Dopamine receptor D5 deficiency results in a selective reduction of hippocampal NMDA receptor subunit NR2B expression and impaired memory

Rodrigo Moraga-Amaro a, Hugo González b, Valentina Ugalde b, Juan Pablo Donoso-Ramos a, Daisy Quintana-Donoso a, Marcelo Lara c, Bernardo Morales c, Patricio Rojas c, Rodrigo Pacheco b,d,* , Jimmy Stehberg a,**

a Laboratorio de Neurobiología, Centro de Investigaciones Biomédicas, Universidad Andres Bello, 8370146, Santiago, Chile
b Laboratorio de Neuroinmunología, Fundación Ciencia & Vida, Núñoa, 7780272, Santiago, Chile
c Laboratorio de Neurociencias, Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, 9170022, Santiago, Chile
d Laboratorio de Neuroinmunología, Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas, Universidad Andres Bello, 8370146, Santiago, Chile

*Corresponding author. Laboratorio de Neuroinmunología, Fundación Ciencia & Vida, Av. Zañartu 1482, Núñoa, 7780272, Santiago, Chile.
**Corresponding author. Laboratorio de Neurobiología, Centro de Investigaciones Biomédicas, Universidad Andres Bello, Av. República 217, Santiago 8370146, Chile.
E-mail addresses: rpacheco@cienciavida.org (R. Pacheco), jstehberg@unab.cl (J. Stehberg).

1. Introduction

The Dopaminergic system plays key roles in many aspects of brain function, including movement coordination, reward, endocrine regulation, cognition and emotion (Jaber et al., 1996; Jackson and Westlind-Danielsson, 1994; Sibley, 1999). The dopaminergic system is mainly investigated as a modulator of motivational behaviors, addiction and reward (Berridge, 2007; Everitt and Robbins, 2005; Goodman, 2008; Kelley and Berridge, 2002; Schultz, 1998; Wise and Bozarth, 1987; Zhang et al., 2007). The importance of dopamine in relation to cognition is well documented, with an essential role in learning and memory (Bliss and Collingridge, 1993; Robbins, 2003). A role for the hippocampal dopaminergic system has been demonstrated in several learning paradigms, including passive avoidance (Bernabeu et al., 1997), win-shift positive reinforcement learning (Packard and White, 1991) and spatial navigation (Gasbarri et al., 1996), while the prefrontal dopaminergic...
system has been associated to aversive memory storage (Gonzalez et al., 2014), associative learning (Puig et al., 2014a), working memory (Clark and Noudoost, 2014) and other memory paradigms (Puig et al., 2014b). Dysfunction of the dopaminergic system in the prefrontal cortex or the hippocampal formation leads to altered learning and impairments in short and long term memory, including spatial learning and goal-directed behaviors in rodents and non-human primates (Whishaw and Dunnett, 1985; Williams and Goldman-Rakic, 1995). Dopamine is also critical in cognitive processes that are disrupted in several diseases affecting the central nervous system (Nieoullon, 2002; Robbins, 2003), including schizophrenia and major depression (Meisenzahl et al., 2007; Millan, 2006; Nestler and Carlezon, 2006). A role for dopamine in memory may be explained by its involvement in synaptic plasticity. Studies have reported that dopaminergic inputs are necessary for long-term changes in synaptic efficacy in different brain areas, including the cortex and hippocampus (Gurden et al., 1999; Huang et al., 2004). Moreover, dopamine has been shown to be necessary for activity-dependent synaptic plasticity, for triggering learning-associated immediate-early gene expression (Lisman and Grace, 2005; O’Carroll and Morris, 2004) and for N-methyl-D-aspartate (NMDA) receptor activation (Hersi et al., 2000).

Dopamine receptors include D1R and D5R, which often are coupled to signal transduction machinery and pharmacological properties. Based on their sequence homology, signal transduction machinery and pharmacochemical properties, dopamine receptors have been classified into two types. Type I dopamine receptors include D1R and D5R, which often are coupled with stimulatory Gα subunits (Gs), while D2R, D3R, and D4R constitute type II dopamine receptors, which are generally coupled with inhibitory Gα subunits (Gsi) (Sibley et al., 1993). Importantly, it has been shown that type I dopamine receptors are expressed in cognitive-related structures, including the hippocampus and prefrontal cortex (Dubois et al., 1986; Huang et al., 1992; Kohler et al., 1991). In this regard, there is extensive pharmacological evidence for a role of type I dopamine receptors in cognitive processes (Castner and Williams, 2007; El-Ghundi et al., 2007) and for synaptic plasticity and LTP/LTD establishment (Dalley and Everitt, 2009; Kusuki et al., 1997; Lemon and Manahan-Vaughan, 2006; Otmakhova and Lisman, 1998; Shepherd and Huganir, 2007; Swanson-Park et al., 1999). Furthermore, pharmacological evidence has shown the involvement of type I dopamine receptors in frequency-modulated discrimination memory (Schicknick et al., 2008), working memory in the win-shift eight-arm radial maze (Packard and White, 1991), in the T-maze (Amico et al., 2007) and in visual attention (Chudasama and Robbins, 2004).

Although current pharmacological evidence points toward a relevant role for type I dopamine receptors in memory, available drugs do not discriminate between D1R and D5R. Some studies have attempted to determine whether D1, D5 or both receptors are involved in memory. however, Sarinana and colleagues using knockout animals lacking either D1R or D5R confined to granule cells of the dentate gyrus, showed that granule cells containing D1R but not D5R are necessary for context memory and context generalization in a fear memory paradigm (Sarinana et al., 2014). The idea that D1R alone is involved in memory is also supported by a previous study in which pharmacological modulation of memory and LTP were attributed only to D1R but not to D5R activation (Granado et al., 2008). Conversely, a study performed by Hersi and colleagues using a mixture of antisense and in vivo-dialysis approaches showed that hippocampal acetylcholine release is modulated by D5R and not D1R (Hersi et al., 2000), which is significant as hippocampal acetylcholine is well known to be relevant for memory (Blake et al., 2014) and LTP induction (Segal and Auerbach, 1997).

In addition, studies using knockout mice have been inconclusive on the differential roles of D1R and D5R in memory. For instance, El-Ghundi and colleagues reported that D1RKO mice show longer escape latencies in the Morris water maze (MWM) paradigm but not in contextual learning (El-Ghundi et al., 1999), suggesting that D1R is involved in certain cognitive tasks. With regard to the possible role of D5R in memory using genetic studies, there are no studies testing memory directly on D5RKO mice. Thus, whereas D1R is clearly associated with some aspects of memory, the role of D5R in memory remains controversial.

The present study aims at determining whether the D5R is involved in memory by testing D5RKO mice in a battery of behavioral tests, including motivational, memory and attention tasks. We found that D5RKO mice have unaffected motivation, but show significant impairments in memory and possibly, in attention. Moreover, our results demonstrate that D5R deficiency results in significant impairment in hippocampal LTP, which may be explained by a selective reduction in the expression of the NR2B NMDA receptor subunit in this brain structure.

