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

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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.

Keywords: cognition; corridor test; food reward; learning; memory; rodent

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 (Vorhees & Williams, 2014; Buzsaki, 2013). 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 (Fitzgerald, Rosenberg, Oettinger, & Battig, 1985; Vorhees & Williams, 2014; Brown et al., 2012; Chrobal, Hanin, & Walsh, 1987; Hauser, Sontag, Tucha, & Lange, 2012; Preissmann, Bertholet, Sierro, Cabungcal, & Schenk, 2011).

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 (Casarrubea, Sorbera, Magnusson, & Crescimanno, 2010; Xu et al., 2013; Chambon, Wegener, Gravius, & Danysz, 2011; Petrovszki et al., 2013; Kekesi, Petrovszki, Benedek, & Horvath, 2015; Fitzgerald et al., 1985; Chrobal et al., 1987; Gordan et al., 2012; Locklear & Kritzer, 2014; McLean, Woolley, & Neill, 2009; van der Staay, Gieling, Pinzón, Nordquist, & Ohl, 2012; Vorhees & Williams, 2014; Roof & Stein, 2001; Vorhees & Williams, 2014). Other, more sophisticated methods, such as tasks with touch screen or lever press, require very intensive and long-lasting learning sessions (Brown et al., 2012; Chen, Wong, Chapman, & Pemberton, 2009; de Bruin et al., 2013; Dunnett, Fuller, Rosser, & Brooks, 2012; Nemeth et al., 2002; Preissmann et al., 2011; Sabbagh, Heaney, Bolton, Murtishaw, & Kinney, 2012; Bussey et al., 2008; George, Rodriguez-Santiago, Riley, & Abelson, 2015).

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 (Heim et al., 2000). 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 (Petrovszki et al., 2013; Kekesi et al., 2015; Casarrubea et al., 2010; Hauser et al., 2012; Schroeder, Schroeder, Schwegler, & Sabel, 2000; van der Staay et al., 2012). 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 (Kimchi & Terkel, 2004). 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 (Nadal, Rotllaant, Marquez, & Armario, 2005; Wikenheiser, Stephens, & Redish, 2013; Wikenheiser & Redish, 2015; Wikenheiser & Redish, 2011; Monaco, Rao, Roth, & Knierim, 2014; Newman, Climer, & Hasselmo, 2014). 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 (Heim et al., 2000). 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 (Kekesi et al., 2015; Sutcliffe et al., 2007), 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 (Petrovszki et al., 2013). 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 (Petrovszki et al., 2013; Kekesi et al., 2015; Horvath et al., 2015, 2016; Szűcs et al, 2016a, b). 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 (Petrovszki et al., 2013; Kekesi et al., 2015). 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 12h 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 (National Research Council, 1995). 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 (Petrovszki et al., 2013). 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 (Petrovszki et al., 2013; Kekesi et al., 2015; Horvath et al., 2015, 2016; Szűcs et al, 2016a, b).

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: 5x5x5 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 Task 2, respectively) were baited. Altogether, 16 side-boxes extending from the corridor are numbered consecutively (1–16; Fig. 1). Each box is fitted with an infrared LED (light emitting diode) at one side and a photocell at the other side to measure nose-poking activity (or visits as exploration) in the boxes by the disruption of light beams, and the 16 photocell units are interfaced to a microcontroller unit. The apparatus automatically records individual beam breaks associated with each side-box visit with 1 ms time resolution, and stores the information about the location, the duration and the number of visits by a custom-made software (L.P.). Based on our preliminary experiments, all emitters and receivers were located 1 cm above the floor and from the entrance of the side-box to detect the visits into the side-boxes to the food rewards. This location of the sensors ensures that whenever a rat interrupts the infrared beam, its nose should necessarily be located within the side-box. The apparatus was installed in a room where no other activities took place during the testing procedure. Before each trial the experimenter inserted the food rewards into the side boxes. Trials commenced by placing the rats into the same starting point within the corridor (Fig. 1); thereafter, the experimenter immediately left the room. The animals were allowed to explore the corridor and collect food rewards for 5 min (cut-off time). The number of food rewards eaten was detected at the end of each trial by the experimenter. The apparatus was cleaned with 70% alcohol after each rat. Experiments were recorded using an infrared video device (WCM-21VF, CNB, China) equipped above the apparatus. When the animals had eaten all the available food rewards, the video recordings were analyzed manually by an experimenter to collect data about the time required to complete the task, while the animals which did not eat all of the rewards, had 300 s eating time (cut off time), and they had only pre-eating exploratory activity (Table 1).

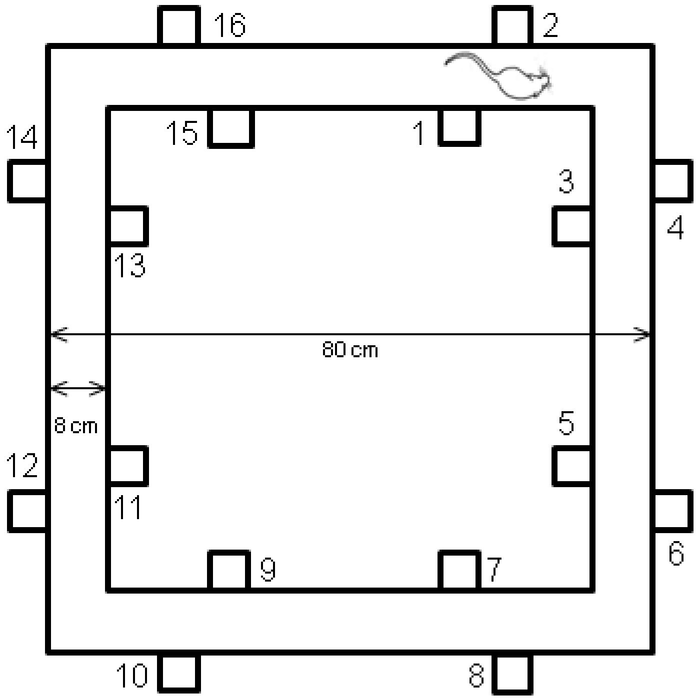


Figure 1. Ground plan of the corridor with 16 side-boxes equipped with photo beams with a rat at the starting point. The height of the apparatus is 50 cm.

2.3.Test procedures

2.3.1. Series 1: Short-term behavioral protocol

The goal of this series was to reveal the difficulty for the animals to acquire Task 1 (the entire outer and inner side-boxes were baited; i.e. 16 food rewards), repeated two times with 1 min apart. In order to evaluate the ability of this system to detect the well-known sex-dependent differences in motor activity, 14 male and 15 female healthy rats were involved in this series. Furthermore, differences in the behavior of another (second) group of healthy male rats (n=14) and the new “schizophrenia-like” male rats (n=15) were tested together and then compared. The control animals in this series were involved in all of the behavioral tests, but they were not isolated and injected as the ones in the new substrain. To be sure that the new automated scoring method was consistent with the manual one, we compared their scores. The analysis of video records was performed by a trained observer uninformed of the computer-based data.

