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ORIGINAL ARTICLE

Novelty-related behavior of young and adult dopamine transporter knockout rats: Implication for cognitive and emotional phenotypic patterns

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Attention deficit hyperactivity disorder (ADHD) is a neuropsychiatric disorder characterized by a developmentally inappropriate, pervasive and persistent pattern of severe inattention, hyperactivity and impulsivity. Despite onset in early childhood, ADHD may continue into adulthood with substantial impairment in social, academic and occupational functioning. A new animal model of this disorder was developed in rats with genetic deletion of the dopamine transporter (*DAT*) gene (dopamine transporter knockout rats; DAT-KO rats). We analyzed the behavior of DAT-KO rats for a deeper phenotypical characterization of this model. We first tested rats of the 3 genotypes at different ages (preadolescent, adolescent and adult), in a novelty-seeking test using a black/white box (Experiment 1). After that, we tested adult rats in a novelty-preference test using a 3-chamber apparatus with different shapes (Experiment 2). Experiment 1: as evidenced by analysis of time spent in the novel environment, adult DAT heterozygous (DAT-HET) rats show an increased curiosity-driven exploration compared with wild-type (WT) controls while DAT-KO rats did not recognize novelty. The locomotor activity data show a minimal difference between genotypes at adolescent age while the preadolescent and adult DAT-KO rats have significantly increased activity rate compared with WT and DAT-HET subjects. Experiment 2: in this case, due to more clearly evident spatial differences, time spent in novel environment was not significantly different among genotypes. During first 10 minutes, DAT-KO rats showed a decreased hyperactivity, apparently related to curiosity and attention to the new environments. In conclusion, DAT-KO rats may show some inattention while more novelty-seeking traits appear in DAT-HET rats.

KEYWORDS
ADHD, adolescence, DAT-HET (heterozygous), explorative curiosity, hippocampus, inattention, juvenile, novelty preference, novelty seeking, prefrontal cortex

1 | INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental syndrome that affects not less than 2.2% of children worldwide, although considerable variation exists among different countries.[1] ADHD is diagnosed more frequently in males than in females (up to 4 to 1), but diagnosis in females typically occurs at an older age than in males and might be more prone to detection failure.[2] ADHD has a multifactorial origin and the typical manifestation includes failing to pay close attention to details, difficulties in listening and in sustaining attention, difficulties in own organization and in following instructions, as well as hyperactive behaviors that include restlessness and excessive running, climbing and talking.[3]

This disorder coexists with comorbid diagnoses: pervasive developmental disorders, anxiety and mood disorder, attachment disorders, learning impairment; in many cases there are also other problems: family conflict, bullying or child abuse may be present while chromosomal, metabolic, neurological or somatic disorders can

show up with ADHD-like symptoms.[4] One certain feature about ADHD is the alteration in monoaminergic transmission, mainly in brain dopamine (DA) activity, as well as dysregulation of fronto-striatal circuits.[5,6]

Historically, psychostimulant medications—dexamphetamine or methylphenidate (MPH)—are the most effective psychopharmacological treatments for ADHD.[7] Psychostimulants increase extracellular levels of DA and other monoamines by blocking the respective monoamine transporters (amphetamines also rise monoamine outflow through the transporters). Psychostimulants have beneficial effects both on clinical symptomatology, with a paradoxical reduction of hyperactivity and cognitive deficits, as well as on anatomical and physiological abnormalities.[8,9]

Animal models exploiting disruption of dopamine transporter (DAT) have so far focused on mice. DAT null mutant (−/−) mice, with a complete absence of DAT, exhibit extreme phenotypes such as lack of DA reuptake from the synaptic cleft, growth retardation, anterior pituitary hypoplasia, dwarfism, early life mortality and exorbitant hyperactivity.[10] The biomedical research continuously exploits modern strategies to create new animal models of ADHD, first by replicating the biochemical mechanism within brain areas involved in this disorder and thereafter by performing a deeper endophenotype characterization. We presently use an innovative animal model of ADHD, the DAT-knockout (DAT-KO) rats.[11,12] The goal of our study is a characterization of curiosity-related phenotype. We tested rats with all 3 genotypes for DAT: while the +/+ genotype (wild type [WT]) is a control, we wish to ascertain between the +/− (DAT-heterozygous [DAT-HET]) and −/− (DAT-KO) which are the animals that best model ADHD, at least respecting the criterion of face validity. Our experimental protocols assessed different aspect of curiosity-related behavior: in a first novelty-seeking test with slight visual discrepancy between familiar and novel environments, rats were assessed at 3 different developmental ages (preadolescent, adolescent and adult); in a second novelty-preference experiment, rats were tested in easier spatial-novelty conditions with environments differing by their shapes.

