Cognitive training improves the disturbed behavioral architecture of schizophrenia-like rats, "Wisket"

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ABSTRACT

Translational schizophrenia research depends on the relevance of animal models supported by reliable tests. Human data suggest that the intensive cognitive training in schizophrenia improves the memory impairments and decreases the chance of acute psychiatric remission. Here we examined the effects of a 10-day long training session in the behavioral architecture of a new schizophrenia-like rat substrain (Wisket) in a narrow square corridor with food rewards (AMBITUS). The instrument was designed to model the natural environment of rats and enable the simultaneous recording of multiple behavioral parameters. For the compact visualization of differences between the Wisket and control animals in several parameters (behavioromics), color-coded grid plots were applied. The Wisket animals exhibited an altered pattern and/or amount of locomotion, exploratory and food collecting activity at the first few days, revealing impaired motivation, attention, anxiety and learning ability (face validity). Most of the parameters normalized with training, except for the decreased exploratory activity. This resembles the effects of cognitive behavioral therapy in human schizophrenics providing a significant support for the predictive validity of this substrain as an animal model of schizophrenia. This study also highlights the importance of behavior tests that investigate the egocentric learning ability during reward-based tasks.

1. Introduction

The success of translational schizophrenia research depends on the relevance of animal models of the human disease. Animal models play an important role in schizophrenia research, thus testing their validity is crucial. While several symptoms that lie at the core of the diagnosis in humans (e.g. hallucinations, delusions, etc.) cannot be modeled in animals, there is a host of other characteristic symptoms that can be reliably reproduced this way [1]. Most of the animal models of schizophrenia are based on pharmacological or genetic manipulations that are often limited to affect only a single neurotransmitter system or one specific genetic locus, while, in fact, schizophrenia is a multifactorial disorder [2]. To achieve a high constructive validity, we developed a complex, chronic, "multiple hit" rat model, named Wisket (the selective breeding originated from Wistar strain after postweaning ISolation and subchronic KETamine treatment) [3]. This model shows several symptoms of schizophrenia, such as disturbed sensory gating, pain sensitivity and thermoregulation, electroencephalographic alterations and changed opioid and cannabinoid receptor functions (as face validity) [3–9]. Furthermore, decreased exploratory activity and cognitive

dysfunctions have also been described in short-term paradigms of learning tests [3,4,7]. Memory formation is one of the most important capabilities of the central nervous system, which requires the co-operation of various mental abilities depending on different systems within the brain [10,11]. Beside the positive and negative symptoms, cognitive deficit is a hallmark of manifest schizophrenia, and also a reliable predictor of outcome [12,13]. Unfortunately, the antipsychotic drugs are ineffective in the treatment of negative symptoms and cognitive impairments. Recent data suggest that patients with schizophrenia may regain memory function close to normal after intensive cognitive training for a prolonged period [14–18]. It is well-known that the cognitive functions significantly depend on the behavioral activity, which is impaired in schizophrenic patients, too [19–21], and a close relationship was detected between reinforcement learning and general psychosocial functioning in schizophrenia [22].

For detailed characterization of the exploratory activity and learning abilities of rodents, we constructed the AMBITUS system, a combination of the hole-board and corridor tests [7]. It has been proven that control, but not Wisket animals, can find most of the food rewards after a few trials. The AMBITUS system was further developed to give

information about the locomotor activity of the animals, too. Thus, AMBITUS provides data on a wide variety of behavioral parameters related to the locomotor and exploratory activities and cognitive functions, which necessitates compact imagining, for which we introduced color-coded grid plots. The goal of this study was to reveal the short- and long-lasting effects of a 10-day long training session applying different tasks of the new substrain.

2. Materials and methods

2.1. Animals

Male Wistar (control group) and Wisket rats were involved in the study. The experiments were carried out with the approval of the Hungarian Ethical Committee for Animal Research (registration number: XIV/03285/2011 and XIV/1248/2018) and in accordance with the guidelines set by the Government of Hungary and EU Directive 2010/63EU for animal experiments.

Animals were kept with a 12 h light/dark cycle under conditions of controlled temperature (22 ± 1 °C) and air humidity (55 ± 10%). The experimental procedures were performed between 8 AM and 4 PM, under dim lighting. Before the cognitive tests, the animals were food-deprived for two days, but water was freely available. Moderate food restriction was maintained throughout the experiments (for 10 days) with decreased amount of food (10–15 g/day) until the last trial of the day. The rats' body weight was carefully controlled during the whole experiment.

2.2. Interventions in Wisket rats and baseline behavioral tests for both groups

Based on our earlier studies, both control and Wisket rats, after weaning at 3 weeks of age, were tested in the tail-flick (TF) test (48 °C hot water) to assess their basal acute heat pain sensitivity (Table 1.) [3–9]. Then the Wisket animals were housed individually for 28 days, and were treated with intraperitoneal 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. Subsequently, the animals were re-housed (3 or 4 animals/cage), and 1 week of recovery followed, with no treatment. During this time, control animals were socially reared with no ketamine treatment.

Starting at the age of 9 weeks, all of the animals were involved in TF and the sensory gating (pre-pulse inhibition: PPI) tests. The PPI of the acoustic startle response was measured as described previously [3]. Briefly, after 10 min habituation in Plexiglas startle chambers (12x17x15.3 cm) rats were exposed to two different trial types: the pulse alone (PA), in which a 40 ms 95 dB white noise burst was presented; and the prepulse - pulse pair (PP) in which prepulse stimuli (20 ms, 76 dB) were followed by the startle stimulus with a latency of 150 ms. Both types of stimuli were applied 20 times in random pattern.

The interstimulus intervals ranged from 7 to 13 s. PPI was calculated as percentages using the following equation: PPI (%) = [1 − (*startle response for PP*) / (*startle response for PA*)] × 100.

Furthermore, the locomotor and exploratory activities along with the reward consumption were assessed in the AMBITUS with Task-1 and Task−2 (see below) on two consecutive days (−2nd and -1st, as pre-phase) repeated two times with 1 min apart (1st and 2nd trials; Table 1; Fig. 1C).
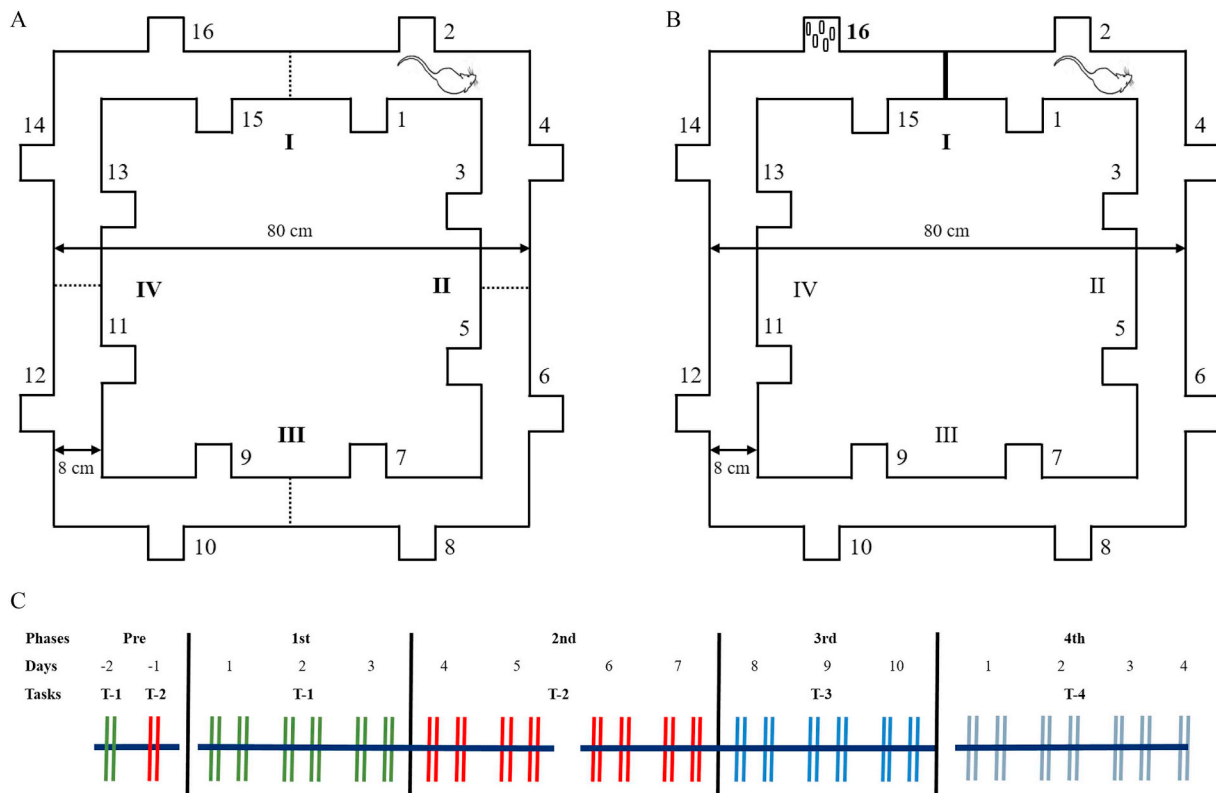
2.3. The AMBITUS apparatus