2.3.2. Series 2: Long-term behavioral protocol

One week after the short-term behavioral protocol, the healthy male rats from the first group were involved in Series 2 (for 6 days). A long-term paradigm was performed to disclose the benefit of this system for the characterization of the development of learning capability during Day 1 and 2 (8 trials) with Task 1 (16 food rewards), and for the detection of the learning flexibility with Task 2 between Days 3–6 (15 trials). During Task 2 only the inside boxes were baited (8 food rewards); therefore, it preserved similar behavior but allowed us to manipulate reward contingency. With this paradigm, we could examine how changes in the task structure influence the exploratory activity and reward collecting. All of the rats performed two sessions (two trials/session) of tasks per day, one in the morning and one about 3 hours later (i.e., four trials/day, except for the 3rd day, when 3 trials were performed). The training was interrupted for 2 days between Day 3 and 4 (Trial 11 and 12).

2.4. Data and statistical analysis

Table 1 shows the manifold, partially dependent parameters which were either recorded automatically by photocells or manually by the experimenter (at the end of the experiment or in the video-recordings) or were calculated from the baseline data by a custom-made software (L.P.). Thus, the exploratory activity was evaluated as the latency, number, and duration of visits into the side boxes revealing the motivation to search. Furthermore, latencies to complete the task, the number of the eaten food rewards, and the learning capacity (LC) calculated from these parameters are the measures of efficiency of the memory. The cognitive processes were also characterized by working and reference memories based on earlier studies (Table 1) (Kekesi et al., 2015; Sontag et al., 2013; Fitzgerald et al., 1985; Woldeit and Korz, 2010). Working (short-term) memory is responsible for the transient holding, processing, and manipulation of information that is an important process for reasoning and for the guidance of decision making and behavior; reference (long-term) memory represents knowledge for aspects of a task that remain constant between trials. The efficacy and accuracy of the automated system was verified by comparing the output results with those manually calculated by an observer.

Table 1. List of variables detected automatically (A), manually (M), or calculated (C).

Variable	**Definition/explanation**
Overall exploration (number of side-box visits; A)	The total number of the disruptions of light beams
Pre-eating exploration (A)	The number of the disruptions of light beams until completing food collection (eating all rewards or up to total duration)
Post-eating exploration (A)	The number of the disruptions of light beams after completing food collection
Duration of pre-eating exploration (A)	The duration of the disruptions of light beams until completing food collection
Duration of post-eating exploration (A)	The duration of the disruptions of light beams after completing food collection
The mean duration of one exploration (C)	The ratio of exploration time and number of exploration
Latency of the first exploration (A)	The time passed till the first side-box visit
Frequency of exploration (C)	The ratio of the number of exploration and the time
Food rewards eaten (M)	The number of rice portions eaten
Time to complete the task (s; M)	The time required to eat all the rice, or total trial duration (300 s) if not all of the rewards were eaten
Learning capacity (LC, %; C)	[(Number of food rewards eaten)x(cut-off time {300s}x100]/ [number of food rewards)x(time required to complete the task {s}]
Working memory (WM, %; C)	[(Number of food rewards eaten)x100]/(number of rewarded visits and unrewarded revisits into previously rewarded boxes until completing Task 2)
Reference memory (RM, %; C)	[(Number of rewarded visits and unrewarded revisits into previously rewarded boxes until completing Task 2)x100]/(total number of visits and revisits until completing Task 2)

Data are expressed as means ± SEM. Behavioral data were analyzed using factorial ANOVA with sex (male vs female), location of the visits (inner vs outer side), and trials. In case of the experiments with the new substrain, the group (healthy vs “schizophrenia-like” rats) was also used as a factor. Post hoc comparisons were performed by using the Newman-Keuls test. To investigate the correlation of exploratory behavior detected automatically and counted by an observer, the Spearman Correlation was performed in 132 cases selected randomly. Only probabilities lower than 0.05 were considered significant. For the analyses, STATISTICA Version 12 (Statsoft Inc., Tulsa, OK) was used.

