor detection (Sobel, Thomason, et al., 2001), suggesting men have a higher olfactory threshold than women and compensate by modifying their sampling behavior. Both men and women performed equally well at estimating the diffuser location though. Hence, modulating sniffing behavior results in successful navigation regardless of detection ability.

No significant relationships were found between self-reported olfactory ratings and how well participants identified the target diffuser. Sniffin' Sticks are a battery of tests using known quantities of odorants contained in felt-tip pens that determines olfactory discrimination and thresholds (Hummel, Sekinger, et al., 2005). Subsequent studies could use the Sniffin' Sticks test to quantitatively determine the olfactory abilities of participants prior to beginning the experiment.

SOD had no impact on sampling behavior. A trend did exist where participants with higher SOD had more accurate estimates of the target diffuser's location, but it was not significant. In addition, a trend suggested that men may have been better at estimating the diffuser's location than women but significant differences were not found either. It is possible that too few diffusers were included in this study, resulting in high variance for the estimates.

Searching for and Following the Odor Plume

PID measurements with isopropanol showed that the plume dispersed quickly longitudinally and laterally. Less than 1 ppm of isopropanol was detected by the PID between 7-8 m from the diffuser at the end of 250 seconds. Only within 4-5 m were isopropanol levels above the 1 ppm threshold. The dispersal pattern, although not necessarily the volatilization rate, of anise seed oil should closely match that of the isopropanol odor plume. The odor detection threshold for anethol, the main component of anise seed oil, is 15 ppm when mixed with air (Czerny, Christlbauer, et al., 2008; Santos, Figueiredo, et al., 1998). The anise seed oil odor plume did not disperse quickly enough to reach the back of the experimental arena (8 m from the diffuser) when participants began each trial. This is supported by observing that initial hand raising, signaling the anise seed odor was above 15 ppm and perceptible, began around 6 m from the target diffuser.

It can be inferred that participants originally casted little when far away because they had yet to detect the scent and were in the *search* phase of navigation. As participants closed the distance, they detected trace amounts of odors mixed with clean air, resulting in more casting as they attempted to isolate the odor plume and learn about its general shape and structure. This is in concordance with other literature that suggest casting is a response to odor plume loss and attempts to re-locate the odor (DeBose & Nevitt, 2008; Kenen & Cardé, 1994). Casting peaked approximately 4 m from the target diffuser. Within 1-4 m from the target diffuser, participants then began casting less as they approached. This may be because the odor gradient was strong and enough information about the odor plume had been learned to *follow* it without losing the odor. Therefore a more direct heading was possible. This behavior is seen in both clown fish (*Amphiprioninae*; Elliot, Elliot, et al., 1995) and gypsy moths (Vickers & Baker, 1994). The rest of the discussion will refer to distances greater than 4 m from the target diffuser as the *search* phase. Within 4 m of the diffuser, participants were considered in the plume *following* phase.

Sampling Behavior in Response to Spatial Movement

Participants increased the length and number of samples taken per meter as they approached the diffuser during the *search* phase. When people were far away, more than 4 m, small pockets of the odor may have been detectable, but it was unclear from which diffuser it originated. Thus, people may have begun to sniff more and longer to detect odors more easily (Noam Sobel, Khan, et al., 2000). Once completely within the odor plume, less than 4 m, people then started to sniff less in number and shorter in length as they *followed* the plume because each sniff carried enough information to know the direction of the odor gradient.

Sampling frequency decreased as participants approached the target diffuser during the *search* phase, then rose as participants neared the diffuser in the plume *following* phase. This suggested that participants preferred sniffing slower, rather than faster, when *searching* for the odor. This result contradicts studies in dogs and rats that show sampling frequency is higher during *search* and then drops once an odor is found (Khan, Sarangi, et al., 2011; Thesen, Steen, et al., 1993). However, Verhagen, Wesson, et al. (2015) showed in rats, that an increase in sniffing frequency did not increase the amount of odor detection ability or increase activation in the olfactory bulb. In fact, sniffing frequency immediately decreased once an odor was detected, which was also seen in the current study. Rajan, Clement, et al. (2006) suggested that an increase in sniffing frequency was a behavioral reaction to excitement in anticipation of nearing the odorant source. Participants in this study may have also increased sniffing frequency once they were *following* the plume because they knew they were close to the target diffuser. Verhagen, Wesson, et al. (2015) also suggested rapid sniffing allowed rats to quickly detect changes in odor concentration. This is important when nearing an odorant source where crossing the edge of an odor plume may result in a sudden decrease in odor concentration signifying the need to cast in the opposite direction (Nauwelaerts, Scholliers, et al., 2004). This same strategy may have been used by participants in this task to help identify when they had walked beyond the edge of the plume when *following* it. Sniffing quickly would not have been useful when the plume shape had yet to be discovered while *searching*.

Sampling intensity linearly increased as participants approached the diffusers while *searching* and *following* the plume. Studies are controversial about the effects of sniffing intensity on the perception of odor intensity. Some support the conclusion that sniffing intensity increasing perception of an odor (David G. Laing, 1983; N. Sobel, Thomason, et al., 2001). Other studies have concluded that sniffing intensity may have no effect on perceived odor strength (Teghtsoonian & Teghtsoonian, 1978). Because sniffing intensity continued to increase during this navigation task, it must have been useful in some way or no changes would have occurred. Sniffing more intensely, thereby increasing flow rate, may have helped to isolate the odor and identify it (Mainland & Sobel, 2006). As participants *followed* the plume, they may have tried to detect fine changes within the odor plume by both sniffing faster and more intensely.

Stereo Olfaction and Sampling

Each participant had their one nostril obstructed (left or right) and neither obstructed, during each of the three trials. No difference in final estimation of the target diffuser was detected between these three conditions. This may seem contrary to what was seen in previous literature discussing the impacts of stereo olfaction in humans. However, in von Békésy (1963) experiment, people's heads were held stationary while estimating the angle to an odorant ball held in front of them. This showed the importance of stereo olfaction in a stationary task, but not during active navigation. Participants in this study often stood in one place sniffing—it was likely they were able to perceive differences in odor concentration between their right and left nostrils during the stereo olfaction trials. While using one nostril though, navigation was still possible. This suggests humans can also use a spatio-temporal odor concentration comparison strategy where they sniff in multiple locations before forming a decision on where to move. Porter, Craven, et al. (2006) also showed that people were able to successfully follow an odor trail when using only a single nostril. Their accuracy suffered and they were slower, but ultimately they completed the task. Similarly, both rats and fruit fly larvae employed the spatio-temporal strategy to successfully follow a trail or odor plume (Gomez-Marin & Louis, 2012; Khan, Sarangi, et al., 2011). Homing pigeons were also able to find their way home, but those with the right nostril occluded stopped more often on the way (Gagliardo, Filannino, et al., 2011).

No bias occurred of moving towards one side of the room was observed during the mono condition trials. However, when moles (*Scalopus aquaticus*) search for earthworms with a single nostril blocked they are biased towards the side with the unobstructed nostril (Catania, 2012). Participants in this study were observed turning their heads such that the unobstructed nostril faced the diffusers which were placed linearly before them. In contrast, earthworms were distributed radially from the start point and moles had no cues to which direction the reward was located. Thus, any bias that may have occurred during *search* or plume *following* in this study was masked by intentional head turning towards the diffusers to compensate for lack of stereo olfaction.

Although no difference existed between number of samples taken among the different experimental trials, during the stereo condition participants sampled shorter, less frequently, and less intensely. When people sampled at their natural pace, they were gathering enough information about the odor to make decisions on where to navigate (Laing, 1983). This hypothesis is supported by results from the logistic regression showing participants were more likely to signal they perceived an odor during the stereo trials. However, once a nostril was obstructed participants needed to modify their sampling behavior to gather the same amount of information. This included sniffing for longer, sniffing more frequently, and increasing the flow speed by sniffing more intensely. Sniffing longer has been shown to improve olfactory percept (Sobel, Khan, et al., 2000) and may have been employed as a strategy to maintain their baseline olfactory threshold for detecting the anise seed oil. Increasing sniff intensity also helps improve odor detection when airways are restricted, like when one nostril is obstructed (Hornung, Chin, et al., 1997; Youngentob, Stern, et al., 1986). The modulation of sampling behavior between stereo olfactory and non-stereo olfactory trials supports navigation models that show stereo olfaction provides more information (Boie, Connor, et al., 2018).

Implications

In conclusion, humans could track an odor plume to its source. This active sampling strategy to both find and follow an odor plume supports the hypothesis that stereo olfaction is not a requirement for successful olfactory navigation. Obstructing one nostril had no effect on the overall ability to locate the odorant. However, doing so resulted in less frequent, longer sniffs, and more intense sniffing, demonstrating that people modulate their sampling strategies to optimize olfactory information gain. Women demonstrated they had a lower olfactory threshold by detecting the odor faster than men, but they were no better or worse than men at finding the odorant. This research contributes to the greater understanding of active sampling and sampling behavior during olfactory navigation in mammals.

Chapter 4
Decoding the odor plume: how humans navigate using information from virtual odors

Introduction

Odor plumes move in unpredictable ways and are complex structures filled with empty spaces adjacent to filaments with highly concentrated areas of odor (Crimaldi & Koseff, 2001). Animals that must discover the odor gradient created by plumes to successfully navigate to the odorant. Studies have shown that animals make movement decisions in a time frame that would not allow them to fully sample and built a complete picture of an odor plume's structure (Atema, 1996). Instead animals must be using probabilistic estimates of an odor plume's structure in order to successfully navigate (P. Moore & Atema, 1988; Vergassola, Villermaux, et al., 2007).

Moths and flies display a behavior called odor-modulated optomotor anemotaxis where neurons trigger the animals to fly upwind and increase their speed in the presence of an attractive odor until they reach the source (Álvarez-Salvado, Licata, et al., 2018; Balkovsky & Shraiman, 2002). This innate behavior is predicted as the probability that an attractive odorant is most likely located upwind of a flying insect's current position.

Vertebrate animals may also have similar innate prototypes of odor plumes that helps guide them towards an odorant (Baker, Dickinson, et al., 2018; Hoss & Makin, 1999). Navigation decisions may be improved by combining information they learn from actively sampling the plume with knowledge of a prototypical odor plume. If this prototype hypothesis is true, even without a true odor stimulus, when presented with information matching the statistics of an odor plume, the brain should be able to process the information as if it were truly sampling an odor. The corresponding series of decisions and behaviors should reflect behaviors during true olfactory navigation.

Virtual Odors

I tested this hypothesis using a virtual odor in a virtual navigation environment. Such tasks are well established in rodents and humans including spatial and olfactory studies (Chai & Jacobs, 2010; Dinh, Walker, et al., 1999; Hartley, Maguire, et al., 2003; Stankiewicz, Legge, et al., 2006; Thurley & Ayaz, 2017). The benefit of using a virtual environment is the ability to precisely record movement behavior and sampling behavior and then correlate them directly with the measurements of a known, virtual odor plume (Baker, Dickinson, et al., 2018; Radvansky & Dombeck, 2018). This current study used humans to further understanding of how humans move and modulate their sampling behavior while following an odor plume, complementing the findings from Chapter Three.

The odor cue an auditory cue will be used instead. Von Bekesy (1963) showed that both olfactory and auditory modalities have the same onset curve for percent concentration of odor as well as sound intensity. In addition, both olfactory and auditory cues are detected by paired sensors and hence can be used in stereo. Substituting an odor cue with an auditory cue is different enough that an auditory cue should not trigger neural responses related to perception of an odor, but shares enough properties with odor stimuli that it may still elicit the same navigation patterns seen in true olfactory navigation. This forces the participants to attend to information presented in a the auditory modality, but higher processes in the brain should recognize the information pattern matches an odor plume and treat it as such instead of as an audio wave.

Navigation and Sampling Behavior

This experiment investigated movement patterns and sampling behavior during three phases of olfactory navigation: the *search* for the odor plume, *following* the odor plume, and navigation to re-contact the odor if the plume is *lost*. *Searching* in a *de novo* environment where no odor cues have yet to be discovered is a complex task that has not been well studied. How humans *search* for an odor will be discussed in this chapter as well as why individuals have different types of *search* patterns.

A simple one-to-one comparison between consecutive samples before deciding which direction to travel during *plume* following is not necessarily the best strategy because odor plumes in nature rarely occur as smooth gradients to follow to the odor source. Instead, a better strategy may be to take samples from multiple locations before making a more informed decision on the direction up the odor gradient (Vergassola, Villermaux, et al., 2007). This chapter addresses the number of samples and the frequency of samples humans make as they navigate through a virtual odor plume. People should attend to whether the average concentration of the plume is increasing or decreasing to successfully navigate up the gradient to find the origin of the plume. Further, their sampling methods should closely match that of the experiment in Chapter Three, where initially their sampling frequency may be high during *search*, but slows down when they find the odor plume. Once the plume is discovered though, sampling frequency should once again increase.

Often in nature, animals may find a plume, then walk beyond the plume's edge, losing contact with the odor. Casting then occurs as the animal corrects its trajectory by broadly sweeping back towards the direction it came from as it attempts to find the plume again. Movement patterns following the loss of a plume has been observed in diverse species (Álvarez-Salvado, Licata, et al., 2018; Kenen & Cardé, 1994; Khan, Sarangi, et al., 2011). How vertebrates, such as mammals, alter their sampling behavior in response to losing contact with an odor plume, has been studied less. In addition, this chapter also investigated how sampling behavior changes when a person loses contact with a plume. A study in rats by Khan, Sarangi, et al. (2011) showed that when rats encounter a gap in an odor trail, their sniffing frequency remains the same as when they were following the odor trail. Thus, it is predicted that humans also will not modify their sampling frequency upon losing contact with an odor plume.

Individual Differences

This study examined how sex differences and sense of direction (SOD) affected navigation and sampling behavior. Men use distal cues and are better at using cardinal directions to navigate than women (Astur, Ortiz, et al., 1998; Chai & Jacobs, 2010). In this experiment, which uses an open virtual arena with no cues, men are expected to perform better than women overall. In addition, spatial ability will likely also impact how well people perform (Maguire, Burgess, et al., 1999; Moffat, Zonderman, et al., 2001). Those with higher spatial skills should navigate to the source of the odor plume more accurately or faster than those with lower spatial ability.

Methods

Subjects

Participants were 178 University of California, Berkeley students between the ages of 18 to 28 (20.4 ± 1.89, mean ± st. dev) from the Department of Psychology Research Participation Pool. All volunteers provided written consent prior to beginning the experiment. For their participation in the study they were given class credits. This research was approved by the Committee for the Protection of Human Subjects, the Institutional Review Board for the University of California, Berkeley.