A rectangular corridor was constructed of clear Plexiglas on black floor with an external diameter of 80 cm, width of 8 cm and height of 50 cm, where the rats could move around between the walls forward and backward [7] (Fig. 1A–B). Each of the four corridors has four side-boxes (2 on the internal wall, 2 on the external wall) of equal size (5 × 5 × 5 cm) with food reward (puffed rice: 20 mg); altogether 16 side-boxes belong to the entire system. Each side-box was equipped with an infrared LED (light emitting diode) at one side and a photocell at the other side to measure the exploratory activity (or visits) into the boxes, while the locomotor activity was detected by infrared beams located midway in each corridor, at 1 ms time resolution.

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. 1A–B); 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: 300 s), and the number of food rewards eaten (Eat/Cn) was recorded at the end of each trial. The apparatus was cleaned with 70% alcohol after each trial. Experiments were recorded using an infrared video device (WCM-21VF, CNB, China) fastened above the apparatus. When the animals had eaten all the available food rewards, the video recordings were analyzed offline to determine the time required to complete the task (eating time: Eat/T).

2.4. 10-day long experimental paradigm

One week after the routinely executed behavioral tests, two groups of the animals (Wistar and Wisket; $n = 8$/group) were involved in a 10-day long experiment in the AMBITUS system in three phases defined by the task the animals had to perform (Task-1-3, Table 1; Fig. 1A,C).

In phase 1 (Day 1–3, 12 trials) all the internal and external boxes were baited with puffed rice (Task-1: 16 rewards), in phase 2 (Day 4–7, 16 trials) only the internal boxes (Task-2: 8 rewards), while in phase 3 (Day 8–10, 12 trials) only the external ones (Task-3: 8 rewards) were baited. All of the rats performed two sessions (two trials/session, 1 min apart) of tasks per day, one in the morning and another about 3 h later (4 trials/day; Fig. 1C). The training was interrupted for 2 days between days 5 and 6 (between trials 20 and 21). Altogether, 44 trials were completed, including the pre-phase.

Table 1
Experimental protocol for selective breeding and behavioral testing.

Age (weeks)	4	4	5–7	8	9		10–11			14
Phases						Pre	1st	2nd	3rd	4th
Days						−2, −1	1–3	4–7	8–10	1–4
Control trained group	Tail-flick test	Social rearing (3–4 rats/cage)			Tail-flick test	Pre-pulse inhibition test	AMBITUS Task-1	AMBITUS Task-1 Task-2 Task-3		AMBITUS Task-4
Control non-trained group							No testing			
Wisket trained group		Social isolation	Social isolation + ketamine treatment	Social rearing			AMBITUS Task-1 Task-2 Task-3			
Wisket non-trained group							No testing			

Fig. 1. Ground plan of the corridor (Ambitus) with 16 side-boxes. The height of the apparatus is 50 cm. During task-1, 2 and 3 in the middle of the 4 corridors (I-IV) dashed lines indicate the photo beams (A). The line in the middle of the corridor I. represents the gate that closed the corridor during Task-4 (B), with the 5 rewards in box 16. The black rat silhouette indicates the starting point of the animals. C: Time scale of the AMBITUS test paradigm.

2.5. Single-box rewarded paradigm

To demonstrate whether the previously trained control and Wisket rats are able to learn a novel task faster than rats not exposed to the 10-day long experiment in the AMBITUS, two other groups of animals (non-trained Wistar and Wisket) were also involved at similar age (*n* = 8/group; Table 1). Thus, a 4-day long test with a new task (Task-4, phase 4) was introduced 17 days after the completion of the 10-day long paradigm.

During Task-4, a door was fixed in the middle of the corridor I., therefore, the animals' movement was partially restricted (Fig. 1B). In phase 4 (Day 1–4, 14 trials) the 16th external box was baited with 5 pieces of puffed rice (Task-4: 5 rewards in a single-box; Fig. 1B,C). Therefore, during the task the animals had to run the whole AMBITUS to reach the rewards. The animals were allowed to explore the corridors and collect food rewards for 5 min (cut-off time: 300 s), and the number of food rewards eaten (Eat/Cn) was recorded at the end of each trial. As in the 10-day long experiment, all of the rats performed two sessions (two trials/session, 1 min apart) of tasks per day, one in the morning (1–2 trials) and another (3–4 trials) about 3 h later (4 trials/day), except on Day 4, when only two trials were performed.

2.6. Data and statistical analysis

Tables 2–4 show the definitions, the equations and the results of the statistical analyses of manifold, partially dependent parameters, which were either recorded automatically by photocells or manually by the experimenter, or were calculated from the baseline data by a custom-made software (LP). The parameters were analyzed up to consumption all the food rewards (pre-eating) and during the whole 5-minute period (total). The pre-eating values were calculated to 5 min to be independent from eating time (Eat/T; see Tables for equations). In the case of those animals that did not eat all of the rewards in 300 s, the pre-

eating and the total behavioral parameters were equal.

Data are expressed as means ± SEM. Data obtained during the 10-day long training were analyzed by repeated measurements ANOVA. The repeated measurements were trials within a single day. The factors were: group, day and side (external or internal, if available). To reveal the behavioral differences between the two groups during the different tasks, separate analysis was performed for all of the experimental phases (pre-, 1st-3rd phases).

To reveal the significant changes with the repetition during the single-box rewarded paradigm, and to reveal the delayed effect of practice, only the mean of the 1st and 2nd trials of each day were analyzed by factorial ANOVA. The factors were: group (Wistar vs. Wisket), condition (trained vs. non-trained groups) and days (1–4).

Only probabilities lower than 0.05 were considered significant. For the analyses, STATISTICA 13.1 (Dell Inc. Round Rock, Texas, US) was used.

Color-coded grid plots (2D grid with a color-coded scale for a 3rd variable) of parameter matrices were created for the 44 samples, as the ratio of data obtained from Wisket and control animals, to generate a compact representation of the results, where each trial is represented by a colored square. Green color indicates that in the given trial the two groups behaved similarly. Yellow to brown colors mean that the Wisket rats showed a significantly higher value in the given parameter and in the given trial (e.g. eating time, which was typically longer). In contrast, colors toward the dark blue end of the spectrum indicate lower values in the new substrain (e.g. exploration). The original time-course data for both groups are presented in graphs attached to the grid-plots.

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

3. Results

In agreement with our recent studies [3–6,8], Wisket rats show

decreased pain sensitivity indicated by longer tail-flick latency (TF) at both 3 (Wistar: 4.2 ± 0.21, Wisket: 5.6 ± 0.56 s; $p < .05$) and 9 (Wistar:7.2 ± 0.52, Wisket:16.5 ± 1.19 s; $p < .001$) weeks of age, impaired sensory gating (Wistar:52 ± 6.3, Wisket:26 ± 8.3%; $p < .05$), cognitive function and exploratory activity during the pre-phase (Tables 2–3 and Fig. 2–3). Regarding the body weight of the animals, due to the food restriction, the weight of the animals did not increase significantly in either group, however, the new substrain had lower body weight during the whole investigated phases (Wistar: 285 ± 8.4, Wisket: 207 ± 6.2 g; p < .001).

3.1. Results obtained during the 10-day long training session

3.1.1. Locomotion-related parameters

Most of the animals entered in each of the four corridors, therefore, there were no significant differences in the number of discovered corridors (DC) between the two groups at any phases (Table 2. row 0). The locomotor activity up to the consumption all of the available food rewards (pre-eating period: Loco/Bef) did not differ significantly between the two groups (Table 2 and Fig. 2, row 1), but when considering the entire trial (300 s, Loco/Tot), it was significantly lower in the Wisket rats during the 3rd phase (Day 8–10; Table 2. and Fig. 2, row 2). The locomotor activity decreased when the trial was repeated after one-minute break in both groups (especially in the first few days), indicating that the animals showed decreased interest for the corridor after this short delay. The amplitude of this zig-zag pattern of activity was higher in the Wisket rats compared to the controls. This is confirmed by the significant group-trial interactions, which led to a striped pattern in the grid-plot (Table 2. and. Fig. 2. rows 1–2.). A low level of locomotor activity was detected during the 1st phase, but as the number of the available food-rewards decreased (from 16 to 8), it increased and was maintained afterwards.