2. Materials and methods

2.1. Animals

D5RKO mice were kindly donated by Dr. David Sibley, generated originally from the 129svJ strain (Hollon et al., 2002) and then backcrossed in the C57BL/6 genetic background for more than ten generations with C57BL/6 mice purchased from Jackson Laboratory (Bar Harbor, ME). Eighteen D5RKO mice and nineteen wild-type (WT) littermates, all of them 10–12 weeks old males, weighing between 25 and 35 g were used for behavioral procedures. Beginning 2 weeks prior to behavioral tests and until the end of the experiments, mice were housed individually in plastic home cages in a temperature controlled room, under a 12 h/12 h illumination cycle (lights on at 8:00) and ad libitum access to standard rodent food pellets and tap water. The studies were performed according to protocols approved by the bioethical committee of Universidad Andrés Bello, Chile. Behavioral testing consisted of a battery of tests that followed a sequence from the least to the most stressful. The sequence for the first group of mice began with spontaneous alternation in the T-test, object-based attention test (OAT), Morris water maze test (MWM), Porsolt forced swim test (FST), tail suspension test (TST) and finally, sucrose preference test, (SPT). The SPT was performed to measure anhedonia, at least 1 week after termination of previous tests, to ensure that sucrose withdrawal did not affect other behavioral measures. In a second group of animals, behavioral tests were performed in the following order: Open Field (OF), Object location memory (OLM), the Novel Object recognition memory (ORM), homecage locomotion, MWM, FST and SPT. In a third group of animals, behavioral essays where performed in the following order: OF, OLM, ORM, homecage locomotion, MWM and FST. In all experiments mice behavior was analyzed off-line from digital video recordings by a blinded investigator.

2.2. Genotyping

Genotyping of WT and D5R mutant alleles were performed as previously described (Hollon et al., 2002) with few modifications. The following oligonucleotide primers were used: Primer 1 (5′-ACT CTC TTA ATC TTC GAC ACC TTC-3′) and primer 2 (5′-GGA GAT ACC GCG CAG GAT CTG AAC-3′) were used to amplify the WT allele, whereas primer 3 (5′-TGA TCA ACT ACT GCC CCG GGC GTA-3′), which was unique to the neomycin cassette, was used with primer 1 to amplify the recombinant allele. The PCR reaction was...
performed using 200 ng of genomic tail DNA in a 20 μl reaction (50 mM KCl, 10 mM Tris–HCl, 1.5 mM MgCl2, 0.2 mM dNTP, 0.5 μM of each forward and reverse primer with 2.5 U TaqDNA polymerase). The initial cycle of amplification was as follows: denaturation at 94 °C for 1 min, primer annealing at 55 °C for 2 min, and extension at 72 °C for 2 min. The reaction was then carried for 30 cycles, each consisting of 94 °C for 45 s, 55 °C for 1 min, and 72 °C for 1 min. The extension time at 72 °C for the final cycle was 10 min. The PCR products were subsequently analysed in 1.5% agarose electrophoresis. Whereas the predicted mutant fragment is 468 bp in length, the expected WT fragment is 630 bp.

2.3. General health condition and neurological function

A series of preliminary evaluations for general health, including sensory and motor function were conducted for each mouse to avoid erroneous conclusions. All animals were checked visually for any major health problem or gross motor defect that could affect performance in behavioral tasks. No animals were excluded from the experiments. Exclusion criteria included being deaf, malformations, dwarfism, blindness, fur or whisker abnormalities, and atypical neurological reflexes, which were tested in each mouse including eye blink, ear and whiskers twitch and grasping reflex. For whisker reflexes, whiskers were gently touched by a cotton swab and rapid ear movement was defined as a positive reflex. To test the eye blink reflex, mouse’s eyes were approached with a cotton swab and a blink was defined as a positive reflex. Lastly, to measure the grasping reflex, animals were gently suspended by the tail and were balanced forward towards the corner of a surface. If the animals extended their limbs to reach the surface, this was counted as a positive reflex.

2.4. Morris water maze

The Morris Water Maze (MWM) was modified from the original description (Morris, 1984). An 80 cm diameter round pool filled with water to 40 cm height was used to determine potential spatial memory deficits in D5RKO mice. Each animal was given five swim trials daily for four consecutive days. Five different start positions were used for each trial. Animals were introduced into the pool in the same order each day, but the starting point and animal order were randomized daily. Mice were given 60 s to reach the non-visible submerged platform. If they had not reached the platform by the end of the allotted time, they were placed on the platform for 5 s before starting the next trial. Latency to reach the platform was measured. Every twenty-four hours mice were put in the pool again with the platform in the same location and mice were allowed to search for the platform in order to measure memory retrieval. On the fifth day, the platform was removed and the time the mice spent in the quadrant where the platform was located was measured. The swimming trajectory and speed were also assessed from digital videos recorded for offline analysis.

2.5. Object location memory

We used the Object Location Memory (OLM) paradigm as a second test to assess potential spatial memory deficits in the D5RKO mice. We used the protocol described before (Ampuero et al., 2013) with minor modifications. First mice were habituated to the cage for 3 min per day, for 3 consecutive days. For the training phase, two similar objects were used (two 100 ml schott beakers) which were placed in distinct corners of the cage (Plexiglas box of 50 cm × 30 cm × 30 cm). To enhance object exploration, the cage was inserted in a dark box under dim red light. Animals were placed in the cage to explore the objects for 3 min (counted from the time the animal touched any of the objects for the first time) and allowed to freely explore the two objects. Exploration included any interaction with the object using the head, whiskers or forearms, excluding standing on the object, defecating on it, or touching it with the tail, torso or hindlimbs. In the test phase, 24 h later (to measure long term memory), one of the objects (randomly chosen) was moved to a new corner and the same animals were allowed to explore the objects for another 3 min counted from the time they touched any of the objects. Exploration time of each object, latency to touch the first object and total exploration time were measured for each phase. Animals which did not explore more than 5 s in total on every phase were excluded from the study. On the test phase, object exploration was scored as D2-index, in which the exploration time of the object whose position changed was divided by the total object exploration time, as previously described (Ennaceur and Delacour, 1988).

2.6. Spontaneous alternation in the T maze

Spontaneous alternation in the T maze has been extensively used to measure working memory and is dependent on limbic structures such as prefrontal cortex and septum (Lalonde, 2002). The method used here has been described elsewhere (Jaffard et al., 1981; Lainiola et al., 2014) with minor modifications. The T maze was made of acrylic and consisted of three arms (50 cm × 10 cm, walls 20 cm height) with a 30 cm start compartment and two other perpendicular compartments (30 cm each). Mice were placed in the start compartment facing outwards (not towards the arms) with access to both arms. Once the mouse entered an opened arm, a door was used to close the arm for 30 s, forcing the animal to explore it. After the end of the 30 s the animal was placed again in the start compartment and the trial was repeated. Animals are expected to alternate, remembering the arm they already explored to explore the new one. Each correct alternation is given a score of 1 and no alternation, zero. Alternation score was measured as the average of 4 trials.

