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COLORcation: A new application to phenotype exploratory behavior models of anxiety in mice

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HIGHLIGHTS

- *COLORcation* analyses exploration patterns and provides a heat map of mouse activity.
- *COLORcation* provides new parameters to track activity and locomotion of the test animals.
- The results demonstrate the use of *COLORcation* in different anxiety paradigms.

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ABSTRACT

Background: Behavioral analyses in rodents have successfully delineated the function of many genes and signaling pathways in the brain. Behavioral testing uses highly defined experimental conditions to identify abnormalities in a given mouse strain or genotype. The open field (OF) is widely used to assess both locomotion and anxiety in rodents. In this test, the more a mouse explores and spend time in the center of the arena, the less anxious it is considered to be. However, the simplistic distinction between center and border substantially reduces the information content of the analysis and may fail to detect biologically meaningful differences.
New method: Here we describe *COLORcation*, a new application for improved analyses of mouse behavior in the OF.
Results: The application analyses animal exploration patterns in detailed spatial resolution (e.g. 10×10 bins) to provide a color-encoded heat map of mouse activity. In addition, *COLORcation* provides new parameters to track activity and locomotion of the test animals. We demonstrate the use of *COLORcation* in different experimental paradigms, including pharmacological and restraint-based induction of stress and anxiety.
Comparison with existing method(s): COLORcation is compatible with multiple acquisition systems, giving users the option to make the most of their raw data organized text files containing time and coordinates of animal locations as input.
Conclusion: These analyses validate the utility of the software and establish its reliability and potential as a new tool to analyze OF data.

1. Introduction

Mouse behavioral profiling relies on a defined set of experiments which aim to find differences between mouse strains or genotypes (Beckers et al., 2009). Most such experiments provide a quantitative or qualitative score that can be used as a proxy for specific neurological functions (Crawley, 2008). The open field (OF) test is widely used to assess both locomotion and anxiety in rodents (Prut and Belzung, 2003; Crawley, 2008). In this test, the animal is placed in an arena and its location is monitored over time. Initially the assay was done by manual monitoring of animal location in an arena subdivided by square markings on the floor. Nowadays, automated video-tracking systems provide location coordinates throughout the experiment (Gould et al., 2009), however many analyses do not take full advantage of this extensive raw data.

Here we describe *COLORcation*, a program analyzing OF data as a batch and enabling description of group behavior by heat maps

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instead of multiple tracking images of single animals as used in most studies. *COLORcation* analyses raw data from video tracking software, virtually dividing the arena into bins used as spatial units for analysis. This spatial annotation provides detailed localization information throughout the experiment rather than general regional assessments. *COLORcation* also calculates velocity at any given time point, and can then ignore pausing events (rest time) to accurately report the median velocity of animal movements throughout a trial. Finally, *COLORcation* provides the user with a summary file containing all calculated parameters for each animal and per group.

2. Materials and methods

2.1. Animals

Animal procedures were in accordance with Weizmann Institute of Science animal care committee regulations. 2–3 months-old C57BL6 male mice (body weights 20–23 g) were used. The mice were kept at 24.0 ± 0.5 °C in a humidity-controlled room under a 12-h light–dark cycle (lights on at 7:00 PM) with free access to food and water.

2.2. Open field

We assessed motility and anxiety-like behaviors in the open field (OF) paradigm (Neufeld-Cohen et al., 2010b). Animals were tested over 10 min in a 50 × 50 cm rectangular OF arena, an illumination of 120 lux and background noise of 65 dB. The total distance moved (cm), the time spent (global, center, border; in seconds), center/border ratio, mean speed (cm/s), and percentage of time spent moving versus rest in the different user-defined areas were recorded using VideoMot2 software (TSE System, Germany).

2.3. Pharmacological treatment

The benzodiazepine receptor inverse partial agonist FG7142 (*N*-methyl-ß-carboline-3-carboxamide; Sigma–Aldrich, cat# E006-100MG) was used for pharmacological induction of anxiety as described previously (Evans and Lowry, 2007). The drug was dissolved in 1 ml ethanol (96%) and subsequently diluted in 10 ml 1x PBS and injected intraperitoneally (IP) at a final concentration of 5 mg/kg of body weight 30 min before the behavioral test. This dosage induced anxiety-related behaviors without affecting general locomotion or inducing seizures (Little et al., 1984; Löscher and Stephens, 1988). We compared two groups of animals, FG7142-treated ($n = 8$) versus vehicle control ($n = 10$).

