



Research report

Rats can track odors, other rats, and themselves: implications for the study of spatial behavior

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Abstract

In order to demonstrate that rats solve dead reckoning (path integration) tasks in which they return to a starting location using self-movement (idiothetic) cues, it is necessary to remove external (allothetic) cues. Odor cues, especially those generated by a rat on a single passage, are difficult to control and they can potentially serve as a cue to guide a homeward trip. Because it is presently unknown whether rats can track the cues that they themselves leave, as opposed to the odor trails left by other rats, we investigated this question in the present study. A tracking task was used in which rats: (1) followed a scented string from a refuge to obtain a food pellet located on a large circular table; (2) followed odors left on the table; (3) followed odors left by the passage of another rat; or (4) followed odors left by themselves. Groups of rats were presented with strings scented with either the rat's own odor (Group Own), a conspecific's odor (Group Other), or another scent, vanilla (Group Vanilla). After training, a series of discrimination tests were given to determine the nature of the stimulus that controls scent tracking. The results indicated that Own, Other, and Vanilla groups were equally proficient in discriminating and following their respective odors. The rats were also able to follow odor trails on the table surface as well as a trail left by the single passage of another rat or their own passage. This is the first study to demonstrate that rats can discriminate between conspecific odors and their own odor left during a single passage. The results are discussed in relation to their implications for experimental methodology and olfactory contributions to spatial navigation in general and dead reckoning in particular. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Scent tracking; Olfactory discrimination; Self-conspecific odor cues; Spatial navigation

1. Introduction

Rats possess a remarkable olfactory system that they can use for solving a variety of laboratory-based olfactory discriminations. They can detect differences in urine odor between genetically identical mice maintained on different diets [20], and respond differentially to conspecific odors of reward and nonreward [3]. Many researchers are aware of the potential use of olfactory cues as an aid in solving spatial problems [14,15]. When rats are trained in a simple T-maze, they are able to use conspecific odor cues to control re-

sponding [16], provided that the conspecific has made a number of passages [8].

Although it is recognized that rats usually do not use odor cues in solving spatial tasks if they are able to use visual cues [10,11,14,18], the extent to which they will use olfactory cues when visual cues are unavailable or irrelevant is not known. In particular, it is not known whether they can discriminate their own trails from those left by other rats. For example, such an ability would be useful to a rat foraging in novel terrain in darkness, because it would be able to return to its starting location by following the trail that it left on its outward trip. In laboratory tasks directed toward studying dead reckoning, rats are tested under very similar conditions [1,7,14,26,27]. They are typically required to return to a starting point by integrating

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self-movement cues generated on the outward trip. In such studies, olfactory cues are difficult to control, especially those olfactory cues left by the rat itself on its outward trip. At present, however, it is not known whether a rat can distinguish its own odor from that of a conspecific, or whether it can follow the odor trail that it leaves on a single passage. This was investigated in the present study.

The rats were trained to track conventional odors, the odors of an identified conspecific, or self-generated odors to solve a spatial task. Rats were trained to track strings scented with vanilla [25], conspecific-generated odors, or self-generated odors in order to reach a food pellet. Subsequent tests determined whether the rats could discriminate among the odors in order to track a target odor, whether the animals could track a trail left on the table surface, and whether they could track multiple versus single passages of a conspecific or themselves.

2. Materials and methods

2.1. Animals

Twelve adult female Long–Evans rats (University of Lethbridge vivarium), weighing 250–300 g, were housed in groups of four in wire mesh cages. The colony room was maintained at 20–21 °C and a 12/12 h light/dark cycle.

2.2. Feeding

At the beginning of the experiment, feeding was restricted to maintain the animals at 85% of their free feeding weight. Large food pellets (750 mg Bio-Serv, PO Box 450, Frenchtown, NJ) were used as reward during testing. Rats reliably carry these pellets to a refuge for eating [24]. After testing each day, the rats were fed with LabDiet Laboratory Rodent Pellets in their home cage to supplement the food obtained on the table.

2.3. Apparatus

The open field was a 204 cm diameter circular wooden table painted white. The table was elevated 75 cm above the floor. Eight 11.5 cm diameter holes were arrayed around the perimeter of the table [27]. The table was constructed such that a cage can be affixed beneath any of the holes from which the rat can exit. The apparatus was located in a large room that could be made completely dark. A camera equipped to record under both light and dark conditions (using infrared wavelengths) was positioned perpendicular to the apparatus. The experimenter used an infrared spotter to monitor the rat's behavior during testing.