3.1.2. Exploration-related parameters

The Wisket rats explored only a fraction of the side-boxes (exploratory area: EA; max. 8–8 on both sides; Table 2. and Fig. 2. rows 3–5), thus, they showed lower values on both sides during the pre- and 1st phases compared to the controls. The introduction of Task-2 during the 2nd phase resulted in decreased EA on the external side in both groups. Since the controls explored almost all of the internal side-boxes during phase 1, this parameter could not increase any further with the introduction of Task-2, while an enhancement in the internal side exploration was detected in Wisket rats. During Task-3 both groups explored most of the external side-boxes.

In agreement with our recent study [7], no significant differences were detected in the duration of a single exploration between the two groups (about 1 s), therefore, the parameters related to the number of explorations were analyzed. In contrast to locomotion, the exploratory activity was significantly lower in the Wisket rats in all phases of the experiment (Table 2. and Fig. 2. rows 6–11). The overall pre-eating exploratory activity continuously increased in both groups, especially if a new task was introduced (Table 2. and Fig. 2. row 6). The introduction of Task-2 or 3 led to an increase in the pre-eating exploratory activity toward the rewarded side-boxes with similar pattern for both groups (but lower level for the Wiskets; Table 1. and Fig. 2. rows 6–8, 15), but the side-preference were less pronounced, when the whole 5-minute period was analyzed (Table 2. and Fig. 2. row 16), thus as the animals consumed all of the rewards, they started to investigate the contralateral side, as well. The ratio of overall exploratory and loco-motor activities was significantly lower in the Wisket group in the pre-, 1st and 2nd phases compared to controls, thus, these animals investigated fewer side-boxes during their locomotion, indicating attention deficit (Table 2. and Fig. 2. rows 12–13).

Another marked difference between the groups was that the Wisket rats had higher preference for the external side-boxes as first exploration in the 1st and 2nd phases (Table 2. and Fig. 2. row 14), and the latency of the first exploration was also longer in this group up to the last two days (Table 3. and Fig. 3, rows 1–3). The number of visited corridors up to the first exploration (skipping) was higher in Wisket rats during the pre- and first phases, showing a zig-zag pattern with high amplitude, which suggests impaired attention in these animals (Table 3. and Fig. 3. row 5). However, this difference between the two groups lessened with the training.

The pattern of repetitive explorations also depended on the location of the food rewards: the animals repeatedly explored the food-rewarded side-boxes, but the Wisket rats showed a lower degree of repetitive behaviors compared to controls, especially into the internal side-boxes (Table 3. and Fig. 3. rows 6–11).

3.1.3. Parameters related to reward consumption

All of the control rats were able to successfully learn Task-1 by the end of the third day, while only half of the new substrain ($n = 4$) consumed all of the food rewards. Thus, both the number of consumed food rewards (Eat/Cn; Table 2. and Fig. 2. row 17) and the time required to complete the task (Eat/T Table 3. and Fig. 3. rows 4) differed significantly between the two groups during this phase. The Wisket rats consumed significantly fewer rewards during the first few days of the second phase as well, however, they caught up with controls by the end. However, their eating time was longer for all of the phases, and the Wisket rats caught up with the control group only on the last day 10. The introduction of new tasks caused temporary decrease in the food consumption in both groups.

Regarding the learning capacity (LC), the new substrain had lower values up to the third phase (Table 2. and Fig. 2. row 18). The introduction of Task-2 led to an enhancement, while Task-3 caused a temporary decrease in LC in both groups.

3.2. Results obtained during the single-box rewarded paradigm

During this task the statistical analysis of both the exploratory and food consumption related parameters show significant effects of group and condition (trained vs non-trained) (Table 4, Fig. 4A–E). Thus, the non-trained animals had lower level of exploratory activity and eating behavior compared to the trained ones, and the Wisket non-trained animals had the lowest values in these respects. Regarding the effects of the 4-day practice, the exploration of non-baited boxes decreased during the days, while the baited box exploration increased primarily in the non-trained control animals on day 4, and this behavior was accompanied with enhanced LC value in this group, too.

4. Discussion

This study firstly proved that the beneficial behavioral effects of cognitive training can be replicated preclinically, revealing the predictive validity of this schizophrenia animal model. At the beginning of the training Wisket rats showed altered locomotion pattern accompanied by a low degree of exploratory activity, inattentive behavior and slow performance, which ultimately added up to impaired learning ability. However, in spite of the permanently decreased exploration, most of the parameters reached levels close to normal with training. The introduction of a new behavioral task (single-box rewarded paradigm) 2 weeks after the 10-day training revealed that both the control and Wisket rats had long-term improvement in their motivation and cognitive functions.

Quantitative motor abnormalities, both paucity and excess, are common in schizophrenia, so much so that motor symptoms are used as diagnostic criteria [19,20]. Altered activity has also been noted in several rat models of schizophrenia, but these changes were observed in open field [4,23–26]. In the AMBITUS system, the most obvious finding was the large amplitude with which locomotor activity fluctuated within one session in the Wisket animals, especially during the first part of the experiment. The low level of locomotion after 1 min delay may

Table 2

Definitions, abbreviations, means and significances of the different parameters detected in the AMBITUS system, and shown in Fig. 2.