3. Results

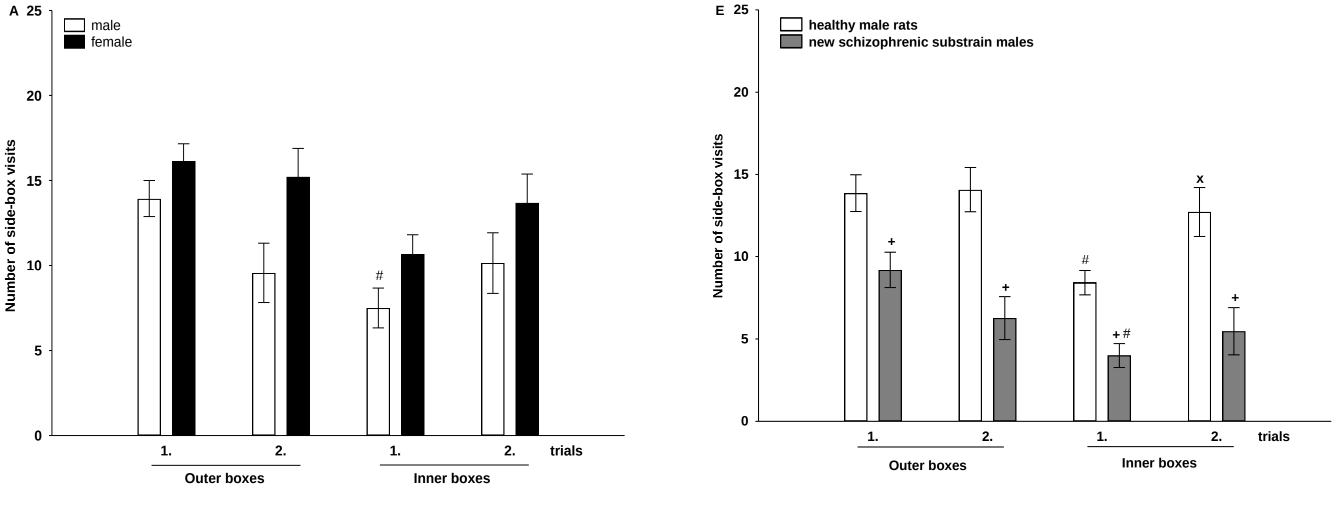
3.1. Short-term protocol

3.1.1. Male *vs* female groups

Since only 2 male and 4 female animals (out of the 29) ate all the food rewards in the first trial, and 2 females and 5 males in the second one, most of the animals did not have any post-eating exploration; thus, only pre-eating exploratory activity (before eating all of the food rewards) was analyzed in this series. ANOVA analysis of the pre-eating visits showed statistically significant effects of sex ($F_{(1,108)}= 12.53$, $p<0.001$), location ($F_{(1,108)}= 8.73$, $p<0.005$), and trial and location interaction ($F_{(1,108)}= 9.82$, $p<0.005$; Fig. 2A). The mean total inspection number was 25±2.5 and 24±4.6 for males, and 30±2.0 and 33±3.8 for females in the first and second trials, respectively; suggesting a high level of activity of the animals of both sexes with a higher degree of exploratory activity in females. The animals of both sexes explored the outer boxes more frequently compared to the inner ones in the first trial, but not in the second one. The minimum and maximum number of visits was 10 and 44, respectively, in the first trial, while the minimum decreased to 1 in the second trial, but the maximum value increased up to 52. Since the duration of explorations provided the same ANOVA results as their number, the mean duration of one exploration (about 1 s) did not differ between the groups and trials (data are not shown). Regarding the latency of the first visit, ANOVA analysis showed a significant effect of sex ($F_{(1,108)}= 6.31$, $p<0.05$) thus, the latency was shorter for the female rats (Fig. 2B). Regarding the eating behavior, ANOVA analysis did not show statistically significant effects, but the female animals ate slightly more rice in the second trial on both sides (Fig. 2C). As it was mentioned above, only a few animals ate all the rice; thus, the mean time to collect food rewards was close to the cut-off time (300 s) in both trials, with no significant differences between trials, locations, and sexes (for the first: 281±7.0 s vs for second: 277±9.3 s). Regarding the learning capacity (LC), ANOVA analysis did not show statistically significant effects, but a slight improvement was observed in the second trial (from 61±10.2 to 76±11.6%), which was primarily due to the increased number of the eaten rice (Fig. 2D).

3.1.2. Male healthy *vs* “schizophrenia-like” groups

Regarding the comparison of healthy animals to a new “schizophrenia-like” substrain, ANOVA analysis of the pre-eating visits showed statistically significant effects of the group ($F_{(1,108)}$= 51.83, p<0.001), the location ($F_{(1,108)}$= 14.54, p<0.001), and the trial and location interaction ($F_{(1,108)}$= 6.38, p<0.05; Fig. 2E). The mean total inspection number was 22±1.6 and 28±3.6 for controls and 13±1.2 and 12±1.7 for the new substrain in the first and second trials, respectively, suggesting a very low level of activity of the animals in the new substrain. The animals of both groups explored the outer boxes more frequently compared to the inner ones in the first trial, but not in the second one. Regarding the latency of the first visit, ANOVA analysis showed a significant effect of the group ($F_{(1,108)}$= 8.24, p<0.005), the location ($F_{(1,108)}$= 7.76, p<0.01), and the trial ($F_{(1,108)}$= 6.23, p<0.05); thus, the latency was longer for the new substrain, especially in the second trial (Fig. 2F). Regarding the eating behavior, ANOVA analysis showed statistically significant effects of the group ($F_{(1,108)}$= 155.61, p<0.01) and the trial ($F_{(1,108)}$= 12.37, p<0.001); thus, rats in the new substrain ate fewer rice in both trials on both sides (Fig. 2G). Regarding the eating time, only a few animals ate all the rice; thus, the mean time to collect food rewards was close to the cut-off time (300 s) in both trials; ANOVA showed significant differences between the groups ($F_{(1,108)}$= 12.10, p<0.001), the trials ($F_{(1,108)}$= 6.64, p<0.05), and the location and trial interaction ($F_{(1,108)}$= 6.64, p<0.05); thus, rats in the control group showed decrease in the eating time at the second trial but not the rats in the new substrain. Regarding the learning capacity (LC), ANOVA analysis showed statistically significant effects of the group ($F_{(1,108)}$= 78.01, p<0.001) and the trial ($F_{(1,108)}$= 7.83, p<0.05); thus, significant improvement was observed in the second trial in the control group (from 109±14.7 to 160±20.2%) but not in the new substrain group (from 6±2.1 to 29±10.2%; Fig 2H).



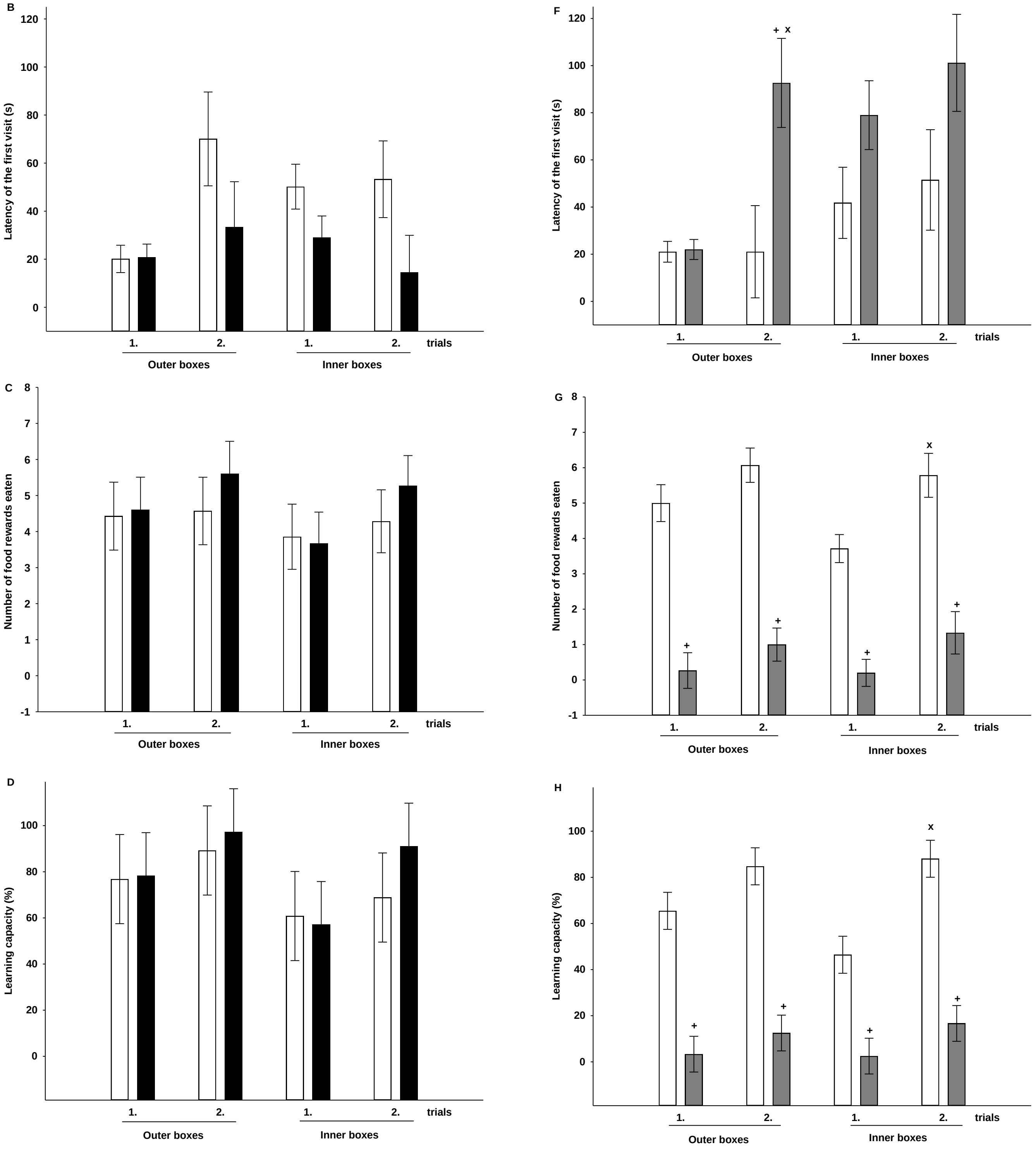
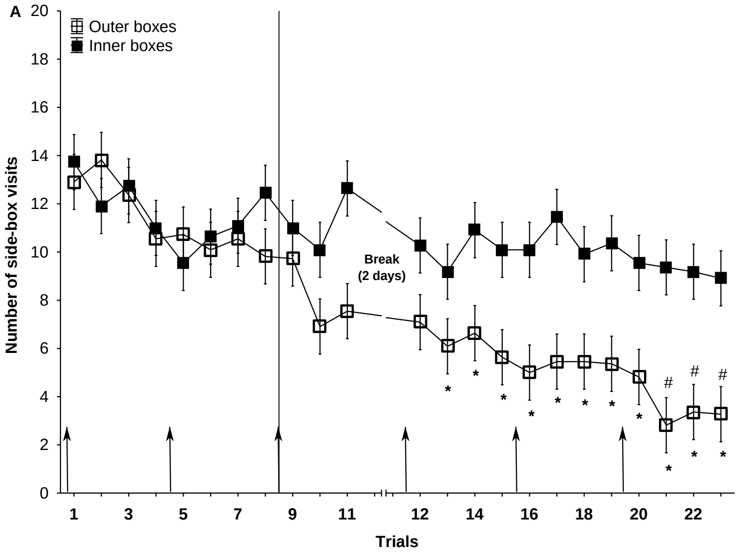