The strings used in this study were 100% cotton butcher twine approximately 2 mm in diameter [25]. Overhand knots were tied at each end of the string so that thumbtacks could hold the string in position on the table. Vanilla odor was placed on strings by dipping the string in pure vanilla extract and then the excess fluid was wiped off. A string was scented with rat odor by gently rubbing the string on a rat's body and tail for 15–30 s. Neutral strings were placed on the table without any odors added to them. Each string was kept in a different jar and handled with a new pair of rubber gloves each time, minimizing odor contamination between strings. In addition, the strings were frequently replaced.

2.4. Procedure

Prior to training and testing, rats were habituated to the table for 6 days in which each day the rat could leave the home base and retrieve five food pellets located randomly around the table. After habituation, rats were randomly assigned to one of the three groups. Each group was assigned a different odor to track. The Own group was required to track a string scented with their own odor. The Other group was required to track a string scented with another rat's odor. The Vanilla group was required to track a string scented with vanilla. A trial was counted as correct if the rat left the home base, followed the target string to the end, and obtained the food pellet (see Fig. 1a). If the rat followed one of the other strings present on the table, the trial was recorded as an error and the rat was allowed to explore the table until it obtained the food pellet. Rats were given three trials per day across all training and testing conditions. During training and testing, string locations were changed randomly from trial to trial and across days, thereby eliminating positional cues and making distal spatial cues irrelevant for task performance (see Fig. 1b). The table was rotated between rats and wiped down with Windex cleaner every day after testing.

2.5. String tracking

2.5.1. Habituation and training

Throughout training, the string to be tracked plus two neutral strings were placed on the table such that they radiated from the rat's home base. Training proceeded in three phases. The first phase (10 days) required rats to track a 50 cm string under light conditions. The second phase (5 days) extended the length of the strings to 100 cm. The third and final phase (8 days) of training required each group to track a 100 cm string under infrared light, a wavelength in which rats cannot see [4]. The table was rotated between subjects and cleaned with Windex at the end of each testing day.

2.5.2. Two-odor string tracking

The first phase of discrimination testing examined the rat's ability to discriminate between two-scented strings and an unscented string. Each group was exposed to a set of strings three times a day under dark conditions. A set of strings included: the string that the rat was trained to track, a string that one of the other groups was trained to track, and a neutral string. For example,

a rat trained to track vanilla would be exposed to: (1) vanilla scented string, other rat scented string, and a neutral string; (2) vanilla scented string, own scented string, and a neutral string. This phase of discrimination testing required 2 days to pair each group's training scented string with the other two group's training scented strings. Rats received a total of 6 days on the two-scented strings discrimination test resulting in 3

