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Research report

Is it safe? Voles in an unfamiliar dark open-field divert from optimal security by abandoning a familiar shelter and not visiting a central start point

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ABSTRACT

Open-field behavior is a common tool in studying exploration and navigation, as well as emotions and motivations. However, it has been suggested that this behavior might be parsimoniously interpreted as directed to optimize security, with no need to interpret the animal's mental state. This latter view was challenged here by providing voles with presumably sense of optimal security. For this, voles were introduced into a dark open-field inside a familiar shelter in which they previously lived in their home cage. Voles then emerged either to locomote only in the vicinity of the shelter, or to travel further out to explore the entire arena and only later to return to the shelter. While their staying near the shelter confirms the notion of optimizing security, their traveling further out along the perimeter negates this notion. This divergence of behavior under the same security conditions illustrates that open-field behavior, which is a multi-faceted and dynamic process, is also affected by an emotional component. That is, safety is a subjective emotional state dictated by various inputs and, therefore, the resulting dynamic behavior, which is the ultimate output of the central nervous system, may vary beyond the possibility of being parsimoniously interpreted by only one factor. In a similar vein, we show that the impact of the start point on the paths of locomotion is not an intrinsic property of that point, but depends on its physical location.

1. Introduction

The open field is the most widely used test in animal psychology [33], and has been applied to the study of spontaneous activity in various species (for example, [8,16,30,36,40]). In this test an animal (usually a rodent) is introduced into a plain, either illuminated [21] or dark [3] arena, and observed for periods ranging from acute exposure of a few minutes [17] to repeated exposures of several hours [31]. Open-field behavior in rodents is commonly regarded as a fundamental index of general behavior, and is a predictive of locomotor scores in other novel environments [33]. It has long been assumed that the open-field test also reveals the emotional-motivational state of the tested animal [1,7,9,23]. Indeed, it was argued that the test evaluates emotional reaction more than exploratory motivation, thus contributing to the fallacy of taking general motor patterns as the sole expression of any specific motivation [26]. However, in contrast to traditional theories that invoke motivational explanations, it was also suggested that open-field behavior is merely an attempt to optimize security [35]. Moreover, it was suggested that the parsimony of this optimization theory is that "*rats' behavior can be fully accounted for without speculating about their mental state*" [35]. One target of the present study was to challenge the notion of optimizing security by providing social voles (*Microtus socialis*) with a familiar shelter in a relatively safe environment, and testing whether they would still be motivated to explore and travel in the environment.

The open-field test has been extensively used in studying exploration and navigation [2,3,16,25,34,39,40], despite being criticized as "*a poor and explicitly aversive environment with excess light and open spaces that does not elicit exploratory motivation*" [21]. Exploration in an open field may be regarded as a set of round trips that start and end at a home base, the location where rodents stay for extended periods and visit most frequently [18]. It was illustrated that in establishing the location of their home base, rodents mainly rely on visual cues, and they establish it near salient cues [12,24,38]. Nevertheless, it was noted that the start point, at which the animal is introduced into the test environment affects the organization of exploration, as manifested in repeated visits to that point [28,38]. In exploration and navigation, paths of progression are generally attached to landmarks [4,5,10,11,14,19,41]. More so, in the lack of physical landmarks, animals may deposit scent marking, or even move objects to landmark their way [29]. Since rats had accurately

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returned to the point of entry at the perimeter both when it lacked any salient cue, and when such a cue was located in another location, it was claimed that the importance of the point of entry is even superior to that of environmental cues [28]. In the present study, voles were confronted with a conflict between a familiar shelter at the start point in the center of the open field, and the arena walls. The shelter and the walls were the only salient environmental cue, and we could therefore discriminate between whether the voles favored the sheltered familiar central start point or the arena walls that surrounded the open field. Two questions were thus posed in the present study: (i) with the provision of presumed optimal security, would it be possible to parsimoniously explain the behavior of the voles as solely directed to optimize security; and (ii) what would be the impact of a central start point, located far from the arena walls, on the paths of progression?

2. Methods

2.1. Animals

Social (Guenther's) voles (*Microtus socialis guentheri*) are burrow-dwelling rodents with an elongated trunk (11 cm long, plus a 2 cm tail; weight 37–50 g), short legs, small eyes, and short external ears. In summer they are mainly nocturnal, whereas in the winter they are active around the clock. High year-round fecundity and early maturation result in large vole populations that are heavily predated upon by owls and other predators. Accordingly, voles minimize their above-ground activity by not moving far from their burrow openings, and when alarmed they dash back into the burrows along well-worn routes [27]. Voles were selected for the present study in light of the above properties, assuming that they would be sensitive to the availability of a familiar shelter. Sixteen captive-bred male voles were kept in groups of 4–7 in metal cages (64 cm × 121 cm × 43 cm) with a transparent glass jar inside that they used for shelter. Before testing, vole cages were placed for 3 weeks in an air-conditioned room (24 °C) with 12/12 h inverted light/dark cycle (lights on at 21:30). Voles were daily fed *ad-libitum* with standard rodent pellets, sunflower seeds and fresh vegetables.