Parameter/Definition	Significance F;(df);p	Days -1;0 (4 trials) C	W	Days: 1-3 (Task-1: 12 trials) C	W	Days: 4-7 (Task-2: 16 trials) C	W	Days: 8-10 (Task-3: 12 trials) C	W
0. DC: Discovered corridors: number of different corridors entries during 5 min (max 4)	Mean ± SEM	3.8±0.10	3.8±0.11	3.7±0.08	3.7±0.10	4.0±0.01	3.8±0.06	4.0±0.00	4.0±0.00
	Trial			4.21;(3,126);<0.01					
	Group/Trial			3.45;(3,126);<0.05					
1. Loco/Bef: Locomotion Before: (number of entries into corridors until all reward collection)x300/(Eat/T)	Mean ± SEM	15.7±1.35	16.5±1.62	12.8±0.55	12.2±0.76	27.0±0.65	23.9±1.14	28.9±0.65	28.6±0.91
	Trial	134.99;(1,28);<0.001		18.89;(3,126);<0.001		9.66;(3,168);<0.001		6.83;(3,126);<0.001	
	Day	5.50;(1,28);<0.05							
	Group/Trial			10.79;(3,126);<0.001					
2. Loco/Tot: Locomotion-Total: number of entries into corridors up to 5 min	Mean ± SEM	15.6±1.18	16.5±1.58	13.4±0.62	13.0±0.81	18.5±0.56	16.5±0.70	22.5±0.62	18.8±0.64
	Group							5.55;(1,42);<0.05	
	Trial	116.95;(1,28);<0.001		17.04;(3,126);<0.001		20.53;(3,168);<0.001		16.84;(3,126);<0.001	
	Day	7.37;(1,28);<0.05							
	Group/Trial			8.49;(3,126);<0.001		2.90;(3,168);<0.05			
	Day/Trial					2.52;(9,168);<0.01			
3. EA/EI: Overall explored area: number of different box visits until all reward collections (max. 16)	Mean ± SEM	12.7±0.53	8.6±0.62	14.8±0.33	9.2±0.65	12.1±0.24	9.6±0.35	12.7±0.25	10.9±0.27
	Group	24.02;(1,28);<0.001		15.14;(1,42);<0.001		13.63;(1,56);<0.001		28.91;(1,42);<0.001	
	Trial	4.23;(1,28);<0.05		3.85;(3,126);<0.05				4.44;(3,126);<0.01	
	Day					4.31;(3,56);<0.01		59.26;(2,42);<0.001	
	Group/Trial			3.17;(3,126);<0.05		3.92;(3,168);<0.01			
4. EA/E: Externally explored area: number of different external box visits until all reward collections (max. 8)	Mean ± SEM	6.2±0.31	5.2±0.42	7.4±0.17	4.7±0.33	4.1±0.24	2.7±0.23	8.0±0.01	7.9±0.0
	Group	5.58;(1,28);<0.05		14.17;(1,42);<0.001		10.24;(1,56);<0.005			
	Trial	15.87;(1,28);<0.001		3.38;(3,126);<0.05					
	Day	6.27;(1,28);<0.05				14.97;(3,56);<0.001			
	Group/Trial			2.72;(3,126);<0.05					
	Day/Trial					2.18;(9,168);<0.05			
5.EA/I: Internal explored area : number of different internal box visits until all reward collections (max. 8) internal box visits until all reward collections (max. 8)	Mean ± SEM	6.5±0.31	3.4±0.36	7.4±0.17	4.5±0.34	8.0±0.02	6.9±0.21	4.7±0.25	2.9±0.27
	Group	38.44;(1,28);<0.001		15.86;(1,42);<0.001		7.30;(1,56);<0.01		27.92;(1,42);<0.001	
	Trial			3.30;(3,126);<0.05		5.01;(3,168);<0.005		4.78;(3,126);<0.005	
	Day							61.09;(2,42);<0.001	
	Trial			3.30;(3,126);<0.05		5.01;(3,168);<0.005		4.78;(3,126);<0.005	
	Day							61.09;(2,42);<0.001	
	Group/Trial			2.85;(3,126);<0.05		4.27;(3,168);<0.01			
	Day/Trial	5.05;(1,28);<0.05							
6. Exp/Bef/EI: (overall number of box visits until all reward collection)x300/(Eat/T)	Mean ± SEM	39.5±4.35	13.8±1.40	57.1±2.24	32.0±3.29	84.9±2.20	61.4±3.28	97.1±2.31	75.3±2.71
	Group	28.50;(1,28);<0.001		10.72;(1,42);<0.01		9.89;(1,56);<0.005		16.83;(1,42);<0.001	
	Trial			5.66;(3,126);<0.005		3.53;(3,168);<0.05		2.76;(3,126);< 0.05	
	Day	8.42;(1,28);<0.01						6.24;(2,42);< 0.005	
	Group/Day	6.60;(1,28);<0.05							
7. Exp/Bef/E: (number of external box visits until all reward collection)x300/(Eat/T)	Mean ± SEM	16.7±1.55	8.7±0.83	28.5±1.17	16.7±1.70	24.0±1.46	13.4±1.48	64.7±1.32	58.6±1.77
	Group	18.19;(1,28);<0.001;		9.02;(1,42);<0.005		11.70;(1,56);<0.005			
	Trial	10.83;(1,28);<0.005		6.51;(3,126);<0.001				7.23;(3,126);<0.005	
	Day					9.46;(3,56);<0.001			
	Group/Day	7.31;(1,28);<0.05						2.83;(6,126);<0.05	
	Day/Trial					2.08;(9,168);<0.05			
8. Exp/Bef/I: (number of internal box visits until all reward collection)x300/(Eat/T)	Mean ± SEM	22.9±3.12	5.1±0.82	28.6±1.14	15.3±1.62	60.9±1.44	48.0±2.52	32.4±1.82	16.7±1.76
	Group	32.66;(1,28);<0.001		12.43;(1,42);<0.005		5.57;(1,56);<0.05		27.85;(1,42);<0.001	
	Trial			5.04;(3,126);<0.005		11.06;(3,168);<0.001			
	Day	15.64;<0.001						30.82;(2,42);<0.001	
	Group/Day	8.73;(1,28);<0.01							
9. Exp/Tot/EI: overall number of box visits up to 5 min	Mean ± SEM	35.3±2.94	13.9±1.42	40.9±1.38	25.2±2.37	31.7±0.73	25.7±1.15	36.7±0.89	30.6±0.93
	Group	41.73;(1,28);<0.001		8.49;(1,42);<0.01		7.10;(1,56);<0.05		16.97;(1,42);<0.001	
	Trial			6.07;(3,126);<0.001				16.01;(3,126);<0.001);	
	Day	8.99;(1,28);<0.01						19.41;(2,42);<0.0001	
	Group/Day	6.00;(1,28);<0.05							

(continued on next page)

Table 2 (*continued*)

10. Exp/Tot/E: number of external box visits up to 5 min	Mean ± SEM	16.3±1.31	8.7±0.82	21.6±0.77	13.9±1.32	13.4±0.45	10.3±0.61	23.3±0.45	20.8±0.53
	Group	22.60;(1,28);<0.001		6.78;(1,42);<0.05		7.16;(1,56);<0.01		7.02;(1,42);<0.05	
	Trial	5.54;(1,28);<0.05		8.42;(3,126);<0.001		4.06;(3,168);<0.01		6.44;(3,126);<0.001	
	Day					4.85;(3,56);<0.005			
11. Exp/Tot/I: number of internal box visits up to 5 min	Mean ± SEM	18.9±1.83	5.2±0.89	19.2±0.66	11.2±1.08	18.3±0.40	15.4±0.65	13.4±0.60	9.8±0.55
	Group	54.25;(1,28);<0.001		10.53;(1,42);<0.005		5.14;(1,56);<0.05		21.53;(1,42);<0.001	
	Trial	20.92;(1,28);<0.001				3.55;(3,168);<0.005;		18.22;(3,126);<0.001	
	Day							44.92;(2,42);<0.001	
	Group/Day	7.61;(1,28);<0.05							
12. Ratio of exploration and locomotion before: Exp/Loco/Bef/EI = (Exp/Bef/EI)x100/(Loco/Bef)	Mean ± SEM	3.0±0.31	1.1±0.19	5.0±0.21	2.4±0.23	3.3±0.08	2.5±0.11	3.4±0.08	2.9±0.21
	Group	34.29;(1,28);<0.001		20.49;(1,39);<0.001		11.76;(1,54);<0.005			
	Trial	33.25;(1,28);<0.001		4.58;(3,117);<0.0051;		7.80;(3,162);<0.001;			
	Day	10.79;(1,28);<0.005				5.31;(3,54);<0.005;		3.75;(2,42);<0.05	
	Group/Trial	8.53;(1,28);<0.01							
	Day/Trial							2.46;(6,126);<0.05	
13. Ratio of exploration and locomotion up to 5 min: Exp/Loco/Tot/EI = (Exp/Tot/EI)x100/(Loco/Tot)	Mean ± SEM	2.6±0.25	1.0±0.16	3.8±0.23	1.8±0.17	1.9±0.07	1.6±0.09	1.8±0.08	1.8±0.07
	Group	33.36;(1,28);<0.001		12.68;(1,39);<0.001					
	Trial	31.23;(1,28);<0.001				8.50;(3,162);<0.001		19.00;(3,126);<0.001	
	Day	7.39;(1,28);<0.05							
	Group/Trial	7.01;(1,28);<0.05				6.24;(3,162);<0.001			
	Day/Trial							2.40;(3,162);<0.05;	
	Group/Day/Trial	5.54;(1,28);<0.05							
14. Pref/Exp/1st: the side of the firstly visited box (1 for internal; 2 for external)	Mean ± SEM	1.4±0.09	1.7±0.09	1.4±0.05	1.7±0.05	1.2±0.04	1.4±0.04	1.7±0.05	1.8±0.04
	Group			8.18;(1,33);<0.01		4.04;(1,52);<0.05			
	Trial							4.29;(3,126);<0.01	
	Day							27.89;(2,42);<0.001	
15. Pref/Exp/Bef: Side preference before: The ratio of number of explorations into the external vs all of the boxes until all reward collections	Mean ± SEM	44.5±2.64	62.5±3.70	50.2±0.58	54.7±2.20	26.7±1.31	22.6±1.61	68.4±1.41	79.8±1.66
	Group	32.86;(1,27);<0.001				4.20;(1,52);<0.05		30.77;(1,42);<0.001	
	Trial	29.22;(1,27);<0.001				5.19;(3,156);<0.005		6.11;(3,126);<0.001	
	Day	45.05;(1,27);<0.001				24.42;(3,52);<0.001		54.18;(2,42);<0.001	
	Day/Trial			2.41;(6,108);<0.05		3.24;(9,156);<0.005			
	Group/Day/Trial					1.95;(9,156);<0.05			
16. Pref/Exp/Tot: Side preference total: The ratio of the number of explorations into the external vs all of the boxes up to 5 min	Mean ± SEM	46.7±2.33	62.4±3.73	53.1±0.53	56.3±2.18	41.4±0.78	38.3±1.19	64.7±0.90	69.8±1.10
	Group	22.45;(1,27);<0.001						13.65;(1,42);<0.001	
	Trial	27.05;(1,27);<0.001				5.55;(3,156);<0.005		12.35;(3,126);<0.001	
	Day	33.18;(1,27);<0.001		3.28;(2,36);<0.05		13.05;(3,52);<0.001		40.49;(2,42);<0.001	
	Group/Trial	4.33;(1,27);<0.05							
	Group/Day					3.06;(3,52);<0.05			
17. Eat/Cn: Number of rewards eaten from all baited boxes (max. 8 or 16)	Mean ± SEM	9.1±0.53	2.1±0.49	14.6±0.38	7.3±0.77	7.9±0.09	6.6±0.26	8.0±0.00	7.9±0.03
	Group	94.77;(1,28);<0.001		17.61;(1,42);<0.001		6.90;(1,56);<0.05			
	Trial			3.20;(3,126);<0.05		5.21;(3,168);<0.005			
	Group/Day	10.91;(1,28);<0.005							
	Day/Trial	6.38;(1,28);<0.05							
18. LC: Learning capacity (%): [(Eat/Cn)x(300)x100]/[number of reward rewards)x(Eat/T)]	Mean ± SEM	129.6±19.12	22.9±6.43	225.4±11.62	96.7±12.50	607.5±18.80	471.0±27.45	659.9±17.12	602.7±21.00
	Group	29.46;(1,28);<0.001		18.40;(1,42);<0.001		5.17;(1,56);<0.05			
	Trial			9.23;(3,126);<0.001		7.71;(3,168);<0.001		17.39;(3,126);<0.001	
	Day	13.62;(1,28);<0.001						6.55;(2,42);<0.005	
	Group/Day	5.45;(1,28);<0.05							
	Day/Trial			2.62;(6,126);<0.05				3.27;(6,126);<0.01	