2.4. Restraint stress experiment

The mice were tested in the OF under four conditions ($n = 16$–18 for each condition): (i) unstressed: no additional stressor other than the challenge of the test ("NAIVE"); (ii) following an habituation procedure that included handling and transferring the mice to the experimental room over 3 days before the test ("HABITUATED"); (iii) immediately following 15 min of acute restraint stress ("IMMEDIATE"); and (iv) 24 h following the noted acute restraint stress ("DELAYED"). Acute restraint stress was performed as described in Neufeld-Cohen et al. (2010a).

2.5. Comparison of COLORcation and Ethovision

Animals ($n = 10$; C57BL6 mice, 2 months old) were tested over 10 min in the same rectangular OF arena (50 × 50 cm) described above (provided by TSE) under illumination of 120 lux and background noise of 65 dB. Movies of 10 min were acquired and analyzed off-line using the Ethovision XT11 software (Noldus Information Technology, The Netherlands). The animal movements were based on the center-point tracking. The total distance traveled (cm), the distance traveled in the center area (cm; 25% of the total area), the time spent in the center area (in seconds), and the number of visits in the center area were calculated and both individual and group heat maps generated. Individual raw tracking data were exported and analyzed using *COLORcation* with the same center area definition and the corresponding parameters, individual and group heat maps generated.

Table 1
List of *COLORcation* output parameters.

Parameter	Description and units
File name	Source file
Group	Source file's folder/group
Center	Time spent in center (s)
Walls	Time spent in walls (s)
Corners	Time spent in corners (s)
PauseCenter	Pausing time in center (s)
PauseWalls	Pausing time in walls (s)
PauseCorners	Pausing time in corners (s)
VelocitiesLow	Lower quartile (.25) of velocity (cm/s)
VelocitiesMid	Median velocity (cm/s)
VelocitiesHigh	High quartile (0.75) of velocity (cm/s)
VelocitiesMean	Average velocity (cm/s)
MeanPauseTime	Average pausing time (s)
NumOfPause	Number of pauses (s)
DistCovered	Distance covered (cm)
DistCent	Distance covered in center (cm)
DistWall	Distance covered in walls (cm)
DistCorn	Distance covered in corners (cm)
1st Center loc	Time to visit center (min)
NumVisitsCenter	Number of visits in center region
NumVisitsWalls	Number of visits in walls regions
NumVisitsCorners	Number of visits in Corner regions
AccelerationLow	Lower quartile (0.25) of acceleration (cm/s^2)
AccelerationMid	Median acceleration (cm/s^2)
AccelerationHigh	High quartile (0.75) of acceleration (cm/s^2)
AccelerationMean	Average acceleration (cm/s^2)

2.6. Algorithm and overview of the procedure

COLORcation is a MATLAB based analysis tool which reads the individual raw tracking files of animal locations to assemble a database of the given experiment. Any organized excel file (.xlsx) containing the *x*, *y* coordinates of animal locations over time can be used as an input. In such case the user will need to provide his data frame rate, field size and pixel-to-cm ratio. We used TSE VideoMot2 raw data files in this paper, which already contain the calibration index and the video frame rate, both required for the analysis. Additional input parameters are user-provided, including the initial mouse position in the arena and the field size used in the assay (in cm). Given files containing coordinates of animal locations over time, *COLORcation* matches each coordinate to a corresponding spatial bin and then calculates the total time spent in each bin of the arena. The average time spent in each bin is then used to produce a heatmap representation for the group. Bins are then assigned to a region—center, walls and corners and the average time spent in each region is calculated as well. Additional parameters such as velocity (in cm/s) and acceleration (in cm/s^2) (lower/median/upper quartiles; average), mean pausing time, number of pauses, distance covered in each region, latency to explore the central compartment and number of visits in every compartment are also calculated. Finally, the analysis tool summarises all parameters (Table 1) for each mouse and group into an Excel summary file. Cluster analysis is done using the "Clustergram" function in MATLAB with euclidean distance calculation. The program can be run on any PC after installation of the MATLAB runtime environment.