In this study, we present evidence on how the environment novelty of a test chamber, created by various parameters (ie, spatial compared with more emotional ones), may play different roles in curiosity-driven exploration between DAT genotypes and at diverse ages. We started with a focus on developing ages (Experiment 1): our interest was on how the number of DAT alleles changes the explorative choice of animals and its interaction with the locomotor behavioral change during the adolescent maturation. The second focus of this study was to compare the adult rats in 2 slightly differing novelty tasks (Experiment 2), which differ for rooms' structure: specifically, we run a novelty-seeking with visual cues vs a novelty-preference with spatial elements (see also Reference [13]). This allowed to evaluate different components of rats' explorative behavior because of the putative involvement of prefrontal cortex (PFC) (in the "visual" novelty-seeking task) vs the hippocampus (in "spatial" novelty-preference task). By use of the DAT-KO animal in these different tasks, we sought to clarify different novelty-related behavioral profiles, which could in turn be ascribed to activation of distinct forebrain circuits.

2 | MATERIALS AND METHODS

All experimental procedures have been approved by the ISS animal welfare survey board on behalf of the Italian Ministry of Health (_formal license to G. Laviola, veterinary surveillance by G. Panzini_). Procedures were carried out in close agreement with the directive of the European Community Council (2010/63/EEC) and with the corresponding Italian law guidelines. We have tried to minimize animals' suffering and to use as few animals as possible, according to the 3Rs principle. Calculation of the correct number of animals per group was performed according to Ricceri & Chiarotti[14] using the software G∗POWER (freeware from Heinrich-Heine-Universität, Düsseldorf).

2.1 | First experiment (BWB)

2.1.1 | Subjects

Experimental subjects used in the novelty-seeking task were 24 adult _Wistar-Han_ rats, born in our colony, with different genotypes for DAT (in detail: 8 WT, 8 DAT-HET and 8 DAT-KO), that were 120 days old and whose body-weight was approximately 320 g. Young animals, at preadolescent and adolescent age (N = 45), came from the breeding of 8 male and 15 female DAT-HET subjects (1 female did not give birth): an average of 3 male rats per litter were used, with separate litters assigned to be tested at either age. Thus, nonsibling rats were tested either when preadolescent (day 27) or adolescent (day 34).

After birth (P0), animals were culled on PND 2 ± 1 to 4/5 males and 3/4 females per litter and stayed with their DAT-HET mother. At weaning on the 21st day of life, a small sample of tissue was taken from the ear in order to mark rats' identity and for the purpose of genotyping. This sample was genotyped according to the procedure described elsewhere.[11] Rats have been housed in pairs of 2 same-sex siblings inside Makrolon III cages. Rats were maintained in an air-conditioned room (T 21°C ± 1°C, relative humidity 60% ± 10%) with a 12 hour reverse dark-light cycle (light turned off at 7:00 AM). These subjects had food (ALTROMIN-R, Rieper SpA, Vandoies, Italy) and tap water ad libitum.

2.1.2 | Experimental apparatus

The experimental apparatus used for the _novelty-seeking_ test is a plexiglass box with smooth walls and floor (70 × 30 × 35 cm) composed of 2 different environments distinguished by the end walls' colors. In detail, the walls on the long sides are gray whereas those placed on the short side of the maze are distinguished by color (black or white). This rectangular maze in its center, thus at a distance of about 35 cm from the end walls of the maze, has a dividing panel in which there is an opening with a partition (door), allowing the experimental subject to pass from one compartment to the other, if required.

On both long sides of the maze there are 2 aluminum bars equipped with 8 photocells connected by cables to a computer. The software in use is _Cage controller 1.27 for Dark Light for Rat and Mouse_ (PRS Italia, Rome, Italy). The software allows to observe: (1) subject's activity rate in the novel environment, namely the locomotor activity in relation to the time spent in it; (2) time spent in each compartment; (3) transitions (number of times a subject crosses the door between the 2 compartments). Data were divided into partial

bins every 5 minutes, generating 6 bins out of every 30-minute session. Data were analyzed for the first day and for the test day too.

2.1.3 | Experimental protocol

During the first 3 days, habituation sessions were necessary for rats to familiarize with one of the 2 environments. They were gently placed inside 1 of the 2 maze's environments and left free to explore it for 30 minutes a day, without being able to enter the other compartment. The familiar room was always the black side while the white room was left unknown. Thus, when the door on the central panel is eventually opened, this compartment results new.

On day 4, or TEST day, rats were placed inside the almost familiar environment and, after just 5 minutes, the door was opened allowing them to reach and discover the unknown (ie, novel and slightly nonpreferred) environment. Hence, since the door remained open all the residual session time, they were able to freely choose where to spend their time during 25 minutes. Each box was cleaned between rats with ethyl alcohol diluted at 33% in water. Habituation and test was carried out under red illumination.

We evaluated locomotor activity rate (ie, the number of beam interruption within the novel chamber per time unit), time spent in the novel environment and transitions (ie, the entering from the familiar to the novel chamber), with a 2 factors *split-plot* analysis of variance (ANOVA) model: 3-level (WT, HET, KO) genotype × 6-level (bins) time. For the examined developmental stages, the same model was used, with the addition of the "age" (between-subjects, 2-level) variable. Post hoc analyses were run with the Tukey HSD test. The animals' average number was 7/8 per group.

