Automated Classification of Rat Social Behavior

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Introduction

Several psychopathologies affecting social behavior such as autism disorders, major depression disorder and schizophrenia are detrimental diseases for which currently no cure exists and much of the pathology is still unknown. The development of valid animal models and reliable test paradigms are crucial elements in the process of understanding the underlying mechanisms of these disorders. Rodents are, similar to humans, very social animals. This aspect, however, has been neglected in behavioral neuroscience and drug discovery. While the quantification of rodent social behavior has long relied on manual observations, recent advanced software and/or hardware technologies e.g. [1-5] make it possible to automatically monitor multiple animals and their social interactions. This requires a different approach in analyzing the data that is generated by these systems. Here, we propose a method to analyze social interactions of rat pairs. Since this method is not relying on subjective human interpretation, but on computerized data, it is more objective and less labor intensive. Also, we will argue here that our method is very valid in ethological terms, because criteria differentiating various behaviors are based on the occurrence (using individual frequency distributions) of the animal’s own behavior.

Methods

Pairs of male rats of 5 weeks old were placed in a relatively large PhenoTyper® cage of 90 cm x 90 cm (Noldus Information Technology, the Netherlands) and allowed to freely interact (i.e. social interaction test). At this age rats are known to display the highest levels of social (play) behavior (e.g. [6, 7]). All testing was done during the dark phase (that is the active phase) of the animals under red light conditions and all animals were habituated to human handling. Before each social interaction test, animals were marked red or black (permanent marker, Edding, Germany) for the software to recognize both individuals based on their marking. The black marking is visible in the video, while the red marking is not visible (due to the infrared light conditions, see below). In this way both animals experience the same practical procedure, but the software recognizes a marked and an unmarked animal; a similar procedure is used by [8, 9]. Incorrect identity swops (in the initial version of the software this was about 50% of the samples) made by the software were manually corrected after video tracking. The experiments were performed in adherence to the legal requirements of Dutch legislation on laboratory animals (Wod/Dutch ‘Experiments on Animals Act’) and were reviewed and approved by an Animal Ethics Committee (‘Lely-DEC’).

From each social interaction test session, top view video recordings were made with a top-unit placed on top of the PhenoTyper, containing an infrared sensitive camera (CCD 1/3” SONY SUPER HAD CCD black/white) and IR-filter (type Kodak 87C). In the development of our method we were inspired by Golani and coworkers that have for long been working on automatic classification of rodent behavior, e.g. [10]. Their approach involves the Gaussian expectation maximization method to search for natural modes in the data based on frequency distributions (see [11] for an extensive explanation of this method). After tracking the animal from video recordings with automatic tracking software (EthoVision XT 8.0, Noldus Information Technology, the Netherlands), raw data containing x- and y-coordinates was exported and further analyzed in MatLab® R2012b (The MathWorks, United States). First, raw track data was smoothed using a robust LOWESS filter with a 1-s time window in order to remove noise. Then, parameters such as the distance moved, speed of movement and distance between two animals were determined. The tracking data was divided between movement bouts and stops (i.e. arrests). Therefore, data was filtered with a repeated running median using a one dimensional median filter with order 7, 5, 3 and 3 respectively. Based on visual inspection of the tracks of the animals and video images the threshold for a stop was set at 0.07 cm between 2 samples lasting at least 0.16 seconds. Next, a density estimator...
(i.e. smoothed frequency distribution) of the maximal velocity of each movement bout was made. On these density estimator plots the best Gaussian curves to represent the empirical data were fitted using an expectation maximization (EM) method. Subsequently, the intersection of these curves determined the different “modes” or velocity categories in which the animals moved. Additionally, the same analysis was done on the distance between the animals, with the difference that this parameter was calculated for each sample in the track data. Also, for this distance between parameter the intersections of the Gaussian curves that best represented the empirical data were used to determine the different “modes” of proximity. Thereafter, we combined both movement and proximity modes together to obtain specific approach and avoidance behaviors characterized by velocity and distance.

Results and Discussion

Our method revealed that in our setup the movement of juvenile rats (when freely socially interacting) can be divided between movements with either low velocities or high velocities (average threshold of 34 cm/s). In addition, we found that distance between rat pairs can be classified in 3 different proximities categories: in -very-close proximity, in proximity and not in proximity (average thresholds: 11 and 31 cm, respectively). Combining these two modes (velocity with proximity) we could clearly identify for example behavioral bouts in which the animals are moving with high velocity and in close proximity, representing chasing behavior. Currently, we are also investigating social interactions of adult rat pairs with this method and we are applying our method on existing models used to examine social deficits, for example phencyclidine induced social impairments.

Using automated social parameters based on coordinates of the animals is not completely new. Sams-Dodd (1995) used a similar method to create the automated parameter ‘social interaction’ [8]. This parameter was made with a fixed threshold; animals were regarded to be in proximity when their center of gravity points are within 20 centimeters from each other. He mentioned that: “selection of this criterion value of 20 cm is based on systematic variation of this parameter from 0 to 50 cm” and “the value of 20 cm resulted in the least variation in the data”. It is exactly this variation that we now use to classify the different categories of proximity. Important benefit of this approach is that the threshold is not created artificially by limiting variation, but based on the animal’s own behavior and natural variation within this behavior. In addition, it is now also possible to combine the information represented by speed of movements of the animals with the categories of proximity.

The next step is to apply this method on data from continuously monitored group-housed rats to study their social behavior displayed in a home-cage environment for more precise registration and thus enhanced differentiation of rodent social behavior. This is also beneficial for increasing the translational value of social behavior when employed as an indicator for assessing treatment efficacy in animal models for psychopathology involving social behavior.

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References