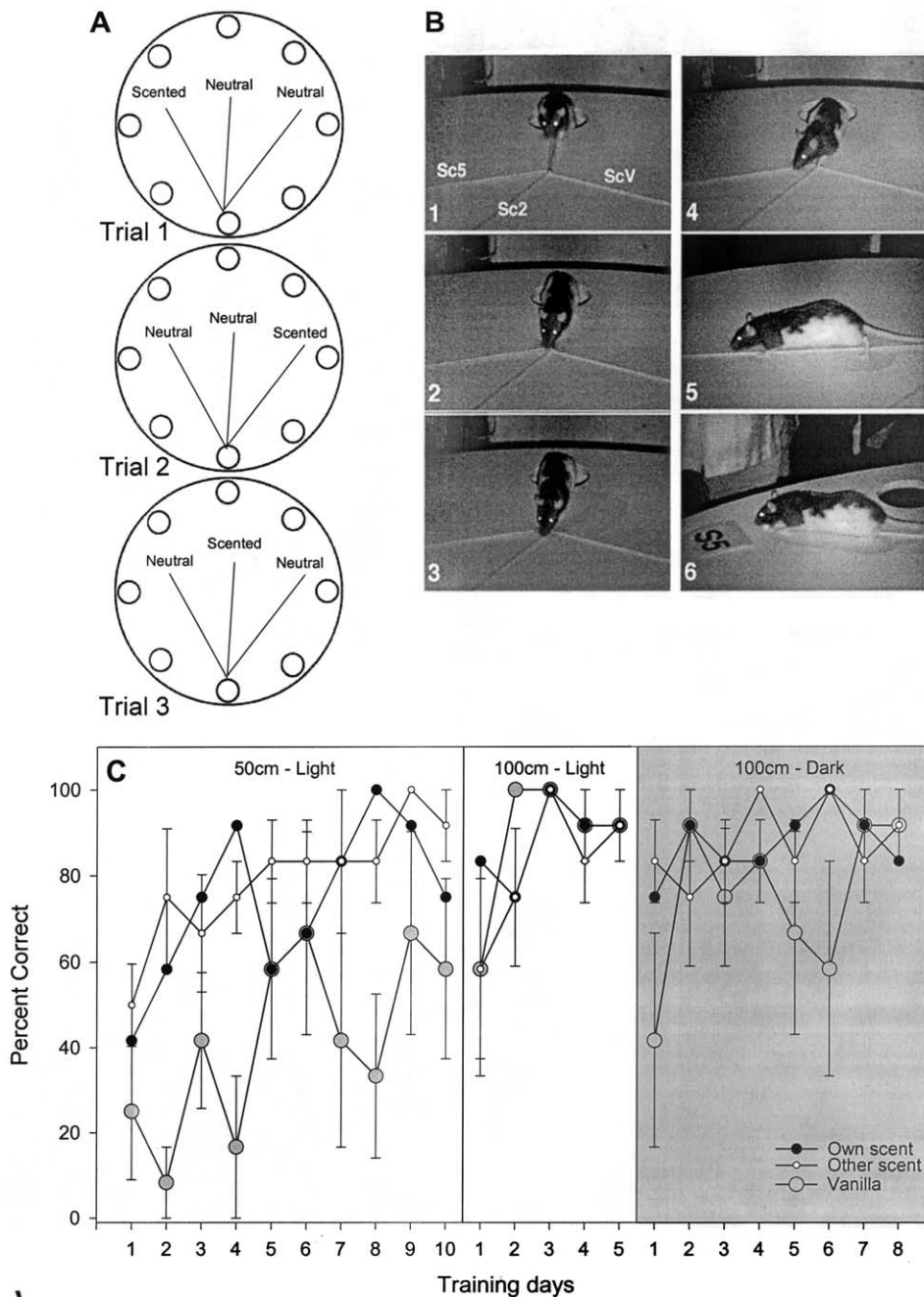


Fig. 1. Panel A presents a schematic of the string placement across a typical training session. Each of the strings radiated from the home base, and the portion of the string farthest from the home base is shifted between trials. Panel B is a series of photographs illustrating the behavior of a rat as it exits from the home base, chooses a string, follows the chosen string, and subsequently retrieves the food. Panel C presents the average percent correct for Own (black circles), Other (white circles), and Vanilla (gray circles) groups across the three training phases. Error bars reflect the standard error associated with each group's performance on each training day.

days per testing pair. The table was rotated between subjects and cleaned with Windex at the end of each testing day.

2.5.3. Three-odor string tracking

The second phase of discrimination testing examined the rat's ability to discriminate among the three-scented strings. Each group was exposed to their training string and the other two groups' training strings. Rats were presented with the three-scented strings three times per day for 8 days under dark conditions. The table was rotated between subjects and cleaned with Windex at the end of each testing day.

2.6. Table tracking

The next stage of testing examined the importance of the string in tracking odors by placing the odor cues directly on the table. Each rat's string that was associated with reinforcement during training and testing was lightly rubbed on the table in a path from the home base to a location 100 cm away from the home base. Rats were tested under both light and dark conditions with probe trials in which the food was absent. The table was rotated between subjects and cleaned with Windex if the addition of an odor trail would overlap any of the previously laid odor trails at the end of each testing day.

2.7. True tracking

The final phase of testing assessed the rat's ability to track odor trails left by another rat or by themselves. Rats have a natural tendency to follow walls in an open field. This feature of rat behavior was used to generate specific paths on the table for each group of rats to follow. Rats trained to track their own odors were permitted to follow the 200 cm by 30 cm plywood barrier out and back, thereby depositing an odor trail on the table in a controlled path. After the animals followed the barrier for the required number of times, it was removed. The intensity of the odor trail was manipulated by varying the number of times that the rat followed the barrier. Rats were initially trained to track the path left by three trips across the table. Subsequently, rats were trained to track a path left by one trip across the table. The barrier connected to a hole on the opposite side of the table created a $\pm 22.5^\circ$ (+ indicating right, – indicating left side of table with respect to home base) angle with a line that would connect two holes opposite one another. Rotating the table 135° clockwise (the hole that was linked to the home base by the barrier assumed the location of the home base) would require a positional response in a

different direction than the one produced while generating the scent trail. Arranging a series of trials in which the barrier is located in different directions and the table is either rotated or not controls the use of positional cues.