2.2 | Second experiment (D/L shapes)

2.2.1 | Subjects

The experimental subjects used in the novelty-preference task with different shapes were 31 adult *Wistar-Han* rats born in our colony, of different genotypes for DAT gene (15 WT, 8 DAT-HET and 8 DAT-KO). These animals came from breeding of 4 male and 8 female DAT-HET subjects: offspring was culled and weaned as above: body weight of animals was approximately 420 g and they were 120 days old. After weaning from their DAT-HET dam, rats were housed in groups of 3 inside Plexiglas cages (33 × 13 × 14 cm), located in an air-conditioned room (same as above). Unformal observation of pups at this age allows to discriminate those of DAT-KO genotype. When possible, we housed 1 putative DAT-KO subject with 2 non-KO siblings, to avoid potential carryover biases of a DAT-KO behavior over subjects of other genotypes. Water and food pellets were available ad libitum.

2.2.2 | Experimental apparatus

The experimental apparatus used for the *novelty-preference* test with shapes is a plexiglass box composed of 3 different rooms, with smooth walls and smooth floor (70 × 30 × 35 cm): the walls are gray and the middle starting chamber (10 × 30 × 35 cm) gives access to 2 end chambers (30 × 30 × 35 cm). This apparatus has 2 end rooms that differ for their shapes (D and L), so that the D-shaped environment was a "familiar" room and the L-shaped environment was the "new room." The rooms are separated with doors, which can be open

(or closed) to allow the experimental subject to pass (or not) from one room to another. Each apparatus was provided with 8 infrared photobeams, placed on the long wall a few centimeter above the floor (same as in Experiment 1).

2.2.3 | Experimental protocol

The experimental protocol required 3 consecutive days: during the first day ("habituation"), all experimental subjects were put in the central room with one door opened toward the D-shaped end room: rats can freely go to explore this D-shaped environment and thereafter will consider it like a familiar one. The second day ("training"), all experimental subjects received 2 injections of vehicle, 10 minutes before being placed into the familiar end side and immediately after the 30 minutes of exposure. The third day ("test"), rats were put in the central room but now the apparatus had all doors open, toward both D-shaped (familiar) and L-shaped rooms (new environment), for all the 30 minutes of session duration. The floors and walls of each chamber were cleaned between each animal with water and ethanol (2:1), and test was carried out under red illumination.

We evaluated the same parameters as of Experiment 1 (locomotor activity rate, time spent in the novel environment, transitions), with a 2 factors *split-plot* ANOVA model: 3-level (WT, DAT-HET, DAT-KO) genotype × 6-level (bins) time. Post hoc analyses were run with the Tukey HSD test.

2.3 | Methodological remark

Spending time at chance within these apparatuses would imply (1) for black/white box (BWB), a 45.8% to 46.1% of time in either chamber plus a 7.7% to 8.3% under the central door (ie, with hindpaws in one chamber and forepaws in the other one); (2) for D/L shapes, a 37.5% to 38.4% in each end-side chamber plus a 23% to 25% in the center. To be more precise about time spent in the central start chamber, just a 7.7% to 8.3% would be spent in the very middle of the start chamber, while equivalent amounts would be spent under the door connecting it to either the Novel or the Familiar end-side chambers. It is possible to set 2 thresholds for time spent at chance vs when fully in the Novel (not comprising "ambiguous" time, spent under the door connecting the central start chamber with the Novel end-side). Therefore, thresholds are: more than 37.5% to 38.4% of time (114 seconds with 5-minute bins) for 3-chamber novelty-preference (D/L shapes) vs more than 45.8% to 46.1% of time (138 seconds with 5-minute bins) for 2-chamber novelty-seeking (BWB) paradigms, respectively.[15]

3 | RESULTS

3.1 | Experiment 1 (BWB novelty seeking)

3.1.1 | Adolescent and preadolescent subjects

Activity rate

Statistical post hoc analysis shows a significant difference between the preadolescent and the adolescent KO subjects' locomotor activity ($P < .05$): while the former was elevated over WT control level, the latter did not differ (see Figure 1A).

Time spent in the novel environment

Time spent in novelty exploration, after comparison between genotypes of the 2 ages, is apparently not significant (time × genotype × age, $F_{74,4}$ = 0.735, P = .5708). The post hoc analysis carried out about this interaction shows, however, a meaningful preference, compared with chance level (138 seconds per bin), showing that more time was spent by preadolescent and especially adolescent WT subjects in the novel chamber (P < .05); the comparison between preadolescent (but not adolescent) DAT-HET subjects and chance level (138 seconds per bin) is also significant, denoting a novelty aversion especially in the central 10 minutes of the test (P < .05). KO rats never differed reliably from chance level (see Figure 1B).

Transitions

Number of transitions is significantly greater in preadolescent KO subjects compared with the adolescent ones (transitions, time × genotype × age, $F_{10,185}$ = 4.299, P = .0001) throughout the duration of the task. Post hocs show a significance in number of transitions for preadolescent but not adolescent KO compared with WT subjects, except in the first 5 minutes of test (_not shown_).

3.1.2 | Adult subjects

Activity rate

Locomotor activity in the novel environment (Figure 2A) has a significant trend denoting that KO rats are more highly aroused by novelty than WT and DAT-HET subjects (activity rate, time × genotype, $F_{5,10}$ = 22.476, P = .078).

