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Research paper

Proximity as a predictor of social behavior in rats

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HIGHLIGHTS

- A simple method for estimating social behavior of a single rat is proposed.
- The method measures the time a rat attempts to interact with a co-specific.
- The method does not require special conditions or special training from experimenter.
- The measure is stable under several conditions and sensitive to at least one drug.

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ABSTRACT

Background: In studies that measure social behavior of a freely interacting pair rats social behavior of one rat is strongly influenced by the behavior of the other. This prevents evaluating social behavior of one single rat.
New method: We assessed the motivation to interact socially in a modified open-field, by measuring the time a rat attempted to interact with a co-specific separated by a grid in a birdcage outside of the apparatus. We propose time in front of the birdcage is an indicator of social behavior.
Results: We showed that the focal rat allocates more time in front of the birdcage, interacting with another rat through the grid. Also, that the presence of the other rat that attracts the focal rat. Habituation to the apparatus, repeated testing and illumination condition did not alter the proximity measures of rats. Finally, treatment with chlordiazepoxide (3.0 mg/kg) either increased the time spent in front of the cage by males and females or (5.6 mg/kg) increased the proximity measure of females.
Comparing with existing method: Our method prevents partners from influencing the target rat's social behavior; existing methods do not. Also, it is more sensitive to the effect of chlordiazepoxide than the broadly used method proposed by File and Hyde (1978).
Conclusions: Proximity is an advantageous measure: it allows the assessment of only one focal animal without the interference of a partner; it is simple to take; it requires little interpretation skills or training from the experimenter, no special equipment or conditions.

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1. Introduction

There are not many tests for the study of sociability of laboratory animals. Mice have been studied in different ways which involve placing the animals in proximity. For example, the interactions occurring between pairs or groups of mice placed together in standard cages or specialized arenas have been reported (*e.g.*, Terranova and Laviola, 2005; Bolivar et al., 2007). Or, alternatively, the aim of study is the social behavior of pairs of mice separated by barrier which prevents physical contact (*e.g.*, Kudryavtseva, 2003; Crawley et al., 2007; Moy et al., 2007; Silverman et al., 2010). This latter alternative seems more advantageous since the animal under study is not capable of physical interaction but still can engage in sensory interaction (smell, sound, *etc.*). Like many other rodents, the rat is a social animal and even a brief period of isolation may cause a broad range of effects in these animals (*e.g.*, Morgan et al., 1975; Wongwitdecha and Marsden, 1996; Morato and Brandão, 1997). There are not many reports in the literature on the study of interaction between two rats (Silverman, 1965; Latané, 1969; File and Pope, 1974; File and Hyde, 1978). Of these, the test reported by File and Hyde (1978) is one of the most used since its introduction almost forty years ago (for a review, see File and Seth, 2003) and one that has been influential to others reporting sophisticated and complex calculations to assess social behavior (Spruijt, 1991; Spruijt et al., 1992; Casarrubea et al., 2015). In this study (File and Hyde, 1978), however, the rats were tested in pairs which were

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then analyzed as a unit, making it difficult to measure the social behavior of just one of the rats since the behavior of one member of the pair influences the behavior of the other member. Since all studies reporting on rat social behavior were conducted in pairs of subjects that interact, it possible that the social interaction measures of one rat turn out to be different when the animal is tested with other partners. Considering there are no reports in the literature that allow investigating sociability in only one experimental rat, without actually engaging in full social behavior with another rat, we propose a test with very simple measurements that yields a predictor of rat tendency to interact socially, similar to the partition test used in mice (Kudryavtseva, 2003). The proposed test allows recording measurements of a single subject, without much interference of a co-specific. For such, in a modified open-field apparatus, we measured proximity between two rats, as the time a rat spent in front of a grid, on the other side of which there was a co-specific of the same sex. We present data and arguments showing that proximity is a reliable predictor of the tendency to interact socially with a co-specific. In addition to the main goal (demonstrating that the time spent in front of a grid separating the focal animal from a co-specific is a simple and reliable measurement of a rat's tendency to interact socially), more specific objectives were: (1) compare, in the same animals, the measurements we are proposing with measurements obtained using the method reported by File and Hyde (1978); investigate (2) analysis of the experimental setting and eventual differences in the proximity measures of male and female rats, (3) the stability of the measurements obtained under different conditions such as light versus dark, naïve versus habituated rats, one versus repeated measurements, and drug effects. Some other behaviors that occurred along the experiments, unrelated to social interaction, were also recorded in order to have a better measurement of rat behavior when social interaction is available.