Virtual Odor

The virtual odor plume in the environment was created by measuring the spatiotemporal structure of an acetone-air mixture delivered at 10 cm/s into the center of a wind tunnel. The plume was imaged using PLIF at 15 Hz within a 20 cm x 40 cm window for eight minutes. The normalized concentration distribution of the plume was then calculated (Crimaldi, 2008) to create a grid of 400 x 200 with values ranging from 0-1, no odor to maximum odor concentration for each image of the odor plume. The normalized measurements of the plume were then graphically represented and the structure of the plume could be seen. The structure

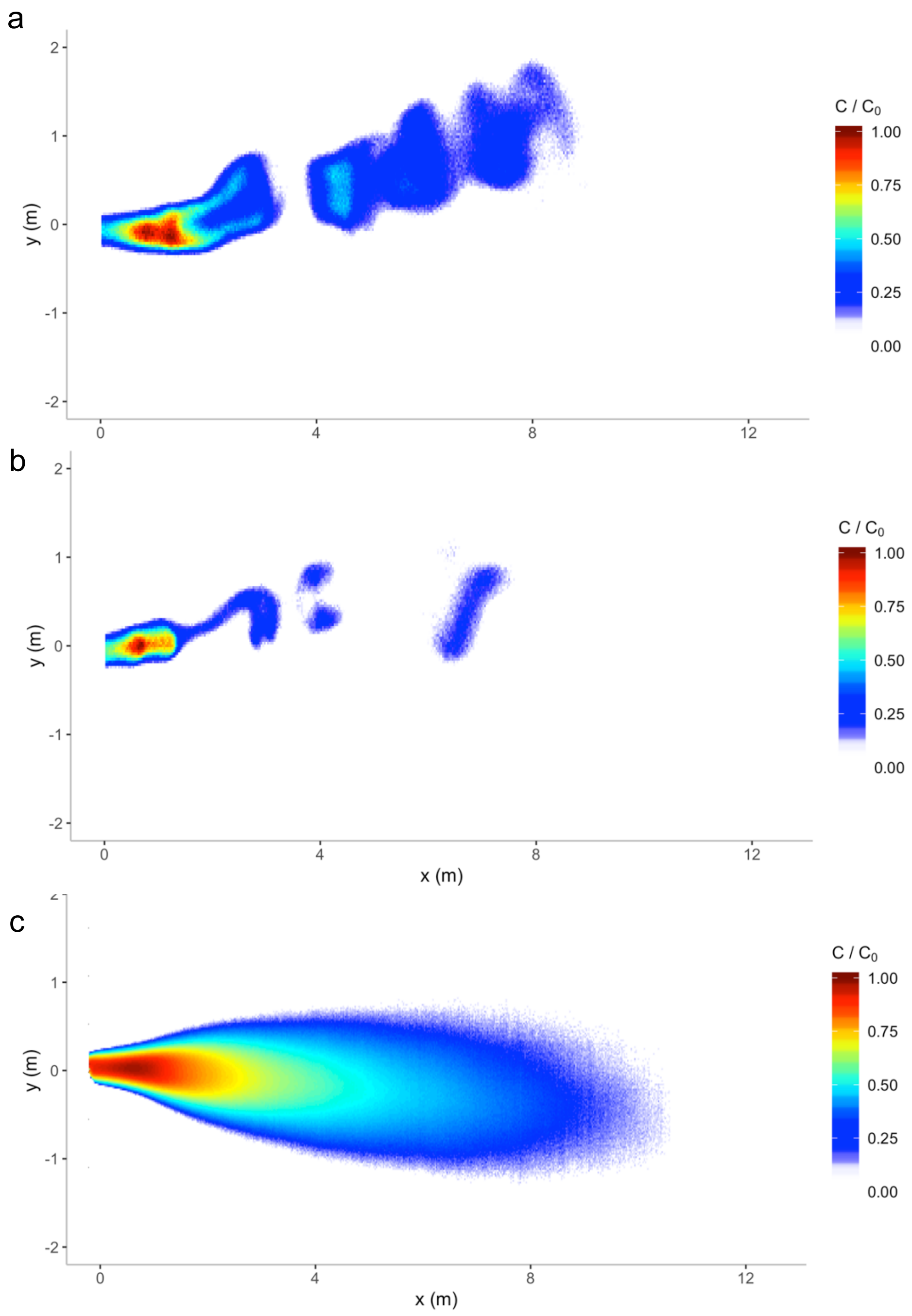

Figure 4.1. (a, b) Two frames taken from PLIF measurements of an acetone and air mixture odor plume injected at 10 cm/s to a wind tunnel, flowing from left to right. Colors represent normalized concentration distribution. Plume measurements by McHugh (2017). Scaled to 12 m in length. (c) The normalized mean concentration of the acetone plume over the eight minute duration of PLIF measurements. The most concentrated parts of the odor plume were approximately 2 m origin of the odorant. Unpublished image courtesy of Erin Connor from the Crimaldi laboratory.

of the plume was highly variable and at various points in time could look entirely different (Figure 4.1). The structure and concentrations of the plume were highly intermittent with many areas absent of any odor. The plume was measured by Margaret McHugh and Dr. John Crimaldi (McHugh, 2017).

In the virtual environment, the plume was linearly increased in scale such that it had a maximum length of 12 m. It was then placed in various locations and orientations within the environment for participants to find. Although this odor plume may seem large seem large, viewing Figure 4.1c reveals that over half of the length of the plume showed little odor concentration (< 5%). Therefore, the odor would only predominately be detectable between 6-12 m from the odor source, with the highest concentration of the plume found within 6 m from the source of the odor. The plume was not visible to participants during trials.

Virtual Environment

The environment was created by Fredrik Ludvigsen using the Unity game engine and presented on a 24” Dell monitor. The virtual environment was an in-game 30 m x 30 m flat surface overlaid with a checkerboard of one m^2 gray and white squares. No objects were located in the virtual environment during experimental trials. Virtual movement within the game was fixed at 2 m/s. Participants were instructed to find the origin of the virtual odor plume where it was the most heavily concentrated (Figure 4.2).

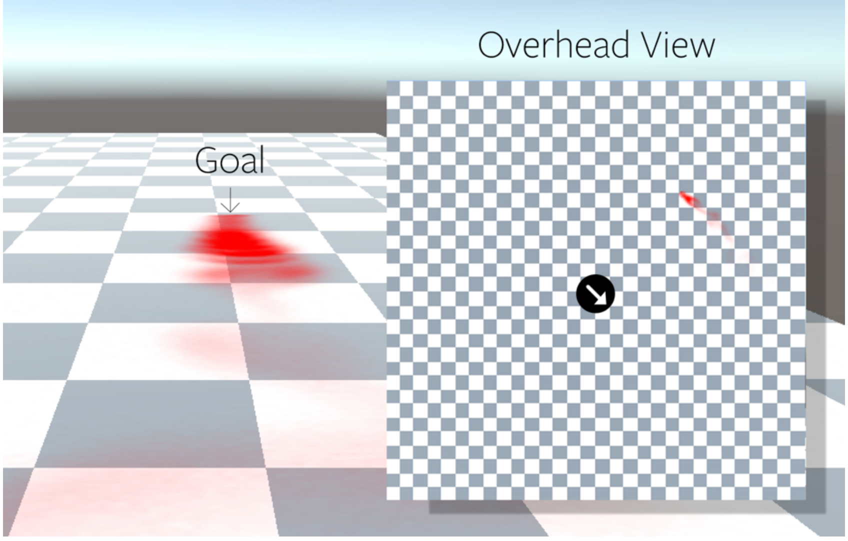

Figure 4.2. A screenshot of the virtual environment with a superimposed overhead view. The odor plume is shown in red for this figure, but is hidden from participants during trials. The goal was to stand at the origin of the odor plume, shown by the arrow in the figure. The participant’s starting location is the black circle in the overhead view. An arrow in the circle shows the starting direction of the participant. Circle is not proportional to actual size player in virtual environment. Odor plume size is shown to scale in the overhead view.

Participants moved using the keyboard (W: forward, S: back, A: step left, D: step right) and changed directions by moving the mouse to turn the field of view in the direction they wished to travel. They submitted their estimates of where the virtual odor plume originated by standing at the desired location and pressing the enter key.

Participants interacted with the virtual odor by sampling the environment. This was achieved by pressing the space bar. For each sample the program calculated the average value (0-1) of the PLIF plume captured within the area of an isosceles triangle (height = 28 cm, base = 20 cm, area = 400 cm^2) where the apex was centered on the participant's current location (Figure 4.3). Once the average value was calculated, a 440 Hz tone ("A" note) was played back to the participant through headphones (Philips Closed Back Stereo Headphones) for up to 750 ms where the audio intensity was linearly proportional to the odor concentration. For example in Figure 4.3, if the average "concentration" of odor sampled in the triangular area was calculated at 0.62 then the tone would be replayed at 62% of the maximum audio level of the computer. The computer audio level was set to the same level for all participants. Participants were screened prior to the experiment to ensure none had hearing disabilities. Instructions for the keyboard and mouse controls were provided on a printed piece of paper for participants to refer to throughout the experiment.

Figure 4.3. Schematic (not to scale) showing the triangular area (height = 28 cm, base = 20 cm, area = 400 cm^2) used to calculate average concentration of the odor plume during sampling.

This task had three experimental conditions. In the stereo condition (control group), the triangular area was bisected and the odorant concentration for each respective half of the triangle was treated as a sensor area and was calculated—the audio intensity was played back to the corresponding right and left sides of the headphones (Figure 4.3). In the crossed conditions, the calculated concentration for each sensor was played back to the opposing side of the headphones. In the mono condition, the average concentration of the odor is calculated for the entire isosceles triangular area and both the right and left sides of the headphones were played the same audio intensity.

Procedure

Preceding the experiment, participants completed a survey. They reported their gender, age, and the maximum number of hours per week, on average, they had ever spent playing computer games or video games, whether it was currently or in the past. Possible responses were given in the form of eight choices from 0 hours to 7+ hours. This question was created to identify participants that may have once played games heavily, but no longer played as often at the time answering the survey. Their scores were then used to bin them into groups of novice (0-1 hours), average (2-4), or experienced gamers (5-7+). The survey also contained the Santa Barbara Sense of Direction Scale (Hegarty, Richardson, et al., 2002) used in Chapter Three. SOD scores were also binned into low (scores 2-3), average (scores 4-5), and high (scores 6-7).

Participants were randomly assigned an experimental condition: stereo (control group, n = 65), mono (stereo effects removed, n = 60), or crossed (right and left stimuli reversed, n = 53). Participants were not told there were experimental groups or their experimental group assignment. They were only given information that they might notice the audio stimuli could sound strange but that this was expected and a part of the experiment.

A guided tutorial of the virtual environment was given where participants were introduced to the controls. During this time, in addition to self-reported gaming experience, the researcher assigned a score for each individual for their gaming level "novice", "average", "experienced". This was determined by how well a participant was able to walk back and forth between two trees around a circular fountain placed in the center of the environment during the tutorial (Figure 4.4). Gaming experience was judged by the behavioral criteria listed in Table 4.1.

Table 4.1. Criterion used by researchers to rate a participant's gaming experience.

Rating	Criterion
Novice	- Voiced their discomfort with the controls and required additional assistance explaining the controls. - Had difficulty understanding they needed to move the mouse to change their field of view and ergo their direction of movement, resulting in pointing the field of view to the sky or ground while moving. - Needed time to stop and review the sheet with instructions. - Were unable to navigate between the trees or around the fountain without frequently stopping, turning, then moving again in a repetitious manner.
Average	- Only glanced down at their hands occasionally to check if they were positioned correctly. - Were able to walk between the two trees and the perimeter of the fountain with little trouble. - Had slight trouble keeping the field of view on the screen parallel with the ground and occasionally pointed the field of view up or down but quickly adjusted.
Experienced	- Immediately placed their hands in the correct keyboard position without prompting. - Seamlessly used both the mouse and keyboard in conjunction to walk between the trees and around the fountain. - Kept the field of view parallel to the ground at all time.

Following the gaming experience assessment, participants were able to view a visible odor plume within the environment. They were told to sample three locations within the odor plume (once at each of the three gray rocks seen in Figure 4.4) to gain experience and knowledge about the relationship between the audio intensity and the odor concentration as the plume fluctuated. The odor plume was then made invisible for the remainder of the experiment. Participants were told the goal of each trial was to find the origin of the plume where odor was the most concentrated (i.e. the audio tone was the loudest), face upwind respective to the odor plume direction, and press the enter key to submit their guess of where the plume was located. Following the researcher-guided tutorial, the participant was left to complete two more tutorials on their own where no data was collected. The experiment began at the conclusion of the three tutorials.

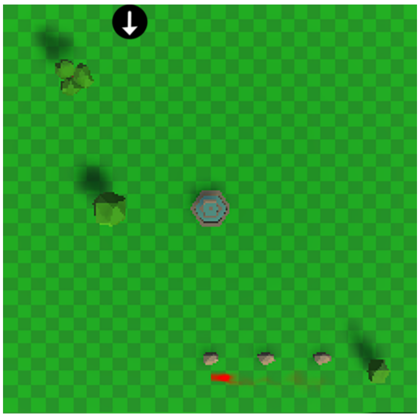

Figure 4.4. Overheard view of the guided tutorial used to determine gaming experience and allow participants to see the odor plume once. All tutorials had a green colored checker board. Participants were asked to walk between the two trees in the upper left hand corner and walk around the circular fountain in the center of the map. Participant starting location and direction faced is represented by the black circle with an arrow (not to scale).

The experiment consisted of eight trials each which contained one odor plume. All trials had odor plumes of the same size, but varied in their orientation and location pseudorandomized in the environment. Participants began each trial facing a pseudorandomized direction such that the origin of the plume was never located directly in front or behind them. During the eight trials the plume was located twice in each of four quadrants of the map to ensure the participant was not biased towards searching in a region. All participants were given the same trial order. Trial times were recorded, but participants did not have a time limit. Each trial concluded when the participant estimated the origin of the plume by pressing the enter key. Immediately after placing their estimate, an overhead map (as seen in Figure 4.2 and Figure 4.4) of the participant's final location, direction faced, and the plume in red was shown to the participant to both provide feedback on their accuracy and provide reinforcement. In total, the experiment was approximately 60 minutes long.

Analyses

Three metrics were used to assess how well participants were able to locate the origin of the odor plume. Location estimation accuracy of the plume's origin was determined by the distance from the participant's final location to the origin of the plume. Angle accuracy was found by calculating the difference between the vector of the participant's final heading and the vector the plume's downward gradient. The participant's angle was subtracted from 180 degrees to check if the participant was facing towards the origin of the odor, where minimizing this angle represented facing the correct direction. Trial duration in seconds was recorded as well.

Two types of navigation behavior were of interest, the paths participants traveled as they moved throughout each trial and also their sampling behavior. These two behaviors are related to each other and as will be shown, information gained from sampling the environment affected a participant's subsequent decisions on where to move next.

Each trial could be separated into three distinct phases of navigation: *search*, plume *following*, and *loss* of the plume. During the *search* phase, the plume had yet to be detected. The participant actively moved and sampled the environment. Plume *following* was a distinct phase that began when the plume was detected and ended when participants concluded the trial by estimating the location of the origin of the plume. This distinction was made because it was possible for the participant to detect the plume then *lose* contact with the plume by walking more than 15 m from the origin of the plume. If the participant detected the plume, subsequently *lost* it, and then searched the environment again before locating the plume again and ultimately providing their estimate of the odor source location, only the final period of time was labeled as plume *following* behavior.

