Short communication

Object recognition impairment and rescue by a dopamine D2 antagonist in hyperdopaminergic mice

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HIGHLIGHTS

- Heterozygous mice for dopamine transporter (DAT+/-) exhibit higher levels of synaptic dopamine.
- Here we confirmed that D2 antagonism can interfere in object recognition.
- We observed in DAT+/- a natural phenotype of impaired novel object memory recognition.
- The injection of haloperidol at 0.05 mg before object exposition restored object recognition.
- This effect could be explained by restoring D2 activity to optimal levels, acting on memory acquisition.

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ABSTRACT

Genetically-modified mice without the dopamine transporter (DAT) are hyperdopaminergic, and serve as models for studies of addiction, mania and hyperactive disorders. Here we investigated the capacity for object recognition in mildly hyperdopaminergic mice heterozygous for DAT (DAT +/-), with synaptic dopaminergic levels situated between those shown by DAT-/- homozygous and wild-type (WT) mice. We used a classical dopamine D2 antagonist, haloperidol, to modulate the levels of dopaminergic transmission in a dose-dependent manner, before or after exploring novel objects. In comparison with WT mice, DAT +/- mice showed a deficit in object recognition upon subsequent testing 24 h later. This deficit was compensated by a single 0.05 mg/kg haloperidol injection 30 min before training. In all mice, a 0.3 mg/kg haloperidol injected immediately after training impaired object recognition. The results indicate that a mild enhancement of dopaminergic levels can be detrimental to object recognition, and that this deficit can be rescued by a low dose of a D2 dopamine receptor antagonist. This suggests that novel object recognition is optimal at intermediate levels of D2 receptor activity.

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Dopamine (DA) is a neurotransmitter related to complex behaviors, such as: reward perception, social interaction [1,2], and is also linked to memory consolidation both in humans and rodents [3]. Alterations in DA synaptic regulation are related to a large variety of mental diseases, such as schizophrenia, hyperactivity, mood disorders, and Parkinson disease [4,5].

DA has many receptor subtypes, but they are basically divided in D1 and D2 families [3]. DA, mainly through D1 receptors, elicits the onset of the late phase of long term potentiation in the hippocampus [6], control plasticity-induced protein synthesis [6], and enhance the persistence of hippocampus-dependent memories [7].

The involvement of both dopamine receptors families with learning and memory is widely reported for working memory [3], spatial learning [3,6], aversive memory [7], reward-related learning [8] and cognitive flexibility [9]. In particular, impairment in object recognition has been induced by D2 activity suppression due to haloperidol IP injection [10], by D1 activity suppression trough...
IP injection of SCH-23390 [11], or by D1 activity facilitation via SKF81297 microinjection in the prefrontal cortex [12].

More specifically, the lack of D2 receptors was associated to odor discrimination in mice [13]. The impairment of D2 activity was related to sleep regulation and memory consolidation, through the down-regulation of plasticity factors and reduction of rapid eye movement (REM) sleep amount [10]. The antagonism of D2 receptors also led to electrophysiological changes during object exploration [14]. Furthermore, mice lacking D2 receptors do show memory consolidation deficits [15]. On the other hand, animals with high levels of synaptic DA, namely knockout mice for the DA transporter (DAT-KO), show impairment in the Morris water maze task [9], and also impairment of spatial memory in the Y-maze [16]. These animals presented, however, less immobility time in the forced swimming task [17], and exhibited an increase in locomotion, reversed by D2 receptor blocking [17].

DAT-KO mice were initially generated to study the influence of hyperdopaminergia in physiological and behavioral parameters, and in response to dopaminergic drug administration [13]. DAT–heterozygous (defined here as DAT+/−) mice, expressing only one copy of the DAT gene, were also investigated [18]. Clearance of dopamine released in the synapse takes thrice more time in DAT+/− mice than in wild-type (WT) mice [18].

