Quantifying exploratory behavior in mice: the vertical dimension

Yair Wexler[1], Ilan Golani[2], Liad Shekel[1] and Yoav Benjamini[1]

[1]Department of Statistics and Operations Research, Tel Aviv University, Tel Aviv, Israel. [1]yairwex@gmail.com.
[2]Department of Zoology, Tel Aviv University, Tel Aviv, Israel.

Introduction

Studies of exploratory behavior in rodents are conventionally based on tracking animal movement in a flat arena. Most studies of both forced and free exploration restrict the animal to horizontal locomotion. In this presentation we add the vertical dimension to the traditional 2D setup, and suggest how to quantify vertical movement. We focus on free exploration, where the animal is allowed to enter and leave the arena to an adjacent home cage, connected to it through a doorway, as it pleases.

A previous study on BALB/c mice behavior in a novel flat circular arena [1][2] provides evidence that this behavior consists of sequences of repeated motion away from and back to specific points of reference. In measuring this behavior in reference to a polar coordinate system whose origin is at the arena center, the angular and radial dimensions are explored via near-wall excursions from the doorway and incursions, which are performed from the wall towards center (Figure 1). The growth in both extent and complexity of these sequences has been extensively documented.

For example, the first excursions are straightforward and end with the animal leaving the arena after reaching a certain maximal angular distance from the entry point. However, as the maximal excursion distance from home is extended, more complex patterns appear. Excursions may now end in the vicinity of the doorway rather than in complete exit ("cage-skip"), or in other cases the mouse may reach a certain point and then backtrack part of the way before setting off again to extend the excursion further ("shuttle"). Shuttles such as this are also exhibited in incursions towards the center.

Quantifying movement in the vertical dimension

In the aforementioned and other open field setups, exploration of the vertical dimension was limited to rearing and jumping. However, surrounding the arena with a vertical wire grid on which the mouse can climb freely revealed sequences of repeated motion ("ascents") growing in a similar fashion to the horizontal ones (Figure 1). At first, the ascents are strictly vertical. The mouse climbs up a certain distance before descending all the way to the floor along the same route. Degrees of freedom are added to the motion on the grid as the maximal height per ascent gradually extends – by performing vertical shuttles, by progressing on the wire horizontally after reaching a certain height and later by climbing freely in diagonals involving vertical and angular change all around the grid.

Quantifying the growth in extent of the ascents (Figure 2) is based on methods previously used for quantifying near-wall excursions and incursions to the center [3]. The dependence of the maximal height per ascent on time (scaled by activity) is estimated using percentile LOESS smoothing. The comparative variables are the time to reach certain thresholds (e.g. 20% percent of the maximal height) and the growth rate at those thresholds, where growth rate is defined as the slope of the derivative at a particular point on the smoothed function.

So far this experiment was performed only with BALB/c mice, but considering the large differences observed in these two measures in pilot comparisons, we expect that significant differences will characterize ascents across strains, genders and preparations.

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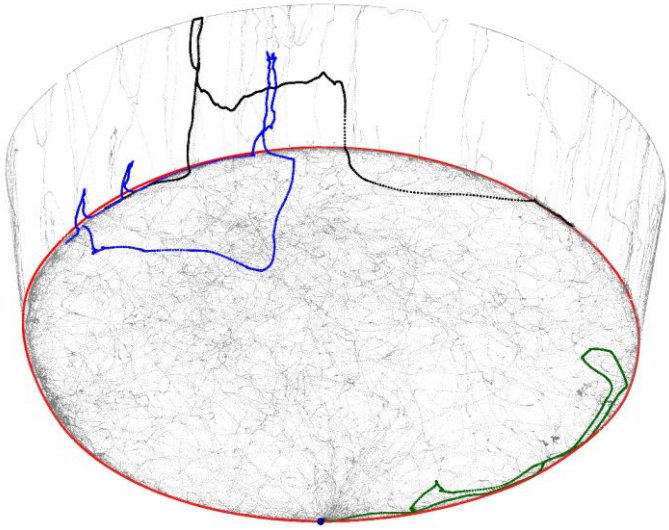

Figure 1. A 3D plot of exploratory patterns on the arena floor and the vertical grid. The arena boundary is highlighted in red, and the dark blue point marks the entrance. The three highlighted routes demonstrate addition of degrees of freedom to the mouse locomotion, sorted by time. Each is taken from a different entry. The earliest (dark green, starts from the left) is a simple near-wall excursion, starting and ending in the doorway. The second (blue, starts from the leftmost point and continues along the wall) includes three simple ascents growing in extent, followed by an incursion towards the center. The latest (black, starts on the right) demonstrates a more complex ascent, containing movement in the vertical and the angular dimensions, as well as a small vertical shuttle.