2.7. Object recognition memory

To assess memory recognition in D5RKO mice, we used the Novel Object Recognition memory paradigm (ORM) as described before (Ampuero et al., 2013) with modifications. Animals were placed in the same training cage as the OLM (Plexiglas box of 50 cm × 30 cm × 30 cm) for 3 consecutive days, 3 min per day to habituate the animals to the cage. To enhance object exploration during training, the cage was inserted in a dark box under dim red light. On the training day, mice were introduced to the cage and allowed to freely explore for 3 min two identical objects (100 ml Schott beakers) placed in distinct corners of the training cage. The 3 min exploration time were counted from the time the animals touched the objects for the first time. At the test phase 24 h after training (to measure long term memory), one of the objects (randomly chosen) was removed and replaced with a new object. Mice were allowed to explore the two objects for 3 min, counted from the time they first touched one of the objects. Exploration time for each object, latency to touch the first object and total object exploration in each phase were measured. For the test phase, object exploration was scored as D2-index, in which the novel object exploration time, was divided by the total object exploration time, as described before (Ennaceur and Delacour, 1988). Animals that did not explore both objects for at least 5 s in total were excluded from the study.
For the Object-based Attention Test (OAT), the protocol was adapted from a previous study (Alkam et al., 2011) with minor modifications. A Plexiglas box (50 cm long, 30 cm wide, 30 cm high) was divided into two chambers (25 cm long, 15 cm wide, 30 cm high each); the exploration and test chambers. Dividing walls were made from opaque Plexiglas with a sliding door, which allowed access from the exploring chamber to the test chamber. The chambers were cleaned after every mouse with ethanol 70% to eliminate the odor left from the previous mouse. The general procedure consisted of three different phases: a habituation phase, an acquisition phase and a retention phase. On the habituation phase, mice were individually subjected to a single habituation session of 10 min, during which they were exposed to both empty chambers, in order to familiarize them with the chambers. On the acquisition phase animals were subjected to a 3-min session, during which, five objects fixed to the floor (A, B, C, D, and E) were placed separately in the center arena of the exploring chamber. Objects A, B, D, E were located at every corner and C was placed at the center of the chamber. All objects were made of glass and had no color or smell; they were different in shape but roughly similar in size. Mice were allowed to explore the objects for 3 min. Before beginning the trial, the experimenter randomly assigned any two of the five objects for video recording (e.g., object A and object D). The object-exploration time was defined as the time spent exploring any of the two randomly designated objects. The five objects were then withdrawn from the exploring chamber upon the completion of the training session. On the retention phase, which immediately followed the acquisition phase, an object used in the training session (one of the two randomly designated objects for video recording during the training session, e.g. object A) was placed in the test chamber at its original position together with a novel object F of different shape but similar color and size. The time spent exploring each object was measured. Given that mice were allowed to explore and remember the 5 objects during the acquisition phase for only 3 min, which is just enough to learn the objects, normal mice will explore more the novel object than the familiar one. However, attention deficits will decrease the probability of the animal recognizing the previously explored objects, thus treating both objects as novel and spending equal amounts of time exploring them.

2.9. Openfield test (OF)

Thigmotaxis (avoidance of open spaces) is used as a measure of anxiety-like behavior in rodents, and was evaluated in the OF test, together with exploratory activity and locomotion. We the same protocol described before (Moraga-Amaro et al., 2014). Animals were placed at the center of a Plexiglas rectangular box (50 × 30 × 30 cm) and allowed to explore for 5 min. The behavior was recorded digitally for subsequent off-line analysis by a blinded investigator, using 10 × 10 cm virtual squares on the floor of the OF. The number of grooming events, number and latency of rearing, distance traveled (measured as the number of transitions along the squares), average locomotion speed (measured as transitions traveled, divided by time moving), time spent in the periphery (thigmotaxis) or at the center of the OF and duration of locomotion, were measured. Also, transitions and time in center were also measured in time (per minute).

2.10. Homecage locomotion

To measure locomotion at their homecage, mice were recorded for 3 min for offline analysis, and scores were measured for the duration of locomotion.

2.11. Forced swimming test

The Forced Swimming Test (FST) employed here was essentially similar to that described elsewhere (Porstelt et al., 1978). Mice were individually placed in a transparent Plexiglas cylinder (30 cm diameter × 50 cm height) filled with water at 25 ± 2 °C for 5 min. Water was changed after every trial. Following initial vigorous swimming activity, swimming attempts normally cease and the animals adopt a characteristic immobile floating posture. Mice were scored for the total time of immobility defined as the cessation of limb movements by a blinded trained observer. Immobility included movements necessary to balance the body and keep the head above the water, movements associated to drifting or single bouts of mobility. All trials were digitally recorded for subsequent off-line analysis.

2.12. Tail suspension test

The Tail Suspension Test (TST) was performed as previously described (Steru et al., 1985) with minor modifications. In short, mice were suspended for 6 min from a lever by the tail 30 cm above the floor and 40 cm away from the nearest object. The tail was immobilized onto the lever with adhesive tape and a protective rubber was attached around the pressure zone to avoid any physical damage. The duration of escape attempts (escape time) and latency to surrender for the first time were measured throughout a 6 min time period and recorded by a blinded trained observer.

2.13. Sucrose preference test

The Sucrose Preference Test (SPT) is widely used to measure anhedonia (Bolanos et al., 2008; Willner et al., 1987). The original protocol was modified using a two-bottle choice system as a variation of the protocol published previously (Otmakhova and Lisman, 1996). Water bottles were replaced with a 5% sucrose solution for 3 days. On the night of day 3 their bottles were removed (water deprivation overnight) and then given again bottles for 3 days, but now filled with water. On day 7 (test day) one water bottle was replaced with a solution of 5% sucrose, confronting each mouse with a choice of water and sucrose for 10 min. Total liquid consumption of each of the solutions was measured. After the test, mice were returned their regular water bottles.