Yet, in addition to neurochemical alterations leading to a mild DA increase at the synaptic level, only a handful of studies have described memory alterations in DAT+/− mice [19,20]. Previous studies revealed that DAT+/− mice show impairment in pattern completion in a partial cue environment [19], and exhibit decreased anxiety-related behaviors [20]. The mild hyperdopaminergic DAT+/− mice present biochemical changes [18] related to memory impairment that could be reversed by D2 down regulation [19].

The present work aimed to investigate the relation between D2 activity and novel object recognition in mild hyperdopaminergic DAT+/− mice. To that end, we investigated DAT+/− mice trained in the object recognition (OR) task, with assessment at basal levels of dopamine transmission as well as under the influence of different doses of haloperidol, (0.05 and 0.3 mg/Kg) before or after the training session of the task.

A total of 75 adult (2–5 months) male mice were used, comprising 39 WT (C57Bl-6 strain) and 36 heterozygous (DAT+/− strain, on C57Bl/6j background). The animals were housed in cages (2–4 animals/cage), under a 12h/12h light/dark cycle, with lights on at 07:00, and food and water ad libitum. Animals were daily handled for 5 min for 10 sessions prior to the experiments, in order to decrease stress responses WT and KO littermates were generated form C57Bl/6j-129/Sv hybrid DAT heterozygotes as previously described [18]. Mice were genotyped by PCR using sense WT (5′-CCGCTCTACCACTAGTAAACA-3′), sense KO (5′-TGACGCTTCTCGTGC-3′) and a common antisense primer (5′-CTCCACCTCTACAGCATAAC-3′). The procedures applied in the study followed guidelines of the National Institutes of Health and were approved by the Edmond and Lily Safra International Institute of Neuroscience of Natacl Ethics Committee (protocol number 08/2010).

Animals were submitted to an OR task, based on the novelty exploration tendency of rodents. The task was performed in a circular arena (50 cm diameter and 30 cm high) in a room with dim and well-spread light, so as to avoid producing shadows in the apparatus. Animals were naïve to the apparatus when exposed. We employed 6 different objects presented over 2 consecutive days (two sessions); 4 objects (A–D) were presented during the initial exploration session (First session, 10 min); and 2 unfamiliar objects (E and F) replaced 2 familiar objects (C and D) during the second session, 24 h later (testing session, 10 min). In order to evaluate memory recognition, an object preference ratio was calculated (time spent with E and F/A and B objects). Notice that if the animal follows the natural behavior, they will spend more time exploring novel objects [14,15] (ratio E and F/A and B > 1), if they had impairment in OR they will explore similarly the familiar and the novel objects (ratio E and F/A and B = 1).

Based on the D2 influence on learning and memory [8–10], we used haloperidol to induce OR impairment. At the time point of 30 min before exploration (B.E.) of the object, as well as immediately after exploration (I.A.E.), animals were injected with haloperidol (0.3 mg/Kg and 0.05 mg/Kg) or vehicle, and were then allowed to behave freely in their home cages until the second exploration session. In order to differentiate whether a possible memory deficit could be due to memory acquisition, or specifically related to the memory consolidation phase, we performed injections of haloperidol at low dose both before and after object exploration. The injection of 0.3 mg/Kg haloperidol before the exploration phase results in partial deficit of movement that impairs object exploration [17,21]. Therefore, we could not determine whether the decrease in object recognition was due to impaired consolidation, or to a deficit in locomotor activity during the exploration phase. For this reason, we did not investigate animals injected with 0.3 mg/Kg haloperidol before the exploration. In contrast, the use of the 0.3 mg/Kg dose after the exploration could not affect the mobility neither during the acquisition phase nor during the test/evocation phase, and therefore we set out to investigate this condition.

The parameter of object exploration considered the time animals spent with the whiskers or front paws in contact with one of the objects for at least 0.5 s, with a 0.5 s as a minimum interval bout. On test session, by presenting half of objects as novelty, animals should spend more time with novel objects, giving rise to unequal exploration time between novel and familiar objects [22]. By recording videos with a Panasonic camera and Animap 9.21 free software, we defined the preference ratio as the exploration duration of novel objects (E and F) divided by the time spent exploring the objects that were the same as in training in sessions (objects A and B).

