Measuring Social Behavior in Drug Discovery

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Abstract

Disturbed social interaction is seen in many psychiatric and neurological disorders. The majority of animal models used in the pharmaceutical industry, however, do not allow assessment of social behaviors. This may due to the beliefs that measuring social behavior is too labor intensive, lacks robustness, or is too complicated to measure. Here, we describe two models of social domination: Dominance Submissive Behavior and Social Isolation-Induced Aggression. These models can be useful to estimate efficacy for compounds that may be beneficial for treatment of depression, mania and disorders with symptoms of enhanced aggressive behavior such as Alzheimer's disease and schizophrenia. We demonstrate, by showing data of training sessions and effect imipramine on established submissive behavior, that Dominance Submissive Behavior can be studied in a simplified setting with automatic color recognition video tracking. In addition, we show that the level of aggressive behavior in mice can be easily assessed with simple "real time" latency measurement. However, the example of effect of buspirone indicates that detailed analysis of drug-induced effects can be partially automated, but require manual inspection of behavior of video analysis played back on reduced speed. Finally, training data from both models indicate that selection of animals that show reliable dominant/submissive or aggressive behavior is essential for drug testing.

Introduction

Disruption of social behaviors is symptoms that occur in many psychiatric and neurological disorders. Yet, the use of animal models allowing assessment of social behavior in drug discovery is limited. Here, we evaluate two models that may allow estimation of efficacy profile of compounds in preclinical drug discovery. Dominant submissive behavior models tax hierarchy formed through social interactions and can be based on naturally competition for various resources [1]. Dominant submissive behavior has been described for rodents in special designed arenas. Dominant behavior has been shown to be attenuated by antimanic drugs, whereas submissive behavior can be reduced by antidepressant drugs [2]. Here, we evaluate effects of the antidepressant drug imipramine in standard behavioral equipment (open field) with video tracking analysis approach.

Aggressive behavior has been described in disorders such as schizophrenia and Alzheimer's disease [3,4]. These symptoms are distressing for the patients, but also for caregivers and family members. For Alzheimer's disease, aggression is often the catalyst for institutional care [4]. Various animal models have been described. Here, we evaluate the development of aggression social isolated animals and compare various analysis methods to detect the effects of 5-HT_{1A} receptor partial agonist buspirone in aggressive mice.

Methods

Animals

Male Han Wistar rats (Janvier, France, weighing between 250-300g) housed in groups of four were used for Dominant Submissive Behavior. Male Swiss OF1 (residents, purchased form Charles River, Germany) and NMRI (intruders, purchased from Janvier, France) 6 weeks of age at arrival were used for social isolation-induced aggression. Residents were single housed for 4-6 weeks before the start of an experiment. Intruders were housed in groups of five. Animals were kept under standard laboratory conditions (21±1 °C air temperature and 55±15% humidity), with free access to food (except for Dominant Submissive Behavior) and water. Experimental procedures were approved by Abbott's Animal Welfare Officer, and were performed in

accordance with the recommendations and policies of the U.S. National Institutes of Health "Principles of laboratory animal care" (1996 edition), and performed in an AALAAC certified institute.

Dominant Submissive Behavior

Training: Animals were tested in pairs (same composition throughout the experiment) derived from different home cages, once a day, five days a week. A test session lasted for 5 min and started by placing the pairs in an open field arena (60 x 60 cm) with a centrally inserted beaker glass (50 ml, 6 cm diameter in a hole in the floor plate) with condensed milk (Cat milk, Attika, < 0.4% lactose). Animals were color marked on their head and neck. During the session, a video recording was made of the central area of the arena. The time the color mark covered the beaker glass was analyzed by video tracking (EthoVision XT, Noldus Information Technology, Wageningen, The Netherlands) and taken to indicate time at the milk beaker. In order to make the analysis less time consuming, the daily recorded videos were merged to a single video. Analysis of the video in 5 min bins allowed analysis of single pairs. After each test, animals received laboratory chow ad libitum for only 1 hour, but had free access to chow during the weekends.

