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Comparative Evaluation of Behavioral Tests Efficiency in Learning of Dopamine Transporter Knockout Rats

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Abstract—**Objective:** The dopamine transporter knockout (DAT-KO) rats are an optimal model for studying attention deficit hyperactivity disorder (ADHD). This model has tremendous potential for biotranslational research to guide the development of new approaches to ameliorating the symptoms of human neuropsychiatric disorders. However, studies of learning and memory in DAT-KO rats have difficulties and limitations due to their pronounced hyperactivity. The aim of this study was to reveal the most effective behavioral tasks for experiments at DAT-KO rats. We analyzed the dynamics of tracks in different mazes as an indicator of task successful learning **Methods:** During long-term experiments we tested several behavioral paradigms such as 8-Arm Radial Maze, Hebb-Williams maze, T-maze, Red Box apparatus. The comparison of learning differences of DAT-KO and WT rats was made. **Results and Discussion:** DAT-KO rats were able to learn in all selected maze types, but they learned significantly less efficiently than WT rats. DAT-KO rats also showed perseverative patterns, such as repeatedly entering error zones and returning to previously visited areas. However, in experiments involving manipulative behavioral tasks, this perseverative activity was useful for successful task performance. The configuration of the tracks during exploration of the maze also differed between the two groups of animals, reflecting the rate and efficiency of learning. **Conclusions:** Choosing and developing the most adequate methodological approaches can help to evaluate the novel pharmacological approaches to ADHD treatment. Selecting appropriate behavioral tests for hyperactive rats is also essential for creating training techniques for patients with ADHD.

Keywords: dopamine transporter, knockout rats, hyperactivity, animal model

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INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is the pathology most often diagnosed in childhood (Posner et al., 2020; Shrestha et al., 2020). This neurodevelopmental disorder is polygenic and affects up to 12% of children and more than 4% of adults (Danielson et al., 2018; Faraone et al., 2021). In adults, ADHD symptoms are remaining approximately at 50% of patients. The main symptoms of ADHD are hyperactivity, inattention and impulsivity, but significant links have been found between ADHD symptoms and physical health (asthma, sleep problems) (Galéra et al., 2022; Grace, 2016). Moreover, the patients with ADHD also demonstrate decreased abilities to learn new tasks, working memory impairment and reduced motivation (Tripp and Wickens, 2009; Volkow et al., 2011; Asherson et al., 2016; Arjona Valladares et al., 2020; Jang et al., 2020; Ortega et al., 2020; Luo et al., 2023; Salari et al., 2023; van der Plas et al., 2025;). It is generally accepted that ADHD is connected with the dopamine (DA) system dysfunction (Viggiano et al., 2004). The dopamine system is involved in the modulatory control of reward evaluation, cognition, locomotion (Wise, 2004), and formation of memories for reward–cue associations (Dalley et al., 2005; Bromberg-Martin et al., 2010; Ko et al., 2013; Tripp and Wickens, 2024). This system also is very important for realization such forms of complex behavior as decision making (Bardgett et al., 2009) and social behavior (Homberg et al., 2016). Abnormal DA system functioning also occurred during development of different neurological and psychiatric disorders, such as Parkinson disease, schizophrenia, and bipolar disorder (Lee et al., 2024; Wu et al., 2025).

There are different hypotheses about ADHD nature which include genetic and environmental factors interaction that contribute to this pathology. One of the most promising approaches to ADHD treatment is the creating the animal's models for study this disease. Some models may allow to investigate not only ADHD, but also other neurological disorders (Creed and Goldberg, 2018; Dougnon and Matsui, 2022; Cabana-Domínguez et al., 2023; Lee and Yoon, 2023).

The genetic models of ADHD include spontaneously hypertensive rat (SHR), the Naples high excitability (NHE) rat, the dopamine transporter (DAT) knock-out mouse and rat**s**, the SNAP-25 deficient mutant coloboma mouse, mice expressing a human mutant thyroid hormone receptor, a nicotinic receptor knock-out mouse, and a tachykinin-1 (NK1) receptor knock-out mouse (Russell, 2011; Regan et al., 2022; Kim et al., 2024). In these models different behavioral tasks used for revealing attention abnormalities. One of the such task is the 5-Choice serial reaction time task (Fizet et al., 2016). This task also may be used for measuring the motor impulsivity (Higgins and Silenieks, 2017; Fox, 2004). However, our experience allows us to suppose that these tasks may be very difficult to use for spontaneously hyperactive DAT-KO rats. A strain of mice with knockout of DAT gene (DAT-KO mice) was created (Ide et al., 2018; Giros et al., 1996; Gainetdinov et al., 1999). After it the strain of DA transporter knockout (DAT-KO) rats that have no DA reuptake and thus elevated extracellular DA levels was developed (Leo et al., 2018). The strain of DAT-KO rats is the most useful model for investigation of cognitive impairments in ADHD due to abilities of these animals to learn the complex behavioral tasks.

In the present study we have compared different behavioral tasks in order to select the most appropriate ones for DAT-KO rats training. We tested several cognitive tasks such as 8-arm radial maze, Hebb-Williams HW maze, T-maze, Red Box apparatus and compared successfulness of task solution by DAT-knockout rats. Here, we focused on the tracking, perseverative activity, and tactics that animals choose to explore mazes as indicators of potential task learning success. The main objective of the study was to identify the most significant criteria of task successfulness learning in order to determine the most appropriate behavioral settings for training hyperactive rats.

MATERIALS AND METHODS

Animals

All experiments were conducted on male DAT-KO and wild-type (WT) rats (3–4 months old) in accordance with the guidelines established by FELASA and RusLASA concerning the care and use of laboratory animals. A total of 84 rats were used in the four series of experiments: 39 dopamine transporter knockout (DAT-KO) rats and 45 control wild-type (WT) rats. Animals were sourced from the Vivarium of St. Petersburg State University. Before the experiments, rats were maintained in IVC cages (RAIR IsoSystem World Cage 500; Lab Products, Inc., Seaford, DE, USA) with free access to food and water, at a temperature of 22°C, 50–70% relative humidity and a 12 h light/dark cycle. Experiments were carried out between 2 and 6 pm. We videotaped the animal's behavior and analyzed its parameters using a video tracking system (EthoVision XT, Noldus Information Technology, VA, USA).