Figure 2. Behavioral parameters of healthy male and female rats (A–D) and of healthy and new (“schizophrenia-like”) substrain rats (E–H) during the short-term protocol. A and E: Pre-eating exploration. B and F: latency of the first exploration. C and G: the number of food rewards eaten. D and H: learning capacity. The symbols denote significant differences by Newman-Keuls post-hoc test between the outer and inner boxes (#); the first and the second trials (x), and the healthy group and the group of “schizophrenia-like” rat substrain (+).

3.2. Long-term protocol

The food restriction process resulted in a significant decrease in the body weight (from 300 ± 5.9 g to 286 ± 6.7 g). 3 animals out of 14 showed inactivity up to the end of the 8 trials (i.e. they showed very low exploratory activity, and did not eat any food reward); thus, they were excluded from further analysis. Regarding the number of pre-eating visits during the whole period, ANOVA analysis showed statistically significant effects of location ($F_{(1,460)}$= 81.70, p<0.001), trial ($F_{(22,460)}$= 6.91, p<0.001), and their interaction ($F_{(22,460)}$= 2.31, p<0.001). The introduction of Task 2 (food rewards only in the inner boxes) caused preference of the inner side, i.e., the number of the pre-eating visits decreased in the outer side (Fig. 3A), while the analysis of post-eating exploration showed no significant differences in the discrimination of the side, but the trial showed significant effects ($F_{(22,459)}$= 2.00, p<0.01), (Fig. 3B). Thus, a learning capability in the avoidance of unbaited boxes can be seen during the trials of Task 2, resulting in the rats' moving less redundantly over the days through the corridor. Regarding the total and pre-eating frequency of exploration, the ANOVA analysis showed statistically significant effects of the trial ($F_{(22,230)}$= 3.07, p<0.001), the phase (pre-eating *vs* overall) ($F_{(1,230)}$= 583.87, p<0.001), and their interaction ($F_{(22,230)}$= 6.60, p<0.001). Thus, the overall and pre-eating exploratory activities overlap in the first few trials, whereas later, the pre-eating frequency was significantly larger and increased by the trials. Additionally, the frequency of overall activity showed a tendency to slightly decrease by repetition (Fig. 3C).



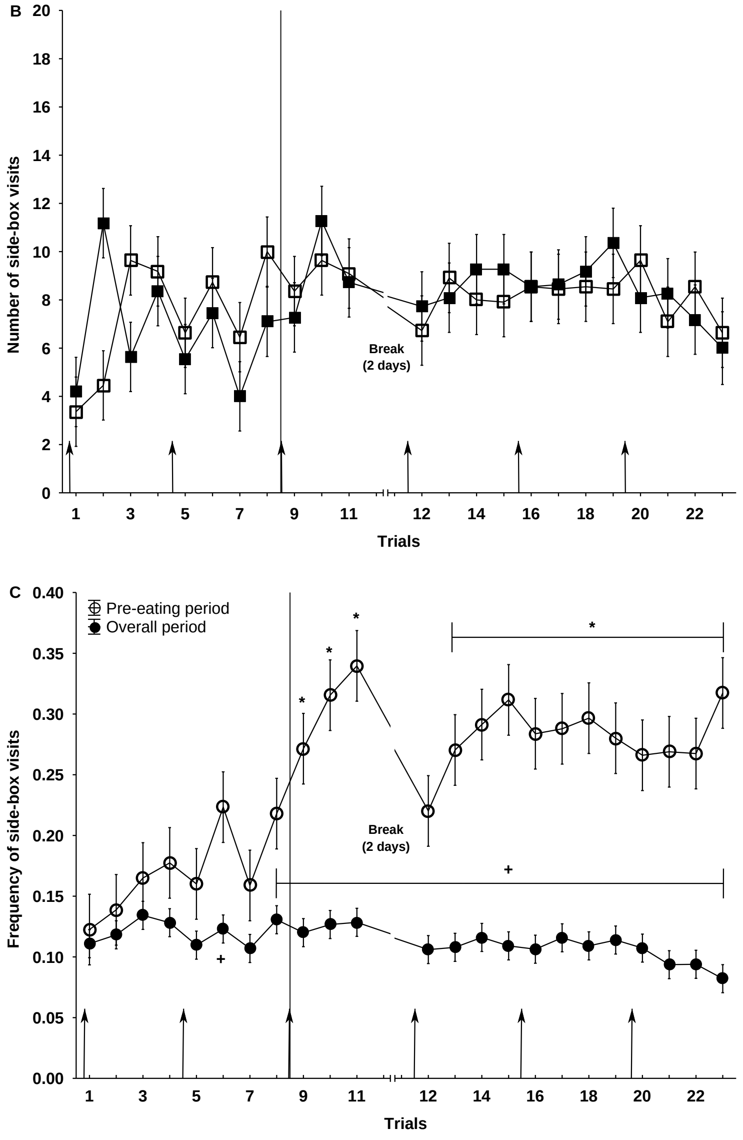


Figure 3. Changes in the exploratory activity before (A) and after (B) completing the task. C: Pre-eating and overall frequencies of side-box visits. The symbols sign significant differences by Newman-Keuls post-hoc test between the inner and outer sides (#); compared to the first trial (*); and between the phases (+). Arrows show the days. The reference line signs the introduction of Task 2.