First we measured the influence of haloperidol (0.3 and 0.05 mg/Kg) in the WT mice strain during OR task (Fig. 1). We executed two different statistical analyses; one-way ANOVA followed by Bonferroni correction was performed to analyze the difference among groups submitted to vehicle or haloperidol. We also performed the difference between the group (vehicle or haloperidol) and the ratio = 1 (described above), t test against 1, to find out if the preference ratio presented by WT groups was significant different of the hypothesis of non-recognition of objects. To verify possible effects of haloperidol in the motor and motivation system [8,17] we measured the total distance traveled and the total time spent in object exploration.

We applied Shapiro-Wilk normality tests to assess the distribution of the data. For the comparison within strains, in which treatment is the only independent variable, we used one-way ANOVAs of three dependent variables (Preference Ratio, Total Exploration Time and Total Distance Traveled). For the comparison between strains we used a Two-way ANOVA with strain and treatment as independent variables, followed by Bonferroni post-hoc tests. The comparison against one was performed by a paired t-test (alpha = 95%, all data with Bonferroni correction). The descriptive statistics comprise normality test values (W), mean ± SEM, p values and degrees of freedom (DF).

First we applied the Shapiro-Wilk normality tests with all data set groups to verify if the data followed the normal distribution in order to choose correctly parametric or non-parametric tests. W values for the Wild Type groups were: Vehicle 30 min B.E., W = 0.94; Halo0.05 mg/Kg 30 min B.E. I.A.E. W = 0.95; Halo 0.3 mg/Kg I.A.E, W = 0.93; Vehicle I.A.E., W = 0.97; Halo 0.05 mg/Kg I.A.E, W = 0.95 (Shapiro-Wilk p summary values p > 0.05). For the
DAT +/- animals, Shapiro-Wilk normality test W values were: Vehicle 30 min B.E., W = 0.89; Halo0.05 mg/Kg 30 min B.E. I.A.E. W = 0.81; Halo 0.3 mg/Kg I.A.E. W = 0.90; Vehicle I.A.E., W = 0.86; Halo 0.05 mg/Kg I.A.E. W = 0.77. (Shapiro-Wilk p summary values p > 0.05). Therefore, in this we used only parametric tests to analyze all comparisons Fig. 1.

We applied ANOVA to compare the preference ratio among WT groups. ANOVA comparison of preference ratio revealed a significant difference among groups (F = 11.74, df = 4.38 and p < 0.0001) (Fig. 2A). The Bonferroni’s post-hoc showed significant preference ratio decreases of haloperidol 0.3 mg/Kg in comparison to the other groups (see details in Table 1). The higher dose of haloperidol was the only treatment that led to impairment in OR, supported by the t-test against 1, t = 1.74, p = 0.6. The other groups showed significant preference ratio higher than 1: Vehicle I.A.E. (t = 4.57 df = 6, p = 0.019); Haloperidol 0.05 mg/Kg I.A.E. (t = 4.14 df = 7, p = 0.021); Vehicle 30 min B.E. (t = 11.13 df = 7, p < 0.0001); and Haloperidol 0.05 mg/Kg 30 min B.E. (t = 11.05 df = 7, p < 0.0001).

The second part of the study tested if the mild disbalance of the DA levels in DAT +/- mice strain had influence in OR task. Therefore, we analyzed the effect of haloperidol (0.3 and 0.05 mg/Kg) during OR task.

We applied one-way ANOVA to compare all the groups of DAT +/- (Fig. 2B). The ANOVA comparison of preference ratio revealed a significant difference among groups (F = 12.44, df = 4.35 and p < 0.0001). Bonferroni post hoc revealed that the treatment with haloperidol (0.05 mg/Kg) injected 30 min before exploration led to a higher preference ratio in comparison to the other groups (see details in Table 1). When we performed the comparison to 1, DAT +/- with haloperidol 0.05 mg/Kg 30 min B.E. was the only group to present difference (t = 5.621, df = 6, p = 0.007), demonstrating to be the only group with a preference ratio compatible with novel OR. The other comparisons between 1 and DAT +/- groups revealed no difference: Vehicle I.A.E. (t = 0.73 df = 6, p > 0.99); Haloperidol 0.05 mg/Kg I.A.E. (t = 0.39, df = 7, p > 0.99); Haloperidol 0.3 mg/Kg I.A.E. (t = 0.26 df = 6, p > 0.99); and Vehicle 30 min B.E. (t = 2.095, df = 6, p = 0.4).

