Short communication

Extinction memory is facilitated by methylphenidate and regulated by dopamine and noradrenaline receptors

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HIGHLIGHTS

- Methylphenidate (MPH) facilitates the consolidation of extinction memory.
- The blockade of the β-noradrenergic receptors reversed the effect induced by the MPH.
- The blockade of the D1/D5 dopamine receptors reversed the effect induced by the MPH.

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ABSTRACT

Extinction is defined as the learned inhibition of retrieval and is the mainstay of exposure therapy, which is widely used to treat drug addiction, phobias and fear disorders. The psychostimulant, methylphenidate (MPH) is known to increase extracellular levels of noradrenaline and dopamine by blocking their reuptake and studies have demonstrated that MPH can modulate hippocampal physiology and/or functions including long-term potentiation (LTP), learning and memory. However, the influence of MPH on fear extinction memory has been insufficiently studied. Here we investigate the effect of MPH infused into the CA1 region of the hippocampus on extinction memory in animals normally incapable of showing contextual fear conditioning (CFC) extinction because of weak training, and the possible mechanisms through which it acts during this process. For this, male Wistar rats with infusion cannulae stereotaxically implanted in the CA1 region were submitted to a weak extinction protocol in a CFC apparatus. Animals that received intra-CA1 infusion of MPH (12.5 μg/side) 20 min before the extinction training (Ext Tr) expressed less freezing behavior than Veh-treated animals during both Ext Tr and extinction retention Test (Ext Test). Additionally, the administration of MPH + Timolol (1 μg/side) or MPH + SCH23390 (1.5 μg/side) intra-CA1 20 min before the Ext Tr blocked the enhancing effect of the MPH on extinction learning. These results suggest that MPH in the CA1 region of the hippocampus is able to induce the consolidation of extinction memory and this process occurs through both β-adrenergic and D1/D5 dopaminergic receptors.
functions including long-term potentiation (LTP), learning and memory [6–10]. However, the influence of MPH on fear extinction memory has been insufficiently studied. Taking into account the clinical application of extinction, reinforces the need to understand its mechanisms better, particularly those that underlie its modulation by pharmacological agents [2,11,12]. Here we investigate the effect of MPH infused into the CA1 region of the hippocampus on extinction memory in animals normally incapable of showing CFC extinction because of weak training, and the possible mechanisms through which it acts during this process. This could be useful to better understand exposure therapy and increase its effectiveness.

For this, male Wistar rats (Crl:CD:WI; 3 months-old, 300–330 g) purchased from Centro de Modelos Biológicos e Experimentais (CeMBE) of the Pontifical Catholic University of Rio Grande do Sul, Porto Alegre – Brazil, were housed four to a cage and kept with free access to food and water, under a 12-h light/dark cycle (lights on at 7:00 a.m.) and temperature of the animals’ room maintained at 22–24 °C. All experimental procedures were performed in accordance with Animal Committee on Ethics in the Care and Use of Laboratory Animals of the Pontifical Catholic University of Rio Grande do Sul. Under deep anesthesia (75 mg/kg ketamine plus 10 mg/kg xylazine; intraperitoneally) animals were bilaterally implanted with stainless steel 22-gauge guide cannulae aimed 1 mm above the CA1 region of the dorsal hippocampus (anterior, −4.2 mm; lateral, ±3.0 mm; ventral, −1.8 mm from bregma) [13] and fixed to the skull with dental acrylic. Animals were allowed 7 days to recover from surgery before experimental procedures and after that were handled once daily for 3 consecutive days. The CFC apparatus was a chamber within a ventilated sound attenuating box (Panlab®. Barcelona, Spain) with aluminum walls (35 × 35 × 35 cm), a transparent plastic front lid and a floor of parallel stainless-steel grid bars connected to a device to delivery the footshocks. The chamber was cleaned with 70% ethanol between animals. Freezing behavior (defined as the absence of all visible movement except for respiratory-related movements) was scored and converted into a percentage of time. On Day 1 (CFC training session), animals were placed into the conditioning chamber for 2 min. Then three electrical scrambled footshocks (0.5 mA, 2 s) were delivered with a 30-s interval between them. Animals were removed from the conditioning chamber 30 s after the last foot shock and placed back in their home cages. On Day 2, animals were placed in the same conditioning chamber for a 10-min Ext Tr session of CFC, without the footshocks. On Day 3, animals were placed again in the same apparatus for a 3-min Ext Test, without the footshocks [14]. Administration of drug into the CA1 region of the hippocampus occurred 20 min before or immediately after the extinction training session. The animals were gently restrained by hand, and an infusion needle (30 gauge) was fitted tightly into the guides, extending 1 mm from the tip of the guide cannulae. The infusion needle was attached to a polyethylene tubing (PE10, Plastics One), connected to a 10-µL Hamilton microsyringe, and infusion was performed at a rate of 1.0 µL/60 s. The infusion needle was left in place for one additional minute after the microinfusion to minimize backflow and then carefully withdrawn and placed on the other side. All treatments were bilateral. The drugs were freshly dissolved in sterile saline 0.9% and the doses used were methylphenidate (MPH; 12.5 µg per side, Novartis®); the β-adrenergic receptor antagonist, Timolol (Tim; 1.0 µg per side, Sigma-Aldrich); and the D1-family dopamine receptor antagonist, SCH-23390 (SCH; 1.5 µg per side, Sigma-Aldrich), microinfused in a total volume of 1.0 µL per side into the CA1 region. The number of animals for each experimental group was between 10 and 12 animals. Correct cannulae placements were verified 2–4 days after the end of the last behavioral procedure. Animals were infused with a 4% methylene blue solution over 30 s into the CA1 region of the dorsal hippocampus (1.0 µL/side) at the coordinates mentioned above. After 30 min, the animals were sacrificed by excess anesthesia and the brains were removed and kept in 10% formalin. The extension of the spread of the dye was considered to represent an estimate of the amount of drug infused. Cannula placement was considered correct when the spread was ≤1 mm from the intended infusion site; this occurred in 98% of the animals [4,14].