Time spent in the novel environment

Exploration in the novel chamber is significantly longer for DAT-HET rats compared with the WT and KO ones (Figure 2B); this, in the first 5 minutes after door opening and between 20 and 25 minutes of the test (time × genotype, $F_{2,38}$ = 15.012, P < .0001). Instead, time spent in the novel chamber by the KO subjects is significantly lower than the chance level (namely, a slight aversion), unlike WT ones, only at the end of the test.

Transitions

Post hoc analysis displayed that transitions performed by the KO rats are significantly more numerous compared with WT and DAT-HET rats during the whole task (transitions, time × genotype,
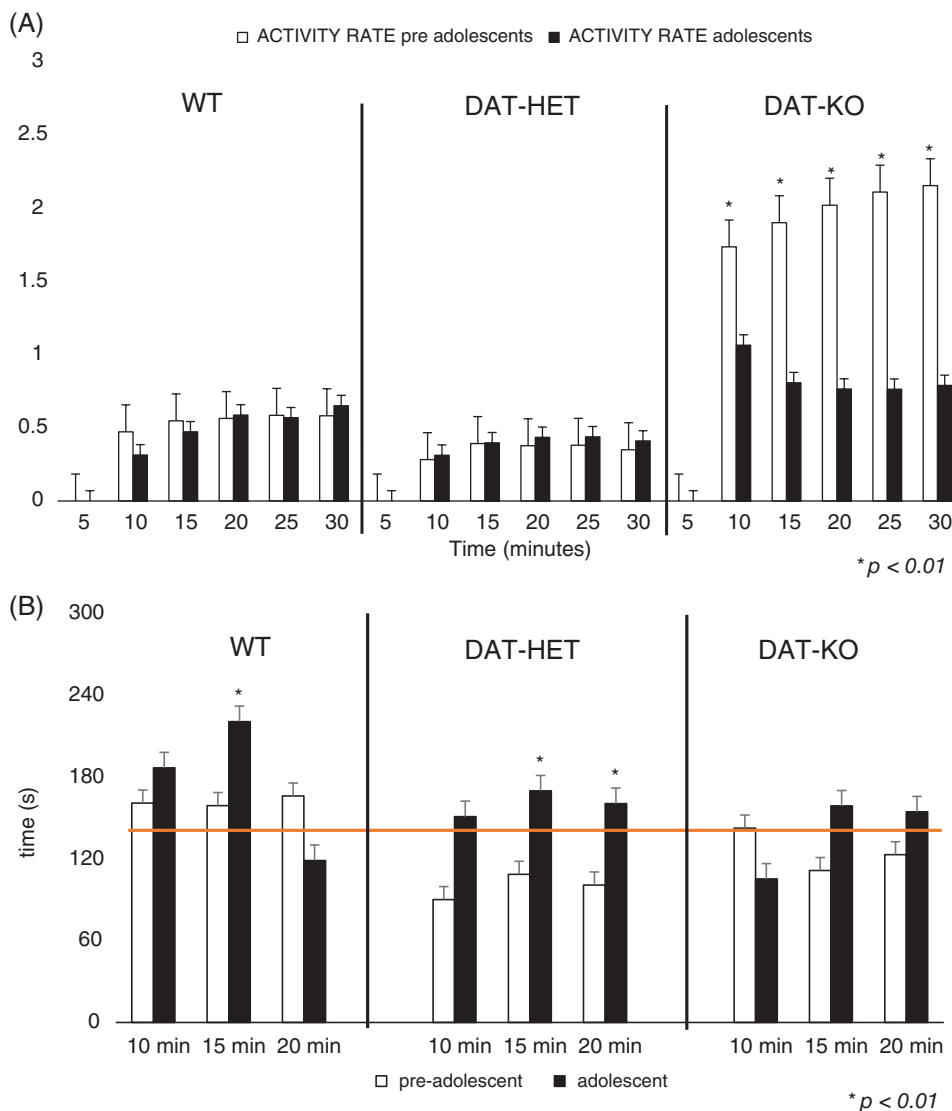

FIGURE 1 A, Activity rate (number of beam interruptions per second) in a BWB test for novelty seeking: after 5 minutes the door was opened and rats could move between 2 familiar and novel rooms that differ for end wall color. Data show a different locomotor behavior in relation to age and genotype (n = 7/8 per group). DAT-KO rats show an increment of activity rate over the control; their activity rate during adolescence does not show a significant difference compared with DAT-HET and WT subjects. B, In a BWB novelty-seeking test, juvenile and adolescent rats have different behaviors: WT rats overtake the chance level but DAT-HET ones during preadolescent age remain under chance level. About DAT-KO rats, they seem to show their own inattentive behavior because they are unable to show curiosity: the time spent in novelty remains around chance level (orange line)

(A)

(B)