We also measured the influence of haloperidol in WT or DAT +/- by using the strains and haloperidol dosage as independent variables. The Two-way ANOVA (Strain: F (1, 65) = 21.78, p < 0.0001; Treatment: F (4, 65) = 41.65, p < 0.0001; Interaction: F (4, 65) = 4.39, p = 0.072) revealed that both treatment and strain has influence in the preference ratio of the animals (Fig. 2C). Finally, we applied the Bonferroni post hoc to be able to compare where in each treatment both strains have different preference ratios. Bonferroni post hoc showed in haloperidol 0.3 mg/Kg I.A.E. group similar results considering WT and DAT +/- preference ratios (p > 0.05). Both strains presented ratios similar to 1 indicating that both strains have impairment in OR. Haloperidol at 0.05 mg/Kg 30 min B.E. also induced a similar preference ratio considering the two strains (p > 0.5). Nevertheless, in this case both strains revealed a preference ratio higher than 1 indicating that they are able to recognize novel objects. Regarding the comparison of WT and DAT +/- groups: vehicle I.A.E., haloperidol 0.05 mg/Kg I.A.E. and vehicle 30 min B.E. revealed a difference in the preference ratio (see details in Table 1). Among those three treatments, WT animals presented a preference ratio higher than 1 and DAT +/- similar to 1 (Fig. 2C).
Table 1

<table>
<thead>
<tr>
<th>Wild-Type Mice</th>
<th>ANOVA</th>
<th>F</th>
<th>P Value</th>
<th>DF</th>
</tr>
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<tr>
<td>11.74</td>
<td></td>
<td></td>
<td>0.0001</td>
<td>(4.38)</td>
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<tr>
<td>Bonferroni</td>
<td></td>
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<tr>
<td>Vehicle I.A.E x Halo 0.3 mg/Kg I.A.E.</td>
<td>Mean ± SEM; N</td>
<td>2.28 ± 0.28; N = 7</td>
<td>1.23 ± 0.13; N = 8</td>
<td>P &lt; 0.01</td>
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<tr>
<td>Halo 0.05 mg/Kg B.E x Halo 0.05 mg/Kg I.A.E.</td>
<td>2.73 ± 0.15; N = 8</td>
<td>1.66 ± 0.16; N = 8</td>
<td>P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Vehicle B.E x Halo 0.3 mg/Kg I.A.E.</td>
<td>2.20 ± 0.10; N = 8</td>
<td>1.23 ± 0.13; N = 8</td>
<td>P &lt; 0.01</td>
<td></td>
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<tr>
<td>Halo 0.05 mg/Kg B.E x Halo 0.3 mg/Kg I.A.E.</td>
<td>2.73 ± 0.15; N = 8</td>
<td>1.23 ± 0.13; N = 8</td>
<td>P &lt; 0.001</td>
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<tr>
<td>DAT+/−-Mice</td>
<td>ANOVA</td>
<td>F</td>
<td>P Value</td>
<td>DF</td>
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<tr>
<td>12.44</td>
<td></td>
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<td>0.0001</td>
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<td>Bonferroni</td>
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<tr>
<td>Vehicle I.A.E x Halo 0.3 mg/Kg I.A.E.</td>
<td>Mean ± SEM; N</td>
<td>2.25 ± 0.22; N = 7</td>
<td>1.07 ± 0.10; N = 7</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Halo 0.05 mg/Kg B.E x Halo 0.05 mg/Kg I.A.E.</td>
<td>2.25 ± 0.22; N = 7</td>
<td>0.96 ± 0.10; N = 8</td>
<td>P &lt; 0.001</td>
<td></td>
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<tr>
<td>Halo 0.05 mg/Kg B.E x Halo 0.3 mg/Kg I.A.E.</td>
<td>2.25 ± 0.22; N = 7</td>
<td>0.97 ± 0.12; N = 8</td>
<td>P &lt; 0.001</td>
<td></td>
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<tr>
<td>Halo 0.05 mg/Kg B.E x Vehicle B.E</td>
<td>2.25 ± 0.22; N = 7</td>
<td>1.38 ± 0.18; N = 7</td>
<td>P &lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