The 8-Arm Radial Maze

In this experiment 10 DAT-KO and 15 control WT were used. The 8-arm radial maze consisted from the central arena and 8 open arms. The central area of the maze was 40 cm in diameter, 8 arms were 37 cm long and numbered 1 to 8 clockwise. At the end of each arm the food reinforcement was placed (Brown and Giumetti, 2006). The rats were trained to visit each arm only once. Repeated visits to a previously visited arm were considered incorrect. In addition to recording the distance travelled, the duration of the task and the number of errors made during the test (Kurzina et al., 2020), we compared the trajectories of knockout and control rats at different stages of the learning.

The Hebb-Williams (HW) Maze

The experiment involved 10 rats DAT-KO and 10 WT control rats. HW maze was made in accordance to parameters of Pritchett and Mulder (Pritchett and Mulder, 2004). The HW maze consisted of a square area 75 × 75 cm, with start and goal boxes and the interior walls. The walls of the maze were moveable and it was possible to create different routes through the maze and different layouts were used to diminish habituation's influence. Rats were trained to find the optimal way from the start box to a goal box to obtain food reinforcement. The ways from error

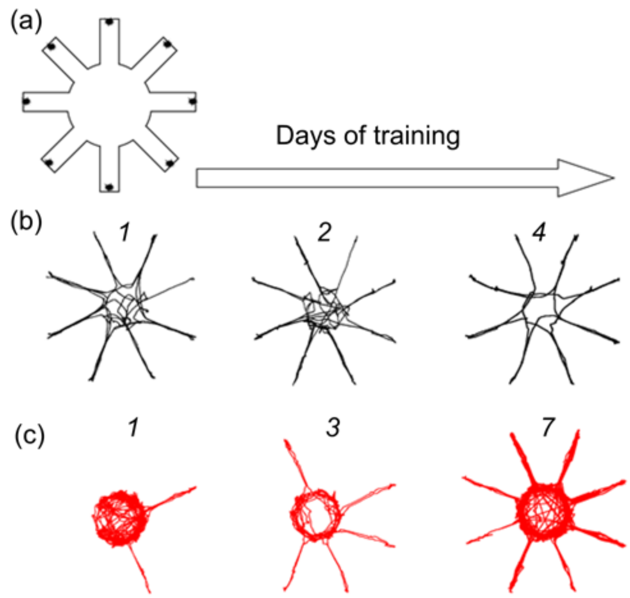

Fig. 1. Examples of tracks in the (a) 8-arm maze recorded during the experiments: (b) for wild-type rats and (c) for dopamine transporter knockout (DAT-KO) rats. Asterisks indicate the location of food reinforcements at the ends of the maze arms; *1*, *2*, *3*, *4*, and *7*—days of training.

zones not leading to the finish were marked. We tested several different configurations of the HW maze's inner walls. In addition to analyzing the distance travelled, task duration, number of errors and number of returns to the starting box (Kurzina et al., 2022), we analyzed the configuration of DAT-KO and WT rats tracks.

T-Maze

A total of 10 DAT-KO and 10 WT rats were included in this experiment. The T-maze had the following parameters: a starting area of 50 × 16 cm and two arms of 50 × 10 cm each. The rats were trained to choose the arm of the maze that contained reinforcement. The arm with food reinforcement was randomly selected for each rat (Deacon, 2006). Our experiment consisted of training the rats to make the correct choice (training) followed by retraining (re-training). Retraining was done by changing the reinforced arm of the maze to the opposite arm. For example, a rat was trained to choose the right arm of the maze, and in the retraining stage, the reinforcement was placed to the left arm of the maze. In addition to formally counting and analyzing the number of errors (Belskaya et al., 2024), we compared the configuration of DAT-KO and WT rats trajectories during learning and re-learning.

Red Box Apparatus

In this experiment 9 DAT-KO and 10 control WT rats were used. Red Box apparatus made after Gilbert and Kesner (Gilbert and Kesner, 2003) consisted of a box with nontransparent red plexiglas walls. A removable guillotine door divided the apparatus into a small starting compartment (40 × 40 cm) and an experimental compartment (85 × 40 cm) in which the objects were presented. Objects were placed on the food wells, which contained reinforcement. The rats were trained to move the familiar objects (cubes) with food reinforcement and not to move non-rewarded novel objects (other geometric forms), which never had reinforcement under them. In our previous study (Kurzina et al., 2021), we analyzed the distance travelled by the animals, the duration of the task, and the number of erroneous trials (i.e. when familiar objects with reinforcement were not moved and new objects without reinforcement were moved). The present study focuses on a comparative analysis of track configurations in DAT-KO and control WT rats.

RESULTS

We found that the DAT-KO rats were able to learn tasks in all selected maze types, but the learning rate and the efficiency of learning was significantly lower than that for the WT rats. It should be noted that along with hyperactivity, DAT-KO rats showed perseverative patterns when solving spatial tasks: returns to previously visited areas of the maze, multiple entries into error zones. In this study, the tracks of the animals in the 4 types of mazes were analyzed. The data showed that DAT-KO rats used different from WT rat's spatial exploration strategy which was reflected in longer trajectories in the maze.

8-Arm Maze

In our experiments in 8-arm radial maze (Fig. 1a) we revealed that DAT-KO rats were able to learn behavioral task but their performance was significantly worse than in WT rats. The knockout rats took more time to learn the task rules in the 8-arm maze, the duration of task performance and distance travelled was significantly longer (Kurzina et al., 2020). Knockout animals needed more days of training to reach the training criterion of finding all eight reinforcements at the end of each maze arm (Figs. 1b, 1c).