2.14. Electrophysiology of hippocampal slices

Acute hippocampal slices were prepared from 4 to 5 weeks old WT and DS-RO male littermates. 400 µm thick brain slices were obtained by cutting the brains in a Leica Vibratome VT1200 (Leica, Germany) in ice cold dissection solution (250 mM sucrose, 4 mM KCl, 2 mM CaCl2, 7 mM MgCl2, 125 mM NaHPO4, 2 mM NaHCO3, pH 7.4) bubbled with 95% CO2, 5% O2 until the end of the experiment. Slices are left afloat for one hour at room temperature in ACSF (124 mM NaCl, 4 mM KCl, 2 mM CaCl2, 1.8 mM MgCl2, 125 mM NaHPO4, 2 mM NaHCO3, pH 7.4). Before recording all slices were cut between CA1 and CA3 layers to prevent seizure-like volleys. Extracellular field potentials (fEPSP) were elicited by 200 μs long current pulses in the Shaeffer's collateral fiber and recording in CA1 stratum radiatum with a differential AC Amplifier Model 1800 A-M Systems (Sequim, WA USA). Glass pipettes were pulled from borosilicate tubing in a P-87 Puller (Sutter Instruments, Novato CA) having 1–2 MOhm resistance and filled with ACSF. Acquisition and analysis were performed with custom written routines in Igor Pro (WaveMetrics, USA) using an acquisition card PCI 6221 (National Instruments). Input–Output relationships were obtained by averaging 3 rounds of 200 μs current pulses ranging 5–100 μA,
delivered every 15 s. Paired pulses were obtained by spacing the separation between two 200 μs current pulses, applied every 15 s, by a high frequency stimulation. Current amplitude was chosen to be 50% of maximal fEPSP amplitude. Inter stimulus intervals were 20, 40, 80, 160, 320, 640, 1280 and 2560 ms.

For LTP induction a baseline of at least 20 min consisting of 200 μs current pulses, applied every 15 s were obtained before high frequency stimulation. Current amplitude was chosen to be 50% of maximal fEPSP amplitude. High frequency stimulation consists of theta burst stimulation (TBS), corresponding to 4 trains of 10 stimuli at 100 Hz separated by 400 ms. Bath application of 5 μM SCH23390 started 10 min before TBS for a total of 20 min, as previously described (Rozas et al., 2015).

2.15. Determination of NMDA and AMPA glutamate receptor expression in the hippocampus

Animals were sacrificed by cervical dislocation and brains were quickly removed and placed in ice-cold PBS. The brains were dissected and the hippocampi extracted under a magnifying glass (KL 1500 LCD) using Dumont No.5 forceps. To determine expression levels of NMDA and AMPA receptors in hippocampal tissue of WT and D5RKO mice, hippocampi were homogenized in ice-cold RIPA buffer (0.1% SDS, 150 mM NaCl, 1% Triton 100X, 1.5% sodium deoxycholate), with protease and phosphatase inhibitor cocktails (Roche, Mannheim, Germany), and proteosome inhibitor MG132 (Sigma–Aldrich, St. Louis, MO, USA) in a glass homogenizer for 10 min. Hippocampal lysates were centrifuged at 10,000 g for 10 min at 4 °C and insoluble pellets were discarded. Finally, protein concentration was determined in lysate supernatants by using the bicinchoninic acid (BCA) method (Thermo Scientific, Rockford, IL, USA). Proteins (25 μg per sample) were resolved by a 10% SDS-PAGE, transferred to polyvinylidene difluoride membranes (PVDF, Thermo Scientific, Rockford, IL, USA). Afterward, GluA1 (Clone N355/1; UC Davis/NIH NeuroMab Facility Cat# 73-327, RRID:AB_10672980), GluA2 (Clone L21/32; UC Davis/NIH NeuroMab Facility Cat# 73-002, RRID:AB_10674575), NR1 (Clone N308/48; UC Davis/NIH NeuroMab Facility Cat# 73-327, RRID:AB_10672980) subunits of NMDA and D5RKO mice, hippocampi were homogenized in ice-cold RIPA buffer (0.1% SDS, 150 mM NaCl, 1% Triton 100X, 1.5% sodium deoxycholate), with protease and phosphatase inhibitor cocktails (Roche, Mannheim, Germany), and proteosome inhibitor MG132 (Sigma–Aldrich, St. Louis, MO, USA) in a glass homogenizer for 10 min. Hippocampal lysates were centrifuged at 10,000 g for 10 min at 4 °C and insoluble pellets were discarded. Finally, protein concentration was determined in lysate supernatants by using the bicinchoninic acid (BCA) method (Thermo Scientific, Rockford, IL, USA). Proteins (25 μg per sample) were resolved by a 10% SDS-PAGE, transferred to polyvinylidene difluoride membranes (PVDF, Thermo Scientific, Rockford, IL, USA). Afterward, GluA1 (Clone N355/1; UC Davis/NIH NeuroMab Facility Cat# 73-327, RRID:AB_10672980), GluA2 (Clone L21/32; UC Davis/NIH NeuroMab Facility Cat# 73-002, RRID:AB_10674575), NR1 (Clone N308/48; UC Davis/NIH NeuroMab Facility Cat# 73-327, RRID:AB_10672980) subunits of NMDA and AMPA receptors were detected by using specific primary mouse monoclonal antibodies (all from NeuroMab, UC Davis/NIH, Davis CA, USA) followed by a secondary HRP-conjugated anti-mouse IgG antibody (Rockland, Gilbertsville, PA, USA). For more details about specificity and immunoreactivity of the primary monoclonal antibodies used here please visit: http://neuromab.ucdavis.edu/catalog.cfm. Immunodetection was carried out with SuperSignal West Pico chemiluminescent substrate (Thermo Scientific, Rockford, IL, USA). Membranes were stripped and re-probed with mouse monoclonal anti-β-actin antibody (Sigma–Aldrich, St. Louis, MO, USA) followed by the secondary HRP-conjugated anti-mouse antibody as a control for sample loading. Protein bands were quantified and normalized relative to the loading control band with ImageJ software (National Institute of Health, Bethesda, MD, USA).

2.16. Statistical analyses

All values are expressed as mean ± SE. All data sets were found to be normally distributed using the Kolmogorov–Smirnov normality test. In consequence, statistical analyses were performed using two-tailed unpaired Student’s t-test when comparing two groups of data. Multiple group comparisons were performed using ANOVA followed by a Bonferroni post hoc test (GraphPad Software). P values < 0.05 were considered significant.