Sample frequency was the number of spacebar presses per second during the three navigation phases. Sample number was also recorded. Metrics for these two sampling behaviors were averaged by distance to the plume origin binned to the nearest meter (e.g. four samples on average at 10 m from the origin of the plume).

The number of casts performed during the plume following phase was quantified by identifying each time a participant changed their direction of travel by more than 45 degrees. Average cast number during plume *following* by distance to the origin to the plume were reported.

Data were initially parsed and cleaned in Python 3.6.1. All statistical analyses were performed in R 3.5.1. Linear mixed models (LMMs; *lme4* package) were performed to test for a learning order effect where participants were included as a random effect and conditional r^2 values are reported. Multiple linear regressions models were used for analyses (*lm* package) with adjusted r^2 values reported. Any pairwise comparisons from ANOVAs, linear models, and LMMs were performed with Tukey HSD adjustments (*emmeans*) and reported if significant. Goodness of fit chi-squared tests were performed using the package *chisq.test* in R. Unless otherwise stated, $\alpha = 0.05$ for all statistical analyses. Average values and standard deviations are reported throughout the text.

Results

Estimating the Origin of the Plume

Only researcher assigned gaming experience scores were used in analyses. During the experiment's pilot sessions, some participants' responses to the survey claimed they had played less 1 or less hours of games per week, considered a novice gamer, did not match their obvious familiarity with the keyboard controls in the game once the guided-tutorial trial began. It became clear they had reported their current time spent playing video games instead of answering how often they had played in the past. Thus, although self-reported computer game experience was collected using the survey, the data were not used in the final analyses.

Participants were able to accurately estimate the origin of the virtual plume although there was a learning effect. As trials progressed, participants' estimates were closer to the source by an average of 10 cm per trial ($\beta = -0.10$, $t_{1245} = -4.72$, $p < 0.001$) and trial times diminished by approximately 6 seconds per trial ($\beta = -6.03$, $t_{1245} = -4.93$, $p < 0.001$). Progression of the trials had no effect on angle accuracy ($\beta = 0.86$, $t_{1245} = -1.58$, $p = 0.12$). To negate learning effects, results were averaged across all eight trials for each participant and average distance, angle, and trial time per person were obtained.

A two-way ANOVA was conducted with experimental condition and sex as factors. There was no interaction between condition and sex (Table 4.2, Figure 4.5). Participants in each of the three experimental conditions performed equally well at finding the origin of the odor, facing the odor source, and how fast they completed the trials regardless of experimental group (stereo, mono, or crossed cues). Men were more accurate at estimating the location of the odor than women, facing the odor source with a smaller angle error, and completed their trials faster.

Table 4.2. Table of average values and standard deviations. Two-way ANOVA between experiment condition and sex.

	Distance to Source (m)	Angle to Source (degrees)	Trial Time (s)
Stereo	2.22 ± 1.22	67.86 ± 29.91	164.23 ± 71.35
Mono	2.69 ± 1.44	65.75 ± 29.89	151.16 ± 56.98
Crossed	2.50 ± 1.25	61.14 ± 26.53	166.02 ± 78.28
	$F_{2,172} = 0.83$, $p = 0.44$	$F_{2,172} = 1.54$, $p = 0.22$	$F_{2,172} = 1.23$, $p = 0.30$
Female	3.076 ± 1.40	73.37 ± 27.82	173.54 ± 76.02
Male	1.96 ± 1.0	58.01 ± 28.05	148.42 ± 59.73
	$F_{1,172} = 35.33$, $p < 0.001$*	$F_{1,172} = 5.49$, $p = 0.02$*	$F_{1,172} = 6.74$, $p = 0.01$*
Condition x Sex	$F_{2,172} = 0.77$, $p = 0.46$	$F_{2,172} = 0.19$, $p = 0.83$	$F_{2,172} =0.05$, $p = 0.96$

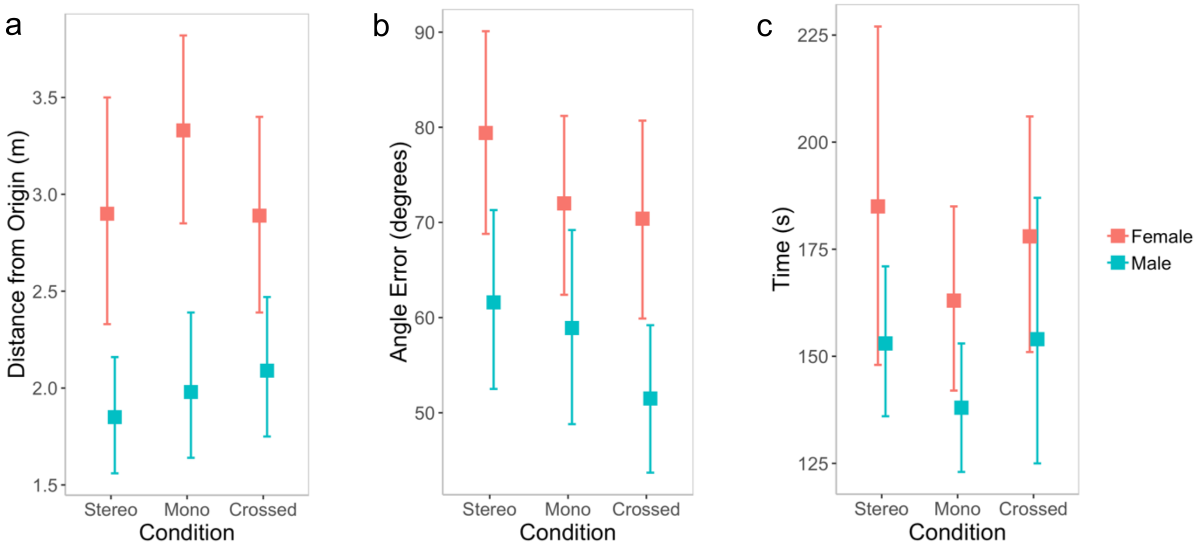

Figure 4.5. Three figures showing the average final distance from the odor source, final angle, and total trial time for men and women in each experimental condition. Means and 95% confidence intervals are shown. (a) The average final distance from the source participants were when they were guessing where the origin of the odor. No significant difference exists between conditions. However, men were better at estimating the location of the odor source (b) Angle error of direction faced by the participant from where the source of the odor. No significant differences between conditions. Men were significantly better at facing the origin of the odor. (c) The average time in seconds it took to complete a single trial. Women were slower at completing each trial. No time differences exist between experimental conditions.

The poor performance of correctly facing the origin of the plume is likely an artifact of the game controls. Participants tended to side step right and left when moving towards the source of the odor because it was easier to move this way than to move the mouse back and forth to change the angle of movement. An extreme real world example of this situation would a person with their back to an odorant and following the odor concentration while walking backwards towards the odorant and casting.

Time was a poor proxy for judging how well participants estimated the location of the plume's origin. Some participants concluded each trial quickly, but their estimated distance to the odor plume was large because they concluded the trial upon finding the edge of the plume instead of the origin of the plume. Other participants concluded the trial quickly because they stumbled directly onto the origin of the plume by accident. In contrast, some participants used a methodical strategy to find the source which could take a long time, but resulted in high accuracy for their final location estimation. However, some participants took a long time to complete a trial because they struggled with the controls. For these reasons, the remainder of the analyses only report final distance from the origin of the plume as the metric for performance.

Men were more experienced with gaming (2.92 ± 0.28) than women (2.04 ± 0.62, $t_{176} = -12.56$, $p < 0.001$), where 1 represented novice gamers and 3 were experienced gamers. In a two-way ANOVA with sex and gaming experience, sex was the only significant factor for predicting estimated location of the odor plume ($F_{2,172} = 7.83$, $p = 0.005$). Gaming experience was not significant ($F_{2,172} = 2.89$, $p = 0.06$) with no interaction effects ($F_{2,172} = 0.10$, $p = 0.75$). T results of the two-way ANOVA show that it was sex that predicted how well participants performed and not gaming experience.

Overall, having a higher SOD resulted in more accurate estimations of the plume's origin. A two-way ANOVA with sex and SOD as factors revealed that both sex ($F_{1,172} = 26.50$, $p < 0.001$) and SOD ($F_{2,172} = 3.30$, $p < 0.04$) had an effect on final distance to the odor source with no interactions ($F_{2,172} = 1.13$, $p = 0.33$). Individuals with high SOD estimated the odor location (1.92 ± 0.89) with higher accuracy than those with low SOD (3.15 ± 1.45). Those with average SOD also outperform those with low SOD (2.29 ± 1.23; Figure 4.6b). Men had higher SOD scores (2 ± 0.50) than women (1.63 ± 0.51; $T_{176} = -4.81$, $p < 0.001$; Figure 4.6a).

As a final test to investigate possible confounding interactions between sex, SOD, and gaming experience, a three-way ANOVA between the factors was performed on how well individuals could find the odor. Se x continued to have a main effect ($F_{1,172} = 5.54$, $p < 0.02$) while SOD had an almost significant ($F_{2,172} = 3.00$, $p = 0.05$). Gaming experience had no effect at all ($F_{2,172} = 2.15$, $p = 0.12$). A single interaction effect was found between gaming experience and SOD ($F_{2,172} = 4.00$, $p = 0.02$). This showed that for people with low SOD, having more gaming experience caused them to perform worse, not better. Overall, it appeared that sex was the dominating factor for performance on this task followed by SOD.

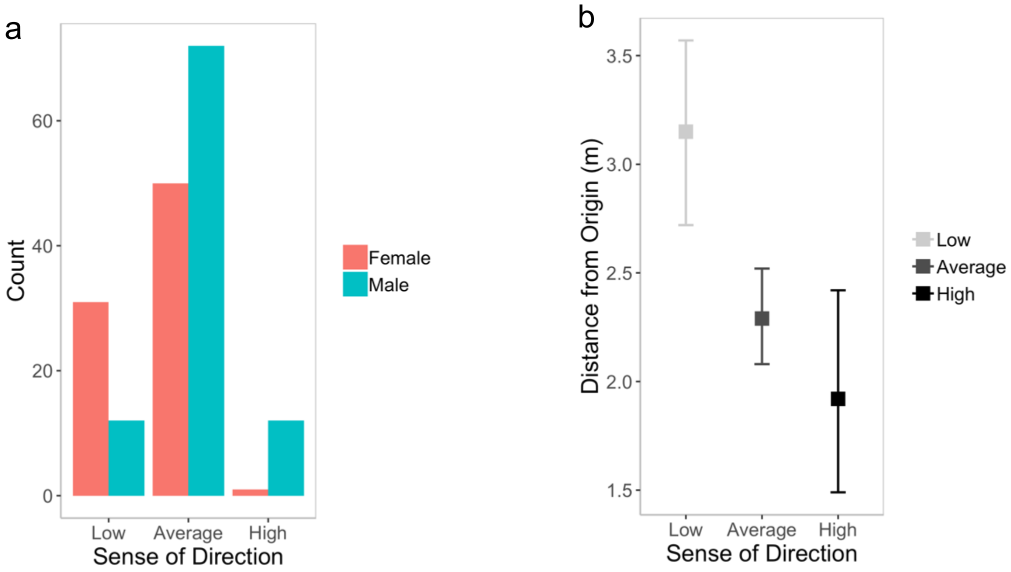

Figure 4.6. Two figures showing the distribution of SOD and performance related to SOF. (a) A histogram of the distribution of men and women and SOD. Most individuals have a low or average SOD. Men have significantly higher SOD than women. (b) Means and 95% confidence intervals of final distance from the odor source are shown. People with high SOD and average SOD outperform those with low SOD.

Searching for the Odor Plume

Search strategies, defined by their spatial geometry, varied among individuals—the five most common were *spiral, random walk, transect, irregular* and *diagonal* (Figure 4.7). The *spiral strategy* consisted of circling the environment as participants searched for the plume. *Random walks*, seemed to follow no perceivable pattern. *Transect* was defined as linear paths across the environment with 90 degree turns at the edges of the environment map. The *irregular* search strategy was characterized by multiple tight switchbacks whilst walking around the map without intersecting a previously explored area. This strategy was most often used in conjunction with both the spiral and transect strategy. Lastly, the *diagonal* strategy began by moving to one corner of the environment, crossing to the opposing corner, then returning to the center before moving outwards to an unvisited corner and crossing the environment again. If this failed to find the plume, a spiraling strategy inwards was often employed. If participants walked by chance straight towards the plume without any search pattern, these trials were excluded from the search strategy analysis. These trials were easy to characterize based on their short, generally linear paths which made it impossible to discern which of the five search strategies was being used. In total, 271 trials were excluded, however all participants were represented by at least one trial where they exhibited a search strategy.

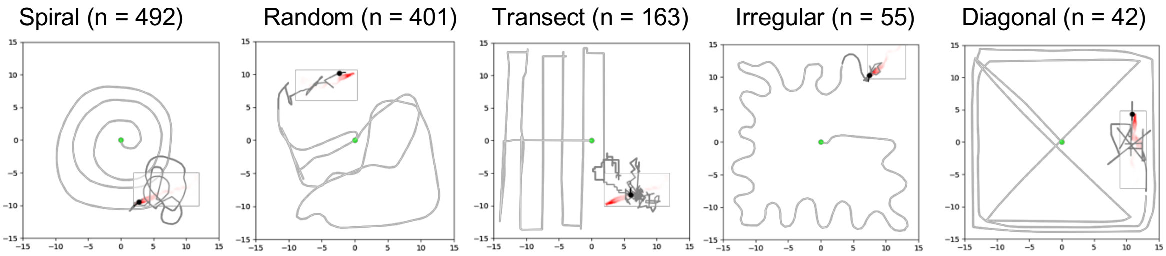
Spiral (n = 492) Random (n = 401) Transect (n = 163) Irregular (n = 55) Diagonal (n = 42)

Figure 4.7. Examples of individuals' trials as they attempted to locate the source of the odorant. Gray lines represent a person's path. The green circle represents their starting location. A still shot of the odorant plume is shown in red. The person's final location is shown as a black circle with a short black line showing their final heading direction. Navigation took place in two distinct phases, search (light gray) and plume following (dark gray).

A chi-square test showed that sex differences existed amongst the search strategies ($\chi^2(5, 1424) = 40.67$, $p < 0.001$). Women were more likely to use the transect search strategy while men were more likely to use the diagonal and irregular strategies (Figure 4.8). Similarly, people with high SOD preferred the diagonal and irregular strategies and those with low SOD preferred the transect strategy ($\chi^2(5, 1424) = 19.96$, $p = 0.007$). A two-way ANOVA with sex and SOD showed that only sex had an influence on how fast participants found the odor plume during the *search* phase (Sex: $F_{1,1147} = 28.70$). Men (42.43 ± 51.82 s) were faster than women (65.23 ± 75.65 s) at first contacting the plume during the search phase. There was no main effect of SOD ($F_{2,\ 1147} = 0.90$, $p = 0.41$) nor an interaction between the two factors ($F_{2,1147} = 2.38$, $p = 0.09$).

