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Characterization of exploratory activity and learning ability of healthy and "schizophrenia-like" rats in a square corridor system (AMBITUS)

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HIGHLIGHTS

- Semi-automated square corridor system with food rewards (AMBITUS) was developed.
- The system was characterized for investigation of motor and learning abilities.
- Healthy rats had high level of learning capacity and flexibility.
- "Schizophrenia-like" rats showed impaired exploration and learning capacity.
- The manual and automated scoring of the visits into the side-boxes showed significant correlation.

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ABSTRACT

The rodent tasks with food rewards are useful methods to evaluate memory functions, including hole-board and corridor tests. The AMBITUS system (a square corridor with several food rewards), as a combination of these tests, was developed for the investigation of a variety of parameters associated with exploration and cognitive performance in rodents. Experiments were performed to characterize these behaviors in healthy rats and a new "schizophrenia-like" rat substrain with impaired learning ability to reveal the reliability in tests related to these functions.

A square corridor was constructed with equally spaced sites along each wall (4 inside and 4 outside) resulting in 16 side-boxes for food rewards. Photocells at each box recorded the visits into the side-boxes (as exploratory activity), while the eating parameters were obtained from video records. The animals were exposed to two types of tasks repeatedly in two series: all (16) or only the inside (8) boxes (Task 1 or Task 2, respectively) were baited. Most of the rats acquired Task 1, and their performance improved by repetition, but the new substrain showed decreased exploration and learning capacity. The introduction of Task 2 caused prompt preference of the baited inner side-boxes, and gradually improved working and reference memory during the trials. The manual and automated scoring of the visits into the side-boxes showed significant ($r = 0.97$) correlation.

The results proved that healthy animals could perform the simple tasks in the square corridor after a few repetitions. The semi-automated AMBITUS system might be appropriate to detect cognitive flexibility after different manipulations, and it provides immediate, online assessment of exploratory behavior of a large number of animals within a short period of time, and it reduces the possibility of experimenter bias.

1. Introduction

Memory is one of the most important fundamental neurological activities which requires the cooperation of mental abilities depending on different systems within the brain [1,2]. Evaluation of chemically-induced neurotoxicity in laboratory rodents or animal models of several diseases associated with learning and memory decline (e.g., depression, Parkinson disease, senile dementia or brain trauma, schizophrenia, and autism) are frequently expected to test locomotor activity, learning, and memory functions [2–7].

Several methods are available to determine the learning ability of rodents during relatively short time period and/or repeated short trials, including novel object recognition, hole-board, and several mazes, e.g., Y maze, T maze, Morris Water maze, Barnes maze, or radial mazes with different number of arms ([2,4,5,8–17]). Other, more sophisticated methods, such as tasks with touch screen or lever press, require very intensive and long-lasting learning sessions [3,7,18–24].

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It is very important that the experimental setups need to be designed within the normal range of behavior, and the experimenter has to be sure that the animals are given the opportunity to solve the problems with which the test procedure confronts them [25]. The recognition tasks with food rewards in hole-board test are based on the natural behavior of the rodents to explore novelty; thus, these tests are applied frequently for the characterization of the nose-poking activity into the holes (as exploration) and memory functions of rats [6,8,11,14,16,26]. It is well-known that in contrast to a hole-board with an open field, where the rats can show significant anxiety, rats prefer the narrow tunnels [27]. Based on this assumption, some laboratories have applied a circular corridor with or without a few side-boxes containing food rewards to evaluate the exploratory activity and/or learning abilities with a video-tracking system [28–33]. Unfortunately, both methods may require time-consuming data collection and/or they need long training sessions, or the commercial video-tracking systems (e.g., EthoVision XT) require special softwares which can analyze precisely the behavior of the animals.

If cognitive function and behavioral activity of a large number of animals have to be investigated routinely, simple and fast tests are required. Heim et al. have developed the "COGITAT" system for the automatic registration of nose-poking activity of rats on hole-boards using photo beams [25]. We developed a new instrument named the AMBITUS system (AMBITUS is a Latin term literally meaning "to go round"), which is a square corridor with side-boxes along the inner and outer sides; thus, it is a combination of hole-board and corridor tests (with several food rewards but without open field). The system permits the detailed and automatic recording of the visits of the animal into the side-boxes (exploratory behavior) and in concert with the video records, eating behavior can also be determined. Some important features are: 1) the number and the duration of visits into the side-boxes are recorded automatically and saved for later data processing, 2) the data files can be analyzed with a software developed for it, and the results are exported to a spreadsheet file, 3) the system is unaffected by insufficient contrast between the rat and the background, as well as by lighting conditions, as it could be the case with some video-based methods, and 4) it has the great advantage of using very low cost electric components for the automatic registration of the exploratory activity.