To test whether the involvement of D2 receptors in the motivational and motor systems played a role during the exploration of objects in the test session, we measured the total exploration time and the total distance traveled. We applied ANOVA to compare the total exploration time among WT groups. ANOVA comparison of total exploration revealed a significant difference among groups (F = 17.59, df = 4, 39 and p < 0.0001), but the comparison of the total distance traveled in the WT groups revealed no significant difference (F = 2.372, df = 4, 39 and p = 0.0712). We applied the same comparison to the DAT+/− groups. The comparison of total exploration time revealed no difference (F = 1.805, df = 4, 35 and p = 0.153). When we compared the total distance traveled, a significant difference was detected (F = 4.684, df = 4, 35 and p = 0.0045). Finally, we applied the Two-Way ANOVA to compare both strains. We found a significant interaction regarding total exploration (Strain: F (1,65) = 11.27, p = 0.0013; Treatment: F (4, 65) = 8.398, p < 0.001; Interaction: F (4, 65) = 9.301, p < 0.001), while the comparison of total distance traveled did not reveal any significant interaction (Strain: F (1, 65) = 27.30, p < 0.0001; Treatment: F (4, 65) = 6.746, p < 0.0001; Interaction: F (4, 65) = 1.236, p = 0.304).

The present study shows that DA D2 antagonist haloperidol was able to both decrease and increase memory formation, depending on the mouse strain, and on the time of injection. Specifically, we replicated WT mice data, in which haloperidol 0.3 mg/Kg after exploration led to an impairment in a memory task related to OR [10,14]. We also showed that mild hyperdopaminergic DAT+/− mice have a basal impairment in this parameter. Thereafter, in DAT+/− mice, we demonstrated that halo injection before (0.05 mg/kg), but not after exploration (0.05 or 0.3 mg/kg), was able to recover OR capacity to similar levels of their WT correspondents.

Dopamine D1 and D2 receptors are linked to attention and memory consolidation and to both mesolimbic and mesocortical dopaminergic pathways [3,6]. Rossato et al. reported that D1 family receptor modulates the long-term memory storage persistence in the inhibitory avoidance task [7], as well D1 participate in the spatial memory process [23]. Similarly, the systemic injection and intra-accumbens of D2 antagonist impair spatial memory. Both D1 and D2 suppression results in impairment to recognize changes in the environment [24].

The use of DAT-KO mice as a model to study the behaviors associated with dopamine is widely described. DAT-KO mice exhibit hyperlocomotion [18] and this phenotype can be reversed by haloperidol injection [17,20]. DAT-KO animals also exhibit a preference for the borders of an open field [20], and a decrease of immobility time in the forced swimming task [17]. When maintained in isolation, DAT-KO mice exhibit increase in reactivity and aggression when submitted to social interactions [2]. DAT-KO mice also present deficits in odor recognition [13] and impairment in the normal wake-sleep cycle [25]. Using DAT-KO, Morice et al. showed that these animals had impairment in the water-maze paradigm, and long-term depression [9]. In contrast, only a few studies have been published on DAT+/− mice, a mild model of hyperdopaminergia. In this model, a mild disbalance of dopamine results in several cognitive changes [17,19,20]. DAT+/− mice exhibit less immobility time during the forced swimming test [17], higher time spent in the center of an open field [20], and more time spent in the open arms in comparison to WT mice in a plus maze [19] or zero maze [20]. In addition, these heterozygous mice showed an increase in the exploration of objects placed in an open field [17], and in the frequency of object exploration [20]. DAT+/− mice showed impaired pattern completion in an environment with few spatial cues, which could be reversed by haloperidol [19]. However, no impairment of OR has been described [19,20].