3. Results

3.1. DSR deficiency results in spatial memory impairment

Due to the lack of drugs discriminating between D1R and D5R and the lack of genetic studies to specifically evaluate the role of DSR in memory, the relative contribution of DSR in learning and memory is unclear. Therefore, we first addressed the question of whether genetic deficiency of DSR has an impact on spatial memory. For this purpose we compared the performance of DSR-deficient mice with WT littermates in the MWM test, which is commonly used to measure spatial memory (Morris, 1984). WT and D5RKO mice were trained for four consecutive days to allow learning the location of the platform. DSRKO mice displayed a slower learning performance than WT, showing a significant difference in performance at days 2–4, with greater latency to find the platform compared to WT, suggesting learning impairments (Fig. 1A, F = 18.45; p < 0.001; WT day 4 = 16.4 ± 2.9; D5RKO day 4 = 35.4 ± 3.7). At the test trial 24 h after training, in which the platform was removed, DSRKO mice spent significantly less time in the quadrant where the platform was located before removal (Fig. 1B, p < 0.01; WT = 31.0 ± 5.1; D5RKO = 12.9 ± 2.9), and showed a swimming trajectory that did not present a specific search strategy (see Fig. 1C). The total distance that DSRKO mice swim in the test was significantly less than that of WT (Fig. 1D, p < 0.05; WT = 1958.0 ± 95.4; D5RKO = 1454.0 ± 160.5), but swimming speed was not significantly different (Fig. 1E, p > 0.05; WT = 10.9 ± 0.5; D5RKO = 10.7 ± 1.1). These results suggest that DSR deficiency results in impaired spatial memory in mice. In fact, on day 4 of training, the average distance swam by D5RKO mice was 165.1 ± 60.0 cm, while the average linear distance between the platform and every entrance point was 25.5 ± 3.9 cm. This means that DSRKO animals could have traveled linearly at least 3 times from any entrance point to the platform and back, which clearly demonstrates that their increased latency to find the platform cannot be attributed to swimming impairments. This is further supported by the fact that DSRKO mice spent significantly less time in the quadrant where the platform had been previously located (Fig. 1B) and their swimming trajectory reflected no search strategy for the platform (Fig. 1C). Thus, learning impairments in D5RKO cannot be attributed to motor impairments rendering DSRKO mice incapable of swimming towards the platform.

3.2. DSR deficiency results in impaired object location memory

To test the effects of DSR deficiency in another hippocampal-dependent memory paradigm (Barker and Warburton, 2011), DSRKO mice were trained on the OLM, using a dark chamber (with red light) to enhance direct physical exploration of the objects. As can be seen in Fig. 2A, DSRKO mice explored the 2 objects equally during training (p > 0.05; F = 1.424; WT OBJ1 = 63.3 ± 13; WT OBJ2 = 71.8 ± 0.9; DSRKO OBJ1 = 49.6 ± 0.6; DSRKO OBJ2 = 48.5 ± 0.8). At the test 24 post training, DSRKO failed to explore more the object which had been changed to a different location (Fig. 2B, p < 0.01; WT = 0.59 ± 0.03; DSRKO = 0.39 ± 0.05) unlike WT mice, suggesting again impairments in spatial memory.

Interestingly, both during training and test, DSRKO mice showed increased latency to begin exploring (Fig. 2C, p < 0.05; WT = 13.4 ± 2.1; DSRKO = 33.4 ± 9.0, and Fig. 2D, p < 0.05; WT = 10.3 ± 3.2; DSRKO = 52.0 ± 15.7), a non-significant decrease in total exploration time during training (Fig. 2E, p > 0.05; WT = 13.4 ± 2.1; DSRKO = 9.7 ± 1.3) and a significant decrease during the test (Fig. 2F, p < 0.05; WT = 15.8 ± 2.7;
D5RKO = 8.7 ± 1.3). This may be explained by increased anxiety or decreased exploration due to a lack of perceived environmental novelty (as the chamber used during training was the same as the one used during the test). To test these two hypotheses, D5RKO mice were either trained in the object recognition memory task but the test was performed in a novel environment (see Section 3.3.), or tested in the OF to assess possible anxiety-like behavior (see Section 3.6).

3.3. D5R deficiency results in impaired object recognition memory

To test whether D5R deficiency results in non-hippocampal dependent memory impairments, D5RKO mice were trained for the object recognition task (Winters et al., 2004). However, to ensure that the lack of context novelty did not affect exploration, different novel contexts were used for both the training and test. When D5RKO mice were exposed to the two novel objects they explored them equally (Fig. 3A, F = 2.037; p > 0.05; WT OBJ1 = 7.6 ± 1.3; WT OBJ2 = 7.9 ± 1.8; D5RKO OBJ1 = 4.1 ± 0.9; D5RKO OBJ2 = 5.3 ± 0.8), but during the test 24 h after the training, they failed to explore more the novel object compared to WT mice (see Fig. 3B, p < 0.01; WT = 0.65 ± 0.03; D5RKO = 0.38 ± 0.07), suggesting that D5R deficiency results in impairments in novel object recognition memory.

It must be noted that D5RKO mice showed a non-significant increase in latency to explore at training (Fig. 3C, p > 0.05; WT = 12.6 ± 3.0; D5RKO = 28.6 ± 14.7) which was significant at test (Fig. 3D, p < 0.05; WT = 7.6 ± 1.8; D5RKO = 40.7 ± 13.7) and a non-significant decrease in total exploration at training (Fig. 3E, p > 0.05; WT = 15.4 ± 2.8; D5RKO = 9.4 ± 1.7) and test (Fig. 3F, p > 0.05; WT = 14.0 ± 1.8; D5RKO = 9.2 ± 1.6). Given that D5RKO mice show decreased exploration (albeit being non-significant), the possibility that a lack of sufficient time to explore the objects may contribute to the impairments in object recognition memory cannot be ruled out.

3.4. D5R deficient mice display impaired attention

Since attention also involves hippocampal function (Levin et al., 2011) and is required for memory (Broersen, 2000), we next evaluated whether D5R deficiency results in altered attention. For this purpose we compared the attention span of D5RKO and WT mice
by using the OAT. During the training trial, WT mice as well as
D5RKO mice spent similar times exploring object 1 and object 2
(Fig. 4A, F = 1.390; p > 0.05; WT OBJ1 = 6.8 ± 1.0; WT
OBJ2 = 6.6 ± 0.9; D5RKO OBJ1 = 3.3 ± 0.8; D5RKO OBJ2 = 4.1 ± 1.2).
However, in the test trial, while WT mice explored the new
object for a longer time than the familiar object, D5RKO mice
explored both, the new and the familiar objects for similar times
(Fig. 4B, F = 8.431; p < 0.05; WT New = 11.0 ± 1.2; WT known = 6.3 ± 0.9;
D5RKO New = 8.4 ± 0.9; D5RKO Known = 4.1 ± 1.2). Thus, these
results suggest that D5R deficiency in mice may result in attention
deficit. Nonetheless, given that OAT involves multiple-object
recognition, deficits in this task may also be explained by motiva-
tional deficits, impairments in motor function or in recognition
memory. We have found in the previous experiment that D5RKO
mice show impairments in ORM as assessed 24 h after training.
In consequence, it is possible that D5RKO mice failed in the OAT for
having short-term memory impairments in object recognition
memory.
It must be noted yet once again that D5RKO mice showed a significant decrease in total object exploration during the training (Fig. 4C, p < 0.05; WT = 13.3 ± 1.9; D5RKO = 7.4 ± 1.7) but no differences at the test (Fig. 4D, p > 0.05; WT = 17.33 ± 1.9; D5RKO = 15.7 ± 1.5). That this decrease in total exploration during training may have also contributed to the decreased exploration of the novel object cannot be ruled out.