The present study characterized the behaviors related to the exploratory activity and cognitive functions (task acquisition and learning flexibility) of healthy rats in the AMBITUS system to reveal its reliability for the detection of these parameters. Since it is well-known that female animals show higher level of motor activity compared to male ones [11,34], we tested this phenomenon, too. Recently a complex animal model has been developed by selective breeding based on behavioral alterations after combined subchronic ketamine treatment (NMDA-receptor antagonist) and postweaning social isolation [14]. These animals showed several signs of schizophrenia, i.e., disturbed sensory gating, motor activity, pain sensitivity, cognitive function and thermoregulation; electrophysiological alterations in electroencephalographic waves; changes in the signalling properties of the cannabinoid and opioid receptors in different brain regions [11,14,35–38]. The evaluation of these animals in this test may give further evidence about the reliability of this test tool for the investigation of animals with disturbed cognitive function.

2. Methods

2.1. Animals

Healthy Wistar rats 2 to 3 months of age (mean weight of male rats: 279 ± 9.5 g and female rats: 174 ± 3.6 g) and the new substrain of male rats (205 ± 6.2 g) were involved in the study [11,14]. The healthy animals were purchased from the animal house of the Biological Research Centre, Szeged, Hungary. Animals were treated in accordance with the guidelines set by the Government of Hungary and EU Directive 2010/63EU for animal experiments. Group-housed (3 rats/cage) animals were kept with a 12 h light/dark cycle under conditions of controlled temperature (22 ± 1 °C), and before starting the two series, the animals were food deprived for two days (no any food was provided during this period), but water was freely available. The additional food restriction process remained throughout Series 2 (for 6 days) with decreased amount of food (10–15 g/day) after the last trial of the day [39]. The rats' body weight was carefully controlled during the whole experiment. The experimental procedures were performed between 8:00 a.m. and 4:00 p.m. in a room with dim white light. The order of testing remained the same throughout the experiment.

2.1.1. Selective breeding process

The paradigm for selective breeding has previously been described [14]. Briefly: after weaning at 3 weeks of age, rats were tested with the tail-flick (TF) test (48 °C hot water) to assess their basal acute heat pain sensitivity, and then they were housed individually for 28 days. The animals were treated intraperitoneally (i.p.) with ketamine (Calypsol, Gedeon Richter Plc., Budapest, Hungary; 30 mg/kg, 4 ml/kg, daily, 5 times/week, 15 injections in total) from 5 to 7 weeks of age. Then the animals were re-housed (3–4/cage), and 1 week of recovery followed with no treatment. Starting at the age of 9 weeks, the acute heat pain sensitivity, the sensory gating, the cognitive functions and the motor activity were assessed. Starting from a population of outbred Wistar rats, animals with the highest level of disturbances in these parameters were used for selective breeding throughout several generations [11,14,35–38].

2.2. The AMBITUS apparatus

A square corridor was constructed of a clear Plexiglas on black floor with an outer diameter of 80 cm, width of 8 cm and height of 50 cm (Fig. 1). The rats can move around the track between the walls in forward and backward directions. Each wall (4 inside and 4 outside) has two equally spaced sites (side-boxes: 5 × 5 × 5 cm) with one possible food reward (puffed rice: 20 mg). In the present the animals were exposed to two types of tasks: all (16) or only the inside (8) boxes (Task 1 or

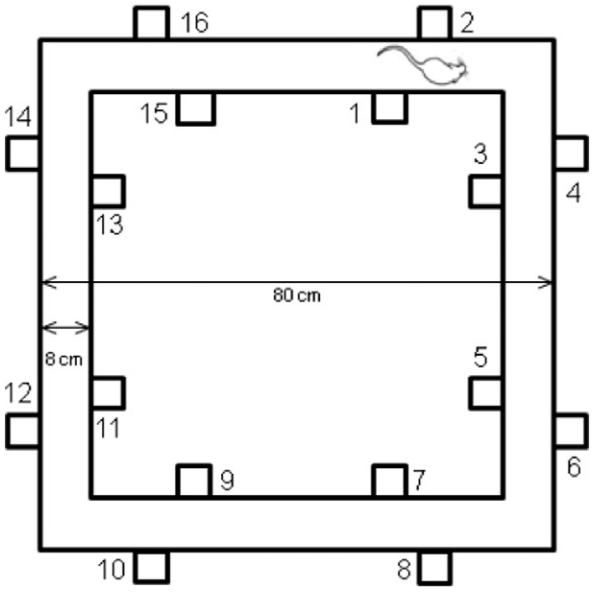

Fig. 1. Ground plan of the corridor with 16 side-boxes equipped with photo beams with a rat at the starting point. The height of the apparatus is 50 cm.