We showed that the 8-arm maze, in which the animal must enter each arm of the maze for reinforcement once

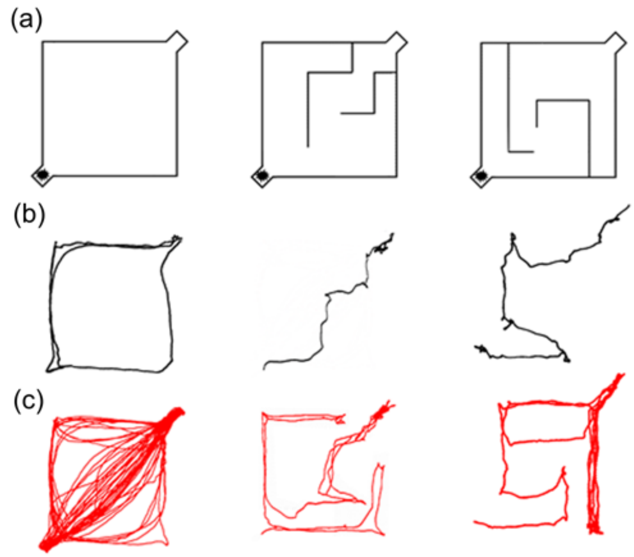

Fig. 2. Examples of Hebb-Williams maze apparatus with different configurations of the (a) inner walls, and tracks of (b) WT and (c) DAT-KO rats during experiments. Asterisks indicate the location of food reinforcements in the target box.

avoiding repeated incorrect arm entries, was the most difficult task for DAT-KO rats. As can be seen in Fig. 1, knockout animals repeatedly entered the maze arms despite on previously have taken reinforcement. The track shape and length also reflects that knockout animals often visited arms at an angle of 135° to the previously visited arm, and even ran in a straight line from one arm to the opposite arm (Fig. 1c). It was clearly different from the tactic of WT rats which selected maze arms at a 45° to the previously visited arm, i. e. selected an adjacent arm in order to efficiently obtain reinforcement (Fig. 1b). We revealed that DAT-KO rats demonstrated pronounced perseveration and formed behavioral tactics different from those in control animals. The dynamics of tracks showed that WT rats are able to form optimal behavioral tactic to the end of training, whereas DAT-KO rats even at final stage of learning failed to choose optimal tactics of reinforcement getting. This qualitative analysis of rat's behavior in the spatial maze enables us better understand behavioral features of hyperactive rats. So, DAT-KO rats were able to learn spatial task in open 8-arm maze but they demonstrate deficit in working memory and their behavioral tactics significantly differ from behavioral tactics in control animals. We suppose that high levels of DA and subsequently high level of hyperactivity in DAT-KO rats lead to perseveration and influenced on formation not optimal behavioral tactics.

HW Maze

In the next series of experiments, we used the HW maze to compare rats' spatial learning abilities. DAT-KO rats were found to be able to learn this task, but again their performance was less successful compared to WT rats. DAT-KO rats showed marked hyperactivity in comparison with WT animals and took longer to reach the target box. WT rats learned the task rules faster than DAT-KO rats, which showed a deficit in finding the correct path to the target. In addition, DAT-KO rats also showed an increased number of returns that indicated perseverative activity during task performance. We hypothesize that due to the hyperactivity of DAT-KO rats, their behavioral task performance was significantly worse than that of WT rats (Kurzina et al., 2022; Volnova et al., 2023).

The examples of rat tracks in the HW maze shown in Fig. 2 clearly illustrate the differences between the knockouts and the WT animals. When pre-tested in an arena without interior walls (Fig. 2 left), WT rats explore the maze and avoid entering the open part of the maze and found the food reinforcements relatively quickly (Fig. 2b). The hyperactivity of DAT-KO rats is expressed in a continuous, chaotic movement through the maze arena, with multiple entries into the target zone and returns to the start even without trying to pick up food (Fig. 2b). It should be noted that the need to perform the behavioral task slightly reduced the hyperactivity of DAT-KO rats compared to their behavior in the maze without interior walls (Fig. 2b). However, the spatial exploration tactics of the maze differed between control and knockout rats. When testing in HW maze with variable wall configurations, WT rats always chose the optimal path (Fig. 2b), whereas DAT-KO animals path was longer, (Fig. 2c). DAT-KO rats also made returns and entered error zones before reaching the target box and receiving food reinforcement (Fig 2c). We demonstrated that while WT rats found the target box quickly and accurately, dopamine transporter knockout DAT-KO rats took additional turns and reversals. We suppose that DAT-KO rats use different from WT rats strategies, such as following the walls of the maze which helps hyperactive rats may be with the involvement of vibrissae apparatus navigate more effectively.

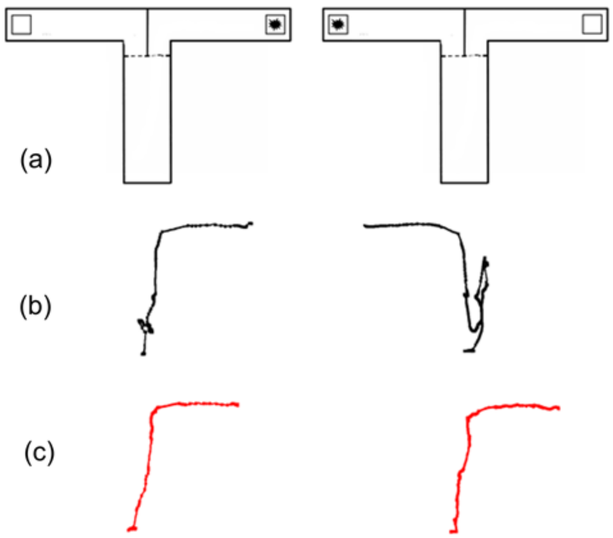

Fig. 3. Examples of (a) T-maze configurations during learning (left) and re-learning (right) stages of experiment; tracks made by (b) WT and (c) DAT-KO rats. Asterisks indicate the location of food reinforcements in the maze arm, an empty square indicates the absence of reinforcement.