3.5. DSR deficiency results in unaffected working memory

Spontaneous alternation in the T maze has been extensively used to measure working memory in rodents and is dependent on limbic structures such as prefrontal cortex and septum (Lalonde, 2002). As can be seen in Fig. 4E, D5RKO mice showed no significant differences with WT mice in spontaneous alternation using the T-maze test, suggesting unaffected working memory (Fig. 4E, p > 0.05; WT = 0.8 ± 0.1; D5RKO = 0.7 ± 0.2).

3.6. DSR deficiency results in decreased locomotion but no effects on anxiety

To assess whether D5R produces effects in locomotion, exploration and anxiety, D5RKO mice were exposed to the OF. D5RKO mice spent the same amount of time at the center and at the periphery (no increased thigmotaxis) as WT mice (see Fig. 5A, p > 0.05; WT = 29.4 ± 6.0; D5RKO = 19.2 ± 6.7; and Fig. 5B, p > 0.05; WT = 270.6 ± 6.0; D5RKO = 280.8 ± 6.7) suggesting no effects on anxiety-like behavior. However, D5RKO mice showed significantly less total locomotion (Fig. 5C, p < 0.01; WT = 148.1 ± 15.0; D5RKO = 87.4 ± 10.5). To determine whether this decrease in locomotion is due to motor problems, the average speed of locomotion was measured, taking the distance traveled, divided by the duration of movements. There was no difference between D5RKO and WT mice in locomotion speed (Fig. 5D, p > 0.05; WT = 1.4 ± 0.1; D5RKO = 1.5 ± 0.1), suggesting that the decrease in locomotion in D5RKO is not due to a motor deficit. Interestingly, when the total locomotion in the OF was measured by minute, WT mice showed increased locomotion during the first minute, which decreased over time, becoming significantly lower at the fourth and fifth minute (Fig. 5E, p < 0.05; WT 1MIN = 35.1 ± 5.6; WT 4MIN = 23.2 ± 2.6; WT 5MIN = 23.0 ± 3.6). This effect was not seen in D5RKO mice, showing significantly less locomotion compared to WT over the first 2 min, but remaining constant over the 5 min measured.

The total duration of locomotion for D5RKO mice was significantly lower than for WT (Fig. 5F, p < 0.001; WT = 92.2 ± 4.7; D5RKO = 56.7 ± 5.7). This means that D5RKO mice not only traveled a smaller distance but also spent less time moving, which is congruent with having no differences in speed. Given that D5RKO mice did not show increased anxiety-like behavior in the OF and do not show noticeable motor problems that could affect their locomotion speed, it is possible that the decrease in total locomotion may be associated to a difference in the response to environmental novelty, which would also explain the differences with WT mice in locomotion over time found in the OF. To assess this issue, D5RKO mice were placed at their homecage and their locomotion was measured. D5RKO showed no differences in the total duration of locomotion inside their homecage compared to WT (Fig. 5G, p > 0.05; WT = 38.2 ± 3.8; D5RKO = 34.0 ± 2.3). This suggests that D5RKO mice may show decreased locomotion in arousing environments (such as the OF), but not at their homecage.

3.7. DSR deficiency has no impact on depression-like symptoms

Spatial memory impairments are commonly associated with hippocampal dysfunction. Since depression has been extensively associated with hippocampal alterations (Malykhin and Coupland, 2015; O’Leary and Cryan, 2014), it is possible that D5R deficiency is also associated with depressive-like symptoms. Depressive-like symptoms in D5RKO mice could explain learning deficits by a
Fig. 5. D5RKO mice showed decreased locomotion but no effects on anxiety or depression. A. D5RKO showed no differences in time spent at the center of the openfield compared to WT. B. There were no differences between groups in thigmotaxis. C. D5RKO showed decreased locomotion expressed as a decrease in number of transitions traveled. D. D5RKO mice showed no difference on locomotion speed. E. WT mice (open bars) showed increased locomotion during the first minute, which decreased over time, becoming significantly lower at the fourth and fifth minute. D5RKO (closed bars) showed a significant decrease in locomotion compared to WT during the first 2 min, but did not show significant changes in locomotion over time. F. D5RKO animals showed deceased duration of locomotion in the Openfield. G. When returned to their homecage D5RKO showed similar duration of locomotion than WT. H. D5RKO mice show similar immobility time as WT mice in the tail suspension test. J. D5RKO mice show similar immobility time as WT mice in the tail suspension test. N: WT = 9 (open bars) and D5RKO = 9 (closed bars). E: One way ANOVA (within groups), *p < 0.05, **p < 0.01, ***p < 0.001; Two-way ANOVA (between groups), #p < 0.05, ##p < 0.01. A-D, F-J: t-test, *p < 0.05, **p < 0.01, ***p < 0.001.
lack of motivation. For this purpose we evaluated depressive-like symptoms using the three most accepted tests to measure depressive-like symptoms in rodents; the FST, TST and SPT. Results showed that D5RKO mice remained immobile for similar times as WT littermates when evaluated in the FST (Fig. 5H, p > 0.05; WT = 96.9 ± 14.6; D5RKO = 85.1 ± 17.4) and the TST (Fig. 5I, p > 0.05; WT = 106.2 ± 10.7; D5RKO = 111.2 ± 12.5). Since FST and TST involve locomotor activity, we also included the SPT, which evaluates depressive-like symptoms (anhedonia) without significant requirement of locomotor activity. Results show that D5RKO mice display decreased sucrose preference than WT mice in the SPT (Fig. 5J, p < 0.05; WT = 59.1 ± 6.2; D5RKO = 26.7 ± 8.1). However, given that there is no evidence of motor dysfunction in D5RKO mice and that FST and TST are the most commonly used tests to assess depressive-like symptoms in rodents, the data altogether suggests that D5R deficiency may not be associated with depressive behaviors in mice. It is unknown whether D5R deficiency induces effects in the taste system that could explain the difference in the SPT.

3.8. D5R deficiency results in attenuated long-term potentiation

Spatial memory and attention are associated to synaptic plasticity in the hippocampus (Lynch, 2004). In order to understand how the lack of D5R affects the function of hippocampal circuits, we studied long-term potentiation (LTP) in D5RKO mice as a way to understand changes in synaptic plasticity. Field excitatory postsynaptic potentials (fEPSP) in CA1 stratum radiatum were elicited by stimulation of Schaffer’s collaterals. In order to study the intrinsic activity of the circuit, input—output (I–O) relationships were obtained by application of increased amplitude current pulses (Fig. 6A). The position of the I–O curve for D5RKO showed no difference compared to WT, suggesting no alteration of circuit excitability. Pair pulse stimulation at several inter stimulus intervals (ISI) were performed to study plastic properties of synapses, such as facilitation or depression. Pair-pulse ratios with respect to ISI show no differences between WT and D5RKO (Fig. 6B). Altogether this evidence shows that the intrinsic circuit properties were not affected by the deletion of D5R.