Rats trained to track vanilla were required to track a rat that had its feet dipped into vanilla and then allowed to follow the barrier. Other rats were trained to track an odor trail left by a target rat or themselves. The table was rotated between subjects and cleaned with Windex if the addition of an odor trail would overlap any of the previously laid odor trails at the end of each testing day.

3. Results

3.1. String tracking

3.1.1. Training

Fig. 1c presents group mean percent correct across each phase of training. The analysis of variance (ANOVA) conducted on mean percent correct for the first phase of training, 50 cm strings under light conditions, revealed a significant effect of day [$F(9,81) = 4.138$, $P < 0.05$], while group main effect and Group by Day interaction were not significant. The ANOVA conducted on mean percent correct for the second phase of training, 100 cm strings under light conditions, revealed a significant effect of day [$F(4,36) = 3.509$, $P < 0.05$], while group main effect and Group by Day interaction were not significant. The ANOVA conducted on mean percent correct for the third phase of training, 100 cm strings under dark conditions, failed to reveal a significant main effect of group or day and Group by Day interaction. Post hoc analysis of the days effect revealed a significant linear trend [$F(1,9) = 20.626$, $P < 0.05$] across the first phase of training. While a linear trend across days was not significant for the second phase of training, a significant quadratic trend [$F(1,9) = 9.836$, $P < 0.05$] in days was observed. The failure to find a significant effect of day across the third phase of training precluded a trend analysis.

3.1.2. Two-odor string tracking

The left panel of Fig. 2 presents group mean percent correct on the two-odor discrimination problems. One should recall that rats had to discriminate among three strings: the string presented during training, a string scented with an odor used during training by one of the other groups, and a neutral string. During testing, none of the rats tracked the neutral string. The Vanilla group tracked vanilla at a level significantly different from chance only when another rat's odor was present [$t(3) = 3.904$, $P < 0.05$]. The Own group significantly

Two and Three Odor Discrimination Probes

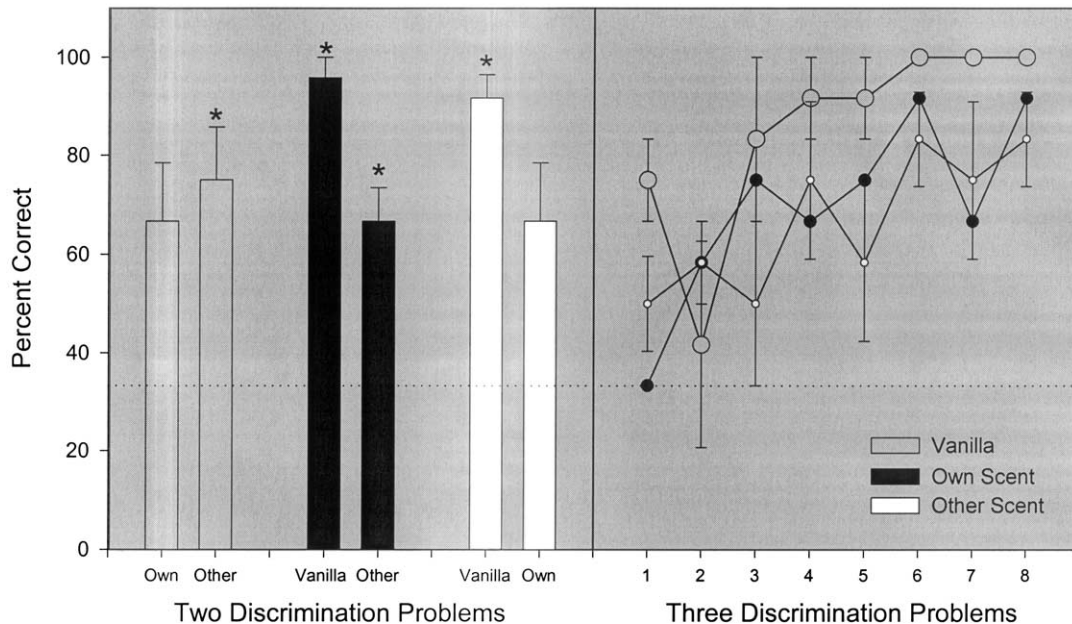


Fig. 2. The left panel presents Vanilla (gray bars), Own (black bars), and Other (white bars) group mean percent correct (i.e. correctly choosing the string presented during training) on two-odor discrimination problems. The labels on the x -axis of the left panel indicate the scented string that was paired with each group's training string. For example, the Vanilla group chose the vanilla string significantly more than chance when it was presented with a string that was scented with another rat's odor but not when presented with a string scented with the rat's own odor. The right panel presents mean percent correct for each group during acquisition of the three-odor discrimination problems. The dotted lines reflect chance performance for the two- and three-odor discrimination problems. Single sample t -tests were used to evaluate two-odor discrimination performance relative to chance. Asterisks indicate conditions in which group performance was significantly different from chance, or different from 33.3%. Error bars reflect the standard error associated with each group's performance on each testing day.