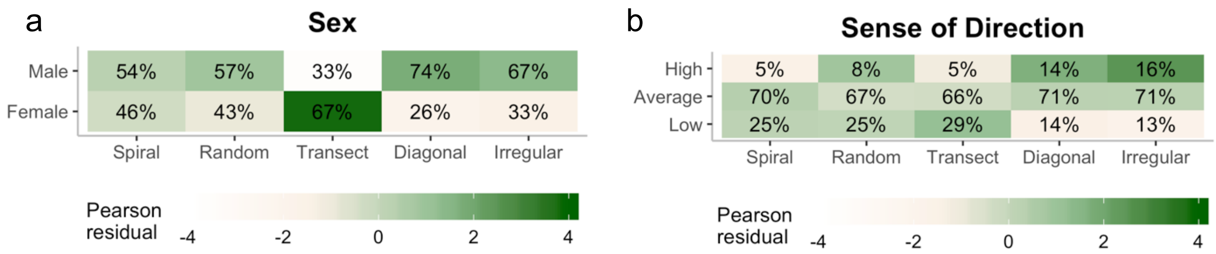

Figure 4.8. Plots showing the results of a chi-square test for search strategy. Colors represent the magnitude of Pearson residuals from the chi-square test darker green is a higher residual score. Values in the plot are the relative proportion of sex or SOD groups that used the search strategy. (a) Comparing men's and women's search strategies. Men are more likely to use the diagonal and search strategies while women prefer the transect strategy. (b) Sense of direction and search strategy preference also show a relationship. Individuals with high SOD prefer the irregular and diagonal strategies. Those with low SOD choose the transect strategy while people with average SOD do not have an overall preference.

The type of search strategy used had an impact on how quickly individuals could find the plume. Most of the search strategies were equally effective, finding the plume in approximately the same amount of time. However, the transect strategy was the slowest with an average time of 94.80 ± 75.82 s ($F_{4,1128} = 25.90$, $p < 0.001$) compared to the other strategies (spiral 38.02 ± 38.13 s, random 55.97 ± 82.47 s, diagonal 57.55 ± 39.12 s, irregular 39.72 ± 24.02 s).

Casting

As participants approached the origin of the odor, they began to cast more (Figure 4.9a). Participants in the study began to increase casting when they first encountered the odor plume approximately 15 m away from the origin. The average number of casts per meter to the odor source was fit to a multiple regression quadratic model. ($\beta = -0.44$, $t_{31} = -19.43$, $p < 0.001$; $\beta^2 = 0.01$, $t_{31} = 15.38$, $p < 0.001$, adj. $r^2 = 0.95$).

A one-way ANOVA showed that all experimental groups had an equal number of total casts during plume following (45.46 ± 32.36, $F_{2,175} = 0.66$, $p = 0.52$). In a two-way ANOVA with sex and SOD scores, men had significantly more casts on average (47.17 ± 26.86) than women (29.1 ± 26.30; $F_{1,175} = 16.01$, $p < 0.001$). SOD had no impact on how many total casts were made ($F_{2,175} = 0.30$, $p = 0.74$) and there were no interactions ($F_{2,175} = 0.55$, $p = 0.58$).

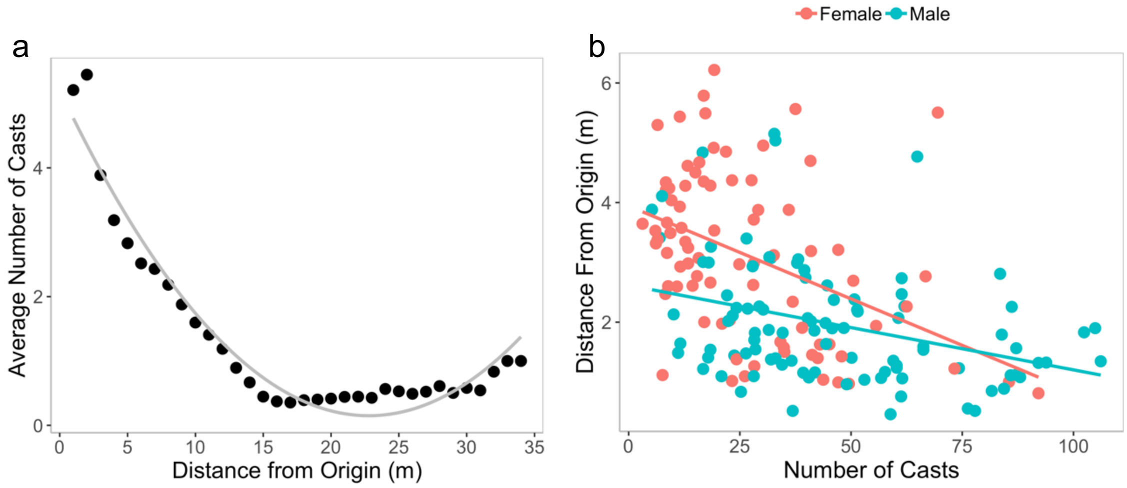

Figure 4.9. Figures showing the relationship between number of casts and distance from odor source. (a) Average number of casts per meter from the odor source. The number of casts increases as distance to the source diminishes. A slight drop in the number of casts occurs at the closest distance once people locate the odor source. (b) Plot showing as cast number increases for both men and women, final distance to the odor source diminishes. Casting helps people navigate an odor plume to its source. Men cast more than women on average.

Casting improved a person's ability to find the odor source (Figure 4.9b). Participants who casted more while navigating through the plume had a final distance that was closer than those who casted less. On average, a single cast predicted a decrease in the final distance to the plume's origin by 2 cm ($\beta = -0.02$, $t_{187} = -6.94$, $p < 0.001$; Adj. $r^2 = 0.21$; $F_{1,176} = 48.21$, $p < 0.001$).

Sample Frequency during Navigation Phases

Linear multiple regression models with distance and experimental conditions as variables to predict sampling frequency were created. If the odor had yet to be detected during the *search* phase, sampling frequencies showed no change respective of distance to the odor source (Appendix Q) Pairwise comparisons showed that participants in the crossed condition had a slower sampling frequency than the stereo or mono group participants (Table 4.3). Search sampling frequency can be seen by the dashed lines in Figure 4.10a.

Once the odor was detected and plume *following* began, the sampling frequency dropped with decreasing distance to the odorant in all three experimental groups as long as the participant remained within 15 m of the plume where odor was detectable (Appendix R). This can be seen by the thicker solid lines shown in Figure 4.10a. An interaction was found between distance and participants in the mono experimental group which showed that individuals in the mono class started with a higher sampling frequency than the other groups when they first found the odor plume. They then sharply decreased their sampling frequency until it matched the average frequency of the crossed condition group. Participants in the crossed group continued to sample at a slower frequency (2.11 ± 1.32 Hz) than participants in the stereo condition (2.40 ± 1.18 Hz) and mono condition (2.37 ± 1.19 Hz).

The plume could be *lost* by walking in the beyond 15 m of the plume after it was detected, represented by the thinner, solid lines in Figure 4.10a. Comparing the average sampling frequencies amongst the experimental conditions and whether participants were *searching* for the plume, *following* the plume, or *lost* the odor plume yielded significant differences using a two-way ANOVA. An interaction between experimental group and navigation location existed (condition: $F_{2,34587} = 1583.04$, $p < 0.001$; navigation phase: $F_{2,34587} = 311.68$, $p < 0.001$, interaction: $F_{4,34587} = 21.90$, $p < 0.001$). Pairwise analyses revealed that in the stereo group sampling frequency on average was slower during plume *following* compared to when the plume was *lost*. Furthermore, if the plume was *lost*, sampling frequency returned to the same frequency as during *search*. Participants in the mono group also had slower sampling frequencies during plume *following* compared to their search phase and when they *lost* the odor plume. However, their sampling frequency during the *lost* phase was higher than during the *search* phase. Lastly, unlike the stereo and mono groups, individuals in the crossed condition group maintained the same sampling frequency on average both when *following* and while *lost* – this sampling frequency was slowest during the *search* phase.

Table 4.3. Average sample frequency in Hz per meter from the plume origin and standard deviations during each phase of navigation.

	Navigation Phase		
	Search	Follow	Lost
Stereo	3.23 ± 1.27	2.4 ± 1.18	2.99 ± 1.32
Mono	3.59 ± 1.31	2.38 ± 1.27	3.59 ± 1.44
Crossed	2.82 ± 1.37	2.11 ± 1.32	2.37 ± 1.27

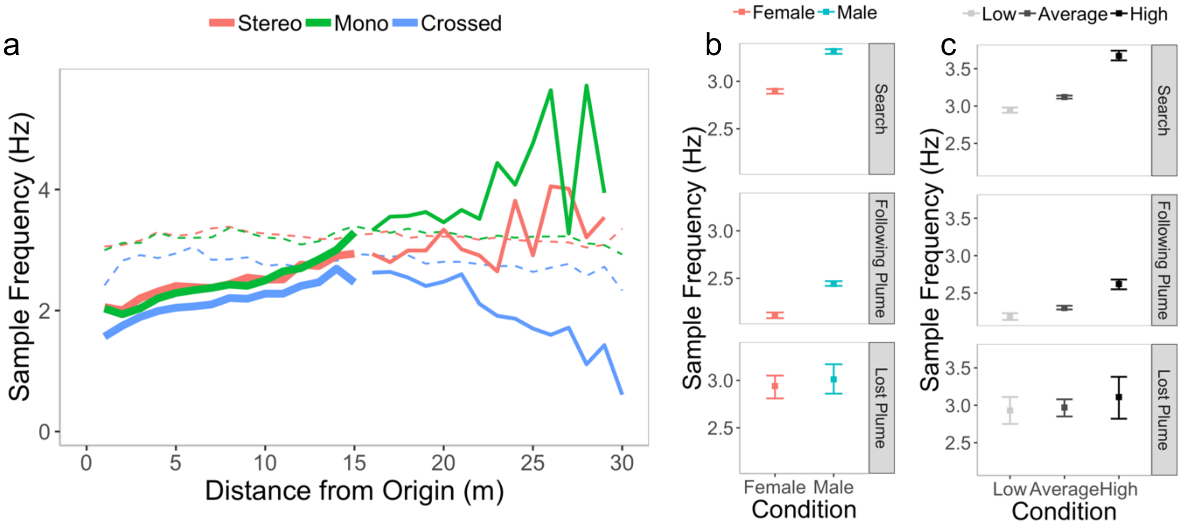

Figure 4.10. Figures describing sampling frequency as a response to distance from the origin, sex, and SOD. (a) Sampling frequency during the *search*, *following*, and *lost* phases by distance to the odorant. Averages of sample frequency for each meter from the odor source are graphed. Thick, solid colored lines represent behavior of different experiment groups during the plume *following* phase. Dashed lines represent behavior during the *search* phase. Thin solid colored lines represent the *lost* plume phase. Sampling frequency drops as individuals approach the odor source. Minimal changes in sampling frequency occur during the search phase. (b) The difference in sampling frequencies between men and women while following the plume, after losing the plume, and during search. Mean have a higher sampling frequency than women. (c) Individuals with high SOD also had significantly higher sampling frequency than those with average or low SOD.

A two-way ANOVA with the three navigation phases and sex as factors was created to compare sampling frequencies (sex: $F_{2,34587} = 744.56$, $p < 0.001$; navigation phase: $F_{2,34587} = 1698.57$, $p < 0.001$, interaction: $F_{4,34587} = 10.21$, $p < 0.001$; Figure 4.10b). An interaction was present. Women had slower sampling frequencies in comparison to men for all navigation phases except when they *lost* the plume. Here, women and men have the same sampling frequency.

Table 4.4. Average sampling frequencies (Hz) and standard deviations in men and women during the three navigation phases.

	Navigation Phase		
	Search	Follow	Lost
Female	2.9 ± 1.30	2.11 ± 1.21	2.94 ± 1.42
Male	3.32 ± 1.32	2.44 ± 1.23	3.01 ± 1.47

A two-way ANOVA analysis showed differences in sampling frequency occurred between groups of SOD scores for each of the three navigation phases (SOD: $F_{2,34587} = 219.01$, $p < 0.001$; navigation phase: $F_{2,34587} = 1637.32$, $p < 0.001$, interaction: $F_{4,34587} = 7.62$, $p < 0.001$). A significant interaction was found. In the *search* and *following* phases, participants with low SOD had the lowest sampling frequency, followed by average SOD participants, and then high SOD participants. During the *lost* phase, all participants sampled with the same frequency regardless of their SOD (Figure 4.10c).

Table 4.5. Average sampling frequencies (Hz) and standard deviations for participants with low, average, and high SOD during the three navigation phases.

	Navigation Phase		
	Search	Follow	Lost
Low SOD	2.95 ± 1.36	2.19 ± 1.24	2.93 ± 1.31
Average SOD	3.12 ± 1.29	2.3 ± 1.24	2.97 ± 1.29
High SOD	3.67 ± 1.36	2.62 ± 1.14	3.11 ± 1.12

Number of Samples during Navigation Phases

A two-way ANOVA was conducted with the experimental groups and navigation phases pertaining to *search* for the plume, *following* the plume, or *losing* the plume on the effects of average number of samples per meter. The results showed a significant interaction between the two factors (condition: $F_{2,34587} = 46.10$, $p < 0.001$; navigation phase: $F_{2,34587} = 1692.74$, $p < 0.001$, interaction: $F_{4,34587} = 30.86$, $p < 0.001$). Each group sampled more when they were *following* plume compared to when they were *searching* for the plume or if the plume was *lost*. During the *search* for the plume, all three experimental groups did not differ in the average number of samples. When *following* the plume the participants in the mono and crossed condition groups sampled less than the participants in the stereo condition group. The mono group also had a higher average number of samples than the crossed group. When the plume was *lost*, the average number of samples for all three experimental groups did not differ. Furthermore, once the plume was *lost* the number of times each group matched the average number of samples during the *search* phase.

Table 4.6. Average number of samples per meter from the plume origin and standard deviations during each phase of navigation.

	Navigation Phase		
	Search	Follow	Lost
Stereo	7.04 ± 11.9	27.7 ± 50.9	4.67 ± 4.41
Mono	7.32 ± 9.86	21.7 ± 31.1	4.67 ± 3.34
Crossed	6.45 ± 10.3	20 ± 32.0	4.25 ± 3.88

A multiple regression with number of samples regressed with distance to the plume origin and experimental group showed that participants in all three experimental groups increased the number of samples during plume *following* as they approached the origin of the plume (Appendix U; Figure 4.11). During *search* for the plume and when the plume was *lost*, the number of samples did not change by distance to the origin of the plume (Appendix T, Appendix V).

