Behavioural Brain Research 326 (2017) 303–306

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Short communication

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

Cristiane R.G. Furini[a,b], Jonny A.K. Behling[a], Carolina G. Zinn[a], Mara Lise Zanini[c], Eduardo Assis Brasil[a], Luiza Doro Pereira[a], Ivan Izquierdo[a,b,*], Jociane de Carvalho Myskiw[a,b,*]

[a] Memory Center, Brain Institute, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Av. Ipiranga, 6690–2nd Floor, 90610-000, Porto Alegre, RS, Brazil
[b] National Institute of Translational Neuroscience (INNT), National Research Council of Brazil, Brazil
[c] College of Chemistry, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Av. Ipiranga, 6681, 90619-900. Porto Alegre, RS, Brazil

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.

ARTICLE INFO

Article history:
Received 25 January 2017
Received in revised form 16 March 2017
Accepted 18 March 2017
Available online 21 March 2017

Keywords:
Extinction memory
Methylphenidate
Dopamine receptors
Noradrenaline receptors
Contextual fear conditioning
Hippocampus

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.

Extinction is defined as the learned inhibition of retrieval [1]. It is the mainstay of exposure therapy, which is widely used to treat drug addiction, phobias and fear disorders such as post-traumatic stress disorder (PTSD) [2,3]. Until recently, it was believed that extinction requires retrieval involving memory destabilization and relevant protein synthesis-dependent processes in hippocampus and amygdala [2]. However, new findings have shown that retrieval performance is not necessary for the initiation, maintenance or spontaneous recovery of extinction [4]. The psychostimulant, methylphenidate (MPH; Ritalin®, Concerta®) is known to increase extracellular levels of noradrenaline and dopamine by blocking their reuptake [5–7]. In addition to its use to treat the attention deficit and hyperactivity disorder (ADHD), studies have demonstrated that MPH can modulate hippocampal physiology and/or

* Corresponding authors at: Av. Ipiranga, 6690 – IPB – 2nd Floor, HSL – Pontifical Catholic University of Rio Grande do Sul (PUCRS), 90610-000, Porto Alegre, RS, Brazil.
E-mail addresses: izquier@terra.com.br (I. Izquierdo), jociane_carvalho@hotmail.com (J. de Carvalho Myskiw).

http://dx.doi.org/10.1016/j.bbr.2017.03.027
0166-4328/

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 (CrlCembe: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].

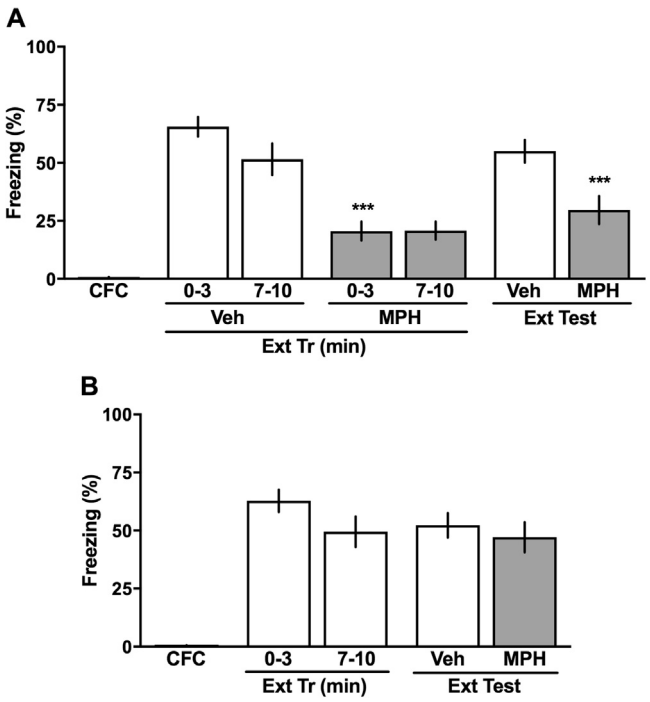

Fig. 1. Effect of MPH given into the hippocampus before or after the extinction training session. Animals were subjected to a CFC task. After 24 h they received intra-CA1 infusion of Veh (saline 0.9%) or MPH (12.5 (g per side) 20 min before (A) or immediately after (B) the Ext Tr of CFC, and then were 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.