discriminated between their own odors when another rat's odor [$t(3) = 15.077$, $P < 0.05$] or vanilla odor [$t(3) = 4.949$, $P < 0.05$] was present. The Other group demonstrated significantly differential responding only when vanilla was the other odor [$t(3) = 12.191$, $P < 0.05$].

3.1.3. Three-odor string tracking

Each group's mean percent correct on the three-odor discrimination test is presented in the right panel of Fig. 2. Upon transfer to the three-odor discrimination test, the Vanilla group was responding significantly different from chance, while both Own and Conspecific groups were responding at chance. By the last day of testing, all groups were responding significantly different from chance [$t(11) = 13.412$, $P < 0.05$, collapsed across groups]. An ANOVA conducted on daily mean percent correct choices revealed a significant effect of day [$F(7,63) = 3.731$, $P < 0.05$], while the main effect of group and Group by Day interaction was not significant. Post hoc analysis of the days effect revealed a significant linear trend [$F(1,9) = 18.845$, $P < 0.05$] across the three-odor discrimination testing sessions.

3.2. Table tracking

When the string component was removed from the tracking task, all rats tracked the odor trail (left by rubbing the string on the table) under light and dark conditions without any errors. Tracking was also observed on probe trials when the food pellet at the end of the odor trail was absent.

3.3. True tracking

Fig. 3 presents the data from the final phase of testing in which rats followed paths generated by a rat's movement across the table. The left panel of Fig. 3 presents the mean percent correct for each group tracking, a trail left by three trips of a conspecific across the table. The ANOVA failed to result in a significant effect of groups, days, or Group by Days interaction. This is in contrast to the tracking elicited by one trip of the conspecific across the table (Fig. 3, right panel). The ANOVA based on the one trip tracking revealed a significant effect of groups [$F(2,9) = 7.681$, $P < 0.05$], day [$F(6,54) = 14.104$, $P < 0.05$], and Group by Day interaction [$F(12,54) = 7.221$, $P < 0.05$]. Rats never per-

formed significantly different from chance when tracking three-odor trails. By the last day of training with one odor trail, rats were following the odor trail significantly different from chance [$t(11) = 7.040$, $P < 0.05$, collapsed across groups].

4. Discussion

The behavioral observations and data analyses support the hypothesis that rats track olfactory cues while navigating to a location in the environment. Rats can be trained to track either vanilla, self-generated odor cues, or conspecific-generated odor cues. Subsequent discrimination tests demonstrated that animals use specific olfactory cues to locate food in the environment. The rat's ability to track these odors is not dependent on the availability of visual cues or using strings as a substrate for the odor trails. The ability to self-track is not only a remarkable discrimination ability but could also be a potential problem when rats are trained to solve spatial problems.

Olfactory cues can be used in two ways to locate food in an environment. First, olfactory cues can be used to mark the location of the food. Work by Lavenex and Schenck [10,11] has demonstrated that learning the location of a food source is enhanced when the food source is associated with a local olfactory cue

(i.e. almond extract). Other work has demonstrated that rats prefer to use distal visual cues relative to local olfactory cues when foraging for food [14]. Second, olfactory cues can serve as a stimulus that the rats track as they move through an environment. Our study is the first to demonstrate that rats respond differentially to a set of olfactory trails deposited by the single passage of a rat based on food-odor associations when place information is absent or made irrelevant.