Abbreviations: Exp: exploration; Bef: before eating all of the food reward; C: control; Cn: count; df: degree of freedom; E: external side-boxes; Eat/T: time to collect all of the available rewards; I: internal side-boxes; Tot: total time of the trial (5 min); W: Wisket. The blue color signs the significant effects of group or group/trial interactions marked in Fig. 2.

suggest that these animals lost their interest within a short period, while its high level after a longer delay may imply that Wisket rats forgot the environment.

While Wisket rats had lower level of exploratory activity during all phases, the pre-eating exploration increased during the training days in both groups, suggesting a gradually enhanced motivation in Wistar and Wisket rats, too. The perseverative behavior (repetition of explorations) was also generally lower in the Wisket animals, which might be due to their lower level of exploratory activity in general. While several studies demonstrated increased perseveration in schizophrenic patients [27–29], our data are in agreement with recent human data, which showed decreased repetitive behavior in patients characterized by a dominance of positive psychotic symptoms [30]. As the method is based on the rats' natural tendency to explore their environment, factors such as lack of general exploratory drive and/or the ability to initiate actions (which relate closer to the negative symptoms of schizophrenia than to the cognitive deficits) could affect the investigation of the side-boxes. All these findings, along with the longer latency of the first exploration and more frequent skipping of boxes suggest a low degree of motivation and/or attention in the Wisket rats. These results suggest that Wisket substrain is a good model of schizophrenia in terms of motivation, attention and executive functions [31–33].

The introduction of Tasks-2 or 3 brought about notable side-preference for the baited side in both groups, revealing that the animals learned to skip unrewarded side-boxes. Regarding the food-collecting activity of the animals, the 10-day long training session revealed that

both groups could acquire these simple tasks, learn the new paradigms and improve their performance over time. Ultimately, the Wisket rats were able to collect all the food rewards just like the controls, but they needed more trials. However, the eating time in the Wisket rats were longer than controls until the last day, which was due, at least partially, to the fact that the first visit into a box delayed. In this respect, it is interesting to note that processing speed is the domain that accounts for most of the differences in the neurocognitive performance between schizophrenia patients and healthy controls [34–36].

The long-term repetition of the similar tasks (Task-1-3) shows a simple learning curve that is not necessarily an exhibition of improved cognitive/motivational ability. Therefore, the results obtained during the 10-day session might suggest that the AMBITUS task reveals not 'normalization' of cognitive and motivational parameters, but actually impaired learning acquisition of the tasks in Wisket rats. However, the introduction of a new task after a 17-day long break in the same system robustly demonstrated the long-term beneficial effects of cognitive training primarily in the Wisket but also in the control animals.

Increased stress reactivity has repeatedly been reported in patients suffering from psychiatric diseases [35,37], and it is suggested that cognitive deficits in schizophrenia may arise, at least partially, from the increased stress sensitivity and the consequently decreased motivation/attention [31,35,38]. In accordance with the observation that animals with a higher degree of anxiety prefer the peripheral region in open field [4,39], our data revealed that the external side boxes were recognized and preferred by Wisket rats. This points to a higher

Table 3

Definitions, abbreviations, means and significances of the different parameters detected in the AMBITUS system, and shown in Fig. 3.

Parameter/Definition	Significance	Days -1; 0 (4 trials)		Days: 1-3 (Task-1: 12 trials)		Days: 4-7 (Task-2: 16 trials)		Days: 8-10 (Task-3: 12 trials)	
	F;(df);p	C	W	C	W	C	W	C	W
1. Lat/EI (s): Latency of the first exploration into any boxes	Mean ± SEM	3.8±0.99	32.9±10.55	3.6±0.46	52.3±9.44	2.6±0.17	25.7±6.22	2.3±0.07	2.3±0.14
	Group	6.22; (1,28); <0.05		11.41; (1,42); <0.005		5.02; (1,56); <0.05			
	Trial			2.72; (3,126); <0.05		4.57; (3,168); <0.005			
	Group/Trial			2.78; (3,126); <0.05		4.70; F(3,168); <0.005			
2. Lat/E (s): Latency of the first exploration into an external box	Mean ± SEM	29.0±10.05	65.3±15.89	7.5±0.75	72.2±10.81	48.4±8.50	95.5±11.10	2.9±0.15	4.8±1.52
	Group	4.55; (1,28); <0.05		14.07; (1,42); <0.001		8.15; (1,56); <0.01			
	Trial	13.75; (1,28); <0.001				2.76; (3,168); <0.05			
	Day					9.19; (3,56); <0.001		3.78; (2,42); <0.05	
	Group/Trial					4.56; (3,168); <0.005			
	Day/Trial							2.20; (6,126); <0.05	
3. Lat/I (s): Latency of the first exploration into an internal box	Mean ± SEM	16.4±5.08	69.3±14.22	7.1±1.28	85.3±11.85	2.8±0.17	27.9±6.39	16.1±4.38	84.5±12.50
	Group	12.33; (1,28); <0.005		18.16; (1,42); <0.001		5.31; (1,56); <0.05		31.82; (1,42); <0.001	
	Trial					4.72; (3,168); <0.005		6.73; (3,126); <0.001	
	Day							16.70; (2,42); <0.001	
	Group/Trial					4.85; (3,168); <0.005		3.70; (3,126); <0.05	
	Group/Day							8.99; (2,42); <0.001	
4. Eat/T (s): Time required to consume all available rewards, or 300 s (cut-off time)	Mean ± SEM	245.8±14.35	296.2±2.62	157.8±8.07	244.1±8.61	57.8±3.34	117.7±9.60	49.1±1.58	64.1±5.56
	Group	11.87; (1,28); <0.005		19.49; (1,42); <0.001		9.54; (1,56); <0.005		4.59; (1,42); <0.05	
	Trial			6.52; (3,126); <0.001		4.88; (3,168); <0.005		8.24; (3,126); <0.001	
	Day	13.55; (1,28); <0.001		3.60; (2,42); <0.05				5.59; (2,42); <0.01	
	Group/Day	9.98; (1,28); <0.005							
	Day/Trial			2.49; (6,126); <0.05				3.31; (6,126); <0.005	
5. Skipping: number of corridor entries before the first visit into a side-box	Mean ± SEM	1.6±0.13	4.1±0.49	1.5±0.03	2.6±0.22	1.1±0.04	1.6±0.13	1.2±0.04	1.2±0.05
	Group	26.62; (1,28); <0.001		5.45; (1,41); <0.05					
	Trial			2.78; (3,123); <0.05		3.28; (3,162); <0.05			
	Day	4.73; (1,28); <0.05							

Table 3 (*continued*)