T-Maze

During learning in the T-maze, DAT-KO rats demonstrated pronounced patterns of perseverative activity and learned and performed the behavioral task significantly worse than WT rats (Belskaya et al., 2024), which was consistent with our data for DAT-KO rats in other types of mazes. All WT rats were able to meet the training criteria, whereas only about half of the DAT-KO rats chose the reinforced arm of the maze with minimal errors. Fig. 3 (left) illustrates the tracks of the rats at this stage of the experiment. Re-learning rats in the T-maze revealed another distinctive behavioral feature of DAT-KO rats. Among control WT animals, all rats were able to re-learn and chose a new (reinforced) arm of the T-maze (Fig. 3b, right). In contrast, DAT-KO rats continued to choose the arm that previously contained the food reinforcement (Fig. 3c, right).

It is important to note the different patterns of WT and DAT-KO rats' activity during testing. The rats in the control group demonstrated a small delay before choosing the correct turn into the arm of the maze. The knockout rats, which had learned the correct choice in the previous "learning" stage, ran through the starting box without delay during re-learning, showing pronounced behavioral rigidity. Thus, during re-learning, DAT-KO rats revealed the rigid perseverative activity of choosing the previously learned path even in the absence of reinforcement.

Red Box

In learning non-spatial manipulative object recognition tasks (Red box apparatus) under the conditions of novel object presentations DAT-KO rats were able to learn to move familiar objects and not to move novel once (Fig. 4a). It should be noted that for DAT-KO rats the period of acquisition of task rules was longer than in WT rats. It was also found that DAT-KO rats demonstrated pronounced hyperactivity and perseverative reactions

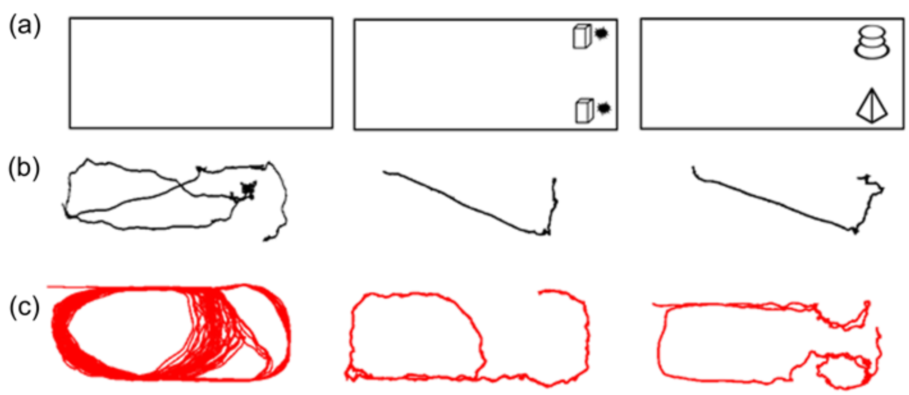

Fig. 4. Examples of (a) Red box apparatus configurations: during the pretest period in the empty arena (left), and during the experimental phase with familiar objects (cubes) and novel objects (various geometric shapes); examples of tracks made by (b) WT and (c) DAT-KO rats during experiments. Asterisks indicate the location of food reinforcements under the familiar objects.

in comparison with control rats. The time of object exploration and distances covered were also longer in knockout rats. During re-testing DAT-KO rats were able to performed task from the first day and even made fewer errors than control animals because the time they needed to run and move first object was shorter (Kurzina et al., 2021).

It should be noted the different ways in which WT and knockout DAT-KO rats explore the Red Box apparatus arena. At the initial stage of training without objects exploration (Fig. 4) WT rats quickly run around the arena (Fig. 4b) while DAT-KO rats many times run around it (Fig. 4). After the objects presentation WT rats choose the most optimal, shortest path to explore familiar objects and receive reinforcement by moving them. When presenting novel objects, WT rats in some cases do not even approach them and tended to leave the arena quickly (Fig. 4b). In contrast, DAT-KO rats tended to investigate both familiar and novel objects for longer time. Knockout rats ran around objects, studying them before moving familiar figures and receiving reinforcement. DAT-KO rats also carefully explore new objects in a similar manner (Fig. 4c). Paradoxically, a high level of perseverative activity positively influences on the ability of DAT-KO rats to learn to move an object and retrieve food from the rewarded familiar objects and not to move the non-rewarded novel objects. This may be due to the longer period of exploration of the objects, which the knockout animals remember well enough. The dynamics of tracks allows us to think that namely motor memory helps WT rats faster learn the task rules, whereas for hyperactive DAT-KO rats longer objects exploration plays a crucial role and facilitates their memory for object’s shape,

DISCUSSION

Here we report the results of our long-term experiments in DAT-KO rats while learning spatial and non-spatial behavioral tasks. It is established that DA is involved in various cognitive processes such as learning, memory and attention. The DA system dysfunction leads to the appearance of neurological and psychiatric disorders such as Parkinson disease, Huntington’s disease, schizophrenia and ADHD (Viggiano et al., 2004; Lee et al., 2024; Wu et al., 2025). The generation of new animal models is necessary to investigate the molecular mechanisms underlying these diseases. The development of the DAT-KO mice, which lack the DA reuptake transporter, has been very fruitful for translational research (Ide et al., 2018). Later, a line of rats with a deletion of the DA transporter gene, the DAT-KO rats, was created (Leo et al., 2018). This animal model has allowed us to study the cognitive impairments associated with DA transporter dysfunction. DAT-KO rats are characterized by elevated extracellular DA levels and marked locomotor hyperactivity and perseverative motor patterns. In addition to hyperlocomotion, DAT KO rats demonstrated reduced social interaction and saccharin preference. Marked stereotypes and gait changes were also observed in these animals (Mallien et al., 2022). It has also been demonstrated that pronounced hyperdopaminergia in DAT-KO rats may significantly contribute to the motivation/effort-cost relationship in rodents (Savchenko et al., 2023). Transporter knockout rats have been shown to be a useful model for ADHD research. Since cognitive impairments in ADHD include impaired spatial memory, our studies provide the characteristics of spatial orientation and manipulative activity in DAT-KO rats.