In the present work we found basal impairment in OR in the DAT+/− mice (without pharmacological interference), differently from previous studies [19,20], but our OR protocol was quite different from the protocol used by Li et al. We used 50% less time of object exploration sessions and twice the number of novel objects than the protocol of that study [19]. The increase in the amount of novel objects, combined with less time to explore them, likely led to the differences between the previously published results and our own. In the Pogorelova protocol, animals were submitted to 7 consecutive days of novel exploration. The animals had 3 daily sessions to explore the same objects. In the fourth day the placement of the objects was changed, and in the sixth day one of the objects was replaced [20]. Most likely, any mild impairment of OR in DAT+/− mice should be reversed by several consecutive contacts with the same objects. Similarly to our results, pattern completion impairment was reversed by haloperidol injection in DAT+/− mice [19].

Secondly, regarding exploration and overall mobility, note that both behaviors are modulated by dopamine. Yet the results in this
study, related to memory in DAT+/− or WT mice, are probably not influenced by an alteration of these two variables. As shown in the results above, the WT 0.3 mg/Kg I.A.E. group exhibited the same levels of total exploration seen in the vehicle I.A.E. group, but they explored differently the new and familiar objects (see Fig. 2A).

Furthermore, we observed no difference among groups comparing 0.3 mg/kg and their respective controls in the mobility parameter (distance traveled). The same basic explanation can be applied to the DAT+/−; the statistics indicate no difference in total exploration time when we compare DAT with vehicle and with halogen 0.05 mg/kg or 0.5 mg/kg. (and only differences between I.A.E and B.E. treatments, treated in sequence) but these groups exhibited differential exploration of the new and familiar objects (see Fig. 2B).

A useful rationale in this case is to explain the results as an increase in potentially stressful stimuli presented to the DAT+/−

animals, since more objects were presented and the animals had less time to be familiarized with the environment. This increase in potentially stressful conditions may be subtle for wild type animals, but not for DAT+/− animals with a mild dopaminergic excess.

In agreement with this hypothesis, it has been demonstrated that the enhanced activity regulation of DAT receptor in WT mice occurs as a synaptic response to restraint stress, and this effect occurs even in haloperidol-treated animals [26]. Since DAT+/− mice have less DAT receptors, they might be more susceptible to this stress-associated phenomenon. Despite the scarcity of pharmacological and neuronal studies in DAT+−/− mice, DATKO littermate mice exhibit a different neurocircuity [27] and a non-dopaminergic acute response to psychostimulant drugs [28].

Future investigation of the neurocircuity of DAT+/− animals shall clarify what are the neural correlates of the differential modulation of its reward system implied by our results.

Interestingly, with regard to motor parameters, we observed that WT mice with injection before exploration and DAT+/− mice with B.E. injection at 0.05 mg/Kg of haloperidol, presented locomotion alterations when compared to both control and to 0.3 mg/kg after exploration. The differences are more evident in DAT+/− mice, which presented, antagonically, a decrease of total exploration in injections B.E. as well as an increase in distance traveled (0.05 mg/kg B.E.). These findings, together with an increase in DAT +−/− (distance traveled) at 0.05 mg/kg after exposure, also suggest, additionally, an increased susceptibility of motor modulation in DAT+−/− animals due to previous injection stress, and to haloperidol action at lower doses.

Object recognition deficits in DAT+−/− were, in fact, the main findings in this study: this finding is supported by related research. There is involvement of D2 receptor activity in neuroplasticity markers impairment associated with OR deficits [10,14]. A high dose of haloperidol (0.3 mg/Kg) lead to a decrease in CAMKII, ZIF-268 and BDNF [10] after object exploration. In the same study above, the haloperidol injected animals that presented impairment in OR had significant decrease in the REM sleep duration [10]; they also revealed a strong correlation between REM sleep decrease and OR impairment [10].