Regarding the data analysis of the learning functions during Task 1 (8 trials), ANOVA of time to collect food rewards showed significant effect of the trial ($F_{(7,160)}$= 4.24, p<0.001; Fig. 4A) but not the number of rice portions eaten (Fig. 4B). As regards the LC, both the location $F_{(1,160)}$= 6.15, p<0.05) and the trial ($F_{(7,160)}$= 6.58, p<0.001; Fig. 4C) showed statistically significant effects; thus, the animals showed improvement in their performance during the trials.

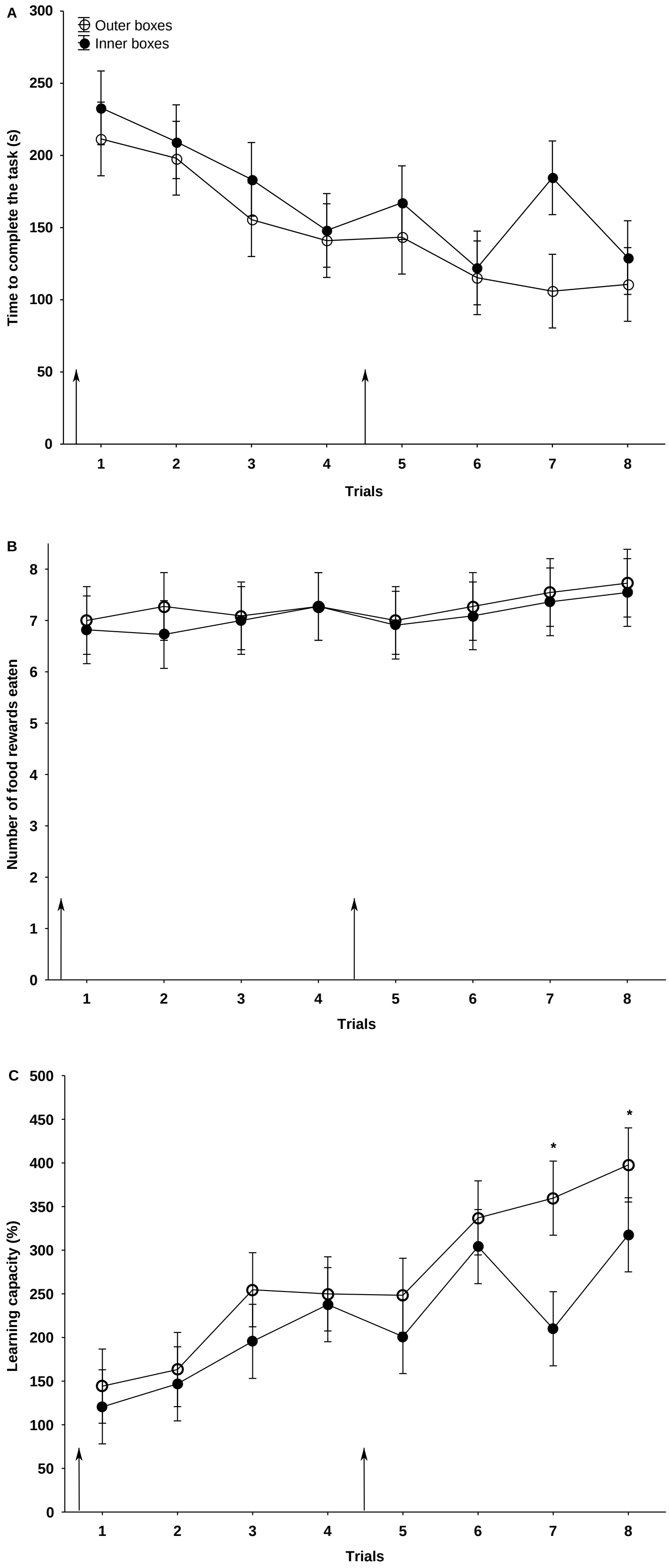
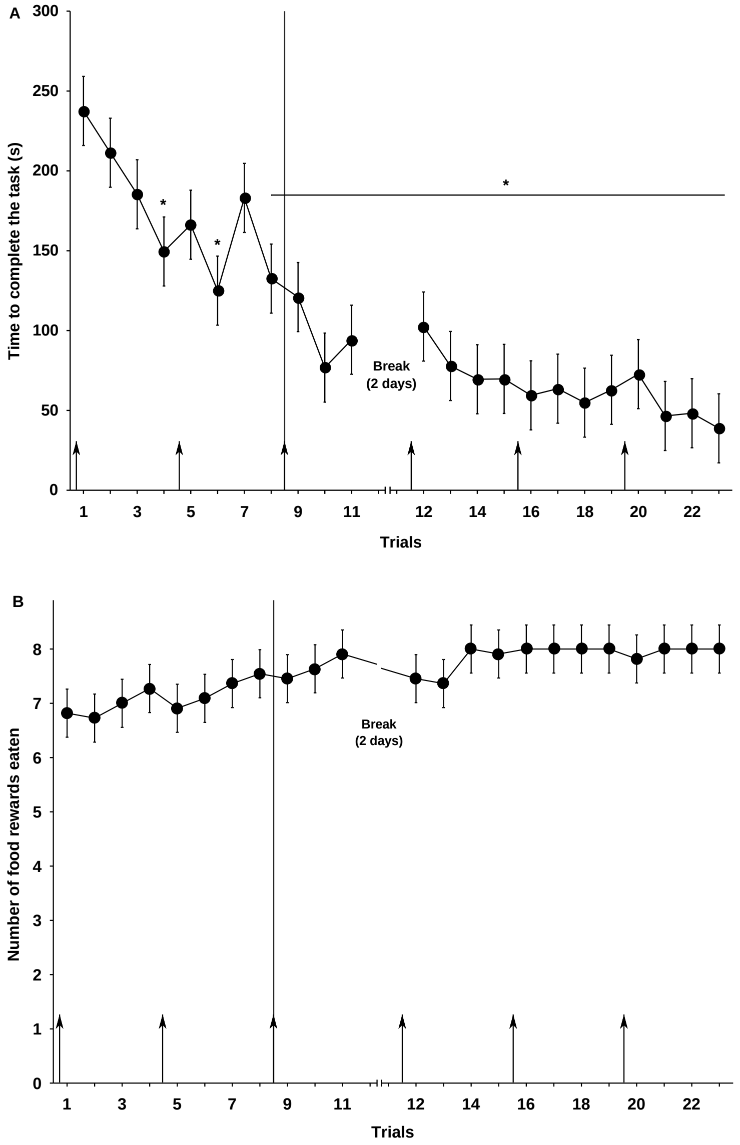


Figure 4. The nature of eating behaviors observed during the first 8 trials, when both sides contained food rewards (Task 1). A: the time to complete the task, B: number of food rewards eaten, C: learning capacity.