2.2. Apparatus

Each vole was tested once in a dark open field. This was a 200 cm × 200 cm arena with PVC white floor enclosed by 50 cm-high opaque Plexiglas walls, placed in a light-proofed and temperature-controlled room (21 °C ± 1). The arena was illuminated by an infra-red light source (Tracksys, IR LED Illuminator; UK) with 830 nm filters that emits light not visible to rodents. All light sources in the room (e.g. indicator lights on the video, camera and air-conditioner) were sealed with opaque tape, maintaining the room in total darkness. A video camera (Ikegami B/W ICD-47E) was placed 2.5 m above the center of the arena, providing a top view that was recorded onto a VCR (JVC HR-J737).

2.3. Procedure

A vole was randomly selected and removed from the cage in the glass jar with its lid closed. It was then gently placed in the arena center in an orientation and location that were preserved over the trials. We then alternated between the two following procedures:

2.3.1. Tests with access to the familiar shelter (jar)

The lid of the glass jar was gently removed and the vole was able to spontaneously exit the jar into the dark arena and then re-enter the jar as it chose. Since the jar had been in the vole's cage for 3 weeks, it bore the odors of the cage and voles, and was therefore considered by us as a familiar shelter.

2.3.2. Tests without access to the familiar shelter (jar)

The lid of the jar was quietly removed and the vole was gently released into the center of the dark arena, at the same point and orientation of the previous test. The jar was then removed so that the voles under this procedure did not have further access to it.

Each vole was tested only once for 30 min. After each session, the vole was returned to its home cage and the arena was cloth-wiped with detergent. Testing took place during the dark phase of vole activity.

2.4. Data acquisition and analysis

Videotapes were analyzed by means of 'Ethovision' software (by Noldus Information Technologies, NL), which tracks the progression of the vole in the arena, providing five times per second the time and the location of the center of the vole image against the background of the brighter arena floor. For video analysis the arena was divided into five concentric 'zones', with the central zone (40 cm × 40 cm) representing the location of the shelter or the start location in tests without shelter. The other concentric zones (20 cm wide each) represent a gradient of diminished proximity to the center, with the fifth being a perimeter zone which comprised traveling along the arena walls and remaining in their vicinity (Fig. 1). Data from Ethovision were then exported to 'Microsoft Excel' program for further analyses of the following parameters: Total traveled distance (m); time spent in zone (s); number of stops over 1 s each in a zone; average travel velocity in a zone.

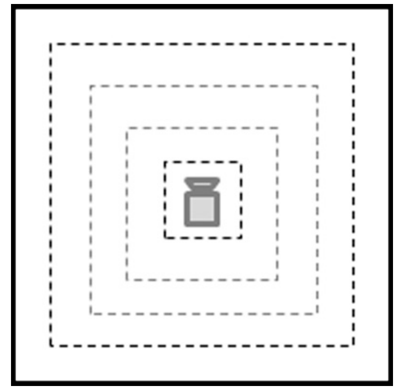

Fig. 1. The test arena (open field). Dashed lines represent the zones for which activity was assessed.

2.5. Statistics

Data of the test groups were compared by means of either student *t*-test for independent sample, assuming unequal variance, or by two-way ANOVA with repeated measures. Alpha level was set to 0.05.

3. Results

3.1. Access to a familiar shelter affects the spatial distribution but not the level of activity

Voles tested with or without access to a familiar shelter did not differ in the total distance they traveled during the 30 min of testing (mean ± SEM, 157 ± 41 m and 203 ± 34 m, respectively; $t_{15} = 0.89$; $p = 0.39$). Nevertheless, these levels of activity substantially varied in their spatiotemporal organization, as follows. Whereas voles that were tested without a shelter spent most of the time at the perimeter of the arena, voles that were tested with a familiar shelter spent more time in the shelter zone. Indeed, a two-way ANOVA comparison revealed no difference in the overall time spent in all zones (between-group effect; $F_{1,15} = 1$, $p = 0.24$), but did reveal a significant effect of zone location (within-group effect; $F_{1,15} = 70$, $p < 0.0001$). The interaction between zone location and test group was also significant ($F_{10,96} = 49$; $p < 0.0001$), indicating that the time spent in the various zones differed between the groups tested with and without shelter. Tukey HSD test revealed that with a shelter, time in the shelter zone was significantly longer than in any other zone, including the perimeter. Time spent in the perimeter zone was second longest, also being significantly longer than that spent in the mid zones. In testing without a shelter, voles mostly remained at the arena perimeter, hurriedly crossing the mid and central zones and spending there minimal, if any, time.

In addition to the spatial distribution of activity, the two groups also differed in the temporal distribution of their activity. The time course of remaining at the perimeter and time spent in the shelter zones in voles tested with access to a familiar shelter underwent a reciprocal change. During the first 5 min, these voles spent significantly more time at the perimeter than in the shelter zone. Then, however, perimeter time declined and shelter time increased, so that on average, voles spent equal time at the shelter and perimeter by 10 min, and by 15 min they spent most of the time in the shel-