The aim of our study was to select the most appropriate behavioral methods to detect behavioral abnormalities in knockout animals and identify alternative behavioral tactics that help them to solve the task in different mazes. We investigated the dynamics of hyperactivity and perseverative patterns during learning in DAT-KO rats. Analysis of the locomotion in all types of the used mazes allowed us to reveal the important indicator of the successfulness of learning. We suppose that formation of the optimal strategy mainly depends on the ability of the animals to learn task rules. Knockout rats were able to learn in all selected maze types, but the learning rate and efficiency of learning was significantly lower than that of WT rats. It was found that in spatial tasks without additional cues such as Hebb-Williams maze, DAT-KO rats were able to learn tasks rules much more slowly than WT rats, and the level of task performance never reached that of control animals. Their tracks also demonstrate the perseverative tendency and strictly differ from WT rats runs. The similar result was obtained when the animals were tested in an 8-arms maze. It is possible to think that DAT-KO rats are not able clearly remember previously visited arm and form the most successful behavioral tactic. The analysis of tracks confirms this assumption. DAT-KO rats demonstrate the large amounts of repetitive tracks unlike WT rats. This fact reflects the deficiency in cognitive maps formation in knockout rats. It should be noted that hyperactivity and perseveration in solving spatial tasks were very high in DAT-KO rats and did not decrease during training. The task performance in the 8-arms maze was directly related to the time that the rat remained in the maze and is connected with the enhanced amounts of perseverative reactions (Kurzina et al., 2020).

The perseverative patterns of activity even without reinforcement were found in T-maze and reflect the expressed rigid tactics in these tasks in knockout rats. DAT-KO rats that had learned the rule of choosing the reinforced arm showed pronounced rigid tactics in the re-training experiment, continuing to choose the arm without reinforcement (Belskaya et al., 2024). Paradoxically, the perseverative activity of knockout rats in solving non-spatial behavioral tasks in the Red Box setup involving manipulative behavior facilitated task learning (Kurzina et al., 2021). DAT-KO rats not only successfully manipulated familiar objects while receiving food reinforcement, but also effectively discriminated them from novel non-reinforced objects what clearly shows the tracks analysis. The DAT-KO rats more often run around the objects than control animals. Knockouts spent more time exploring novel objects during training and were able to retain task rules in memory for three months. Thus, the behavioral inflexibility of DAT-KO rats resulted in making significantly fewer errors during task performance than WT rats.

Pharmacological correction of ADHD-like behavior in DAT-KO rats is important both for understanding the mechanisms of the observed behavioral abnormalities and for testing new potential drugs. Therefore, we also needed to choose an experimental design that would allow us to assess the effects of drugs that improve behavioral performance in knockout rats. The HW maze was very convenient (Pritchett and Mulder, 2004). In this experimental setup, the rats were trained to remember the basic rule (search food reinforcement in the goal box of the maze). The animals then performed the task of searching food in several maze arenas that differed in the position of the inner walls. Layouts of arenas with the same complexity were chosen. This made it possible to test different pharmacological substances on a group of animals at intervals of several days.

In our previous experiments, pharmacological behavioral correction was performed by modulating α2A-adrenoreceptor activity using the α2A-adrenoreceptor agonist guanfacine (Volnova et al., 2023). It reduced DAT-KO rat's hyperactivity, perseverative activity and time spent in error zones of the maze, and improved spatial working memory indicators. Decrease the time spent in error zones and distance travelled were found. Perseverative activity patterns were also reduced. In contrast, the yohimbine, an α2A-adrenoreceptor antagonist, impaired these parameters (Kurzina et al., 2022). Our results support the efficiency of guanfacine as an effective pharmacological treatment for hyperactivity. Both guanfacine and atomoxetine (the norepinephrine reuptake transporter) are currently known to be used for the treatment of ADHD. Thus, all behavioral techniques used revealed significant abnormal behavioral characteristic of DAT-KO rats. We observed motor hyperactivity, reduced task efficiency, difficulties in performing spatial navigation tests in mazes, and pronounced perseverative behavior patterns. In our opinion, tests that combine spatial navigation with manipulative behavior and object discrimination are the most promising.

The perseverative activity observed in DAT-KO rats may be reduced by including objects in the spatial task, which may interrupt hyperlocomotion due to the rats exploring the objects and the additional motor task of food retrieving. The imposition of objects also slows down the rate of running. In this context, the Red Box apparatus seems to us the most appropriate for training hyperactive rats. Moreover, as mentioned above, DAT-KO rats were able to keep in memory this behavioral task up to three months (Kurzina et al., 2021). This apparatus also allows to change behavioral tasks applying different objects or their interposition. It is possible to reveal differences in learning object-object and object-place tasks. We suppose that in this apparatus the investigation of spatial memory in DAT-KO rats may be carried with minor design changes when adding transparent door before the start chamber. The behavioral tasks in HW and T-mazes can also be used to train hyperactive rats because they include not only spatial tasks, but also retrieving food from food wells. However, the manipulative component is less pronounced in these tasks.

We suggest that such an integrated approach may also improve the learning performance of children with ADHD and increase the translational potential of DAT-KO rats model application. The tracks analysis allows quickly detect the ability of animals to learn the task rules and is an indicator of task validity.

CONCLUSION

Animal models contribute to our understanding of the role of neurochemical, genetic and environmental factors in the development of ADHD. The data presented above proved that DAT-KO rats are a valid model for studying the pathological mechanisms underlying ADHD. The behavioral techniques used helped to select the most appropriate training methods for the knockout animals. At the same time, the use of spatial and non-spatial tests made it possible to identify the specific features of the DAT-KO rat's behavior. We suppose that there is a need to develop and test more effective teaching methods for children and adults with ADHD. The translational significance of the data obtained consists in the fact that analyzing of the behavior of hyperactive DAT-KO rats can help us to develop the adequate approaches to learning ADHD patients and select the most effective training methods.

