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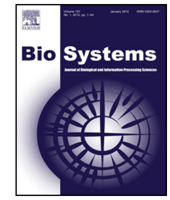

Simulation of behavioral profiles in the plus-maze: A Classification and Regression Tree approach

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ABSTRACT

This article introduces a simulation model of rat behavior in the elevated plus-maze, designed through a Decision trees approach using Classification and Regression algorithms. Starting from the analysis of the behavior performed by a sample of 18 Sprague-Dawley male rats, probabilistic rules describing behavioral patterns of the animals were extracted, and were used as the basis of the model computations. The model adequacy was tested by contrasting a simulated sample against an independent sample of real animals. Statistical tests showed that the simulated sample exhibits similar behaviors to those displayed by the real animals, both in terms of the number of entries to open and close arms as well as in terms of the time spent by the animals in those arms. However, the performance of the model in parameters related to the behavioral patterns was partially satisfactory. Given that previous attempts in the literature have neither include this kind of patterns nor the time as a crucial model parameter, the present model offers a suitable alternative for the computational simulation of this paradigm. Compared with antecedent models, the present simulation produced similar or better results in all the considered parameters. Beyond the goal of establish an appropriate simulational model, extracted rules also reveal important regularities associated to the rat behavior previously ignored by other models, i.e. that specific rat behaviors in the elevated plus-maze are time dependent. These and other important considerations to improve the model performance are discussed.

1. Introduction

At the core of the behavioral neurosciences, the modeling of processes related to variations in the animal behavior, highlights the Elevated Plus-Maze as an important target for recent computational research projects (Salum et al., 2000; Giddings, 2002; Tejada et al., 2010). The Elevated Plus-Maze (EPM) is one of the most used and well validated paradigms for the analysis of rodent anxious behavior (Buccafusco, 2009; Lister, 1987; Pellow et al., 1985) and given its structure, the EPM also offers the possibility to develop simulational models of some behavioral parameters that could improve the understanding of the behavioral response in rats (Salum et al., 2000) and their underlying processes.

The standard EPM consists of two open arms, two closed arms and a central area where the animal can choose to enter at any of those four arms. The entire maze is elevated from the ground; and the test procedure usually involves the analysis of the free moving animal during five minutes, starting with the animal in the central area position. The frequency of entries and time spent on every arm are usually registered. Commonly, high frequencies of entries and longer time lapses spent in open arms are associated with low anxiety states (Brenes-Sáenz et al., 2006); while other Q3 behavioral measures like grooming and rearing are recorded for a better characterization of the behavioral response (Holmes et al., 2000).

So far, computational modeling approaches to rat behavior in the EPM have shown partially satisfactory results (Miranda et al., 2009; Salum et al., 2000). In a seminal reference, Salum et al. (2000) based their proposal on the approach/avoidance theory of Montgomery (1955), and introduced the use of a neural network in which nodes corresponds to every possible position of the animal in the maze. Following Montgomery's statements, every node in the network was associated with a set of w_{ij} values which represents the tendency (w) of change from a position i to a position j. In this system, the node of the network that represents the actual position of the animal in a given state gets a value of 1, while all the other nodes maintain a value of 0. All the weights of the network were computed by a few algorithms directed to estimate those tendencies (Salum et al., 2000). Also, a random adjustment was added to introduce the effect of variation in the exploratory motivation exhibited by some animals. This later adjustment was not based on data, but on assumptions introduced by the authors, as were

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the case with other parameters that were also re-adjusted for the network to reach an acceptable performance.

Later, a second computational proposal tried to improve the performance of this seminal model using an empirical based initiative (Giddings, 2002) not derived from theory driven algorithms, but from the analysis of the probabilities observed in the performance of a group of real rats. Giddings (2002) analyze the evidenced probabilities of seven different situations that frequently occur in the maze such as: a rat moving toward the entry of an open arm, toward the end of an open arm, toward the entry of a closed arm or toward the end of a closed arm, among others. Then, the empirically registered probabilities associated to those situations, direct the changes in the movements of a virtual rat through a simulation process (Giddings, 2002). Nevertheless, besides the important claim in favor of a more empirically based approach, the segregation of rat behavior in seven different situations was established based on the author ad hoc judgment and not as result of any empirical data analysis. In addition, the effect of time was not considered; while some relevant model parameters were corrected using a trial and error strategy until it reaches an acceptable result.

A third approach was proposed by Tejada et al. (2010) using Markov chains. Conceiving the antecedent models as evidence about the plausibility of modeling the rat behavior in the EPM as a probabilistic problem of event transitions, Tejada et al. (2010) proposed a Markov chain model. The states in this model correspond to places in the maze, while transitions represent movements to adjacent locations. Using this interesting approach, the authors verify that the proposed model reproduced the generic features of the exploration transition patterns of real rats in an EPM. But the model does not include the influence of time on the patterns of transition among different locations nor the display of animal behavior (i.e. grooming and rearing) as actual parameters.