There are two important implications of the present study, one is methodological and the other theoretical. From a methodological perspective, odor-mediated navigation has been difficult to observe [18] or only observed under restricted experimental conditions [12,14,8]. Our results demonstrate that the rats can use self-generated, conspecific-generated, or other odors to solve spatial problems under a range of experimental conditions. These results were observed under light and dark conditions, immediately after the table was cleaned or after several rats were tested on the table. This finding that rats can detect and respond to their own odor opens the possibility that the animals might use self-odor cues to solve many spatial problems that might be thought to be mediated by visual cues. For example, in maze tests in which animals must alternate between arms of the maze [5] or sequentially make a number of choices [19], the ability to detect whether the choice has been previously made could provide an

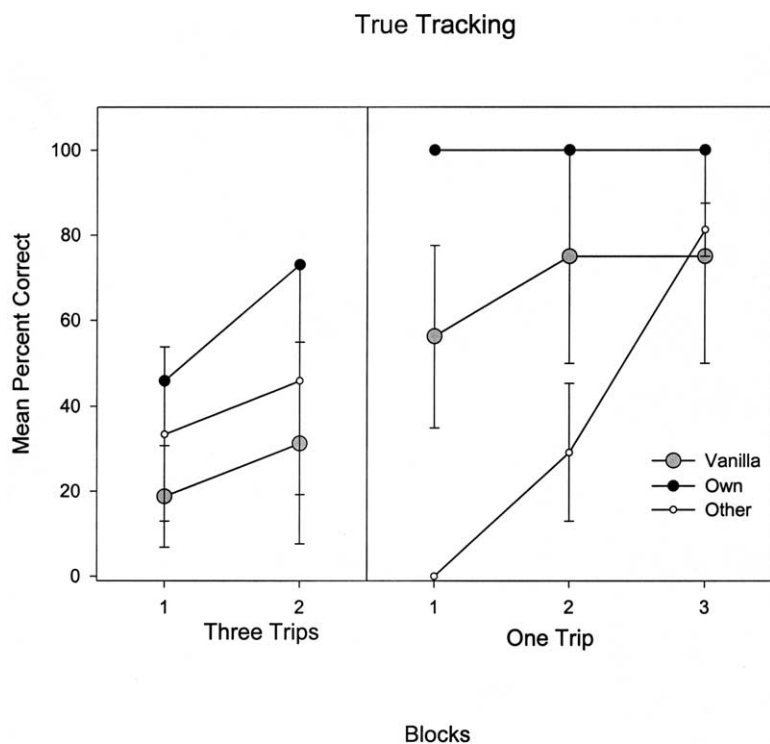


Fig. 3. The left panel presents mean percent correct for Vanilla (gray circles), Own (black circles), and Other (white circles) groups on the three-trip true tracking during the first two blocks of 3 days. The right panel presents group mean percent correct on one trip true tracking during the three blocks of the days. Error bars reflect the standard error associated with each group's performance on each testing day.

essential cue for the task's solution. In typical studies of deductive reckoning, an animal has to return to a location from which it started an excursion, and at this point, olfaction could provide an important cue indicating the return route if the animal were able to detect its outward trail. For example, odor-mediated navigation may confound explanations of behavior observed in deductive reckoning experiments that fail to control for self-generated, conspecific-generated, or other odor cues (i.e. food trails). This is especially important when examining the effects of brain damage on deductive reckoning [1,13].

From a theoretical perspective, the finding that rats are adept at following and discriminating odor trails provides a necessary link between olfaction and spatial behavior that may be essential to understanding possible spatial functions of the hippocampal formation, one of the targets of olfactory pathways. The present results open the possibility of investigating not only the ability of a rat to follow an odor trail but also higher-order neural processes that allow a rat to distinguish between kinds of odor trails and the significance of these trails. Potential targets of future investigations include the hippocampus and orbital frontal cortex. Anatomical studies have linked the hippocampal formation to the olfactory system [2,9,21], and electrophysiological and behavioral data support a role for the dentate gyrus in sniffing behavior as well as detecting species-relevant odors such as those left by a potential predator [23]. Whereas a central thrust of behavioral investigations have been directed toward its role in spatial behavior guided by visual cues [17,22], the results of the present investigation raise the possibility that it derives some of its spatial functions from olfactory-related spatial behavior. It is also possible that the orbital frontal cortex may play a role in some higher-order aspects of odor tracking. A number of lines of investigation have demonstrated that olfactory discriminations and conditional olfactory discriminations are impaired subsequent to orbital frontal lesions, while intensity judgments are spared [6].

In conclusion, the novel and important finding of the present paper is that not only can rats follow an odor trail but they can also follow the trail left by the passage of an identified conspecific or left by themselves. While this is a remarkable discriminative ability, the tasks used to illustrate the behavior provide a new set of methods for investigating higher order cognitive functions related to olfactory information. Finally, the results confirm the often-expressed suspicion that olfactory cues are important for animal navigation; therefore, experimental control is necessary in certain testing situations.

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