ABBREVIATIONS AND NOTATION

ADHD—attention deficit hyperactivity disorder;

DA—dopamine;

DAT—dopamine transporter;

DAT-KO—dopamine transporter knockout;

WT—wild type;

HW—Hebb-Williams maze.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All studies were conducted in accordance with the principles of biomedical ethics as outlined in the 1964 Declaration of Helsinki and its later amendments. They were also approved by the Ethics Committee for Animal Research of St. Petersburg State University (St. Petersburg, Russia), protocols no. 131–03-10 dated November 22, 2021 and no. 131–03-6 dated April 25, 2025.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

AUTHOR CONTRIBUTION

Conceptualization—NPK; methodology—ADB; formal analysis and investigation—AAG; original draft preparation—NPK, AAG, ADB; writing (review and editing)—ABV, RRG; funding acquisition—ABVolnova; supervision—RRG. All authors have read and agreed to the published version of the manuscript.

REFERENCES

Arjona Valladares, A., Gómez, C.M., Rodríguez-Martínez, E.I., Barriga-Paulino, C.I., Gómez-González, J., and Diaz-Sánchez, J.A., Attention-deficit/hyperactivity disorder in children and adolescents: An event-related potential study of working memory, *Eur. J. Neurosci,* 2052, vol. 20, pp. 4356–4369.
https://doi.org/10.1111/EJN.14767

Asherson, P., Buitelaar, J., Faraone, S.V., and Rohde, L.A., Adult attention-deficit hyperactivity disorder: Key conceptual Isues, *The Lancet Psychiatry*, 2016, vol. 3, pp. 568—578.
https://doi.org/10.1016/S2215-0366(16)30032-3

Bardgett, M.E., Depenbrock, M., Downs, N., Points, M., and Green, L., Dopamine modulates effort-based decision making in rats, *Behav. Neurosci.,* 2009, vol. 123, pp. 242–251.
https://doi.org/10.1037/a0014625

Belskaya, A., Kurzina, N., Savchenko, A., Sukhanov, I., Gromova, A., Gainetdinov, R.R., and Volnova, A., Rats lacking the dopamine transporter display inflexibility in innate and learned behavior, *Biomedicine,* 2024, vol. 12, p. 1270 .
https://doi.org/10.3390/BIOMEDICINES12061270

Bromberg-Martin, E.S., Matsumoto, M., and Hikosaka, O., Dopamine in motivational control: rewarding, aversive, and alerting, *Neuron,* 2010, vol. 68, pp. 815–834. https://doi.org/10.1016/J.NEURON.2010.11.022

Brown, M.F. and Giumetti, G.W., Spatial pattern learning in the radial arm maze, *Learn. Behav.*, 2006, vol. 34, pp. 102–108. https://doi.org/10.3758/BF03192875

Cabana-Domínguez, J., Antón-Galindo, E., Fernàndez-Castillo, N., Singgih, E.L., O'Leary, A., Norton, W.H., Strekalova, T., Schenck, A., Reif, A., Lesch, K.P., Slattery, D., and Cormand, B., The translational genetics of ADHD and related phenotypes in model organisms, *Neurosci. Biobehav. Rev.*, 2023, vol. 144, p. 104949. https://doi.org/10.1016/J.NEUBIOREV.2022.104949

Creed, R.B. and Goldberg, M.S., New developments in genetic rat models of Parkinson's disease, *Mov. Disord.*, 2018, vol. 33, pp. 717–729. https://doi.org/10.1002/MDS.27296

Dalley, J.W., Lääne, K., Pena, Y., Theobald, D.E.H., Everitt, B.J., and Robbins, T.W., Attentional and motivational deficits in rats withdrawn from intravenous self-administration of cocaine or heroin, *Psychopharmacology (Berl),* 2005, vol. 182, pp. 579–587. https://doi.org/10.1007/S00213-005-0107-3

Danielson, M.L., Bitsko, R.H., Ghandour, R.M., Holbrook, J.R., Kogan, M.D., and Blumberg, S.J., Prevalence of parent-reported ADHD diagnosis and associated treatment among U.S. children and adolescents, 2016, *J. Clin. Child Adolesc. Psychol.*, 2018, vol. 47, pp. 199–212. https://doi.org/10.1080/15374416.2017.1417860

Deacon, R.M.J., Appetitive position discrimination in the T-maze, *Nat. Protoc.*, 2006, vol. 1, pp. 13–15. https://doi.org/10.1038/NPROT.2006.3

Dougnon, G. and Matsui, H., Modelling autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD) using mice and zebrafish, *Int. J. Mol. Sci.,* 2022, vol. 23, p. 7550. https://doi.org/10.3390/IJMS23147550

Faraone, S. V., Banaschewski, T., Coghill, D., Zheng, Y., Biederman, J., Bellgrove, M.A., Newcorn, J.H., Gignac, M., Al Saud, N.M., Manor, I., et al., The World Federation of ADHD International Consensus Statement: 208 Evidence-based conclusions about the disorder, *Neurosci. Biobehav. Rev.,* 2021. vol. 128, pp. 789–818. https://doi.org/10.1016/J.NEUBIOREV.2021.01.022

Fizet, J., Cassel, J.C., Kelche, C., and Meunier, H., A review of the 5-choice serial reaction time (5-CSRT) task in different vertebrate models, *Neurosci. Biobehav. Rev.*, 2016, vol. 71, pp. 135–153. https://doi.org/10.1016/J.NEUBIOREV.2016.08.027

