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Short communication

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

Arthur S.C. França[a], Larissa Muratori[b], George Carlos Nascimento[c], Catia Mendes Pereira[d], Sidarta Ribeiro[a], Bruno Lobão-Soares[e,*]

[a] Brain Institute, Federal University of Rio Grande do Norte, RN 59056-450, Brazil
[b] Biochemistry Department, Federal University of Rio Grande do Norte, Brazil
[c] Medical Engineering Department, Federal University of Rio Grande do Norte, Brazil
[d] Edmond & Lily Safra International Institute of Neuroscience of Natal, Brazil
[e] Biophysics and Pharmacology Department, Federal University of Rio Grande do Norte, RN 59078-970, Brazil

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.

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

* Corresponding author.
E-mail addresses: brunolobaosoares@gmail.com, brunolobaosoares@hotmail.com (B. Lobão-Soares).

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IP injection of SCH-23390 [11], or by D1 activity facilitation via SKF81297 microinfusion 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 12 h/12 h 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/SvJ hybrid DAT heterozygotes as previously described [18]. Mice were genotyped by PCR using sense WT (5′-CCCGTCTACCCATGAGTAAAA-3′), sense KO (5′-TGACCGCTTCCTCGTGC-3′) and a common antisense primer (5′-CTCCACCTTCCTAGCACTAAC-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 Natal 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 AMcap 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.

2 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, F, 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