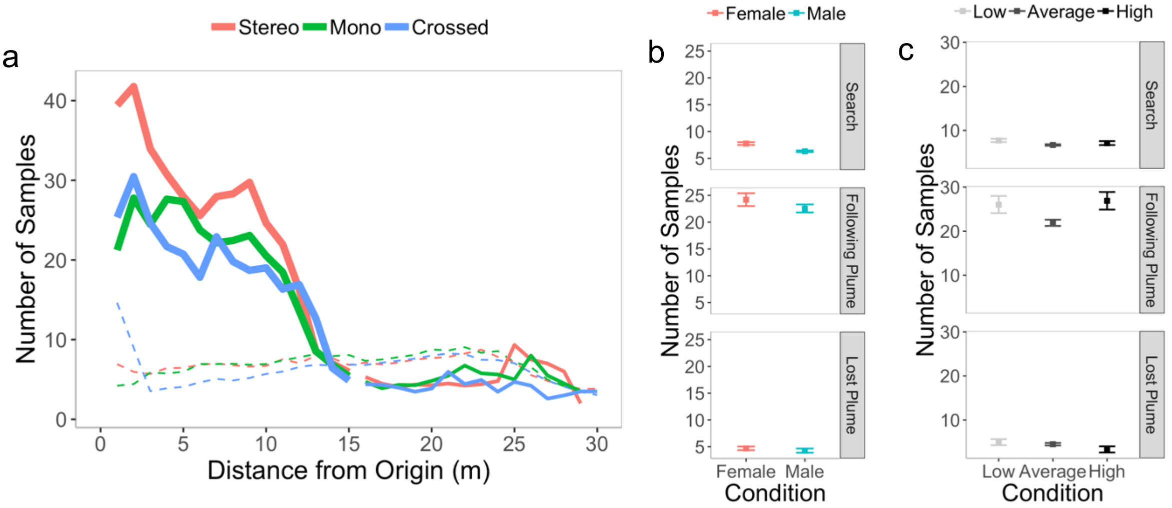

Figure 4.11. Figures describing number of samples as a response to distance from the origin, sex, and SOD. (a) Thick, solid colored lines represent behavior of different experiment groups during the plume *following* phase. Dashed lines represent behavior during the *search* phase. Thin solid colored lines represent the *lost* plume phase. Number of samples increased as the distance to the odorant decreased during plume following. During the *search* phase, if the odor is not detected, no changes in sample number occur. Note that although sample frequency is decreasing, the number of samples are increasing. (b) In absolute numbers, women sampled the environment more often than men throughout *searching* for the plume, during plume *following*, and even if the plume is *lost*. (c). The number of samples based on SOD shows that those with average SOD show a lower number of samples as they *search*, *follow*, and after they *lose* the plume.

Women sampled the environment more than men when *searching* for the plume, *following* the plume, and if the plume was *lost*. There was no interaction between sex and number of samples during *search*, *following*, or *loss*. (Two-Way ANOVA, sex: $F_{1,34590} = 30.03$, $p < 0.001$; navigation phase: $F_{2,34590} = 1691.94$, $p < 0.001$, interaction: $F_{4,34590} = 0.27$, $p = 0.76$, Figure 4.11b).

Table 4.7. Average number of samples per meter from the plume origin and standard deviations during each phase of navigation in men and women.

	Navigation Phase		
	Search	Follow	Lost
Female	7.75 ± 12.9	24.2 ± 46.0	4.69 ± 3.95
Male	6.3 ± 8.35	22.5 ± 33.6	4.25 ± 3.75

A two-way ANOVA with SOD and navigation phases showed found an interaction between the two factors (SOD: $F_{2,34587} = 25.84$, $p < 0.001$; Navigation phase: $F_{2,34587} = 1687.31$, $p < 0.001$, Interaction: $F_{4,34587} = 8.57$, $p < 0.001$). SOD had no effect on the number of samples made during the *search* phase. During plume *following* individuals with average SOD had lower numbers of samples than both the low SOD and high SOD groups which did not differ between their numbers of samples. This result showed that the number of samples taken does not necessarily translate to having higher or lower SOD. What individuals did with the information they gained by sampling was more important. If the plume was *lost,* SOD also had no effect on the number of samples. As before, for all SOD groups, if the plume was lost the number of samples matched the number made during *search*.

Table 4.8. Average number of samples per meter from the plume origin and standard deviations during each phase of navigation in participants with low SOD, average SOD, and high SOD.

	Navigation Phase		
	Search	Follow	Lost
Low SOD	7.72 ± 13.6	26 ± 54.0	4.93 ± 4.77
Average SOD	6.7 ± 9.69	21.9 ± 33.9	4.5 ± 3.62
High SOD	7.1 ± 9.01	26.9 ± 34.8	3.27 ± 2.72

Sampling Influences Navigation Decisions

The previous section showed how changes in sampling behavior differed among the navigation phases. Figure 4.11a shows that participants increased the number of samples significantly when they were navigating within the plume. However, 47.4% of the time participants sampled the environment, they made no movement afterwards implying they took time to think prior to choosing an action. Information gained during sampling itself was somehow used to inform decision making during navigation.

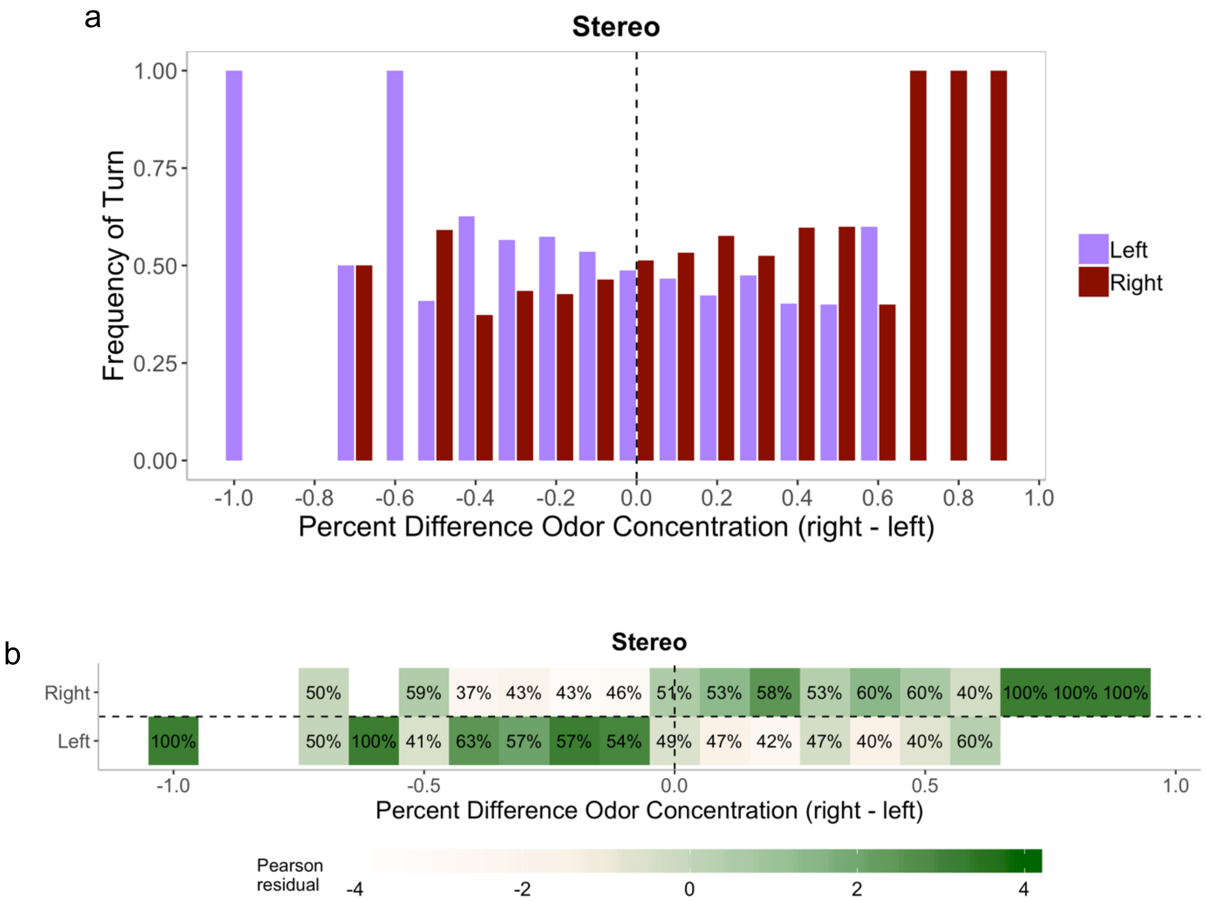

Figure 4.12. Right and left turns for participants in the stereo condition. (a) Horizontal axis represents the difference in percent odor concentration (right side – left side). Negative values represents that the left side had higher stimuli intensity and vice versa. On the vertical axis is the relative frequency of a left or right turn. Participants turned more often towards the side with greater stimuli. (b) Results of a chi-squared test with higher Pearson residuals represented by darker shades of green. Numbers in the plot represent the proportion of right or left turns for the given percent difference in odor concentration.

For participants in the stereo and crossed groups, differences in odor concentration between the right and left sides could have informed the direction to turn and the magnitude of the turn. Indeed, individuals in the stereo responded to differences in stimuli intensity between right and left side of the headphones by turning towards the respective side that had the higher stimulus. Figure 4.12a shows the relative frequency of left and right turns. To right of the dotted line, red bars (right turns) can be seen dominating the lavender bars (left turns) and vice versa for the area to the left of the dotted line. Figure 4.12b shows the results of a chi-squared test demonstrating that higher than expected counts of right turns are experienced when the right side has a higher concentration of odors and vice versa ($\chi^2(12, 52390) = 81.215$, $p < 0.001$).

Participants in the crossed experiment condition responded by turning to the opposite side that had a greater stimulus intensity (Figure 4.13). A chi-squared analysis of turning response to differences in odor concentration between the right and left sides showed the inverse findings from participants in the stereo condition ($\chi^2(14, 41367) = 55.916$, $p < 0.001$). Participants in the crossed condition realized the inputs were reversed and could compensate accordingly (Figure 4.13).

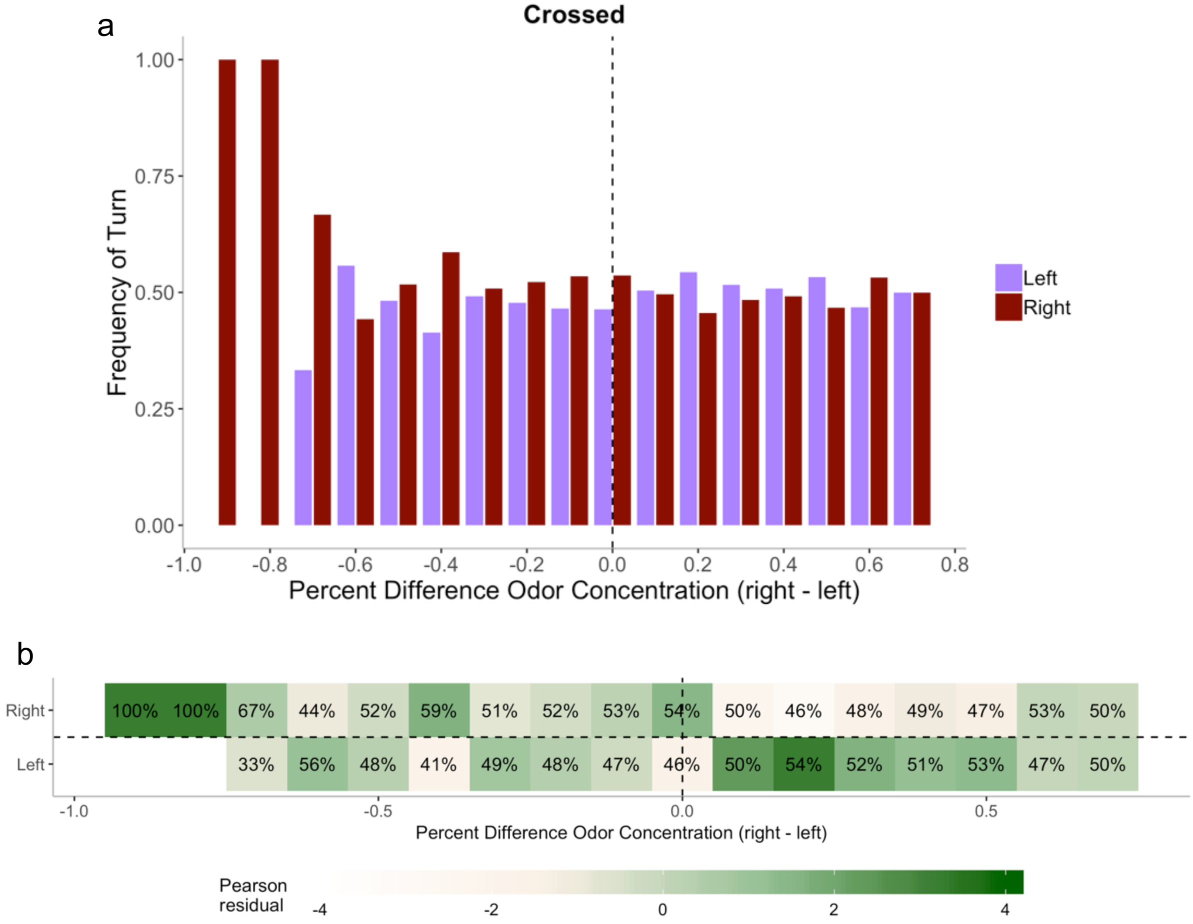

Figure 4.13. Right and left turns for participants in the crossed condition. Any odor concentrations that differed between the right and left sensors were switched such that the corresponding intensity of audio was played to the opposite ear. (a) Horizontal axis represents the difference in percent odor concentration (right side – left side). Negative represents the left side had higher odor concentration and vice versa. On the vertical axis is the relative frequency of a left or right turn. In the crossed condition, individuals are more likely to turn the opposite direction of the sensor with the highest concentration. (b) Results of a chi-squared test with higher Pearson residuals represented by darker shades of green. Numbers in the plot represent the proportion of right or left turns for the given percent difference in odor concentration.

Lastly, analyses of the mono condition group demonstrated people were not biased towards turning to the right or left after sampling if the stimulus intensity did not differ between the two sides ($\chi^2(10, 50258) = 8.2762$, $p = 0.60$; Figure 4.14). Therefore participants were actively attending to differences in odor concentration as they sample the environment for cues of which direction to follow the plume.