Arrows show the days. The symbol * signs significant difference from the first trial by Newman-Keuls post-hoc test.

Regarding the whole long-term protocol (8 trials with Task 1 followed by 15 trial with Task 2), the eating parameters were analyzed only the inner side-boxes, since during trials from 9 to 23, only these boxes were baited. The ANOVA analysis regarding the time required to complete the collection from the inner boxes showed statistically significant effects of the trial ($F_{(22,230)}$= 7.07, p<0.001); thus, it decreased with the series from 238±19 s up to 39±2 s (Fig. 5A). The ANOVA analysis of the number of the rice eaten did not show significant effects of the trial (Fig. 5B); thus, the animals ate almost all the rice even in the first trial, suggesting that the animals remembered the first series. The ANOVA analysis of the LC showed significant effects of the trial ($F_{(22,230)}$= 9.65, p<0.001; Fig. 5C). The introduction of Task 2 on Day 3 did not cause significant decrease in the LC, even some improvement was achieved by repetitions, which might be due, at least partially, to the fewer food rewards. However, only the last trial showed significant improvement compared to Trial 9 suggesting a prolonged plateau during Task 2.



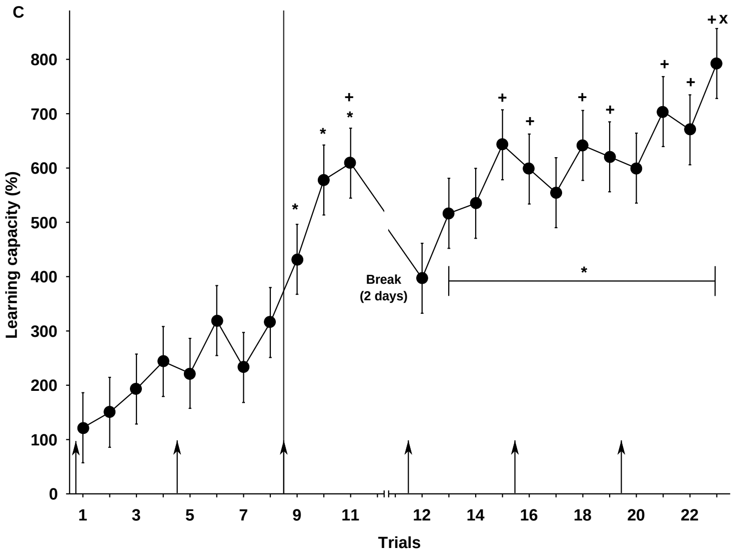


Figure 5. Changes in the time required to complete the task (A), the number of food rewards eaten (B), and the learning capacity (C) in the inner side during the total long-term protocol (23 trials). Arrows show the days. The reference line signs the introduction of Task 2. The symbols sign significant difference from the first trial (*); from Trial 8 (+); and from Trial 9 (x) by Newman-Keuls post-hoc test.

Regarding the WM during Task 2, it showed a tendency to increase with trials (from 70±5.5% up to 90±2.1% ; $F_{(14,150)}$= 1.59, p=0.09; Fig. 6A); the RM showed significant enhancement ($F_{(14,150)}$= 3.53, p<0.001; Fig. 6B). However, the linear trend analysis showed significant correlations (p<0.001) with trials for both parameters (r=0.47 *vs* r=0.63 for WM and RM, respectively) suggesting improvement during the trials.

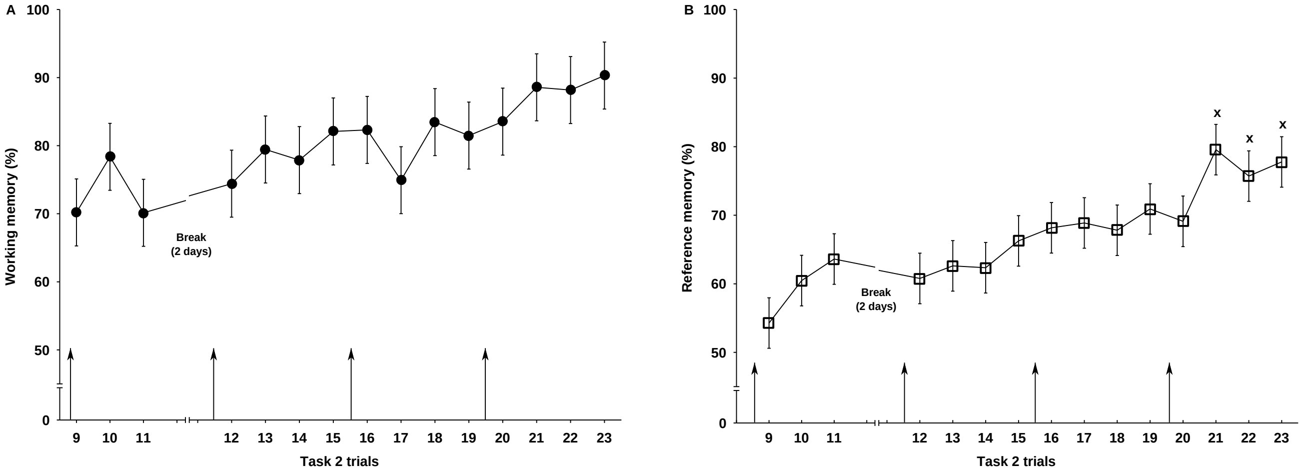


Figure 6. Working (A) and reference memory (B) during Task 2 (Trials 9-23). Arrows show the days. The symbol x signs significant difference from Trial 9 by Newman-Keuls post-hoc test.

Regarding the correlation analysis between manual and computer-based data acquisition on exploratory behavior, significant correlation was shown (r=0.97) with -0.53±0.16 mean difference between these values. The strict parallel analysis of the manual and the computer-recorded values revealed that the underestimation of the number of visits by the observer was due to the very short inspection time of the boxes in some occasions, which were overlooked by the investigator.