The obtained data are expressed as mean ± SEM and were analyzed statistically by one-way ANOVA followed by Newman–Keuls Test using GraphPad Prism software. Differences between groups below $P < 0.05$ were considered statistically significant.

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

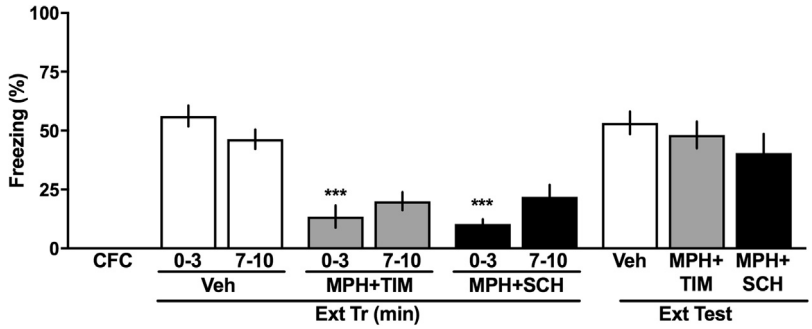

Fig. 2. Effect of MPH plus Timolol and MPH plus SCH23390 given into the hippocampus before the extinction training session. Animals were subjected to a CFC task. After 24 h they received intra-CA1 infusion of Veh, MPH plus Timolol (12.5 μg per side plus 1.0 μg per side) or MPH plus SCH-23390 (12.5 μg 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.

per side) or MPH (12.5 μg per side) plus SCH-23390 (1.5 μg per side), 20 min before the Ext Tr of CFC and 24 h later they were subjected to a 3-min Ext Test [14]. As shown in Fig. 2, animals that received MPH plus Tim and MPH plus SCH into the CA1 before the Ext Tr, expressed less freezing behavior compared with the Veh-treated animals during the Ext Tr. However, during the Ext Test, all the groups (Veh, MPH plus Tim and MPH plus SCH) exhibited similar levels of freezing, indicating that the blockade of the β-adrenergic receptors and the D1-family receptors reversed the effect induced by the MPH.

Here we verified that the MPH infused bilaterally into the CA1 region of the hippocampus was able to induce the consolidation of extinction memory in a protocol that is too weak by itself to induce the learning of extinction [14], and that this process occurs through both β-adrenergic and D1-dopaminergic family receptors. Previous studies reported that the systemic administration of MPH affects learning and memory of different behavioral tasks [8,15–18]. Concerning extinction process, MPH (2.5–10 mg/kg) was reported to enhance the extinction of fear memory when administrated before extinction session [19]. Regarding its administration into brain structures related with memory, the infusion of MPH in the prefrontal cortex of rats improved working memory performance [20], in the basolateral amygdala or *nucleus accumbens* enhances fear memory consolidation [21] and in the lateral amygdala enhances cue-reward learning [18]. Although the effects of MPH have been amply investigated, its corresponding cellular and molecular mechanisms of action, specifically those related with memory process, are still controversial and few have studied the connection of MPH to the noradrenergic or dopaminergic systems [see 22]. Recent studies propose that noradrenergic and dopaminergic systems in hippocampus could interact supporting a polysynaptic effect of MPH on hippocampal synapses [23,24]. In hippocampal slices of mice, MPH was able to induce LTP, in a protocol that is too weak to induce LTP by itself, and the inhibition of D1-dopamine or β-noradrenergic receptors reduced the average LTP amplitude induced by the MPH protocol. Similar results were observed on *in vivo* LTP on the perforant path of hippocampus [9]. Also, in hippocampal slices of young rats acute application of MPH enhances LTP in CA3-CA1 synapses in a dose-dependent way, and the MPH-dependent increase of LTP involves both β-noradrenergic and D1/D5 dopamine receptors [10]. Also, recently it has been described a new molecular mechanism at the perforant pathway-hippocampal CA1 synapses that are associated to the erasing of retrieved memories and which involves the adenosine A1 receptors [25].