6. Rep/Cn/Bef/EI: Overall repetition before: (number of repetitive visits until all rewards consumed) x300/(Eat/T)	Mean ± SEM	19.9±2.28	5.0±0.77	20.6±1.09	14.7±1.74	12.7±0.84	11.7±1.04	15.1±1.34	12.4±1.33
	Group	39.34; (1,28); <0.001							
	Trial							4.97; (3,126); <0.005	
	Day	9.23; (1,28); <0.01						13.59; (2,42); <0.001	
	Group/Day	4.93; (1,28); <0.05							
	Day/Trial	11.36; (1,28); <0.005							
	Group/Day/Trial	10.70; (1,28); <0.005						2.20; (6,126); <0.05	
7. Rep/Cn/Bef/E: (number of repetitive visits into external boxes until all rewards consumed)x300/(Eat/T)	Mean ± SEM	7.5±1.04	3.4±0.46	10.2±0.67	8.0±0.97	1.2±0.24	1.6±0.44	12.1±0.90	10.4±1.04
	Group	18.58; (1,28); <0.001							
	Trial	8.80; (1,28); <0.01		3.51; (3,126); <0.05		3.08; (3,168); <0.05		2.74; (3,126); <0.05	
	Day					5.96; (3,56); <0.005		4.87; (2,42); <0.05	
	Group/Trial					3.27; (3,168); <0.05			
	Day/Trial	11.00; (1,28); <0.005				3.14; (9,168); <0.005			
	Group/Day/Trial	7.98; (1,28); <0.01				3.38; (9,168); <0.001			
8. Rep/Cn/Bef/I: (number of repetitive visits into internal boxes until all rewards consumed)x300/(Eat/T)	Mean ± SEM	12.4±1.75	1.5±0.46	10.1±0.56	6.7±0.83	11.6±0.71	10.0±0.86	3.1±0.70	2.0±0.51
	Group	39.08; (1,28); <0.001		4.47; (1,42); <0.05					
	Trial							8.65; (3,126); <0.001	
	Day	18.12; (1,28); <0.001						28.15; (2,42); <0.001	
	Group/Trial							4.17; (3,126); <0.01	
	Group/Day	8.62; (1,28); <0.01							
	Day/Trial							6.02; (6,126); <0.001	
	Group/Day/Trial	4.24; (1,28); <0.05		2.91; (6,126); <0.01				3.58; (6,126); <0.005	
9. Rep/Cn/Tot/EI: Overall repetition total: number of repetitive visits up to 5 min	Mean ± SEM	22.5±2.60	5.3±0.88	26.1±1.15	16.0±1.78	19.3±0.63	16.3±0.89	24.0±0.75	19.7±0.80
	Group	41.14; (1,28); <0.001		6.25; (1,42); <0.05				(1,42); 9.84; <0.005	
	Trial			4.83; (3,126); <0.005				(3,126); 14.79; <0.001	
	Day	13.96; (1,28); <0.001						(2,42); 8.36; <0.001	
	Group/Day	7.61; (1,28); <0.05							
10. Rep/Cn/Tot/E: number of repetitive visits into external boxes up to 5 min	Mean ± SEM	10.1±1.14	3.5±0.48	14.3±0.65	9.3±1.03	9.2±0.38	7.7±0.51	15.3±0.45	12.9±0.53
	Group	25.78; (1,28); <0.001		4.74; (1,42); <0.05				6.84; (1,42); <0.05	
	Trial			7.65; (3,126); <0.001		4.68; (3,168); <0.005		6.77; (3,126);<0.001	
11. Rep/Cn/Tot/I: number of repetitive visits into the internal boxes up to 5 min	Mean ± SEM	12.4±1.59	1.8±0.58	11.8±0.55	I 6.7±0.78	10.1±0.41	8.7±0.49	8.7±0.44	6.9±0.40
	Group	49.80; (1,28); <0.001		8.19; (1,42); <0.01				8.40; (1,42); <0.01	
	Trial							18.44; (3,126); <0.001	
	Day	25.17; (1,28); <0.001						16.76; (2,42); <0.001	
	Group/Trial							3.17; (3,126); <0.05	
	Group/Day	9.88; (1,28); <0.005							

Abbreviations: Bef: before eating all of the food reward; C: control; Cn: count; df: degree of freedom; E: external side-boxes; I: internal side-boxes; Tot: total time of the trial (5 min); W: Wisket The blue color signs the significant effects of group or group/trial interactions marked in Fig. 3.

degree of anxiety in this substrain compared to controls. Increasing familiarity with the experimental conditions across trials and/or increasing mastery of the task led to a gradual increase in pre-eating exploratory activity and LC in both groups. The fact that from about the seventh day Wisket rats consumed the same amount of rewards as controls may reflect that the learning impairment was greatly overcome by the repetition of the tasks. Since cognitive training also improves cognitive performance in schizophrenic patients, and it can decrease the chance of acute psychiatric admission, too [13–18,30], we suggest the predictive validity of this model in this respect. Several brain structures (e.g. prefrontal and temporal cortices, subcortical nuclei) and neurotransmitter systems (e.g. dopaminergic, glutamatergic,

serotoninergic and GABAergic) disturbed in schizophrenia, are involved in the cognitive functions, too. The beneficial effects of cognitive training might be associated with changes in these systems, and their disruption in schizophrenia may be improved by cognitive remediation [17,18,38,40–42]. Therefore, further imaging, electrophysiological and/or molecular biological studies are warranted to explore the structural and functional changes in Wisket rats, and their potential improvements after cognitive training.

The lack of appropriate controls – a Wisket group without isolation housing and ketamine; and a Wistar group with isolation housing and ketamine – to determine the contribution of the different intervention to the face validity of the model might be seen as a limitation of this

Table 4
Definitions, abbreviations, means and significances of the different parameters analyzed during Task-4, and shown in Fig. 4.

Parameter/Definition	Significance	Trained/1-4 days mean 2-trials		Non-trained/1-4 days mean 2 trials	
	F;(df);p	C	W	C	W
1. EA/NR: percentage of non-rewarded explored area: (number of different non-rewarded box visits until all reward collections)x100/15 (number of non-rewarded boxes)	Mean ± SEM	22.9±2.85	23.1±3.70	26.6±3.51	10.7±2.35
	Group	11.21;(1,112);<0.005			
	Condition				
	Day	33.63;(3,112);<0.001			
	Group/Condition	11.81;(1,112);<0.001			
2.EA/R: percentage of explored rewarded area: (number of rewarded box visit until all reward collections [0 or 1])x100	Mean ± SEM	89.1±4.88	85.9±5.13	64.1±6.82	26.6±5.49
	Group	12.79;(1,112);<0.001			
	Condition	55.17;(1,112);<0.001			
	Group/Condition	9.16;(1,112);<0.005			
3. Exp/Bef/NR: (number of non-rewarded box visits until all reward collection)x300/[(Eat/T)x15]	Mean ± SEM	1.5±0.23	1.3±0.27	0.7±0.11	0.2±0.04
	Group	4.97;(1,112);<0.05			
	Condition	43.25;(1,112);<0.001			
	Day	20.42;(3,112);<0.001			
	Condition/Day	8.10;(3,112);<0.001			
4. Exp/Bef/R: (number of rewarded box visits until all reward collection)x300/(Eat/T)	Mean ± SEM	33.9±2.66	26.2±2.75	11.4±2.10	3.3±0.96
	Group	12.53;(1,112);<0.001			
	Condition	104.50;(1,112);<0.001			
5. Exp/Tot/NR: (number of non-rewarded box visits up to 5 min)15	Mean ± SEM	0.6±0.06	0.6±0.08	0.7±0.09	0.2±0.04
	Group	19.54;(1,112);<0.001			
	Condition	7.32;(1,112);<0.01			
	Group/Condition	22.79;(1,112);<0.001			
	Day	17.36;(3,112);<0.001			
6. Exp/Tot/R: number of rewarded box visits up to 5 min	Mean ± SEM	6.5±0.39	5.7±0.46	5.2±0.66	1.6±0.38
	Group	21.44;(1,112);<0.001			
	Condition	30.87;(1,112);<0.001			
	Group/Condition	8.66;(1,112);<0.005			
	Condition/Day	3.44;(3,112);<0.05			
7. Eat/Cn: Number of rewards eaten (max. 5)	Mean ± SEM	4.4±0.24	4.0±0.31	3.0±0.35	1.0±0.24
	Group	16.56;(1,112);<0.001			
	Condition	56.79;(1,112);<0.001			
	Group/Condition	7.31;(1,112);<0.01			
8. Eat/T (s): Time required to consume all rewards, or 300 s (cut-off time)	Mean ± SEM	80.20±14.81	109.9±17.91	195.3±15.96	273.3±8.20
	Group	13.34;(1,112);<0.001			
	Condition	89.27;(1,112);<0.001			
9. LC: Learning capacity (%): [(Eat/Cn)x(300)x100]/ [5x(Eat/T)]	Mean ± SEM	685.4±55.42	559.5±57.67	208.5±39.13	59.5±18.78
	Group	8.93;(1,112);<0.005			
	Condition	112.85;(1,112);<0.001			