FIGURE 2 A, Activity rate in a BWB novelty-seeking test in adults rats with different genotypes for DAT (n = 8 per group). In DAT-KO rats, we observe a significant increase in locomotor activity as a function of time compared with other 2 genotypes. DAT-HET rats show an equivalent level to control WT with a decrease of locomotor activity over time, which represents a decrease in the initial excitation after they preferred exploring the new room. B, Time spent in the novel environment during a BWB test for novelty seeking. Explorative curiosity is higher than chance (orange line) for DAT-HET during the first 5 minutes after door opening. Adult DAT-KO rats spend less time than the other genotypes in novelty exploration, confirming that they are not able to discriminate between the 2 environments, initially, and develop a slight aversion thereafter
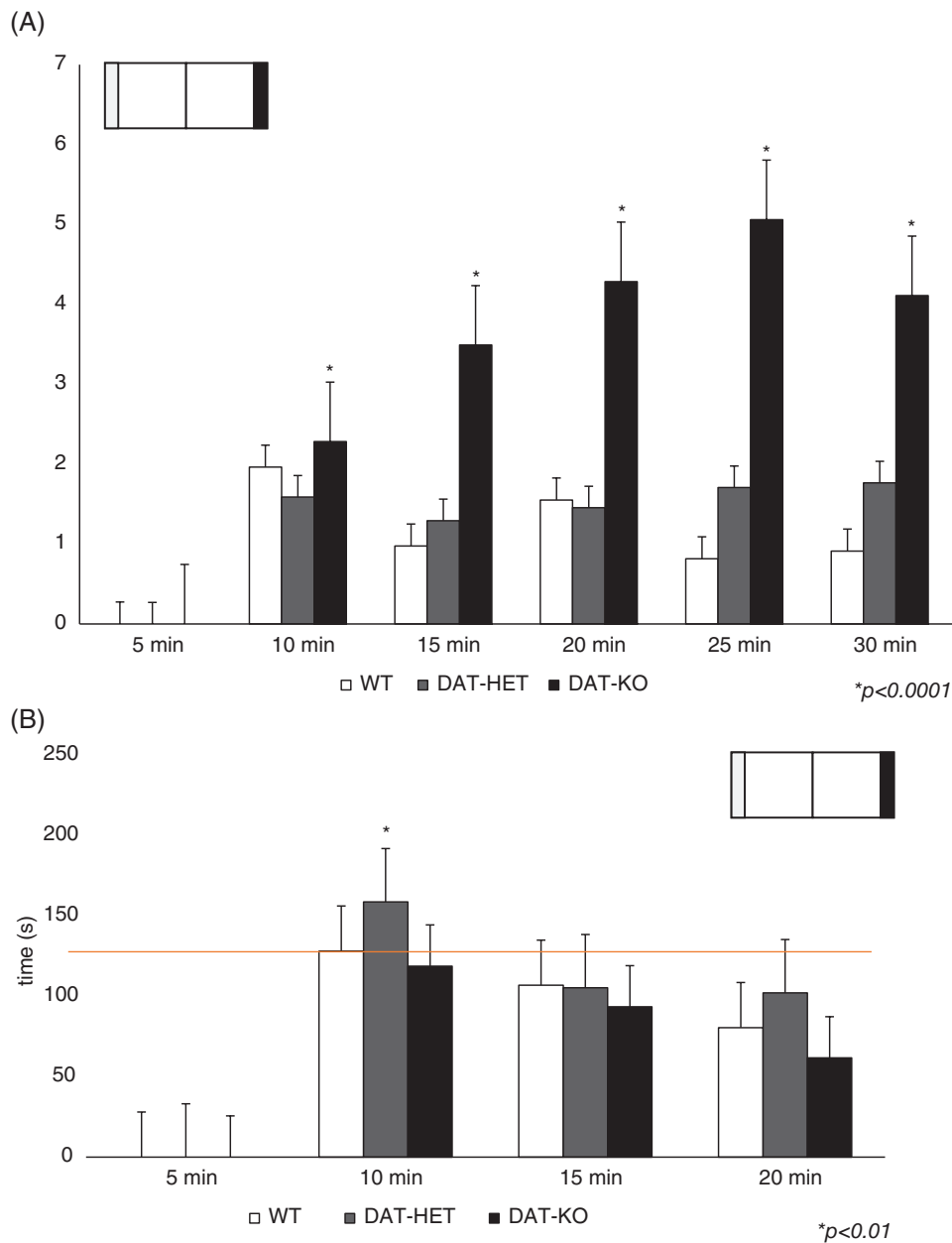
$F_{2,37}$ = 2.726, P = .020). Even the DAT-HET subjects perform a slightly higher number of transitions than the WT ones (*data not shown*).

3.2 | Experiment 2 (D/L shapes novelty preference)

Activity rate

ANOVA for the activity rate shows effects of genotype (activity rate, genotype × time, $F_{10,140}$ = 4.975, P < .0001). The threshold obtained with Tukey was 0.15 (df = 140; K = 7). The profile for the activity rate showed that, during first 10 minutes, the KO genotype shows a less pronounced hyperactivity (Figure 3), putatively due to curiosity and attention for the new environments (P < .05); this is somewhat opposite compared with the other 2 genotypes, which show a peak of activity during the first 10 minutes, especially DAT-HET rats (P < .05). Deeper analysis of actual behavior, expressed by DAT-KO

rats in a novel chamber, is warranted to ascertain the real nature and extent of their attention and/or curiosity toward it.