Twenty pairs (from 40) showed robust and stable dominant submissive behavior and were selected for drug treatment. Submissive animals received imipramine (10 mg/kg) or vehicle once per day and examined for consecutive 3 weeks.

Social Isolation-induced aggression.

Resident mice were tested during a training sessions. A training session started by placing an intruder in the home cage of the resident, and lasted for 5 min or shorted when the intruder was attacked and the intruder showed submissive behavior. Resident mice were selected for aggression after 3-4 training sessions (one session per day), and animals with short attack latency were selected for drug treatment studies. A drug testing session started by placing the resident in a test arena (open field 28 x 37 cm). During the first 5 min, locomotor activity was analyzed using EthoVision XT. A naïve intruder was introduced for the next 5 min of the session, which was recorded on video. Analysis of behavior (latency to attack, and number of bites) was performed manually with play back at half speed. Behavior as also manually analyzed and categorized in exploratory behavior, social interaction and inactivity using The Observer XT (Noldus Information Technology). Buspirone was tested at 0.03, 0.1 and 0.3 mg/kg.

Results

Stable and robust dominant submissive behavior was observed in approximately 50% of the tested pairs. Dominant animals continued to spend more time at beaker after vehicle treated animals, whereas submissive behavior who received imipramine did not differ from the animal that showed increased time at the beaker before treatment. We demonstrated that dominant submissive behavior can be measured in standard behavioral equipment (open field). Furthermore, merging video files reduced time spent for analysis.

Aggression levels, in terms of latency to attack varied strongly between residents. Training data over consecutive days indicated that animals that show initial low attack latencies, strongly decrease attack latencies over time, whereas animals that show long attack latencies do not show decreased latencies over time. Approximately 50% of the animals showed consistently very low attack latencies and were selected for drug testing. Video tracking revealed that locomotor activity was dose dependently reduced by buspirone during the first 5 min before the introduction of the intruder. Buspirone increased latencies to attack, although a large variation of the data was seen for this parameter. Number of bites was less variable, and reduced by the highest dose of buspirone. Buspirone tended to increase the time spent inactive, but had no effect on social interaction time or exploration time.

Discussion

Here, we demonstrated that dominant submissive behavior can be studied in standard behavioral equipment (open field). Video recording and analysis allows behavior testing in multiple arenas simultaneous. In order to enhance sensitivity of the recording, only the center of the open field, including the beaker glass was recorded. This enhances the ratio background to target (colored spot on the animals), and allows more flexibility in light settings. This is particularly of interest when several arenas are in use, placed next to each other. Video recording results in large data files. In order to allow analysis over night, we merged the videos and rendered and tracked the videos overnight. Video analysis with software that allows using a "play list" would be beneficial and further reduce analysis time.

The data indicate that dominant submissive behavior is established only approximately 50% of the pairs tested. A selection of animals is essential for drug testing. As an alternative, animals could be tested in larger groups (3-4 rats) from the same holding cage, to estimate hierarchy in the home cage. As such, submissive and dominant animals could be identified in each group (ongoing evaluation). Color identification of 3-4 animals is, however, technically more demanding.

Simple measurement of attack latency during training sessions is adequate to select highly aggressive animals for drug testing. However, analysis of the buspirone sessions indicated that latency to attack has high variability and the number of bites is a more robust read out. Analysis of the number bites requires the videos to be played at reduced speed. Video tracking data indicate that buspirone reduces locomotor activity, whereas manual analysis of social interaction, exploratory behavior and inactivity does not reveal an effect of buspirone. The analysis have been performed without intruder (video tracking) and with intruder (manual analysis), which may explain the difference. Alternatively, video tracking may be more sensitive. Nevertheless, number of bites was reduced by 60% by the highest dose buspirone, leaving social interaction unaffected.

Dominant Submissive Behavior and Social Isolation-Induced aggression can be reliably measured with standardized laboratory equipment (Open field). Read out are stable and robust, however, a selection of animals is necessary. Automatic analysis of the behavior increases speed of analysis, but can not be applied for all parameters.

References

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