Application of theta burst stimulation (TBS) to induce LTP produced an increase in fEPSP slope in WT, which was almost completely abolished in D5RKO mice (Fig. 6C). This evidence suggests that most of the TBS-induced LTP depends on the presence of D5R. Interestingly, the magnitude of the impairment in the fEPSP slope of D5RKO mice is stronger than the reduction of fEPSP slope previously reported for D1RKO mice (Granado et al., 2008).

Furthermore, when LTP was induced in presence of the D1R/D5R inhibitor SCH23390 there was a non-significant decrease in LTP induction in WT compared to control conditions (Fig 6D), although it has been reported previously that SCH23390 has no effect on early LTP (Rozas et al., 2015). In D5RKO mice the antagonist also showed no significant alteration on TBS-induced LTP (Fig. 6D). Taken together, these results indicate that D5R deficiency in mice results in impaired synaptic plasticity, which is manifested by decreased TBS-induced LTP, effect that was not sensitive to acute administration of SCH23390. Moreover, this data suggests that D5R deletion caused a circuit remodeling probably involving NMDAR subunits that impairs the development of early LTP.

3.9. D5R deficiency results in a strong and selective reduction of expression of the NMDA subunit NR2B

Since the precise contribution of hippocampal NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunits plays a major role in synaptic plasticity and memory (Paolletti et al., 2013), we next addressed the question of whether D5R deficiency involves alterations in the expression of NMDA or AMPA receptors subunits. Accordingly, we determined the protein expression of NMDA subunits NR1, NR2A and NR2B and the AMPA subunits GluA1 and GluA2 in the hippocampus of WT and D5RKO mice. The results show that whereas GluA1, GluA2, NR1 and NR2A (Fig. 7A) subunits were expressed at similar levels in the hippocampus of WT and D5RKO, the NR2B subunit was dramatically decreased in the hippocampus of D5R deficient mice (Fig. 7A and B). Therefore, these results indicate that D5R deficiency in mice results in a strong and selective reduction in the hippocampal expression of the NR2B subunit of NMDA receptors.

4. Discussion

The findings presented in this study indicate that D5R deficiency results in impaired spatial memory without inducing depression-like symptoms. Furthermore, the memory deficits observed in D5RKO mice may be associated with important hippocampal alterations including attenuated LTP and a selective reduction in the expression of the NMDAR subunit NR2B. Moreover, these results represent the first genetic evidence indicating the involvement of D5R in memory and they also give a functional and molecular association linking D5R with synaptic plasticity and NMDAR subunit composition in the hippocampus.

The evidence presented here indicates that D5R deficiency results in impaired memory and is in agreement with previous studies using pharmacological approaches. In this regard, the microinjection of the D1R/D5R antagonist SCH23390 into the hippocampus impairs long term memory in the MWM (da Silva et al., 2012). ORM, inhibitory avoidance (Furini et al., 2014; Rossato et al., 2013) and fear conditioning (Inoue et al., 2000), while the hippocampal microinjection of the D1R/D5R agonist SKF38393 has been shown to enhance long term memory in the MWM (da Silva et al., 2012). Acute application of this agonist to brain slices increases LTP in CA3–CA1 synapse (Huang and Kandel, 1995), consistent with improvements in memory. Indeed, even intraperitoneal administration of D1R/D5R antagonist SCH23390 has shown to increase the escape latency in the MWM (Stuchlik et al., 2007). In a similar way, the acute application of SCH23390 decreases in vivo late but not early LTP (Lemon and Manahan-Vaughan, 2006). Further pharmacological evidence has shown the involvement of type I dopamine receptors in frequency-modulated discrimination memory (Schicknick et al., 2008), working memory in the win-shift eight-arm radial maze (Packard and White, 1991) and in the T-maze (Amico et al., 2007) and in visual attention (Chudasama and Robbins, 2004). Interestingly, a study based on intracellular signaling coupled to D1R and D5R in the hippocampus has attempted to dissect the contribution of these receptors to memory consolidation. Since hippocampal D1R and D5R activate different signaling pathways involving cAMP-dependent protein kinase (PKA) and protein kinase C (PKC) respectively, Furini and colleagues induced memory consolidation blockade of ORM and inhibitory avoidance using SCH-23390, which was reverted by co-injections of both the PKA activator 8Br-cAMP or PKC activator PMA (Furini et al., 2014). Thus, these authors proposed that both D1R and D5R contribute to memory consolidation (Furini et al., 2014). Both D1R and D5R are expressed in hippocampus and dentate gyrus, where D1R but not D5R has been implicated in fear conditioning (Sarinana et al., 2014).

At the circuit level, the intrinsic excitability of glutamatergic response measured as the position of I–O and ISI curves were not affected on D5RKO mice compared to WT, similar to what has been reported for D1RKO (Granado et al., 2008), suggesting that the activity or presence of these receptors is not critical for setting the
basal circuit excitability.

LTP is usually divided into a non-protein synthesis dependent early LTP and a protein synthesis dependent LTP. While a role for D1R and D5R in late LTP is well accepted (Granado et al., 2008; Hu et al., 2010; Huang and Kandel, 1995; Yang et al., 2005), their role in early LTP remains controversial (Mockett et al., 2004). There are studies that have reported that administration of D1R/D5R agonists or antagonists affect early LTP (Otmakhova and Lisman, 1996) while others have not found effects (Rozas et al., 2015).

Genetic studies using D1RKO mice found a small reduction in early LTP (Gangarossa et al., 2012; Granado et al., 2008). In this regard, D1R expression in the hippocampus is low (Gangarossa et al., 2012), which is consistent with the small reduction in early LTP found in D1RKO mice (Granado et al., 2008). Conversely, D5R is widely expressed in the hippocampus (Khan et al., 2000), which is consistent with the virtually complete loss of LTP in D5RKO mice found in this work. This decrease in LTP can be explained by long-term circuit modifications, which may not occur after the acute blockade of D1R/D5R by SCH23390. However, it is also possible that the strong LTP disruption in D5RKO mice obtained here may not allow us to detect the effects of SCH23390 administration.

Here we show that genetic deficiency of D5R results in a significant attenuation of hippocampal LTP. Consistently, D1R/D5R antagonism attenuates hippocampal LTP (Lemon and Manahan-Vaughan, 2006; Wiescholleck and Manahan-Vaughan, 2014), while D1R/D5R stimulation induces slow onset potentiation.

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Fig. 6. D5R deficiency results in impaired long-term potentiation. A. Circuit excitability in D5RKO mice is similar to WT. Slope of fEPSP in response to 200 ms current pulses form 5–60 μA for WT (open circles) and D5KO mice (filled circles). B. Paired-pulse ratio at ISI values 20–2000 ms for WT (open circles) and D5RKO mice (filled circles). C. LTP in CA1 layer is significantly decreased after TBS stimulation on Schaeffer’s collateral fibers for the D5RKO (filled circles) compared to WT (open circles). In the inset are shown representative fEPSP before (1) and 30 min after TBS (2). D. D1/D5 inhibitor SCH23390 (black line) does not alter TBS-induced LTP. Values represent mean ± SEM from five mice per group (A and B). ***p < 0.0001.
(Navakkode et al., 2007) and a lower threshold for LTP (Lemon and Manahan-Vaughan, 2006), suggesting that synaptic plasticity may be impaired after D1R/D5R antagonism. The absence of TBS-induced LTP in D5RKO mice independently of SCH23390 administration can be explained by the decreased expression of glutamatergic NMDAR subunit NR2B, which is critical for LTP induction.