Time spent in novel environment

ANOVA about time spent in the new chamber is not significant (time × genotype, $F_{10,70}$ = 0.521; P = .8696); as Tukey is protected against false positives, we anyway performed the post hoc analysis and the threshold obtained with Tukey was 25 (dF = 70; K = 7). As expected, the control animals show a foreseeable behavior with a greater curiosity for the new room during the first 10 minutes; thereafter, they habituate to this environment and explore it for decreasing quantities of time. The time course shows that (compared with BWB in Experiment 1) the KO rats changed drastically own behavior and spent more time to explore the new room (see Table 1; chance level 114 seconds per bin). This behavior can represent a proof that for KO rats it is not so difficult to recognize the new room provided it is
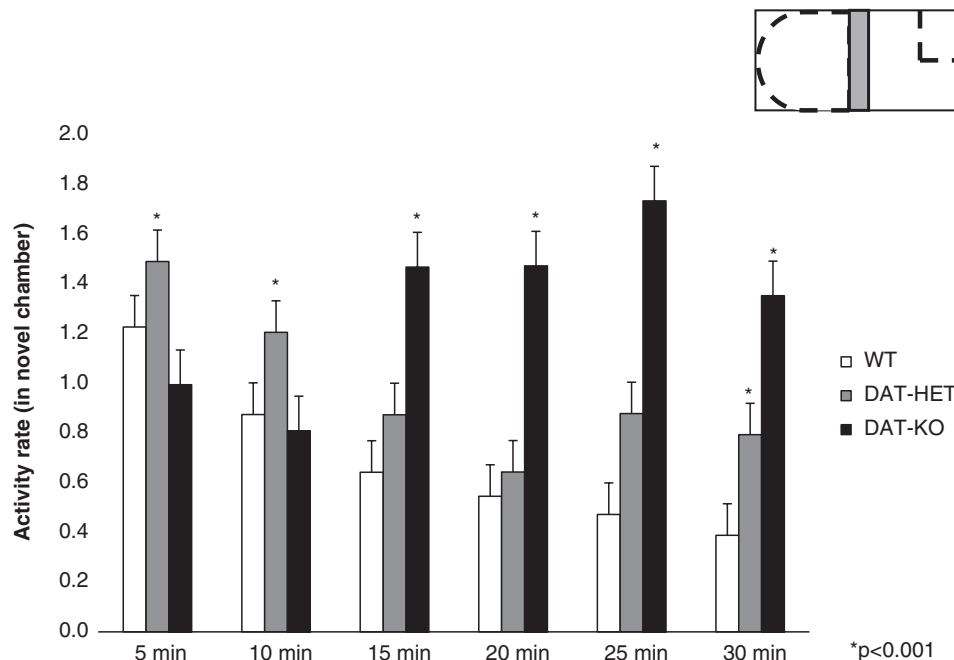
FIGURE 3 Activity rate in a novelty preference task with 2 end rooms that differ for spatial conformation. During the first 10 minutes, DAT-KO rats did not show a difference with control; after 10 minutes, DAT-KO rats show more activity in the novel room with respect to the other 2 genotypes. DAT-HET rats show a clear difference with controls only during first 10 minutes of the test, being apparently more excited. DAT-KO rats did recognize the difference between rooms, and they were interested to explore the novel room (see Table 1), confirming intact skills when the shapes are simpler to discriminate

of a clearly different shape, with a greater back and forth locomotor activity between new and familiar environments.

Transitions

The analysis for transitions showed a data profile similar to the activity rate (*not shown*). ANOVA showed an effect of genotypes (transitions, genotype × time, $F_{10,140} = 5.604$; $P < .0001$). The threshold obtained with Tukey was 0.92 (dF = 140; $K = 7$). Profiles were confirming that WT rats showed a peak of transition during first 10 minutes, according to expectations; the profiles of DAT-HET rats imitate the controls but with higher back and forth activity than controls.

4 | DISCUSSION

Historically, adolescence was considered like a transition period between childhood and adult life.[16] During this phase of life, the central nervous system still undergoes its development and shows changes related to an increase in the number of circuits and their interconnection. In adolescent brain, these are established and refined with axonal overgrowth and plasticity, followed by a phase of dramatic pruning occurring in human teenagers.[17] Adolescence is a crucial period because PFC matures relatively slowly and therefore later, while subcortical regions mature earlier and relatively more quickly.[18] This particular developmental mismatch causes individuals

TABLE 1 Time spent in novel environment during Experiment 2 (D/L shapes novelty preference)

	5 min	10 min	15 min
WT	136.2 ± 10.6	148.2 ± 23.5*	119.2 ± 29.1
DAT-HET	142.9 ± 7.8*	139.5 ± 12.6*	130.7 ± 20.6
DAT-KO	178.3 ± 14.8*	158.3 ± 9.8*	99.7 ± 42

*$P < .05$ compared with chance (114 seconds).

at this age range to have tendencies to seek for novel experiences, even at the risk of physical or social harm.[19] Such a profile might be expected to worsen if "their capacity to assess risk or to compute outcome probably is underdeveloped."[20]

Adolescent humans are therefore at risk of developing psychiatric symptoms contiguous to their sensation seeking, like ADHD and addictive behaviors. Changes due to maturation of the neurotransmitter systems have an influence on behavior that, during adolescence, may take them in the need of sensations: novelty seeking is defined as "the need for varied, novel, and complex sensations and experiences".[21] Compared with adults, also adolescent rats display elevated levels of basal locomotor and explorative activity, greater "impulsive choice,"[22] as well as a propensity to sensation seeking and risk taking.[23,24] There are alterations within neurotransmission systems, more specifically for the DAergic one. Thus, studies on rodent adolescence do confirm a similarity with human adolescence[25] and support possibility to use animal models that recapitulate adolescence-related alterations.