4. Discussion

The main goal of this study was to determine the suitability of the AMBITUS system for the analysis of exploratory activity and cognitive functions of rats. The movement sensors can automatically detect spontaneous visits into the side-boxes giving objective information about the exploratory activity. In concert with the detection of the eating activity by the analysis of the video records, several parameters related to learning and memory functions could also be determined. The detailed analyses of the individual parameters related to task performance justified that most of the healthy rats learned the pattern and showed cognitive flexibility using this system. The number of the visits recorded by the examiner correlated well with that detected by the photocells, showing the accuracy of this method. The sex (male vs female) and strain dependent (naive Wistar vs the new substrain) differences are in good agreement with the earlier studies (Kekesi et al., 2015; Sutcliffe et al., 2007, Petrovszki et al., 2013) suggesting that the system can be an appropriate method for investigating the exploratory activity and the learning ability of rats.

4.1. Methodological aspects

A number of behavioral paradigms have been developed to assess spatial learning and memory performance in rodents. Different mazes and hole-boards with food rewards are commonly used in this regard. Rats show spontaneous exploratory activity in new environment, which may correspond to a form of information acquisition (Vorhees & Williams, 2014). Searching for food is also a naturally motivated behavior; thus, rodents are able to learn to retrieve food rewards located in different setups (Post et al., 2011; Vorhees & Williams, 2014). Some researchers have used a circular corridor with a few (3) side boxes (only in the outer side) with food rewards to investigate the learning capability of rodents (Wikenheiser et al., 2013; Wikenheiser & Redish, 2015; Wikenheiser & Redish, 2011). The AMBITUS system is a combination of the hole-board and the corridor tests; since similarly to the hole-board, it offers the animals the opportunity of exploring a new environment and finding several food rewards, but no open field is available. Altogether, the corridor combined with the advantages of the hole-board could quantify the activities of rats and reflect the degree of learning ability and memory as well. There are other automatic scoring methods as well, but they require the purchase of expensive hardware and/or software; i.e., an automated registration of hole-poking activity in a hole-board was developed earlier containing 25 holes equipped with 3 photocells in each hole at 3 different levels (Hauser et al., 2012; Post et al., 2011; Heim et al., 2000; Sontag et al., 2013).

The short-term protocol was designed to test a simple task (Task 1) acquisition. The most efficient strategy in this task requires the subjects to run directly from box to box, collecting food rewards at each site. It was found that normal rats were able to learn the task to a high level of performance during a short period of time, and they exhibited some improved performance at the second trial, suggesting that this simple task could be acquired easily. Regarding the differences between male and female rats, the female rats had enhanced exploratory activity, but no differences in cognitive functions were observed. In most tests of allocentric navigation in rodents, male animals show an advantage compared with female animals, while in some egocentric navigation tasks, female rats performed better than male rats (Vorhees & Williams, 2014), or no sex differences were observed; thus, the sex differences depend on the specific task parameters and/or configurations (Kekesi et al., 2015; Roof & Stein, 2001; Vorhees & Williams, 2014; Sutcliffe, Marshall, & Neill, 2007; Locklear & Kritzer, 2014). The new substrain of rats developed in our laboratory showed decreased exploratory activity and learning ability in the AMBITUS system, which is in agreement with the results obtained recently in novel object recognition and the hole-board tests (Petrovszki et al., 2013; Kekesi et al., 2015), and it suggests that this system can be applied to reveal the abnormalities in these parameters of rats with different impairments. However, it should be considered that alterations in vision and motor ability could also influence the results in the new substrain. It is well-known that albino rats have highly limited vision (Prusky et al., 2002); therefore, the Wistar albino animals can navigate without detailed visual information. Regarding the motor ability, we did not notice any motor impairment, and the investigation of the motor activity in their homecage with telemetric system proved that only a minor hypoactivity could be detected in this substrain (Horvath et al., 2015). As regards their motivation in the homecage, our preliminary study suggested that these animals showed similar behavior in the sucrose preference test to the control animals (unpublished data). Therefore, it can be supposed that the lower activity of the new substrain might be due to the new circumstance in the AMBITUS system, as was detected in the holeboard test, too (Kekesi et al., 2015). However, it should be considered that food preference is not a perfect measure of food motivation; therefore, further studies are required to clarify the causes of the differences in body weight. The lower body weight of the new substrain could also be an important factor influencing the obtained results. However, it should be mentioned that the animals with lower body weight show higher level of motor activity, generally. This is also right for Gunn rat, another substrain with schizophrenia like symptoms (Tsuchie et al., 2013). Furthermore, our preliminary studies showed that the food restriction for two days should be enough for animals to evoke hunger, and the low amount of food rewards cannot lead to satiation in either group. Therefore, it is supposed that the lower level of motor activity and the number of the eaten food rewards detected in the animals of the new substrain might not be related to their lower body weight. Even, the correlation analysis of body weight with exploratory and eating activities of the new substrain showed a slight, non-significant inverse correlation between these parameters ($r=-0.22$ and $r=-0.12$, respectively). Over the course of the 2-day training with 4 trials per day during the long-term behavioral protocol, we could conclude that control Wistar rats became proficient in Task 1. Furthermore, pre-eating exploration was more frequent, but it decreased thereafter, suggesting that the animals recognized the completion of the task. The paradigm applied in this study was based on several preliminary data. Earlier experiments in hole-board and our pilot study in this device showed that the activity of the animals was the highest during the first 5 min, and this period seemed to be sufficient for the animals to complete the task (Kekesi et al., 2015; Fitzgerald et al., 1985; Chambon et al., 2011). Furthermore, the four trials (in two sessions) per day were acceptable for the animals with high throughput (25 animals daily), but the massed version of the trials (3 repetitions with 1 min interval) did not show enhanced results, and even decreased activity could be observed (Vorhees & Williams, 2014). 8 trials for two days in two blocks each day seemed to be enough for the animals to acquire the Task 1 with gradually decreased time required to complete the task. Thus, healthy rats showed clear improvement in the learning capacity during the investigated period. The 24 or 72 h time lag between the tests did not decrease the performance of the animals significantly, and even a one-week break between the short- vs long-term protocols caused slightly higher degree of LC (89±16.6 vs 119±18.0 %; p=0.12), revealing that the animals could remember the experimental condition, and also suggesting the simplicity of the task.