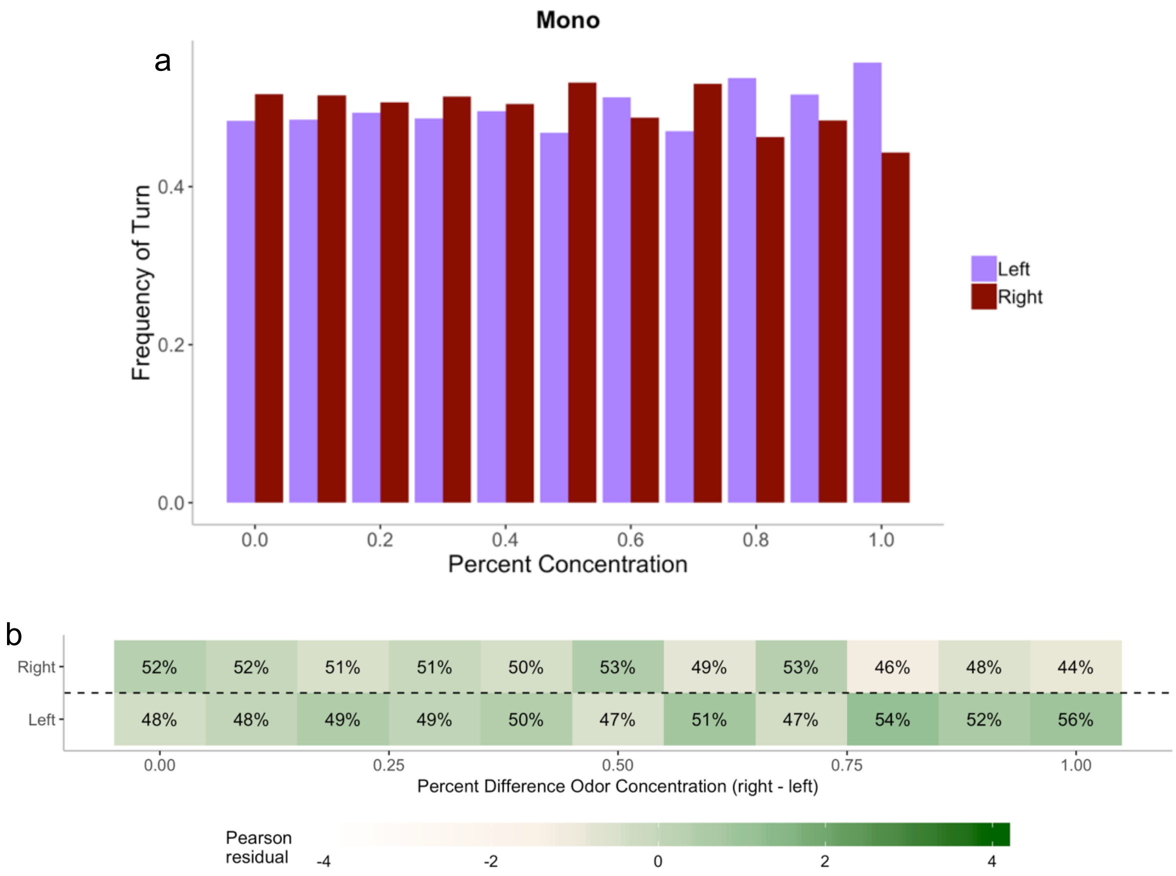

Figure 4.14. Right and left turns for participants in the mono condition. (a) The relative frequency of right and left turns in response a sample of the environment. There is no turning response bias. (b) Results of a chi-squared test with higher Pearson residuals represented by darker shades of green. Numbers in the plot represent the proportion of right or left turns for the given percent difference in stimuli intensity. Participants were equally likely to turn right or left when sampling.

Participants could detect an overall change in the gradient of odor concentration during the *following* phase or they never would have found the origin of the plume. Changes in stimuli intensity between samples informed a participant of whether they were approaching the origin of the plume or walking farther away. The number of consecutive samples made while moving away from the origin of the plume or standing in place before correcting and turning back towards the odor source were counted. On average, individuals sampled 7.18 ± 10.68 times before realizing they were moving in the incorrect direction and changed their trajectory to walk towards the plume's origin. Figure 4.15 shows a plot of the average change in concentration for up to 60 consecutive samples taken before participants corrected their path. If there was no change in stimuli intensity between samples, participants would continue to walk away from the odor source or stand in the same location. A linear regression with number of consecutive samples regressed against average concentration change showed there was a positive relationship between the average percent change in concentration and the number of consecutive samples taken (β = 24.497, t_{56}

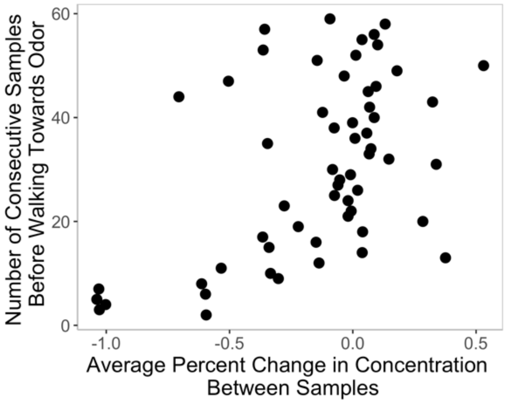

Figure 4.15. Horizontal axis is the average change in concentration of the odor (negative is diminished concentration). Vertical axis represents the number of consecutive samples taken while initially walking farther from the plume's origin before turning and going towards the odor source. Individuals who took greater less than 20 samples before correcting their trajectories were able to detect diminishing odor concentrations leading to their change in behavior.

$= 4.422$, $p < 0.001$; Adj. $r^2 = 0.25$; $F_{1,56} = 19.55$, $p < 0.001$). The greater the negative magnitude of change in stimuli during the samples, the less steps taken before the person reversed their trajectory.

Figure 4.16a shows a heat map of all individual's locations during plume following for all eight trials. The heatmap created by participants in the *following* phase closely matched the true time-averaged concentration of the odor plume (Figure 4.16b).

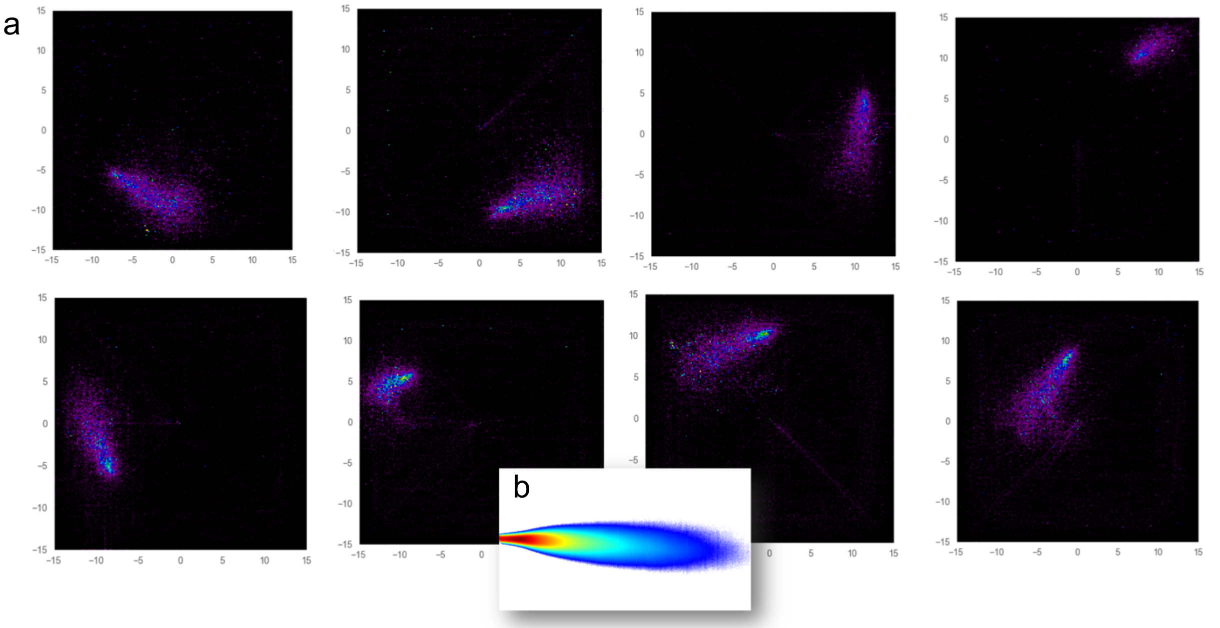

Figure 4.16. (a) Heatmap of all 178 participants' trajectories during plume following during each of the eight trials. The cumulative paths taken give a clear impression of the plume's average mean concentration, direction, and shape as seen by the true mean average concentration plume overlaid at the bottom (b).

Discussion

Little is known about how organisms learn an odor plume's structure and gather information about the statistics of its fluctuating odor concentrations. Here, I have proposed that mammals may have a prototype of an odor plume that assists them with navigation. To test this hypothesis, odor stimuli were removed and replaced with an auditory cue that scaled in audio intensity with true odor concentration from an odor plume measured using PLIF. The results of this experiment suggested that even without olfactory stimuli, the behaviors elicited are identical to search in a real olfactory plume. Therefore, at least in humans, innate knowledge of the structure of an odor plume. As in a real plume, people modulated their sampling behavior while gathering information about the virtual odor in the form of stereo or non-stereo cues. Participants in the non-stereo experimental groups were able to locate the origin of the virtual odor just as accurately as individuals from the stereo group.

Navigating the Plume

Search strategies were captured differed among men, women and participants with different SOD. Women and participants who had lower SOD preferred the *transect* strategy. The *transect* strategy eventually lead to the discovery of the plume but at the cost of time. At the same time, the presence of the grid pattern in the virtual environment did not influence participants to walk in a transect. Both humans and rats use a *transect* strategy when exploring novel environments (Avni, Tzvaigrach, et al., 2008; Yaski, Portugali, et al., 2011). Because this strategy also manifests in other species, the *transect* method is probably a reliable strategy used to explore new spaces. This meticulous search strategy may be an egocentric strategy for exploring a space (MacFadden, Elias, et al., 2003). It does not require building of an allocentric cognitive map, but is an egocentric strategy (Wang & Spelke, 2000).

Men and individuals with higher SODs were more likely to use an *irregular* search strategy, combining a *spiraling* and *transect* strategy simultaneously without overlapping their previous path. This was a more complex strategy and may have required a better SOD. This is the first experiment to demonstrate that exploratory behavior in an open arena varies depending on people's SOD and sex.

Casting is a behavior that occurs whenever an odor is lost (Dusenbery, 1992; Svensson, Strandh, et al., 2014; Vickers, 2001). Participants increased casting behavior as they approached the origin of the plume, and those who casted more were better at estimating the location of the plume. However, studies of other animals reveal that casting should decrease as animals approach an odorant (Elliot, Elliot, et al., 1995; Vickers & Baker, 1994). This is the result that was also found in Chapter Three.

One explanation for the increase in casting is that the shape of the virtual plume was thin and long, rendering it easy for the participant to lose the odor if they approached it from any direction other than directly upwind from the origin. Unlike a naturalistic setting, there were no visual or mechanosensory cues, such as wind, which could help a participant decipher from which direction the odor was originating (Gomez-Marin, Duistermars, et al., 2010). Combining cues from multiple modalities, such as visual cues, wind strength, and wind direction, are important for odor navigation (Wasserman, Aptekar, et al., 2015). Without this extra information, plume *following* was challenging and the plume may have been easy to lose and required casting more often. In addition, the virtual plume which was measured in a turbulent environment. A study on cockroaches in laminar and turbulent odor plumes also showed that cockroaches casted under much more turbulent conditions (Willis, 2005). Eels also casted more the closer they get to the odorant (Carton & Montgomery, 2003).

It initially appeared as if individuals performed poorly on this task because the final distance to the plume's origin on average was 2.47 ± 1.32 m. On the contrary, participants performed quite well. A look at the time-averaged plume concentration from Figure 4.1c shows that the highest concentration of the odor was approximately 2 meters away from the origin of the plume. Thus, people were very accurate at finding the location with the most intense stimuli. Under naturalistic conditions, animals would have likely switched to other cues, such as visual cues, to find the odorant at close distances. Even animals such as nocturnal geckos use a combination of olfaction, audition, and vision to capture prey (Chou, Leong, et al., 1988). It is therefore interesting that navigating using information from an odor plume remains highly precise in this artificial task where no visual cues were given. This result may support a hypothesis introduced by Jacobs (2012) that olfaction originally evolved as a means for navigation. Olfactory neurons began to emerge before other sensory modalities like vision (Goldsmith, 1990). Therefore, having highly accurate spatial navigation abilities using only the olfactory system would have conferred an evolutionary advantage.

Stereo Olfaction

Stereo olfaction assists odor localization in diverse species, including humans, and impairing stereo olfaction results in poorer localization abilities (Borst & Heisenberg, 1982; Gaudry, Nagel, et al., 2012; Von Bekesy, 1963). Boie, Connor, et al. (2018) showed that when an individual samples while standing in one location, having two sensors sampling an environment increases the information gained about plume structure. Participants in the present experiment sometimes stood in a single place sampling multiple times before making a decision to move or turn. In this case, having two sensors revealed which direction had the higher concentration of odors. Participants in the crossed and stereo groups correctly turned towards the direction with highest stimuli while the mono group was not biased to turn in either direction. It was somewhat surprising that when some participants in the crossed condition were asked whether they noticed the reversed audio stimuli after the experiment, none were cognizant of the reversal yet overall they were making appropriate navigation choices by turning towards the side with greater stimuli. Overall, no difference in the final estimate to the plume's origin was found among the stereo, mono, and crossed experimental groups. Therefore, although stereo sensors provide instantaneous information about plume structure, temporal and spatial comparisons during active sampling were used to drive navigation choices. This result matched results from Chapter Three and from other animal species such as rats who can still navigate even if one nostril is occluded (Baker, Dickinson, et al., 2018; Khan, Sarangi, et al., 2011).

Individual Differences

Men outperformed women in this task regardless of experimental condition. Evolutionary history suggests that men were primarily the hunters in ancestral hominids and searched great distances for prey (Hawkes, 1996; Lovejoy, 1981). Women commonly gathered resources instead of hunting actively, generally not moving over as great of distances as their male counterparts (Gurven & Hill, 2009; Isaac, 1978). Men, therefore, required better spatial abilities to help them return to their communities (Cashdan, Marlowe, et al., 2012; Silverman, Choi, et al., 2000). This is not to say women have lower spatial cognitive abilities. Rather the two sexes have different spatial advantages, with men predicted to have better long range navigation skills than women who are thought to excel in short range navigation (Ecuyer-Dab & Robert, 2004). Therefore, men may have navigated better in the virtual environment because they were more adept at orienting without visual cues.

Although primary literature has not been published correlating the SOD scale and hunter-gatherers, in this study men had higher SOD than women. However, across all the different experimental condition groups, individuals with higher SOD performed better on the task. Therefore, having higher SOD does not only benefit men.

Sampling Behavior

In general, participants in all three conditions showed that they were sampling much more once they were in the plume *following* phase. They gathered more information about the virtual odor by increasing the number of samples per meter. A recent study by Boie, Connor, et al. (2018), using the same plume data as in this study suggested that maintaining a location in the virtual plume and sampling repeatedly is not as effective as moving elsewhere and sampling again to increase certainty about one's location within the odor plume. In this task, participants averaged seven samples in an unchanging or falling odor gradient before they recognized they were walking in the wrong direction and corrected their trajectory. This correction was in response to an average of 1% fall in odor concentration within the seven steps. The ability to detect even 1% difference in odor concentration and react accordingly has been shown in other studies of human odor perception (Zahn, DiSpirito, et al., 2001).