About ADHD, in several human studies adults show a significant reduction in volume of the orbital-frontal cortex, OFC,[26] as well as with concurrent hypofunction of dorsal anterior cingulate, dACC.[27] The rat, at adolescent age, shows a peak of expression of DA D1 and D2 receptors in subcortical targets such as dorsal striatum[28] and nucleus accumbens.[29,30] As far as the nigrostriatal system is concerned, a reduced basal rate of DA release, and a reduced pool of readily releasable DA, have been reported in peri-adolescent rats.[31] As adolescent rats have a larger, yet non-used, DA storage when compared with adults,[31] neurons are able to release more DA *only* if the condition is appropriately stimulant. During adolescence, DA system undergoes a remodeling process such as proliferation and maturation of axon terminals and synapses (Zoratto et al., manuscript in preparation).[16]

Adolescent, generally, have an overexpression of dopaminergic, adrenergic, serotoninergic and endocannabinoid receptors.[32] Many

data show imbalance between excitatory and inhibitory neurotransmission during the adolescent-to-adult age transition. Levels of GABA increase linearly through adolescent phases in rat forebrain,[33] and also the DA-receptor binding shifts during adolescence.[34,35] As correct function of all these systems (DA, glutamate and GABA) is basic for correct function of cognitive and emotional brain's processes,[36] we aimed in the present study to evaluate how adolescent transition could be modified as function of the number of *DAT* gene copies in our new animal model.

The first preclinical genetic model of ADHD, DAT-KO mice, was obtained 20 years ago. Many behavioral studies confirmed that genetically engineered mice lacking functional DAT show a spontaneous behavioral hyperactivity compared with their WT controls.[37–39] Davids et al[37] showed that DAT-KO mice have a cognitive impairment, environment-dependent hyperactivity and increased stereotypy with an absence of normal exploration behavior. However, generally rats remain a most preferable model in behavioral neuroscience due to their more developed cognitive skills, allowing operant testing as it was performed on other ADHD rat models like SHR and NHE rats.[40] Just recently DAT-KO rats were developed (see[12]), however, to be validated as a promising model for ADHD, they should be tested for hyperactivity, altered attentional skills and potential deficits in executive functions.

In this study, we would like to evaluate the explorative choice of animals (between DAT genotypes and at different ages) during 2 different experimental tasks (allowing to compare the novelty-related emotional and motivational parameters). The profile of developing rats for time spent in a novel environment confirms what is known for adolescent subjects, that are always more curious compared with preadolescent and to adult ones.[41,42] Time spent in the novel environment was related to genotypes: we can observe that only WT rats (both preadolescent and adolescent) spend much more than half of their time in the novel chamber, being thus much more curious than adults; the DAT-KO subjects failed to pay attention to environmental (visual) differences, and hardly spent more than chance level in either environment. Interestingly, preadolescent DAT-HET rats displayed a novelty aversion. The analyses made on activity rate show a developmental difference only in DAT-KO rats: intriguingly, they have significantly more hyperactivity during preadolescence while the activity rate decreases dramatically at adolescence. This difference can be ascribed to different maturation of central nervous system since, during the transition from preadolescence to adulthood, the limbic system and PFC mature differentially. While adolescent behavior typically shows less habituation, due to innate curiosity and age-related hyperactivity, DAT-KO adolescents paradoxically exhibited less restless arousal, consistently with calming effects of psychostimulants.[11,12]

Neurobiological models of adolescent brain development postulate an imbalance between early maturation of limbic structures involved in processing of reward (ie, dorsal and ventral striatum) on the one hand, and delayed maturation of top-down control by the PFC on the other hand.[43] The noteworthy point is a low locomotor activity in DAT-KO adolescent rats: the literature explains classical DAT-KO behavior with reduced/absent reuptake of DA, causing a spontaneous hyperactivity; but, in our study, the fact that DAT-KO adolescents did not show this hyperactivity at all can be interpreted
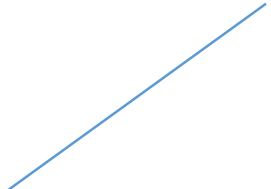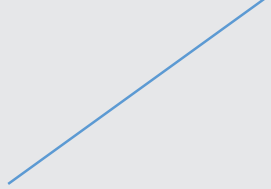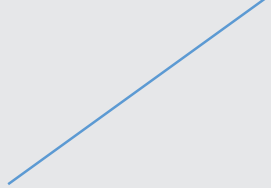
as an indirect indication of the low basal DA release at this age,[31] leaving a reduced DA tone also in the DAT-KO genotype. In other words, during adolescence, the basal release of DA is far less than at other ages and this is why subjects need to seek for more interesting stimuli to reach gratification.[19]