Hippocampal glutamatergic synapses have been shown to be crucial for LTP and memory (PaOLETTI et al., 2013). Accumulating evidence indicates that two kinds of ionotropic glutamate receptors, NMDA and AMPA receptors, are dynamically regulated and subjected to activity-dependent long-term plasticity in the adult hippocampus (Hunt and Castillo, 2012). These receptors form heterotetrameric assemblies; whereas AMPA receptors are composed by symmetric dimers of GluA2 and either a GluA1, GluA3 or GluA4 subunit (Greger et al., 2007; Mayer, 2005). NMDA receptors typically associate NR1 subunits with NR2 subunits or a mixture of NR2 and NR3 (PaOLETTI et al., 2013). Thus, the precise subunit contribution to the composition of hippocampal NMDA and AMPA receptors plays a major role in synaptic plasticity and memory.

The results presented here show that D5R deficiency results in a strong and selective reduction in the hippocampal expression of the NMDA subunit NR2B, without affecting the expression of NMDA subunits NR1 and NR2A or AMPA subunits GluA1 and GluA2 (Fig. 7). In this regard, there is strong evidence indicating that Ca2+-/calmodulin-dependent protein kinase II (CaMKII) interacts more strongly with the C-terminal domain of NR2B subunit than with that of NR2A subunit of NMDA receptors (Barria and Malinow, 2005). Since CaMKII plays a pivotal role in LTP induction (Lisman et al., 2012), the NR2B–CaMKII interaction has major implications for synaptic plasticity. Furthermore, several studies in vitro and in vivo have indicated that a change in the NR2A-to-NR2B ratio affects subsequent NMDA receptor-dependent synaptic modifications. It has been shown that D1R/D5R agonist SKF38393 is able to induce LTP only when NMDA receptors have the NR2B subunit present (Stramiello and Wagner, 2008). Moreover, changes in the NR2A-to-NR2B ratio, either through pharmacological means (Xu et al., 2009) or activity-dependent alterations (Lee et al., 2010; Philpot et al., 2007) regulate both the magnitude and sign of subsequent plasticity, leading to changes in LTP. In agreement with a critical role of NR2B in LTP, when the NR2A-to-NR2B ratio is enhanced, stronger stimulation (i.e. a higher stimulation frequency) is required to induce LTP (Kopp et al., 2006). In addition, activation of D1R/D5R produces an increase in amplitude of NMDA EPSPs composed of NR2B subunits, while it may induce a decrease when NR2B subunit is absent (Varela et al., 2009). This is consistent with our data showing a decrease in NR2B expression in D5RKO mice, which showed decreased LTP. Thus, the precise contribution of NR2A and NR2B to the composition of hippocampal NMDA receptors plays a major role in synaptic plasticity and memory. The present results suggest that D5R deficiency leads to a reduction of hippocampal NR2B, which may explain at least in part, the impairments in both, hippocampal LTP and memory that have been observed in this study.

In a study of Barkus and colleagues (Barkus et al., 2012), it was shown that NR1 hypomorph mice which have decreased NMDA receptor expression show a similar pattern of deficits in memory as found here in D5RKO mice. These authors also found unclear results in anxiety and changes in spontaneous homecage behavior. However, they found important differences in depressive-like symptoms in males and hyperactivity (Barkus et al., 2012). Thus, it is possible to suggest that D5RKO cognitive impairments, including hippocampal and non-hippocampal dependent memory deficits, may be attributed to a decrease in NMDA-dependent synaptic plasticity due to decreased NR2B expression in the hippocampus, while the loss of exploratory behavior and decreased locomotion without clear signs of anxiety, depression or motor dysfunction, may be more related to direct consequences of D5R deficiency.

In that respect, D5RKO mice showed decreased locomotion when exposed to the OF (Fig. 5C and F) but not in their homecage (Fig. 5G). Also D5RKO mice showed decreased yet constant locomotion when analysed by each minute in the OF, not showing the decrease in locomotion found over time in WT (Fig. 5E). Given that D5RKO mice did not show increased anxiety in the OF (Fig. 5A and B), or differences in locomotion speed that may suggest motor problems (Fig. 5D), it is possible to suggest that the decrease in locomotion and exploration may be due to changes in the response to contextual novelty (hence the change in behavior according to the novelty or arousal associated to the context, like homecage versus novel contexts).

Another alternative is that D5RKO mice, due to their almost complete loss of LTP and decreased expression of NR2B, they may act demented-like with severe loss of navigational capacity and cognition. D5RKO showed increased latency to explore, taking significantly longer time to begin exploring (Figs. 2C, D and 3D), and a significant decrease in total exploration time (Figs. 2E, F and 4C). Thus, they may not to learn at all in the MWM, ORM and OLM, they may have decreased locomotion and exploration and increased latency to explore.

Although the present evidence demonstrates that D5RKO mice show severe learning and memory deterioration that may be
associated to impairments in synaptic plasticity, further studies are required to determine the mechanisms subjacent to the decrease in exploration and locomotion found in D5RKO. In agreement with the decreased locomotion observed here in D5RKO mice, previous studies have shown that D5RKO mice present a reduced locomotion in response to cocaine (Elliott et al., 2003), while not displaying noticeable motor deficits (Holmes et al., 2001).

Based on the present results, D5R deficiency does not appear to affect motivation (SPT excluded), or working memory. It has been suggested that polymorphisms of the D5R gene may be involved in attention deficit disorder (Daly et al., 1999). To test whether D5R deficiency results in attention deficits we tested attention in D5RKO mice using the OAT, which requires recognition memory. The ORM deficits found here in long-term memory, may explain D5RKO failure in the OAT. Even though attention deficit was found here in D5RKO mice, this deficit cannot be solely attributed to attention. Thus, the present study was not able to determine whether D5RKO mice show attention deficits in a conclusive manner. Further studies are required to determine whether the deficits found in the OAT are attributable to short-term memory deficits or are in fact attentional.

5. Conclusions

The findings presented here demonstrate a relevant contribution of D5R in memory. This genetic evidence indicates the involvement of D5R in spatial memory and in hippocampal synaptic plasticity. Our results show that D5R deficiency results in LTP impairments that are not dependent on D1R antagonism. They also demonstrate a significant reduction in the expression of NMDA subunit NR2B, suggesting a functional collaboration between D5 and hippocampal glutamatergic pathways. This new data could be useful for future therapies for disorders involving alterations in memory and the dopaminergic system.

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