Abbreviations: Bef: before eating all of the food reward; C: control; Cn: count; df: degree of freedom; Exp: Exploration; NR: non-rewarded; R: rewarded; Tot: total time of the trial (5 min); W: Wisket. The blue color signs the significant effects of group, condition or group/condition interactions.
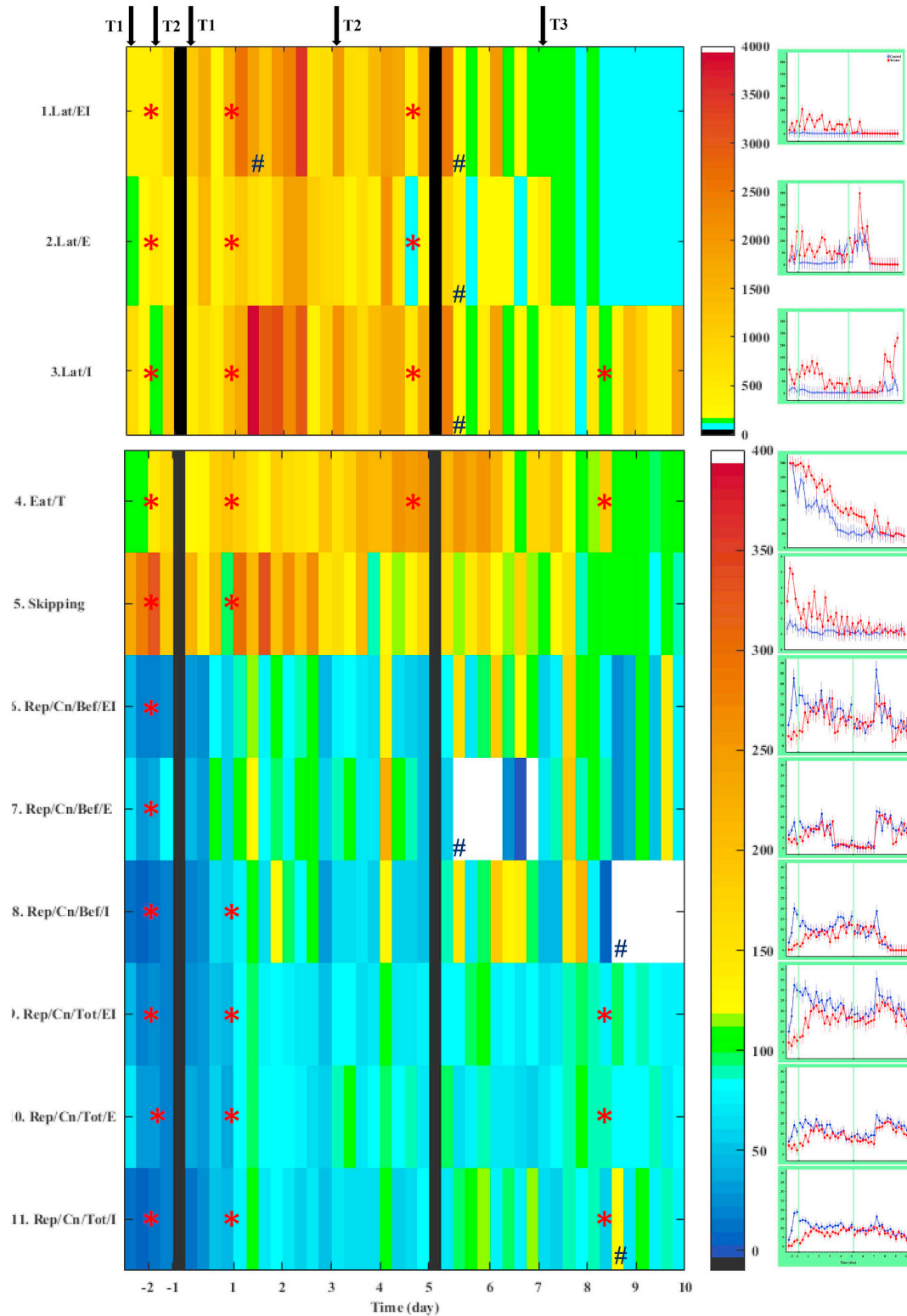
study. However, as far as the validity of the animal model itself is concerned, this is not really an issue. In our previous papers we provided ample evidence that the new substrain after the complex treatment has the highest validity as a schizophrenia model compared to the appropriated control groups [3], and from that time we concentrated to the characterization of this model from several aspects [4–9]. The contribution of the genetic or environmental factors separately to the different signs is indeed an interesting theoretical question, however, it also raises ethical concerns. and, from an animal welfare point of view,