In this study, sampling frequency for all three experimental groups was between 2.8-3.4 Hz during the *search* phase. Once the plume was discovered, all groups decreased their sampling frequencies. However, if the plume was then *lost*, sampling frequency increased. This suggests that during active search sampling frequencies are high, but when making decisions on where to navigate, frequency decreases. Dogs also slow their sampling frequency when deciding which way to follow an odor (Thesen, Steen, et al., 1993). Contrary to the current study though, dogs then increased their sampling frequency after a decision had been made. However, dogs in the Thesen experiment followed an odor trail and could only travel left or right, thus did not need to make more decisions once the trail was found. Participants in the virtual task were given an open area to explore, with no visual or wind cues to help them locate the direction of the odor plume, thus participants were constantly exploring to gather information and make decisions regarding which way to travel. Thus, participants required more time to decide between samples on where to move next which was reflected in the slower sampling frequency.

This study also offers new insight on how people changed their sampling frequency in response to *losing* the virtual plume after it was initially found. Participants in the stereo group increased frequency to their baseline level if the virtual plume was *lost*. In the mono condition group, when the plume was *lost,* participants increased their sampling frequency above the levels used during the *search* phase. Participants in the mono group obtained less information per sample than participants in the stereo group. Once participants in the mono group were *lost* they sampled more quickly in order to maximize information gain from the surrounding area. Rats which have one nostril sewn shut cast more broadly if a trail is lost, indicating their behavioral strategy is also adapted to sample widely for more information (Khan, Sarangi, et al., 2011).

The crossed condition group searched at a lower sampling frequency than the stereo and mono groups during the *search* phase. Although they further decreased their sampling frequency during plume *following,* if they *lost* the odor plume, their sampling frequencies remained as slow as during *following*. It was unclear why this occurred. The number of men and women, as well as the SOD scores of participants in the crossed group matched those in the stereo and mono group, thus the result was not a result of sex or SOD differences. One possibility is that crossing sensors is very confusing (Catania, 2012; Martin, 1965). Participants in the crossed condition received detailed information about the odor plume but the right and left sensors were inverted. Although they were able to compensate for the difference, as seen by their correct turning behavior, it may have taken extra cognitive processing time to make decisions. This was reflected by having a slow sampling rate as they processed the information.

Finally, participants in the virtual task were unable to modulate their sampling behavior as they would do in real life, as seen in Chapter Three. They could only vary the frequency and number of samples because the length of the sample and intensity of the sample were fixed. However, the ability to modulate these two sampling properties played an important role for participants during non-stereo trials in Chapter Three. Wesson, Verhagen, et al. (2015) revealed that increasing sniffing frequency does not enhance information processing. Thus, by not allowing participants in the virtual task to vary sampling length or sample intensity, it forced them to learn about the odor plume in different ways. One such strategy may have been to predominately follow the odor plume edge by casting between the bulk of highly concentrated odors and the clean air outside of it (Finelli, 2000). This would explain why participants increased their rate of casting as they approached the plume's origin as well as why those who casted more were also more accurate at estimating the origin of the plume.

Implications

Overall, this experiment supports the hypothesis that humans may have a prototype of odor plume structure. They are capable of using information that matches the structure of a real odor plume, but presented in a different modality to navigate. The resulting behavior matches behavior seen during ordinary olfactory navigation. In fact this behavior is so robust that even when the information was presented as stereo, mono, or crossed cues, all three experimental condition groups performed equally well. People also modulated their sampling behavior, exactly as they would do in a real-world scenario. Therefore, people may use a generic template of an odor plume to make basic navigational decision. They then modify their sampling and movement behavior appropriately as they continue to gather other information about the plume structure from the environment.

Chapter 5
Conclusions

Studies of olfactory neuroscience in mammals has exponentially grown since the characterization of mammalian olfactory receptor genes by Buck and Axel in 1991. However, research on olfactory navigation has focused on invertebrates and birds. The goal of this dissertation was to study how mammals navigate through odor plumes by studying their movement patterns and sampling behaviors. The studies demonstrated how observations of mammalian behaviors can give insight to an odor plume's complex structure. Chapter Two described a naturalistic field experiment with dogs and revealed how odor dispersion increases under windy, hot, and dry conditions leading dogs to stray farther from a human's original scent trail while tracking the scent. Chapter Three investigated sampling behaviors people use during olfactory navigation, revealing that humans are also efficient olfactory navigators. Lastly, in Chapter Four, a virtual odor experiment provided detailed, quantitative data about the relationship between stimuli concentration, sampling behavior, and navigation decisions. In all three of the experiments, individual dogs and people displayed unique sampling patterns and search strategies as they followed odors through the environment. In the virtual task, men and individuals with high sense of direction (SOD) more accurately estimated the location of an odor plume's origin. Similar trends in men and those with high SOD were also found in the real world navigation task. Stereo olfaction or the lack thereof had no effect on participants' abilities to locate odorants, supporting the hypothesis that most animals may rely on a spatio-temporal strategy for olfactory navigation.

Navigation in Dispersing Odor Plumes

Odor plumes can spread over great distances outdoors when subject to meteorological conditions (Bursik, 2001; Crimaldi & Koseff, 2001; Drescher, Lobascio, et al., 1995). In Chapter Two, search and rescue (SAR) dogs could follow an odor trail hours after a person had walked through an area under varying weather conditions. Variables, such as wind, low humidity, and high temperatures resulted in rapid odor dispersion as discernible by the greater distances dogs followed odors from the person's original trail. In these dry, hot, windy conditions, odors appear to disperse quickly laterally. Thus, if the original odor trail was lost, the alternative was to find the edge of odor plume, which in these conditions could be quite far from the trail. Observing how animals navigate while following an odor can give coarse information about the general structure of the odor plume (Willis, 2005). This concept was supported by the results of Chapter Four which demonstrated that tracking the trajectories of 178 people following the virtual odor also revealed patterns that closely matched the true time-averaged odor plume structure (Figure 4.16).

The age of a scent affects how SAR dogs are able to trail is unknown and has not been formally tested. The time frame in which Chapter Two's dog study took place was relatively short, 3 hours at the most between when the trail was laid and when the dogs searched. Waiting 24 hours, days, or even weeks between laying the trail and having the dogs search could reveal different behaviors for odor trail following. In this study, the results showed that the current prevailing conditions had the greatest impact on where the dogs searched. This may not be the case for an older trail. During longer time frames, trails may be crossed by different people, resulting in what SAR call "cross contamination" (Lorenzo, Wan, et al., 2003; Oesterhelweg, Kröber, et al., 2008). The weather may also change dramatically during this time and the trail may be subject to large changes in temperature, humidity, and wind, or experience precipitation.

How long term meteorological changes may affect odor dispersion is currently unknown. Experiments on soil pesticides showed that pesticides sprayed on moist soil were carried by vapors out of the soil when temperatures rose; the concentration of pesticides in the air then increased 2-3 times (Harper, White, et al., 1976). If an odor trail is created during cold, humid conditions, ground moisture may retain odor molecules for a long time so long as no hot, dry days occurred between when the odor was deposited and when an animal following the odor encounters it. However, in the event that several hot days occur in the interim, odor molecules may have entirely dissipated from areas exposed to sunlight, but under shaded areas, like near vegetation or structures, odor molecules may remain trapped. Thus, the odor trail an animal, such as a dog, follows may be more akin to multiple point source odors linked together like a game of connect-the-dots. Future studies of long-scale temporal changes in odor trails will require both computational modeling and experimental studies to increase the understanding of how odors behave in these conditions.

Knowledge of the effects meteorological conditions have on odor trails was a useful finding for SAR. A critical time to search for a person who has gone missing may be in the morning as the sun is beginning to rise. Indeed, SAR teams report that mornings were often a time to set out and search for people. Dogs seemed more invigorated and "on the trail" at these times. Future studies of SAR success based on the age of trails and the effects of long term environmental conditions would be valuable to the SAR community.

Unfortunately, in this field experiment, the audio recordings of dogs' respiratory patterns was unusable. A future replication of this experiment would ideally find a way to gather these recordings to compare the sampling patterns of dogs to existing literature on other search dogs and mammals such as rats following trails.

The temperature effect in Chapter Two, parallels the results from Chapter Three's real world indoor olfactory navigation task. Higher temperatures in the room also resulted in worse performance identifying the correct diffuser with the anise seed oil. High temperatures lead to increased turbulence in the experimental room (Carr, Connor, et al., 1973; Drescher, Lobascio, et al., 1995) which in turn caused the odors carried by air to disperse faster. Temperature was difficult to control within the room. Future experiments would benefit with better temperature as well as humidity control. An ideal study would take place within a wind flume with controlled release of odorants where researchers could also be certain if the air flow is laminar.

Individual Differences Affect Odor Navigation

Men and people with high SOD may be better at olfactory navigation tasks. Although this was true for the virtual experiment in Chapter Four, this was not reflected in the real world odor experiment in Chapter Three. Men are better than women at using cardinal directions and distal cues for spatial navigation whereas women tend to use landmarks to cue them (Sandstrom, Kaufman, et al., 1998; Silverman, Choi, et al., 2000). Men may have had an advantage in the virtual environment due to the larger size of the virtual environment and lack of perceptible landmarks. In contrast, in the real world task, men and women were placed into a room with many visual features and also 12 visible locations from which the odorant was emanating. Thus, the male advantage may have been eliminated.

However, there was a small trend where both men and those with higher SOD still performed better on average in the real world trial. Therefore, a replication of the task with a higher sample size may reveal same results seen during the virtual task. In addition, more diffusers may reduce high variation seen for estimates of the diffuser's location. This, in turn, may also show that SOD and sex do have an effect on how well people perform when tracking an odor plume.

A future odor navigation study could also include a large array of containers where only one is a target location with essential oils volatizing in air. This setup would simulate the task set by the virtual experiment. There would need to be enough containers to render checking each one individually infeasible, yet allow people to walk around all of them searching for the odor. Here, it would be interesting to see if men and those with high SOD tend to estimate the location of the odor with higher accuracy. In addition, an analysis of their search strategies in this task could also be compared to the ones seen in the virtual experiment to examine if the same *spiral, random walk, transect, irregular* and *diagonal* search strategies appear naturally.

Stereo Olfaction and Navigation

Limiting access to stereo cues was not detrimental to navigation in either the virtual environment or in the real world experiment. In all experimental conditions where people either used stereo cues, mono, crossed, or had a single nostril obstructed, participants found the origin of the odor equally well. This was achieved by modulating their sampling behavior. Although stereo olfaction is used by people, it is not an important factor for accurate navigation. Dogs and humans use a spatio-temporal strategy to compare differences in odor concentration from different locations in an odor plume to navigate similar to other animals (Baker, Dickinson, et al., 2018; Gershow, Berck, et al., 2012; Gomez-Marin & Louis, 2012).

However, it is possible that no significant results were found in the real world experiment due to the low number of diffuser options—there were only 12 possible locations. Based on literature about flies and moles (Catania, 2012; Gomez-Marin, Duistermars, et al., 2010), a bias towards the left or right was expected when the opposite nostril was obstructed. This was not seen as participants advanced through the grid area. Instead, a future experiment which uses double or triple the number of diffusers, may reveal a left or right bias when one nostril is obstructed

It was also unclear from the real world navigation data if there were differences between sampling using either only the right of left nostril that stem from neurophysical differences between the right and left hemispheres of the brain for olfactory perception. Studies have shown that a right-side dominance for familiar odors is present in both people and birds (Savic & Berglund, 2000; Vallortigara & Andrew, 1994). Dahmani, Patel, et al. (2018) suggested a correlation between increased right hippocampal size performance, olfactory identification, and navigational learning. People in the real world task could have behaved differently when their right nostril was unobstructed because they would have been faster to recognize the anise seed oil odor and also the right hippocampus may have been activated more to assist in navigating to the diffuser. However, this is confounded by knowledge that a left or right lateralization bias for recognizing odors also coincides with handedness in individuals (Hummel, Mohammadian, et al., 1998). Some participants may have exhibited different sniffing patterns depending on their handedness.

An additional confound may have been nasal cycle. Human nasal passages have a cyclical pattern of unilateral partial obstruction due to differing blood flow in each passage resulting in higher airflow through one nostril over the other at any given time (Principato & Ozenberge, 1970). This has an effect on odor perception with people reporting higher odor intensity when sniffing using the more open nasal passage (Noam Sobel, Khan, et al., 1999). Airflow velocity for each nasal passage was not measured for each participant, nor was handedness recorded. Therefore, it is impossible to say if differences between the right and left in sniffing intensity and length was because of a partially obstructed nasal passage or due to lateralization differences. Future studies should include measurements of airflow velocity for each nostril prior to trials and hand preference.

Sampling an Odor Plume

Chapters Three and Four focused on the different sampling strategies that were used by people during olfactory navigation. The virtual environment findings complemented the results from real odor plume experiment. Participants in the virtual odors experiment could not vary their sampling behavior much beyond the sample number and frequency, nor did they have any wind cues to help then orient in the correct direction. In turn, the only way to gain more information about where they were in the odor plume was to consistently step outside of the plume and re-locate it by following the edge of the odor plume (Finelli, Pentcheff, et al., 1999). This resulted in a high number of casts because of the thin, elongated shape of the plume in the experiment.

However, in real life, people and other land vertebrates can use their diaphragms to control a sniff by modifying how long, frequently, and intensely they are sampling and odor (Kepecs, Uchida, et al., 2006). In the experiment in Chapter Three, instead of casting more, participants altered their sniffing behavior to gather information about the odor plume. They sniffed longer and at lower frequencies *searching* for the odor. This behavior matched how participants behaved when navigating through the difficult, thin, odor plume in the virtual environment. There, participants also slowed their sampling as they processed the information and tried to make decisions on where to move next.

In contrast, once participants were well within the odor plume in the real world experiment, approximately within 4 m away, the odor gradient and plume structure was known; sniffing length shortened and frequency increased. This same behavior of decreasing frequency when deciding where to navigate and then increasing frequency while *following* the odor to the source is exhibited by both dogs and rats when they find a trail (Khan, Sarangi, et al., 2011; Thesen, Steen, et al., 1993).

The virtual experiment used a planar image of an odor plume to represent the structure of an odor plume. In this study a triangular, planar area was used to simulate a sniff such that it would gather a similar amount of information during a single sniff. A person generally inspires around 200 cm^3 of air per sniff (D. G. Laing, 1982). However, sniffing pulls air predominately from beneath the nose and around the body in humans (Heist, Eisner, et al., 2003; King Se, Inthavong, et al., 2010) instead of air from in front of the face, as in the virtual study. Sampling behavior in a virtual environment could vastly be improved using 3D plume data to create a 3D sampling area true to human sniffs. Currently, 3D plumes are being imaged by the Crimaldi Laboratory at the University of Colorado at Boulder and could be used in a future virtual study.