The present article tries to take these former models a step further, and describes a simulational model of the rat behavior in the EPM based on a Classification and Regression Trees (CART) approach (Hastie et al., 2009). Starting from a database with locational and behavioral records of real animals in the EPM, this approach uses CART analyses to extract a set of rules that characterizes the different conditional probabilities of animal movements and behavioral transitions at different time intervals through the test length. Then, those sets of rules are used to attribute the movements and behaviors of a virtual sample through a simulational architecture. Hence, the approach tries to improve the gaps in the empirical foundations of antecedent models, and offers a new simulation model that for the first time: (a) introduce the time as a relevant parameter to predict the pattern of the animal performance in the maze, and (b) bring in the behaviors commonly exhibited by the rats in this maze as other kind of potentially valuable parameters. For the validation process, the performance of a simulated sample is contrasted against the performance of a new sample of real animals, not used for the establishment of the model.

2. Methods

2.1. Subjects and housing conditions

Recorded videos of 40 Sprague-Dawley male rats (28 days old) on the EPM were used for this study. The Animals were obtained from LEBI Laboratories (University of Costa Rica) and were housed in the colony room (room temperature at 22 °C ± 2.8 °C, 68–91% of relative humidity, 10 air cycles per hour and 12:12 h light–dark schedule) during 1 week before the behavioral measurement. At the moment of the behavioral measurement, the animals showed a mean weight of 64.08 g ± 3.07 g (mean ± S.E.M.). All the procedures were approved by the Institutional Committee for Animal Care and Use of the University of Costa Rica (Session 3-AE-450).

2.2. Behavioral testing

The EPM apparatus was made of wood and consisted of four arms of equal dimensions (50 cm × 10 cm) connected by a central area (10 cm × 10 cm) and elevated at 50 cm from the floor. Two arms enclosed by walls (40 cm high), were perpendicular to two opposed open arms. To avoid falls, the open arms were surrounded by a Formica rim (0.5 cm high). Testing room was dimly illuminated with two 25 W red bulbs located 150 cm above the maze. At the beginning of the test, each rat was placed in the central area facing to a predefined closed arm. Each animal experienced the EPM for 5 min. All testing sessions occur between 8 a.m. and 11 a.m. and were digitalized as individual videos for posterior analysis. The maze was cleaned with 70% alcohol between rat sessions to reduce odor cues.

2.3. Data codification

Following the procedure of Salum et al. (2000), the EPM area was divided in 13 zones for a better characterization of the transitions among different locations. Behavioral and positional data registered in each video were codified using two different modalities: (a) locational transitions of the animal through different EPM areas were codified using an automatic video-tracking system (Stoelting, Any-maze 4.63 using the location of the 88% of the animal body as the criteria to determine the current area), while (b) behavioral parameters like grooming times/frequencies, rearing time/frequencies, and stretch-attempt postures (as described in Brenes et al., 2009) were codified by trained human observers. Inter-rater agreement was assessed using 30% of all the video recorded data. Agreement reliability was >0.85 for each behavioral category. Given the relevance of both the number of zones used as surface area divisions in the simulation, and the percentage of animal body used as the criteria for the current area determination, these parameters were explored as part of the preliminary analysis (see Section 3). Also, following Giddings (2002) the maze was divided in regions A and B, where A identifies the region that includes the initially predefined closed arm that the animal is facing at the beginning of the test and the open arm to the right; while B identified the two other arms. This was implemented to empirically identify possible bias in arm selection favoring the initially predefined closed arm. Q4

Both kinds of data (behavioral and locational) were coded for every second that the animal spent in the maze, and were included as relevant information for the analysis of the behavior of each rat. Later, half of the animal's videos randomly selected were used to implement the simulation model, and the remaining half were used for the validation procedure.

2.4. The simulation model

The implementation of the simulation model begins with the analyses of a database that includes the behavior and position of 18 rats (2 animals were removed after being identified as outliers, using the criteria of Mahalanobis distances higher than χ^2 (17) = 33.40) at every second in the EPM (300 s for each animal). Using this data, computations were made to establish other relevant variables like: *animal movement* (set to 1 if the animal moved to an adjacent area from one second to the next, or 0 otherwise), *current zone location* (set to 0, 1 or 2, if the animal location belongs to the central area, closed or open arms, respectively), *distance from center* (set to 0 when the animal was located in the central area, or to 1, 2 or 3, if the animal was located at the entrance, middle or