The second aim of the study was a comparison in adult rats submitted to novelty in different experimental conditions, namely with chambers which differ for spatial structure rather than for visual cues. We were interested in understanding the DAT-related behavior when the novelty-directed curiosity also activated 2 different cerebral circuits (ie, the PFC in "visual" novelty-seeking task vs the hippocampus in "spatial" novelty-preference task[15]). As expected, the phenotype of WT rats shows a behavioral congruence, with a similar trend during the 2 protocols: during the first 10 minutes of both tasks, WT rats were more excited and more inquiring about the novel room but, with the progress of time, they showed an expected decrease, due to a full exploration and a probable habituation to the novel environment. From a genetic point of view, the DAT-HET rats are interesting because they present only half function of DAT: this suggests that DA turnover, under novelty-induced DA release[44] with a bit less reuptake, is quite altered than for control rats and this may well affect their motivational system. During both experiments, adult animals of the DAT-HET genotype follow the WT behavior (greater curiosity and excitation during first 10 minutes that decreases with advancement of time): the relevant difference between these 2 genotypes was a higher time spent in novel rooms for DAT-HET subjects. Once again, like for adolescents,[41,42] we propose that DAT-HET rats are somewhat seeking for more gratification and/or experience more novelty-induced DA release.

While DAT-KO rats are unable to discriminate and to prefer novelty, if relying on a slight contrast between end wall colors, we wanted to ascertain ability to show novelty preference under easier conditions, and to investigate to what extent the DAT-KO rats may be considered a model of ADHD. Our interesting results clearly show a behavioral change within 10 minutes of exploration after discovery of a novel chamber: this DAT-KO group of rats is able to pay attention and does recognize the novel over the familiar side, confirming that they have intact skills at least if chamber shape is used for a simplification of the task. While different shape of rooms is a clear spatial detail for DAT-KO subject, a slight color difference is insufficient for them to discern the familiar and the novel environment. Regarding DAT-KO rats, in the second experiment (D/L shapes, novelty-preference task), own activity rate during the first 10 minutes was transiently lower than in the first experiment (BWB, novelty-seeking task) but, during the following time bins, activity rate increased over the first task. Results (see Table 2 for a summary) confirm that DAT-KO rats can decrease their activity while paying attention to environments, but immediately after come back to have a locomotion much greater than WT control and DAT-HET rats. Such piece of data is fully supporting previous evidence in mice that novelty-driven hyperactivity and increased stereotypy is a quality of DAT-KO rodents in general.[38,39,45]

We hypothesized that adults would show a modulation of emotion with mature cognitive control in PFC, while adolescents would show prevalent modulations in the amygdala and ventral striatum. The BWB task involved the motivational system and this task can

TABLE 2 Summary Table of results from experiments carried out on rats of three genotypes, at three ages, with two novelty-related protocols. Arrows denote changes in comparisons between pre-adolescents vs. adolescents or between adults of a given genotype vs. adult WT rats

Genotype	Age	BWB, novelty seeking		D/L Shapes, novelty preference
WT	Preadolescent to adolescent	Activity rate:	↔	
		Novelty Time:	↑	
		Transition:	↔	(increase)
DAT-HET	Preadolescent to adolescent	Activity rate:	↔	
		Novelty Time:	↔	
		Transition:	↔	(increase)
	Adult to adult WT	Activity rate:	↔	↑
		Novelty Time:	↑	
		Transition:	↑	↔
				↑
DAT-KO	Preadolescent to adolescent	Activity rate:	↑↑↑	
		Novelty Time:	↔	
		Transition:	↑↑↑	(increase)
	Adult to adult WT	Activity rate:	↑↑↑	↓↑↑
		Novelty Time:	↓	
		Transition:	↑↑↑	↔
				↓↑↑

thus be more appropriate for young animals since, during adolescence, the PFC is not yet mature. The novelty seeking could be higher than at other ages as impulsivity characterizes this phase of development.[22] The second task involved hippocampus because we used different room shapes; in this case, the spatial component of this task could be more appropriate for adults than for adolescents.

5 | CONCLUSIONS

The analysis across ages showed that (1) only adolescent DAT-KO subjects displayed a developmental reduction of hyperactivity and of back-and-forth maze door crossing; (2) juvenile and adolescent WT rats spent largely more than double time in the novel than in the familiar chamber, while DAT-KO subjects hardly spent more than chance level; (3) while preadolescent DAT-HET rats displayed quite a novelty aversion, adult DAT-HET rats spent slightly longer time in the novel environment, indicating a greater novelty seeking.

DAT-KO rats shall be further tested for attentional skills to be considered a model of ADHD. While DAT-KO rats are unable to discriminate novelty if they shall rely only on a subtle difference in end wall color, this group of rats can pay attention and recognize the

novel over the familiar side by shape of rooms. Our data confirm that they have intact skills if a clear spatial detail is used as a simplification of the task. Further studies are warranted and will focus on anxiety- and depression-like profiles, compulsive behavior and stereotypies, associative memory, sociality; this, to verify in depth the phenotype of DAT KO and HET rats.

Conflict of interest

There is no conflict of interest to disclose.

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