it would be difficult to argue for the necessity of extra experiments only to describe the individual contribution of the hits, once it has been proven that the model is valid.
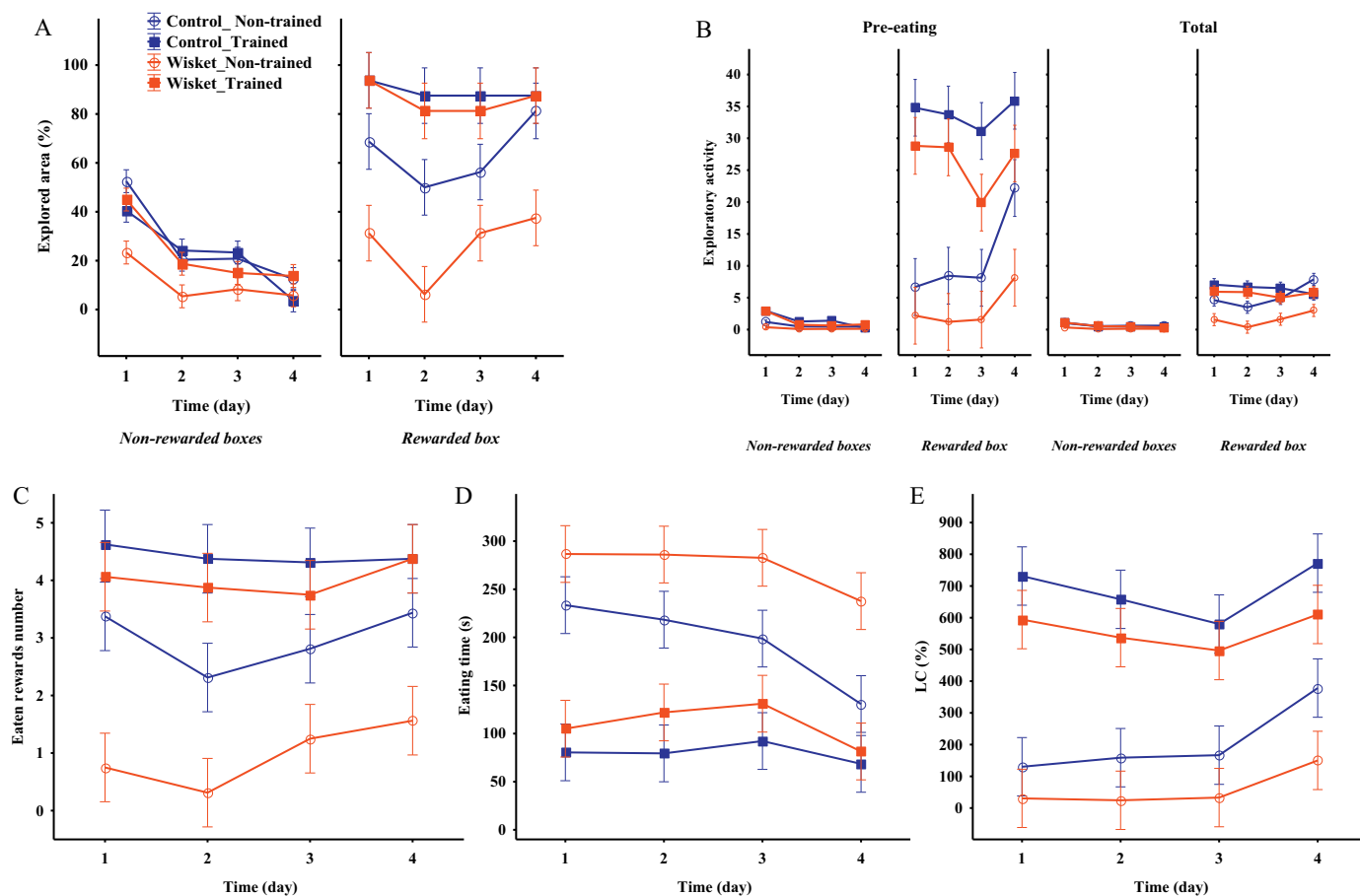
A number of methods have been developed to assess spatial learning and memory performance in rodents including different mazes and hole-boards with food rewards. The automatic recognition of rodent behavior revolutionized the investigation of these animals, but most of the existing solutions are rather expensive and/or sophisticated [43,44]. The goal of the introduction of AMBITUS was to provide a new
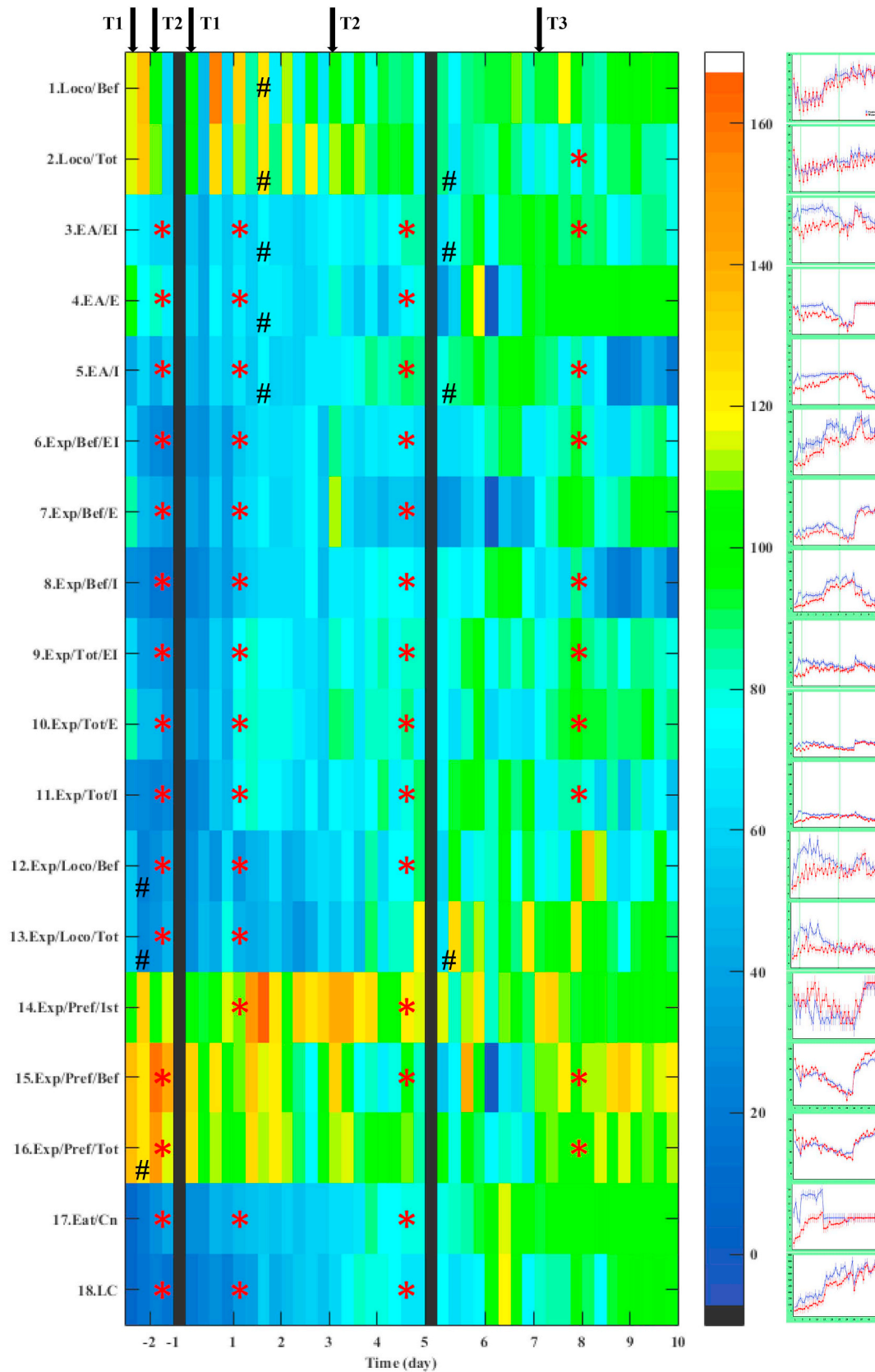
Fig. 2. Grid plot (left side) and original time-course curves (right side: mean ± SEM) for different behavioral parameters obtained in AMBITUS between control (blue) and Wisket (red) animals, with maximal difference up to 170%. 100% [green color] means that the Wisket rats have the same mean value as the controls). Yellow to brown colors mean that the Wisket rats showed a significantly higher value in the given parameter and in the given trial. In contrast, colors toward the blue end of the spectrum indicate lower values in the new substrain. Significant differences between the two groups during the different phases (*) and group/trial interactions (#). The arrows show the introduction of a new task (T1, T2 or T3). The black vertical lines sign several days break between the pre- and 1st phases (one week) and between the 5th and 6th days of training session (two days). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. Grid plot (left side) and original time-course curves (right side: mean ± SEM) for different behavioral parameters obtained in AMBITUS between control (blue) and Wisket (red) animals, with maximal differences larger than 170%. 100% [green color] means that the Wisket rats have the same mean value as the controls). Yellow to brown colors mean that the Wisket rats showed a significantly higher value in the given parameter and in the given trial. In contrast, colors toward the blue end of the spectrum indicate lower values in the new substrain. Significant differences between the two groups during the different phases (*) and group/trial interactions (#). The arrows show the introduction of a new task (T1, T2 or T3). The black vertical lines sign several days break between the pre- and 1st phases (one week) and between the 5th and 6th days of training session (two days). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 4. Time-course curves for the single-rewarded box experiments A: Explored area for rewarded and non-rewarded boxes up to eating all of the food rewards. B: Exploration activity for rewarded and non-rewarded boxes during the pre-eating and for the whole period (5 min, total). The number of eaten rewards (C), the time required to consume all rewards (D), and the learning capacity (D) during the 4 days of Task-4. Significances are indicated in Table 4.

system for behavioral studies with several advantages. First, it detects automatically a wide range of parameters in behaving animals related to locomotor, exploratory and cognitive functions. Second, the tasks are relatively simple and can be completed within a few minutes; therefore, it can be conducted in large groups of animals in a short time period. Third, the method makes it possible to investigate the ability of the animals to acquire, maintain and shift attentional sets as well as to alter behavior under different tasks. Fourth, the AMBITUS test requires an egocentric strategy, thus the animals have to navigate considering both the position of their own body and that of the rewards. Therefore, it is safe to assume that the functioning of brain structures necessary for egocentric navigation learning (e.g., hippocampus, striatum) is reflected in the animals' performance [10,45]. Fifth, the narrow corridor as a more natural environment for the animals compared to open field and the reward-based tasks without any punishment resulted in lower stress and/or anxiety [30,46], suggesting that AMBITUS provides a minimally stressful experimental environment.

Time-course curves are the most frequently used means of showing differences between groups regarding a parameter. The use of the method, however, is limited when lots of parameters are to be studied simultaneously. Using a plethora of parameters has the advantage of minimizing the bias of the investigator toward a particular aspect of the response. The three-dimensional data visualization technique is widely used for the presentation of complex patterns of gene expressions or activity of different brain structures. In this paper, we introduced the color-coded grid plot method for displaying three-dimensional behavioral deviations in Wisket rats. The color code based on the magnitude and direction of the differences between the two groups is a new way

for the simultaneous visual representation of multiple behavioral changes. Our approach also has the advantage of shifting the focus away from binary outcomes (significant vs. non-significant) toward robust effect sizes and the quantification of differences for several parameters (behavioromics). These representations show a rich image of the behavioral architecture of the studied group in a glance.

4.1. Conclusion

Taken together, the results demonstrate that this new rat substrain, developed by "multiple hits" (construct validity), in addition to its previously demonstrated schizophrenia-like symptoms, display several behavioral and cognitive deficits, especially related to attentional/motivational aspects (face validity), but most of the signs showed long-term improvements with a 10-day long training, similar to those observed in patients suffering from schizophrenia (predictive validity). This study also provided data about the reliability of the new instrument, AMBITUS, as a proper system for the detection of locomotor and exploratory activities and different aspects of cognitive functions in rodents. Our experience gained in this study with color-coded figures suggests that this approach is very useful for the simultaneous characterization of changes in various behavioral parameters.

Acknowledgements

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