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.

Acknowledgments

Work supported by grants from the National Research Council of Brazil (CNPq) and CAPES.

References

[1] I.P. Pavlov, G.V. Anrep, Conditioned Reflexes: An Investigation of the Physiological Activity of the Cerebral Cortex, Oxford University Press, S.l.], 1927.
[2] M.R. Milad, G.J. Quirk, Fear extinction as a model for translational neuroscience: ten years of progress, Annu. Rev. Psychol. 63 (2012) 129–151, http://dx.doi.org/10.1146/annurev.psych.121208.131631.
[3] M.R. Milad, B.L. Rosenbaum, N.M. Simon, Neuroscience of fear extinction: implications for assessment and treatment of fear-based and anxiety related disorders, Behav. Res. Ther. 62 (2014) 17–23, http://dx.doi.org/10.1016/j.brat.2014.08.006.
[4] J. de Carvalho Myskiw, C.R.G. Furini, B. Schmidt, F. Ferreira, I. Izquierdo, Extinction learning, which consists of the inhibition of retrieval, can be learned without retrieval, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) E230–233, http://dx.doi.org/10.1073/pnas.1423465112.
[5] C.W. Berridge, D.M. Devilbiss, M.E. Andrzejewski, A.F.T. Arnsten, A.E. Kelley, B. Schmeichel, C. Hamilton, R.C. Spencer, Methylphenidate preferentially increases catecholamine neurotransmission within the prefrontal cortex at low doses that enhance cognitive function, Biol. Psychiatry 60 (2006) 1111–1120, http://dx.doi.org/10.1016/j.biopsych.2006.04.022.
[6] E.J. Dommett, E.L. Henderson, M.S. Westwell, S.A. Greenfield, Methylphenidate amplifies long-term plasticity in the hippocampus via noradrenergic mechanisms, Learn. Mem. 15 (2008) 580–586, http://dx.doi.org/10.1101/lm.1092608.
[7] J.A. Bethancourt, Z.Z. Camarena, G.B. Britton, Exposure to oral methylphenidate from adolescence through young adulthood produces transient effects on hippocampal-sensitive memory in rats, Behav. Brain Res. 202 (2009) 50–57, http://dx.doi.org/10.1016/j.bbr.2009.03.015.
[8] S.A. Carmack, C.L. Block, K.K. Howell, S.G. Anagnostaras, Methylphenidate enhances acquisition and retention of spatial memory, Neurosci. Lett. 567 (2014) 45–50, http://dx.doi.org/10.1016/j.neulet.2014.03.029.
[9] D. Jenson, K. Yang, A. Acevedo-Rodriguez, A. Levine, J.I. Broussard, J. Tang, J.A. Dani, Dopamine and norepinephrine receptors participate in methylphenidate enhancement of in vivo hippocampal synaptic plasticity, Neuropharmacology 90 (2015) 23–32, http://dx.doi.org/10.1016/j.neuropharm.2014.10.029.
[10] C. Rozas, C. Carvallo, D. Contreras, M. Carreño, G. Ugarte, R. Delgado, M.L. Zeise, B. Morales, Methylphenidate amplifies long-term potentiation in rat hippocampus CA1 area involving the insertion of AMPA receptors by