Fox, G.B., Model of attention deficit hyperactivity disorder: five-trial, repeated acquisition inhibitory avoidance in spontaneously hypertensive rat pups, *Curr. Protoc. Pharmacol.,* 2004, vol. 5, p. 5.37. https://doi.org/10.1002/0471141755.PH0537S26

Gainetdinov, R.R., Wetsel, W.C., Jones, S.R., Levin, E.D., Jaber, M., and Caron, M.G., Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity, *Science,* 1999, vol. 283, pp. 397-401. https://doi.org/10.1126/science.283.5400.397

Galéra, C., Cortese, S., Orri, M., Collet, O., van der Waerden, J., Melchior, M., Boivin, M., Tremblay, R.E., and Côté, S.M., Medical conditions and attention-deficit/hyperactivity disorder symptoms from early childhood to adolescence, *Mol. Psychiatry,* 2022, vol. 27, pp. 976–984. https://doi.org/10.1038/s41380-021-01357-x

Gilbert, P.E. and Kesner, R.P., Recognition memory for complex visual discriminations is influenced by stimulus interference in rodents with perirhinal cortex damage, *Learn. Mem.,* 2003, vol. 10, pp. 525–530. https://doi.org/10.1101/lm.64503

Giros, B., Jaber, M., Jones, S.R., Wightman, R.M., and Caron, M.G., Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter, *Nature,* 1996, vol. 379, pp. 606–612. https://doi.org/10.1038/379606a0

Grace, A.A., Dysregulation of the dopamine system in the pathophysiology of schizophrenia and depression, *Nat. Rev. Neurosci.*, 2016, vol. 17, pp. 524–532. https://doi.org/10.1038/nrn.2016.57

Higgins, G.A. and Silenieks, L.B., Rodent test of attention and impulsivity: the 5-choice serial reaction time task, *Curr. Protoc. Pharmacol.*, 2017, vol. 78, p. 549. https://doi.org/10.1002/CPPH.27

Homberg, J.R., Olivier, J.D.A., VandenBroeke, M., Youn, J., Ellenbroek, A.K., Karel, P., Shan, L., Van Boxtel, R., Ooms, S., Balemans, M., Langedijk, J., Muller, M., Vriend, G., Cools, A.R., Cuppen, E., and Ellenbroek, B.A., The role of the dopamine D1 receptor in social cognition: Studies using a novel genetic rat model, *Dis. Model. Mech.*, 2016, vol. 9, pp. 1147–1158. https://doi.org/10.1242/DMM.024752

Ide, S., Ikekubo, Y., Hua, J., Takamatsu, Y., Uhl, G.R., Sora, I., and Ikeda, K., Reward-enhancing effect of methylphenidate is abolished in dopamine transporter knockout mice: A model of attention-deficit/hyperactivity disorder, *Neuropsychopharmacol. Reports,* 2018, vol. 38, pp. 149–153. https://doi.org/10.1002/npr2.12020

Jang, K.M., Kim, M.S., and Kim, D.W., The dynamic properties of a brain network during spatial working memory tasks in college students with ADHD traits, *Front. Hum. Neurosci.*, 2020, vol. 14, p. 580813. https://doi.org/10.3389/FNHUM.2020.580813

Kim, D., Yadav, D., and Song, M., An updated review on animal models to study attention-deficit hyperactivity disorder, *Transl. Psychiatry*, 2024, vol. 14, p. 187. https://doi.org/10.1038/s41398-024-02893-0

Ko, I.G., Kim, S.E., Kim, T.W., Ji, E.S., Shin, M.S., Kim, C.J., Hong, M.H., and Bahn, G.H., Swimming exercise alleviates the symptoms of attention-deficit hyperactivity disorder in spontaneous hypertensive rats, *Mol. Med. Rep.,* 2013, vol. 8, pp. 393–400. ttps://doi.org/10.3892/MMR.2013.1531,

Kurzina, N., Belskaya, A., Gromova, A., Ignashchenkova, A., Gainetdinov, R.R., and Volnova, A., Modulation of spatial memory deficit and hyperactivity in dopamine transporter knockout rats via α2A-Adrenoceptors, *Front. Psychiatry,* 2022, vol. 13, p. 851296. https://doi.org/10.3389/fpsyt.2022.851296

Kurzina, N.P., Aristova, I.Y., Volnova, A.B., and Gainetdinov, R.R., Deficit in working memory and abnormal behavioral tactics in dopamine transporter knockout rats during training in the 8-arm maze, *Behav. Brain Res.,* 2020, vol. 390, Art. ID: 112642. https://doi.org/10.1016/j.bbr.2020.112642

Kurzina, N.P., Volnova, A.B., Aristova, I.Y., and Gainetdinov, R.R., A new paradigm for training hyperactive dopamine transporter knockout rats: influence of novel stimuli on object recognition, *Front. Behav. Neurosci.,* 2021, vol. 15, Art. ID: 654469. https://doi.org/10.3389/fnbeh.2021.654469

Lee, H., Kim, H.F., and Hikosaka, O., Implication of regional selectivity of dopamine deficits in impaired suppressing of involuntary movements in Parkinson's disease, *Neurosci. Biobehav. Rev.,* 2024, vol. 162, Art. ID: 105719. https://doi.org/10.1016/j.neubiorev.2024.105719

Lee, W.S. and Yoon, B.E., Necessity of an integrative animal model for a comprehensive study of attention-deficit/hyperactivity disorder, *Biomedicine*, 2023, vol. 11, Art. ID: 1260 https://doi.org/10.3390/BIOMEDICINES11051260

Leo, D., Sukhanov, I., Zoratto, F., Illiano, P., Caffino, L., Sanna, F., Messa, G., Emanuele, M., Esposito, A., Dorofeikova, M., Budygin, E.A., Mus, L., Efimova, E.V., Niello, M., Espinoza, S., Sotnikova, T.D., Hoener, M.C., Laviola, G., Fumagalli, F., Adriani, W., and Gainetdinov, R.R., Pronounced hyperactivity, cognitive dysfunctions, and BDNF dysregulation in dopamine transporter knock-out rats, *J. Neurosci.,* 2018, vol. 38, pp. 1959–1972. https://doi.org/10.1523/jneurosci.1931-17.2018