Besides corroborating previous studies in the field, data from WT mice were important for the comparison with DAT +−/− results on memory impairment. DAT+−/− mice did not receive major scientific attention as did their knockout counterparts, since they only exhibit a mild hyperdopaminergia and exhibit less phenotypic differences [18]. Nevertheless, in the present study these mice showed a clear impairment in OR. These data are relevant since they indicate that a mild hyperdopaminergia can be detrimental enough to cause attention or memory deficits related to novelty recognition.

It is important to note that the two strains investigated here, WT and DAT+/−, present different basal levels of dopamine [18]. Because of these different basal synaptic dopamine levels, we expected different sensibility to dopamine antagonists in these two mic strains. A higher sensibility was observed in DAT+−/− mice at a low haloperidol dose (0.05 mg/Kg, BE injection), since haloperidol reverted the natural OR deficit phenotype, and notably enhanced object memory formation in this strain. In contrast, in WT we did not detect any difference between haloperidol and vehicle BE, using the ANOVA test. In fact, in this case a trend was revealed by Student’s T test (t = 2.69, df = 14 and p = 0.0176), indicating that haloperidol BE at a low 0.05 mg/kg dose could possibly enhance object recognition in WT mice. We interpret these specific strain variations at low haloperidol doses as different susceptibilities to a minor modulation of dopamine receptors. However, when injected after exploration, haloperidol at the same low dose did not increase OR in DAT +−/− mice during the test session. Together, both results suggest that this impairment could most probably be due to DA imbalance during memory acquisition in DAT +−/− mice. Consequently, since the acquisition phase seems to be directly affected, no further pharmacological intervention after memory acquisition could exert any effect on OR in DAT +−/− mice, as we observed with haloperidol injections after object exploration.

Interestingly, the same drug that caused memory deficits after exploration in WT mice also caused an increase in this parameter in DAT+−/− mice, when used before exploration and at a lower dose. An inverted U-shape activity, in which optimal activity levels enhance performance, is proposed for DA D1 receptor activation, and is related to goal-oriented tasks, memory consolidation and attention [29,30]. In this approach model, both low or high DA receptor activation reduces the performance in these activities, and medium optimal activation produces the best performances. An interesting study conducted in humans indicates the inverted U-shaped, D-2 receptor- based neuroplasticity is also observed [31].

Since we used a drug that is mainly a D2 receptor antagonist, the results can indicate that D2 activation levels may also induce an inverted U-shape performance in mice related to OR. The D2 receptor action may modulate OR both at acquisition and consolidation phases. We infer that in the present study, both high and low D2 DA receptor activation levels led to object memory impairment. The high D2 activation could be relative to DAT +−/− at basal performance, which was reverted by haloperidol in pre-exploration (at 0.05 mg/kg). The low activation case could be that of WT, impaired by haloperidol injection after exploration (at 0.3 mg/kg).

These results indicate that optimal D2 activity levels can be pharmacologically induced, and can potentially be considered a therapeutic target for dopamine-related dysfunctions. Indeed, the use of D2/D3 family antagonists has been described for reducing the impairment of memory deficits induced by amnesic compounds in rodents [32]. This is also supported by the trend for OR facilitation in WT injected with 0.05 mg/kg haloperidol. Altogether, the results indicate that D2 family antagonists in low doses can produce memory enhancement.

In summary, we report here an OR deficit in DAT +−/− mice. Importantly, injection of the D2 DA antagonist before object exploration reverted this phenotype. These results are complemented by an haloperidol-induced decrease of OR in WT mice when injected after the training session. This indicates that D2 DA receptors can modulate OR tasks by acting both in attentional processes during acquisition, and during subsequent memory consolidation. More studies could be performed examining other phenotypic characteristics of DAT +−/− mice, in order to associate these behavioral deficits to molecular and electrophysiological parameters. Furthermore, additional pharmacological studies involving DA agonists and antagonists for different receptors are in order to build a more comprehensive understanding of D2 regulation of attention and memory.
Conflict of interest

None. The authors declare no competing interests.

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