Fig. 1. Effect of MPH given into the hippocampus before or after the extinction training session. Animals were subjected to a CFC task. After 24 h they received intra-CA1 infusion of Veh (saline 0.9%) or MPH (12.5 g per side) 20 min before (A) or immediately after (B) the Ext Tr of CFC, and then were subjected to a 3-min Ext Test 24 h later. (A) Animals that received MPH 20 min before the Ext Tr expressed a decreased freezing behavior when compared with the Veh group, in both Ext Tr and Ext Test sessions. (B) Animals that received MPH immediately after the Ext Tr expressed the same freezing behavior in the Ext Test as the Veh group. The figure shows the percentage of time spent freezing in the first 2 min of the CFC session, in the first 3 min and last 3 min of the Ext Tr and in the Ext Test. Data are expressed as mean ± SEM (n = 10–12 animals per group). ***P < 0.001 vs. Veh group in the first 3 min of the Ext Tr, Newman–Keuls test after one-way ANOVA.

Thus, to verify the effect of MPH given into the hippocampus before or after extinction training, animals were trained in CFC and 24 h later they were placed again for 10 min in the CFC compartment and received no foot shock (Ext Tr). Animals received intra-CA1 infusions of vehicle (Veh; saline 0.9%) or MPH (12.5 µg per side) 20 min before or immediately after the Ext Tr of CFC, and 24 h later they were subjected to a 3-min Ext Test [14]. As shown in Fig. 1A, animals that received MPH into the CA1 before the Ext Tr expressed less freezing behavior compared with the Veh-treated animals during the Ext Tr and during the Ext test, indicating that animals that received intra-CA1 infusion of MPH were able to learn the extinction of CFC, while the Veh–treated animals were not. The animals that received MPH intra-CA1 immediately after the Ext Tr expressed the same freezing behavior in the Ext Test as the Veh group, indicating that none of the groups were able to learn the extinction of CFC (Fig. 1B). To investigate whether the effect of MPH on extinction memory is regulated by β-noradrenergic and D1-dopaminergic receptors, animals received intra-CA1 infusions of vehicle (Veh; saline 0.9%), MPH (12.5 µg per side) plus Tim (1.0 µg
Fig. 2. Effect of MPH plus Timolol and MPH plus SCH23390 given into the hippocampus before the extinction training session. Animals were subjected to a CFC task. After 24 h they received intra-CA1 infusion of Veh, MPH plus Timolol (12.5 μg per side plus 1.0 μg per side) or MPH plus SCH-23390 (12.5 μg per side plus 1.5 μg per side) 20 min before the Ext Tr of CFC, and then were subjected to a 3-min Ext Test 24 h later. Animals that received MPH plus Tim and MPH plus SCH-23390 before the Ext Tr expressed less freezing behavior compared with the Veh-treated animals during the Ext Tr. However, all the groups (Veh, MPH plus Tim, MPH plus SCH-23390) exhibited similar levels of freezing during the extinction retention test. The figure shows the percentage of time spent freezing in the first 2 min of the CFC session, in the first 3 min and last 3 min of the Ext Tr, and in the Ext Test. Data are expressed as mean ± SEM (n = 10–12 animals per group). ***P < 0.001 vs. Veh group in the first 3 min of the Ext Tr, Newman–Keuls test after one-way ANOVA.

In agreement with what happens in LTP into the hippocampus, similar results were seen in the learning of extinction of contextual fear conditioning, since the facilitation of extinction induced by MPH was blocked by the administration of both β-adrenergic and D1/D5 dopamine receptors antagonists. The present results provide an important addition to the knowledge of modulation of extinction memory, demonstrating that the psychostimulant MPH facilitates this process and its effect is regulated by D1-dopamine and β-noradrenergic receptors.

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