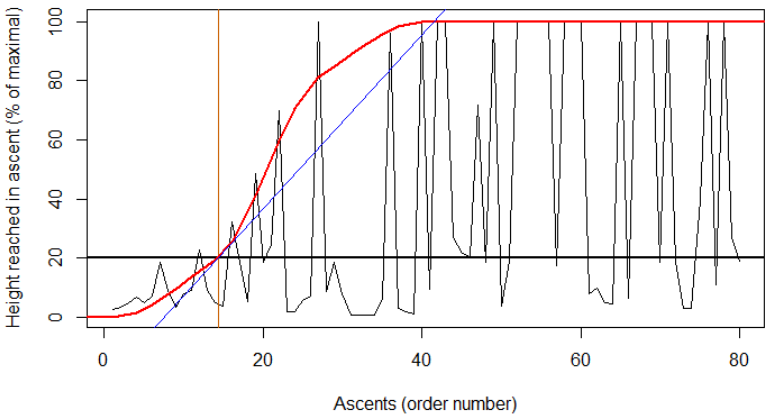

Figure 2. Quantifying the growth in extent in ascents. The red line represents the percentile-LOESS function. The black horizontal line is a certain selected threshold (in this case 20% of maximal height), and the vertical orange line is the time (in activity) to reach the threshold after smoothing. The blue line is the growth rate at the selected threshold.

Experimental setup, protocols and analysis

Except for the addition of the 47.5 cm tall vertical grid, the experimental setup and the protocols are described in details in [1]. 2D tracking was performed using Noldus EthoVision XT 9 [4]. The study was conducted in accordance with the Guide for Care and Use of Laboratory Animals provided by the NIH, ‘‘Principles of Laboratory Animal Care’’ (NIH publication no. 86–23, 1996).

Analyzing wall movement included the following phases:

- Converting the raw tracking data from a Cartesian to a polar coordinate system
- Defining accurate floor-wall boundary to isolate wall coordinates
- Smoothing wall coordinates separately
- Quantifying growth in extent of ascents

Considering the flat image produced by the single camera, analyzing the movement on the grid required an accurate separation of the grid from the floor. For that we use an algorithm which calculates the floor boundary based on estimates provided by the user. The user is required to draw a low resolution polygon on the mouse XY plot where the boundary should be, and the algorithm corrects deviations from the actual boundary and smoothes the polygon. The algorithm does not depend on arena symmetry and was found accurate for all kinds of arena shapes, from ellipses to rectangles, crosses and stars, as long as the boundary is clear to the eye (Figure 3).

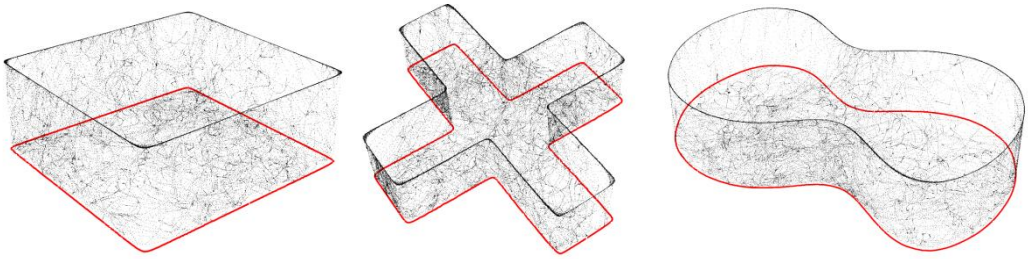

Figure 3. 3D images of mouse movement in different symmetrical and asymmetrical shapes of arenas, using one overhead camera and regular 2D video tracking.

Using the center of the arena as the origin of axes, the arena is divided to sectors by angle from the entry point, and the mouse XY coordinates are converted in the same manner to polar coordinates (r, θ). For each sector i, the separation function provides the boundary radius (R_i) per sector. Points within a specific sector found beyond the boundary $(r > R_i)$ are considered wall coordinates and are transformed to a cylindrical coordinate system (ρ, θ, z), where $z = r - R_i$ and ρ is held constant at R_i. The θ and z coordinates are then smoothed using LOESS algorithm.

Ascents are defined as periods of time between the first and last time stamp in which $z > 0$. They are quantified as explained before. Growth rate and time to reach threshold in two groups of mice may be compared using unpaired two sample t-test.

Floor coordinates $(r \leq R_i)$ are converted back to XY and the movement is analyzed, including position smoothing, retrieval of velocity and acceleration and motion segmentation, in the SEE software [5] (http://www.tau.ac.il/~ilan99/see/help/).

Summary

Conventional horizontal tests of locomotion and exploratory behavior in rodents are limited, and therefore may fail to detect important differences between groups. According to this hypothesis, we suggest addition of vertical locomotion to these tests, as well as methods to quantify it. We believe that this addition may reveal patterns unique to different strains and conditions. The techniques presented enable adding a vertical dimension to any former test setup, without the additional cost and restrictions of a 3D tracking system which requires more cameras and relatively small tanks.

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