Multiple olfactory sensor shapes and sizes have evolved in the animal kingdom. Mead, Wiley, et al. (2003) showed that stomatopods use antennae flicks to sample their environment while tracking an odor plume to a food reward. Reidenbach and Koehl (2011) also showed that crabs with short antennae flick their antennae at much faster frequencies than lobsters which have long antennae. Humans have a rather unique nose shape in comparison to other primates other nose morphologies typically point their nostrils out laterally (Smith, Rossie, et al., 2007). In humans, inhalation pulls air from under the nostrils and by the chest area (Heist, Eisner, et al., 2003; King Se, Inthavong, et al., 2010; Zhu, Kato, et al., 2005), which may not be conducive for long range detection. Future experiments in the virtual environment could test if sampling behaviors and movement patterns change depending on sensors shape and size. The program used in the experiment was modular, and thus can be modified to many different shapes, including linearly to simulate antenna. Perhaps arthropods with long antenna cast less because the wide distance between sensors helps them stay within the plume while arthropods with shorter antenna cast more frequently and move more laterally because it is easier to lose contact with the plume. The virtual environment would also allow for direct comparisons between the sampling behavior and movement patterns that result from using arthropod antennae or vertebrate nose structures in response to the same odor plume.

Robotics and Odor Plumes

Virtual environments offer a unique place to test odor navigation hypotheses, namely by engineers who model robot behavior before running physical tests (Chen & Huang, 2019). Recently, there have been increased efforts to use robots to navigate through odor plumes with the rationale that robots are safer to use when tracking dangerous odor plumes, such as in toxic conditions. Some of these robots use algorithms directly inspired by animal olfactory navigation (Consi, Atema, et al., 1994). Other robots use more complex mathematically-inspired probabilistic algorithms (Martinez, 2007). These algorithms provide new hypotheses on how animals learn about odors which can be tested empirically.

Many of the bio-inspired robots are micro air vehicles, such as drones, which use algorithms inspired by moth and bird swarming behavior (Jatmiko, Jovan, et al., 2011; Neumann, Bennetts, et al., 2015). The interest in aerial odor navigation may be due to the advances in technology of micro air vehicles which make them an appealing system to use. However, is it possible that there is also a bias toward using aerial vehicles due to the lack of research on terrestrial odor navigation? Although micro air vehicles are useful where there is ample room for flight, in tight spaces odor plume navigation may be delegated to terrestrial robots. Thus, it is important to fully understand odor dispersion and navigation closer to boundary layers or in confined spaces by terrestrial animals.

Final Thoughts

This dissertation has shown that mammals have flexible strategies for adapting their behavior to navigate through an odor plume, whether it is by changing their sampling behavior or the way they physically move through space to follow an odor. Chapter Two revealed how large mammals find and follow odor plumes by combining both taxis and sampling behavior outdoors. The dog experiment was one of the first to rigorously investigate how meteorological conditions impact odor navigation over long distances. In addition, it showed dogs have flexible sampling strategies to switch between air sampling and ground sampling to follow odors. This dissertation was also the first to investigate olfactory navigation in humans. Though people rarely think about using their sense of smell to navigate, people are more than capable of doing so. Humans also modulate their sampling behavior to optimize information about an odor plume' structure. Furthermore, the processes for doing so is evolutionary entrained and even without a real olfactory cue, people exhibit odor navigation behavior. In conclusion, the present dissertation offers insight to mammalian olfactory navigation and lays the foundation for future research on mammalian olfaction.

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Appendices

Appendix A. Linear mixed effects model predicting between trails area for average conditions during a trial.

	β	df	t	p
Intercept	10711.98	11.69	11.69	< 0.001*
Relative Humidity	-90.08	10.34	-3.40	< 0.01*
				cond. $r^2 = 0.50$

Appendix B. Coefficients for linear mixed effects model predicting dogs' distance to each meteorological waypoint.

	β	df	t	p
Intercept	12.90	124.16	9.29	< 0.001*
Relative Humidity	-0.10	284.56	-4.96	< 0.001*
Wind Speed	0.65	418.82	2.69	< 0.01*
				cond. $r^2 = 0.11$

Appendix C. Coefficients for mixed effects logistic regression predicting ground sampling from velocity.

	β	z	p
Intercept	-1.28	-3.11	< 0.01*
Velocity (m/s)	-0.25	-6.73	< 0.01*

Appendix D. Coefficients for a linear mixed effects model predicting ground sampling behavior from weather conditions.

	β	df	t	p
Intercept	0.76	5.23	12.42	< 0.01*
Wind Speed (m/s)	0.02	430.18	2.19	0.03*
				cond. $r^2 = 0.35$

Appendix E. Coefficients for a linear mixed effects model predicting velocity from distance from the person's trail for distances less than 40 m.

	β	df	t	p
Intercept	0.95	5.02	4.92	< 0.01*
Distance (m)	0.03	14982.00	13.67	< 0.001*
Distance^2	0.001	14981.33	-13.97	< 0.001*
				cond. $r^2 = 0.33$

Appendix F. Coefficients for a linear mixed effects model predicting velocity from distance from the person's trail for distances more than 40 m.

	β	df	t	p
Intercept	2.21	5.40	4.00	< 0.01*
Distance (m)	-0.02	143.86	-3.62	< 0.001*
				cond. $r^2 = 0.59$

Appendix G. Coefficients for linear mixed effects model predicting dogs' ground sampling behavior.

	β	df	t	p
Intercept	0.76	5.23	12.42	< 0.01*
Wind Speed	0.02	430.18	2.19	0.03*
				cond. $r^2 = 0.35$

Appendix H. Permutations of locations of correct diffuser and conditions per trial during the experiment. Participants who were given only three trials excluded trial 4.

	Diffuser Lane				Trial Condition			
Group	Trial 1	Trial 2	Trial 3	Trial 4	Trial 1	Trial 2	Trial 3	Trial 4
a	7	3	6	10	Stereo	Left Nostril	Right Nostril	Stereo
b	7	6	3	10	Stereo	Left Nostril	Right Nostril	Stereo
c	7	3	10	6	Stereo	Left Nostril	Right Nostril	Stereo
d	7	10	3	6	Stereo	Left Nostril	Right Nostril	Stereo
e	7	10	6	3	Stereo	Left Nostril	Right Nostril	Stereo
f	7	6	10	3	Stereo	Left Nostril	Right Nostril	Stereo

Appendix I. Coefficients for a LMM with correct lane for diffusers and experimental condition (stereo, right nostril, left nostril). Subject and trials crossed included as a random effect. Condition r^2 is reported.

	β	df	t	p
Intercept	2.05	134.04	4.75	< 0.001*
Lane 3	0.61	128.77	1.05	0.30
Lane 10	-0.85	127.65	-1.45	0.15
Left Nostril	-0.60	150.54	-0.99	0.32
Right Nostril	0.59	138.07	-1.05	0.30
Lane 3 x Left Nostril	-0.28	140.24	-0.33	0.74
Lane 10 x Left Nostril	1.21	137.33	1.45	0.15
Lane 3 x Right Nostril	-0.22	109.89	-0.29	0.77
Lane 10 x Right Nostril	0.86	115.93	1.09	0.28
				cond. $r^2 = 0.26$

Appendix J. Results from LMM predicting number of casts in response to distance and condition.

	β	df	t	p
Intercept	-1.23	1822.43	-2.55	0.01
Distance from Diffuser (m)	2.01	1743.96	9.27	< 0.001*
Distance from Diffuser^2	-0.20	1745.00	-8.98	< 0.001*
Left Nostril	-0.97	1770.17	-1.46	0.15
Right Nostril	-0.48	1767.98	-0.72	0.47
Distance x Left Nostril	0.28	1741.21	0.92	0.36
Distance x Right Nostril	0.11	1738.93	0.35	0.73
Distance^2 x Left Nostril	-0.03	1742.12	-0.82	0.41
Distance^2 x Right Nostril	-0.01	1739.85	-0.31	0.76
				cond. r^2 = 0.30

Appendix K. Results from LMM predicting cast number in response to distance.

	β	df	t	p
Intercept	0.67	426.42	1.66	0.10
Distance from Diffuser (m)	1.04	5719.16	6.99	< 0.001*
Distance from Diffuser^2	-0.13	5720.38	-8.29	< 0.001*
Left Nostril	0.30	5761.44	0.66	0.51
Right Nostril	0.43	5763.85	0.95	0.34
Distance x Left Nostril	-0.30	5718.43	-1.43	0.15
Distance x Right Nostril	-0.27	5716.65	-1.27	0.21
Distance^2 x Left Nostril	0.03	5719.39	1.42	0.16
Distance^2 x Right Nostril	0.03	5717.64	1.19	0.24
				cond. r^2 = 0.50

Appendix L. Results from LMM predicting sniff length (ms) in response to distance.

	β	df	t	p
Intercept	873.41	91.26	10.72	< 0.001*
Distance from Diffuser (m)	61.85	12082.47	2.74	< 0.01*
Distance from Diffuser^2	-9.31	12082.88	3.76	< 0.01*
Left Nostril	30.67	12115.06	0.44	0.66
Right Nostril	-162.77	12111.03	-2.35	0.02*
Distance x Left Nostril	3.85	12081.94	0.12	0.91
Distance x Right Nostril	59.33	12080.59	1.82	0.07
Distance^2 x Left Nostril	1.22	12082.16	0.34	0.73
Distance^2 x Right Nostril	-2.90	12080.68	-0.81	0.42
				cond. r^2 = 0.55

Appendix M. Results from LMM predicting sniff frequency (Hz) in response to distance.

	β	df	t	p
Intercept	1.16	347.08	19.06	< 0.001*
Distance from Diffuser (m)	-0.05	19735.99	-2.28	0.02*
Distance from Diffuser^2	0.01	19737.03	3.60	< 0.001*
Left Nostril	0.22	19531.54	3.02	< 0.01*
Right Nostril	0.19	19374.44	2.67	< 0.01*
Distance x Left Nostril	-0.03	19735.75	-0.94	0.35
Distance x Right Nostril	-0.04	19735.42	-1.23	0.22
Distance^2 x Left Nostril	0.001	19736.68	0.19	0.85
Distance^2 x Right Nostril	0.003	19735.34	0.73	0.46
				cond. $r^2 = 0.19$

Appendix N. Results from LMM predicting sniffing audio intensity (dB) in response to distance.

	β	df	t	p
Intercept	48.83	39.81	-35.69	< 0.001*
Distance from Diffuser (m)	-0.34	12086.60	-7.19	< 0.001*
Left Nostril	-0.74	11580.52	-2.02	0.04*
Right Nostril	0.33	11294.20	0.89	0.37
Distance x Left Nostril	0.23	12086.99	3.35	< 0.001*
Distance x Right Nostril	0.12	12083.85	1.75	0.08
				cond. $r^2 = 0.75$

Appendix O. Results from LMM predicting time with hand raised to signal odor perceived in response to distance.

	β	df	t	p
Intercept	2.11	2287.16	2.87	< 0.01*
Distance from Diffuser (m)	1.30	2885.08	3.76	< 0.01*
Distance from Diffuser^2	-0.19	2885.50	-4.98	< 0.001*
Left Nostril	1.22	2862.72	1.17	0.24
Right Nostril	0.03	2879.08	0.03	0.98
Distance x Left Nostril	-0.56	2894.99	-1.09	0.28
Distance x Right Nostril	0.11	2894.77	0.22	0.83
Distance^2 x Left Nostril	0.073	2896.47	1.26	0.21
Distance^2 x Right Nostril	-0.010	2895.09	-0.17	0.87
				cond. $r^2 = 0.21$

Appendix P. Results of a mixed effects logistic regression predicting hand raising which signaled the odor had been perceived. Coefficients are log odds.

	β	odds	z	p
Intercept	0.21	1.24	0.66	0.51
Distance from Diffuser (m)	-0.45	0.64	-51.21	< 0.01*
Left Nostril	0.32	1.37	5.40	< 0.01*
Right Nostril	0.32	1.38	5.32	< 0.01*
Male	1.14	3.12	1.14	0.04

Appendix Q. Multiple regression of sampling frequency regressed against distance and condition during the search phase.

	β	t_{20804}	p
Intercept	3.29	93.83	< 0.001*
Distance (m)	0.00	-1.95	0.05
Mono	-0.07	-1.42	0.15
Crossed	-0.36	-6.60	< 0.001*
Distance x Mono	0.01	1.75	0.08
Distance x Crossed	0.00	-0.84	0.40
		$F_{5,20804} = 85.96$, $p < 0.001$*	Adj. $r^2 = 0.02$

Appendix R. Multiple regression of sampling frequency regressed against distance and condition within 15 m of the source.

	β	t_{12966}	p
Intercept	2.01	53.43	< 0.001*
Distance (m)	0.06	11.76	< 0.001*
Mono	-0.16	-3.03	< 0.01*
Crossed	-0.35	-6.29	< 0.001*
Distance x Mono	0.02	2.65	< 0.01*
Distance x Crossed	0.01	0.83	0.41
		$F_{5,12966} = 141.9$, $p < 0.001$*	Adj. $r^2 = 0.05$

Appendix S. Multiple regression of sampling frequency regressed against distance and condition after losing the odor plume.

	β	t_{859}	p
Intercept	2.22	3.67	< 0.001*
Distance (m)	0.04	0.03	0.20
Mono	-0.47	-0.59	0.55
Crossed	2.17	2.89	< 0.01*
Distance x Mono	0.06	1.37	0.17
Distance x Crossed	-0.14	-3.68	< 0.001*
		$F_{5,859} = 33.51$, $p < 0.001$*	Adj. $r^2 = 0.16$

Appendix T. Multiple regression of sample number regressed against distance and condition during the search phase.

	β	t20804	p
Intercept	6.76	23.60	< 0.001*
Distance (m)	0.02	1.10	0.27
Mono	0.27	0.68	0.50
Crossed	1.84	-4.09	< 0.001*
Distance x Mono	0.04	1.51	0.13
Distance x Crossed	0.08	2.94	< 0.01*
		$F_{5,20804}$ = 11.57, p < 0.001*	adj-r^2 = 0.002

Appendix U. Multiple regression of sample number regressed against distance and condition within 15 m of the source.

	β	t_{12966}	p
Intercept	41.84	34.59	< 0.001*
Distance (m)	-2.09	-13.39	< 0.001*
Mono	-11.14	-6.61	< 0.001*
Crossed	-13.06	-7.41	< 0.001*
Distance x Mono	0.80	3.73	< 0.001*
Distance x Crossed	0.84	3.75	< 0.001*
		$F_{5,12966}$ = 81.32, p < 0.001*	adj-r^2 = 0.030

Appendix V. Multiple regression of sample number regressed against distance and condition after losing the odor plume.

	β	t_{859}	p
Intercept	4.54	2.56	0.01*
Distance (m)	0.01	0.08	0.94
Mono	-2.52	-1.08	0.28
Crossed	0.05	0.02	0.98
Distance x Mono	0.14	1.09	0.28
Distance x Crossed	-0.02	-0.22	0.83
		$F_{5,859}$ = 1.126, p = 0.34	adj-r^2 = 0.001