Neurobiology of Learning and Memory 97 (2012) 271–275

Contents lists available at SciVerse ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

D_1/D_5 dopamine receptors modulate spatial memory formation

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ARTICLE INFO

Article history:
Received 8 November 2011
Revised 19 December 2011
Accepted 4 January 2012
Available online 14 January 2012

Keywords:
Spatial learning
Hippocampus
SCH23390
SKF38393
Reversal learning
Water maze

ABSTRACT

We investigated the effect of the intra-CA1 administration of the D_1/D_5 receptor antagonist SCH23390 and the D_1/D_5 receptor agonist SKF38393 on spatial memory in the water maze. When given immediately, but not 3 h after training, SCH23390 hindered long-term spatial memory formation without affecting non-spatial memory or the normal functionality of the hippocampus. On the contrary, post-training infusion of SKF38393 enhanced retention and facilitated the spontaneous recovery of the original spatial preference after reversal learning. Our findings demonstrate that hippocampal D_1/D_5 receptors play an essential role in spatial memory processing.

1. Introduction

Dopamine regulates value-based decision-making (Sugrue, Corrado, & Newsome, 2005), and through modulation of the effectiveness and significance of stimuli (Wise, 2004), might induce synaptic plasticity (Lisman & Grace, 2005). In agreement with this hypothesis, D_1 dopamine receptors elicit the onset of the late, protein synthesis-dependent phase of long-term potentiation (LTP) in the hippocampus (Huang & Kandel, 2006), control plasticity -induced protein synthesis (Sajikumar & Frey, 2004), and enhance the persistent storage of hippocampus-dependent memories (Rossato, Bevilaqua, Izquierdo, Medina, & Cammarota, 2009).

The participation of the hippocampus in spatial memory formation has been clearly established (Bird & Burgess, 2008; Leutgeb et al., 2005; Martin & Clark, 2007). Nonetheless, knowledge about the molecular requirements of this process is still incomplete. In particular, information about the role played by the hippocampal dopaminergic system is scarce and originates, mainly, from studies with mutant animals. In this regard, it has been shown that D_1 receptor-knockout mice have spatial learning deficits (El-Ghundi et al., 1999; Granado et al., 2008; Tran et al., 2008), although these mutants also show decreased reactivity to external stimuli, increased locomotion, and deficiencies in initiating movement (Smith et al., 1998), which could reflect compensatory processes resulting from the developmental absence of D_1 receptors (Clifford et al., 1998), and therefore, complicate the interpretation of behavioral data. To circumvent these problems, we analyzed the effect of the intra-hippocampal infusion of well-known D_1/D_5 receptors antagonists and agonists on the retention of the long-term memory (LTM) for a spatial preference in the water maze (WM).

2. Material and methods

2.1. Drugs and statistical analyses

SKF38393 and SCH23390 were from Sigma–Aldrich (St. Louis, MO, USA). They were dissolved in DMSO and stored protected from light at −20 °C until use. Right before that an aliquot was thawed and diluted to working concentration with 0.1% DMSO in saline (pH 7.2). The doses utilized were determined based on previous studies showing the effect of these compounds on learning and memory (Rossato et al., 2009). Data were analyzed by two-tailed Student's *t*-test, repeated measures ANOVA, one-way ANOVA followed by post hoc tests, or the Wilcoxon signed rank test, as appropriate.

2.2. Subjects, surgery and drug infusion procedure

Male Wistar rats (3-month-old, 300–350 g) bought at FEPPS (Fundação Estadual de Produção e Pesquisa em Saúde do Rio

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doi:10.1016/j.nlm.2012.01.005

Grande do Sul, Porto Alegre, Brazil) were used. The animals were housed 5 to a cage and kept with free access to food and water under a 12/12 light/dark cycle, with light onset at 7:00 AM. The temperature of the animal room was maintained at 22–24 °C. To implant them with indwelling cannulas, rats were deeply anesthetized with thiopental (i.p. 30–50 mg/kg) and 22-gauge cannulas stereotaxically aimed to the CA1 region of the dorsal hippocampus (A-4.2, L ± 3.0, V-1.8; Paxinos & Watson, 1986). Animals were allowed to recover from surgery for 4 days before submitting them to any other procedure. At the time of drug delivery, 30-gauge infusion cannulas were tightly fitted into the guides. Infusions (1 μl/side) were carried out over 60 s with an infusion pump and the cannulas were left in place for 60 additional seconds to minimize backflow. The placement of the cannulas was verified postmortem: 2–4 h after the behavioral test, 1 μl of a 4% methylene-blue solution was infused as described above and the extension of the dye 30 min thereafter was taken as an indication of the presumable diffusion of the vehicle or drug previously given to each animal. Only data from animals with correct implants were analyzed (96% of all implanted animals). All experiments were conducted blind to the treatment condition of the animals. Every effort was made to minimize the animal's suffering and to reduce the number of animals used. The "Principles of laboratory animal care" (NIH publication N° 85–23, revised 1996) were strictly followed.