activation of β-adrenergic and D1/D5 receptors, Neuropharmacology 99 (2015) 15–27, http://dx.doi.org/10.1016/j.neuropharm.2015.07.003.
[11] N.G. Fiorenza, J. Rosa, I. Izquierdo, J.C. Myskiw, Modulation of the extinction of two different fear-motivated tasks in three distinct brain areas, Behav. Brain Res. 232 (2012) 210–216, http://dx.doi.org/10.1016/j.bbr.2012.04.015.
[12] N. Singewald, C. Schmuckermair, N. Whittle, A. Holmes, K.J. Ressler, Pharmacology of cognitive enhancers for exposure-based therapy of fear, anxiety and trauma-related disorders, Pharmacol. Ther. 149 (2015) 150–190, http://dx.doi.org/10.1016/j.pharmthera.2014.12.004.
[13] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, Academic Press, San Diego, 1986.
[14] J. de Carvalho Myskiw, F. Benetti, I. Izquierdo, Behavioral tagging of extinction learning, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 1071–1076, http://dx.doi.org/10.1073/pnas.1220875110.
[15] S.A. Carmack, K.K. Howell, K. Rasaei, E.T. Reas, S.G. Anagnostaras, Animal model of methylphenidate's long-term memory-enhancing effects, Learn. Mem. 21 (2014) 82–89, http://dx.doi.org/10.1101/lm.033613.113.
[16] C.J. Heyser, M. Pelletier, J.S. Ferris, The effects of methylphenidate on novel object exploration in weanling and periadolescent rats, Ann. N. Y. Acad. Sci. 1021 (2004) 465–469, http://dx.doi.org/10.1196/annals.1308.066.
[17] D. Yonkov, W. Wetzel, H. Matthies, K. Roussinov, Improvement of shuttle-box avoidance by combinations of orotic acid and central stimulants, Psychopharmacology (Berl.) 73 (1981) 399–401.
[18] K.M. Tye, L.D. Tye, J.J. Cone, E.F. Hekkelman, P.H. Janak, A. Bonci, Methylphenidate facilitates learning-induced amygdala plasticity, Nat. Neurosci. 13 (2010) 475–481, http://dx.doi.org/10.1038/nn.2506.
[19] A.D. Abraham, C.L. Cunningham, K.M. Lattal, Methylphenidate enhances extinction of contextual fear, Learn. Mem. 19 (2012) 67–72, http://dx.doi.org/10.1101/lm.024752.111.
[20] R.C. Spencer, R.M. Klein, C.W. Berridge, Psychostimulants act within the prefrontal cortex to improve cognitive function, Biol. Psychiatry 72 (2012) 221–227, http://dx.doi.org/10.1016/j.biopsych.2011.12.002.
[21] X. Zheng, F. Liu, X. Wu, B. Li, Infusion of methylphenidate into the basolateral nucleus of amygdala or anterior cingulate cortex enhances fear memory consolidation in rats, Sci. China Ser. C: Life Sci. 51 (2008) 808–813, http://dx.doi.org/10.1007/s11427-008-0105-x.
[22] K. Koda, Y. Ago, Y. Cong, Y. Kita, K. Takuma, T. Matsuda, Effects of acute and chronic administration of atomoxetine and methylphenidate on extracellular levels of noradrenaline, dopamine and serotonin in the prefrontal cortex and striatum of mice, J. Neurochem. 114 (2010) 259–270, http://dx.doi.org/10.1111/j.1471-4159.2010.06750.x.
[23] A. Borgkvist, T. Malmlöf, K. Feltmann, M. Lindskog, B. Schilström, Dopamine in the hippocampus is cleared by the norepinephrine transporter, Int. J. Neuropsychopharmacol. (2011) 1–10, http://dx.doi.org/10.1017/s1461145711000812.
[24] C.C. Smith, R.W. Greene, CNS dopamine transmission mediated by noradrenergic innervation, J. Neurosci. 32 (2012) 6072–6080, http://dx.doi.org/10.1523/JNEUROSCI.6486-11.2012.
[25] N. Madroñal, J.M. Delgado-García, A. Fernández-Guizán, J. Chatterjee, M. Köhn, C. Mattucci, A. Jain, T. Tsetsenis, A. Illarionova, V. Grinevich, C.T. Gross, A. Gruart, Rapid erasure of hippocampal memory following inhibition of dentate gyrus granule cells, Nat. Commun. 7 (2016) 10923, http://dx.doi.org/10.1038/ncomms10923.