2. Experiment I – comparison of measurements between the test we propose and the one reported by File and Hyde (1978)

The object of this experiment was to study the correlation between proximity measurements produced by the test we propose and by the one proposed by File and Hyde (1978). The experiment also aimed at showing other behaviors occurring in the apparatus.

2.1. Method

2.1.1. Subjects

Ten 60-day old Wistar-derived rats were used. The animals came from the Animal House of the Universidade de São Paulo at Ribeirão Preto and were housed in groups of five in two polypropylene cages (41 × 34 × 17 cm). Throughout the experiment, the animals had rat chow (Nuvilab, Brazil) and tap water *ad libitum*. The animal room was maintained in a 12-h light/12-h dark photoperiod (lights on at 7:00 a.m.) with temperature kept between 24 and 27 °C. Cage cleaning procedures were performed three times a week and wood shavings were used as bedding. All testing was performed between 7:30 and 11:30 a.m. All experiments reported here were approved of by the ethics committee of the University of São Paulo (number 13.1.47.53.2).

2.1.2. Apparatus

For the data collection according to the method described by File and Hyde (1978) an open-field (50 × 50 × 40 cm) lined with black opaque Formica was used. For the test we are proposing, the rats were studied in an open-field (120 × 120 × 40 cm) lined with dark brown opaque Formica. This bigger open-field could be used with four conventional walls (see Experiment II) or three conventional walls and a fourth wall with a 20 × 20 cm opening that contained a bird cage on the outside of the apparatus (34 × 22 × 26 cm), where a co-specific could be placed. Interaction between the co-specific and the focal animal could only happen through the grid (Fig. 1).

2.1.3. Procedure

The animals were submitted to a 3-day period of habituation to the conditions of the animal room. On the fourth day, the animals were tested in pairs (one from each cage) according to the method described by File and Hyde (1978) with modifications: to maintain the same conditions also in the test we are proposing, we did not submit the rats to the 5-day isolation period, as originally described by these authors. The rats were transported to the apparatus in two polypropylene cages (18.5 × 30 × 13.5 cm) and were both placed in the center of the open-field. The behaviors of these two rats were individually recorded in a 10-min session (in disagreement with File an Hyde procedure, which analyzes only the pair of rats). On the fifth day, the same pairs of rats were submitted to the test we are proposing. One of the rats of the pair was taken to the open-field in a polypropylene cage (18.5 × 30 × 13.5 cm) and placed inside the bird cage. Then the second rat (the focal animal) was also taken in a polypropylene cage (18.5 × 30 × 13.5 cm), placed in the center of the open-field and a 10-min session was started. On the sixth day, the latter procedure was repeated, with the former focal animal was now placed inside the bird cage and the other one in the center of the open-field for the start of the 10-min session.

The analysis of rat behavior occurring during the test according to the modified File and Hyde (1978) procedure included only recording the time spent (and not, as performed by the authors, where in the apparatus or how many times) performing the following behaviors: sniffing, nipping, allo-grooming, following, mounting, kicking, boxing, wrestling, jumping on and crawling under or over the partner, independently of which rat performed it (See Table 1 for a list of behavior which were actually recorded in the videos and descriptions). For the test we are proposing, the image of the apparatus on the monitor was divided into 36 20-cm squares, which allowed us to analyze the frequency, duration and place in the apparatus where the behaviors occurred. The 36 squares were grouped in larger areas according to the number of walls surrounding it (see Fig. 1. For a rationale of such a division, see Lamprea et al., 2008). The following behaviors were analyzed: entries into the different squares (later grouped in larger areas), rearing, sniffing, self-grooming and grid gnawing (Table 1).

After each one of these tests, the apparatuses were cleaned with a cloth soaked with a 5% alcohol solution and dried with a paper towel. The sessions were carried out in a room adjacent to the animal room. The test room was illuminated by 60-W bulb placed 2.25 m above the floor of the apparatus, yielding 55 lx at the central floor level. All sessions were recorded by a video camera placed above the apparatuses and connected to a computer in an adjacent room which recorded the videos. The videos were later analyzed using the software X-Plo-Rat (Tejada et al., 2017). This simple to use software was developed in our laboratory and allows the experimenter to record behavior while watching a video. Pressing arrow keys record the movements from one square to the next in the direction indicated by the arrow and pressing and holding alphabetic keys record the time a behavior began and how long it lasted (releasing the key when the behavior is no longer being emitted). After recording, the experimenter could recover where each behavior occurred, how many times and how long it lasted each time. Only one experienced experimenter performed the recording of behavior.

2.1.4. Statistical analysis

Spearman correlation test was used in order to analyze the measurements obtained with File and Hyde modified procedure and the

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