2.3. Training in the spatial version of the water maze (WM)

The WM was a black circular pool (200 cm in diameter) conceptually divided in 4 imaginary quadrants for the purpose of data analysis. The temperature of the water was kept at 21–23 °C. Two centimeters beneath the surface of the water and hidden from the animals' view was a black circular platform (12 cm in diameter) which had a rough surface that allowed rats to climb onto it easily once its presence was detected. The swimming path was evaluated using a video tracking and analysis system. The maze was located in a well-lit white room with several posters and other distal visual stimuli hanging on the walls to provide spatial cues. Rats were handled during 5 min per day for 3 consecutive days prior to training. Animals were trained in the hidden platform (spatial) version of the WM for 5 (long training protocol) or 2 consecutive days (short training protocol), depending on the experiment. On each day, rats received eight consecutive training trials during which the hidden platform was kept in a constant location. A different start location was used on each trial, which consisted of a swim followed by a 30-s stay on the escape platform. The intertrial interval was 30-s. Any rat that did not find the platform within 60-s was guided to it by the experimenter. Drugs were infused at immediately or 3 h after the last trial of each training session. Memory retention was evaluated during a probe test in the absence of the escape platform carried out 24 or 120 h after the last training session. As indicators of memory retention we used the latency to swim over an imaginary annulus (24 cm in diameter) centered at the previous location of the escape platform and/or and the time spent swimming in the target quadrant.

2.4. Reversal learning

Rats were trained in the spatial version of the WM during five days as stated above and, 24 h after the last training session were submitted to eight 60-s long reversal learning trials in which the platform was placed in the opposite quadrant of the pool. Memory retention was evaluated in a probe test carried out 24 or 120 h after the last reversal trial.

2.5. Training in the non-spatial version of the WM

For training in the non-spatial version of the WM, we used the same tank as for training in the spatial version of the task, but heavy black curtains hanged on a ceiling-mounted track were drawn around the maze to occlude distal visual cues. A white plastic disk 10 cm in diameter was mounted on top of the hidden platform to indicate its location. The number of trials and sessions were identical to that for training in the spatial protocol. Drug infusion was performed as stated above.

2.6. Inhibitory avoidance training

Rats were trained in a one-trial, step-down inhibitory avoidance during the light phase of the subjective day (between 9.00 and 11.00 h). The training apparatus was a 50 × 25 × 25 cm plexiglass box with a 5 cm-high, 8 cm-wide and 25 cm-long platform on the left end of a series of bronze bars which made up the floor of the box. For training, animals were gently placed on the platform facing the left rear corner of the training box. When they stepped-down and placed their four paws on the grid, received a 2-s, 0.5 mA scrambled footshock and were immediately withdrawn from the training box. Memory retention was evaluated in a non-reinforced test session carried out 24 h after training. In the test session, the animals were placed back on the training box platform until they eventually stepped down to the grid. The latency to step-down during the test session was taken as an indicator of memory retention.

3. Results

To establish whether D_1/D_5 dopamine receptors are necessary for spatial LTM formation, rats were trained in the spatial version of the WM using a 5 day-long training protocol. Bilateral intra-CA1 infusions of the D_1/D_5 receptor antagonist SCH23390 (5 nmol/side), immediately but not 3 h after every daily training session, blocked the decrease in escape latency seen in control animals (Fig. 1A; $F(2, 80) = 5.303$, $p < 0.05$ for treatment; $F(8, 80) = 2.816$, $p < 0.01$ for the interaction between session and treatment). A probe test in the absence of the escape platform carried out 24 h after the last training session confirmed that intra-CA1 administration of SCH23390 impairs spatial memory retention during a limited post-training time window. In this probe test, rats that received SCH23390 immediately after training showed longer latencies to swim over the previous position of the escape platform (Fig. 1B; $F(2, 20) = 4.336$, $p < 0.05$), and spent less time swimming in the target quadrant (Fig. 1C; $F(2, 20) = 9.796$, $p < 0.01$) than animals that received vehicle or were given SCH23390 3 h post-training. SCH23390 did not affect acquisition of the escape response when given immediately after training in the non-spatial version of the WM (Fig. 1E; $F(1, 40) = 3.157$, $p = 0.11$ for treatment; $F(4, 40) = 1.053$, $p = 0.39$ for the interaction between session and treatment). Moreover, animals that received daily intra-CA1 infusions of SCH23390 for 5 days before being trained in inhibitory avoidance, a hippocampus-dependent learning task, learned the avoidance response normally (Fig. 1F; $Z = -2.803$, $p < 0.01$), suggesting that repeated administration of SCH23390 does not affect the functionality of the hippocampal formation.

We next tested the ability of the D_1/D_5 receptor agonist SKF38393 to improve long-term spatial memory. Because the 5 day-long WM training protocol generates a persistent spatial preference lasting more than 30 days (not shown), initially we used a short WM training protocol (see Section 2.3) to avoid possible ceiling effects. When given in dorsal CA1 immediately after training, SKF38393 (45 nmol/side) decreased the time to swim