Surprisingly, the animals in all groups show higher exploratory activity toward the outer sides in the first trial. We found no explanation for this phenomenon, but it might be suggested that the animals can differentiate between the peripheral and central walls, and they prefer the peripheral wall in the first trials. This phenomenon seems to be similar to a preference of the peripheral holes compared to the central ones in the hole-board test (Kekesi et al., 2015; Wernecke & Fendt, 2015). Since the experiments were performed in dim light without external cues, the learning in the corridor could essentially be considered a form of an egocentric task solved by learning the association between its egocentric body response and the reward (Vorhees & Williams, 2014; Buzsaki & Moser, 2013). Egocentric way finding is characterized by the ability using internal cues (i.e., by feedback from limb movement for the movement of the rat [speed], directions of turns and sequence of turns), optokinetic flow as the animal moves past surrounding objects, and signposts (corners and side-boxes). In contrast to the earlier corridor tests, the corridor in the AMBITUS system has a square shape to provide important egocentric information due to the four corners and the inner and outer side-boxes containing the food rewards. Several brain structures are involved in egocentric navigation learning (e.g., hippocampus, striatum); thus, the functions of these brain structures could be investigated in this system (Vorhees & Williams, 2014; Buzsaki & Moser, 2013). An important property of cognitive processes includes its flexibility, which involves the updating and modifying of previously learned behavioral response strategies and is critical for successful adaptation to a changing environment (George et al., 2015; Placek, Dippel, Jones, & Brady, 2013; Vorhees & Williams, 2014; Mala et al., 2015). Behavioral shifts within one dimension (e.g., shift according to spatial position) is denoted as learning flexibility, which is a broadly-used assay of plastic reward learning and has been proposed as an index for some psychopathology. There are many examples of treatments or lesions of different brain regions that produce small or even no differences during the task acquisition, but significant deficits during the introduction of a new task, which might be due to navigation deficit or inflexibility in learning (Vorhees & Williams, 2014). Differences in the reward contingencies of the two tasks give rise to differences in the strategy of exploration. The results from the second phase of the long-term series (Task 2) revealed that as the animals learned to skip unrewarded feeders, the visit frequency significantly decreased in the empty side-boxes. However, the presentation of the simple Task 2 (only 8 rewards) seemed to cause a clear plateau in LC for about 3 days until it started to improve again, suggesting that the length of the plateau could also be used as a measure of flexibility.

It is well-known that both WM and RM improve with practice (experience) (Markowska & Savonenko, 2002), and our results are in good agreement with this phenomenon, suggesting that Task 2 was also simple for healthy rats, as they were able to learn the new paradigm and to improve their performance over time. Primarily the orbitofrontal cortex and hippocampus are involved in the mediation of learning flexibility; thus, the behavioral effects of lesions in these structures can be characterized by this system (Placek et al., 2013; George et al., 2015; Vorhees & Williams, 2014).

4.2. Limitations

The AMBITUS system records multiple behavioral items simultaneously, which can be displayed immediately after the completion of the experiment. An important disadvantage of this method is that video-records should be checked by an investigator for data acquisition about the time required to collect the food rewards, if the rat ate all of them. However, the analysis of video-records was a fast process, required maximum 5 min/records, and together with the automatic registration of exploratory behavior, it significantly decreased the time required for data collection, and it could also prevent some mistakes originated through an oversight in case of manual data collection.

It has repeatedly been hypothesized that anxiety related behavior and cognitive processes may interact in a fundamental manner (Vorhees & Williams, 2014). Most of these behavioral tests are accompanied by different levels of stress, which might be due to exposure to a novel environment, open field and/or food restriction (Post et al., 2011; Vorhees & Williams, 2014). Since no habituation phase was applied in our paradigm, the novel environment in the first trial should have significant influence on the results, i.e., the stress to the novelty can negatively influence memory formation, but the curiosity of the corridor led to a high level of exploratory activity (Sandi & Pinelo-Nava, 2007). Increasing familiarity with the experimental conditions across trials and/or increasing proficiency at performing the task led to gradual increase in LC. Since the narrow corridor in the AMBITUS system can simulate the natural environment of the animals; thigmotaxis has no influence on the behavior of the rats. However, it has to be mentioned that stress is not always negative, i.e., stress in relation to performance has an inverted U-shape function, too little as well as too much is counterproductive to performance, but in the midrange, there are levels of stress that optimize performance (Vorhees & Williams, 2014). Most rodents show high levels of learning in the AMBITUS system, suggesting that the task has not been overly stressful. A restricted daily feeding procedure leading to reduced body weight during the experiments had to be established to motivate the animals to perform the task (Post et al., 2011; Vorhees & Williams, 2014). Appetitive mazes generally require more days of testing, which might be subject to satiation effects and reduced motivation. The low weight of rice in our experiment together with the 4 trials/day could not lead to satiation, since the number of rice portions eaten and the LC did not decrease in the last trials during a day. Similarly to other learning methods applying food-rewards, the restricted interest in rodents can also influence the results (Sontag et al., 2013; Vorhees & Williams, 2014). It was observed that the activity of some animals decreased on the second occasion in the short paradigm, and 3 animals out of 14 did not show any activity during the long-term period. Thus, the rats' activity can be a confounding factor in spatial learning tasks, which may lead to the difficulty of selection bias that is a common problem in appetitive tasks, where there can be significant dropout rates. However, this device might be appropriate for the investigation of restricted interest of animals, too.

Similarly to other food-rewarded tests, the animals can see and/or smell the rewards when they are close to the entrance of the boxes, which can also influence the results. However, it has to be considered that the albino rats have highly limited vision (Prusky et al., 2002). Very important errors might have been the tail or foot entering into the side-boxes and scored as visits. Fortunately, since the foot is below the body, it never happened that the feet were entering the side-boxes accidentally, which might occur in the hole-board test. Furthermore, since the position of the photocell is 1 cm above the floor and the distal part of the rat's tail is thinner, this error has not been observed, too.

The AMBITUS may be adapted to many other experimental conditions, e.g., more and/or longer learning trials, habituation periods without food rewards, providing more palatable food or various configurations of stimuli. The structural analysis of the exploratory activity can give further data about exploratory strategy of the animals (Casarrubea et al., 2010), thus the separate analysis of each side-box or clustering of the inner and outer ones may give several possibilities for the investigation of spatial learning. Some other parameters can also be considered in the future, e.g., the number of different side-boxes inspected, revisited boxes or repetitions can reveal the perseverative behavior of the animals (Fitzgerald et al., 1985; Vorhees & Williams, 2014). Our preliminary data showed that no significant differences could be observed in these parameters between healthy male and female animals during the short-term paradigm (data are not shown).

4.3. Conclusion

In conclusion, the AMBITUS system, containing photocells for automated recording of exploratory behavior and video-tracking for the detection of the eating activity, is a fast, efficient, objective and reliable way to assess the activity and learning capacity and memory functions of rats after minimal training sessions. The advantages of this new system include the shape (square corridor without open field), the large number of food rewards with different locations, and the possibility for separate analysis of each box or in the chain of inner and outer ones that ensures the investigation of spatial learning functions and learning flexibility applying different protocols. The software working with data obtained by photocells gives immediate data acquisition, the assurance of experimenter-independent measurements, and the reproducibility of results. The repeated use of the AMBITUS system can also be applied in the same animals to assess time-dependent effects such as in neurodegenerative and aging models and to evaluate the effects of different treatments. In summary, the AMBITUS system may be a valuable tool to screen for the effects of compounds or diseases which are believed to act on rat exploratory activity and memory capacity during a short time period, but additional studies are required to validate the AMBITUS system with other protocols.

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