Luo, N., Luo, X., Zheng, S., Yao, D., Zhao, M., Cui, Y., Zhu, Y., Calhoun, V.D., Sun, L., and Sui, J., Aberrant brain dynamics and spectral power in children with ADHD and its subtypes, *Eur. Child Adolesc. Psychiatry,* 2023, vol. 32, pp. 2223–2234. https://doi.org/10.1007/S00787-022-02068-6

Mallien, A.S., Becker, L., Pfeiffer, N., Terraneo, F., Hahn, M., Middelman, A., Palme, R., Creutzberg, K.C., Begni, V., Riva, M.A., Leo, D., Potschka, H., Fumagalli, F., Homberg, J.R., and Gass, P., Dopamine transporter knockout rats show impaired wellbeing in a multimodal severity assessment approach, *Front. Behav. Neurosci.*, 2022, vol. 16, Art. ID: 924603. https://doi.org/10.3389/FNBEH.2022.924603

Ortega, R., López, V., Carrasco, X., Escobar, M.J., García, A.M., Parra, M.A., and Aboitiz, F., Neurocognitive mechanisms underlying working memory encoding and retrieval in attention-deficit/hyperactivity disorder, *Sci. Rep.,* 2020, vol. 10, Art. ID: 7771. https://doi.org/10.1038/S41598-020-64678-X

Posner, J., Polanczyk, G. V., and Sonuga-Barke, E., Attention-deficit hyperactivity disorder, *The Lancet*, 2020, vol. 395, pp. 450–462. https://doi.org/10.1016/S0140-6736(19)33004-1

Pritchett, K. and Mulder, G.B., Hebb-Williams mazes, *Contemp. Top. Lab. Anim. Sci.,* 2004, vol. 43, pp. 44–45.

Regan, S.L., Williams, M.T., Vorhees, C. V., Review of rodent models of attention deficit hyperactivity disorder, *Neurosci. Biobehav. Rev.,* 2022, vol. 132, pp. 621–637. https://doi.org/10.1016/J.NEUBIOREV.2021.11.041

Russell, V.A., Overview of animal models of attention deficit hyperactivity disorder (ADHD), *Curr. Protoc. Neurosci.,* 2011, vol. 9, p. 9.35. https://doi.org/10.1002/0471142301.NS0935S54

Salari, N., Ghasemi, H., Abdoli, N., Rahmani, A., Shiri, M.H., Hashemian, A.H., Akbari, H., and Mohammadi, M., The global prevalence of ADHD in children and adolescents: a systematic review and meta-analysis, *Ital. J. Pediatr.,* 2023, vol. 49, p. 48. https://doi.org/10.1186/S13052-023-01456-1

Savchenko, A., Belozertseva, I., Leo, D., and Sukhanov, I., Hyperdopaminergia in rats is associated with reverse effort-cost dependent performance, *J. Psychopharmacol.,* 2023, vol. 37, pp. 1238–1248. https://doi.org/10.1177/02698811231211225

Shrestha, M., Lautenschleger, J., and Soares, N., Non-pharmacologic management of attention-deficit/hyperactivity disorder in children and adolescents: a review, *Transl. Pediatr*., 2020, vol. 9, pp. 114–124. https://doi.org/10.21037/tp.2019.10.01

Tripp, G. and Wickens, J., Using rodent data to elucidate dopaminergic mechanisms of ADHD: Implications for human personality, *Personal. Neurosci.,* 2024, vol. 7, Art. ID: e2. https://doi.org/10.1017/PEN.2023.12

Tripp, G. and Wickens, J.R., Neurobiology of ADHD, *Neuropharmacology,* 2009, vol. 57, pp. 579–589. https://doi.org/10.1016/J.NEUROPHARM.2009.07.026

Van der Plas, N.E., Noordermeer, S.D.S., Oosterlaan, J., and Luman, M., systematic review and meta-analysis: predictors of adult psychiatric outcomes of childhood attention-deficit/hyperactivity disorder. *J. Am. Acad. Child Adolesc. Psychiatry*, 2025, Art. ID: S0890-8567(25)00215-1. https://doi.org/10.1016/J.JAAC.2025.04.012

Viggiano, D., Vallone, D., and Sadile, A., Dysfunctions in dopamine systems and ADHD: Evidence from animals and modeling, *Neural Plast.,* 2004, vol. 11, pp. 97–114. https://doi.org/10.1155/NP.2004.97

Volkow, N.D., Wang, G.J., Newcorn, J.H., Kollins, S.H., Wigal, T.L., Telang, F., Fowler, J.S., Goldstein, R.Z., Klein, N., Logan, J., Wong, C., and Swanson, J.M., Motivation deficit in ADHD is associated with dysfunction of the dopamine reward pathway, *Mol. Psychiatry,* 2011, vol. 16, pp. 1147–1154. https://doi.org/10.1038/mp.2010.97

Volnova, A., Kurzina, N., Belskaya, A., Gromova, A., Pelevin, A., Ptukha, M., Fesenko, Z., Ignashchenkova, A., and Gainetdinov, R.R., Noradrenergic nodulation of learned and innate behaviors in dopamine transporter knockout rats by guanfacine, *Biomedicines*, 2023, vol. 11, p. 222. https://doi.org/10.3390/biomedicines11010222

Wise, R.A., Dopamine, learning and motivation, *Nat. Rev. Neurosci.,* 2004, vol. 5, pp. 483–494. https://doi.org/10.1038/nrn1406

Wu, D., Chang, Z., Wang, Y., Jiang, Z., Wang, R., and Wu, Y., High-order network degree revealed shared and distinct features among adult schizophrenia, bipolar disorder and ADHD, *Neuroscience*, 2025, vol. 568, pp. 154–165. https://doi.org/10.1016/j.neuroscience.2024.12.063